CURRENT APPROACHES IN TRANSPLANT IMMUNOLOGY

İbrahim PIRIM

CURRENT APPROACHES IN TRANSPLANT IMMUNOLOGY

Editorial Board:
Prof. Dr. Ibrahim PIRIM

Review:
Seyma Nur CELIK, Derya EROGLU

CURRENT APPROACHES IN TRANSPLANT IMMUNOLOGY

2024, İZMİR/TURKEY

Genel Koordinatör / General Coordinator
Öğr. Gör. Yaşar ALDIRMAZ

Editör / Editor
Prof. Dr. İbrahim PIRIM

Sertifika No / Certificate No
48888

Tasarım / Design
Büşra UYAR
Muhammed Sami TEKİN

Basım / Printing
Necmettin Erbakan University Press (NEU PRESS)
Yaka Mah. Yeni Meram Cad. Kasım Halife Sok. B Blok
No: 11 Meram / KONYA / TÜRKİYE
0332 221 0 575 - www.neupress.org

ISBN
978-625-94338-7-5

E-ISBN
978-625-94338-4-4

Tüm Hakları Saklıdır. Bu kitabın basım, yayın, satış hakları İKÇÜ Yayınevinin izni alınmadan kitabın tümü ya da bölümleri herhangi bir bilgi depolama ve erişim sistemiyle veya mekanik, elektronik, fotokopi, manyetik ya da başka yöntemlerle çoğaltılamaz.

All Rights Reserved. All or parts of this book cannot be reproduced by any information storage and access system or by mechanical, electronic, photocopying, magnetic or other methods without the permission of IKCU Publishing House.

Contents

SECTION 1 - TRANSPLANT IMMUNOLOGY
NOMENCLATURE OF HUMAN LEUKOCYTE ANTIGENS

1.1 Introduction 1
1.2 Human Leukocyte Antigens 2
1.3 Naming Human Leukocyte Antigens 5
1.4 Conclusion 15
1.5 References 16

INVESTIGATION OF HUMAN LEUKOCYTE ANTIGEN EPITOPES IN ORGAN TRANSPLANTATION

2.1 Introduction 19
2.1.1 Antibody (Immunoglobulin, IgG) 20
2.1.2 Antigen 21
2.2 Antibody-Antigen Binding 22
2.3 Conclusion 40
2.4 References 41

REJECTION MECHANISM AND BIOMARKERS IN KIDNEY TRANSPLANTATION

3.1 Introduction 43
3.2 Human Leucocyte Antigens (HLA) 44
3.3 Allorecognition 47
3.3.1 Direct Recognition 48
3.3.2 Indirect Recognition 50
3.3.3 Semi Direct Recognition 51
3.4 Mechanisms Of Rejection 52
3.5 Transplantation And Biomarkers 56
3.6 Conclusion 62
3.7 References 63

THE EMERGING ROLE OF NON-HLA ANTIBODIES IN TRANSPLANTATION

4.1 Introduction 67
4.2 Minor Histocompatibility Antibodies 68
4.3 Antibodies Against Anti-Angiotensin Receptor 69
4.4 MIC Antibodies 70
4.5 Collagen Antibodies 71
4.6 K-A 1 Tubulin Antibodies 72
4.7 Anti Lg3 Antibodies – Perlecan 72
4.8 Antibodies Against Myosin and Vimentin 73
4.9 Conclusion 74
4.10 References 75

miRNAs IN ORGAN TRANSPLANTATION

5.1 Introduction 83
5.2 miRNA Biosynthesis and Function 84
5.3 The Detection of miRNA Expression Profile 87
5.4 The Role of miRNA in Kidney Transplantation Patients 88
5.5 Conclusion 90
5.6 References 91

OVERVIEW OF THE COMPLEMENT SYSTEM IN KIDNEY TRANSPLANTS

6.1 Introduction 95
6.2 Complement System 96
6.3 Complement Activation Pathways 99
6.3.1 Alternative Complement Pathway 99
6.3.2 Lectin Complement Pathway 100
6.3.3 Classic Complement Pathway 101
6.4 Activation of The Complement System in Kidney Transplantation 104
6.5 Immunsuppressive Agents That Provide Inhibition of The Complement System 114
6.6 Conclusion 120
6.7 References 121

INFLAMMATION AND KIDNEY DISEASES

7.1 Introduction 127
7.2 Exogenous Inducers 133
7.3 Endogenous Inducers 136
7.4 Inflammatory Mediators 142

7.5 Conclusion 148
7.6 References 148

A BIOMARKER IN RENAL TRANSPLANTATION: DONOR – DERIVED CELL FREE DNAS

8.1 Introduction 153
8.2 What Is A Cell Free DNA? 154
8.3 The Isolation And Analysis Of cfDNAs 156
8.4 Donor Derived Cell Free DNA and Transplantation 157
8.5 cfDNA And Renal Transplantation 159
8.6 Conclusion 160
8.7 References 161

EXOSOME IN ORGAN TRANSPLANTATION

9.1 Introduction 165
9.2 Extracellular Vesicle Types 166
9.3 Extracellular Vesicle and Transplantation 172
9.4 Investigation Of Extracellular Vesicles In Biologic Materials 177
9.5 Conclusion 190
9.6 References 190

THE FUNCTION OF WNT SIGNALING IN KIDNEY TRANSPLANTATION

10.1 Introduction 195
10.2 Canonical β-Catenin/Wnt Signaling Pathway 197
10.3 Non-Canonical Wnt Signaling Pathway 199
10.4 Relationship Between Kidney and Wnt Signalling Pathway 200
10.5 Wnt Signaling Pathway in Transplantation 205
10.6 Conclusion 208
10.7 References 208

IMMUNE CHECKPOINT INHIBITORS (ICI) TREATMENTS IN SOLID ORGAN TRANSPLANTATION PATIENTS WITH CANCER

11.1 Introduction 213
11.2 Cancer and The Immune System 214
11.3 Immunotolerance and Immunosurveillance 214
11.3.1 Immunoediting 215
11.3.2 Immune Checkpoint Inhibitors 215
11.3.3 Anti-PD-1 Monoclonal Antibodies 217
11.3.4 Anti-PDL-1 Monoclonal Antibodies 217
11.3.5 Anti-CTLA-4 Monoclonal Antibodies 218

11.4 Cancer and ICI After Transplantation 219
11.4.1 How Safe Are ICIs for Cancer Patients Undergoing Organ Transplantation? 221
11.4.2 Mechanisms and Risk Factors Of Rejection Stimulated By Ici 222
11.5 Use of ICI in Common Organ Transplants 226
11.5.1 Kidney Transplantation 226
11.5.2 Liver Transplantation 228
11.6 The Utilization of ICIs in Rare Transplantion Cases 230
11.6.1 Lung Transplantation 230
11.6.2 Heart Transplantation 231
11.6.3 CAR T-Cell 231
11.7 Conclusion 232
11.8 References 233

SECTION 2
MESENCHYMAL STEM CELLS: SOURCES, REGENERATION POTENTIAL AND USAGE IN CELL THERAPY

12.1 Introduction 243
12.2 Sources Of Mesenchymal Stem Cells 245
12.3 Differentiation Potential Of Mesenchymal Stem Cells 247
12.3.1 Osteogenic and Chondrogenic Differentiation 248
12.3.2 Neuronal Differentiation 249
12.3.3 Adipogenic Differentiation 250
12.3.4 Myogenic Differentiation 251
12.3.5 Endothelial Cell Differentiation 252
12.4 MSC Usage In Cell Therapy 253
12.5 Conclusion 257
12.6 References 258

MESENCHYMAL STEM CELL AND IMMUNE SYSTEM

13.1 Introduction 271
13.2 Immune System Cells And Mesenchymal Stem Cells 273
13.2.1 Adaptive Immune Cells 273
13.2.1.1 T Cells 273
13.2.1.2 B Cells 276
13.2.2 Native Immune Cells 279
13.2.2.1 Naturel Killer Cells 279
13.2.2.2 Dendritic Cells 285
13.2.2.3 Monocytes and Macrophage 288
13.2.2.4 Neutrophils 290

13.2.2.5 Mast Cells 292
13.3. Immun Molecules and MSCs 293
13.3.1 Indoleamine- 2,3 Dioxygenase (IDO) 293
13.3.2 Human Leukocyte Antigen-G (HLA-G) 294
13.3.3 Prostaglandin E2 (PGE-2) 295
13.3.4 Inducible Niitric Oxide Synthase (INOS) 296
13.3.5 Interleukin 10 (IL- 10) 297
13.3.6 Other Mediators 297
13.4 Extracellular Vesicles (EVs) and MSC Vesicles 297
13.5 Conclusion 306
13.6 References 307

KEY TRANSCRIPTION FACTORS IN MESENCHYMAL STEM CELL DIFFERENTIATION

14.1 Introduction 215
14.2 Mesenchymal Differentiation 316
14.2.1 Adipogenic Differentiation 316
14.2.2 Osteogenic Differentiation 318
14.2.3 Chondrogenic Differentiation 321
14.2.4 Myogenic Differentiation 324
14.2.4.1 Skeletal Muscle Cells 324
14.2.4.2 Cardiomyocytes 326
14.2.4.3 Smooth Muscle Cells 327
14.2.5 Endotelial Cell Differentiation 329
14.3 Alternative Splays in Mesenchymal Stem Cell Differentiation 333
14.3.1 Alternative Splays and Regulation Mechanisms 334
14.3.2 Direction of Stem Cells with AS 336
14.3.3 Control of Osteogenic Transformation by AS 337
14.3.4 Regulation of Chondrogenic Differentiation by AS 339
14.3.5 Regulation of Adipogenic Differentiation by AS 339
14.4 References 341

EPIGENETIC CHANGES IN MESENCHYMAL STEM CELLS DIFFERANTIATION

15.1 Introduction 349
15.2 Epigenetic Mechanisms 351
15.2.1 DNA Methylation 351
15.2.2 Histone Modifications 353
15.2.3 Chromatin Remodeling 356
15.2.4 Non-Coding RNA Gene Regulation 358
15.3 Conclusion 361
15.4 References 362

EXTRACELLULAR MATRIX DYNAMICS IN THE DEVELOPMENT OF MESENCHYMAL STEM CELL

16.1 Introduction 367
16.2 MSC Self- Renewal and Differentiation 368
16.3 Extracellular Matrix in MSC Microenvironment 369
16.4 Extracellular Matrix Role in MSC Development and Differentiation ..370
16.4.1 In Adipose Tissue 371
16.4.2 In Chondrogenic Tissue 372
16.4.3 In Osteogenic Tissue 372
16.5 Conclusion 373
16.6 References 373

POTENTIAL APPLICATIONS OF MESENCHYMAL STEM CELLS IN KIDNEY DISEASES

17.1 Introduction 375
17.1.1 Totipotent Stem Cells 376
17.1.2 Pluripotent Stem Cells 376
17.1.3 Multipotent Stem Cells 376
17.1.4 Unipotent Stem Cells 376
17.2 Characteristics Of MSC Treatment 379
17.2.1 Source 379
17.2.2 Potency 380
17.2.3 Route Of Delivery 380
17.2.4 Dose 380
17.2.5 Timing 381
17.2.6 Homing 381
17.2.7 Engraftment 381
17.2.8 Safety Profile 382
17.2.9 Proliferation / Thriving 382
17.3 Extracellular Vesicles (EVs) 382
17.4 Studies on Kidney Disorders Conducted with MSC or MSC Derived EVs
....383
17.4.1 AKI 383
17.4.2 Experimental Studies on Msc 384
17.5 Clinical Trials on Mscs 385
17.5.1 Studies with EVs 385
17.5.2 Renovascular Diseases 386
17.5.2.1 Experimental Studies On MSCs 386
17.5.2.2 Clinical Trials on MSCs 386
17.5.2.3 Studies With EVs 387
17.5.3 CKD 388

17.5.3.1 Experimental Studies On MSCs 388
17.5.3.2 Clinical Trials On MSCs 388
17.5.3.3 Studies With EVs 388
17.5.4 Diabetic Nephropathy 389
17.5.4.1 Experimental Studies On MSCs 389
17.5.4.2 Clinical Trials On MSCs 389
17.5.4.3 Studies With EVs 389
17.5.5 Lupus Nephritis 389
17.5.5.1 Experimental Studies On MSCs 390
17.5.5.2 Clinical Trials On MSCs 391
17.5.5.3 Studies On EVs 391
17.5.6 Kidney Transplantation 392
17.5.6.1 Experimental Studies On MSCs 392
17.5.6.2 Clinical Trials 393
17.5.6.3 Studies With EVs 394
17.6 Conclusion 395
17.7 References 396

MESENCHYMAL STEM CELL AND CRISPR TECHNOLOGY

18.1 Introduction 409
18.2 General Mechanism 410
18.2.1 Adaptation 412
18.2.2 Expression And Maturation Phase (Biogenesis) 413
18.2.3 Intervention Phase 414
18.3 Current Use of CRISPR-Cas 9 Technology 415
18.4 Conclusion 419
18.5 References 420

BRIEF INTRODUCTION ON ADSCS, WJMSCS, SSCS AND STEMNESS PROPERTIES, STEM CELL SENESCENCE AND REGENERATIVE POTENTIAL 425

METHODS FOR THEIR ISOLATION, CHARACTERIZATION AND DIFFERENTIATION TOWARD DIFFERENT PHENOTYPES 435

Improving the Vocational Competency in the Potential Use of Mesenchymal Stem Cells

in Kidney Transplants and Patients

"Erasmus+ Small Scale Partnership in Vocational Education and Training"

2022-1-TR01-KA210-VET-000084758

https://msctx.ikcu.edu.tr

"The European Commission's support for the production of this publication does not

constitute an endorsement of the contents, which reflect the views only of the authors,

and the Commission cannot be held responsible for any use which may be made of the

information contained therein."

SECTION 1

TRANSPLANT IMMUNOLOGY

NOMENCLATURE OF HUMAN LEUKOCYTE ANTIGENS

Tülay KILICASLAN AYNA[1,2,3]
[1]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[3]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

1.1 INTRODUCTION

The major histocompatibility complex (MHC) is essential in the organism's defense against foreign antigens. Human leukocyte antigens (HLAs) are MHC molecules that were initially discovered in leukocytes in humans. These molecules, which have garnered growing attention since their identification, are among the most genetically diverse genes in humans. The HLA complex, situated on the p-arm of chromosome 6, encompasses areas for class I, II, and III. The HLA-A, -B, and -C genes located in the class I region, as well as the HLA-DR, -DQ, and -DP genes located in the class II region, are the most genetically diverse genes within the complex. In January 2016, a grand total of 14,232 HLA alleles were officially reported, consisting of 10,574 alleles belonging to the HLA class I category and 3,658 alleles belonging to the HLA class II category. Given the polymorphic nature of HLA, a systematic nomenclature was required. With an increase in the num-

ber of alleles, the comprehension and utilization of the HLA naming system undergo some modifications. The correlation between patient and donor HLA type compatibility in solid organ transplants and bone marrow stem cell transplants significantly impacts the success of the graft and the survival of the patient. Furthermore, research has demonstrated a correlation between certain HLA alleles and several diseases. Furthermore, the nomenclature of an HLA allele conveys specific details like the methodology employed to study the allele, its expression properties, and the specific area within the gene where the polymorphism occurs. Consequently, it is believed that the historical progression of HLA and HLA allele data will be valuable to professionals in areas such as organ transplantation, infection, and auto-immune illnesses.

1.2 HUMAN LEUKOCYTE ANTIGENS

The human major histocompatibility complex (MHC) resides in a specific area of around 4 megabases on the short arm of chromosome 6, known as 6p21.3 (Figure 1). The MHC region, which contains the human leukocyte antigen [HLA] genes, has been the subject of extensive research for the past four decades due to its significant involvement in disease susceptibility and transplant compatibility [1,2].

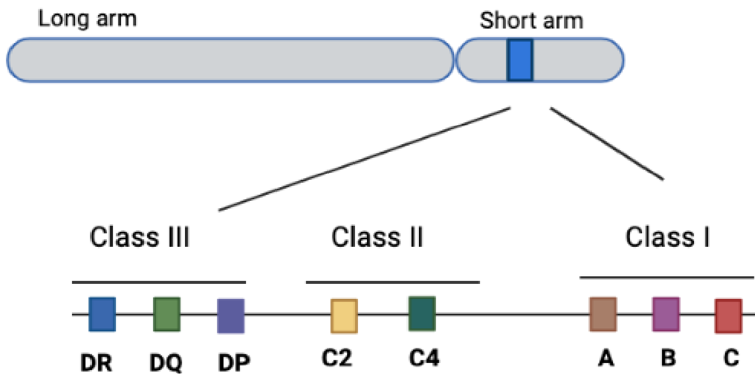


Figure 1: Gene map of the HLA region

The identification of HLA genes was achieved by experiments conducted on domestic mouse and rabbit strains. In 1916, Little and Tyzzer conducted tumor transplantation experiments among different mouse breeds and observed that tumors may be successfully transplanted between certain strains, while being rejected in others. In 1927, Bover noted that tissue transplantation carried out on identical twins did not result in rejection. These observations suggest that the genetic makeup of individuals plays a crucial role in determining tissue compatibility between donors and recipients[1,2]. In 1933, Haldane contended that the immune response responsible for rejecting the transplanted tumor is initiated by regular cellular antigens rather than antigens peculiar to the tumor. In the late 1930s, Gorer identified four blood group antigens, referred to as “antigens I, II, III, and IV.” He observed a correlation between the presence or absence of these antigens and the development or rejection of tumors.

In the 1940s, Medavar et al. showed that rabbits rejected grafts because their immune systems attacked foreign tissue. Subsequently, Snell and his colleagues selectively developed many strains of laboratory mice (known as congenic lines) that were almost genetically identical, with the exception of a specific genetic region responsible for the rejection of foreign tissues. Snell designated these genes responsible for regulating tissue rejection as histocompatibility (H) genes[4]. Subsequent research has demonstrated that several genes responsible for tissue rejection are situated on the same chromosome. The current designation for this area is MHC. It is also known as HLA since 1975, was initially identified in leukocytes in humans. The HLA complex consists of HLA class I regions close to the telomere, Class III located in the middle, and class II regions located close to the centromere. This region contains a total of 220 genes, 21 of which are immune system-related genes[4].

The significance of class I genes HLA-A, B, C and class II genes HLA-DR, DQ and DP is well-established in the context of solid organ and bone marrow transplantations. The significance of these genes arises from the proteins they encode. Cell surface-expressed Class I HLA molecules impede the activity of exogenous peptides, such as viral proteins, that originate from within the organism. Class II HLA molecules are significant in presenting extrinsic peptides, which are acquired through endocytosis, to helper T-cells. Although they also have a role in the presentation to cytotoxic T-cells. B2 microglobulin, derived from a gene located on chromosome 15, plays a crucial role in the presentation of antigens by Class I HLA molecules. The DNA area encoding HLA-A and C class I genes comprises 8 exons, while the HLA-B region comprises 7 exons. The initial exon is responsible for encoding the leader peptide, whereas exons 2 and 3 encode the α1 and α2 domains, which constitute the antigen-binding portion of the class I

molecule. This section is the most genetically diverse region in the class molecules. Consequently, it serves as the foundation for typing class I molecules. Class II molecules, in contrast to class I HLA molecules, are formed by the combination of two distinct genes situated on chromosome 6. The genes are referred to as alpha (A) and beta (B) genes. Gene A is composed of 5 exons, while gene B is composed of 6 exons. The A1 and A2 domains are produced from the A gene, while the B1 and B2 domains are produced from the B gene. The a1 and b1 domains in class II molecules function as locations for binding antigens. The second exon of the genes encodes these domains, which form the most polymorphic portion of class II molecules. Thus, exon 2 is regarded as the fundamental exon in the typing of class II molecules[5,6].

1.3 NAMING HUMAN LEUKOCYTE ANTIGENS

A systematic nomenclature is required for the polymorphism in the genes that encode HLA molecules. The regulations pertaining to this classification system are commonly referred to as HLA nomenclature[7]. The initial investigation into the historical progression and terminology of HLA commenced in the latter part of the 1950s. In 1958, Dausset, Payne, and Van Rood published three investigations on the HLA complex. All three papers shared the characteristic of identifying antigens in human leukocytes by utilizing serum acquired from women who had received blood transfusions or had given birth multiple times. Among these three researchers, Dausset was the one who initially discovered an antigen and subsequently termed it MAC. The term was formed by combining the initials of three individuals who willingly took part in Dausset's research[8]. The antigen was detected in 60% of the French population. In the conclusion of the research, Dausset stated that the examination of leukocyte antigens could have signi-

ficant implications in tissue transplantation, particularly in the context of bone marrow transplantation. Dausset was awarded the Nobel Prize in Physiology and Medicine in 1980 for this discovery, which he shared with Snell and Benacarraf. In 1962, Van Rodd and Payne conducted a serological test on leukocytes obtained from 100 individuals using the serum of 60 women who had given birth multiple times. The public antigens, currently referred to as Bw4 and Bw6, were identified and designated as 4a and 4b [8]. Currently, it is understood that these formations are situated inside sections 79-83 of HLA molecules that are encoded by the B locus, as well as some A locus products such as A*23 and A*24. There exist distinct amino acid sequences located at locations [9]. In 1964, Payne identified three antigens which are today referred to as HLA-A1, HLA-A2, and HLA-A3. These antigens were originally designated as leukocyte antigen (LA)-1, LA-2, and LA-3. Furthermore, the inaugural discussions on HLA commenced in 1964, alongside these aforementioned breakthroughs. The inaugural workshop was hosted at Duke University, marking the commencement of the HLA meetings. The event brought together 16 laboratories and 23 individuals. During the discussion, it was determined that HLA typing of 8 distinct cells would be conducted using seven distinct approaches. There was a total of 16 workshops. In 1964, Bach, Bian, and Vas et al. conducted a study to investigate alterations in cell proliferation by combining leukocytes from two distinct persons. The HLA nomenclature discussion began to form during the second workshop [7,10]. The rationale behind this decision is the inconsistency in the nomenclature of the same antigen across various researchers. For instance, the antigen currently referred to as HLA-A2 was designated as LA-2 by Dausset MAC, Payne, and Bodmers, as 8a by Van Rood, and as Te2 by Terasaki [8].

1967 Workshop The term "HLA haplotype" refers to combinations of HLA antigens that are encoded by genes located on the same chromosome. This expression was initially introduced by Cepellini. The gene region was initially designated as HL-A. The nomenclatures of H Dausset's Hu-1 and Payne's LA were merged, ensuring recognition to the pioneering researchers in this field. The "World Health Organization (WHO) Nomenclature Committee for the "Factors of the HLA System" committee was founded in 1968 [3]. To date, the committee has released a total of 19 main reports. Initially, these publications documented HLA alleles that were identified by serological methods. However, more recently, they have started reporting genes and alleles that were identified through nucleotide sequences. The initial identification of the HL-A antigens by serological methods in 1968 resulted in the naming of the first 8 antigens. The following are the HL-A types: HL-A1, HL-A2, HL-A3, HL-A4, HL-A5, HL-A6, HL-A7, HL-A8. In 1969, Dausset, Terasaki, and Walford made the initial discovery of the first antigens that are currently encoded from the C locus. Simultaneously, the Scandinavian antigen detection group also discovered analogous antigens. Given that this particular antigen was the initial antigen identified, it was designated as AJ. During the 4th workshop convened in 1970, an additional four antigens were designated. The following are the HL-A types: HL-A10, HL-A11, HL-A12, and HL-A13. The HL-A9 was absent from the list throughout this time frame. There has been an observed occurrence of cross-reactivity between the two constituent antigens of HL-A9. However, during the 5th meeting, the committee presented the notion of extensive specificity for A9. Wide range of applicability. The individual elements are referred to as splits. Consequently, the HLA nomenclature incorporated the terms "public antigen" and "split antigen".

Four phases have been recognized in the process of designating histocompatibility antigens during this workshop[1]. A laboratory provides a report on the specificity[2]. If multiple reference laboratories confirm this specificity, a provisional number with the prefix "w" is assigned[3]. The HL-A number is assigned if all reference laboratories unanimously agree on this new specificity[4]. The specificity is determined through chemical and molecular analyses[8].

In 1975, it was discovered that the specific characteristics of the histocompatibility system, as assessed by serological methods, were dictated by two distinct genes. Furthermore, each gene was shown to have several variations, known as alleles. Consequently, two genes were assigned distinct names. The decision has been made to eliminate HL-A. The two genetic areas are designated as HLA-A and HLA-B. The nomenclature of the antigens identified thus far has been updated based on their respective gene regions. The antigens HL-A1, HL-A2, HL-A3, HL-A9, HLA10, and HL-A11 are referred to as HLA-A1, HLA-A2, HLA-A3, HLA-A9, HLA-A10, and HLA-A11, respectively. Similarly, the antigens HL-A7, HL-A7, HL-A8, HL-A12, and HL-A13 are designated as HLA-B5, HLA-B7, HLA-B12, and HLA-B13.

The technique of mixed lymphocyte culture (MLC) has shown to be highly influential. Amos developed cell proliferation research in 1964. In this study, we assessed the varying rates of cell proliferation when class I HLA antigens were mixed with suitable cells and allowed to grow. It was believed that there was an additional genetic location, apart from HLA-A and B, which had HLA markers that triggered the response to MLC, and it was designated as Dw. In 1977, the public was introduced to Bw4 and Bw6. The term used to refer to it is "epitope". These epitopes are found on many HLA-B antigens,

as previously stated. In the early 1980s, it was discovered that the HLA region had six distinct polymorphic sequences (Table 1). In 1984, Ekkehard Albert and Wolfgang Mayr made the discovery of DP, also known as HLADQ. The serological techniques identified the presence of DQw1, DQw2, and DQw3. Two novel DR antigens, DR52 and DR53, were designated in the same year [7]. DR52 and DR53, which are produced by secondary DR genes, are alternatively referred to as pubic antigens. These compounds are co-expressed with specific DR antigens. The correlation between secondary DR antigens and class I pubic antigens remained unclear for an extended period of time. Nevertheless, contemporary knowledge confirms that DR molecules consist solely of a solitary α chain gene, and all DR molecules possess an identical α chain. There exists a total of nine DRB1 genes. Out of these genes, DRA1, DRB1, DRB3, DRB4, and DRB5 produce proteins that are expressed in the membrane, but DRB2, DRB6, DRB7, DRB8, and DRB9 genes are not expressed. Genes are thought to be created by the process of gene duplication, when a single ancestral gene is duplicated, and new mutations arise as a result of this duplication. Regarding the expression of a DRB1 molecule, there are four distinct DR heterodimers that can be present on the surface of a cell. The four types are DRA/DRB1, DRA/DRB3, DRA/DRB4, and DRA/DRB5. The numbers 11 and 12.

Table 1: Polymorphisms in HLA class I molecules triplets

Position	HLA-A	HLA-B	HLA-C
9	FSTY	DHY	DFSY
12	sV	sV aM Av	sV hunting
14	R.	R.	R.W.
17	gr gS	Gr	gr sR
41	A.	HORSE	A.
45	Me Kme	Ee Te Ge Ke Ma GeV	Ge
56	GRE	g	g
62	Rn Qe Well Lq Ge	Rn Re Ge Re	Re
66	rKv rNm gKv rNm	qKy qlc qls qly qlf rNm	qKy qNy
70	aqs aHs	aQa tNt aKA baton rQa	rQa
74	DNH iD	DY	D aD
76	An Vd En Es	Es En Ed Etc Vg	Vs VN
80	GTI rla	rla rNI RTI rTa	rNI rKI
82	aLr 1 Rg	aLr irg Ilr	irg
90	IN	A.	NAME
105	P.S.	P.	P.
107	G.W.	g	g
127	NC	N	N
131	R.	RS	R.
138	T	T	KT
142	IT	I	I
144	tKr tKh tQr	tQr tQI sQr	tQr
147	W	W.L.	W.L.
149	aVh aah aAr tah	aAr	aAr
151	vHa aHv aHAe aRv aRw arrr aHa	aRv aRe	plus aRe Search
156	LWRQ	WLDR	LRW
158	HUNTING	HORSE	A.
163	RT Dt E	LET	PHONE
166	Dg Ew	Ew Es Dg	ew
171	YH	YH	Y
177	Meat	Dt Et Dk	Kt Meat
180	Q	QE	Q
184	dp In	dp	eP eH

186	KR	K	K
193	Av Pi	PiPv _	PvPl _
199	A.	HUNTING	A.
207	GS	g	g
246	AS Va	A.	A.
248	V	V	VM
253	Ee Ke Qe	Well	Well Eq.

The expression of DRA/DRB3, DRA/DRB4, or DRA/DRB5 heterodimers in all DR haplotypes relies on the presence of the DRA/DRB1 heterodimer. The DRB haplotypes that were expressed are displayed in Table 2 (Table 2). Pseudogenes in the HLA area were additionally identified in 1987. These genes are non-coding. Currently, numerous pseudogenes have been identified, such as HLA-H, I, K, L, P, T, U, V, W, X, and Y. The initial sequencing investigations of HLA genes commenced in the current year[13]. The HLA class I allele that was initially sequenced is HLA-B7.2 (as per the 2010 HLA nomenclature: HLA-B*07:01:02), while the HLA class II allele is HLADRA0101 (as per the 2010 HLA nomenclature: HLADRB1*01:01). Four distinct HLA-A2 alleles (A*0201, A*0202, A*0203 and A*0204) were discovered throughout this era. Each allele differs by at least one amino acid. During this time, a total of 12 class I HLA alleles and nine class II HLA alleles were designated. As the number of alleles grew, HLA typing started being referred to as four digits[14]. A three-person preliminary evaluation team of HLA alleles, comprising Bodmer, Marsh, and Parham, was established in 1989 to assess the growing number of alleles resulting from the broad adoption of molecular techniques. Significant HLA sequences have begun to be reported to the nomenclature committee[15]. In 1990, the HLA allele labels were extended to five digits in order to facilitate the differentiation of alleles with distinct synonymous alterations in coding DNA sequences (exons). While synonymous substitutions typically

entail alterations in nucleotides, they are considered silent mutations as they do not result in any changes to the amino acid sequence. Nevertheless, there are instances where these mutations that have the same meaning can impact the processes of transcription, splicing, mRNA transport, and translation, ultimately altering the observable characteristics of an organism. In this scenario, these are not silent mutations[7].

Table 2. DRB genes expressed in the cell membrane.

	DRB1*	**DRB3***	**DRB4***	**DRB5***
DRB1*01	X			
DRB1*15/16	X			X
DRB1*03	X	X		
DRB1*04	X		X	
DRB1*11/12	X	X		
DRB1*13/14	X	X		
DRB1*07	X		X	
DRB1*08	X			
DRB1*09	X		X	
DRB1*10	X			

In 1995, the HLA nomenclature committee extended the nomenclature to include seven digits, enabling the identification of alleles based on variations in introns or 3′-5′UTR. In this era, the internet network started to gain popularity and the information included in the HLA sequence database was made available to the public in 1995 through the website of the tissue typing laboratory of the Imperial Cancer Research Fund (ICRF). Additionally, in 1996, the Anthony Nolan Research Institute (ANRI) also published this information on their website. The "International ImMuno GeneTics (IMGT)/HLA" database, established in 1998, offers valuable data for the examination of nucleotide and protein sequences of HLA molecules. The database can be accessed at www.ebi.ac.uk/imgt/hla. The provided information is revised every three months[16,17].

The IMGT/HLA data bank receives help from tissue typing laboratories, corporations specializing in this domain, organizations like ASHI and EFI, as well as bone marrow donor banks. Starting in the early 2000s, the asterisk symbol was employed to differentiate tissue typing tests conducted by molecular techniques from those performed using serological methods. In 2002, the A*02, B*15 allele family was introduced. For cases involving more than ninety-nine allele numbers, the second number series was utilized to extend the first number series. The B*95 allele series was employed for the B *15 allele family, while the A*92 allele series subsequent to A*0299 was utilized for the A*02 allele family. The HLADP allele family of Class II molecules was also introduced in this period [18]. The HLA nomenclature system had enhancements in 2010. One method is to insert the colon (:) symbol between two digits in the alleles. This facilitates the assessment of alleles that have a length of up to eight digits. For instance, the allele denoted as HLA-A*01010101 prior to 2010 was modified to HLA-A*01:01:01:01 after 2010. Furthermore, the suffix appended to the allele conveys insights regarding protein expression. Examining this letter system, we see that

- The N allele represents a null allele, which is an allele that is not expressed.

- L (Low allele): This allele has a reduced amount of cell surface expression.

- S (Soluble allele): This term refers to a specific allele of a protein that is capable of being secreted but does not have any expression on the cell surface.

- The C allele, also known as the cytoplasmic allele, refers to the allele found in the cytoplasm that does not manifest itself on the cell surface.

- An aberrant allele is an allele that raises uncertainty regarding its expression in the protein.

- Q (Expression problematic allele): The mutation seen in this allele is the one that has been demonstrated to impact the regular level of expression [13].

The HLA-A*30:14L variant exhibits a mutation at codon 164, which codes for cysteine. This mutation is crucial for the formation of the disulfide link in α2 and results in reduced expression levels relative to the normal allele. Hence, the suffix L is appended. Some alleles have been found to lack cysteine amino acids at positions 101 and 164, as stated. The suffix Q was appended to establish a bracket for the expression levels of these alleles. Through more investigations of these alleles, the inserts have the potential to undergo alterations. Furthermore, alleles that possess identical peptide binding domains are denoted with the P suffix, while HLA alleles that exhibit identical nucleotide sequences for exons encoding peptide binding domains are denoted with the G suffix. Since 2010, the molecular technique has led to the removal of the w suffix in the study of HLA-C, and it is now represented as C* [14,19]. In January 2016, the IMGT/HLA data indicated that there was a total of 14,232 HLA alleles, with 10,574 belonging to class I and 3,658 belonging to class II. The information regarding these data is presented in Table 3 (Table 3).

Table 3. International imMunoGeneTics/Human leukocyte antigens data.

a) Class I HLA data										
Genes	**A.**	**B.**	**C.**	**TO**	**F**	**g**				
alleles	3,356	4,179	2,902	21	22	51				
proteins	2,372	3,095	2,067	8	4	17				
null alleles	155	131	101	one	0	2				
b) Class II HLA data										
Genes	**DRA**	**DRB**	**DQA1**	**DQB1**	**DPA1**	**DPB1**	**DMA**	**DMB**	**DOA**	**DOB**
alleles	7	1,976	55	900	43	630	7	13	12	13
proteins	2	1,442	33	615	21	518	4	7	3	5
null alleles	0	50	one	23	0	16	0	0	one	0
c) Data for the DRB gene family										
Genes	**DRB1**	**DRB2**	**DRB3**	**DRB4**	**DRB5**	**DRB6**	**DRB7**	**DRB8**	**DRB9**	
alleles	1,860	one	69	17	24	3	2	one	one	
proteins	1,357	0	55	10	20	0	0	0	0	
null alleles	44	0	one	3	2	0	0	0	0	

1.4 CONCLUSION

Currently, molecular techniques are widely utilized in HLA typing facilities for tissue type. The DNA sequence analysis approach yields typing data consisting of six to eight digits. The HLA compatibility between the patient and the donor is a crucial element that significantly impacts the success rate, particularly in bone marrow stem cell transplantation from unrelated donors. HLA compatibility is crucial in organ transplantation, particularly in kidney, lung, and heart transplantation, and has been recognized for a significant duration. Recognizing the significance of HLA typing in the healthcare sector, HLA meetings, first attended by 23 individuals, have evolved into expansive gatherings involving numerous prominent companies and thousands of participants.

1.5 REFERENCES

1. Salvadori L de C, Santana FC, Marcos EV. Frequency of alleles oath haplotypes of the human leukocyte antigen system in Bauru, São Paulo, Brazil. rev bras Hematol Hemoter 2014;36(2): 108-14. https://doi.org/10.5581/1516-8484.20140026

2. Maiers M , Gragert L, Madbouly A, Steiner D, Marsh SG, et al. 16 (th) IHIW: global analysis of registry HLA haplotypes from 20 million individuals : report from the IHIW Registry diversity Group. Int J Immunogenet 2013;40(1): 66-71. https://doi.org/10.1111/iji.12031

3. Thorsby E. A short history of HLA tissue Antigens 2009;74(2):101-16. https://doi.org/10.1111/j.1399-0039.2009.01291.x

4. Shiina T, Inoko H, Kulski JK. An update of the HLA genomics region, locus information oath disease associations: 2004. Tissue Antigens 2004;64(6): 631-49. https://doi.org/10.1111/j.1399-0039.2004.00327.x

5. Goldberg AC, Rizzo LV. [MHC structure oath function - antigen presentation _ Part 1]. Einstein (Sao Paulo) 2015;13(1):153-6. https://doi.org/ 10.1590/S1679-45082015RB3122

6. Goldberg AC, Rizzo LV. [MHC structure oath function - antigen presentation _ Part 2]. Einstein (Sao Paulo) 2015;13(1):157-62. https://doi.org/10.1590/S1679-45082015RB3123

7. Marsh SGE. HLA nomenclature. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FH, eds. HLA Complex in Biology Oath Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.79-85.

8. Thorsby E. History of HLA. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FHJ, eds. HLA Complex in Biology Oath Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.42-58. https://doi.org/10.1111/j.1399-0039.2009.01291.x

9. Lutz CT. human leukocyte antigen Bw4 and Bw6 epitopes recognized by antibodies oath natural killer cells _ curr Opin Organ Transplant 2014;19 (4):436-41. https://doi.org/10.1097/MOT.0000000000000103

10. Amos DB. Human histocompatibility locus HL-A. Science 1968;159(3815):659-60. https://doi.org/10.1126/science.159.3815.659-a

11. Tait BD. genetics structure oath functions of the major histocompatibility complex. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FHJ, eds. HLA Complex in Biology Oath Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.61-77.

12. RJ D. HLAMMATCHMAKER: a molecular based donor selection algorithm for highly alloimmunized patients. PG - 493-7 FAU - Duquesnoy, R J. CLSI Tissue Typing Laboratory, Division of Transplantation Pathology and Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.: Transplant Proc.; 2001.p. 493–7. https://doi.org/10.1016/s0041-1345(00)02108-4

13. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, et al. nomenclature for factors of the HLA system. tissue antigens 2010; 75:291 -455. https://doi.org/10.1111/j.1399-0039.2010.01466.x

14. Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Dupont B, et al. nomenclature for factors of the HLA system, 1990. Immunobiology 1991;182(3-4):334-45. https://doi.org/10.1016/S0171-2985(11)80669-8

15. Marsh SG, Bodmer JG. HLA class II region nucleotide sequences, 1994. Eur J Immunogenet 1994;21(6):519-51. https://doi.org/10.1111/j.1744-313x.1994.tb00223.x

16. Robinson J, Malik A, Parham P, Bodmer JG, Marsh SG. IMGT/HLA database --a sequence database for the human major histocompatibility complex. tissue Antigens 2000;55(3):280-7. https://doi.org/10.1034/j.1399-0039.2000.550314.x

17. Robinson J, Waller MJ, Parham P, Bodmer JG, Marsh SG. IMGT/HLA Database--a sequence database for the human major histocompatibility complex. nucleic Acids Res 2001;29(1):210- 3. https://doi.org/10.1093/nar/29.1.210

18. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, et al. nomenclature for factors of the HLA system, 2002. Tissue Antigens 2002;60(5):407-64. https://doi.org/10.1034/j.1399-0039.2002.600509.x

19. Robinson J, Halliwell JA, McWilliam H, Lopez R, Parham P, et al. The IMGT/HLA database. nucleic Acids Res 2013;41(Database issue): 1222-7. https://doi.org/10.1093/nar/gks949

INVESTIGATION OF HUMAN LEUKOCYTE ANTIGEN EPITOPES IN ORGAN TRANSPLANTATION

Tülay KILICASLAN AYNA[1,2,3], Ibrahim PIRIM[1,2,3]

[1]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[3]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

2.1 INTRODUCTION

Human leukocyte antigen (HLA) compatibility molecules are molecules that are encoded by genes located on the short arm of chromosome 6 in humans and are expressed on the cell surface [1]. These molecules are important in presenting protein antigens to immune system cells. HLA Class I molecules are involved in antigen presentation to CD8+ T cytotoxic cells; Class II molecules are responsible for antigen presentation to CD4+T helper cells. In solid organ and bone marrow transplantations, HLA compatibility between the patient and the donor is also important for the success of the transplant. Kidney transplantation is the most common solid organ transplantation in our country. Before kidney transplantation, HLA antibodies specific to the donor's HLAs should be carefully investigated in the patient [2,3]. Because the presence of these antibodies causes hyperacute and acute rejections.

At the same time, HLA antibodies may develop against donor HLAs in the post-transplant period [4]. This study aimed to shed light on the understanding of HLA antibody reactions. First of all, the two basic molecules that play the leading role in these reactions are antibodies and antigens.

2.1.1 Antibody (Immunoglobulin, IgG)

HLA antibodies are of the IgG isotype, which makes up 80% of the immunoglobulins (Ig) in the blood. These molecules consist of two heavy and two light polypeptides. The heavy chain in the Ig isotype gives the molecule its name. 60% of the light chains are in the k form and 40% are in the l form. The molecule binds to the antigen molecule with the region referred to as Fab. The first 100-110 amino acids in both the heavy and light chains constitute the variable region and play an important role in binding to antigen. In addition, amino acid residues called the complementary determining region (CDR) in the heavy and light chains are important in binding with antigen. Heavy and light Ig molecules contain three CDRs (CDRH1, CDRH2, CDRH3 and CDRL1, CDRL2 and CDRL3) in their variable regions. Especially CDRH3 plays a decisive role in binding with the antigenic molecule. Other CDR residues provide binding support (Figure 1) [5,6].

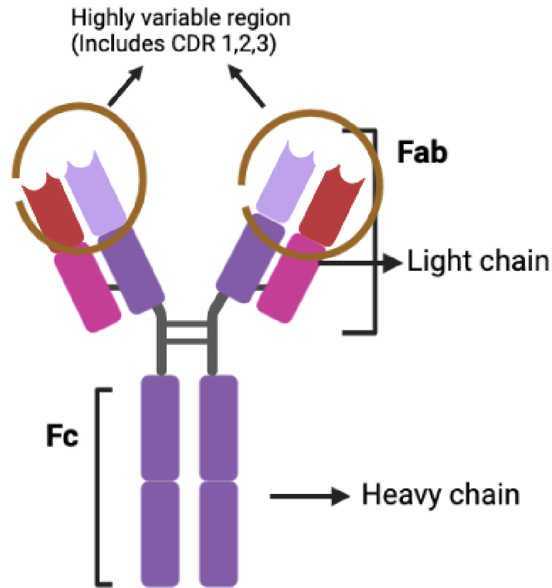


Figure 1: Antibody structure

2.1.2 Antigen

Molecules that are foreign to an organism are generally referred to as antigens. Parts of an antigen that show more than one antigenic feature are called epitopes (antigenic determinants). HLA molecules are one of the most polymorphic molecules known in humans. Therefore, it has more than one epitope. Generally, three types of epitope terms are used. While the structural epitope covers 15-22 residues in the binding region of the antigen and antibody, the functional epitope covers 2-5 residues that make direct contact with the CDR regions in the antibody. Cryptic epitopes are known as hidden epitopes. While these epitopes do not normally cause an antigenic stimulus, they are epitopes that emerge as a result of the breakdown of antigens taken into the cell by phagocytosis in lysosomes (Figure 2) [5,6].

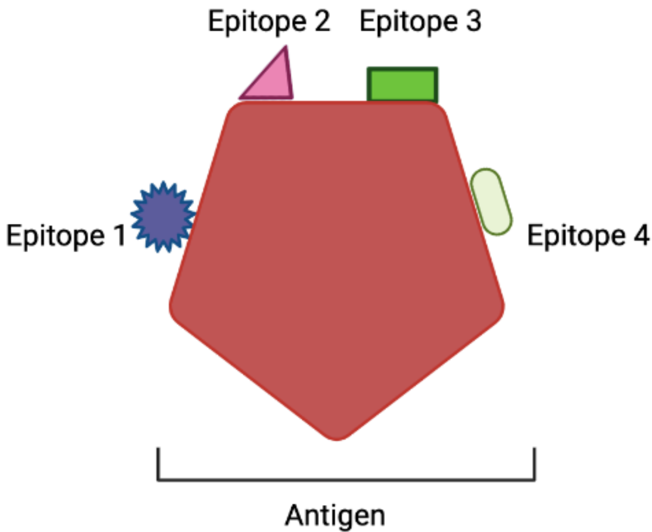


Figure 2: Antigen and epitope

2.2 ANTIBODY-ANTIGEN BINDING

Interactions between antigen and antibody molecules are important in binding. These interactions are established between amino acids that form polypeptide chains. Noncovalent bindings are important in reactions against antigen. It is known that 70-120 H bonds play a role in average antibody-antigen binding. In addition to H bonds, ionic bonds, "van der Waals" bonds and hydrophobic interactions, the structural harmony of the antigen and antibody is also important. As a result of the research, it was determined that the entire contact area on the antigen in humoral immune reactions was 690-900 angrom (Å). The binding groove of the HLA molecule, seen from above, also has a binding area of 750 Å. As a result of binding with antigen, changes occur in the three-dimensional structure of the antibody molecule, which plays an important role in the progression of the reaction [5,7,8].

In kidney transplantation, the similarity of graft survival rates between transplants from HLA fully compatible donors and transplants from low HLA compatible donors has led researchers to focus on the regions where HLA epitopes interact with antibodies [9]. In this context, the HLA "matchmaker" program started to be used by Duquesnoy in the early 2000s. 9-11HLA "matchmaker" is a computer program based on polymorphisms in the amino acid sequence of immunogenic epitopes that can elicit alloantibodies. There are basically two different versions of this program. The first version of the program was called triplet mismatch (tmm) and focused on HLA Class I molecules [12,13]. This is due to the fact that the immune reactions of T-cytotoxic cells stimulated by HLA Class I molecules cannot be controlled by immunosuppressive drug treatments. To determine the role of HLA Class I molecules in the immune reaction, the residues of the molecule that interact with the antibody were detected by X-ray crystallography studies. The polymorphisms of these residues are determined separately for the HLA-A, HLA-B and HLA-C loci. Accordingly, 30 polymorphic residues were detected in the HLA-A locus, 24 in the B locus and 19 in the C locus [11].

In the publication reported by Duquesnoy, polymorphic residues in the HLA Class I molecule are seen in Table 1 (Table 1) [11]. Amino acids that are in a position to reach the antibody are in the alpha-helix and beta loop parts of the protein [13,14]. The HLA "matchmaker" program is based on the triple aminoocide sequence of the tmm (incompatible) version and the antibody-interacting residues of the Class I antigen (residues refer to amino acids at certain positions). It determines how many TMMs there are between the recipient-donor pair by comparing the triplets of the patient and the donor[11,15].

Table 1. Polymorphic triplets in HLA Class I molecules.

Position	HLA-A	HLA-B	HLA-C
9	FSTY	DHY	DFSY
12	sV	sV aM Av	sV hunting
14	R.	R.	R.W.
17	gr gS	Gr	gr sR
41	A.	HORSE	A.
45	Me Kme	Ee Te Ge Ke Ma GeV	Ge
56	GRE	g	g
62	Rn Qe Well Lq Ge	Rn Re Ge Re	Re
66	rKv rNm gKv rNm	qKy qlc qls qly qlf rNm	qKy qNy
70	aqs aHs	aQa tNt aKA baton rQa	rQa
74	DNH iD	DY	D aD
76	An Vd En Es	Es En Ed Etc Vg	Vs VN
80	GTI rla	rla rNI RTI rTa	rNI rKI
82	aLr 1 Rg	aLr irg llr	irg
90	IN	A.	NAME
105	P.S.	P.	P.
107	G.W.	g	g
127	NC	N	N
131	R.	RS	R.
138	T	T	KT
142	IT	I	I
144	tKr tKh tQr	tQr tQI sQr	tQr
147	W	W.L.	W.L.
149	aVh aah aAr tah	aAr	aAr
151	vHa aHv aHAe aRv aRw arrr aHa	aRv aRe	plus aRe Search
156	LWRQ	WLDR	LRW
158	HUNTING	HORSE	A.
163	RT Dt E	LET	PHONE
166	Dg Ew	Ew Es Dg	ew
171	YH	YH	Y
177	Meat	Dt Et Dk	Kt Meat
180	Q	QE	Q
184	dp In	dp	eP eH
186	KR	K	K

193	Av Pi	PiPv _	PvPI _
199	A.	HUNTING	A.
207	GS	g	g
246	AS Va	A.	A.
248	V	V	VM
253	Ee Ke Qe	Well	Well Eq.

Triplet amino acid residues are named as shown in the figure (Figure 3). Monomorphic residues may not be specified in these triplets. This program has two basic principles. The first of these is that each HLA antigen has different polymorphic triplets; The second is the view that patients may not produce antibodies against epitopes carrying triplets of their own HLA antigens. Due to this feature, it is stated that it will increase the chance of transplantation in highly sensitive patients.

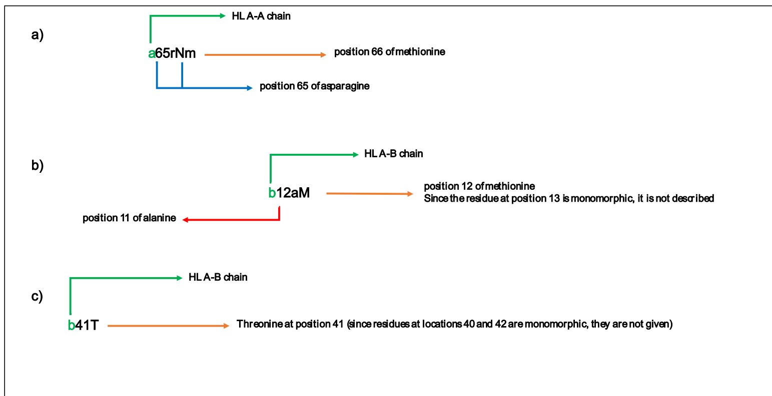


Figure 3: Nomenclature of polymorphic triplets in HLA molecule

The example from Duquesnoy's study in 2001 is shown in Table 2 (Table 2) [11]. Accordingly, HLA tissue type; Conside-ring that the highly sensitive kidney patient with HLA-A2, A31, B42, B53, Cw2 and Cw7 has 2 donors; HLA tissue type of the first donor; The tissue type of the second donor is A2, A30, B51, B60, Cw4, Cw7, and the tissue type of the second donor is A2, A32, B8, B55, Cw3, Cw6. When the HLA compatibility

between the patient and his two donors is evaluated, it is seen that both donors have similar Class I HLA compatibility with the patient. Table 2 compares the triplets in the antibody binding region of one B antigen of the patient and two donor candidates (Table 2).

Table 2. Investigation of triplet mismatches for HLA-60 and B-8 in a patient with tissue type HLA-A2,-A31,-B42,-B53,-Cw2,-Cw7

Patient	A2	A31	B42	B53	Cw2	Cw7	Donor 1 HLA B60	B8	Donor 2 HLA B60	B8
HLA Allele	A*0201	A*3101	B*4201	B*5301	Cw2*0202	Cw7*0701	B*4001	B*0801	B*4001	B*0801
Polymorphismler										
9	F	T	Y	Y	Y	D	H	D	NA	NA
12	sV	sV	sV	aM	aV	aV	aM	aM	NA	NA
14	R	R	R	R	R	R	R	R	NA	NA
17	gR	gR	gR	gR	gR	gR	gR	gR	NA	NA
41	A	A	A	A	A	A	T	A	NA	NA
45	Me	Me	Ee	Te	Ge	Ge	**Ke**	Ee	NA	NA
56	G	R	G	G	G	G	G	G	NA	NA
62	Ge	Qe	Rn	Rn	Re	Re	Re	Rn	NA	NA
66	rKv	rNv	qly	qlf	qKy	qKy	**qls**	qlf	NA	NA
70	aHs	aHs	aQa	tNt	rQa	rQa	tNt	tNt	NA	NA
74	H	İD	D	Y	D	aD	Y	D	NA	NA
76	Vd	Vd	Es	En	Vn	Vs	Es	Es	NA	NA
80	gTI	gTI	rNI	rIa	rKI	rNI	rNI	rNI	NA	NA
82	Irg	Irg	Irg	aLr	IRg	Irg	IRg	IRg	NA	NA
90	A	A	A	A	D	A	A	A	NA	NA
105	A	S	P	P	P	P	P	P	NA	NA
107	W	G	G	G	G	G	G	G	NA	NA
127	K	N	N	N	N	N	N	N	NA	NA
131	R	R	R	S	R	R	R	R	NA	NA
138	T	T	M	M	T	T	M	M	NA	NA
142	T	I	I	I	I	I			I	I
144	tKh	tQr	tQr	tQr	tQr	tQr	NA	NA	**sQr**	tQr
147	W	W	W	W	W	L	NA	NA	L	W
149	aAh	aAr	aAr	aAr	aAr	aAr	NA	NA	aAr	aAr
151	aHv	aRv	aRv	aRv	aRe	aRa	NA	NA	aRv	aRv

156	L	L	D	L	W	L	NA	NA	L	W
158	A	A	A	A	A	A	NA	NA	A	A
163	T	T	T	T	E	T	NA	NA	E	T
166	Ew	Ew	Ew	Ew	Ew	Ew	NA	NA	Ew	Ew
171	Y	Y	Y	Y	Y	Y	NA	NA	Y	Y
177	Et	Et	DT	Et	Et	Et	NA	NA	**Dk**	Dt
180	Q	Q	E	Q	Q	Q	NA	NA	E	E
184	A	P	P	P	Eh	Ep	NA	NA	P	P
186	K	K	K	K	K	K	NA	NA	K	K
193	Av	Av	Pl	Pv	Pv	Pl	NA	NA	Pl	Pl
199	A	A	A	A	A	A	NA	NA	A	A
207	S	S	G	G	G	G	NA	NA	G	G
246	A	s	A	A	A	A	NA	NA	A	A
248	V	V	V	V	V	V	NA	NA	V	V
253	Q	Q	E	E	E	Eq	NA	NA	E	E

NA: Not applicable Uncommon triplets are shown in bold and underlined

As a result of the intra-locus and inter-locus comparison of the B60 antigen of the first donor, there were 6 tmm; It appears that the second donor is not complete with the B8 antigen. Apart from the mentioned antigens, it is also 0 tmm with A32, A74, B35, B56 and B59 antigens [11]. Additionally, when all HLA antigens of the donors and the patient's HLA antigens were compared in terms of tmm, a total of 14 [A2 (0 tmm), A30 (4 tmm), B51 (1 tmm), B60 (6 tmm), Cw4 (3 tmm) were detected between the first donor and the patient), while Cw7 (0 tmm)] tmm is present, the number of tmm between the second donor and the patient is 0 [A2 (0 tmm), A32 (0 tmm), B8 (0 tmm), Cw3 (0 tmm) and Cw6 (0 tmm)] is. In this case, the suitable donor is donor 2. Once the HLA tissue type that the patient can accept is determined by panel reactive antibody (PRA) tests, the HLA "matchmaker" program also determines the additional tissue type that can be accepted. The HLA tissue type of a highly sensitive patient and the HLA antigens with a negative PRA result are shown in Table 3 (A1, A33, B8, Cw8) (Table 3) [11].

Table 3. Acceptable HLA antigens (negative reactions) detected as a result of testing with a cell panel of highly sensitive patients, and triplets of these antigens incompatible with the HLA "matchmaker".

Patient	A2	A11	B7	B36	Cw2	Cw7	A11	A33	Negative reactions B8	Cw8
Position										
9	Y	Y	Y	Y	Y	D	F	T	Y	Y
12	sV	sV	sV	aV	aV		sV	sV	**aM**	aV
14	R	R	R	R	R	R	R	R	R	R
17	Gr	Gr	Gr	Gr	Gr	Gr	Gr	Gr	Gr	Gr
41	A	A	A	A	A	A	A	A	A	A
45	Me	Me	Ee	Ee	Ge	Ge	Me	Me	**Te**	Ge
56	G	G	G	G	G	G	G	G	G	G
62	Qe	Qe	Rn	Rn	Re	Re	Qe	Rn	Rn	Re
66	rNv	rNv	qIy	qIc	qKy	qNy	rNv	rNv	**qIf**	qKy
70	aQs	aQs	aQa	tNt	rQa	rQa	aQs	**aHs**	tNt	rQa
74	D	D	D	Y	D	AD	D	**ID**	D	D
76	Vd	Vd	Es	En	Vn	Vs	Vd	Vd	Es	Vs
80	gTL	gTL	rNl	rIa	rKl	rNl	rNl	gTL	rNl	rNl
82	lRg	lRg	lRg	aLr	lRg	lRg	lRg	lRg	lRg	lRg
90	D	D	A	A	A	D	A	A	A	A
105	P	P	P	P	P	P	S	S	P	P
107	G	G	G	G	G	G	G	G	G	G
127	N	N	N	N	N	N	N	N	N	N
131	R	R	S	S	R	R	R	R	S	R
138	T	T	M	M	T	T	T	T	M	T
142	I	I	I	I	I	I	I	I	I	I
144	tKr	tKr	tQr	tQr	tQr	tQr	tKr	tQr	tQr	tQr
147	W	W	W	W	W	L	W	W	W	W
149	aAh	aAh	aAr	aAr	aAr	aAr	aAh	aAr	aAr	aAr
151	aHa	aHa	aRv	aRv	aRe	aRa	**aHe**	aRv	aRe	**aRt**
156	Q	Q	L	L	W	L	L	L	L	L
158	A	A	T	T	A	A	A	A	A	A
163	R	R	T	T	E	T	**dT**	T	L	T
166	Ew	Ew	Ew	Ew	Ew	Ew	Ew	Ew	Ew	Ew
171	Y	Y	Y	Y	Y	Y	Y	**H**	**H**	Y
177	Et	Et	Et	Et	Et	Et	Et	Et	Et	Kt
180	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
184	Dp	Dp	Dp	Dp	eH	eP	dP	dP	dP	eH
186	K	K	K	K	K	K	K	**R**	K	K
193	Pi	Pi	Pi	Pi	Pv	Pl	Pi	**Av**	Pv	Pv

199	A	A	A	A	A	A	A	A	A	A
207	G	G	G	G	G	G	G	S	G	G
246	A	A	A	A	A	A	A	S	A	A
248	V	V	V	V	V	V	V	V	V	V
253	E	E	E	E	E	E	E	Q	E	E

Uncommon triplets are shown in bold and underlined

Here, firstly, according to the PRA result, the triplets of antigens that the patient can accept are determined. As seen above, although there was tmm [A1 (2 tmm), A33 (10 tmm), B8 (4 tmm), Cw8 (1 tmm)], the PRA result was determined to be negative. According to the HLA "matchmaker" program, the patient's HLA type is 0 tmm with HLA-A69, B39, B67. By determining the tmm level of the HLA antigens that cause a negative PRA result, other HLA antigens with similar tmm are determined. This patient also has low TMM with HLA-A32, A36, A74, B42, B54, B55, B59, B64, B65. Thus, the number of HLA antigens that the patient can accept increases. This means that patients with high PRA have an increased chance of transplantation [11]. Recognition of several different HLA antigens by a single antibody is known as cross-reactivity between HLAs. Cross-reactions arise from common regions in the amino acid sequences between cross-reactive group (CREG) antigens. To put it another way, HLAs have an epitope set consisting of more than one epitope. Some of these epitopes are specific to that HLA molecule, while others may be shared by other HLA molecules[6,16]. As a result, an antibody specific to an A epitope will create an immune reaction with other HLA molecules carrying the A epitope. By comparing these epitopes, the HLA "matchmaker" program also contributes to the determination of the donor with less TMM. The HLA "matchmaker" program results of two different patients (patient X and Y) with the same CREG in Duquesnoy's study are shown in Table 4 (Table 4)[11].

Table 4. Tmm results of two different patients (patient X and patient Y) in the same CREG with HLA "matchmaker" program

Patient X HLA type	Incompatible tmms despite not having the same CREG	Patient Y HLA type	Incompatible tmms despite being the same CREG
A2	A68 (2) A69 (1)	A2	A68 (2) A69 (1) A29 (4) A30 (5) A31 (3) A33 (4) A74 (0)
A31	A29 (3) A30 (4) A32 (0) A33 (2) A74 (0) B7 (2) B8 (0) B27 (6) B54 (1) B55 (0)	A32	B7 (4) B8 (3) B27 (7) B42 (3) B54 (1)
B42	B56 (0) B60 (6) B61 (4)	B55	B56 (0) B60 (9) B61 (5)
B53	B35 (0) B49 (4) B50 (4) B51 (1) B52 (2) B57 (3) B58 (2) B70 (1)	B35	B49 (5) B50 (4) B51 (2) B52 (3) B53 (1) B57 (4) B58 (3) B70 (1)

HLA: Human leukocyte antigens; CREG: Cross-reactive group; tmm: triplet mismatch.

In recent years, the second version of the HLA "matchmaker" program, eplet, has started to be used. A comparison of Class I and Class II HLAs is made with the Eplet version. In addition to TMM where structural epitopes are evaluated, the eplet program focuses on functional epitopes. Here, polymorphic residues and other residues in the immediate vicinity are evaluated. There are two different epitope structures in structural epitopes. The first of these is known as one patch. Here, for the epitope to be antigenic, there must be at least one nonself residue and this part must come into contact with the CDR regions of the antibody. The second one is called two patches. There may be two different situations in the two-patch epitope model. Both patches may contain at least one nonself residue, or one patch may have one or more nonself residues while the other has self-residues [7,9].

Although the humoral mechanisms are not fully understood, it is known that each individual has a B cell repertoire with low avidity for epitopes of receptors expressed on their own cells. These epitopes do not cause activation of B cells and antibody production. However, when nonself residues occur, self-residues contribute to B cell activation [10]. In the immune reaction, the distance between neighboring residues in patch patterns is important. This distance is 3- 3.5 Å. Additionally, in the two-patch model, the distance between patches should be around 7.5-14 Å. According to Eplet version, Class I molecules (HLA-A, B, C) have a total of 530 patches. Most of these patches are in the α1 and α2 regions of the molecule. There are 192 patches between residues 62-73, 91 patches between residues 76-83, and 122 patches between residues 142-152. Patches of some residues also include pockets where the HLA molecule presents antigen. The residues in the 3 and 3.5 Å patches given by Duquesnoy in his study in 2006 and those that are polymorphic are shown in Table 5 (Table 5). When Class II molecules were evaluated in terms of their patches, 74 patches were identified in DR, 103 in DQ and 64 in DP. The patches of both Class I and Class II molecules are mostly on the top and side surfaces of the molecule, although a small number of patches are on the bottom or bottom of the HLAs [7,17,18].

Table 5. Polymorphic and monomorphic residues of HLA Class I antigens at position 3.0-3.5. Å

Sequence position	Class I locus	Molecular location	Surface exposure	Positions in the 3 Å patch	Positions in the 3,5 Å patch
1	C	On the side	+	**1** 2	**1** 2 3
6	C	On the side	+	5 **6** 27	5 **6** 27
9	ABC	On the side	±	8 **9** 10	8 **9** 10 23
12	BC	On the lower side	+	**11** **12** 13 **21**	**11** **12** 13 **21** 92 **94**
14	C	On the side	+	**14** 15 16 19 39	**14** 15 16 **17** 18 19 39
17	A	On the side	++	**14** 16 **17** 18	**14** 15 16 **17** 18
21	C	On the lower side	±	20 **21** 22 39	**14** 19 20 **21** 22 23 39
30	B	On the lower side	+	29 **30** 31 21 1	29 **30** 31 210 211
32	B	On the lower side	±	31 **32** 33	27 31 **32** 33 48
35	C	On the side	±	**35** 36 48 85 3	**35** 36 46 48 853
41	B	On the side	++	40 **41** 42	40 **41** 42 43
44	A	On the side	++	43 **44** **45**	36 42 43 **44** **45** 61
45	B	On the side	+	**44** **45** **46**	35 **44** **45** **46** 64
46	B	On the side	+	35 **45** **46** 47	35 44 **45** **46** 47 48
56	A	On the side	+	55 **56** 57 59	54 55 **56** 57 58 59
62	A	At the top	++	61 **62** **63**	59 61 **62** **63** 64 **65** **66**
63	ABC	At the top	±	59 62 **63** 64 P2	59 61 **62** **63** 64 **65** **66** P2
65	ABC	At the top	++	61 64 **65** **66** **69**	61 62 64 **65** **66** 68 **69**
66	ABC	At the top	++	**65** **66** **70** P2	**62** 64 65 **66** 68 **69** **70** P1 P2
69	B	At the top	++	**65** 68 **69** **70** **73**	**65** **66** 68 **69** **70** 72 **73**

70	ABC	At the top	+	**66** **69** **70**	**66** 68 **69** **70** 72 **73**
71	B	On the side	+	**70** **71** 72	68 **69** **70** **71** 72 **73** 75
73	AC	At the top	+	72 **73** **77**	**69** **70** 72 **73** **77**
76	A	At the top	++	75 **76** **77** **80**	72 **73** 75 **76** **77** 79 **80** P9
77	ABC	At the top	±	**73** **76** **77** 78 P8 P9	**73** 75 **76** **77** 78 79 **80** P8 P9
79	ABC	At the top	++	78 **79** **80**	**76** 78 **79** **80** **82**
80	ABC	At the top	++	**76** **79** **80** 84	**76** 78 **79** **80** **82** **83** 84
82	AB	On the side	+	**80** **82** 86 87	**79** **80** **82** **83** 86 87 88 89
83	AB	At the top	++	**82** **83** 84 86	**79** **80** **82** **83** 84 85 86
90	ABC	On the side	++	89 **90** 91	88 89 **90** 91
94	BC	On the lower side	±	93 **94** **95** 119	93 **94** **95** 118 119
103	BC	On the side	±	2 **103** 104	2 **103** 104 108 110
105	A	On the side	++	104 **105** 106	**1** 104 **105** 106 **107**
107	A	On the side	++	160 **107** 108 169	**105** 106 **107** 108 169 173 **180**
109	A	On the side	+	108 **109** 110	108 **109** 110 111 112
113	BC	On the lower side	+	102 112 **113** **114**	98 102 112 **113** **114**
114	ABC	On the lower side	±	**113** **114** 115 126	98 **113** **114** 115 125 126
116	ABC	On the lower side	±	115 **116** 123 124	115 **116** 123 124
127	A	On the side	++	**127** 128 132	**127** 128 129 132 133 134
131	B	On the side	++	129 130 **131** 132	129 130 **131** 132
138	C	On the side	++	137 **138** 139 141	137 **138** 139 140 141

142	A	At the top	++	**138** 141 **142**	**138** 139 141 **142** **144** **145** 146
143	B	At the top	±	**142** **143** **144** P9	141 **142** **143** **144** 145 146 P9
144	A	On the side	+	**144** **145** 148 141 **144** **145** 146	133 141 **142** **144** **145** 146 148
147	BC	At the top	±	146 **147** 148	**151** P8
149	A	At the top	++	**145** 148 **149** **150**	**145** 146 148 **149** **150** **151**
150	A	At the top	+	**149** **150** **151**	146 148 **149** **150** **151**
151	A	At the top	++	**150** **151** **152**	148 **149** **150** **151** 154 155
152	ABC	At the top	±	**151** **152** 155	**150** **151** **152** 154 155 P7
158	AB	At the top	++	157 **158** 159	154 155 157 **158** 159
161	A	At the top	++	157 **161** 162	157 159 **161** 162
163	A	At the top	+	162 **163** **167**	159 162 **163** **166** **167** P1
166	A	At the top	++	162 165 **166** **167**	162 163 165 **166** **167** 169 170
167	AB	At the top	++	**163** **166** **167**	**163** 165 **166** **167** 169 170
173	C	On the side	++	169 172 **173** 174	169 170 172 **173** 174 176
177	ABC	On the side	++	176 **177** **178**	176 **177** **178**
178	B	On the side	+	**177** **178** 181	176 **177** **178** **180** 181

Polymorphic positions are shown in bold and underlined.

HLA-A25 and HLA-A26; It is one of the subgroups of the HLA-A10 antigen (split). They are molecules that are very similar to each other in terms of molecular structure. Both have the 150TAH patch. 79-83 in the HLA-A25 molecule. The amino acids are arginine, isoleucine, alanine, leucine and arginine,

respectively. IgG antibody to this antigen is at 150 TAH patch with VH CDR3 and at 79-83 TAH patch with VL CDR3. is bound to residues. The distance between the two patches is 14 Å. In the HLA-A26 molecule, 79-83 as well as the 150TAH patch. The amino acids are glycine, threonine, leucine, arginine and glycine. The distance between the two patches is more than 14 Å. In this case, even if the HLA-A25-specific antibody (monoclonal antibody) binds to the HLA-A26 antigen, the immune reaction does not occur. This is due to the inability to achieve the necessary structural change for the antibody to bind to the C1q molecule (which activates the antibody-dependent complement pathway). This feature is called cytotoxicity-negative absorption-positive (CYNAP) [7,18].

In another publication, it was determined that the HLA-A3-specific antibody formed as a result of pregnancy binds to the 62QE patch of HLA-A3. It has been determined that this antibody gives an immune reaction with all antigens containing the 62QE patch except A30 and A31. When the epitope properties of the HLAs tested with anti-HLA A3 monoclonal antibody with the HLA "matchmaker" program were had alanine amino acid in the 56th position (second patch), while there was arginine amino acid in A30 and A31. These two examples demonstrate the effectiveness of the second patch in immune reactions. This similar patch feature in CREGs is important in humoral reactions [7,14].

The Eplet program evaluates "overlapping" groups of polymorphic patches shared by HLAs. While the results are evaluated in the Eplet program, HLA typing, which is studied with high resolution, is also important in understanding these patch properties and antibody reactions. For example, HLA-A23 and HLA-A24 are subgroups of HLA-A9. If an antibody specific to the 63 EEK and 65 GK patches in the A*2301, A*2402,

A*2403 and A*2407 alleles develops, an immune reaction occurs with all four alleles. While the 66 EGKQ patch in A*2407 has glutamine (Q) at position 70; A*2301, A*2402, A*2403 contain histidine (H) at position 70 of the same patch. In this case, the monoclonal antibody against A*2407 is specific for 70Q and reacts only with A*2407 [19,20].

In many countries, as in our country, only DRB1 genes from HLA Class II molecules are typed before kidney transplantation. In addition, when DRB1, DRB3, DRB4, DRB5 molecules are compared between the recipient and donor with the eplet program, DRB3, DRB4, DRB5 molecules significantly affect the number of incompatible eplets (representing single or double patches) in the recipient-donor pair. HLA-DQ typing, one of the Class II molecules, is also gaining importance. As a matter of fact, in recent years, many publications have reported that anti-HLA-DQ antibodies are formed after kidney transplantation [21,23]. In his study in 2008, Duquesnoy showed that DQ eplets, as well as DR genes, contribute to incompatibility in the eplet program (Table 6) [24]. In recent years, the importance of HLA typing at the allele level with high-resolution typing methods, rather than at the antigen level, and the contribution of allele-level results

to the understanding of HLA epitopes has been emphasized. HLA-A, B, C, DR, DQ genes are highly polymorphic. As seen in Table 6, high-resolution tissue typing tests will provide more information about the eplet structures in HLA epitopes (Table 6) [25,26].

Table 6. Comparison of incompatible eplets of the patient with Class II typing with different donors

Patient Serologic typing	Molecular Typing								
	DRB1	DRB3/4/5	DQB1	DQA1	Eplet total	DRB1 eplets	DRB3/4/5 eplets	DQB1 eplets	DQA1 eplets
DR 15	DRB1*1501	DRB5*0101	DQB1*0502	DQA1*0102					
DR18	DRB1*0302	DRB3*0101	DQB1*0402	DRB1*0401					
Donor									
DR1	DRB1*0101	NONE	DQB1*0501	DQA1*0101	9	5	0	2	2
DR4	DRB1*0401	DRB4*0101	DQB1*0301	DQA1*0302	42	8	14	9	11
DR7	DRB1*0701	DRB4*0101	DQB1*0202	DQA1*0202	41	10	14	10	7
DR8	DRB1*0801	NONE	DQB1*0402	DQA1*0401	4	4	0	0	0
DR9	DRB1*0901	DRB4*0101	DQB1*0303	DQA1*0302	36	6	14	5	11
DR10	DRB1*1001	NONE	DQB1*0501	DQA1*0101	12	8	0	0	2
DR11	DRB1*1101	DRB3*0202	DQB1*0301	DQA1*0501	22	3	2	9	8
DR12	DRB1*1201	DRB3*0202	DQB1*0301	DQA1*0501	26	7	2	9	8
DR13	DRB1*1301	DRB3*0101	DQB1*0603	DQA1*0103	12	2	0	7	3
DR14	DRB1*1401	DRB3*0202	DQB1*0503	DQA1*0104	11	4	2	2	3
DR15 (self)	DRB1*1501	DRB5*0101	DQB1*0602	DQA1*0102	6	0	0	6	0
DR16	DRB1*1601	DRB5*0202	DQB1*0502	DQA1*0102	2	0	2	0	0
DR17	DRB1*0301	DRB3*0101	DQB1*0201	DQA1*0501	17	0	0	9	8
DR18 (self)	DRB1*0302	DRB3*0101	DQB1*0402	DQA1*0401	0	0	0	0	0

In addition, in recent years, especially for patients with high levels of HLA antibodies, tissues that they can accept have been determined by using single HLA antigen-coated beads [single antigen beads (SAB)]. The HLA alleles covering these beads were tested with high-resolution methods. Obtaining more specific results with this feature will contribute to the recognition of antibodies and graft survival [27,29]. Recognition of epitopes by HLA-antibodies with the SAB technique plays a key role in determining acceptable HLA mismatches. It is also important in determining the importance of the HLA

"matchmaker" program in terms of transplantation. Resse et al. In a study published by, HLA "matchmaker" eplets and the results of the SAB study were evaluated. In this study, the immunization of a patient registered on the waiting list for heart transplantation with HLAs passed from her husband to her child during pregnancy is discussed. The patient's HLA-A*02:01, A*24:02, B*35:01, B*51:01, C*04:01, her husband's HLA-A*02:01, A*26:01, B* 18:01, B*58:01, C*06:02. In SAB analyses, mean fluorescence intensity (MFI) values were found to be high in beads covered with B*58:01, C*06:02, B*57:01 and B57:03 alleles.

These results were compared with the HLA "matchmaker" program. A double patch feature consisting of 65 RNA and 82 ALR eplets was detected in all alleles that developed antibodies. Apart from these alleles, there are many alleles that are available in the SAB technique and have the 65RNA eplete. However, these do not have the 82ALR eplete. Additionally, the 82ALR eplet is also present in the patient's A*24:02 and B*51:01 alleles. Another issue in the study is that A*25:01, A*32:01 and B*15:16 alleles carrying 65RNA and 82ALR were evaluated as negative with SAB. When the amino acid residues of these alleles were compared with B*58:01 using the HLA "matchmaker" program, it was determined that isoleucine had changed to threonine amino acid at the 94th residue. It is thought that this change may have an effect on antibody binding by affecting the epitope structure [10].

Lomago et al. In a study published by et al., it was determined that eplete-specific antibodies reacted with different alleles in a patient who received a kidney transplant from his cousin. In the patient who was lymphocytotoxic "cross match" negative before the transplant, anti-HLA antibody, which reacted with B*44:02 but did not react with B*44:03, was de-

tected by the SAB technique approximately one month after the transplant. When the patient's tissue typing was studied with high resolution, B*44:03 antigen was determined. With the HLA "matchmaker" program, it was determined that there was a 156DA eplet in B*44:02 and that this eplet was also found in the C*07:04 allele in the donor. The 156 DA eplet is also shared by a group of HLA-B alleles (B*08:01, B*37:01, B*41:01, B*42:01, B*44:02). The antibody in the recipient's serum was found to react with all these alleles [30].

Pregnancy is an attractive model for investigating de novo anti-HLAs. It has been determined using SAB that Class I HLA antibodies develop in 30% of first pregnancies. Until January 2016, 81 HLA-ABC epitopes confirmed by antibody studies were listed on the international epitope registry website (www.epregistry.com.br). Of these, 62 are specific to a single eplet (single patch), and 19 are specific to pairs of eplets (two patches). It was determined that the A*01:01 allele, which was homozygous A*02:01 typing and passed from the father to the child during pregnancy, reacted with all HLA-A alleles carrying 166DG and 90D, except for A2, A68 and A69. It has been determined that A2, A68 and A69 alleles share 138 MI eplets and this eplet plays an important role in antibody antigen binding. In another HLA antibody study; A*23:01, A*24:03, A*25:01 and self-alleles in the SAB panel of antibodies immunized with A*02:01, which were passed from father to child during the pregnancy period when A*24:02, A*32:01 typing was done. It was found to react with all A alleles except A*24:02 and A*32:01. When the results were evaluated with the HLA "matchmaker" program, it was seen that all alleles carried 144 TKH and 62GE epitopes, but a reaction was detected with alleles carrying 79GT epitopes as well as these epitopes. That is, the 79GT eplet is essential for the immune reaction [31].

In a study investigating Class II antibodies after pregnancy, antibodies induced by the DRB1*01:01 allele, which were typed as DRB1*08:01 and DRB1*15:01 and passed from father to child during pregnancy, were evaluated with the SAB and HLA "matchmaker" program. Here, it has been determined that the epitope structure showing two patches is important in the formation of immune reactions. Strong immune reactions were detected in two patches with alleles carrying 67LQ and self-residue 60Y [26].

2.3 CONCLUSION

As a result, it has been known for many years that HLA compatibility between the patient and the donor affects the success of bone marrow and solid organ transplants. For this reason, studies in the field of HLA, which started in the 1960s, continue increasingly. In addition to high-resolution HLA typing, the determination of allele-specific antibodies and the development of HLA analysis programs will also contribute to this field.

2.4 REFERENCES

1. Salvadori Lde C, Santana FC, Marcos EV. Frequency of alleles and haplotypes of the human leukocyte antigen system in Bauru, São Paulo, Brazil. Rev Bras Hematol Hemoter 2014;36(2): 108-14. https://doi.org/10.5581/1516-8484.20140026
2. MaiersM, Gragert L, Madbouly A, Steiner D, Marsh SG, et al. 16(th) IHIW: global analysis of registry HLA haplotypes from 20 million individuals: report from the IHIW Registry Diversity Group. Int J Immunogenet 2013;40(1): 66-71. https://doi.org/10.1111/iji.12031
3. Thorsby E. A short history of HLA. Tissue Antigens 2009;74(2):101-16. https://doi.org/10.1111/j.1399-0039.2009.01291.x
4. Shiina T, Inoko H, Kulski JK. An update of the HLA genomic region, locus information and disease associations: 2004. Tissue Antigens 2004;64(6): 631-49. https://doi.org/10.1111/j.1399-0039.2004.00327.x
5. Goldberg AC, Rizzo LV. [MHC structure and function - antigen presentation. Part 1]. Einstein (Sao Paulo) 2015;13(1):153-6. https://doi.org/10.1590/S1679-45082015RB3122
6. Goldberg AC, Rizzo LV. [MHC structure and function - antigen presentation. Part 2]. Einstein (Sao Paulo) 2015;13(1):157-62. https://doi.org/10.1590/S1679-45082015RB3123
7. Marsh SGE. HLA nomenclature. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FH, eds. HLA Complex in Biology and Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.79-85.
8. Thorsby E. History of HLA. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FHJ, eds. HLA Complex in Biology and Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.42-58.
9. Lutz CT. Human leukocyte antigen Bw4 and Bw6 epitopes recognized by antibodies and natural killer cells. Curr Opin Organ Transplant 2014;19 (4):436-41. https://doi.org/10.1097/MOT.0000000000000103
10. Amos DB. Human histocompatibility locus HL-A. Science 1968;159(3815):659-60. https://doi.org/10.1126/science.159.3815.659-a

11. Tait BD. Genetic structure and functions of the major histocompatibility complex. In: Mehra NK, Kaur G, McCluskey J, Christiansen FT, Claas FHJ, eds. HLA Complex in Biology and Medicine: A Resource Book. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. p.61-77.

12. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, et al. Nomenclature for factors of the HLA system. Tissue Antigens 2010;75:291-455. https://doi.org/10.1111/j.1399-0039.2010.01466.x

13. Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Dupont B, et al. Nomenclature for factors of the HLA system, 1990. Immunobiology 1991;182(3-4):334-45. https://doi.org/10.1016/S0171-2985(11)80669-8

14. Marsh SG, Bodmer JG. HLA class II region nucleotide sequences, 1994. Eur J Immunogenet 1994;21(6):519-51. https://doi.org/10.1111/j.1744-313x.1994.tb00223.x

15. Robinson J, Malik A, Parham P, Bodmer JG, Marsh SG. IMGT/HLA database--a sequence database for the human major histocompatibility complex. Tissue Antigens 2000;55(3):280-7. https://doi.org/10.1034/j.1399-0039.2000.550314.x

16. Robinson J, Waller MJ, Parham P, Bodmer JG, Marsh SG. IMGT/HLA Database--a sequence database for the human major histocompatibility complex. Nucleic Acids Res 2001;29(1):210- 3. https://doi.org/10.1093/nar/29.1.210

17. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, et al. Nomenclature for factors of the HLA system, 2002. Tissue Antigens 2002;60(5):407-64. https://doi.org/10.1034/j.1399-0039.2002.600509.x

18. Robinson J, Halliwell JA, McWilliam H, Lopez R, Parham P, et al. The IMGT/HLA database. Nucleic Acids Res 2013;41(Database issue): 1222-7. https://doi.org/10.1093/nar/gks949

REJECTION MECHANISM AND BIOMARKERS IN KIDNEY TRANSPLANTATION

Tülay KILICASLAN AYNA[1,2,3], Ibrahim PIRIM[1,2,3]

[1]*Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey*

[2]*Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey*

[3]*Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey*

3.1 INTRODUCTION

Organ transplantation is an important option in the treatment of organ failure. Transferring healthy tissues and organs instead of tissues or organs that cannot perform their normal function is called transplantation. Allogeneic transplantation, which refers to transplantation between genetically different individuals of the same species, is a treatment option that is increasingly used in organ failures [1]. Kidney transplantation is the most performed solid organ transplantation in our country and in the world [2,3]. The use of both cadaveric donors and living donors as organ sources enables many patients with end-stage renal failure to undergo transplantation. The ability of the transplanted organ to perform its normal functions not only improves the patient's quality of life, but also contributes to the country's economy. A good understanding of transplantation immunology is important for long-lasting kid-

ney survival. In this context, tissue compatibility between the patient and donor, the mechanisms of immune system cells to recognize the foreign organ, and the cells and molecules involved in this process should be taken into consideration. Detection of anti-human leukocyte antigen antibodies by the combined use of cellular and solid phase techniques developed in recent years is an important innovation that prevents hyperacute rejections. However, long-term rejections and graft failures still remain a problem for organ transplant centers. Scientists continue to attempt to find new biomarkers to understand the immunological relationship between the graft and the patient. In this context, the expression levels of some genes in the patient, gene polymorphism differences between the patient and the donor, and changes in protein expression levels are evaluated in blood and urine samples. In addition, for the last few years, free donor DNAs that pass into the blood and urine due to damage to the graft tissue have been investigated for this purpose. This study aimed to provide information about the types of immune responses in which T and B-cells play a fundamental role, renal rejection mechanisms and new biomarkers.

3.2 HUMAN LEUCOCYTE ANTIGENS (HLA)

AB blood group, HLA compatibility, and HLA-specific antibody profile of the recipient significantly affect kidney transplant success[4,5]. To accomplish kidney transplantation, the recipient and donor pair must be compatible in A, B, and O blood groups[6]. Another parameter that affects the success of transplantation is HLA compatibility between couples. The HLA region is located on chromosome 6 and is 4 Mb in size (Figure 1). This area includes 0.1% of the human genome's genes, with over 20 of them connected to the immune system. HLA is the area of the human genome with the most

disease-related genes. In terms of transplantation, the relevant genes in HLA are in the class I (HLA-A, B, and C) and II (HLA-DR, DQ, and DP) sites[7]. The relevance of these areas stems from their great polymorphism. According to 2018 statistics, the total number of HLA alleles is 18,181. There are 4,857 class II alleles and 13,324 class I alleles. The relevance of these areas stems from their great polymorphism. According to 2018 statistics, the total number of HLA alleles is 18,181. There are 4,857 class II alleles and 13,324 class I alleles. In such a diverse HLA allele pool, organ recipients' chances of finding a fully compatible donor are quite low[8]. The proteins encoded from the HLA region mentioned above are transmembrane proteins. Of these, class I HLA proteins encode a large alpha (α) chain. The alpha chain forms three alpha domains by amino acid folding. The peptide attachment gap is formed by α1 and α2. Class I molecules function in the presentation of 8-10 amino acid intrinsic peptides to CD8+ T-cells. The other alpha domain in the Class I molecule, α3, interacts with the CD8 molecule in cytotoxic T-cells[4]. This interaction strengthens the relationship between the class I molecule and the T-cell. In addition, it is responsible for the stabilization of the β2 microglobulin α chain in the cell membrane, encoded from a gene on chromosome 15 in the class I molecule[4]. Since this feature of β2 microglobulin is known, some recent studies have aimed to silence the β2 microglobulin gene in the allograft in order to increase the survival of the transplanted organ (allograft) after transplantation. The short-term results of allogeneic lung transplantations in pigs are promising[9]. There are two genes (α and β genes) that encode class II HLA molecules. The α and β chains synthesized separately come together to form a class II molecule. Both chains contain two domains. The peptide attachment gap is formed by α1 and β1 domains. By binding 10-20 amino acid peptides here, antigenic determinants

are presented to CD4+ T helper cells. Extracellular (external) peptides bind here, and it has been determined that they are associated with the class II β2 domain of CD4, one of the surface molecules of helper T-cells. Another difference between the two classes is related to the cell group in which they are expressed. Solely antigen-presenting cells (APCs) produce class II molecules, whereas class I molecules are present on the surface of every cell with a nucleus. There are pockets at the base of the peptide attachment gap of HLA molecules. The side chains of the amino acids of the peptide antigens enter these pockets and enable the peptides to adhere to the groove of the HLA molecule. There are amino acids that antigenic peptides contact with the T-cell receptor (THR). In this way, the peptide in the antigen binding region of HLA comes into contact with THR. The way by which HLA class I and II molecules attach antigens is different from each other. Proteosomes degrade Class I molecules in the cytoplasm and bind short peptides arriving at the endoplasmic reticulum (ER) via TAP (transporter associated with antigen processing) pores. The peptide-HLA complex is transported to the cell surface by vesicles. The ER is the site of class II molecule production. It encounters external peptides taken into endosomes in late endosomes. After the peptide fragments bind to the antigen-binding groove of Class II molecules, they are carried to the surface of the cell with vesicles[4]. Dendritic cells (DH), B-cells, and macrophages, also known as APC function for the antigen presentation to recipient T-cells in allogeneic transplantation. Among these cells, some features of DCs make them the most efficient DCs initiating T-cell responses. DCs are strategically located in places where microorganisms and foreign antigens commonly enter and in tissues where microorganisms can colonize. They express receptors [toll-like receptors (TLR), mannose-binding lectin receptors, etc.] that

enable them to capture microorganisms. These cells migrate from the epithelium and tissues via the lymphatics to the T-cell areas of the lymph nodes. DCs express high levels of peptide-HLA complexes, costimulators and cytokines necessary to activate naïve T lymphocytes. Due to this feature, they cause 100-1,000 times more T-cell activation than other APCs [4,10]. One DC can activate 100-3,000 T-cells [11].

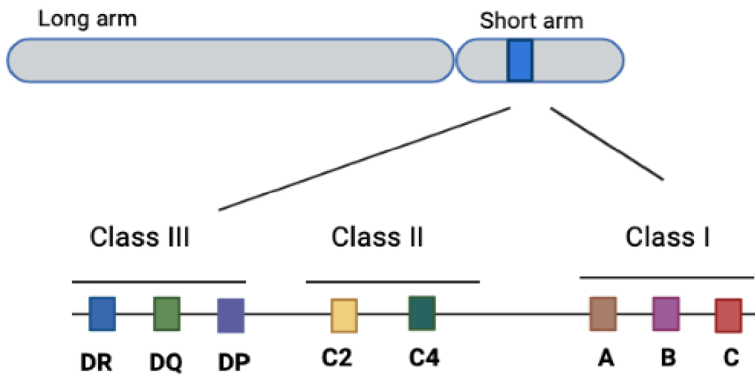


Figure 1: HLA Gene Map

3.3 ALLORECOGNITION

Allorecognition begins with the sensitization phase. At this stage, the antigenic peptide is introduced to T-cells in secondary lymphoid organs by PHCs. The effector cells formed by this recognition reach the graft through blood and lymph channels and initiate rejection, which is the second stage of allorecognition. Studies in this field have shown that allograft rejection usually occurs by humoral (or antibody-based), cellular (or lymphocyte-based), or both mechanisms. Recognition of the allograft in transplantation occurs in three different ways. These are direct recognition, indirect recognition and semi-direct recognition [12,13].

3.3.1 Direct recognition

This is a transplant-specific immune recognition. It is the process by which recipient T-cells recognize intact HLA molecules donor cell surface, such as endothelial cells, and donor-derived PHCs. It is known that the mixed lymphocyte culture test is a test that can show the clinical results of direct diagnosis. When recipient cells recognize donor cells as foreign, they proliferate, and the cytokine pool in the environment is associated with the immune reaction. It is stated that this test can be used as an indicator of acute rejection. Approximately 1-10% of the total T lymphocytes in the bloodstream are expected to be involved in immediate identification. Two different models are proposed for direct recognition that peptide dependent and independent [12,13]. For example, it has been proposed that alloreactive T-cells may identify variation sites in the donor's HLA molecule its own, irrespective of the allopeptide, when donor and recipient HLA vary physically [13]. In the other model, it is suggested that there must be a classical presentation for effective allorecognition. In other words, when the recipient and donor HLAs are different, allo-HLA is directly recognized independently of the allopeptide, whereas when the recipient and donor HLAs are similar, it is thought that the donor HLA-allopeptide structure directly causes T cell alloreaction. In direct recognition, the maturation of immature DCs and their transfer to secondary lymphoid organs is important. Inflammatory signals and signals resulting from ischemia and reperfusion injury are important in the maturation of these cells, also called passenger leukocytes. This process causes chemokine and cytokine expression and the production of reactive oxygen species within the allograft; Stimuli to DCs activate MyD88 and TRIF signaling pathways, especially through the activation of TLRs. This enables the migration of

DCs to secondary lymphoid organs [13]. The degree of ischemia-reperfusion injury was decreased by the knockout of TLRs in the allograft [14]. Mature DCs migrate to lymph nodes via the lymphatic system and stimulate T-cells in the paracortex with peptide-independent and peptide-dependent patterns. When CD4+ T-cells are activated through Class II HLA molecules, they enable the activation of many cells such as B-cells, cytotoxic T-cells, and phagocytic cells. Activated cells and immune molecules such as antibodies and cytokines are transferred to the allograft. Since DCs also express class I HLA antigens, they can activate CD8+ cytotoxic T-cells independently of the peptide, or alloreactive T cells are activated by presenting the peptides escaping from the endosome to the cytoplasm to CD8+ T-cells in a peptide-responsive way by class I molecules. CD8+ T-cells were demonstrated to influence destruction of endothelial cells and ultimately acute rejection through direct interaction with the allograft. Synthesis of donor-specific antibodies from plasma cells formed by activating B-cells in allorecognition is important in allograft pathology. Because these antibodies bind to the graft's endothelial and other cells, causing cell damage through the stimulation of complement proteins and, eventually, the formation of membrane attack complexes in the allograft, or even if the antibody does not cause complement activation, the graft is attacked by cells with antibody receptors such as natural killer (NK) cells and macrophages. Cells may be destroyed. There are also some factors that affect direct recognition in allograft rejection. The first of these is negative selection in the thymus. T-cells recognize their own HLAs and self-peptides during maturation and interact weakly with these molecules. Strongly interacting T-cells are eliminated by negative selection. This makes it easier for T-cells to distinguish allo-HLAs from recipient HLAs. The second is viral infections. Activation of T-cells

by viral infections causes changes in cell surface molecules and the pool of cytokines and chemokines, which can ultimately induce the activation of alloreactive T lymphocytes. This is called a heterologous immune response. It was showed by several researchers that after viral infections T cells can be activated [13,15,16]. The third is the number of allograft-derived passenger leukocytes. Roughly, as the number of ASH (especially DH) to activate T-cells increases, the number of T-cells to be activated also increases. The number of immune system cells decreases with age. Finally, the HLA expression level of allograft-derived ASHs is also important for direct recognition. Expression of coreceptors becomes important in immune activation that begins with HLA-T-cell receptor (THR) interaction during allorecognition. Coreceptors are divided into two main categories: costimulators (those that activate the immune system) and coinhibitors. The balance between costimulators and coinhibitors determines the intensity and direction of the immune reaction. Despite the HLA-THR interaction, the lack of costimulatory signals enables the development of the tolerance mechanism and immune response called anergy occurs. Again, during these cell-cell interactions, the cytokine content in the environment is also important in determining the direction of the immune response [13].

3.3.2 Indirect recognition

Activation of the T-cell through this pathway is not specific to alloimmunity. It covers the antigen presentation process to T-cells. After recipient APCs digest graft antigens and present the donor's peptide to $CD4^+$ T-cells of the recipient in their lymphoid tissues, alloactivation of T-cells occurs by indirectly activation. Three steps that are incompatible with one another for this mechanism to occur. First, the circulation is exposed to alloantigens and/or donor cell membranes from the al-

lograft, which recipient DCs in the secondary lymphoid tissue can ingest. Second, recipient DCs can phagocytose donor cells as they migrate to secondary lymphoid organs. Thirdly, the recipient's ASHs coming to the graft through circulation can phagocytose alloantigens in the graft and then activate recipient T-cells in secondary lymphoid tissues via circulation [13]. Alloantigen-activated T-cells can activate NK cells, B-cells, CD8+ T-cells, and macrophages through the mechanisms mentioned in direct recognition. Epitope shifting is a process that caused by antigenic change with time [17]. However, epitope shift is important as it causes indirect recognition to persist and polyclonal activation to occur. Chronic rejection is linked to persistent T-cell activation caused by indirect recognition in recipients [13].

T-cell activation and self-restriction are critical mechanisms in the growth of regulatory T-cells (Tregs). Regulatory T-cells (CD25+, FoxP3+, and CD4+) inhibit activation of the T cells. Therefore, it can be evaluated that regulatory T cells with limited CD25+ and FoxP3+ expression can cause chronic rejection [18]. While the number of Tregs was high, the number of Tregs was found to be low in patients with chronic rejection. As a result, Treg cells are important in the emergence of an effective immune response in indirect recognition. Again, at this stage, the character of the costimulatory and coinhibitory signals is also important [13].

3.3.3 Semi direct recognition

It is a mechanism that has been described in recent years for the recognition of allo-HLA and/or peptides. Here, intact allo-HLA molecules are transferred to recipient ASHs. This transfer occurs in two ways: 1) As the recipient ASHs cross the graft endothelium with allo-HLA expression, these molecules

are transferred to the recipient ASHs as a result of cell-cell contact, 2) Allo-HLA in the graft is transferred through exosomes, which are vesicles smaller than 100 nm. It is the transfer of CSFs to recipient ASHs and activating the T-cells of ASHs in secondary lymphoid organs via circulation. Exosomes can transmit allo-HLA both in vitro and in vivo, but may not be enough to activate naïve T-cells [18,19].

3.4 MECHANISMS OF REJECTION

Hyperacute, acute and chronic rejection are three types of rejections. Hyperacute rejection occurs shortly after perfusion of the transplanted organ with the recipient's blood. Donor-specific anti-HLA and anti-ABO antibodies are important in the occurrence of hyperacute rejection. With blood group-identical transplants and the use of new cross-over methods before transplantation, hyperacute rejections are no longer a problem for transplantation clinics [20]. In hyperacute rejection, the mechanism begins with donor-specific antibodies binding to antigens on the donor endothelium and activating complement proteins. After this activation, endothelial damage occurs due to MAC formed in the vascular endothelial cell membrane. On the other hand, the graft is lost as neutrophils migrate to the graft with inflammatory signals and secrete lytic enzymes, and platelets migrate to the damaged area and cause vascular hemorrhage. Acute rejection usually happens throughout the initial months and weeks following a transplant. As mentioned above, it occurs after direct recognition mechanisms. Semi-direct recognition mechanisms also contribute to this process. As a result of CD4+ T helper cell activation, monocyte/macrophage-mediated mechanisms mediated by NK cells. Immunosuppressive agents have been shown to significantly prevent the acute rejection by targeting CD4+ T-cell activation. CD8+ T-cells are able to attack the

graft immediately, causing it to be destroyed, because most of parenchymal and vascular cells carry HLA class I molecules [13]. Approximately 90% of acute rejections are cellular rejections that result in CD4+ and CD8+ activation and perforin and granzyme release. Jia et al. They investigated gene expression changes in kidney biopsies of patients with and without a diagnosis of acute rejection [21]. They found that the expression of 437 of a total of 790 genes increased, while the expression of 353 genes decreased. In this study, the genes most strongly associated with acute rejection were determined to be interferon (IFN)-g, tumor necrosis factor (TNF), B2 microglobulin, and LCK. In the study, they explained that LCK (Src kinase), which plays a role in signal transmission for the cell, plays a key role in the pathogenesis of acute rejection. LCK is a molecule that initiates intracellular signal transduction by interacting with the intracytoplasmic part of CD3 (CD3e), which is a part of the T-cell receptor complex [21]. Additionally, B-cells contribute to acute rejection by producing antibodies [22,23]. C4d complement protein is investigated in graft biopsies in the diagnosis of acute humoral rejection. However, C4d negative results may be associated with acute rejection [23,24]. In acute humoral rejection, vasculitis and mononuclear cell accumulation in the graft are observed. Chronic rejection is the most prevalent reason for graft failure that can be both antigen dependent and independent [13]. Reperfusion injury, early acute rejection attacks, usage of kidney-damaging drug such as calcineurin inhibitors, and inflammation associated with delayed-type hypersensitivity reactions contribute to chronic rejection. It is predicted that new anti-HLA antibody production in transplantation poses a chronic rejection possibility [22,25]. Antibodies against incompatible HLAs of the donor after transplantation; It is known to contribute to graft damage through complement stimulation and Anti-HLA antibody-antigen

binding regulates the migration of immune system cells into the graft. Additionally, binding of antibodies to HLAs on endothelial cells engage intracellular signaling pathways which enhance graft failure [26]. Studies on this subject are limited. Because cultured vascular endothelial cells rapidly lose their HLA expression (especially class II). In studies, HLA expression is evaluated by adding IFN-g and TNF-α to the growth medium or by transferring transactivator genes that will initiate HLA expression to the cells by transfection. HLA-II molecules have not been expressed by most of the vascular endothelial cells. During or post-transplantation process cytokines such as TNF-α, interleukin (IL)-1β, and IFN-g are produced. These cytokines cause increased HLA expression [27]. Studies have found that as a result of the activation of phospholipase Cg1 by binding of class I anti-HLA antibodies to antigens, inositol triphosphate (IP3) and diacylglycerol, called secondary messengers in the signaling mechanism, are formed and as a result, intracellular calcium release is regulated. P-selectin carrying Weibel-Palade body (WPb) vesicles are exocytosed in reaction to variations in internal calcium levels. Quick increase of P-selectin expression on the cell membrane supports the adhesion of platelets, monocytes, and neutrophils. This ensures that myeloid cells such as CD68+ macrophages and neutrophils predominate in the graft. HLA antibody-antigen binding also promotes the activation of protein kinases focal adhesion kinase (FAK), paxillin and Src. In addition to FAK, activation of Rho, a small G protein, regulates actin proteins in the cytoskeleton, ensures the formation of focal adhesions and the formation of stress fibers. One of the first functional changes that arises after endothelial exposed directly to class I anti-HLA antibodies is actin cytoskeleton remodeling, which results in quick and significant stress fiber production [28]. The formation of stress fibers allows mechanical transdu-

ction. These structures are important for cell-cell adhesion in the binding of leukocytes. Researches show that the cytoskeleton is an active organizer of internal signaling pathways and affects the location of many activities and pathways. Also in this process, the activation of the mammalian target of rapamycin (mTOR) and extracellular signal regulated kinase (ERK) contributes to the regulation of the cytoskeleton. It has been determined that the synthesis of Basic Fibroblast Growth Factor (bFGF) and its receptor increases with the binding of Class I anti-HLA antibodies to antigens, and that the cytoskeleton contributes to this regulation. bFGF increases the proliferation of endothelial and smooth muscle cells by binding to FGFR on the endothelial surface. Additionally, since bFGF is a growth factor that rapidly increases vascularization, neovascularization also increases [27,29]. These results show that class I anti-HLA antibodies contribute to the proliferation of smooth muscle cells and endothelial cells in graft vessels and narrowing of the vessel diameter. It has also been determined that the binding of Class I anti-HLA antibodies with antigen activates the PI3 kinase/Akt pathway and increases antiapoptotic Bcl-xL and Bcl-2 production in the endothelium [30]. Less work has been done in this area due to the low expression of HLA class II molecules in culture. In these studies, it has been shown that binding of HLA-II molecules to antibodies triggers the intracellular signaling network, including protein kinases MAPK ERK, PI3K/Akt and mTOR. HLA-II antibodies also stimulated angiogenesis in endothelial cells [30,33].

Additionally, processes independent of donor incompatible HLA antigens are known to contribute to chronic rejection. These are donor brain death, hypertension, toxicity of immunosuppressives, and viral infections. In chronic rejection, extracellular matrix proteins degraded. Fibroblasts undergo

morphological changes and produce the new extracellular matrix. These processes are anticipated to emerge over time within injured organs, resulting in severe transplant malfunction. Growth factors generated by many immune cells can cause arterial constriction. TGF-β can be a reason for fibrosis of the tissues. Thus, not only damage but also the process of repair/remodeling of damage and dysfunction contributes to chronic rejection [13].

3.5 TRANSPLANTATION AND BIOMARKERS

Various biomarkers have been used for years to predict the process after transplantation and to obtain information about the function of the allograft. The first of these is skin grafts. The skin transplanted from the donor to the patient before the kidney transplantation shed light on the period after organ transplantation. Again, Terasaki's detection of DSAs with the cytotoxicity test is an important turning point in transplantation. Nowadays, the use of biomarkers that can contribute to the evaluation of transplantation and the subsequent process is increasing day by day. Among these; Various studies at mRNA and protein levels include T-cell alloreaction tests, adenosine triphosphate (ATP) production feature of T-cells, gene expression studies in allograft biopsy materials, peripheral blood and urine [31]. T-cell alloreaction assays evaluate the amount of IFN-g. The IFN-g ELISPOT technique has been found to evaluate pre and post transplantation T cells which can be related to graft failure. There have been studies in which the predicted association was not found due to the effects of induction treatment. The labor-intensive testing technique and need for donor cells make this study system unsuitable as a biomarker [31].

ATP generation is assumed to be one of the first processes after antigen recognition. This test device, also known as Immuknow, works by magnetically separating and lysing CD4+ cells from peripheral mononuclear cells before detecting ATP levels. Studies have produced inconsistent results regarding the association between ATP levels and infections or rejection. Limitations of the test are the short time interval between collection and storage of study samples, the time impact after transplantation on ATP degrees, and the limitation in interpreting the results [31].

Gene expression (transcriptome) studies can also be used as biomarkers. Analysis of perforin and granzyme expressions is important as indicators of T-cell cytotoxicity. The chemicals responsible for kidney transplant rejection can cause apoptosis of target cells and harm tubular epithelial cells. Studies have shown that high granzyme B and perforin amounts in urine cells are linked to acute rejection. Additionally, intragraft granzyme B transcripts have been found to accumulate significantly in patients who underwent acute failure compared to those none rejected. However, the effect of lysed cells on mRNA stability may impact the results of these studies, and larger study groups are needed for a more accurate evaluation. Another useful marker for gene expression is FoxP3, which is produced by regulatory T-cells and helps prevent rejection. Research has found that an increase in FoxP3 mRNA present in urine cells can potentially reverse allograft rejection in kidney transplant patients, even if the acute rejection symptoms have not improved. On the other hand, when it comes to chronic rejection, clinically tolerant recipients have significantly higher levels of peripheral blood FoxP3 transcripts compared to kidney transplant patients who suffer from chronic rejection. Sequence-based approaches

and a microarray that can detect gene expression levels in graft biopsy are used. In 2017, Halloran et al. discovered that analysis of the biopsies by microarray which associated the best with clinical outcome [32]. The need for biopsy material for evaluation is a limiting aspect of the test. The "kidney solid organ response test (kSORT)" test, which measures the expression of 17 distinct genes in the patient's peripheral blood, is very sensitive and specific in predicting rejection, but it cannot discriminate antibody-mediated rejection and T-cell. A second research showed that combining the kSORT test with the antidonor IFN-g ELISPOT test may be useful in differentiating the rejection phenotype [33]. Among the advantages of the kSORT test, the use of peripheral blood is important. The "Genomics of chronic allograft rejection (GoCAR)" study as a marker of chronic rejection is also one of the new approaches. The researchers aimed to create a gene set that could predict fibrosis-related chronic failure. The study identified 13 genes to predict early graft loss after transplantation compared to histological variables and standard clinical datas [26]. These genes played a role in tumor suppression and growth, membrane repair and cell growth pathways. The limiting aspect of this study stems from the need for graft biopsy material. There has been an increased emphasis on the role of recipient urine in diagnosing rejection in recent years. Detection of CD3e, IFN-induced protein (IP-10) and 18S rRNA expressions in urine samples could distinguish acute humoral and cellular rejections. Extracting mRNA from urine can be technically challenging [31,34].

Mass spectrometry provides an excellent platform for identifying one or more biomarkers of rejection. Most research on the urine proteome have identified numerous molecules, but just a handful (α-antichymotrypsin, uromodulin, b2-mic-

roglobulin, and collagen fragments) have been examined by many groups [31].

Urinary CXCR3 chemokines CXCL9 [interferon-g (MIG)] and CXCL10 [interferon-induced protein-10 (IP-10)] are thought to be potential proteinaceous indicators. Multicenter research found that urine levels of CXCL9 mRNA and protein can be used to detect acute rejection. CXCL10 levels have also been reported to rise with acute rejection and poliovirus (BKvirus) infection. CXCL10 has been demonstrated to sensitively identify acute rejection, with levels increasing before any clinical abnormalities [31]. More recent studies have shown that the combination of urine CXCL10 level and donor-specific antibody strengthens the diagnosis of humoral rejection. Additionally, the urinary CXCL10: Cr ratio was found to be associated with graft loss during humoral rejection [31]. In very modest single-center trials, there was also a substantial connection between urine CXCL9 and T-cell-mediated rejection [34]. Urine CXCL9 and 10 tests are also available on antibody or flow-based platform [31,34]. Solid phase-based studies can be performed to detect de novo DSAs in the diagnosis of humoral rejections [35]. The combination of bioinformatics and multiplex polymerase chain reaction studies is important in this approach. Studies in this area are in their very early stages, but it is thought to be a promising tool for measuring the alloimmune response [31].

In the last few years, in studies investigating donor DNA (cell free DNA) that passes into the circulation and urine as a result of graft damage, the results are correlated with acute rejection and infections. It has been determined that cell-free DNA levels are higher in cases of rejection attacks. Expanding the scope of these studies will contribute to the evaluation of the results [33]. Menon stated that urine-based studies are im-

portant in follow-up after kidney transplantation (Figure 2) [34]. Figure 2 shows biomarkers that can be used in immunological monitoring. The importance of monitoring RNA and CXCL9 in urine regularly every month in the initial time after transplantation, transcriptome studies in biopsy and blood samples, and investigating anti-HLA antibodies are emphasized [34].

Biomarker									
Biopsi-transcriptome				*			*		
PMBC transcriptome				*					
Urinary cell RNA		*	*	*	*	*	*		
Urinary CXCL9		*	*	*	*	*	*		
IFN-ɣ ELISPOT	*						*		
Solid phase anti-HLA antibody	*							*	*
Epitope mismatch	*								
Recipient SNP analysis	*								
Donor SNP analysis	*								
	Before transplant	0d		3m			6m	12m	24m

Figure 1: Biomarkers that can be used in immunological monitoring after kidney transplantation

In recent years, patients who tolerate allograft even though they do not receive immunosuppressive treatment have also attracted attention. These patients are referred to as operationally tolerant patients. Most operationally tolerant patients are noncompliant patients who distance themselves from treatment for a variety of reasons. In a study involving many transplantation centers, the incidence of operationally tolerant patients was determined to be 0.03%. It is extremely important to identify biomarkers that can be used to identify these patients. However, finding a reliable biomarker is very difficult. Numerous confounding factors, such as the rarity of operationally tolerant patients, lack of a control group, personal characteristics, viral infections, HLA compatibility, medications, account for the difficulties in finding biomarkers. Two

different Treg populations are noteworthy in the tolerated allografts in the studies. One of these is the CD4 + CD25 + T regs, known as natural T regs, which show immunosuppressive properties through cell contact. The other one is stimulated Treg (induced-iTreg). CD4+ CD25- iTregs that produce TGF-β are also known as Th3.

Immune reaction to the allograft is studied by Trans-vivo delayed-type hypersensitivity (tvDTH) test and it may discover tolerant kidney recipients. Essentially, The TVDTH test requires inserting recipient APCs, donor antigens, and sensitized recipient T-cells into the ear or footpad of mice. with severe combined immunodeficiency after transplantation. If there is an immune response to the recipient cells, a DTH reaction will occur, appearing as swelling. Haynes et al. determined the importance of IL-17 and IFN-g cytokines in tvDTH responses in patients with rejection, and a picture emerged suggesting that the absence of tvDTH responses in tolerant patients was due to TGF-β, not IL-10, and that Th3 Treg cells increased. However, its use is limited due to the need for high cell numbers for the tvDTH test, the necessity of mice and the difficulties of the technique. Vendetti et al. found that B-cells and nTreg and iTreg cells were found at higher rates in tolerant patient blood and/or allografts. It was determined that there was an increase in the inhibitory molecules CTLA-4, GITR and CD39 in T-cells, and in the inhibitory molecules CD1d and CD5 in B cells. There are also studies where similar or reduced CD3+ T-cell numbers were determined when blood samples of operationally tolerant patients were compared with healthy individuals. The proportions of CD8+Tc memory, CD8+Tc effector, and CD4+ T-cells were similar. FoxP3 transcripts and CD4 + CD25++ Treg cells are comparable in the individuals who have tolerance and is healthy. It was lower in individu-

als with chronic rejection. This is explained by the fact that FoxP3+ T-cell numbers in the blood most likely do not accurately reflect the condition within the graft. Certainly, researches on graft tissues from tolerant kidney patients show that patients with rejection had higher FoxP3 expression in allograft infiltrates than healthy controls. It has also been demonstrated that Treg cells from tolerant individuals had a high rate of DNA demethylation at the FoxP3 region. It was also found that regulatory B-cells (B reg) increased in tolerant patients. In studies conducted with blood samples of stable and tolerant patients' TNF, CD40, NF-kB and granulocyte-macrophage colony-stimulating factor were noted as the best discriminatory genes. However, new studies are needed in this field [36].

3.6 CONCLUSION

Allograft rejection has a complex mechanism in which immune system cells, immune molecules and many cellular proteins participate. Immune recognition is attempted to be controlled with immunosuppressive drugs used after transplantation. However, the drugs used in the treatment are in the critical dose drug group, and it is important to carefully monitor their effectiveness and side effects. Studies investigating transcriptome, proteome, and donor-specific antibodies after transplantation will also contribute to the elucidation of rejection mechanisms, identification of tolerant patients, and treatment.

3.7 REFERENCES

1. Abbas AK, Licthman AH, Pillai S. Cells and tissues of the immune system. Cellular and Molecular Immunology. 7th ed. Philadelphia: Elsevier Saunders; 2012. p.16-34.
2. Edirne T. Organ and tissue transplantations: results and recommended strategies in Turkey. Turkiye Klinikleri J Med Sci. 2004;24(3):261-6.
3. Matesanz R, Mahillo B, Alvarez M, Carmona M. Global observatory and database on donation and transplantation: world overview on transplantation activities. Transplant Proc. 2009;41(6):2297-301. https://doi.org/10.1016/j.transproceed.2009.05.004
4. Abbas AK, Licthman AH, Pillai S. Major histocompatibility complex molecules and antigen presentation to T lymphocytes. Cellular and Molecular Immunology. 7th ed. Philadelphia: Elsevier Saunders; 2012. p.110-36.
5. Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, et al. Heterologous immünity provides a potent barrierto transplantation tolerance. J Clin Invest. 2003;111(12):1887-95. https://doi.org/10.1172/JCI17477
6. Oğuz FS. [Transplantation immunology]. Türkmen A, editör. Transplantasyon Nefrolojisi Pratik Uygulama Önerileri. 1. Baskı. Ankara: Buluş Tasarım ve Matbaacılık Hizmetleri San Tic; 2016. p.27-35.
7. Alelign T, Ahmed MM, Bobosha K, Tadesse Y, Howe R, et al. Kidney transplantation: the challenge of human leukocyte antigen and its therapeutic strategies. J Immunol Res. 2018; 5:5986740. https://doi.org/10.1155/2018/5986740
8. Barten MJ, Schulz U, Beiras-Fernandez A, Berchtold-Hertz M, Boeken U, et al. The clinical impact of donor-specific antibodies in heart transplantation. Transplant Rev (Orlando). 2018;32(4):207-17. https://doi.org/10.1016/j.trre.2018.05.002
9. Figueiredo C, Carvalho Oliveira M, Chen-Wacker C, Jansson K, Höffler K, et al. Immunoengineering of the vascular endothelium to silence MHC expression during normothermic ex vivo lung perfusion. Hum Gene Ther. 2018 Nov 20. Doi: 10.1089/hum.2018.117. https://doi.org/10.1089/hum.2018.117

10. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol. 1999;10(10):2208-14. https://doi.org/10.1681/ASN.V10102208

11. Sönmez M. [Immunobiology of dendritic cell]. Basic Molecular Hematology Course. 2005; 87-91.

12. Ingulli E. Mechanism of cellular rejection in transplantation. Pediatr Nephrol. 2010;25(1): 61-74. https://doi.org/10.1007/s00467-008-1020-x

13. Ingulli E, Alexander SI, Briscoe DM. Immunology of pediatric renal transplantation. In: Avner ED, Harmon WE, Niaudet P, Yoshikawa N, Emma F, Goldstein S, eds. Pediatric Nephrology. 7th ed. London, UK: Heidelberg, Springer; 2015. p.1-51.

14. Kupiec-Weglinski JW, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. Transplant Proc. 2005;37(4):1653-6. https://doi.org/10.1016/j.transproceed.2005.03.134

15. Ford ML. Virally-induced heterologous immunity in renal transplant recipients: important or inconsequential? Am J Transplant. 2016;16(5):1348-9. https://doi.org/10.1111/ajt.13657

16. Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, et al. Heterologous immunity provides a potent barrier to transplantation tolerance. J Clin Invest. 2003;111(12):1887-95. https://doi.org/10.1172/JCI17477

17. Suciu-Foca N, Ciubotariu R, Colovai A, Foca-Rodi A, Ho E, et al. Persistent allopeptide reactivity and epitope spreading in chronic rejection. Transplant Proc. 1999;31(1-2):100-1.

18. Martin-Moreno PL, Tripathi S, Chandraker A. Regulatory T cells and kidney transplantation. Clin J Am Soc Nephrol. 2018;13(11):1760-4. https://doi.org/10.2215/CJN.01750218

19. Morelli AE, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. Blood. 2004;104(10):3257-66. https://doi.org/10.1182/blood-2004-03-0824

20. Kılıçaslan Ayna T, Diler AS, Çarin M. Detection of posttransplant donor spesific antibodies aganist donor HLA antijens by flow cytometric cross-match. J Ist Faculty Med. 2006; 69:93-7.

21. Jia CL, Jia R, Li Y, Li X, Jia Q, et al. LCK as a potential therapeutic target for acute rejection after kidney transplantation: a bioinformatics clue. J Immunol Res. 2018;6451298. https://doi.org/10.1155/2018/6451298

22. Ayna TK, Calışkan Y, Ciftçi HŞ, Türkmen A, Gürtekin M. Long-term effects of antibodies against human leukocyte antigens detected by flow cytometry in the first year after renal transplantation. Balkan Med J. 2013;30(1):37- 45. https://doi.org/10.5152/balkanmedj.2012.071

23. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cellmediated rejection, and the injury-repair response: new insights from the genome Canada studies of kidney transplant biopsies. Kidney Int. 2014;85(2):258-64. https://doi.org/10.1038/ki.2013.300

24. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. Am Soc Nephrol. 1999;10(10): 2208-14.

25. Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. Transplantation. 2012;93(8):751-6. https://doi.org/10.1097/TP.0b013e3182483713

26. Théry C, Duban L, Segura E, Véron P, Lantz O, et al. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol. 2002;3(12):1156-62. https://doi.org/10.1038/ni854

27. Jin YP, Valenzuela NM, Zhang X, Rozengurt E, Reed EF. HLA class II-triggered signaling cascades cause endothelial cell proliferation and migration: relevance to antibody-mediated transplant rejection. J Immunol. 2018;200(7): 2372-90. https://doi.org/10.4049/jimmunol.1701259

28. Jin YP, Singh RP, Du ZY, Rajasekaran AK, Rozengurt E, at al. Ligation of HLA class I molecules on endothelial cells induces phosphorylation of Src, paxillin, and focal adhesion kinase in an actin-dependent manner. J Immunol. 2002;168(11):5415-23. https://doi.org/10.4049/jimmunol.168.11.5415

29. Yeşilyurt E, Fidan I. Dendritic cells and their role in infection. Turkish Journal of Microbiology Society. 2011;41(3):91-102.

30. Valenzuela NM, Reed EF. Antibodies to HLA molecules mimic agonistic stimulation to trigger vascular cell changes and induce allograft injury. Curr Transplant Rep. 2015;2(3):222-32. https://doi.org/10.1007/s40472-015-0065-6
31. Safa K, Magee CN, Azzi J. A critical review of biomarkers in kidney transplantation. Curr Opin Nephrol Hypertens. 2017;26(6):509-15. https://doi.org/10.1097/MNH.0000000000000361
32. Halloran PF, Reeve J, Akalin E, et al. Real time central assessment of kidney transplant indication biopsies by microarrays: the Intercomex study. Am J Transplant. 2017;17(11): 2851-62. https://doi.org/10.1111/ajt.14329
33. Celec P, Vlková B, Lauková L, Bábíčková J, Boor P. Cell-free DNA: the role in pathophysiology and as a biomarker in kidney diseases. Expert Rev Mol Med. 2018;20:e1. https://doi.org/10.1017/erm.2017.12
34. Menon MC, Murphy B, Heeger PS. Moving biomarkers toward clinical implementation in kidney transplantation. J Am Soc Nephrol. 2017;28(3):735-47. https://doi.org/10.1681/ASN.2016080858
35. Kılıçaslan Ayna T, Çiftçi HŞ, Gürtekin M. [Donor-specific antibodies with single antigen beads assay determination: review]. Turkiye Klinikleri J Nephrol. 2011;6(1):17-21.
36. Massart A, Ghisdal L, Abramowicz M, Abramowicz D. Operational tolerance in kidney transplantation and associated biomarkers. Clin Exp Immunol. 2017;189(2):138-57. https://doi.org/10.1111/cei.12981

THE EMERGING ROLE OF NON-HLA ANTIBODIES IN TRANSPLANTATION

Burcu CERCI ALKAC[1,2]
[1]*Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey*

[2]*Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey*

4.1 INTRODUCTION

Advances in surgical methods and the change in the pre-post operation therapy protocols can increase the quality of life of the transplanted patients [1]. However acute or chronic rejection after transplantation damages the graft. MHC molecules are crucial targets of the recipient's immune cells [2]. The main risk factor for antibody-mediated acute rejection is the antibodies determined before or after transplantation which is produced against both HLA and non-HLA molecules [3,4]. Antibodies that is produced against non-polymorphic targets originate as a result of proteins released into the immune system following inflammation, transplantation, or injury [5]. The microenvironment of the graft or rejection may interfere with humoral tolerance to autoantigens. Also, indications for kidney transplants are usually autoimmune and patients produce antibodies against their own antigens [6].

In 1995, the first non-HLA antibodies to donor antigens were identified [7]. Terasaki et al. investigated the factors that effect kidney damage after transplantation [8]. Several

antigenic targets were identified as the targets of non-HLA antibodies (Figure 1) [9,10]. Non-HLA allo- or auto-antibodies cause changes in arterial walls [11]. Immunogenic targets of non-HLA antigens can be produced in activated or damaged cells [12]. Non-HLA antibodies act as complement depended and non-depended antibodies in acute and chronic injury [13].

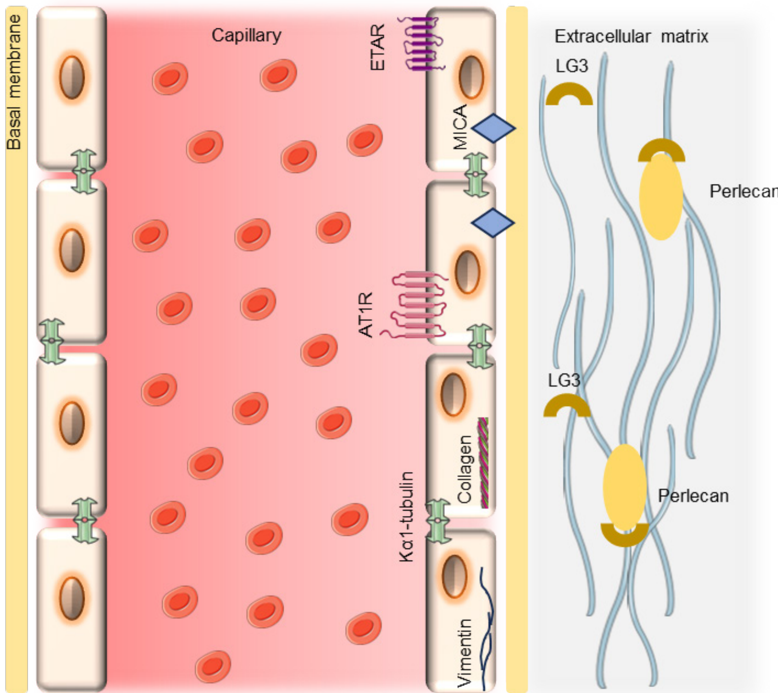


Figure 1: Targets of non-HLA antibodies

4.2 MINOR HISTOCOMPATIBILITY ANTIBODIES

The first studies showing the effect of antigens other than MHC in transplantation were made by Counce et al in the 1950s [14,15]. The first hypothesis regarding the relationship of Minor Tissue Compatibility Antigens (MiHA) to outcomes in bone marrow transplantation was put forward based on a

female patient whose brother had been transplanted. Cytotoxic T cells were isolated from peripheral blood and determined to be against antigens presented on non-HLA-associated donor cells [16].

MiHAs can bind to the same sites with HLA molecules [17]. As a result, it was discovered that miHAs are specific to HLA antigens, indicating that they are MHC-restricted [18]. In a study, it was determined that HA-1 incompatibility was not associated with acute rejection. In another study in which 702 people participated, several mismatches were examined in people who had renal transplantation with HLA-A, B, DR compatibility and graft survival was determined. found to have no effect on [19].

4.3 ANTIBODIES AGAINST ANTI-ANGIOTENSIN RECEPTOR

The angiotensin-1 receptor is a type of G protein that plays role in the homeostasis of the endothelial cells [20,21]. Overactivity of AT1R causes vascular and blood pressure problems [22]. Antibodies against AT1R were first described in pre-eclamptic pregnant women [23]. AT1R antibodies, could be found in the sera of transplant patients with allograft dysfunction, are activated by their target receptors and are not only a biomarker but also a potential cause of allograft damage [24]. AT1R levels are affected by genetic or environmental factors. It has been determined that there are many variants of the AT1R gene [25]. It has been determined that these variants affect the primary protein structure or binding affinity rather than the expression level. As environmental factors, chronic diseases caused by inflammation and infection or ischemia-reperfusion injury can be counted [24].

AT1R antibodies are the most studied non-HLA antibody type [26,27]. It has been determined that both donor specific and AT1R antibodies cause serious damage to the graft [27]. Although AT1R antibodies are IgG1 and IgG3 types, it was determined that vascular rejection is not an indicator of complement aggregation in graft biopsies taken from anti-AT1R positive patients [28]. Intravenous immunoglobulin (IVIG) treatment improved allograft survival in patients with positive AT1R antibody. Several studies have shown that AT1R antibodies are associated with increased AMR and decreased graft survival [23]. In one study, it was stated that there is an increased risk of graft loss in recipients of a kidney transplant containing anti-AT1R antibodies. In some other studies, it was stated that it had no effect on graft damage. Banasik et al. reported that high levels of AT1R antibodies have the effect on increasing graft loss [29].

Yu et al. determined that pre-transplant anti-AT1R antibodies may have negative effects even in low-risk patients after transplantation. For this reason, it is thought that the determination of AT1R antibodies before transplantation may help prevent post-transplant risks [30].

4.4 MIC ANTIBODIES

MIC proteins are coded by MICA and MICB genes and have similarities with HLA A and B molecules [31, 32]. Most of the cells can express MICA however lumphocytes can't [33]. CD4+ and CD8+ T lymphocytes in peripheral blood and causes CD3 and CD28 binding. MICA antibodies have been detected in solid organ transplantaions as kidney and heart [34]. MICA expression in T lymphocytes is induced especially by cytokines such as IL-2, 4 and 15 [35].

MICA antibodies can be produced because of pregnancy, transplantations and transfusions [36]. However, Mizutani et al. They stated that more MICA antibodies were produced in people who had rejection compared to people who had grafts with normal function [37].

Terasaki et al. evaluated the strong alliance between renal rejection and MICA antibodies [8]. There are also studies that showed the existence and role of MICA antibodies in heart transplantation [31].

4.5 COLLAGEN ANTIBODIES

Collagens are fibrous proteins and are the main component of skeletal system [38,39]. Several cells can secrete collagen especially by connective tissue cells [40]. Almost 28 different types of collagen protein were identified encoded by more than 42 different genes. According to the collagen type the amino acid sequence differ [41].

Collagen V is found in tracheal epithelial cells and its expression is increased as a result of immune-related injury or ischemia-reperfusion injury (IRI). Collagen antibodies pose a problem especially in lung transplantations [23]. Graft damage induces expression of matrix metalloproteinases, which modify collagen and make it a prime target for autoantibody production [42]. Production of collagen V antibodies after transplantation causes bronchiolitis obliterans syndrome (CSF) [23].

In renal transplantation collagen (especially type IV) and fibronectin antibodies have been identified [43]. IFN-gamma and IL-17 secreting CD4+ T cells were observed in these patients and the effect of collagen IV on graft rejection was demonstrated [23]. Collagen V-specific CD4+ T cells or collagen V antibodies have been shown to induce CSF in lung transplan-

tation models [23]. Transferring anti-collagen V antibodies to rat recipients results in lung dysfunction and decrease survival rates after transplantation [44].

4.6 K-α 1 TUBULIN ANTIBODIES

KA1T is an epithelial surface gap junction cytoskeleton protein, and while it does not normally pose a problem, it influences inflammation and tissue damage. When KA1T binds to its specific antibody, the expression of increased fibrogenic growth factors increases, cell cycle signaling is activated and KA1T begins to exert its direct negative effect [45].

KA1T antibodies were observed in 67% of lung transplantation cases [45]. Immunization against MHC class I antigens in lung transplanted mouse models has been determined to induce a secondary antibody response against K-alpha1 tubulin and collagen [46]. The attachment of these antibodies to the trachea epithelium results in increased expression of fibrogenic growth factors, activation of cell cycle signaling, and fibroproliferation. All these events cause the formation of CSF [7].

4.7 ANTI LG3 ANTIBODIES – PERLECAN

The risk of acute rejection arises with the presence of autoantibodies after transplantation [47]. It is thought that this may be due to the increase or release of neoepitopes. According to recent data, extracellular matrix components act as neoantigens and induce a humoral response associated with acute and chronic rejections [48]. Increased LG3 levels have been measured in acute vascular rejection after kidney transplantation. It was determined that circulating LG3 levels also increased after transplantation, and this increase was correlated with the increase in antibodies produces against

LG-3 [48]. Perlecan is 500kDa core protein that provides bio-functional diversity to the molecule [49]. Perlecan consists of five different domains, and the C-terminal domain called endorepellin that consists of three laminin-like globular domains (LG) [23]. LG3 is the C-terminal fragment of perlecan and is released during vascular injury and endothelial apoptosis [50].

Growth factors such as chemokines and fibroblast growth factors are potential ligands. Proteoglycans are main components of vascular membranes and play role in remodeling of tissue [51,52]. Pilon et al. shown that the level of LG3 in the circulation and urine is increased in acute, chronic vascular damage and renal dysfunction. Several studies have shown that an increase in serum LG3 levels in kidney transplant recipients causes immune-related vascular damage and kidney dysfunction [44, 54].

4.8 ANTIBODIES AGAINST MYOSIN AND VIMENTIN

Vimentins are involved in stabilizing the structure of cytoplasm as an intermediate filament protein [55, 56]. It has been determined that there are normal phenotypes in mouse models that do not express vimentin, but in special cases damages and anomalies could be detected. Therefore, in many cellular processes' vimentin have special function [57]. Several cells as neutrophils, leukocytes and endothelial cells can express vimentin and in many autoimmune disorders anti-vimentin antibodies can be detected [58,59].

Tissue-specific antigen expression can be explained by the absence of systemic damage during antibody-associated rejection [60]. Vimentin is highly expressed in the intima and coronary arteries, and less expressed in renal tubular and mesenchymal cells [61]. Antibodies produced against vimentin could be observed in heart and vascular diseases. It is unc-

lear whether this association exists independently of HLA alloimmunization. Vimentin immunization is associated with CAV and C3d deposition on the endothelial cell surface [46]. It has also been determined that anti-myosin antibodies seen before transplantation reduce life expectancy below 2 years in people with heart transplantation [62].

4.9 CONCLUSION

The immunosuppressives have an impact on the hyperacute rejection, still anti-HLA antibodies cause many problems in organ transplantation that result lower survival rates and graft lost. The studies on new treatment strategies focused on to overcome this problem. However, as a result of the observation of organ rejections in patients with both donor-specific and antibodies produced against a certain HLA antigen panel and whose cross-match tests were found to be negative, non-HLA antibodies began to attract attention. The reaction of non-HLA antibodies to transplant-related treatment strategies has yet to be fully determined. Studies are being conducted to define mechanisms of non-HLA antibodies using new methods and protocols. The target of researchers are expected to develop new therapy agents, protocols to increase both grafts' and patients' survival.

4.10 REFERENCE

1. Black CK, Termanini KM, Aguirre O, Hawksworth JS, Sosin M. Solid organ transplantation in the 21st century. Ann Transl Med. 2018 Oct;6(20):409. https://doi.org/10.21037/atm.2018.09.68

2. Nakamura T, Shirouzu T, Nakata K, Yoshimura N, Ushigome H. The Role of Major Histocompatibility Complex in Organ Transplantation- Donor Specific Anti-Major Histocompatibility Complex Antibodies Analysis Goes to the Next Stage. Int J Mol Sci. 2019 Sep 13;20(18):4544. https://doi.org/10.3390/ijms20184544

3. Michielsen LA, Zuilen AD, Van Krebber MM, Verhaar MC, Otten HG. Clinical value of non-HLA antibodies in kidney transplantation : Still an enigma ? *Transplantation Reviews*, 2016; *30*(4), 195–202. https://doi.org/10.1016/j.trre.2016.06.001

4. Senev, A., Coemans, M., Lerut, E., Van Sandt, V., Daniëls, L., et al. Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: Clinical presentation and implications for outcome. American Journal of Transplantation, 2019;19(3), 763-780 https://doi.org/10.1111/ajt.15074

5. Reindl-Schwaighofer, R., Heinzel, A., Gualdoni, G. A., Mesnard, L., Claas, F. H., et al. Novel insights into non-HLA alloimmunity in kidney transplantation. Transplant International, 2020;33(1), 5-17 https://doi.org/10.1111/tri.13546

6. Zachary, A. A., Leff, M. S., Walker, J. M. *Transplantation Immunology Methods and Protocols.2nd edition. Humana Press*. 2013; 41-71

7. Sigdel TK, Sarwal MM. Moving beyond HLA: A review of nHLA antibodies in organ transplantation. *Human Immunology*, 2013; *74*(11): 1486–90 https://doi.org/10.1016/j.humimm.2013.07.001

8. Terasaki PI, Ozawa M, Castro R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. *American Journal of Transplantation*. 2007; *7*(2), 408–415. https://doi.org/10.1111/j.1600-6143.2006.01644.x

9. Reinsmoen NL, Lai CH, Heidecke H, Haas M, Cao K, et al. Anti-angiotensin type 1 receptor antibodies associated with antibody mediated rejection in donor HLA antibody negative patients. *Transplantation*. 2010; *90*(12), 1473–7. https://doi.org/10.1097/TP.0b013e3181fd97f1

10. Dragun D, Catar R, Philippe A. Non-HLA antibodies in solid organ transplantation: recent concepts and clinical relevance. *Current opinion in organ transplantation*. 2013; *18*(4), 430–5. https://doi.org/10.1097/MOT.0b013e3283636e55
11. El Hennawy, H. M. Non-HLA Antibodies in Renal Transplantation, Where Do We Stand. J Clin Exp Transplant, 2018;3, 125.
12. Nowańska, K., Wiśnicki, K., Kuriata-Kordek, M., Krajewska, M., & Banasik, M. The role of endothelin II type A receptor (ETAR) in transplant injury. Transplant Immunology, 2022;70, 101505. https://doi.org/10.1016/j.trim.2021.101505
13. Banasik M, Jabłecki J, Boratynska M, Kaminska D, Koscielska-Kasprzak K, et al. Humoral immunity in hand transplantation : Anti-HLA and non-HLA response. *Hum Immunol*. 2014; *75*, 859–62. https://doi.org/10.1016/j.humimm.2014.06.010
14. Kelly, A., & Trowsdale, J. Genetics of antigen processing and presentation. Immunogenetics, 2019;71, 161-170. https://doi.org/10.1007/s00251-018-1082-2
15. Deuse, T., Hu, X., Gravina, A., Wang, D., Tediashvili, G., et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nature biotechnology, 2019;37(3), 252-258. https://doi.org/10.1038/s41587-019-0016-3
16. Dzierzak-Mietla M, Markiewicz M, Siekiera U, Mizia S, Koclega A, Zielinska P, et al. Occurrence and Impact of Minor Histocompatibility Antigens' Disparities on Outcomes of Hematopoietic Stem Cell Transplantation from HLA-Matched Sibling Donors. *Bone marrow research*. 2012; 1-12. https://doi.org/10.1155/2012/257086
17. Granados, D. P., Sriranganadane, D., Daouda, T., Zieger, A., Laumont, C. M., et al. Impact of genomic polymorphisms on the repertoire of human MHC class I-associated peptides. Nature communications, 2014;5(1), 3600. https://doi.org/10.1038/ncomms4600
18. Mo, F., Mamonkin, M., Brenner, M. K., & Heslop, H. E. Taking T-cell oncotherapy off-the-shelf. Trends in immunology, 2021;42(3), 261-272. https://doi.org/10.1016/j.it.2021.01.004

19. Dierselhuis M, Goulmy E. The relevance of minor histocompatibility antigens in solid organ transplantation. *Current Opinion in Organ Transplantation*. 2009; *14*(4), 419-425. https://doi.org/10.1097/mot.0b013e32832d399c
20. Pfeiffer, C. T., Wang, J., Paulo, J. A., Jiang, X., Gygi, S. P., et al. Mapping angiotensin II type 1 receptor-biased signaling using proximity labeling and proteomics identifies diverse actions of biased agonists. Journal of proteome research, 2021;20(6), 3256-3267. https://doi.org/10.1021/acs.jproteome.1c00080
21. Zhang, X., & Reinsmoen, N. L. Angiotensin II type I receptor antibodies in thoracic transplantation. Human Immunology, 2019;80(8), 579-582. https://doi.org/10.1016/j.humimm.2019.04.007
22. El-Arif, G., Khazaal, S., Farhat, A., Harb, J., Annweiler, C., et al. Angiotensin II Type I Receptor (AT1R): the gate towards COVID-19-associated diseases. Molecules, 2022;27(7), 2048. https://doi.org/10.3390%2Fmolecules27072048
23. Zhang, Q, Reed EF. The importance of non-HLA antibodies in transplantation. *Nature Publishing Group*, 2016; *12*(8), 484-95. https://doi.org/10.1038/nrneph.2016.88
24. Philogene, M. C., Johnson, T., Vaught, A. J., Zakaria, S., & Fedarko, N. Antibodies against angiotensin II type 1 and endothelin A receptors: relevance and pathogenicity. Human immunology, 2019;80(8), 561-567. https://doi.org/10.1016%2Fj.humimm.2019.04.012
25. Cao, Y., Kumar, S., Namkung, Y., Gagnon, L., Cho, A., et al. Angiotensin II type 1 receptor variants alter endosomal receptor–β-arrestin complex stability and MAPK activation. Journal of Biological Chemistry, 2020;295(38), 13169-13180. https://doi.org/10.1074/jbc.ra120.014330
26. Lefaucheur, C., Viglietti, D., Bouatou, Y., Philippe, A., Pievani, D., et al. Non-HLA agonistic anti-angiotensin II type 1 receptor antibodies induce a distinctive phenotype of antibody-mediated rejection in kidney transplant recipients. Kidney International, 2019;96(1), 189-201. https://doi.org/10.1016/j.kint.2019.01.030
27. Sorohan, B. M., Ismail, G., Leca, N., Tacu, D., Obrișcă, B., et al. Angiotensin II type 1 receptor antibodies in kidney transplantation: an evidence-based comprehensive review. Transplantation Reviews, 2020;34(4), 100573. https://doi.org/10.1016/j.trre.2020.100573

28. Filippone, E. J., Gulati, R., & Farber, J. L. Noninvasive assessment of the alloimmune response in kidney transplantation. Advances in Chronic Kidney Disease, 2021;28(6), 548-560. https://doi.org/10.1053/j.ackd.2021.08.002
29. Banasik M, Boratyńska M, Kościelska-Kasprzak K, Mazanowska O, Bartoszek D. et al. Long-term follow-up of non-HLA and anti-HLA antibodies: Incidence and importance in renal transplantation. *Transplantation Proceedings*. 2013; *45*(4), 1462–65. https://doi.org/10.1016/j.transproceed.2012.11.025
30. Yu, S., Huh, H. J., Lee, K. W., Park, J. B., Kim, S. J. et al. Pretransplant angiotensin II type 1 receptor antibodies and anti-endothelial cell antibodies predict graft function and allograft rejection in a low-risk kidney transplantation setting. Annals of Laboratory Medicine, 2020;40(5), 398-408. https://doi.org/10.3343/alm.2020.40.5.398
31. Sumitran-Holgersson S. Relevance of MICA and other non-HLA antibodies in clinical transplantation. *Current Opinion in Immunology*. 2008; *20*(5), 607–613. https://doi.org/10.1016/j.coi.2008.07.005
32. Panigrahi A, Gupta N, Siddiqui JA, Margoob A, Bhowmik D et al. Post Transplant Development of MICA and Anti-HLA Antibodies is Associated with acute rejection episodes and renal allograft loss. 2007; 68(5): 6–11. https://doi.org/10.1016/j.humimm.2007.01.006
33. Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stastny P. Identification of MICA as a New Polymorphic Alloantigen Recognized by Antibodies in Sera of Organ Transplant Recipients, *Hum Immunol*. 2000; 61(9): 917-24. https://doi.org/10.1016/s0198-8859(00)00162-2
34. Li L, Chen A, Chaudhuri A, Kambham N, Sigdel T, et al. Compartmental Localization and Clinical Relevance of MICA Antibodies After Renal Transplantation. *Transplantation*, 2010; *89*(3), 312–319. https://doi.org/10.1097%2FTP.0b013e3181bbbe4c
35. Liu, Y., & Zhang, C. The role of human γδ T cells in anti-tumor immunity and their potential for cancer immunotherapy. Cells, 2020;9(5), 1206. https://doi.org/10.3390%2Fcells9051206
36. Andolina, J. R., Walia, R., Oliva, J., Baran, A., Liesveld, J., et al. Non-donor specific anti-human leukocyte antigen (HLA) antibodies are not associated with poor outcome in hematopoietic

stem cell transplant recipients. Human Immunology, 2020;81(8), 407-412. https://doi.org/10.1016/j.humimm.2020.05.006

37. Mizutani K, Terasaki P, Rosen A, Esquenazi V, Miller J, et al. Serial Ten-Year Follow-Up of HLA and MICA Antibody Production Prior to Kidney Graft Failure. *Am J Transplant*. 2005; 2265–72. https://doi.org/10.1111/j.1600-6143.2005.01016.x

38. Scutariu, R. E., Batrinescu, G., Nechifor, G., Popescu, M., & Tenea, A. G. Separation of the collagen protein by ultrafiltration: Effects of concentration on the membrane's characteristics. Polymer Engineering & Science, 2020;60(10), 2487-2495. https://doi.org/10.1002/pen.25486

39. Naomi, R., Ridzuan, P. M., & Bahari, H. Current insights into collagen type I. Polymers, 2021;13(16), 2642. https://doi.org/10.3390/polym13162642

40. Alberts B., Johnson A., Lewis J., Morgan D, Raff M., et al. Molecular Biology of The Cell. 6th Ed. NY, USA, *Garland Science, Taylor & Francis Group*, 2015; 1297-1347. https://doi.org/10.3390%2Fijms161226074

41. Karsdal, M. A., Daniels, S. J., Holm Nielsen, S., Bager, C., Rasmussen, D. G., et al. Collagen biology and non-invasive biomarkers of liver fibrosis. Liver International, 2020; 40(4), 736-750. https://doi.org/10.1111/liv.14390

42. Palladini, G., Ferrigno, A., Richelmi, P., Perlini, S., & Vairetti, M. Role of matrix metalloproteinases in cholestasis and hepatic ischemia/reperfusion injury: A review. World journal of gastroenterology, 2015; 21(42), 12114. https://doi.org/10.3748%2Fwjg.v21.i42.12114

43. Angaswamy, N., Klein, C., Tiriveedhi, V., Gaut, J., Anwar, S., et al. Immune responses to collagen-IV and fibronectin in renal transplant recipients with transplant glomerulopathy. American Journal of Transplantation, 2014; 14(3), 685-693. https://doi.org/10.1111/ajt.12592

44. Soulez M, Pilon E, Brassard N, Qi S, Wu S, et al. The Perlecan Fragment LG3 Is a Novel Regulator of Vascular Rejection. *Circ Res*. 2012; 110(1): 94-104. https://doi.org/10.1161/CIRCRESAHA.111.250431

45. Hachem RR, Tiriveedhi V, Patterson GA, Aloush A, Mohana-

kumar T. Antibodies to K- a 1 Tubulin and Collagen V Are Associated With Chronic Rejection After Lung Transplantation. *Am J Transplant*. 2012; 12(8), 2164-71. https://doi.org/10.1111/j.1600-6143.2012.04079.x

46. Delville M, Charreau B, Rabant M, Legendre C, Anglicheau D. Pathogenesis of non-HLA antibodies in solid organ transplantation: Where do we stand? *Human Immunology*. 2016; *77*(11), 1055–62. https://doi.org/10.1016/j.humimm.2016.05.021

47. Peters, A. L., Rogers, M., Begum, G., Sun, Q., Fei, L., et al. T-cell infiltrate intensity is associated with delayed response to treatment in late acute cellular rejection in pediatric liver transplant recipients. Pediatric Transplantation, e14475 2023. https://doi.org/10.1111/petr.14475

48. Dieudé, M., Cardinal, H., & Hébert, M. J. Injury derived autoimmunity: Anti-perlecan/LG3 antibodies in transplantation. Human Immunology, 2019; 80(8), 608-613. https://doi.org/10.1016/j.humimm.2019.04.009

49. Gubbiotti MA, Neill T, Iozzo RV. A current view of perlecan in physiology and pathology: A mosaic of functions. *Matrix Biology*, 2016; 285–98. https://doi.org/10.1016%2Fj.matbio.2016.09.003

50. Beillevaire, D., Migneault, F., Turgeon, J., Gingras, D., Rimbaud, A. K., et al. Autolysosomes and caspase-3 control the biogenesis and release of immunogenic apoptotic exosomes. Cell Death & Disease, 2022; 13(2), 145. https://doi.org/10.1038/s41419-022-04591-5

51. Katta K, Boersema M, Adepu S, Rienstra H, Celie JW, et al. Renal heparan sulfate proteoglycans modulate fibroblast growth factor 2 signaling in experimental chronic transplant dysfunction. *American Journal of Pathology*. 2013;*183*(5), 1571–84. https://doi.org/10.1016/j.ajpath.2013.07.030

52. Celie J.W.A.M., Rutjes N.W.P, Keuning E.D, Soininen R, Heljasvaara R, et al. Subendothelial heparan sulfate proteoglycans become major L-selectin and monocyte chemoattractant protein-1 ligands upon renal ischemia/reperfusion. *The American Journal of Pathology*. 2007; *170*(6), 1865–78.

53. Pilon, E. A., Dieudé, M., Qi, S., Hamelin, K., Pomerleau, L., et al. The perlecan fragment lg3 regulates homing of mesenchymal

stem cells and neointima formation during vascular rejection. *American Journal of Transplantation*. 2015; *15*(5):1205–18. https://doi.org/10.1111/ajt.13119

54. Riordan EO, Addabbo F, Goligorsky MS. Urine proteomics – prospects for future diagnostics, 2007; *Acta Physiologica Hungarica*. 2007; *94*, 133–141. https://doi.org/10.1556/aphysiol.94.2007.1-2.12
55. Rose ML. Role of anti-vimentin antibodies in allograft rejection. *Human Immunology*. 2013; *74*(11), 1459–62. https://doi.org/10.1016%2Fj.humimm.2013.06.006
56. Paulin, D., Lilienbaum, A., Kardjian, S., Agbulut, O., & Li, Z. Vimentin: Regulation and pathogenesis. Biochimie, 2022; 197, 96-112. https://doi.org/10.1016/j.biochi.2022.02.003
57. Mor-vaknin N, Punturieri A, Sitwala K, Markovitz DM. Vimentin is secreted by activated macrophages. *Nat Cell Biol*. 2003; 5(1): 59-63. https://doi.org/10.1038/ncb898
58. Patteson, A. E., Vahabikashi, A., Goldman, R. D., & Janmey, P. A. Mechanical and non-mechanical functions of filamentous and non-filamentous vimentin. Bioessays, 2020; 42(11), 2000078. https://doi.org/10.1002/bies.202000078
59. Carter V, Shenton BK, Jaques B, Turner D, Talbot D, et al. Vimentin Antibodies : A Non-HLA Antibody as a Potential Risk Factor in Renal Transplantation. *Transplantation Proceedings.* 2005; 654–57. https://doi.org/10.1016/j.transproceed.2004.12.043
60. Holers, V. M. Contributions of animal models to mechanistic understandings of antibody-dependent disease and roles of the amplification loop. Immunological Reviews, 2023; 313(1), 181-193. https://doi.org/10.1111/imr.13136
61. Rampersad, C., Shaw, J., Gibson, I. W., Wiebe, C., Rush, D. N., et al. Early antibody-mediated kidney transplant rejection associated with anti-vimentin antibodies: a case report. American Journal of Kidney Diseases, 2020; 75(1), 138-143. https://doi.org/10.1053/j.ajkd.2019.06.010
62. Morgun A, Shulzhenko N, Unterkircher CS, Diniz RVZ, Pereira Abet al. Pre-and post-trasnplant anti myosin and anti-heat shock protein antibodies and cardiac transplant. *Journal of Heart and Lung Transplantation*. 2004; 2498(3): 204–9. https://doi.org/10.1016/s1053-2498(03)00114-1

miRNAs IN ORGAN TRANSPLANTATION

Aslı OZKIZILCIK KOCYIGIT[1,2]

Medical Biology Department,

[1]İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

5.1 INTRODUCTION

MicroRNAs (miRNA) were first discovered in 1993 in the nematode "Caenorhabditis elegans". These RNA molecules are about 22 nucleotides in length, single-stranded, and do not code for proteins. They are involved in the posttranscriptional regulation of many physiological processes, from development to oncogenesis in cells [1]. By binding specifically to mRNA targets, they either suppress their translation or stimulate their degradation. Due to the tissue and cell specific properties of miRNAs, it has attracted considerable attention as a noninvasive biomarker in the early diagnosis of many diseases [2].

One of the treatment options for patients with end-stage renal disease is kidney transplantation. The allograft may be damaged due to ischemia-reperfusion injury, acute and chronic rejection, infections, and recurrence of kidney disease. Serum creatinine value and proteinuria are checked to monitor this damage. Unfortunately, the increase in these two parameters occurs after kidney damage has progressed. It does not show any symptoms before. In addition, "renal biopsy",

which is the gold standard method, should be performed for the definitive diagnosis of kidney damage. The biopsy procedure is an invasive procedure and has a ~3% risk of complications. For this reason, non-invasive biomarker research is carried out from serum or urine [3].

In expression studies, there are biomarker studies that can be used to monitor the kidney function of many miRNAs from diabetic patients with renal dysfunction [4], patients with chronic renal failure (Trionfini et al., 2014) and even kidney transplant patients [5]. In this section, current data on the use of miRNAs as biomarkers in kidney transplant patients and their contribution to post-transplant therapy will be discussed.

5.2 miRNA BIOSYNTHESIS AND FUNCTION

The first miRNA found was lin-4 isolated from *Caenorhabditis elegans*. miRNAs are small, non-protein-coding RNA fragments about 22 nucleotides long. They are first transcribed from DNA as primary miRNA (pri-miRNA). Then, they are processed to become precursor miRNA (pre-miRNA). They also function as mature miRNAs after the final editing. Almost half of the miRNAs now recognized are located within genes, with the majority being derived from introns. A minority consists of exons that code for proteins, while the remaining units are autonomously produced from a source gene and subject to regulation by their unique promoter [6].

miRNAs are processed in conventional and unconventional ways. The classic method involves the transcription of pri-miRNAs from the appropriate genes by RNA polymerase II, and their transformation into pre-miRNA occurs through the action of a microprocessor complex consisting of the ribonuclease III enzyme Drosha proteins and the RNA-binding protein Digeorge Syndrome Critical Region 8 (DGCR8).

In this case, DGCR8 recognizes N6-methyladenylated GGAC and other patterns, while Drosha chops the double-stranded pri-miRNA at the tip of the characteristic hairpin region. The exportin 5/RanGTP complex transports these small RNAs as pre-miRNA, which are subsequently cleaved by the cytoplasmic RNase III endonuclease Dicer. In this step, the final loop is eliminated and a fully developed, double-stranded miRNA is formed. The 3′ strand and 5′ end of the pre-miRNA are both designated as 3p. Both are capable of ATP-dependent binding to Argonaute (AGO) proteins (AGO1-4). It all depends on the type of cell or the cellular environment to determine which one to attach to. The AGO2 cuts and destroys the unbound strand, also known as the passenger strand. Then, the guide strand joins to AGO2 (Figure 1). The alternative approach can be classified into two categories: Dicer-independent and Drosha/DGCR8-independent. Pre-miRNAs generated autonomously by Drosha/DGCR8 exhibit similarities to Dicer substrates (i.e., mirtrons created from the intron in mRNA during splicing). Drosha is used to convert short hairpin RNAs (shRNA) into dicer-independent miRNAs. To mature, these pre-miRNAs need AGO2 proteins. As a result, AGO2 is fully loaded with pre-miRNA [6,9].

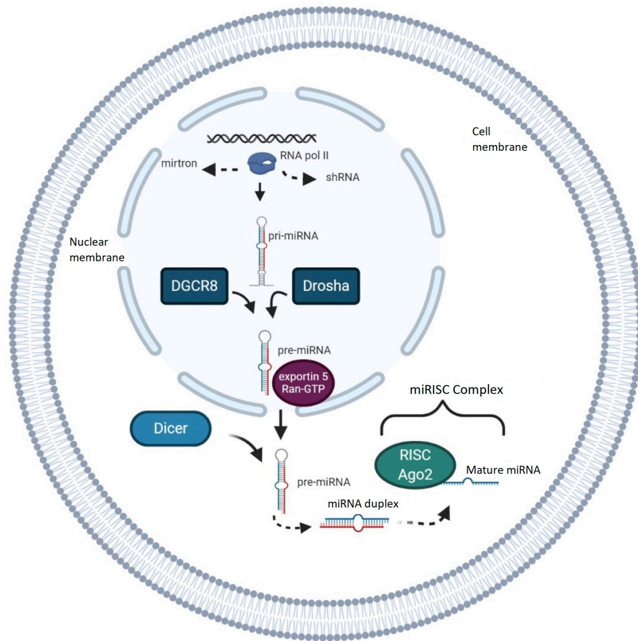


Figure 1: miRNA biosynthesis (created by biorender.com)

Typically, miRNAs attach to the 3′ UTR region of target mRNAs to stop translation. According to research, the 5′ UTR, coding sequences, and promoter regions outside of this region all include miRNA binding sites. According to studies, miRNAs inhibit gene expression when bound to coding and 5′ UTR sequences but enhance transcription when coupled to promoter regions. The guide strand and AGO proteins make up the miRNA-stimulated silencing complex (miRISC), which is essentially a functional structure. The miRNA response element (MRE), a pair of complementary sequences found in target mRNA, is recognized by this complex. Due to the accumulation of GW182 family proteins at that location, the silencing miRISC complex begins to assemble. The GW182 proteins serve as a scaffold for other effector proteins, such as CCR4-NOT, the poly(A)-deadenylase complexes PAN2, and PAN3. PAN2/3

initiates poly(A)-deadenylation of the target mRNA, which is completed by the CCR4-NOT complex. The cap is then cleaved by capping protein 2 (DCP2) and other related proteins. 5′-3′ fragments of exoribonuclease 1 (XRN1) [6,10].

5.3 THE DETECTION OF miRNA EXPRESSION PROFILE

While distinct miRNAs can be identified in various tissues, the same miRNAs can be detected in many tissues. It has become especially crucial to determine the expression of miRNAs in tissues that may aid in illness diagnosis and therapy, as well as to monitor changes in their expression. In plasma, serum, urine, and formalin-fixed tissues, miRNAs were well maintained. Furthermore, they are more sensitive to detection than proteins [11]. In miRNA expression profile research, microarray, real-time pcr, and RNA sequence analysis approaches are used. Microarray assays employ human miRNA chips designed specifically for miRNA research. The analysis is carried out utilizing commercially accessible computer programs. Ready-made commercially available primer assays specific to the relevant miRNAs can be used for real-time PCR analysis, or primers can be generated. RNA sequencing analysis can be used to determine the sequences of novel miRNAs for detection.

Park et al. used the microarray approach to examine the miRNA profile in the kidneys of mice with diabetic nephropathy and found significant alterations in the expression of 137 miRNAs associated with diabetic nephropathy [12]. Using these three approaches, Sonoda et al. assessed the miRNA profile in urine and exosomes in kidney tissue of rats suffering from acute renal damage, as well as observed expression alterations. As a result, they discovered that miR-16, miR-24, and miR-200c levels rose in urine during injury. As a result, they

hypothesized that exosome miRNAs are linked to the TGF-signaling pathway after kidney damage [13]. Timoneda et al. used RNA sequencing to determine which miRNAs were detected in the kidneys of piglets. They discovered 229 miRNAs in total. Hsa-miR-200b-3p, Ssc-miR-125b, and Ssc-miR-23b were identified as the highest expressed miRNAs [14]. Wang et al. used both microarray and real-time pcr technologies in their cell culture miRNA investigation [15].

5.4 THE ROLE OF miRNA IN KIDNEY TRANSPLANTATION PATIENTS

Renal transplantation is the most frequently utilized treatment for renal failure. Post-transplant rejection could not be avoided despite the use of sensitive and modern technological crossover tests prior to transplantation and immunosuppressive medicines after transplantation. Renal biopsy is the gold standard for detecting allograft deterioration after kidney transplantation, but because it is an intrusive procedure, there is always the possibility of consequences. As a result, biomarker research that can predict allograft status in body fluids such as blood and urine of transplant patients has gained prominence as a non-invasive strategy [3].

Biomarker research is looking into miRNAs that are involved in epigenetic control. miR-21, one of the first discovered miRNAs, has been associated with a variety of renal disorders and has been shown to be considerably increased in renal transplant patients as a result of post-transplant damage. miR-155 is another well-studied miRNA. miR-155 has also been found to be abundant in kidney transplant recipients [16]. Vahed et al. examined the levels of miR21, miR-142-3p, and miR-155 miRNA expression in the plasma of 26 renal transplant patients with stable allograft function, 15 healthy controls, and

27 transplant patients with interstitial fibrosis and tubular atrophy. They claimed to have found a link between the expression of these three miRNAs and graft damage, and that they may be used as biomarkers to predict harm [2].

miR-142-5p, miR-155, and miR-223 have been proven in studies to predict T cell-mediated rejection with high sensitivity and specificity [17]. miR-148b-3p, miR-29b-3p, and miR-769-5p have been shown to be considerably downregulated in chronic antibody-mediated rejection [18]. Chen et al. proposed that a miRNA panel found in plasma exosomes (miR-21, miR-210, and miR-4639) is likewise associated with chronic allograft malfunction and can be employed as a useful biomarker panel [19]. Apart from directly targeting miRNAs, the expression levels of Dicer, Drosha, DGCR8, Exportin 5, and Argonaute 2 proteins in the blood were studied in a study targeting proteins involved in miRNA synthesis. The expression of these proteins was dramatically reduced after transplantation compared to before [20].

Urine may be preferred in renal transplant patients since it is non-invasive and provides kidney-specific results. There are additional miRNA investigations in renal transplant patients' urine samples. Maluf et al. (2014) showed that the expression of a miRNA panel in urine changes at a very early stage, allowing it to be utilized as a biomarker [5]. Sonoda et al. revealed in 2019 that the exosome miRNA profile in urine was successful in demonstrating organ failure in rats [13]. Another study found that miR-21 in urine is linked to renal allograft failure, interstitial fibrosis, and tubular atrophy (IFTA) and may be a useful biomarker [21].

Renal transplantation is the transplant with the most miRNA research. In 2017, Hamdorf et al. discovered 40 miRNAs

linked with kidney transplantation [22]. The major goal of these investigations is to identify a non-invasive biomarker that can accurately predict rejection. Its secondary goal is to help individuals undergoing kidney transplants. Immunosuppressive medications given to the patient after transplantation have an influence on miRNA expression as well. The most often used immunosuppressive medicines for the prevention of renal allograft rejection are calcineurin inhibitors such as tacrolimus and cyclosporine A, however they are nephrotoxic when administered for an extended period of time. Vandenbussche et al. studied the effect of Tacrolimus treatment on the expression of miR-21-5p, miR-199a-5p, and miR-214-3p in animal models. These miRNAs have been found to play a role in the development of tacrolimus-induced nephrotoxicity [23]. Pharmacological investigations using miRNAs can be used to guide post-transplant immunosuppressive therapy.

5.5 CONCLUSION

While miRNAs can be employed as biomarkers in the diagnosis of various diseases, its attention as a new generation biomarker has increased in the identification of allograft damage in kidney transplant patients well before the beginning of allograft damage. Today, it has been discovered to be connected with acute or chronic, T cell-mediated or antibody-mediated rejections, and numerous miRNAs that can be utilized as biomarkers have been identified. However, no biomarker exists to substitute kidney biopsy, an invasive approach used more frequently in laboratories. This could be because the same miRNAs work in multiple organs and the organ specificity cannot be determined completely. According to this viewpoint, more exact results can be acquired with more thorough investigations on urine samples from renal transplant patients and may be approved for routine usage.

5.6 REFERENCES

1. Zou DF, Chen Y, Han YX, Chen L, and Tu GJ. Overexpression of microRNA-124 promotes the neuronal differentiation of bone marrow-derived mesenchymal stem cells. Neural Regeneration Research 2014; 9 (12): 1241–1248. https://doi.org/10.4103/1673-5374.135333.

2. Zununi Vahed S, Poursadegh Zonouzi A, Ghanbarian H, Ghojazadeh M, Samadi N et al. Differential expression of circulating miR-21, miR-142-3p and miR-155 in renal transplant recipients with impaired graft function. International Urology and Nephrology 2017; 49 (9): 1681–1689. https://doi.org/10.1007/s11255-017-1602-2.

3. van de Vrie M, Deegens JK, Eikmans M, van der Vlag J, and Hilbrands LB. Urinary MicroRNA as Biomarker in Renal Transplantation. American Journal of Transplantation 2017; 17 (5): 1160–1166 https://doi.org/10.1111/ajt.14082.

4. Nassirpour R, Raj D, Townsend R, and Argyropoulos C. MicroRNA biomarkers in clinical renal disease: from diabetic nephropathy, renal transplantation and beyond. Food and Chemical Toxicology 2016; 98: 73–88. https://doi.org/10.1016/j.fct.2016.02.018.

5. Maluf DG, Dumur CI, Suh JL, Scian MJ, King AL et al. The urine microRNA profile may help monitor post-transplant renal graft function. Kidney International 2014; 85 (2): 439–449. https://doi.org/10.1038/ki.2013.338.

6. O'Brien J, Hayder H, Zayed Y, and Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology 2018; 9: 1–12. https://doi.org/10.3389/fendo.2018.00402.

7. Treiber T, Treiber N, and Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nature Reviews Molecular Cell Biology 2019; 20 (1): 5–20, https://doi.org/10.1038/s41580-018-0059-1.

8. Hitit M, Kurar E, and Güzeloğlu A. MikroRNA Biogenesis (In Turkish). Atatürk University Veterinary Sciences 2015; 10 (3): 211–218. https://doi.org/10.17094/avbd.35776.

9. Ha M and Kim VN. Regulation of microRNA biogenesis. Nature Reviews Molecular Cell Biology 2014; 15 (8): 509–524. https://doi.org/doi: 10.1038/nrm3838.

10. Gebert LFR and MacRae IJ. Regulation of microRNA function in animals. Nature Reviews Molecular Cell Biology 2019; 20 (1): 21–37. https://doi.org/10.1038/s41580-018-0045-7.

11. Pritchard C, Kroh E, Wood B, Arroyo J, Dougherty K. et al. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prevention Research 2012; 5 (3): 492–497. https://doi.org/10.1158/1940-6207.CAPR-11-0370.

12. Park H, Lee S, Kim H, Jeon S, Han D *et al.* Profiling of miRNA expression in mice kidney with diabetic nephropathy. Molecular and Cellular Toxicology 2018; 14 (4): 445–452. https://doi.org/10.1007/s13273-018-0049-6.

13. Sonoda H, Lee B, Park K, Nihalani D, Yoon J *et al* miRNA profiling of urinary exosomes to assess the progression of acute kidney injury. Scientific Reports 2019; 9 (1): 1–11. https://doi.org/10.1038/s41598-019-40747-8.

14. Timoneda O, Balcelis I, Nunez J, Egea R, Vera G *et al.* miRNA Expression Profile Analysis in Kidney of Different Porcine Breeds. PLoS One 2013; 8 (1). https://doi.org/10.1371/journal.pone.0055402.

15. Wang B, Wu B, Liu J, Yao W, Xia D, *et al.* Analysis of altered MicroRNA expression profiles in proximal renal tubular cells in response to calcium oxalate monohydrate crystal adhesion: Implications for kidney stone disease. PLoS One 2014; 9(7): 1–9, https://doi.org/10.1371/journal.pone.0101306.

16. Fan Y, Chen H, Huang Z, Zheng H, and Zhou J. Emerging role of miRNAs in renal fibrosis. RNA Biology 2020; 17 (1): 1–12. https://doi.org/10.1080/15476286.2019.1667215.

17. Ledeganck KJ, Gielis EM, Abramowicz D, Van Craenenbroeck AH, Stenvinkel P, and Shiels PG. MicroRNAs in AKI and kidney transplantation. Clinical Journal of the American Society of Nephrology 2019; 14 (3): 454–468. https://doi.org/10.2215/CJN.08020718.

18. Rascio F, Pontrelli P, Accetturo M, Oranger A, Gigante M *et al.* A type I interferon signature characterizes chronic antibody-mediated rejection in kidney transplantation. The Journal of Pathology 2015; 237 (1): 72–84. https://doi.org/10.1002/path.4553.

19. Chen Y, Han X, Sun Y, He X, and Xue D. A circulating exosomal microRNA panel as a novel biomarker for monitoring post-transplant renal graft function. Journal of Cellular and Molecular Medicine 2020; 24 (20): 12154–12163. https://doi.org/10.1111/jcmm.15861.
20. Celen E, Ertosun MG, Kocak H, Dinckan A, and Yoldas B. Expression Profile of MicroRNA Biogenesis Components in Renal Transplant Patients. Transplantation Proceedings 2017; 49 (3): 472–476. https://doi.org/10.1016/j.transproceed.2017.01.019.
21. Gniewkiewicz MS, Paszkowska I, Gozdowska J, Czerwinska K, Sadowska-Jakubowicz A, *et al.* Urinary microRNA-21-5p as potential biomarker of interstitial fibrosis and tubular atrophy (IFTA) in kidney transplant recipients. Diagnostics 2020; 10 (2): 1–9. https://doi.org/10.3390/diagnostics10020113.
22. Hamdorf M, Kawakita S, and Everly M. The Potential of MicroRNAs as Novel Biomarkers for Transplant Rejection. Journal of Immunology Research 2017; 2017. https://doi.org/10.1155/2017/4072364.
23. Vandenbussche C, Van der Hauwaert C, Dewaeles E, Franczak J, Hennino M, *et al.* Tacrolimus-induced nephrotoxicity in mice is associated with microRNA deregulation. *Archieves of Toxicology* 2018; 92 (4): 1539–1550. https://doi.org/10.1007/s00204-018-2158-3.

OVERVIEW OF THE COMPLEMENT SYSTEM IN KIDNEY TRANSPLANTS

Tülay KILICASLAN AYNA[1,2,3]
Medical Biology Department,
[1]*İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey*

[2]*Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey*

[3]*Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey*

6.1 INTRODUCTION

The immune system comprises three primary components: immunological chemicals, immune system cells, and immune system organs. The complement is a fundamental molecule of both the innate and adaptive immune systems, comprising many proteins. The correlation between the abundance of complement proteins and the equilibrium among various complement proteins is intricately linked to illnesses. The complement system safeguards the host against foreign substances by activation via three distinct mechanisms. The activation process culminates in a shared mechanism, following the involvement of many complement proteins. Kidney transplantation serves as an assessment of the immune system's functionality. The kidney procured from either a living or deceased donor is considered an alloantigen to the recipient, hence triggering an immunological response upon transplantation. Complement proteins also inherently enga-

ge in this process. This review assessed the impact of both the patient and the donor on the complement system in the context of transplantation. The presence of abnormal conditions in the complement proteins is anticipated to initiate the progression towards renal failure in the patient. Additionally, inflammation caused by the disease and the need for dialysis in individuals with end-stage renal failure are also expected to impact complement proteins. Activation of the complement system is particularly common in transplants from cadavers, which can be attributed to donors who have been in intensive care units or have experienced ischemic spells. The donors experience substantial physiological alterations due to hemodynamic instability, hormone dysregulation, and inflammatory reactions. Therefore, alterations in cell characteristics and variations in chemokine and cytokine reservoirs can lead to the activation of complement. The activation of distinct pathways and the synergistic interaction between them enhances the immune response. Assessing the activation of the immune system through the complement window prior to and following transplantation can provide valuable insights for organ transplantation clinics.

6.2 COMPLEMENT SYSTEM

The complement system is one of the basic molecules of the natural immune response. It also serves as a bridge between the natural and acquired immune system. The focus of scientific research at the end of the XIX. century was the defense of the human body against microbial infections. In this context, Jules Bordet supported the "humoral theory" in 1899 by showing that 2 factors (complement and antibody) are important in immune lysis. [1]. Bordet infected mice with bacteria, and 2-3 weeks later, he brought together the bacteria that formed the infection with serum taken from infected mice

under in vitro conditions. In this study, 3 different test setups were prepared. Firstly, when directly incubating serum and bacteria together, he determined that the bacteria agglutinated (clustered) and cell lysis occurred. Secondly, when the serum was pretreated at 56°C and incubated with bacteria, he determined that there was agglutination but not lysis. It was determined that neither agglutination nor lysis occurred when the serum was pretreated at 62°C and then incubated with bacteria. Bordet explained that in the second test setup, agglutination occurs due to the presence of antibodies, but since the complementary molecules are not functional, the cells are not lysed. These immune reactions also gave the name complement to complementary molecules [2]. For this work, he was awarded the Nobel Prize in 1919. The complement system is a large family that includes approximately 40 different proteins. These proteins are divided into two main groups: activating and regulating immunological reactions. Complement proteins are generally denoted by the letter "C", and the numbering of these proteins is made by the World Health Organization according to the order of discovery of the proteins [3]. The complement system functions through 3 pathways (pathways) that begin with different antigenic molecules and different activator complement proteins. Regulatory complement proteins are either soluble (C1-INH, C4BP, Factor H, Factor 1, clasterin, vitronectin, carboxypeptidase) or membrane-bound (CR1, CR59, CR2, CR3, DAF, MCP) [4]. Most of the complement proteins are produced by hepatocytes. It is also synthesized in macrophages, monocytes, lymphocytes, small intestinal epithelium, spleen, lung, kidney, bone marrow and lymphoid tissue. C3, which plays a central role in complement pathways, and C1q, which binds to cytotoxic donor-specific antibodies in antibody-mediated rejection (AMR) in transplantation, are produced in bone marrow, lymphocytes and macrophages.

In serum, the protein with the highest concentration is C3 (3-4 g/L) [5]. While the contribution of C3 produced in the kidney to the circulation is 5%, this contribution was determined to be 16% in transplant patients with a history of acute rejection [6]. Complement components and expression regions expressed in the kidney are shown in Table 1 (Table 1) [7].

Table 1. Complement proteins expressed in the kidney and their expression sites.

Complement components	Expression regions
A) Complement proteins and activation products	
C3 and fragments - iC3b, C3dg and C3d	Glomerular and tubular basement membranes, renal arteries
C4 fragments -C4a and C4b-	Glomerular arteries, mesenchymal cells
C4 binding protein (C4bp)	Mesenchymal cells, subendothelial layer of glomerular basement membrane
C3, C4, C2 and Factor H	Cortical tubules
Factor D and properdin	Glomerular
Factor B	Medulla
B) Regulator proteins	
MCP (CD46), DAF (CD55)	Juktaglomerular apparatus, glomerular capillary, mesenchyme, podocytes, basolateral surface of epithelial distal tubes, peritubular capillary
CD59	Juktaglomerular apparatus, glomerular cells, glomerular basement membrane, proximal and distal tubules, collecting duct, tubular basement membrane, peritubular capillary
Factor H	Glomerular basement membrane, mesenchymal matrix, tubular basement membrane
C) Complement receptors	
CR1 (CD35)	Podocytes
C5aR1, C5aR2, C3aR	Podocytes, proximal tubules

Complement proteins trigger the immune response in 3 different ways. Alternative and lectin pathways are the innate immune system, and the classical complement pathway is the adaptive immune system mechanism. However, since natural antibodies such as anti-ABO antibodies activate the classical complement pathway, it can also be expressed as an innate immune system mechanism (Figure 1).

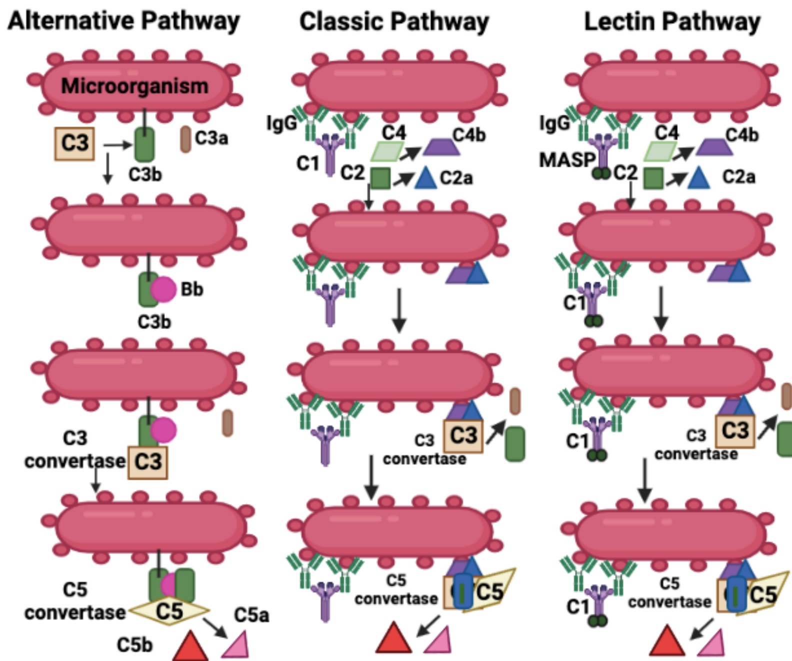


Figure 1: Pathways in complement activation (modified by Abbas et al.)[8]

6.3 COMPLEMENT ACTIVATION PATHWAYS

6.3.1. Alternative Complement Pathway

Activation begins when C3, which is present in high concentration in serum, is induced by microorganisms and is destroyed by proteolytic enzymes in the plasma, and the

C3b fragment is covalently bonded to the membrane of the microorganism. Thiol ester bonds in C3b connect to hydroxyl (OH) or NH2 on the membrane surface. C3b has a very short half-life (100 μsn). Therefore, if microorganisms do not bind to the membrane, they become inactive in a short time. In the presence of Mg^{2+}, this product forms a complex with Factor B, another complement protein. The resulting complex reacts with Factor D in the plasma, cuts Factor B, and the Bb fragment is added to C3b. Another complement protein called properdin ensures the stabilization of C3bBb to the membrane. This double complex, called C3 convertase, allows the C3 in the serum to be separated into C3a and C3b much more effectively. Some of the new C3bs are again bound to the cell membrane, while others are added to C3 convertase. This 3-complex is called C5 convertase and enables the cleavage of C5 complement protein into C5a and C5b fragments. After this stage, the terminal period of activation begins.

6.3.2 Lectin Complement Pathway

This pathway begins with the recognition of microbial molecules, mannose and polymers by molecules such as mannose-binding lectins, ficolins and collectins. These complement proteins split the C4 complement protein into C4a and C4b fragments. C4b binds to the membrane of the microorganism, then C2a is similarly cleaved. Then, C2a is added to C4b, which is covalently bound to the membrane. In this double molecule, it is called the C3 convertase of the lectin pathway. Just like the alternative pathway, it enables the serum C3 complement protein to be fragmented much more effectively. Again, the C3b fragment is added to C3 convertase, forming C5 convertase.

6.3.3 Classic Complement Pathway

In the activation of this pathway; IgM and IgG antibodies, antigen-antibody complexes, pentraxin and polymers play a role. Among the IgG molecules, IgG3, IgG1 and partially IgG2 have the ability to activate complement. The formation of donor-specific antibodies in organ transplants is important in AMRs and allograft survival. It was also stated in the Banff criteria for humoral rejection in 2013. Activation of the classical complement pathway begins with the binding of at least 2 IgG or 1 IgM with the C1q complement protein. With this binding, the conformational change in the C1q molecule enables the C1r and C1s complement proteins, which have serine esterase activation, to bind to C1q. Thus, as in the lectin pathway, C4 and C2 molecules are divided and bound to the target membrane. After the formation of this C3 convertase molecule, the reaction continues as in the lectin pathway [9,10].

Meanwhile, the most important activator of the classical complement pathway is the IgM molecule, which is a member of the Ig molecule [11]. The immune system comprises three primary components: immunological chemicals, immune system cells, and IgM is the initial antibody generated upon B cell activation and is frequently suggested as the primary defense mechanism of the human immune system. IgM is found as a monomeric molecule on naïve B cells and is consistently released as a polymer. Pentameric IgM is the most prevalent IgM polymer in humans, comprising of five monomeric units that are interconnected by the J chain. Conversely, it is established that hexameric IgM is also present in human serum. In many illnesses such as Waldenstrom's Macroglobulinemia, cold agglutin, and recurrent urinary bacterial infections, modest quantities of hexameric IgM have been observed. However, it is also found in the normal sera of humans. Contrary to

the pentamer, the IgM hexamer is composed of 6 individual blocks and does not have the J chain. Despite the passage of several decades after the identification of hexameric IgM, its precise role remains elusive [12]. Hexameric IgM is 10-20 times more effective than pentameric IgM in activating complement. This is due to the ability of the hexameric C1q molecule to bind to hexameric IgM. For this potent complement activation, hexameric IgM is a secret weapon or an undesirable molecule for humans. The question also comes to mind [13]. In 2017, the view that one C1 molecule binds to the classical 2 IgG molecules in the IgG-C1 interaction also changed [14]. It is also suggested that the C1 molecule can bind to 6 IgG molecules [15]. In 2019, it was found that IgG bound to the cell membrane can take a hexameric configuration with C1 and thus initiate complement activation [11].

The glycosylation feature of Iggs is also important in this binding. Glycosylation of arginine (Asn)-297 in the Fc region of the heavy chain of IgG is important in complement activation. This is also important in the formation of the quaternary structure of IgG [16]. Asn-297 is well conserved throughout evolution. It has been determined through mass spectrometry studies that when fucosylation of Asn-297 increases, the C1 binding properties of IgGs increase, and when it decreases, antibody-dependent cell cytotoxicity increases [11]. It has been determined that the ratios of galactose and sialic acid in the carbohydrate tail of IgGs are also different in their anti-inflammatory or pro-inflammatory reactions [15]. Although the initiation phase of the complement protein is different in each pathway, the terminal phase is common. With the formation of C5 convertase, C5 complement protein is divided into C5a and C5b. C5b binds to the cell membrane. Just as other complement proteins trigger one another, C5b triggers C6,

C7, and C8. C8 forms a pore Membrane Attack Complex (MAC) from the cell membrane with a large number of C9. Thus, the selective permeability of the membrane, which is important for cell viability and integrity, is disrupted and the composition of the cytoplasm changes, Na^+ and water enter the cell, causing cell lysis [10].

Complement activation has other consequences besides MAC formation. Anaflatoxins (C3a, C4a, C5a) are released as a result of the breakdown of complement proteins. Some cells have receptors that recognize these anaflatoxins. For example, anaflatoxins binding to C3aR and C5aR in neutrophils and monocytes cause the release of cytokines and chemokines from these cells, the release of free oxygen species and free nitrogen species, and prostaglandins, while also providing the release of vasoactive molecules from mast cells and basophils. Adhesion molecules on the cell surface and cell composition in the environment change, resulting in inflammation. Again, there are R's that recognize complement proteins on the surface of cells that perform phagocytosis, such as neutrophils and macrophages. Of these receptors, CR1 recognizes membrane-bound C3b and C4b, while CR3 recognizes inactive C3b (iC3b) and iC4b. Thus, microorganisms or antigenic structures are destroyed by phagocytosis. C3d, one of the C3 fragments, binds to antigenic structures and is recognized by CR2 receptors on B cells. C3d has the avidity feature. By lowering the activation threshold of B cells, it initiates antibody synthesis and humoral response. The released antibodies bind to the antigen and form immune complexes. Binding of C3b fragments to these immune complexes also activates erythrocytes. The 3-complex formed by the binding of CR1 receptors to erythrocytes is cleared in the liver and spleen. It is recognized by C1q, MBL, ficolins and R's in phagocytes, which

recognize apoptotic signals on the cell membrane. Thus, these cells are cleaned without damaging them[17].

C1 inhibitor (C1INH), one of the complement activators, inhibits the activation of the complement pathway in the first steps. It prevents the formation of the C4bC2a complex in the lectin and classical complement pathway. Factor H and Membrane cofactor protein (MCP, CD46) serve as cofactors for Factor I. With the enzymatic reaction of factor 1, C3b first turns into iC3b and then into C3dg. DAF inhibits the formation of C3 convertase. CD59 and clastrin prevent MAC formation[7].

6.4 ACTIVATION OF THE COMPLEMENT SYSTEM IN KIDNEY TRANSPLANTATION

In kidney transplants, the complement system, which plays an important role in both the innate and adaptive immune systems, is also extremely important for the success of transplantation. The complement system of both the patient and the donor contributes to the outcome of the transplanted kidney. There are two factors that affect the complement process in the patient. First, the patient may be experiencing renal failure as a result of a complement-related disease such as C3 glomerulopathy, membranoproliferative glomerulonephritis Type 1, atypical hemolytic uremic syndrome, IgA nephropathy, diabetes and dyslipoproteinemia. As mentioned before, complement proteins can be produced by different cells in the patient. This is risky in terms of recurrence of the disease even if there is a transplant. Another factor for the patient is dialysis. The majority of patients with end-stage renal failure are treated with hemodialysis (HD) and a very small portion with peritoneal dialysis. According to 2018 data, 2.6 billion people in the world receive dialysis treatment. Bio-

compatibility with dialysis devices is important. Because complement is a circulating protein group, the complement system is an important precursor of bioincompatibility, distinguishing between self and non-self. Cellulose-based dialysis membranes used in the past which were cheap and thin-walled, but immune reactive due to free HO groups. These negatively charged membranes create sites where C3 complement proteins can bind to thiolester groups. During hemodialysis, C3 activation peaks during the first 10-15 minutes. Activation of the terminal pathway results in the formation of C5a, C5b-9 in later stages of dialysis. Cellulose-based membranes appear to trigger complement activation. During a single HD, C5b-9, C3d/C3 ratios increase by 70% in plasma. After cellulose membranes, biocompatibility was improved by using modified cellulose membranes. The free HO groups were replaced by different substitutions, especially acetate. After this, synthetic membranes were developed. Today, these synthetic membranes are widely used [2,18]. The benefit of these membranes is, firstly, to change the pore size, and secondly, to reduce the immune reaction as biocompatibility increases. Even in modern and compatible biomembranes, complement activation still occurs [18].

The alternative pathway and the conventional pathway are activated when the complement control molecule properdin and complement element C1q recognize negatively charged surfaces found in dialysis channels and filters, respectively [19]. Dialysis filters currently include a substantial amount of Mannose-Binding Lectin Serine Protease 2 (MASP2) and ficolin 2, which are responsible for activating the complement system via the lectin pathway [20]. The neutrally charged hydrophobic surfaces of contemporary filters and tube sets play a crucial role in identification and adsorption by complement elements

[21]. Material surfaces rapidly acquire an 8-nm plasma protein coating, equivalent to a single layer, within a few seconds of being exposed to blood and/or plasma. Molecules that cause changes in protein shape and function when they come into interaction with surfaces contain non-antigen-bound IgG, which triggers the classical pathway, and C3, which triggers the other pathway. Complement activation will result in the formation of anaphylatoxins (C3a, C5a), opsonins (C3b, iC3b), and membrane attack complex (C5b-9).

The immune system comprises three primary components: immunological chemicals, immune system cells, and Initially, the activation of complement triggers the enhancement of complement receptor 3 (CR3), which attaches to C3 fragments that have gathered on the surface of leukocytes, resulting in leukopenia. Furthermore, the presence of CR3 on neutrophils plays a crucial role in the creation of platelet-neutrophil complexes, which actively contribute to thrombotic events. In addition, the process of C5a production during hemodialysis (HD) results in the activation of tissue factor and granulocyte colony-stimulating factor in neutrophils, causing hemodialysis patients to become prone to blood clotting. Furthermore, complement activation additionally facilitates the attraction and stimulation of leukocytes, leading to the generation of oxidative burst and the release of proinflammatory cytokines and chemokines. To be more precise, the activation of neutrophils by C5a results in the release of granule enzymes, including myeloperoxidase (Figure 2) [7,18].

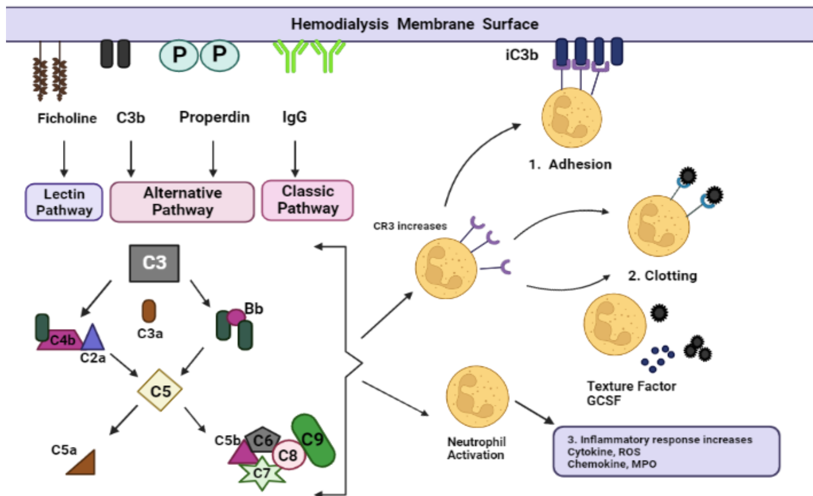


Figure 2: Complement activation during dialysis (modified by Poppelaars F et al.)[18]

Complement activation was shown to be decreased following dialysis in a nonhuman primate (NHP) model, as seen by the rise in complement activation markers levels that followed the treatment before they recovered to normal. Nevertheless, the occurrence of complement activation several times during hemodialysis gives rise to the creation of immune-mediated activity factors and the stimulation of leukocytes, leading to systemic inflammation generated by endotheliopathy[19]. Employing low complement activating filters during hemodialysis and refraining from ultrafiltration in individuals eligible for transplantation may diminish the likelihood of delayed graft function post-transplantation[18]. In patients with end-stage renal failure; The risk of anemia, malnutrition, oxidative stress, endothelial cell dysfunction, immune cell dysfunction, leukopenia, atherosclerosis and myocardial infarction increases. In these diseases, chronic inflammation is important[2]. Considering that chronic inflammation may be triggered in patients who undergo dialysis an average of 3 times a week,

the importance of the complement system draws attention once again. The complement system in the donor also affects the success of kidney transplantation. The donor source is usually either living relative donors or cadaveric donors. Living donor candidates are examined in detail clinically and immunologically, and the transplant is performed in a planned manner in a short time. Cadaveric donors can be donors who have experienced brain death or cardiac death[7]. In our country, kidneys are taken only from brain-dead cadaver donors. The kidneys obtained from these donors undergo significant physiological alterations, such as prolonged periods of cold ischemia (particularly in donors who experienced cardiac death), hemodynamic instability, hormonal imbalance, and inflammatory reactions. These changes ultimately result in a modified cell phenotype in the transplanted kidney. This could lead to the activation of complement. The activation of complement in the deceased donor kidney is the primary reason for graft injury during and after transplantation. Acute rejection is linked to cadaveric donors' systematically higher levels of activated complement components such C3dg and sC5b-9 as compared to healthy persons. Persistent endotheliopathy, inflammation, and atherosclerosis that results in myocardial infarction can coexist with complement activation in donors who have experienced cardiac mortality[22].

Ischemia and reperfusion are the most important reasons that trigger complement activation. Ischemia is a situation when cells does not receive enough oxygen that is typically supplied to the organ by blood flow, resulting in a shift in activity to an anaerobic state. Ischemia triggers complement activation by various processes, one of which is the reduction in blood pH due to anaerobic metabolism. The acidic conditions that arise as a result interfere with the complement

system, leading to a disruption in its regulation. This disruption then promotes the activation of the alternative pathway [23]. Within neutrophils, the anaphylatoxin C5a has the ability to stimulate sodium-proton exchangers, resulting in an elevation of glycolytic flux. This ultimately leads to the creation of an acidic milieu outside the cell [24]. Moreover, NH3, a nucleophile capable of cleaving the thiol ester of C3, is produced by anaerobic metabolism. Therefore, it acts as the catalyst for the alternate pathway. Ischemia also causes alterations in the characteristics of parenchymal and endothelial cells that are detected by the innate immune system. The surface of endothelial cells possesses a structural configuration that actively prevents blood clot formation and inhibits complement activation. This is mostly attributed to the presence of proteoglycans, which create a protective coating called the glycocalyx. Ischemia causes the endothelial cells of the artery wall to produce heparanase and metalloproteinases, which leads to the breaking down and disintegration of the glycocalyx [25]. The result of the dysfunction is a depletion of regulators that control the complement, adhesion systems, and coagulation. These regulators include tissue factor inhibitor, activated protein C, antithrombin, Factor H, C1INH, and C4b binding protein. These are discharged from the cellular membrane. The absence of these regulators renders the endothelial cell surface vulnerable to assault from the contact systems, coagulation, and complement. Cytokines or macrophage-activated cataract (MAC) induces intravascular inflammation and ischemia by activating the endothelium, which then becomes proinflammatory, proadhesive, and procoagulatory (Figure 3) [26].

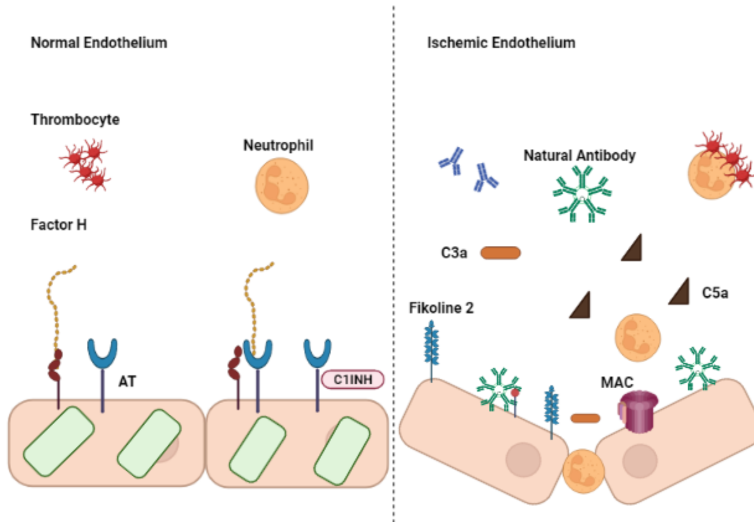


Figure 3: Ischemia reperfusion injury in gromerular capillaries (modified by Biglamia AR et al.) [7]

Although it starts with severe damage, ischemic organ reperfusion triggers a process that is required to accomplish tissue healing. Ischemia causes an increase in the production of fucose and the release of collectin 11 from the basolateral side of renal tubular cells. When locally manufactured complement components are present or during reperfusion, MASP1 and MASP2 attach to collectin11. With ischemia, the levels of C3 synthesized in renal tubular cells increase, and the loss of complement regulators such as MCP and Factor H increases. This facilitates cell death and acute kidney injury. Animal experiments have demonstrated that ischemia reperfusion is a significant factor in the activation of complement. In pig kidney models of ischemia reperfusion, the kidneys were preserved by blocking either the conventional process utilizing C1INH or the lectin pathway prior to reperfusion [7]. Pre-induction renal ischemia reperfusion in mice was protected from renal injury by blocking the alternative pathway with

a monoclonal antibody specific to C3b. It has been shown in mouse models that increasing C3 levels in the graft correlated with increasing cold ischemia time [27,28].

Nauser et al. described complement activation and cellular and humoral immune reactions in kidney allograft [29]. (Figure 4). All three of the complement system's ways are activated when damage-associated molecules, which are expressed in endothelial cells, hypoxic damaged tubules, and perivascular cells during ischemia, are identified by pattern recognition receptors such as ficolin, mannose-binding lectin (MBL), C1q, C3b, and collins. Figure 4 shows the recognition of fucosylated ligands by collectin 11 (Figure 4). Collectin 11 is associated with MASP1, MASP3, and junctional MASP2. In this way, the lectin pathway is activated. After cleavage of C3 and C5, MAC is formed, resulting in inflammatory damage and cell death. If there are alloantibodies against alloantigens expressed on renal endothelial cells, especially against incompatible HLA antigens of the donor, activation of the classical complement pathway can be initiated. Alloantibodies formed against the donor in the patient are investigated through crossover tests performed before transplantation. Alloantibodies, which were first investigated by Terasaki with the lymphocytotoxicity method, are now detected with more sensitive methods using techniques based on the flow cytometry method. Before transplantation, the classical complement pathway is activated in the recipient whose donor has alloantibodies specific to HLA antigens. In short, C1q, C1r and C1s are added to the alloantibody that binds to the HLA antigen in the graft tissue, and the process that ends with MAC formation begins. Alloantigens after transplantation; It can initiate the immune reaction through direct, indirect or semi-direct ways. Rejection may occur hours or even minutes after

transplantation. In recent years, organ transplant immunology laboratory studies and tests investigating serum complement proteins such as C1q and C3d, which play a role in the early steps of complement activation, have been started in order to monitor complement-activating antibodies before and after transplantation [30,31].

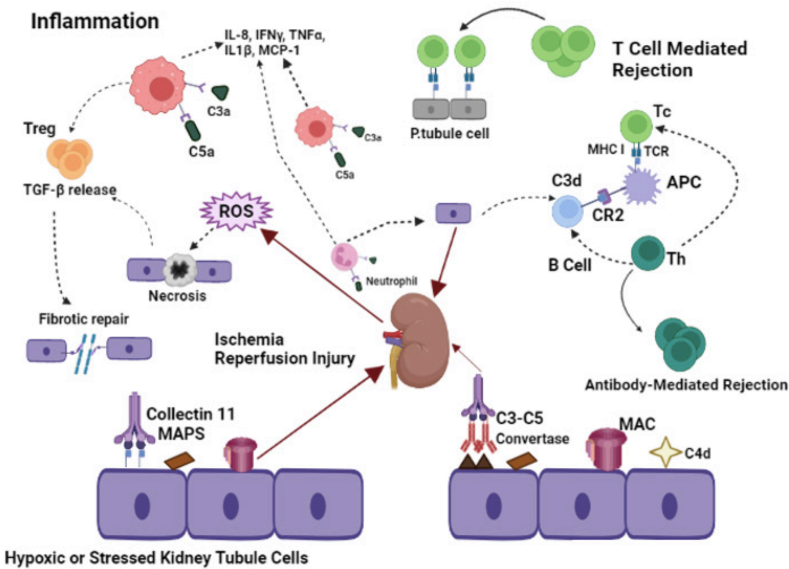


Figure 4: Complement activation pathways and allograft immunology (modified by Nauser CL.)[29]

The immune system comprises three primary components; Immunological chemicals, immune system cells and Furthermore, antigen-presenting cells (APCs), including dendritic cells, monocytes and macrophages from both the recipient and the donor, exhibit the presence of complement components C3 and C5, as well as complement receptors C3aR and C5aR1. C3a and C5a, generated through the activation of the complement system in the area outside of cells, enhance the interaction between T cells and antigen-presenting cells (APCs) by promoting the display of alloantigens and the exp-

ression of molecules that provide further stimulation. In addition, C3a and C5a enhance the development and longevity of CD4+ T cells. Furthermore, APCs facilitate the expansion and specialization of CD4+ and CD8+ T lymphocytes. CD8+ T lymphocytes are responsible for causing cellular rejection in both the intravascular and extravascular compartments. These compartments are specifically characterized as endotheliitis and tubulitis, respectively, in pathological terms. CD4+ T lymphocytes promote B cell proliferation, leading to the generation of antibodies. Furthermore, the B cell's reaction to alloantigen can be directly intensified by complement. This has been documented for antigens that are not related to transplantation. This could enhance the process of antigen presentation through opsonization by C3b and its metabolites C3d and CR2. CR2 is expressed on follicular dendritic cells and B cells within secondary lymphoid tissue. The interaction between the B cell receptor and opsonized antigen reduces the activation threshold of B cells and facilitates the conversion of the donor-specific antibody from IgM to IgG through class switching. De novo alloantibodies formed after transplantation ensure MAC formation through the classical complement pathway. Binding of C3a and C5a to C3aR and C5aR expressed in macrophages and neutrophils causes the release of fibrotic factors, resulting in fibrosis. C4d of a renal biopsy sample is usually evaluated to detect calysic complement activation. In kidney biopsy samples, caution should be exercised when interpreting C4d staining results. Due to the presence of C4d epitopes in C4 and C4 components that are covalently attached to the cell. Thus, rather than C4d accumulating as a result of complement activation, it is possible that the antibody identifies C4 produced by endothelial cells in response to cytokine production[32]. It is worth considering this option. The presence of uncertainty could account for

the variation in the interpretation of C4d data among various sites. Currently, researchers are examining anti-C4d antibodies that target novel epitopes exclusively found in C4d, with the aim of resolving this issue [33]. The polymorphisms of complement proteins of the patient and the donor have been compared in various studies. In this field, the C3 complement protein polymorphism has been evaluated most frequently. There is a single nucleotide difference in the 3rd exon of the gene encoding C3. In this case, 2 different alleles arise, C3F and C3S. The C3S allele is the dominant form. Caucasians carry the C3S allele at a rate of 80%, black races at a rate of 95%, and Asians at a rate of 99% [32]. Again, the relationship between polymorphisms in complement proteins such as C4a, C4b, C5, C5aR, ficolin, MASP2 and MBL and rejection and graft survival was investigated. Generally, no significant difference was found in the results [7,34].

6.5 IMMUNSUPPRESSIVE AGENTS THAT PROVIDE INHIBITION OF THE COMPLEMENT SYSTEM

Since activation of the complement system is a serious threat to the graft during the transplantation process, inhibition of the complement system is important for the survival of the graft. Several agents are now under development for this specific aim (Table 2). These drugs facilitate the improvement of graft quality prior to transplantation, avoiding the occurrence of graft damage caused by complement, and the management of innate and adaptive immune responses [7, 35]. Preclinical investigations have demonstrated that administering complement inhibitors to deceased donors is linked to enhanced graft performance following transplantation. In rats, C3 and C5 convertases were inhibited using the recombinant protein sCR1. It is possible to suppress the adhesion, kalikrein-kinin, and coagulation systems as well as the le-

ctin and conventional pathways of the complement system by using pure C1INH [36].

le 2. Immunosuppressive agents that inhibit complement proteins.

Complex	Content	Target molecule	Mechanism
C1INH	Purified or recombinant protein	C1r, C1s, MASP1, MASP2, Factor B	It blocks classical and lectin pathways by inactivating the serine protease activity of molecules.
Eculizumab	Humanized monoclonal antibody	C5a	Blocks terminal pathway of complement by preventing C5 cleavage
BIVV009	Humanized monoclonal antibody	C1s	Blocks the classical pathway by inactivating C1s
IdeS	Protease	IgG	C1q binding is prevented by damaging IgG, C binding is preserved
APT070	Recombinant protein	C3 and C5 convertases	Inhibits C3 and C5 convertases
Compstatin family inhibitors	Peptide	C3	Binds to C3, preventing it from being cut by C3 convertase
sCR1	Recombinant protein	C3 and C5 convertases	Inhibits C3 and C5 convertases
TT30	Recombinant protein (Chimeric CR2-Factor H)	C3 and C5 convertases of the alternative way	Binds to C3d in target cells and inhibits C3 convertase
C5aR1 antogonist	Peptide	C5aR1	Inhibits signaling by blocking C5aR1
Cobra poison factor	Recombinant protein	C3 and C5	By binding to factor B, it cleaves the alternative pathway C3 and C52 and prevents the formation of convertase.

A viable clinical strategy for suppressing the complement system in transplantation involves modifying the allograft stabilization solution. In a study using mice, the inhibition of C5aR1 in kidney allografts before transplantation greatly en-

hanced the lifespan of the transplants [37]. The researchers inferred from this outcome that modifying the allograft preservation solution could be a viable approach to mitigate complement-induced harm. Nevertheless, there is a lack of clinical data pertaining to this approach. Another intriguing strategy involves inhibiting complement activation by targeting specific regions where C3 fragments accumulate, or the creation of membrane attack complexes (MAC) occurs at the site of inflammation and tissue damage. The suppression of this process has already been accomplished in experimental models of tissue injury, such as by utilizing the chimeric TT30 (CR2-Factor H) protein [38,39]. Pretreating the kidneys with TT30 before transplanting preservation in rats effectively reduced ischemia reperfusion damage. When examining the suppression of complement, numerous potential options have been identified, although only two medications are now employed in clinical settings. C1INH and the anti-C5 monoclonal antibody eculizumab compounds. Eculizumab selectively hinders the final stage of the complement system by obstructing the splitting of C5 into C5a and C5b, thus impeding the creation of MAC. This medication is utilized for the management of ABO incompatibility and antibody-mediated rejection (AMR) in individuals who are sensitive to HLA [7]. Different results are seen in publications regarding organ transplants in which the complement inhibitors in question are used. Eculizumab was used in patients who underwent living donor kidney transplantation and had previous HLA-specific DSA, and it was observed that the probability of AMR at 3 months (7.7%) was reduced compared to the probability of AMR in the group receiving plasmapheresis treatment due to DSA (41.2%) [40]. However, there was no disparity in graft survival in the histologic occurrence of AMR between the 2 groups at the 2-year follow-up. In a study conducted at multiple centers, the

effectiveness of nine-week prophylactic eculizumab treatment was compared to standard of care (SOC) treatment, which includes intravenous immunoglobulin (IVIg) and plasmapheresis. The study found that eculizumab was more effective in patients with circulating C1q-binding HLA-DSA compared to patients with non-C1q-binding HLA-DSA. The efficacy of eculizumab treatment, in terms of reducing the occurrence of antibody-mediated rejection (AMR), was only detected in patients with C1q-binding HLA-DSA after roughly 80 months. The significant data indicate that antimicrobial resistance (AMR) mediated only by complement-dependent effector pathways may be susceptible to anticomplement therapy [41]. This concept could provide a partial explanation for the variable outcomes observed in AMR patients treated with eculizumab, as the extent to which IgGs can bind to complement remained mostly unclear in the majority of investigations. Eculizumab targets the complement system's terminal pathway rather than inhibiting complement components early in activation, which could account for these contradictory outcomes. The data suggest that inhibiting complement at the C5 level does not stop early-stage complement activity. The growing interest lies in inhibiting the initial phases of complement activation, as indicated by these observations. The recombinant version of CIINH, or a preparation enriched in human plasma, has effectively been utilized in preclinical models to suppress allogeneic and xenogenic humoral immune responses [42,43]. Controlled clinical investigations have utilized plasma-derived C1INH that has been enhanced to increase its effectiveness for HLA desensitization and the management of AMR. Early efficacy and safety studies have shown that using C1INH in addition to standard of care (SOC) is a trustworthy and possibly useful treatment. In a preliminary experiment, C1INH was administered alongside high-dose IVIg to six patients with

treatment-resistant AMR. At the six-month mark, these patients exhibited enhancements in glomerular filtration rate in comparison to their initial levels and were observed to have lower levels of C1q-binding DSA than the control group. AMR patients obtaining standard of care (rituximab, IVIg, plasmapheresis, and) and those getting C1INH as an extra treatment were compared in a randomized, placebo-controlled experiment. The study found no significant differences in AMR histopathology or renal function between the group receiving C1INH and the placebo group, as assessed throughout the 20-day follow-up period [35,44,45]. Nevertheless, upon examining a subset of 14 patients who underwent 6-month protocol biopsy, it was found that there were no cases of transplant glomerulopathy in the C1INH group. In contrast, 43% of patients in the placebo group exhibited transplant glomerulopathy. In summary, these first findings indicate that C1INH has a significant function as a supplementary treatment to standard of care (SOC) for managing antibody-mediated rejection (AMR) and preventing transplant glomerulopathy. The latter is the primary risk factor for long-term failure of transplants [46]. A different approach to hinder the initial phases of complement activation involves directing the anti-C1s monoclonal antibody BIVV009 towards C1s. A Phase I trial was conducted to assess the efficacy of BIVV009 as a short-term therapy for late acute or chronic AMR. Comparing the index biopsy samples with the one-month protocol biopsy samples revealed a notable decrease in the buildup of C4d. Nevertheless, there was no histological resolution of antibody-mediated rejection (AMR) and no improvement in glomerular filtration rate (GFR) found 50 days following treatment [7]. Cp40 has a high affinity for binding to C3. CP40 has promise for application in clinical settings, particularly for ABO-incompatible kidney transplantation and the treatment of periodontal disease [47,48]. An alternative

method involves utilizing IdeS, a genetically engineered endopeptidase produced from Streptococcus pyogenes, to weaken the activation of the complement system and decrease its binding to Fc receptors. IdeS enzymatically cleaves the immunoglobulin G (IgG) antibodies at the hinge region, resulting in the removal of a heavy chain from the IgG molecule. The act of severing the second heavy chain results in the formation of F(ab)2 and Fc fragments. Following the initial splitting, the IgG molecule loses its capability to attach to C1q, but it retains the ability to attach to the Fc receptor. The combined Phase I-II clinical trial yielded encouraging outcomes, demonstrating that IdeS effectively eradicated diminished DSAs and enabled HLA-incompatible transplantation un 24 out of 25 patients with remarkable sensitivity [7].

A subset of transplanted patients may not exhibit complement-mediated rejection, even when they have detectable donor-specific antibodies (DSA) and functioning complement proteins in their plasma. This phenomenon is referred to as accommodation, and its mechanism remains incompletely understood. Nevertheless, it has been documented that the excessive production of genes encoding both oxygenase 1 Bcl-2 and Bcl-X leads to an augmentation of the anti-inflammatory condition in non-human primate (NHP) models and receivers. The genes' products control the NF-κB transcription factor, leading to a decrease in the expression of proinflammatory cytokines and chemokines. Nevertheless, the active participation of supplement in the process of accommodation was verified. Typically, in this timeframe, grafts tend to exhibit C4d positivity, which suggests that accommodation is primarily triggered by components involved in the final stage of complement inhibition. Eculizumab was administered to HLA-mismatched and presensitized animals in a heart transplant paradigm to

induce terminal pathway inhibition and accommodation [7]. Accommodation was also induced experimentally using cobra venom factor (CVF). CVF can form a highly stable convertase CVF-Bb that cleaves both C3 and C5. Since CVF-Bb is not inactivated by complement regulators, treatment with CVF leads to depletion of C3 and C5, leading to sustained activation of the alternative pathway and transient inhibition of the complement system [49].

B cells, which are the basic cells of the adaptive immune response, carry the CR2 receptor, and CR2 binding to C3d is important in the development of the humoral immune response. Stimulation of B cells by CR2 as well as B cell surface receptors is important in an effective humoral response. It has been shown that when B cells are stimulated through both receptors, an antibody response that is 1,000 times more effective than stimulation with only the B cell receptor is generated. These data indicate that inhibiting CR2 could be a viable approach to controlling the adaptive immune response in transplantation [35,50].

6.6 CONCLUSION

Since the importance of the complement system for the patient and the allograft is known, in recent years experimental studies have been carried out in this field both in the diagnosis and control of rejections and in order to eliminate the destructive effects of proteins. In this review, it appears that the process of end-stage renal disease and the clinical status of the donor affect the activator and regulatory complement proteins. For this reason, information about the complement system of both parties is important before transplantation. This information will give the clinic preliminary information about the allograft or the patient and clarify the limits of im-

munosuppressive treatment. New studies by accelerating the development of new agents for complement proteins that are still in the experimental stage or by increasing the number of patients in clinical studies will also be beneficial in prolonging allograft life.

6.7 REFERENCES

1. Nesargikar PN, Spiller B, Chavez R. The complement system: history, pathways, cascade and inhibitors. Eur J Microbiol Immunol (Bp).2012;2(2):103-11. https://doi.org/10.1556/eujmi.2.2012.2.2
2. Reis ES, DeAngelis RA, Chen H, Resuello RRG, Ricklin D, et al. Therapeutic C3 inhibitor Cp40 abrogates complement activation induced by modern hemodialysis filters. Immunobiology. 2015;220(4):476-82. https://doi.org/10.1016/j.imbio.2014.10.026
3. Nonaka M. Evolution of the complement system. Subcell Biochem. 2014; 80:31-43.
4. Barnum SR. Complement: A primer for the coming therapeutic revolution. Pharmacol. Ther. 2017; 172:63-72. https://doi.org/10.1016/j.pharmthera.2016.11.014
5. Tichaczek-Goska D. Deficiencies and excessive human complement system activation in disorders of multifarious etiology. Adv Clin Exp Med. 2012;21(1):105-14.
6. Michielsen LA, van Zuilen AD, Muskens IS, Verhaar MC, Otten HG. Complement polymorphisms in kidney transplantation: critical in graft rejection? Am J Transplant. 2017;17(8):2000-7. https://doi.org/10.1111/ajt.14199
7. Biglarnia AR, Huber-Lang M, Mohlin C, Ekdahl KN, Nilsson B. The multifaced role of complement in kidney transplantation. Nat Rev Nephrol. 2018;14(12):767-81. https://doi.org/10.1038/s41581-018-0071-x
8. Abbas A, Lichtman A, Pillai S. Effector Mechanisms of Humoral Immunity, Cellular and Molecular Immunology. 7th ed. Philadelphia: El-sevier; 2011. p.269-92.

9. Khan MA, Shamma T. Complement factor and T-cell interaction during alloimmune inflamation in transplantation. J Leukoc Biol. 2019;105(4):681-94. https://doi.org/10.1002/JLB.5RU0718-288R

10. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement system part I - molecular mechanisms of activation and regulation. Front Immunol. 2015;6;262:1. https://doi.org/10.3389/fimmu.2015.00262

11. de Taeye SW, Rispens T, Vidarsson G. The ligands for human IgG and their effector functions. Antibodies (Basel). 2019;25;8(2):30. https://doi.org/10.3390/antib8020030

12. Sharp TH, Boyle AL, Diebolder CA, Kros A, Koster AJ, et al. Insights into IgM- madiated complement activation based on in situ structures of IgM-C1-C4b. Proc Natl Acad Sci U S A. 2019;116(24):11900-05. https://doi.org/10.1073/pnas.1901841116

13. Petrušić V, Živković I, Stojanović M, Stojićević I, Marinković E, et al. Hexameric immunoglobulin M in humans: desired or unwanted? Med Hypotheses. 2011;77(6):959-61. https://doi.org/10.1016/j.mehy.2011.08.018

14. Stowell SR, Winkler AM, Maier CL, Arthur CM, Smith NH, et al. Initiation and regulation of complement during hemolytic transfusion reactions. Clin Dev Immunol. 2012; 2012:307093. https://doi.org/10.1155/2012/307093

15. Dekkers G, Treffers L, Plomp R, Bentlage AEH, de Boer M, et al. Decoding the human immunoglobulin g-glycan repertoire reveals a spectrum of fc-receptor and complement-mediated-effector activities. Front Immunol. 2017;2; 8:877. eCollection 2017. https://doi.org/10.3389/fimmu.2017.00877

16. Subedi GP, Hanson QM, Barb AW. Restricted motion of the conserved immunoglobulin G1 N-glycan is essential for efficient Fc? RIIIa binding. Structure. 2014;7;22(10):1478-88. https://doi.org/10.1016/j.str.2014.08.002

17. Beltrame MH, Catarino SJ, Goeldner I, Boldt ABW, de Messias-Reason IJ. The lectin pathway of complement and rheumatic heart disease. Front Pediatr. 2015;21; 2:148. https://doi.org/10.3389/fped.2014.00148

18. Poppelaars F, Faria B, Gaya da Costa M, Franssen CFM, van Son WJ, et al. The complement system in dialysis: a forgotten

story? Front Immunol. 2018;25; 9:71. https://doi.org/10.3389/fimmu.2018.00071

19. Kerr PG, Huang L. Review: membranes for haemodialysis. Nephrology (Carlton). 2010;15(4):381-5. https://doi.org/10.1111/j.1440-1797.2010.01331.x
20. Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N Engl J Med. 1977;7;296(14): 769-74. https://doi.org/10.1056/NEJM197704072961401
21. Hempel JC, Poppelaars F, Gaya da Costa M, Franssen CFM, de Vlaam TPG, et al. Distinct in vitro complement activation by various intravenous iron preparations. Am J Nephrol. 2017;45(1):49-59. https://doi.org/10.1159/000451060
22. Damman J, Seelen MA, Moers C, Daha MR, Rahmel A, et al. Systemic complement activation in deceased donors is associated with acute rejection after renal transplantation in the recipient. Transplantation. 2011;27;92(2):163-9. https://doi.org/10.1097/TP.0b013e318222c9a0
23. Sim E, Sim RB. Enzymic assay of C3b receptor on intact cells and solubilized cells. Biochem J. 1983;15;210(2):567-76. https://doi.org/10.1042/bj2100567
24. Denk S, Neher MD, Messerer DAC, Wiegner R, Nilsson B, et al. Complement C5a functions as a master switch for the ph balance in neutrophils exerting fundamental immunometabolic effects. J Immunol. 2017;198(12):4846-54. https://doi.org/10.4049/jimmunol.1700393
25. Farrar CA, Tran D, Li K, Wu W, Peng Q, et al. Collectin-11 detects stress-induced L-fucose pattern to trigger renal epithelial injury. J Clin Invest. 2016;2;126(5):1911 25. https://doi.org/10.1172/JCI83000
26. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators Inflamm. 2014; 2014:694312. https://doi.org/10.1155/2014/694312
27. Castellano G, Intini A, Stasi A, Divella C, Gigante M, et al. Complement modulation of anti-aging factor klotho inischemia/reperfusion injury and delayed graft function. Am J Transplant. 2016;16(1):325- 33. https://doi.org/10.1111/ajt.13415

28. Delpech PO, Thuillier R, SaintYves T, Danion J, Le Pape S, et al. Inhibition of complement improves graft outcome in a pig model of kidney autotransplantation. J Transl Med. 2016;23;14(1):277. https://doi.org/10.1186/s12967-016-1013-7
29. Nauser CL, Farrar CA, Sacks SH. Complement recognition pathways in renal transplantation. J Am Soc Nephrol. 2017;28(9):2571-8. https://doi.org/10.1681/ASN.2017010079
30. Zhang R. Donor-specific antibodies in kidney transplant recipients. Clin J Am Soc Nephrol. 2018;6;13(1):182-92. https://doi.org/10.2215/CJN.00700117
31. Kılıçaslan Ayna T, Pirim İ. [Rejection mechanisms and bioindicators in kidney transplantation]. Turkiye Klinikleri J Intern Med. 2019;4(1):13-24.
32. Hamer R, Molostvov G, Lowe D, Satchell S, Mathieson P, et al. Human leukocyte antigen- specific antibodies and gamma- interferon stimulate human microvascular and glomerular endothelial cells to produce complement factor C4. Transplantation. 2012;93(9):867-73. https://doi.org/10.1097/TP.0b013e31824b3762
33. Lee H, Han E, Choi AR, Ban TH, Chung BH, et al. Clinical impact of complement (C1q, C3d) binding De Novo donor-specific HLA antibody in kidney transplant recipients. PLoS One. 2018;14;13(11):e0207434. https://doi.org/10.1371/journal.pone.0207434
34. Delanghe JR, Speeckaert R, Speeckaert MM. Complement C3 and its polymorphism: biological and clinical consequences. Pathology. 2014;46(1):1-10. https://doi.org/10.1097/PAT.0000000000000042
35. Grafals M, Thurman JM. The role of complement in organ transplantation. Front Immunol. 2019;4; 10:2380. https://doi.org/10.3389/fimmu.2019.02380
36. Mohebnasab M, Eriksson O, Persson B, Sandholm K, Mohlin C, et al. Current and future approaches for monitoring responses to anti-complement therapeutics. Front Immunol. 2019;8; 10:2539. https://doi.org/10.3389/fimmu.2019.02539
37. Lewis AG, Köhl G, Ma Q, Devarajan P, Köhl J. Pharmacological targeting of C5a receptors during organ preservation improves kidney graft survival. Clin Exp Immunol. 2008;153(1):117-26. https://doi.org/10.1111/j.1365-2249.2008.03678.x

38. Rich MC, Keene CN, Neher MD, Johnson K, Yu ZX, et al. Site-targeted complement inhibition by a complement receptor 2-conjugated inhibitor (mTT30) ameliorates post-injury neuropathology in mouse brains. Neurosci Lett. 2016;23;617:188-94. https://doi.org/10.1016/j.neulet.2016.02.025

39. Ruseva MM, Ramaglia V, Morgan BP, Harris CL. An anticomplement agent that homes to the damaged brain and promotes recovery after traumatic brain injury in mice. Proc Natl Acad Sci USA. 2015;17;112(46):14319-24. https://doi.org/10.1073/pnas.1513698112

40. Stegall MD, Diwan T, Raghavaiah S, Cornell LD, Burns J, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant. 2011;11(11):2405- 13. https://doi.org/10.1111/j.1600-6143.2011.03757.x

41. Sicard A, Ducreux S, Rabeyrin M, Couzi L, McGregor B, et al. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. J Am Soc Nephrol. 2015;26(2):457-67. https://doi.org/10.1681/ASN.2013101144

42. Tillou X, Poirier N, Le Bas-Bernardet S, Hervouet J, Minault D, et al. Recombinant human C1-inhibitor prevents acute antibody-mediated rejection in alloimmunized baboons. Kidney Int. 2010;78(2):152-9. https://doi.org/10.1038/ki.2010.75

43. Kirschfink M. C1-inhibitor and transplantation. Immunobiology. 2002;205(4-5):534-41. https://doi.org/10.1078/0171-2985-00152

44. Viglietti D, Gosset C, Loupy A, Deville L, Verine J, et al. C1 inhibitor in acute antibody-mediated rejection nonresponsive to conventional therapy in kidney transplant recipients: a pilot study. Am J Transplant. 2016;16(5):1596-603. https://doi.org/10.1111/ajt.13663

45. Montgomery RA, Orandi BJ, Racusen L, Jackson AM, Garonzik-Wang JM, et al. Plasma-derived C1 esterase inhibitor for acute antibody-mediated rejection following kidney transplantation: results of a randomized double-blind placebo-controlled pilot study. Am J Transplant. 2016;16(12):3468-78. https://doi.org/10.1111/ajt.13871

46. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cell-mediated rejection, and the

injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. Kidney Int. 2014;85(2):258-64. https://doi.org/10.1038/ki.2013.300

47. Ricklin D, Mastellos DC, Reis ES, Lambris JD. The renaissance of complement therapeutics. Nat Rev Nephrol. 2018;14(1):26-47. https://doi.org/10.1038/nrneph.2017.156
48. Mastellos DC, Yancopoulou D, Kokkinos P, Huber-Lang M, Hajishengallis G, et al. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. Eur J Clin Invest. 2015;45(4):423-40. https://doi.org/10.1111/eci.12419
49. Vogel CW, Fritzinger DC. Cobra venom factor: structure, function, and humanization for therapeutic complement depletion. Toxicon. 2010;15;56(7):1198-222. https://doi.org/10.1016/j.toxicon.2010.04.007
50. Heeger PS, Kemper C. Novel roles of complement in T effector cell regulation. Immunobiology. 2012;217(2):216-24. https://doi.org/10.1016/j.imbio.2011.06.004

INFLAMMATION AND KIDNEY DISEASES

Tülay KILICASLAN AYNA[1,2,3], Ibrahim PIRIM[1,2,3]

[1]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[3]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

7.1 INTRODUCTION

Inflammation is a cascade of biological responses that are initiated in circumstances such as exposure to damaging stimuli, infection, and tissue damage [1]. Here, the objective is to eliminate the damaging stimulus and rectify the consequent damage. There exist various forms of inflammatory reaction, namely acute or chronic. If a condition manifests abruptly and resolves quickly, it is referred to as acute inflammation. Conversely, if it develops gradually and persists for an extended period, it is classified as chronic inflammation. The symptoms of inflammation are typically categorized into three main groups:

1. The area exposed to external trauma may experience abnormal circumstances such as edema, redness, and pain.

2. Tissue damage in a specific part of the body can lead to a significant increase in temperature.

3. Chronic inflammation can result in dysfunctions in the corresponding organs.

Considerable advancements have been achieved in comprehending the cellular and molecular mechanisms underlying the acute inflammatory response. The mechanisms underlying the development of persistent local inflammation in chronic infection and autoimmune disorders remain partially elucidated. Nevertheless, there is limited understanding regarding the etiology and mechanisms behind systemic chronic inflammation, which manifests in several diseases such as type 2 diabetes and cardiovascular disorders. Systemic chronic inflammation is believed to be unrelated to traditional causes of inflammation, such as infection and tissue damage. Instead, it is believed to be linked to tissue dysfunction, which is assumed to be caused by an imbalance in one of the organism's physiological systems (Figure 1) [1].

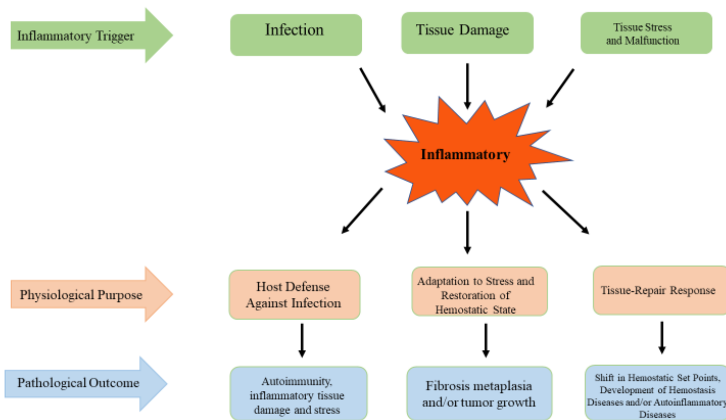
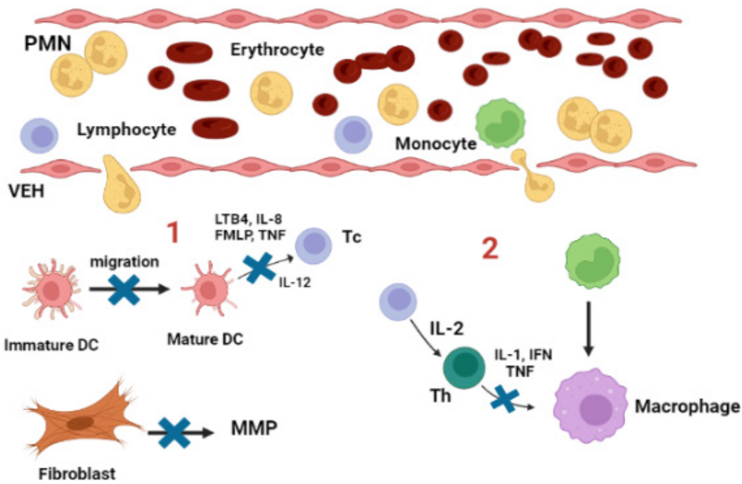


Figure 1: Physiologic and pathological reasons and results of inflammation

There is a prevailing belief that a manageable inflammatory reaction is advantageous to an individual (such as inflammation as a response to bacterial infection), but can be detrimental if not well regulated. Thus, it is hypothesized that

there exists a physiological parallel to the pathological inflammatory state. While the physiological basis for inflammation resulting from infection is clear, the physiological counterparts of various other forms of inflammatory responses are only seen in pathological states, and their underlying processes remain poorly comprehended. Inflammation is believed to occur as a response to many factors, with the purpose of restoring homeostasis. Hence, it is most effectively comprehended within a wider framework, the origin of inflammatory reactions. This section will address the occurrence of inflammation and the chemicals that play a role in this process [3].

During the acute inflammatory response triggered by infection or tissue damage, blood components constantly migrate to the site of the infection or damage. The initiation of this process is prompted by receptors of the innate immune system, such as Toll Like Receptors (TLRs) and NOD Like Receptors (NLRs) [4]. The initial identification of the infectious agent is facilitated by macrophages and mast cells that originate from the tissues. This identification process triggers the release of various substances that promote inflammation, such as chemokines, cytokines, vasoactive amines, eicosanoids, and products of proteolytic cascades. As a result, a localized inflammatory fluid is produced. Leukocytes and plasma proteins, which are typically located in the blood vessels, migrate to the tissues outside the blood vessels at the location of the infection via postcapillary vessels (Figure 2).



PMN: Polymorphonuclear leukocytes- the majority of this group consists of neutrophils, DH: Dendritic cell, VEC: vascular endothelial cell MMP: Matrix metalloproteinases, LTB4: Lökotrien B4, FMLP: N-formyl-methionyl-leucyl-phenylalanine

Figure 2: Leucocytes in inflammation

Endothelial activation in blood arteries facilitates the selective egress of neutrophils from the bloodstream, while impeding the departure of erythrocytes. The selectivity is achieved via the inducible interaction between selectins on endothelial cells chemokine receptors and integrins that are located on leukocytes, occurring in the extravascular areas and on the outer layer of endothelial cells [5]. Neutrophils are activated by direct interaction with pathogens and by the action of cytokines generated by cells residing in the tissue after reaching the affected tissue area. Neutrophils endeavor to eliminate pathogens that enter by producing harmful compounds contained in their granules, including cathepsin G, reactive nitrogen species and reactive oxygen species (ROS), elastase, and proteinase 3. These highly potent compounds do not distinguish both host and microbial groups, inducing injury to host tissues [6]. Following the successful removal of infectious

organisms, a phase of resolution and repair occurs in an acute inflammatory response. This process is facilitated by macrophages, which initially dwell in the tissue and then migrate into the inflamed tissue [7]. The change from pro-inflammatory prostaglandins to anti-inflammatory lipoxins is a critical factor in the resolution of inflammation. Lipoxins facilitate the attraction of monocytes instead of neutrophils, which eliminate deceased cells and start tissue regeneration [8]. Macrophages and transforming growth factor-β (TGF-β) create growth factors, along with resolvins and protectins, which are a type of lipid mediators. These substances play a crucial role in reducing inflammation and promoting tissue healing [8,9]. If the initial acute inflammatory response fails to eradicate the pathogen, the inflammatory process persists and develops additional characteristics. Neutrophil invasion is substituted by macrophages and, in the event of infection, by T cells. If the collective impact of these cells remains inadequate, it leads to a persistent state of inflammation characterized by the development of granulomas and tertiary lymphoid tissues [10,11]. A granuloma is a formation that develops when monocytes and macrophages gather around lymphocytes. The characteristics of this infection may vary depending on the existing effector T cells [12]. Chronic inflammation can arise not just from persistent infections, but also from tissue damage caused by self-antigens or non-degradable foreign substances. It is important to acknowledge that our understanding of the mechanisms of inflammation caused by infection is significantly more advanced compared to other types of inflammatory processes. The topic of whether the information gathered on infection-related inflammation can elucidate inflammation induced by different etiologies remains uncertain. Undoubtedly, infection-induced inflammation, while essential, may be an exceptional circumstance. In overall, the fundamental mechanisms of

systemic chronic inflammatory illnesses remain unclear, yet it is evident they do not follow the typical progression pathway from acute to chronic inflammation [1].

The inflammation that occurs is orchestrated by an extensive spectrum of molecules which create complex regulatory systems. It is advantageous to classify the messages into functioning clusters and distinguish among the substances that trigger inflammation and those that support it to effectively examine these intricate systems. The main causing agents are stimuli that trigger the inflammatory response. Afterwards, they trigger the activation of specific sensors that enable the production of unique groups of mediators. These molecules modify the functional state of organs and tissues, which serve as agents of inflammation, in order to adapt to the particular conditions brought about by the inflammatory stimulus. Consequently, a standard inflammation circuit includes substances that initiate inflammation, receptors that detect these substances, molecules that propagate the inflammatory response, and components that carry out the effects of inflammation. Every factor has a distinct function in determining the particular form of inflammatory reaction (as depicted in Table 1, Figure 3a, and b) [1].

Inducers ➡ Sensors ➡ Mediators ➡ Effectors

Figure 3a: Basic parts in the pathway of development of the inflammatory reaction

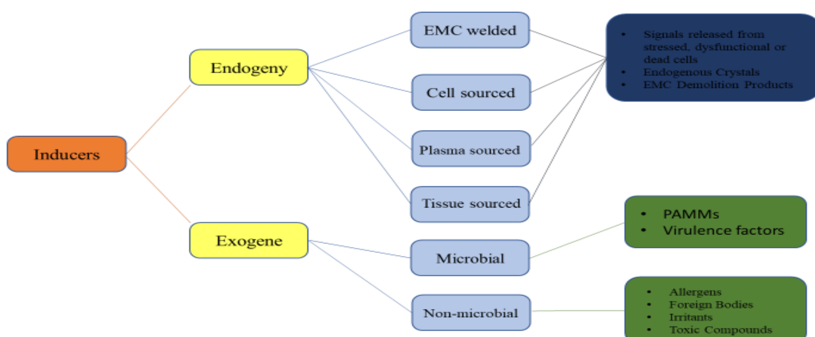


Figure 3b: Inflammation both exogenous and endogenous inducers

Table 1: Some examples of inflammatory pathways

Some examples of inflammatory pathways			
Inducers	**Sensors**	**Mediators**	**Effector**
Lipopolysaccharide	TLR4	TNF-α, IL-6, PGE_2	Endothelial cells, hepatocytes, leukocytes, hypothalamus and others
Allergens	IgE	Vasoactive amines	Endothelial cells and smooth muscle cells
Monosodium urate crystals and calcium pyrophosphate dihydrate crystals	NALP3	IL-1β	Endothelial cells, hepatocytes, leukocytes, hypothalamus and others
Collagen	Hageman Factor	Bradykinin	Endothelial cells and smooth muscle cells

7.2 EXOGENOUS INDUCERS

Externally produced stimulants can be categorized as microbial and non-microbial. Microbial stimulants can be classified into two primary categories.

1. Pathogen-associated molecular patterns (PAMP)

2. Virulence factors

PAMPs refer to a specific and well-defined collection of

conserved molecular patterns that are present in all bacteria, whether they are harmful or commensal. These patterns are identified by receptors in the host organism. The second category of microbial inducers is exclusive to pathogens and encompasses a range of virulence factors [13]. Specific receptors do not directly detect virulence factors, unlike PAMPs. Upon entering the cell, they elicit the inflammatory response by exerting detrimental effects on the cell. Nevertheless, the customary functions of virulence factors can be identified using specialized sensors. Gram-positive bacteria, like staphylococci, produce exotoxins that can form pores. These pores allow K+ ions to enter the cell, which triggers the formation of inflammasomes. Inflammasomes then release cytokines like IL-1 and IL-18, leading to inflammation. This process is illustrated in Figure 4 (Figüre 4). The range is from 14 to 16.

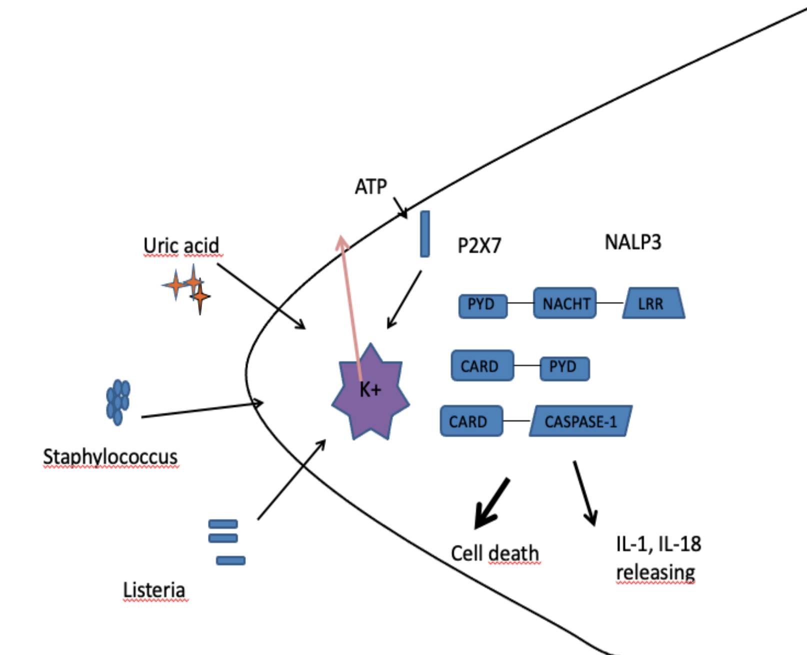


Figure 4: Migration of K+ ions and inflammation

Microbial agents that trigger inflammation are not limited to pathogens alone. Within commensal bacteria, it serves as a significant reservoir of inflammation-inducing agents that are identified by TLRs [17]. These bacteria actively decrease the activation of TLRs through several ways. Mice defective in A20, an essential TLR inhibitor, exhibit severe TLR-dependent inflammation as one example of this event [18].

The factors encompassed are agents that provoke inflammation, substances that cause allergic reactions, irritants, foreign objects, and poisonous substances that are not of microbial origin.

Some triggers might provoke irritation to the mucosal epithelium, while others mimic the detrimental impact of parasites. The two types of antigens generate an inflammatory response that exhibits notable characteristics. The disclosure of sensors for allergens is still largely secret. Non-digestible particulates that are too large for macrophages to digest or that harm the membrane surrounding the ingested particulates are referred to as foreign bodies. Foreign substances such as silica and asbestos particles can trigger an inflammatory response. The aberrant situation in the tissues is indicated by the presence of foreign substances that are of a significant size and the absence of inhibitory receptors, such as CD47, which are typically present on the body's own cells and prevent their engulfment by phagocytes. These large foreign particles form a structure that can be engulfed by phagocytes, but it is not classified as a phagosome. The macrophage forms a granuloma to contain a foreign material when it is too large for a digestive capsule to form. The specific sensor responsible for initiating this response in macrophages remains unidentified [19]. The NALP3 inflammasome is triggered when a macrophage comes into contact with foreign substances, irrespective of their size and ability to be engulfed [20].

7.3 ENDOGENOUS INDUCERS

Endogenous inflammatory inducers are signals that are generated by tissues that are under stress, injured, or experiencing some form of malfunction. The characteristics of these signals are not clearly characterized. Functional classifications are determined mostly by the type and severity of tissue anomalies. Firstly, the inducers that are involved in acute inflammation will be discussed. Inflammation is a prominent characteristic of necrosis. During necrotic cell death, the cell membrane is compromised, causing the express of specific cellular components such as ATP, uric acid, and K+ ions [21,22]. ATP attaches to purinoceptors (specifically P2X7) located on the outer layer of macrophages. This attachment leads to the entry of potassium ions (K+) and can work together with other signals to trigger the activation of the NALP3 inflammasome (as shown in Figure 4) [15]. ATP also activates sensory neurons and convey information about tissue damage to the nervous system [23]. The RAGE receptor, also known as the receptor for advanced glycation end products or AGER, is bound by HMGB1 and S100A12 and works in concert with TLRs to initiate an inflammatory reaction (Figure 5) [24].

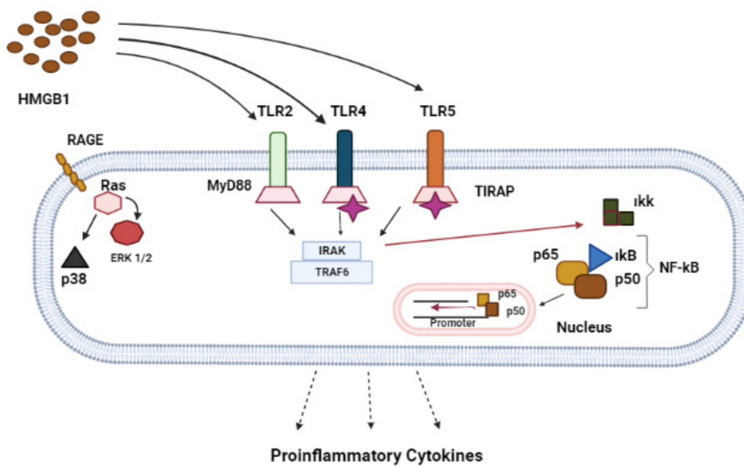


Figure 5: Effect of HMGB1 on inflammatory response via RAGE and TLR

While it is commonly believed that intracellular proteins are released spontanously when the cell membrane of necrotic cells is ruptured, it is important to acknowledge that several intracellular proteins can be secreted through a non-canonical pathway that is independent of the endoplasmic reticulum and Golgi apparatus. Inflammasomes are responsible for controlling unconventional secretion, as evidenced by a recent study that demonstrated active caspase 1 facilitates this process [25]. Given this discovery, it is imperative to investigate whether inflammatory intracellular proteins are released from necrotic cells in a passive manner or if they are secreted by this caspase-1-dependent method. Due to their lack of metabolic activity, necrotic cells rely on ATP for caspase-1-dependent secretion. A clear understanding of the pro-inflammatory role of intracellular proteins and necrotic cell death would be possible if caspase 1 is accountable for the excretion of proteins within cells with inflammatory characteristics. HMGB1, which is clearly produced by TLR4-stimulated macrophages

without necrotic cell death, provides a great example in this setting and suggests that it may be involved in the unconventional Caspase-1-dependent release way.

Typically, the basement membrane acts as a barrier between epithelial cells and mesenchymal cells. When this barrier is disrupted in injured tissues, it leads to interactions between epithelial and mesenchymal cells. The existence of tissue injury is indicated by these interactions, which in turn trigger tissue healing responses. However, the mechanisms by which these aberrant interactions are detected remain poorly understood. The surface epithelium acts as a barrier, separating the internal components from the external surroundings. The disruption of the protective layer in the intestine allows friendly bacteria to reach the Toll-like receptors (TLRs) on macrophages situated in the underlying tissue, known as the lamina propria. This leads to the activation of TLRs, which in turn triggers the body's response to repair the damaged tissue in the intestine [17,26]. The vascular endothelium becomes damaged, which permits plasma proteins and platelets to enter the extravascular spaces [5]. In response to collagen and other extracellular matrix (ECM) components, the plasma-derived mediator of inflammation known as Hageman factor (Factor XII) is stimulated. In addition to acting as an indicator for vascular injury, stimulated Hageman factor initiates four enzymatic processes that produce inflammatory molecules. The steps mentioned are the kallikrein-kinin process, the complement cascade, the fibrinolytic pathway, and the coagulation flow (Figure 6) [27]. Furthermore, platelets undergo activation when encountering collagen, leading to the production of diverse inflammatory mediators such as thromboxanes and serotonin1.

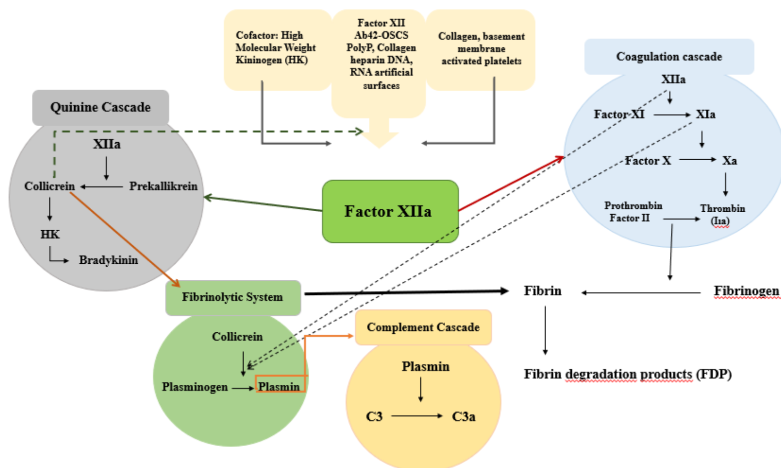


Figure 6: Contribution of Factor XII to inflammation

Another category of naturally occurring stimulants is particularly pertinent to persistent inflammatory disorders. The class of inducers comprises advanced glycation end products (AGEs), calcium pyrophosphate dihydrate, oxidized lipoproteins (including low and high-density lipoproteins), and monosodium urate. Connective tissues play a role in facilitating the creation of these crystals by providing a surface that is conducive to crystal nucleation. For instance, the development of monosodium urate and calcium pyrophosphate dihydrate crystals in joints and surrounding tissues causes the inflammatory disorders called gout and pseudogout, respectively[21]. Macrophages identify these tiny particles when they get to a certain size and treat them similarly to how they treat foreign things. The engulfment of these crystals leads to the stimulation of the NALP3 inflammasome, which in turn triggers the activation of IL-1 (including IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Rα). It induces the synthesis of Caspase-1 substrates, such as IL-37 and IL-38, which belong to the same family[20,28]. AGEs are formed through the process of non-enzymatic glycation, which occurs when long-lasting proteins

like collagen react with sugars. These products can cause the proteins they attach to undergo cross-linking, resulting in a steady decline in the functionality of these proteins. Furthermore, advanced glycation end products (AGEs) are identified by receptors for advanced glycation end products (RAGE), which possess inflammatory properties either independently or in conjunction with Toll-like receptors (TLRs). Advanced glycation end products (AGEs) can build up in the body when there are high levels of glucose and oxidative stress, which can occur in both type 1 and type 2 diabetes[1]. By oxidizing protein and lipid parts, phagocytes generate reactive oxygen species (ROS) that convert both low- and high-density lipoproteins into inflammation messages[29]. Another category of internal triggers for inflammation includes degradation by-products of the extracellular matrix (ECM) produced during tissue dysfunction or injury. The most extensively researched element of the extracellular matrix (ECM) in this particular situation is the glycosaminoglycan hyaluronate (HA) (Figure 7). Hyaluronate often exists as an inactive polymer with a high molecular weight under normal circumstances. Tissue injury induces the degradation of hyaluronate into smaller fragments with low molecular weight, which possess inflammatory properties. These fragments activate TLR4 and stimulate the tissue repair process. Additionally, this transition is believed to be reliant on ROS (reactive oxygen species)[30]. Consequently, multiple internal mechanisms that trigger the inflammatory response rely on reactive oxygen species (ROS).

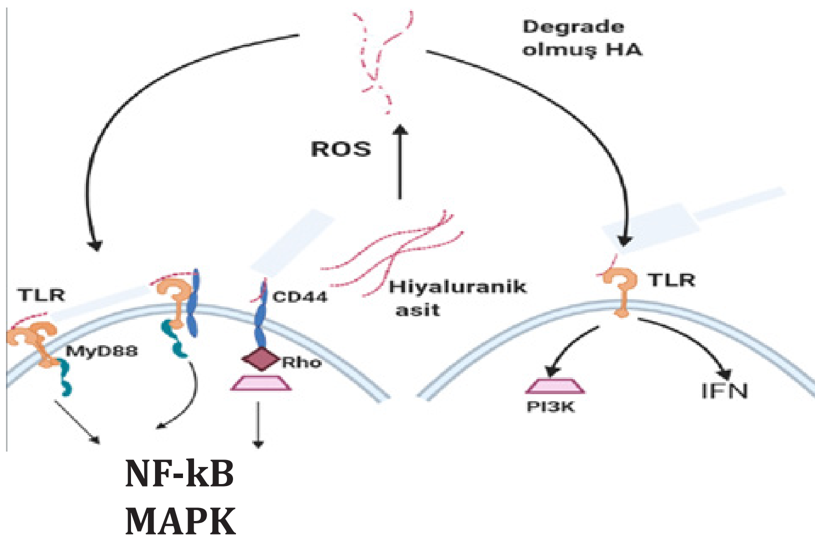


Figure 7: Contribution of ECM to inflammation

Although the number of internal factors that because inflammation increases, the available research on this topic exhibits significant inconsistencies. The primary reason for this is the complex challenges involved in accurately describing this category of signals. Misidentification of a factor as an inducer often occurs when recombinant proteins are contaminated with small amounts of microbial ligands for Toll-like receptors (TLRs) or nucleotide-binding oligomerization domain (NOD) proteins. Crucially, numerous naturally occurring substances that trigger inflammation are likely to have the desired effect in living organisms only when they are combined in specific ways and possibly only when the tissues are not working properly or are damaged. Ischemia, hypoxia, elevated levels of reactive oxygen species (ROS), and changes in extracellular matrix (ECM) components are often linked to tissue injury or dysfunction. However, these conditions are not typically reproduced in tissue culture settings that have reduced levels of nutrients and oxygen. There is a possibility that there

are additional, yet undiscovered, substances that initiate the inflammatory response in injured and/or strained tissues. Inducers of inflammation stimulate the generation of many inflammatory mediators that modify the functionality of various tissues and organs. Several of these inflammatory mediators have shared effects on the blood vessels and the migration of white blood cells into tissues. These messengers can either be created from plasma proteins or released by cells [10,27]. Mast cells and tissue-resident macrophages together with cells in nearby tissues, have the ability to produce specialized cellular messengers. Certain mediators (serotonin and histamine) are already present and stored in mast cells, basophils, and platelet granules. Additional entities are already generated and distributed within the plasma in an inactive precursor state. During the acute phase response, the level of these mediators in the plasma rises due to an enhanced release of precursor substances by hepatocytes. Additional mediators are generated in reaction to activation by inflammatory inducers. The classification of inflammatory mediators is based on their biochemical features, resulting in seven distinct classes [10,27]: vasoactive amines, vasoactive peptides, complement component fragments, lipid mediators, cytokines, chemokines, and proteolytic enzymes.

7.4 INFLAMMATORY MEDIATORS

Initially, it controls the process of mast cells and platelets releasing their stored substances, as well as the creation of vasoactive substances such as histamine and serotonin. Vasoactive amines exert intricate effects on blood vessels, leading to heightened vascular permeability and either vasodilation or vasoconstriction. Their discharge by mast cells can be extremely harmful in vulnerable organisms, leading to the collapse of blood vessels and the breathing system in the event of

a severe allergic reaction. Moreover, secretory vesicles (like substance P) can maintain the active form of vasoactive molecules, or they can be produced by the proteolytic digestion of inert substances found in the extracellular fluid (such fibrin breakdown products, kinins, fibrinopeptide B, and fibrinopeptide A).

Sensory neurons produce Substance P, which can trigger the degranulation of the mast cell. Vasoactive molecules are produced through degradation by plasmin, thrombin, or Hageman factor. The production of histamine from mast cells is possibly directly or indirectly triggered by these molecules, which also cause vasodilation and enhanced arterial flexibility. In addition to acting as a vascular injury indicator and an inflammatory trigger, the Hageman factor is fundamental to the regulation of both responses. The Hageman factor initiates the kallikrein-kinin cascade, which in turn produces bradykinin. Bradykinin, the primary outcome of this cascade, exerts significant effects on the vasculature and also possesses a potent pro-algesic (pain-inducing) property. Pain plays a vital physiological role in infection by alerting cells to the aberrant state of injured area. In addition, anaphylatoxins, specifically C3a, C4a, and C5a, are produced through distinct modes of complement stimulation. C5a, together with C3a and to a smaller extent C4a, induces the mobilization of monocytes and granulocytes. This initiates the secretion of mast cell contents, thereby influencing the blood vessels. Moreover, phospholipids like phosphatidylcholine found in cell membranes are the source of lipid mediators, including platelet-activating factors (TAF) and eicosanoids. The two types of lipid mediators that are derived from phosphatidylcholine that were previously discussed are arachidonic acid and lysophosphatidic acid, which are produced by cytosolic phospho-

lipase A2 in response to intracellular $Ca2^{+}$ ions. Arachidonic acid undergoes metabolism through cyclooxygenases (COX1 and COX2) to make eicosanoids, specifically thromboxanes and prostaglandins. Alternatively, it can be metabolized by lipoxygenases to produce lipoxins and leukotrienes [1]. Prostaglandins (PGI2 and PGE2) have the effect of causing blood vessels to widen, resulting in vasodilation. Additionally, PGE2 has the ability to increase sensitivity to pain and is a strong stimulator of fever [31]. Lipoxins, which include resolvins and protectins, have the ability to inhibit inflammation and facilitate the process of tissue regeneration [32]. TAF, a lipid mediator, is generated through the acetylation of lysophosphatidic acid. It triggers a range of physiological responses, such as vasodilation, platelet stimulation, heightened vascular permeability, vasoconstriction, and leukocyte recruitment. Furthermore, certain cell types, particularly macrophages and mast cells, generate inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1, IL-6, and others. It activates leukocytes and endothelium and starts the acute phase response, among other functions in the inflammatory response. Furthermore, chemokines are synthesized by several cellular entities as a reaction to inflammatory stimuli. They regulate the process of leukocyte extravasation and direct the movement of leukocytes towards afflicted tissues. Furthermore, many degrading enzymes including as cathepsins, matrix metalloproteinases, and elastin play multiple functions in inflammation, partly by breaking down extracellular matrix (ECM) and basement membrane proteins. Proteases play significant roles in various processes, including host defense, tissue remodeling, and leukocyte migration[1]. The relationship between the characteristics of an aggresive stimulus and the specific facilitator remains uncertain. Moreover, some agents both influence certain tissues and promote the production of other agents.

Comprehending the fundamental reasoning behind this mediator hierarchy [33] is essential. Tissues and cells that are specifically attacked by inflammatory mediators are the effectors of an inflammatory response. It is typical for the body to react to certain substances that cause inflammation, such as TNF-α and IL-1. However, these substances have varying effects depending on the specific tissues and types of cells they interact with. The primary impact of inflammatory messenger molecules is to stimulate the production of an exudate by influencing the blood vessels and the movement of white blood cells. However, these molecules also have significant impacts on metabolic functions and neuroendocrine, as well as the overall preservation of tissue balance. The functions of inflammatory mediators demonstrate a broader involvement of inflammation in regulating tissue balance and responding to detrimental circumstances[34].

Homeostasis refers to the cellular mechanism by which a cell actively regulates and maintains its internal equilibrium in the face of unfavorable external conditions. To achieve this objective, several control methods have been devised[35]. Disruptions in glucose and oxygen levels can lead to deviations from the typical range of homeostasis in some parameters. This can elicit an acute stress response, which can either lead to a temporary adaptation to the unfamiliar circumstances or a more enduring flexible modification in the relevant set points. Chronic and acute inflammations are two distinct types of reactions to adaptation that occur when other natural defenses are not enough.

The reaction to inflammation is believed to function in cases of significant disruptions in homeostasis, including the presence of foreign substances or irritants, tissue injury, and infection. Nevertheless, infection and injury represent the most

severe forms of stimuli that can initiate inflammation, leading to the most intense and prominent inflammatory reactions. This is why they are often recognized as the most prominent manifestations of inflammation. In a broader sense, the presence of tissue abnormalities often triggers an inflammatory response. These inflammatory reactions are expected to be more prevalent but less intense compared to the typical reactions to inflammation triggered by damage or infection. The degree and existence of tissue damage will dictate whether inflammatory reactions can be identified with traditional biomarkers. Modest tissue-specific dysfunctions to serious injury can be considered tissue changes. The magnitude of the inflammatory response can vary considerably. Excessive stress can generally be managed by cells that reside in the tissues, such as macrophages and mast cells. However, if there are more serious problems or damage, additional white blood cells and plasma proteins may need to be sent in from nearby areas. The molecular sensors responsible for detecting signals indicating tissue stress and dysfunction, which differ from the signals associated with infection and injury in the traditional inflammatory response, are not well understood [1]. Transplantation often leads to several stressors and damages, which significantly impact the success of the graft. The hypothesis was initially proposed in 1994 that reperfusion injury of kidney allografts triggers acute rejection and subsequently plays a role in the establishment of chronic immune responses. This led to the emergence of the "damage hypothesis," which suggests that oxidative damage is responsible for the creation of DAMPs, also known as danger signals or alarmins. These signals are emitted by dying cells in the allograft. The DAMPs frequently observed in this scenario include HMGB1, AGE, amyloid-β peptides, heat shock proteins, hyaluronan proteins, and molecules belonging to the S100 family, all of which cont-

ribute to inflammation. DAMPs interact with Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), and receptor for advanced glycation end products (RAGE) that are encoded in the major histocompatibility complex III (MHC III) area in both the graft and recipient dendritic cells (DCs). Binding triggers the activation of transcription factors such as NF-κB, AP-1, and IRF-3. Consequently, the production of pro-inflammatory cytokines and chemokines initiates the maturation and proliferation of dendritic cells (DCs). Therefore, the inherent immune response initiates the adaptive immune response. The migration of immune system cells into the kidneys, blood vessel wall, and perivascular region happens concurrently with the stages of the inflammatory process, including heightened cytokine release, generation of reactive oxygen species (ROS), and the emergence of adhesion molecules. The rejection process is significantly influenced by costimulatory molecules, particularly the CD28/B7 system. The expression of these costimulators is upregulated during ischemia reperfusion injury (IRI) and the initiation of the innate immune response. Ischemia-reperfusion injury (IRI) leads to cerebral death during transplantation due to factors such as the application of cold protecting fluids, surgical trauma, and the release of cytokines like IL-6, TNF-α, and IL-1β. It guides the movement of donor antigen-presenting cells (APCs) towards lymph nodes. It comes into contact with host lymphocytes. Donor dendritic cells (DCs) trigger the adaptive immune response by displaying alloantigens to recipient T cells. Once more, stress ligands have the ability to stimulate NK cells via NKG2D receptors in this particular mechanism. It is believed that IRI leads to an elevation in NKG2D ligands in the transplanted organ after the binding of TLR4-HMGB1, hence playing a crucial role in the activation of NK and T cytotoxic cells[36].

7.5 CONCLUSION

Consequently, gaining a more comprehensive comprehension of the mechanisms behind inflammation would enhance our understanding of the stages of graft survival and rejection following kidney transplantation, as well as in numerous other diseases. Additionally, it will aid in identifying specific molecules that can be targeted for preventative purposes.

7.6 REFERENCES

1. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454(7203):428-435. https://doi.org/10.1038/nature07201

2. Kuprash DV, Nedospasov SA. Molecular and Cellular Mechanisms of Inflammation. Biochemistry (Mosc) 2016;81(11):1237-1239. https://doi.org/10.1134/S0006297916110018

3 Sugimoto MA, Vago JP, Perretti M, Teixeira MM. Mediators of the Resolution of the Inflammatory Response. *Trends Immunol* 2019;40(3):212-227. https://doi.org/10.1016/j.it.2019.01.007

4. Barton GM. A calculated response: control of inflammation by the innate immune system. J Clin Invest 2008;118(2):413-420. https://doi.org/10.1172/JCI34431

5. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol 2007;7(10):803-815. https://doi.org/10.1038/nri2171

6. Nathan C. Points of control in inflammation. Nature 2002;420(6917):846-852. https://doi.org/10.1038/nature01320

7. Sugimoto MA, Vago JP, Perretti M, Teixeira MM. Mediators of the Resolution of the Inflammatory Response. Trends Immunol 2019;40(3):212-227. https://doi.org/10.1016/j.it.2019.01.007

8. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005;6(12):1191-1197. https://doi.org/10.1038/ni1276

9. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 2007;25:101-137. https://doi.org/10.1146/annurev.immunol.25.022106.141647

10. Kumar V, Cotran RS, Robbins SL. Robbins basic pathology; Philadelphia: PA: Saunders; 2003.
11. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 2006;7(4):344-353. https://doi.org/10.1038/ni1330
12. Agita A, Alsagaff MT. Inflammation, Immunity, and Hypertension. Acta Med Indones 2017;49(2):158-165.
13. Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997;91(3):295-298. https://doi.org/10.1016/s0092-8674(00)80412-2
14. Tartey S, Kanneganti TD. Differential role of the NLRP3 inflammasome in infection and tumorigenesis. Immunology 2019;156(4):329-338. https://doi.org/10.1111/imm.13046
15. Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 2006;440(7081):228-232. https://doi.org/10.1038/nature04515
16. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 2008;9(3):310-318. https://doi.org/10.1038/ni1558
17. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004;118(2):229-241. https://doi.org/10.1016/j.cell.2004.07.002
18. Turer EE, Tavares RM, Mortier E, Hitotsumatsu O, Advincula R, et al. Homeostatic MyD88-dependent signals cause lethal inflam-Mation in the absence of A20. J Exp Med 2008;205(2):451-464. https://doi.org/10.1084/jem.20071108
19. Rizki TM, Rizki RM. Lamellocyte differentiation in Drosophila larvae parasitized by Leptopilina. Dev Comp Immunol 1992;16(2-3):103-110. https://doi.org/10.1016/0145-305x(92)90011-z
20. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, et al. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 2008;320(5876):674-677. https://doi.org/10.1126/science.1156995

21. Rock KL, Kono H. The inflammatory response to cell death. Annu Rev Pathol 2008;3:99-126. https://doi.org/10.1146/annurev.pathmechdis.3.121806.151456

22. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81(1):1-5. https://doi.org/10.1189/jlb.0306164

23. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001;413(6852):203-210. https://doi.org/10.1038/35093019

24. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med 2007;13(9):1042-1049. https://doi.org/10.1038/nm1638

25. Keller M, Rüegg A, Werner S, Beer HD. Active caspase-1 is a regulator of unconventional protein secretion. Cell 2008;132(5):818-831. https://doi.org/10.1016/j.cell.2007.12.040

26. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. Proc Natl Acad Sci U S A 2005;102(1):99-104. https://doi.org/10.1073/pnas.0405979102

27. Majno G, Joris I. Cells, Tissues, and Disease. Principles of General Pathology. 2nd Ed. Oxford University Press; 2004.

28. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006;440(7081):237-241. https://doi.org/10.1038/nature04516

29. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, et al. Mechanisms of disease: proatherogenic HDL--an evolving field. Nat Clin Pract Endocrinol Metab 2006;2(9):504-511. https://doi.org/10.1038/ncpendmet0245

30. Jiang D, Liang J, Noble PW. Hyaluronan in tissue injury and repair. Annu Rev Cell Dev Biol 2007; 23:435-461. https://doi.org/10.1146/annurev.cellbio.23.090506.123337

31. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation [published correction appears in Nat Rev Immunol. 2015 Nov;15(11):724]. Nat Rev Immunol 2015;15(8):511-523. https://doi.org/10.1038/nri3859

32. Zhong J, Shi G. Editorial: Regulation of Inflammation in Chronic Disease. Front Immunol. 2019; 10:737. Published 2019 Apr 12. https://doi.org/10.3389/fimmu.2019.00737

33. Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. Front Biosci. 1997;2: d12-d26. Published 1997 Jan 1. https://doi.org/10.2741/a171

34. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, et al. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. Front Immunol 2018; 9:586. Published 2018 Apr 9. https://doi.org/10.3389/fimmu.2018.00586

35. Cannon WB. Organization for physiological homeostasis. Physiological Reviews 1929;(3),399-431. https://doi.org/10.1152/physrev.1929.9.3.399

36. Bunker DLJ. Donor MHC Class I Gene Transfer to Recipient Liver: mechanis (c studies of a novel strategy for transplant tolerance induc (on, University of Sydney, 2014,Thesis

A BIOMARKER IN RENAL TRANSPLANTATION: DONOR-DERIVED CELL FREE DNAs

Aslı OZKIZILCIK KOCYIGIT[1,2]

[1]Medical Biology Department,
İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

8.1 INTRODUCTION

Kidney transplantation is the greatest therapeutic choice for patients with end-stage renal disease, however current therapy options cannot prevent acute and/or chronic rejection after kidney transplantation. A kidney biopsy is required to make an accurate diagnosis of rejection. Kidney biopsy is a painful and challenging procedure for patients. As a result, numerous approaches are employed to monitor graft deterioration, including serum creatinine monitoring, medication level monitoring, and evaluation of the patient's expression levels of kidney-related genes. The measurement of free (extracellular) DNA in the patient's plasma or urine is one of these approaches [1].

Cell free DNA (cfDNA) is a double-stranded helical structure of bare DNA molecules that are actively released from cells or as a result of controlled cell death (apoptosis) or cell necrosis. Transrenal DNA refers to low molecular weight DNA

fragments found in urine that can flow through the kidney [2]. Mandel and Metais discovered it in 1948, and it was first utilized for prenatal diagnosis from maternal blood [1]. There is currently research being conducted on the use of cfDNA technology in the identification or prediction of organ damage [2,3].

The release of DNA occurs as a result of cell death caused by graft damage during organ transplantation. The capacity to discriminate between donor-derived cfDNAs (dd-cfDNA) and the patient's own DNA, in particular, has been a significant advancement in the prediction or diagnosis of allograft rejection. The purpose of this review is to define cfDNA, investigate its relevance to organ transplantation, and add to the knowledge on its usage in kidney transplantation.

8.2 WHAT IS A CELL FREE DNA?

cfDNAs are short DNA fragments of approximately 40-200 base pairs (bp) that pass into body fluids such as plasma or urine due to cell death, such as cell apoptosis or necrosis (Figure 1) [4]. In 1948, Mandel and Metais showed that free DNA is formed as a result of apoptosis and necrosis due to damage [1].

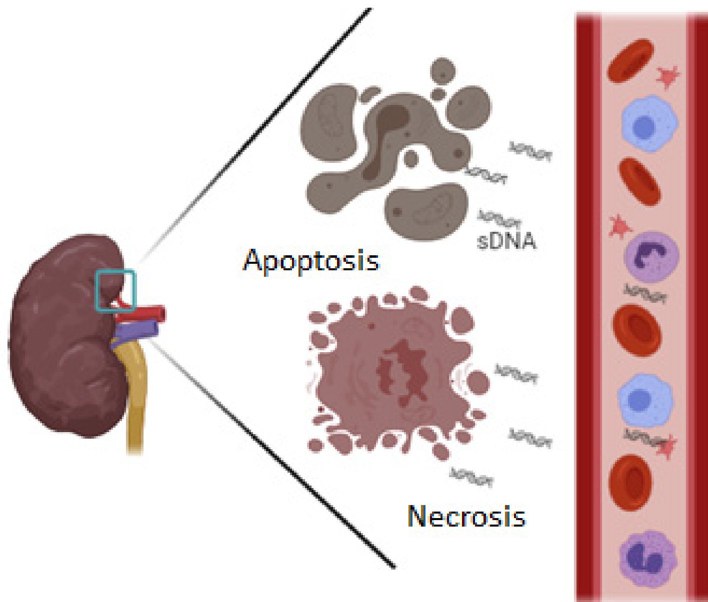


Figure: cfDNA production by cell apoptosis and necrosis (Created by Biorender.com)

The majority of cfDNAs are double-stranded nuclear and mitochondrial DNAs (mtDNA). There are also studies that claim to find small extrachromosomal DNAs, microDNAs, single-stranded, viral, bacterial, or food-borne DNAs. Apart from apoptosis and necrosis, DNA fragments are released via a variety of mechanisms including oncosis, pyroptosis, phagocytosis, active release, neutrophil extracellular trap release (Netosis), and excision repair [4]. The release of DNA fragments from dying cells in the injured kidney is the rejection-related mechanism in organ transplantation.

In blood, cfDNA has a half-life of less than one hour [3]. This half-life may be influenced by factors such as the treatment used and the complexes encountered by cfDNA in the blood. In blood, enzymes such as DNase I, plasma factor VII-activating protease (FSAP), and factor H degrade cfDNA.

The liver and kidney are also involved in the removal of cfDNAs from the body [4].

8.3 THE ISOLATION AND ANALYSIS OF cfDNAs

Noninvasively, cfDNA can be isolated from serum, plasma, and urine samples. Serum has 14 times the amount of cfDNA as plasma [5]. The specific amount in the urine is unknown. In one investigation, the levels identified in urine using two separate kits were compared, and it was discovered that this amount differs from person to person and even within the same person on different days [6]. As a result, if a patient's plasma or urine sample is to be used, the sample amount should be substantial at first. Special blood collection tube systems for cfDNA isolation are already commercially available. After blood sampling into these tubes, cfDNA isolation can be accomplished by centrifuging the plasma or serum. Commercial cfDNA isolation kits are also available. All of these devices were designed to keep contamination and coagulation at bay during isolation [5]. Today's methods include column or magnetic bead separation, phenol-chloroform separation, and filter separation. In the clinic, commercial spin column kits with silica membranes are extensively employed. Although this approach can produce more efficiency and purity, a very high-speed centrifuge is necessary. Furthermore, the risk of cross-contamination in the procedure's steps is significant, and the worker must be experienced. Microfluidic applications are another type of isolation. Although centrifugation is not necessary, vacuum pressure is applied, but additional development is required for routine clinical use [7]. Furthermore, research is being conducted to create tests that can directly evaluate cfDNA from serum or plasma without the need for its isolation. However, their viability for routine use in terms of sensitivity and specificity has not yet been completely verified [5].

Polymerase chain reaction (PCR) and sequence analysis (sequencing) technologies are used to analyze cfDNA. Sigdel et al. reported that using the multiplex PCR approach, they were able to standardize cfDNA analysis in plasma and biopsy samples from kidney transplant patients and achieve positive results [8]. The next generation sequence analysis approach is the most recently employed sequence analysis method (NGS method). The basic idea behind this technology is to detect single nucleotide polymorphisms (SNPs) in patient and donor cfDNAs [9].

8.4 DONOR DERIVED CELL FREE DNA (dd-cfDNA) AND TRANSPLANTATION

After solid organ transplantation, a biopsy of the transplanted organ is performed to check allograft function. Biopsies are conducted when clinical values are suspect or as part of a standard follow-up regimen (protocol biopsy). Protocol biopsies, in instance, are performed solely for monitoring purposes, and there is a risk of hurting the patient. According to Knight et al., protocol biopsies caused significant problems in 1%, extensive hematuria in 3.5%, and had to be repeated in 25% due to insufficient specimens [1]. As a result, noninvasive biomarker research for post-transplant monitoring has grown in popularity in recent years. The dd-cfDNA level in the patient's blood rises as a result of the transplanted organ's damage. It has grown in importance in post-transplant monitoring, particularly because dd-cfDNAs may be differentiated from those of recipients.

Rejection following transplantation is critical for patients with end-stage heart failure. Endomyocardial biopsy is used to monitor the patient after heart transplantation. Following the introduction of sDNA testing, studies on its application as

a biomarker in the prediction of rejection in heart transplant patients have been done. In a multicenter research, Richmond et al. tested sDNA in 241 pediatric and adult heart transplant patients. The level of dd-cfDNA was found to be quite high in patients with post-transplant antibody-mediated rejection (AMR) and acute cellular rejection (ACR), but not statistically significant enough to substitute biopsy. Furthermore, they stated that sample preparation for this test is critical, and that dd-cfDNA can be a valuable biomarker with proper sample preparation protocols [10]. Similarly, at the conclusion of the previous year's multicenter research, they suggested that while this approach did not prevent biopsy, it could provide supportive information [11].

Due to organ damage, lung transplantation is another therapy option. Prediction of rejection is critical because rejection following lung transplantation offers a life-threatening risk. It was indicated in a study involving lung transplant patients that it could be a powerful biomarker in identifying people at risk of developing rejection following transplant. Furthermore, it has been claimed that dd-cfDNA follow-up in the first three months can potentially predict future chronic rejection [12,13].

For many liver conditions, liver transplantation may be the best therapy option. In fact, there is a possibility of rejection following this transplant. As a result, the biomarker feature of dd-cfDNA was also studied in patients undergoing liver transplantation. According to the findings of a multicenter research, the dd-cfDNA test predicts acute rejection earlier and more accurately than standard liver tests [14]. Another study found that in cadaver donor transplants, the brain death features of the donor affected the release of dd-cfDNA and thus its levels in the patient's blood. The dd-cfDNA levels

of the patients were found to be high after transplants from resuscitated donors at the end of the trial. These people have also developed portal hepatitis and a systemic inflammatory reaction. More research is needed to make sense of these findings [15].

8.5 cfDNA AND RENAL TRANSPLANTATION

Allograft rejection can occur as a result of inflammation and result in certain pathological alterations in the kidney. The patient's immune system identifies non-self-antigens and produces tissue damage, which causes inflammation. In this case, both innate and adaptive immune responses are involved. T lymphocytes are the primary immune cells implicated in this response. As a result, hyperacute rejection is defined as rejection that occurs within a few minutes of transplantation. Because of sophisticated, highly sensitive crossmatch assays, hyperacute rejection is now uncommon. Acute rejection occurs after a few days and might be antibody-mediated or T-cell-mediated. Chronic rejections occur more than three months following transplantation and can be antibody-mediated or T-cell-mediated [16].

In biomarker investigations done noninvasively in both blood and urine for the identification of acute and chronic rejection-related graft damage after kidney transplants, dd-cfDNAs yielded successful findings [2,17,18]. In their investigation, Jordan et al. looked at the connection between dd-cfDNA and acute antibody-mediated rejection (AMR) in 90 blood samples and 87 kidney biopsy samples. As a conclusion, they reported that the dd-cfDNA test is more effective when combined with the DSA test, and dd-cfDNA+/DSA+ patients are more likely to have AMR [19]. They used the digital droplet PCR (ddPCR) technology to evaluate dd-cfDNA in a kidney transplant trial

involving 300 patients. At the end of the study, they reported that the cost of this test would be minimal, and that dd-cfDNA might be utilized as a biomarker in the early prediction of allograft injury [20]. In addition to these investigations, another study compared the plasma dd-cfDNA numbers of 189 kidney transplant patients with the proportion (%) and found that the amount of dd-cfDNA in the plasma was more accurate than the percentage ratio [17].

In conclusion, since the discovery of dd-cfDNAs in kidney transplant patients, the results have been significantly related with clinical indications of allograft deterioration and rejection. Studies are being conducted to produce a quick, simple, and cost-effective test process for standardizing this test in the clinic.

8.6 CONCLUSION

The concept of employing dd-cfDNAs to detect allograft deterioration in kidney transplant recipients can be expanded. The current dd-cfDNA detection techniques are not yet standardized as a routine diagnostic method in the clinic. It cannot be utilized on a regular basis since it generates cross-contamination, necessitates expert staff, and is prohibitively expensive. If current research can overcome these obstacles, it will be able to identify allograft damage in plasma or urine at an early stage. Indeed, if it can be further developed and manufactured in the form of small urine strip kits that patients can simply take from the shelves, this sort of transplant patient can be followed even from home.

8.7 REFERENCES

1. Knight SR, Thorne A, and Lo Faro ML. Donor-specific Cell-free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review. Transplantation 2019; 103 (2): 273–283. https://doi.org/10.1097/TP.0000000000002482.
2. Gielis EM, Ledeganck K, de Winter B, del Favero J, Bosmans J. *et al.* Cell-Free DNA: An Upcoming Biomarker in Transplantation. American Journal of Transplantation 2015; 15 (10): 2541–2551. https://doi.org/10.1111/ajt.13387.
3. Bloom RD, Bromberg J, Poggio E, Bunnapradist S, Langone A. *et al.* Cell-Free DNA and active rejection in kidney allografts. Journal of the American Society of Nephrology 2017; 28 (7): 2221–2232. https://doi.org/10.1681/ASN.2016091034.
4. Kustanovich A, Schwartz R, Peretz T, and Grinshpun A. Life and death of circulating cell-free DNA. Cancer Biology and Therapy 2019; 20 (8): 1057–1067. https://doi.org/10.1080/15384047.2019.1598759.
5. Celec P, Vlková B, Lauková L, Bábíčková J and Boor P. Cell-free DNA: the role in pathophysiology and as a biomarker in kidney diseases. Expert Reviews in Molecular Medicine 2018; 20: 1–15. https://doi.org/10.1017/erm.2017.12.
6. Streleckiene G, Reid HM, Arnold N, Bauerschlag D, and Forster M. Quantifying cell-free DNA in urine: Comparison between commercial kits, impact of gender and inter-individual variation. Biotechniques 2018; 64 (5): 225–230. https://doi.org/10.2144/BTN-2018-0003/ASSET/IMAGES/LARGE/FIGURE2.JPEG.
7. Lee H, Park C, Na W, Park KH, and Shin S. Precision cell-free DNA extraction for liquid biopsy by integrated microfluidics. NPJ Precision Oncology 2020; 4 (1). https://doi.org/10.1038/s41698-019-0107-0.
8. Sigdel T, Archilla F, Constantin T, Prins S, Liberto J et al. Optimizing Detection of Kidney Transplant Injury by Assessment of Donor-Derived Cell-Free DNA via Massively Multiplex PCR. Journal of Clinical Medicine 2018; 8 (1): 19. https://doi.org/10.3390/jcm8010019.

9. Dengu F. Next-generation sequencing methods to detect donor-derived cell-free DNA after transplantation. Transplantation Reviews 2020; 34 (3): 100542. https://doi.org/10.1016/j.trre.2020.100542.

10. Richmond ME, Zangwill S, Kindel S, Deshpande S, Schroder J et al. Donor fraction cell-free DNA and rejection in adult and pediatric heart transplantation. Journal of Heart and Lung Transplantation 2020; 39 (5): 454–463. https://doi.org/10.1016/j.healun.2019.11.015.

11. Khush KK, Patel J, Pinney S, Kao A, Alharethi R. et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. American Journal of Transplantation 2019; 19 (10): 2889–2899. https://doi.org/10.1111/ajt.15339.

12. Agbor-Enoh S, Wang Y, Tunc I, Jang M, Davis A. et al. Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation. EBioMedicine 2019; 40: 541–553. https://doi.org/10.1016/j.ebiom.2018.12.029.

13. Bansal S, Fleming T, and Mohanakumar T. The detection of donor-derived cell-free DNA may serve as a biomarker for the early detection of chronic lung allograft dysfunction. EBioMedicine 2019; 40: 13–14. https://doi.org/10.1016/j.ebiom.2019.01.044.

14. Schütz E, Fischer A, Beck J, Harden M, Koch M. et al. Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. PLoS Medicine 2017; 14 (4): 1–19. https://doi.org/10.1371/journal.pmed.1002286.

15. Krenzien F, Katou S, Papa A, Sinn B, Benzing C et al. Increased Cell-Free DNA Plasma Concentration Following Liver Transplantation Is Linked to Portal Hepatitis and Inferior Survival. Journal of Clinical Medicine 2020; 9 (5): 1543. doi: https://doi.org/10.3390/jcm9051543.

16. Seeto RK, Fleming JN, Dholakia S, and Dale BL. Understanding and using AlloSure donor derived cell-free DNA. Biophysical Reviews 2020; 12 (4): 917–924. doi: https://doi.org/10.1007/s12551-020-00713-5.

17. Oellerich M, Shipkova M, Asendorf T, Walson P, Schauerte V. et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study. American Journal of Transplantation 2019; 19 (11): 3087–3099. doi: https://doi.org/10.1111/ajt.15416.
18. Garg N, Hidalgo L, Aziz F, Parajuli S, Mohamed M. et al. Use of Donor-Derived Cell-Free DNA for Assessment of Allograft Injury in Kidney Transplant Recipients During the Time of the Coronavirus Disease 2019 Pandemic. Transplant Proceedings 2020; (4): 1–4. doi: https://doi.org/10.1016/j.transproceed.2020.07.013.
19. Jordan SC, Bunnapradist S, Bromberg J, Langone A, Hiller D. et al. Donor-derived cell-free DNA identifies antibody-mediated rejection in donor specific antibody positive kidney transplant recipients. Transplantation Direct 2018; 4 (9): 1–5. doi: https://doi.org/10.1097/TXD.0000000000000821.
20. Beck J, Oellerich M, Schulz U, Schauerte V, Reinhard L. et al. Donor-Derived Cell-Free DNA is a Novel Universal Biomarker for Allograft Rejection in Solid Organ Transplantation. Transplantation Proceedings 2015; 47 (8): 2400–2403. doi: https://doi.org/10.1016/j.transproceed.2015.08.035.

EXOSOME IN ORGAN TRANSPLANTATION

Tülay KILICASLAN AYNA[1,2,3]

[1]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[3]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

9.1 INTRODUCTION

Extracellular vesicles (EVs) are characterized as membranous structures that are secreted during various stages of cellular development in the majority of cells. The vesicles are enclosed by a phospholipid bilayer that has a composition highly comparable to that of the originating cell. EVs possess a diverse array of chemicals, encompassing proteins (such as cytokines, receptors, or their ligands), nucleic acids (including DNA, mRNA, and miRNA), and lipids. The inside space of EVs provides a favorable milieu for the presence and function of biologically active molecules [1]. Indeed, the initial documentation regarding EVs may be traced back to the 1960s. The release of EVs with a size of 100 nm from chondrocytes was determined by Bonucci and Anderson. During the same time period, Wolf made the determination that platelets release minute EVs, which he referred to as "platelet dust." Trams and his colleagues made the discovery of exosomes derived from the prostate/epididymis in seminal fluid throughout the 1980s.

The study discovered that these vesicles serve the purpose of facilitating not only the maturation of sperm, but also the conveyance of proteins and lipids obtained from the prostate to the sperm membrane [2].

The natural nanoparticles, initially recognized for their ability to eliminate undesirable substances from cells and sometimes referred to as detritus, have currently garnered significant interest as a noteworthy physiological or pathological substance. Regrettably, the aforementioned depiction of debris implies that all particles of this nature are indicative of cellular waste. Over an extended period, this organization discouraged researchers from conducting thorough investigations into extracellular particles, so impeding the exploration of both EVs and non-EV nanoparticles. The historical course of study pertaining to EVs serves as an illustrative instance wherein the utilization of a singular phrase has impeded the progress of an entire scientific domain. At now, the exosome is the most extensively studied category of EVs involved in intercellular communication. The exosome exhibits distinct biophysical and biochemical properties that lend themselves well to regular laboratory investigation [3].

9.2 EXTRACELLULAR VESICLE TYPES

In the context of an undetermined EV population, it is frequently observed that alternative terminologies such as "exosomes," "microvesicles (MVs)," and "microparticles" are employed in place of the term EV. EVs are primarily categorized into three distinct groups based on their size, biological characteristics, and creation mechanism. These groups include exosomes, microvesicles, and apoptotic bodies. Cytokines possess the ability to be secreted by a wide range of cells, and can subsequently be transmitted to recipient cells throu-

gh intercellular contact. Furthermore, these molecules exhibit diverse biological capabilities. EVs are small vesicles enclosed by a membrane that facilitate the transportation of vital biomolecules across cellular boundaries, hence contributing to the maintenance of physiological homeostasis. EVs have the ability to exert their effects by being taken up by target cells and subsequently releasing their cargo within the cytoplasm. Alternatively, EVs can also engage in interactions with receptors expressed on target cells through membrane-bound ligands, thereby initiating downstream intracellular pathways. They have the capability to be secreted by a wide range of cellular types and subsequently transferred to recipient cells through intercellular communication mechanisms. These entities possess the ability to be transferred from one context to another and exhibit a diverse range of biological functionalities. It is not inaccurate to assert that the transfer of these vesicles also alters the destiny of the recipient cell. Biogenesis theory categorizes EVs into two distinct classifications for examination. The entities under discussion are ectosomes and exosomes. Ectosomes are vesicles that are generated by the process of plasma membrane budding. Exosomes are derived from endosomes. The removal of substances from cellular compartments occurs through the process of exocytosis, specifically involving the discharge of multivesicular bodies that contain many vesicles. Empirical evidence indicates that membrane structures, such as the endoplasmic reticulum and nuclear membrane, actively engage in the processes associated with endomembranes. Ectosomes, also known by various other terms such as apoptotic bodies, microvesicles, big oncosomes, migrasomes, and ciliary ectosomes, exhibit distinct subtypes of exosomes based on their biogenesis characteristics. These subtypes include small EVs, amphysomes, and autophagosomes [4]. The establishment of the International

Extracellular Vesicles Association (IESV) in 2011 was prompted by the increasing prominence of membrane structures discharged from cells across various scientific disciplines. The primary objective of the association is to offer reliable and consistent guidelines pertaining to vesicles. The International Society for Extracellular Vesicles (ISEV) has established a set of criteria pertaining to the separation, characterisation, and approval of EVs [5]. In accordance with the guidelines set forth by this organization, EVs are categorized into three distinct classifications. These are:

i. Apoptotic bodies

ii. Microvesicles

iii. Exosomes

Apoptotic bodies are molecular entities that manifest during the process of programmed cell death. The process of enveloping cellular contents with a membrane and executing phagocytosis while minimizing harm to neighboring cells and tissues holds significant importance. The process of its formation occurs through the budding of the cell membrane. The sizes of the particles exhibit a range spanning from 50 to 5000 nm. The identification of this category of electric vehicles can be accomplished through the detection of DNA and histones [6].

MVs, similar to apoptotic bodies, are extruded from the plasma membrane through a process of direct outward budding of the plasma membrane [7,8]. The dimensions of MVs range from 100 to 1000 nanometers, as reported in previous studies [6,7]. Vesicles with a more diverse content are formed through the selective integration of proteins, nucleic acids, and lipids, and subsequently released into the extracellular

environment. The expression levels of these markers are indicative of the traits exhibited by the parental cells [9,10]. In contrast to exosomes, the absence of a particular marker distinguishes MVs.

The release of exosomes has been observed to occur when multivesicular bodies fuse with the plasma membrane. These entities are tiny vesicles that exhibit a diameter spanning from 40 to 100 nanometers, along with a density falling within the range of 1.13 to 1.19 grams per cubic centimeter [11,12]. Specific markers showing their endocytic origin can be used to identify them, including ALG-2 interacting protein X (Alix), tumor susceptibility gene 101 (TSG101), and tetraspanins. However, a combination of these markers is now favored for identification purposes [13].

To enhance comprehension of the dimensions of EVs, a comparative analogy is employed, wherein apoptotic bodies are equated to platelets, MVs to bacteria, and exosomes to viruses. These molecules can be differentiated based on their source, size, and composition (Table 1). Exosomes are commonly included in research investigations owing to their inherent capacity for characterization. The characterization of exosomes is significantly influenced by the presence of surface molecules belonging to the tetraspanin group, namely CD9, CD63, and CD81, which are located on the exosomal membranes. Simultaneously, these surface molecules also fulfill a function in the process of binding and merging exosomes with the recipient cell [14]. To enhance comprehension of the dimensions of EVs, a comparative analogy is employed, wherein apoptotic bodies are equated to platelets, MVs to bacteria, and exosomes to viruses. These molecules can be differentiated based on their source, size, and composition (Table 1). Exosomes are commonly included in research inves-

tigations owing to their inherent capacity for characterization. The characterization of exosomes is significantly influenced by the presence of surface molecules belonging to the tetraspanin group, namely CD9, CD63, and CD81, which are located on the exosomal membranes. Simultaneously, these surface molecules also fulfill a function in the process of binding and merging exosomes with the recipient cell [15].

Table 1: Traits of main extracellular vesicles

Trait	Apoptotic Bodies	Microvesicle	Exosome
Size	1–5 μm	100–1000 nm	30–100 nm
Origin	Cellular debris, plasma membrane blebbing during cell apoptosis	Direct outward budding or blebbing from the plasma membrane	Multivesicular bodies fusion with plasmatic membrane
Morphology	Heterogeneous	Heterogeneous	Cup-shaped
Density	1.16–1.28 g/mL	1.25–1.30 g/mL	1.13–1.19 g/mL
Formation mechanisms	Budding from plasma membrane	Budding from plasma membrane	Exocytosis of multivesicular body
Markers	Membrane permeable (PI positive) Annexin V, DNA, histones	Membrane impermeable (PI negative) integrin, selectin, flotillin-2	Membrane , impermeable (PI negative) CD63, TSG101, Alix, flottilin
Contents	Cytosolic content (protein, RNAs, fragmented DNA) and cellular organelles	Protein, lipid, different RNA species, and DNA	Protein, lipid, different RNA species, and DNA
Pathways	Apoptosis-related pathways	Ca^{2+}-dependent	ESCRT-dependent
Mechanism of release	Rho-associated kinase I and myosin ATPase activity	Relocation of phospholipids to the outer membrane, cytoskeleton rearrangements, generation of membrane curvature, and vesicle release	Constitutive or inducible, depending on the cell type of origin

Timing of release	-	A few tenths of a second	Ten minutes or more
Lipids	Characterized by phosphatidylserine externalization	The lipid contents are primarily derived from plasma membrane, and resemble the parental cells (without BMP)	A major sorting of lipidic molecules from the parental cells (include BMP)
Determinant of controlled contents	The cellular origin and stimuli	No direct correlation	The cellular origin and physiological state of the cell
Size determination and quantification	-	-	Dynamic light scattering
Isolation methods	Ultracentrifugation (10,000–20,000×g)	No standardized methods	Ultracentrifugation (100,000–200,000×g) filtration, density gradient Immunoprecipitation, Immune affinity capture and ExoQuick precipitation methods
Detection methods	Flow cytometry, electron microscopy	Flow cytometry, electron microscopy	Electron microscopy, Western blot for exosome enriched markers
Composition	Cell organelles, proteins, nuclear fractions, coding RNA, noncoding RNA, DNA	Protein, lipids, cell organelles, coding RNA, noncoding RNA, DNA	Protein, lipids, coding RNA, noncoding RNA, DNA

ESCRT: Endosomal Sorting Complexes Required for Transport, BMP: bone morphogenetic protein, PI: propidium iodide, DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, Ca: Calcium, ATP: Adenosine Triphosphate, CD63: Cluster of differentiation 63, TSG101: Tumor susceptibility gene 101, Rho: Ras homology family

9.3 EXTRACELLULAR VESICLE AND TRANSPLANTATION

It is evident that there has been a notable surge in the examination of EVs inside the realm of transplantation, particularly following the year 2016. A query conducted on the Web of Science database utilizing the keywords "extracellular vesicle" and "transplantation" resulted in the identification of 676 scholarly articles published over the past five years. In contrast, a mere 43 publications were found from the period spanning 1991 to 2014 [16]. Human leukocyte antigens (HLA) are the primary molecules responsible for acute or chronic rejection in solid organ transplantations. The recognition of a transplanted allograft by the immune system can be categorized into three distinct mechanisms. One of the mechanisms involved in the recognition of donor cells by recipient T cells is the direct route, also known as direct recognition. In this particular process, the prominence is given to the involvement of donor antigen-presenting cells (APC), with a specific emphasis on dendritic cells (DC). Donor DCs, commonly referred to as passenger leukocytes, migrate to the recipient's secondary organs, such as lymph nodes or spleen, through the circulatory system and lymphatic vessels. Once in these tissues, they undergo allorecognition. The phenomenon of direct recognition involves the prominent manifestation of genetic disparities in the HLAs between the recipient and the donor. The initiation of an immune response occurs when recipient T cells notice the disparity between the donor and recipient HLAs, or the peptides given alongside the donor HLAs. Indirect recognition, the second mode of recognition, involves immune activation that is predicated upon the traditional principle of antigen presentation. The system operates by a similar method as that of a cell infected with a bacterium, wherein it processes antigens and subsequently presents

foreign peptides to host APCs and T cells. In this context, the recipient's APC uptake and process injured cells or subcellular molecules caused by ischemia reperfusion injury of donor cells or a pathological process associated with the allograft. These processed entities are subsequently presented to the recipient's T cells. Although researchers have been aware of these two types of identification for a considerable period, it has become apparent in the past decade that the recipient T cells recognize the allograft by a distinct process. The phenomenon known as semi-direct recognition refers to the initiation of an immune response by the transfer of Human Leukocyte Antigens (HLAs) from donor cells to the surfaces of recipient cells [17]. The process of transmission takes place via three different methods. The topics of interest include trogocytosis, tunneling nanotubes, and exosomes. Trogocytosis refers to the reciprocal exchange of molecules between adjacent cells through direct membrane contact. The phenomenon under consideration was initially documented in 1970 as an integral component of the mechanism via which parasites invade and eliminate host cells. In the year 2002, the term "trogo" was assigned to denote the process of transferring membrane fragments carrying proteins that are bound to the membrane, drawing inspiration from the ancient Greek word "trogo," which translates to "to gnaw"[18]. Tunnelling nanotubes are elongated and slender extensions that develop between adjacent cells. Lateral diffusion facilitates the passage of membrane proteins and lipids between cells. The discovery of these structures took place in 2004[19]. Exosomes facilitate the transport of both membrane molecules and cell content between cells [20]. As previously stated, research on HLA and EVs, which have a crucial impact on solid organ transplantations, commenced in the 1970s. The initial documentation of this phenomenon was made in 1974 by Frelinger et al. They

determined that Major Histocompatibility Complex (MHC) molecules were transmitted between leukocytes[21,22]. In 2006, Brian Dolan and his colleagues discovered that peptide-MHC complexes persisted in APCs for a duration of 2 days following translocation[23]. This offers abundant potential for T cell stimulation. DCs that obtain allogeneic MHC proteins through direct cell-cell interaction have been demonstrated to stimulate alloreactive T lymphocytes both in laboratory settings and in living organisms through indirect recognition [21]. In a study conducted in 2013, Lesly Smith and colleagues showed that in mice with transplanted skin, recipient DCs continue to acquire MHC-class I for a minimum of one month after transplantation. This acquisition is believed to be the primary source of the major alloantigen that triggers CD8+ cytotoxic T cell responses[24]. These investigations illustrate that CD4+ T cells, when indirectly activated, support the direct activation of CD8+ T cells. Additionally, they reveal a semidirect recognition model. Two investigations conducted in 2016 unveiled the significance of EVs in the context of transplantation. One of these is a research conducted on animals by Marino et al. This study revealed that donor DCs had a delayed migration to the lymph nodes following skin transplantation. Nevertheless, two days following transplantation, a significant number of recipient APCs were shown to transport vesicles containing donor MHC class I and II molecules to the lymph nodes. The process of transferring membrane proteins from a donor cell to recipient APCs is referred to as “cross dressing”. This cohort of mice underwent cardiac transplantation, and in this instance, researchers examined the trafficking of donor DCs traveling to the spleen. The study found that just a small quantity of donor DC migrated to the spleen. Quantitative data is provided regarding the cells in the study. Among the $1x10^6$ cells present in the spleen, 100 were identified as donor

DC. Out of the total of $1x10^6$ cells, 90.000 were identified as having the donor MHC and cross-dressing recipient APC [25].

In 2016, Lui and Morelli conducted research in the area of heart transplantation and obtained comparable outcomes to the other team. Although the donor dendritic cells (DCs) migrated to the lymph nodes in limited quantities, it was observed that the recipient DCs, which were covered with proteins from the donor, were there in large numbers. It was revealed that the vesicles participating in this transport have exosome properties [26].

The preceding studies elucidate the reasons behind the increase in research on EVs in the transplantation field, particularly highlighting the prominence of exosomes in immunological responses during transplantation. An essential concern in this context is the cellular phenotype responsible for the secretion of EVs. The secretion of EVs from this cell has the potential to alter the destiny of the target cell and thus impact the individual. DCs are becoming increasingly significant in transplantation because of their involvement in the identification of foreign tissues and the subsequent immune responses. The process of development in allorecognition dendritic cells is very significant. Exosomes generated by mature dendritic cells are believed to stimulate the Th1 arm of the immunological response, leading to the destruction of the allograft by T cell cytotoxicity. Nevertheless, the release of exosomes from immature DCs induces Th2 activation, leading to a relatively benign immunological response that advances at a little slower pace [27]. Although MHC molecules transported by EVs play a crucial role in triggering the immune response during allorecognition, it is worth noting that other membrane molecules and cargo conveyed via these vesicles also hold significant importance. The cargo comprises a diverse assortment

of biomolecules, including mRNA, micro RNA, long noncoding RNA, proteins, and other substances. Transplantation and other illnesses differ in terms of EVs. In transplantation-related illnesses, extracellular vesicles originate from two persons with distinct genetic backgrounds, but in other cases, only vesicles released from the patient's own cells are present. Hence, the specific cell type responsible for delivering and targeting EVs after transplantation holds significant significance.

In the context of organ transplantation, recipient immune system cells release EVs, whereas donor DCs go directly to secondary lymphoid organs to contribute to the immunological response. Additionally, allograft cells also secrete EVs. Typically, it is believed that MHC molecules present in the membrane of EVs from the donor are transported to the membrane of recipient APCs, leading to a process known as semi-direct recognition. Nevertheless, it is also recognized that EVs released mostly by immature donor APCs are taken up by recipient APCs and subsequently stimulate recipient T cells in an indirect manner. Hence, EVs from donors play a crucial role in both direct and indirect recognition [27]. Thus far, we have consistently assessed the impact of EVs in transplantation solely from the perspective of donor EVs. However, in the context of transplantation, the presence of cells from two distinct sources introduces the recipient's immune system into the equation, leading to immunological activation. In this context, the release of EVs from the recipient cells and the recipient's immunological condition assume significant importance. Cells involved in tolerance, particularly regulatory T cells and regulatory immunocytes, are of particular interest. The surface of the cells released by regulatory T cells contains a CD73 receptor. Upon entering the target cell through EVs, this molecule undergoes a conversion from adenosine triphosphate

(ATP) to cyclic adenosine monophosphate (cAMP) adenine within the cell. This inhibits the activation of immunological molecules, guarantees the activation of immune system inhibitors, and triggers tolerance mechanisms. Allo-MHC molecules or the antigenic peptides presented with these molecules are insufficient to initiate immunological responses during the development of adaptive immune responses. The interaction of costimulatory molecules between the two cells is essential. The immune response can be altered by the transport of coinhibitory molecules, such as CTLA4 and PD1L, through EVs. Once transmitted to the target cell, the miRNAs found in Tregs, which are molecules involved in gene expression regulation, as well as the cytokines they carry, have the ability to alter the direction of the immune response. In addition, regulatory complement proteins such as CD46 and CD55 play a crucial role in redirecting reactions towards tolerance [28].

Clinicians in transplantation and other disease pathologies have a significant need for a biomarker that is user-friendly and safe for patients. This biomarker should enable accurate illness diagnosis and provide precise information on the stage of the disease. The search for biomarkers in solid organ transplantation continues in order to monitor the allograft pre- and post-transplantation. Cell-free DNA, molecular microscopy, transplant-related mRNA expressions, miRNA research, non-HLA antibody and protein research are ongoing in blood, urine, and other materials for this specific objective [29].

9.4 INVESTIGATION OF EXTRACELLULAR VESICLES IN BIOLOGIC MATERIALS

Exosomes have the ability to penetrate several bodily fluids, including blood, urine, saliva, bronchoalveolar fluid, and milk. Moreover, they exhibit remarkable stability within these

fluids. From this standpoint, it appears appropriate as a biomarker. However, the limitation lies in the fact that EVs, particularly exosomes, can be utilized in these investigations owing to their characterizability. Due to their small size, exosomes represent a challenge in differentiating them from other molecules present in physiological fluids. To isolate vesicles, an ultracentrifuge is necessary, with long-term centrifuges operating at speeds above about 100.000 g. This approach is highly recommended in exosome research. The excessive cost of ultracentrifuges and the practical challenges associated with accessing ultracentrifuge-equipped facilities significantly constrain scientific study. Exosome isolation can also be achieved by utilizing magnetic beads that are coated with exosome surface molecules like CD9, CD63, CD81, or nonaparticles that are coated with monoclonal antibodies [16]. Once exosomes are separated, one can analyze the content of surface receptor proteins, tissue-specific proteins, mRNA, miRNA, or proteins that are specifically relevant to solid organ donation. The research on EVs in the field of transplantation mostly focuses on the periods following lung, heart, and kidney transplants. Table 2 summarizes research conducted on heart, lung, kidney, and liver transplants using exosomes (Table 2) [30].

Table 2. The results of pre-clinical research investigating the potential of exosomes as a biomarker for different clinical disorders in solid organ or tissue transplantation

Organ	Researcher	Study Group	Material and Methods	Outcomes
Heart	Sukma Dewi, 2017[30]	10 HTx Rs (5 AR + 5 wo AR)	Serum sample 175 microRNAs profiled by qPCR-based microRNA-profiling assay	Cardiovascular allograft failure promotes exosomal miR-142-3p, which inhibits endothelial RAB11FIP2 expression and promotes blood vessel permeability
	Kennel, 2018[30]	10 Rs wo rejection 10 Rs TCMR 8 Rs ABMR 10 healthy controls 10 HF patients wo allograft	Serum sample LC-MS/MS	45 EV proteins were distinguished among the groups. Non-rejection HTx, TCMR, and ABMR expressed 15 EV proteins differently.8 of these 15 proteins are immune-related. Most proteins found were linked to complement activation, adaptive immunity, immunoglobulin components, and coagulation.
	Castellani, 2020[30]	37 Rs in validation cohort 53 Rs in training cohort (33 with no rejection+ 11 TCMR (grade 2R or 3A)+ 7 ABMR (grade 1 or 2 in + positive DSAs))	Serum sample Analysis of sEV surface markers by multiplex flow cytometry	Rs with TCMR and ABMR have higher exosome concentrations and smaller diameters. Exosome surface markers CD3, CD2, ROR1, SSEA-4, HLA-I, and CD41b differentiated controls from ACR, while HLA-II, CD326, CD19, CD25, CD20, ROR1, SSEA-4, HLA-I, and CD41b distinguished controls from ABMR.
	Hu, 2020[30]	4 HTx Rs (1 ABMR)	Serum sample Detection of exosomes subsets specific to donor HLA I; mRNA analysis; Western blot for C4d, flotillin 1, cytochrome c, troponin T.	Troponin protein and mRNA were expressed in exosomes throughout follow-up. In 1 HTxR who experienced ABMR on day 14 endomyocardial biopsy, donor heart exosome subsets contained time-specific C4d protein, which disappeared with therapy. The remaining donor's exosomes did not contain C4d.
Lung	Gunasekaran, 2017[30]	30 bilateral LTxRs (10 AR+10 with BOS, and 10 stable LTxRs)	Serum and BAL fluid samples Western blot for annexin V and lung-associated SAg Col-V, Exosome surface marker analysis by flow cytometry miRNA analysis by TaqMan qRT-PCR	Immune responses cause lung transplant recipients to produce Col-V-containing exosomes. Col-V and Ka1T antigens are found in exosomes from lung transplant recipients with BOS and AR. These people may have Col-V in their blood before AR or BOS. Exosomes from lung transplant individuals with BOS and acute rejection

				(AR) and those with stable lung function had different miRNA expression patterns.
	Gunasekaran, 2018[30]	10 stable LTxRs 10 LTxRs with BOS	Serum sample Western blot for SAg (K-a-1-tubulin [Ka1T] and collagen V [Col-V]), MHC class II molecules, costimulatory molecules CD40, CD80, and CD86, and transcription factors class II MHC trans-activator, NF-kB, hypoxia-inducible factor 1-a, IL-1R-associated kinase 1, MyD88, and 20S proteasome.	BOS exosomes contained lung SAg (K-a-1-tubulin [Ka1T] and collagen V [Col-V]), MHC class II molecules, co-stimulatory molecules (CD40, CD80, and CD86), transcription factors (NF-kB, hypoxia-inducible factor 1-a, IL-1R-associated kinase 1, MyD88, and 20S proteasome), but not stable LTxR. However, both groups had adhesion molecules.C57BL/6 mice vaccinated with BOS exosomes but not stable LTxR had SAg and HLA Ab.
	Mohanakumar, 2019[30]	10 stable LTxRs, 10 with BOS, 5 wo DSA, 5 with de novo DSA, 5 PGD grade 3, 5 PGD grade 0, 15 symptomatic RVIs, 15 LTxRs wo RVI, 10 AR, 10 wo AR	Serum sample Western blot for lung self-antigens K alpha 1 tubulin and collagen-V, costimulatory molecules (CD80, CD86), transcription factors (nuclear factor kappa-light-chain-enhancer of activated B cells, hypoxia-inducible factor 1a, Class II MHC Transactivator), and 20S proteasome	LTxRs with grade 3 PGD, RVI, AR, and DSA contained SAg-containing exosomes, while stable receivers did not LTxRs with grade 3 PGD, AR, and DSA had exosomes with costimulatory molecules 80, 86, MHC class II, and 20S proteasome.
	Sharma, 2020[30]	30 stable time-matched controls 41 with BOS	Serum sample Western blot for lung self-antigens K alpha 1 tubulin, and collagen-V	Exosomes from BOS-afflicted LTxRs (n = 21) have higher lung Sag levels than those from stable LTxRs (n = 10).In a validation analysis, 2-LTxR cohorts with BOS and stable time-matched controls from 2 centers showed elevated lung SAgs-containing exosomes at 6 and 12 months before BOS.
	Gunasekaran, 2020[30]	32 stable LTxRs 35 LTxRs with symptomatic lower- and upper-tract RVIs	Serum sample Western blot for the lung self-antigens, 20S proteasome, and viral antigens for rhinovirus, coronavirus, and respiratory syncytial virus	LTx Rs with RVIs exosomes included lung self-antigens, viral antigens, and 20S proteasome and triggered immune responses to lung self-antigens that caused chronic lung allograft failure in vaccinated animals.

	Bansal, 2020[30]	90 bilateral LTxRs (3 with BOS + 3 with AR + 3 with symptomatic RVIs + 5 stable LTxRs)	Serum sample LC-MS/MS, Western blot to confirm mass spectrometry signatures	2 distinct AR proteins, 4 RVI, 24 BOS, and 8 stable LTxR proteins.AR, BOS, RVI, and stable patients had highly unregulated proteins (31, 2, and 2). LTxRs with AR exosomes contained immunoglobulin, complement control, coagulation, and innate and adaptive immune response proteins. In BOS-LTxR exosomes, immunoglobulin receptors and a carboxypeptidase N catalytic chain were abundant. Exosomes from RVI-LTxRs contained enhanced macrophage-stimulating factor.
	Sharma, 2020[30]	13 stable time-matched controls 6 with BOS 19 pediatric LTxRs	Serum sample Western blot for the lung self-antigens, 20S proteasome, co-stimulatory molecules transcription factors, adhesion molecules, and major histocompatibility complex class II	Compared to stable LTxRs, BOS exosomes had higher quantities of SAgs, donor HLA class I, MHC-II, transcription factors, co-stimulatory molecules, and 20S proteasome. Serial study of SAg-containing exosomes showed that they circulate before BOS.
	Goodlet, 2020[30]	1 LTx Rs with TCMR and SARS-CoV2-infection	Serum sample Western blot for collagen V [COL V] and K**α**1 tubulin [K**α**1T]), HLA-DQ and HLA-DR, and SARS-CoV-2 spike protein	Exosomes contained lung self-antigens and HLA class II molecules before SARS-CoV-2 infection during rejection. After COVID-19 diagnosis, SARS-CoV-2 spike protein exosomes were identified. Exosomes with SARS-CoV-2 spike protein were no longer detectable when viral symptoms resolved, but exosomes with lung self-antigens and HLA class II molecules persisted and spirometric flows decreased, suggesting chronic lung allograft malfunction.
	Itabashi, 2020[30]	34 stable LTxRs 18 LTxRs with BOS	Plasma and BAL fluid samples sEVs were analyzed for SAgs, natural killer cells markers, and cytotoxic molecules. Western blot for Col-V, CIITA, NF-**κ**B, K**α**1T, 20S proteasome, CD56, NKG2D (sc-23869), perforin (sc-373943), and FasL	Exosomes from BAL fluid of LTxRs with BOS showed higher levels of lung SAgs, NK-cell-associated molecules, and cytotoxic molecules, including perforin and FasL, compared to stable LTxRs. In BAL but not in stable and healthy participants, plasma exosomes from LTxRs with BOS included NK cell-associated (CD56,

				NKG2D) and cytotoxic substances Perforin, FasL.
	Bansal, 2021 [31]	Of Rs with RVI, 5 with respiratory syncytial virus (RSV), 5 with coronavirus, 5 with rhinovirus, 5 with influenza virus, 5 with cytomegalovirus (CMV) 5 time-matched stable LTxRs	Exosomes were isolated from lung transplant recipients followed by DNA and RNA isolation from exosomes. Cell signaling mechanisms were studied by co-culturing exosomes with human epithelial cells. Mice were immunized with exosomes and lung homogenates were studied for immune signaling proteins.	Exosomes from lung transplant recipients with RVI carry nucleic acids which are capable of inducing innate immune signaling, endoplasmic reticulum stress, and epithelial mesenchymal transition. Therefore, we propose that RVI can lead to induction of exosomes that initiate the process leading to CLAD in mice models. These novel findings identified the molecular mechanisms by which RVI increases the risk of CLAD.
	Itabashi, 2021 [32]	Plasma and BAL fluid samples were collected from patients 2 to 3 years after undergoing LTx at either of the 2 aforementioned institutions. BOS was diagnosed according to ISHLT criteria. In total, 18 LTxRs with BOS and 34 stable LTxRs were included in this study. For serial study following LTx for 1year posttransplant, the number of patients included in the study were different time points: 3 month (n= 48), 6 month (n=39) and 1 year (n=25).	CCSP levels in BAL fluid and development of antibodies to lung SAgs in plasma were determined by ELISA. Cytokines in BAL fluid were analyzed by 30-plex Luminex panel. Exosomes from BAL fluid or plasma were analyzed for SAgs, natural killer cells markers, and cytotoxic molecules.	We demonstrate that LTxRs with BOS have lower CCSP levels up to 9 months before BOS diagnosis. LTxRs with antibodies to SAgs 1 year posttransplant also developed DSA (43%) and had lower CCSP. BOS with lower CCSP also induced Interleukin-8 and reduced vascular endothelial growth factor. Exosomes from BOS contained increased SAgs, natural killer cells markers, and cytotoxic molecules. We conclude lower CCSP leads to inflammation, pro-inflammatory cytokine production, immune responses to HLA and SAgs, and induction of exosomes. For the first time, we demonstrate that CCSP loss results in exosome release from natural killer cells capable of stimulating innate and adaptive immunity posttransplant. This increases the risk of BOS, suggesting a role of natural killer cell exosomes in CLAD development.
	Ravichandran, 2021 [33]	A murine model of chronic lung allograft rejection was performed as described by Mimura et al13 In brief, a single lung from a B6D2F1 (H2b/d) donor mouse was orthotopically transplanted into a DBA/2(H2d)	To define the mechanisms leading to CCSP decline and chronic rejection, we employed two mouse models: 1) chronic rejection after orthotopic single lung transplantation and 2) anti-major histocompatibility complex (MHC)	In the chronic rejection mouse model, we detected circulating exosomes with donor MHC (H2b) and lung self-antigens and also development of antibodies to H2b and lung self-antigens and then a decline in CCSP. Furthermore, DBA2 mice that received injections of these exosomes

		recipients (10-12 weeks old) weighing 28 to 30 g.	class I-induced obliterative airway disease.	developed antibodies to donor MHC and lung self-antigens. In the chronic rejection mouse model, natural killer (NK) and CD8 T cells were the predominant graft-infiltrating cells on day 14 of rejection followed by exosomes containing NK cell-associated and cytotoxic molecules on day 14 and 28. When NK cells were depleted, exosomes with NK cell-associated and cytotoxic molecules as well as fibrosis decreased. Induction of exosomes led to immune responses to donor MHC and lung self-antigens, resulting in CCSP decline, leading to NK cell infiltration and release of exosomes from NK cells. These results suggest a novel role for exosomes derived from NK cells in the pathogenesis of chronic lung allograft rejection.
	Bansal, 2022 [34]	We retrospectively analyzed 18 LTxRs with BOS, 13 with RAS and 5 time-matched stable LTxRs (controls).	Plasma was collected from LTxRs with BOS (n = 18), RAS (n = 13), and from stable LTxRs (n = 5). Antibodies to lung self-antigens (SAgs) were determined by ELISA. Exosomes were isolated by ultracentrifugation. Donor specific antibodies to HLA were quantified using Luminex. Exosomes were characterized for lung SAgs, transcription factors, 20S proteasome, HLA class I and II, and polymeric immunoglobulin receptor protein using western blot. Exosome miRNA was analyzed using NanoString. The exosome-induced immune response was determined in mice.	LTxRs with RAS, but not BOS, had donor specific antibodies at diagnosis. CIITA, NFkB, polymeric immunoglobulin receptor protein, 20S proteasome, HLA-DQ, and HLA-DR were significantly higher in RAS exosomes than in BOS exosomes. RAS plasma had high levels of proinflammatory cytokines and distinct exosomal miRNA. Immunization of C57BL/6 mice with RAS exosomes showed severe inflammation and peribronchial fibrosis, whereas BOS exosomes induced patchy inflammation and fibrosis. LTxRs with BOS or RAS had exosomes with distinct molecular and immunologic profiles. RAS samples had a higher concentration of proinflammatory factors, HLA class II, lung SAgs, and antibodies to HLA class II molecules, indicating severe allograft injury. Mice immunized with RAS exosomes developed lesions in airways, pleura, interlobular septum, and alveoli, whereas BOS

				exosomes induced mild to patchy inflammation with lung fibrosis.
	Habertheuer, 2022 [35]	Mediastinal and distant lymph nodes, lung grafts and native right lungs were collected until post-operative day 8	Plasma and Pleural fluid collected early after lung transplantation in rats and humans contains donor-specific EVs. Exosomes were isolated from all samples and characterized by NTA, WB, Flow Cytometry and microscopic analyses.	We demonstrate EVs carrying donor antigen preferentially accumulate in mediastinal lymph nodes and colocalize with MHC II expressing cells within 4 h of engraftment. Injection of allogeneic EVs into pleural space of syngeneic lung transplant recipients confirmed their selective trafficking to mediastinal lymph nodes and resulted in activation of T cells in mediastinal, but not peripheral lymph nodes. Thus, we have uncovered an alternative pathway of donor antigen trafficking where pulmonary EVs released into the pleural space traffic to locoregional lymph nodes via pleural lymphatics. This pathway obviates the need for restoration of lymphatics across the bronchial anastomosis for trafficking of donor antigen to draining lymph nodes.
Liver	Zhang, 2019[30]	73 OLTx Rs with AR 63 OLTx Rs wo AR	Serum and liver allograft biopsy Western blot for galectin-9	The rejection group had significantly greater exosome and liver galectin-9 levels.
	Wang, 2022 [36]	60 LT Rs 30 TCMAR 30 wo AR	Serum samples EVs were sequenced by Illmina Hiseq	Serum EV miRNAs (up-regulated: miR-223 and let-7e-5p and miR-486-3p; down-regulated: miR-199a-3p, miR-148a-3p, and miR-152-3p) in AR patients vary significantly from those in non-AR patients and may be promising diagnostic biomarkers for AR in liver transplant patients.
Pancreatic islets	Vallabhajosyula, 2017[30]	5 ITx Rs 5 KTx Rs	plasma and/or urine RNA microarray, western blot, affinity antibody-coupled bead purification of tissue-specific exosomes	A decrease in transplant islet exosome signal was linked to recurrence of islet autoimmunity before the beginning of hyperglycemia.RNA cargo analysis revealed insulin, glucagon, somatostatin, and FXYD2 expression, which was absent

				pre-transplant. KTx: Post-transplant recipient plasma samples showed donor kidney-specific HLA-A2 and HLA-B27 exosomes; Western blot analysis confirmed aquaporin 2 expression; urine donor HLA-A2 exosomes contained renal glomerular protein podocalyxin-1.
	Korutla, 2019[30]	ITx-case report	Serum sample Affinity antibody-coupled bead purification of tissue-specific exosomes, western blot, RT-PCR	A decrease in transplant islet exosome signal was linked to recurrence of islet autoimmunity, which preceded hyperglycemia. Purified transplant islet exosome cargoes showed a decrease in insulin-containing exosomes, but not glucagon-containing ones, and a time-specific increase in GAD65, indicating selective destruction of transplanted **β** cells due to recurrent T1D autoimmunity.
Kidney	Alvarez, 2013[30]	15 KTx Rs	Urine sample Western blot	NGAL expression in exosomes was higher in DGF patients than non-DGF patients.
	Peake, 2014[30]	11 healthy controls 9 CKD pts 14 KTx Rs	Urine sample Expression of exosomal mRNA for the injury biomarkers neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and cystatin C	Day 7 creatinine decrease ratio is shown by urinary NGAL and IL-18. Exosomes contain biomarker mRNA, but they do not predict urinary biomarker levels or the CRR. This may be because exosome packaging of mRNA is selective and not representative of biomarker-producing parent cells.
	Qamri, 2014[30]	213 KTxRs + 14 KPTxRs 60 healthy donors prior to donation	Serum samples CD31+/CD42b- microparticles representing sEV were measured by fluorescence-activated cell scanning	Healthy donor sera and pre-KPTx or KTx recipient sera had similar exosome counts. ESKD patients with diabetes, obstructive/inherited kidney disease, and autoimmune illness reported lower exosomes and SCr by day 60 after KTx.Circulating exosomes increased with AR, but did not change in patients without rejection. After rejection treatment, circulating exosomes decreased rapidly in patients with negative PTC C4d but slowly in those with positive PTC C4d.
	Sigdel, 2015[30]	20 wo AR 10 with AR	Urine samples LC-MS/MS	Three of the eleven urine exosomal proteins specifically engaged in the

				inflammatory and stress response were higher prevalent in AR patients' urine samples.Exosomal AR-specific indicators were also found in unfractionated whole urine, but they were less abundant and not significant for AR.
	Tower, 2017[30]	23 healthy controls 93 KTx Rs with allograft dysfunction	Plasma samples Flow cytometry	The 28 individuals with ABMR had 11-fold more $C4d^+/CD144^+$ microvesicles than KTx Rs without ABMR and 24-fold more than healthy participants. Compared to healthy subjects and those without ABMR, ABMR patients had higher $C4d^+$ and $C4d^+/AVB^+$ microvesicle densities. $C4d^+/AVB^+$ microvesicles linked with ABMR biopsy severity; treatments reduced $C4d^+/CD144^+$ count by 72%.
	Park, 2017[30]	15 KTx Rx wo AR 15 with AR 3 BKV nephropathy 3 chronic AMR Validation cohort 14 KTx Rs, including 7 wo AR 7 with AR	Urine samples A urine-based platform to detect kidney transplant rejection termed iKEA (integrated kidney exosome analysis)	The TCMR-specificity of iKEA was validated by greater $CD3^+$ exosome levels in cellular rejection patients and very low $CD3^+$ EV levels in BKV (BK virus) nephropathy and chronic ABMR patients.
	Zhang, 2017[30]	18 Ktx Rs with ABMR, 8 Ktx Rs with TCMR, 38 Ktx Rs wo rejection	Plasma samples mRNA expression	ABMR patients had significantly higher mRNA transcript levels in plasma exosomes for several genes (gp130, CCL4, TNF**α**, SH2D1B, CAV1, atypical chemokine receptor 1 [Duffy blood group]) among 21 potential genes compared to TCMR and/or control patients. Gene combination scores from gp130, SH2D1B, TNF**α**, and CCL4 genes were considerably higher in ABMR compared to TCMR and no rejection control groups.
	Lim, 2018[30]	22 KTx Rs with stable kidney function 25 TCMR	Urine samples Nano-UPLC-MS/MS, Western blot	In TCMR patients, 17 proteins elevated. Tetraspanin-1 and hemopexin of all potential biomarkers were considerably increased in TCMR patients.

	Sharma, 2018[30]	30 LTxRs (10 with BOS + 10 in stable condition + 5 patients developed DSA + 5 without DSA considered as stable) 8 HTxRs (5 CAV + 3 stable with no evidence of rejection) 15 KTxRs (9 had biopsy-proven TG + 6 had a biopsy that confirmed the absence of rejection pathology and a well-functioning kidney)	Serum samples Western blot for CD9 exosome-specific markers, Tissue-associated lung SAgs, collagen V (Col-V) and K-alpha 1 tubulin (Kα1T), heart SAgs, collagen IV (Col-IV), myosin and vimentin, kidney SAgs, and fibronectin	Exosomes from LTxRs with BOS showed greater expression of Col-V (4.2-fold) and Kα1T (37.1-fold) compared to stable LTxRExosomes from CAV-HTxRs had 3.9-fold more myosin and 4.7-fold more vimentin than stable HTxRs.KTxRs with transplant glomerulopathy had circulating exosomes with 2-fold higher fibronectin and 2.5-fold higher Col-IV expression than stable KTxRs.
	Yang, 2019[30]	28 KTxRs with IF/TA (14 with ABMR + 14 patients wo ABMR)	Serum samples Exosomal biomarkers detected by flow cytometry: (1) anti-CD63, anti-CD4, anti-CXCR5, anti-CXCR3, and anti-CCR6 (2) anti-CD63, anti-CD4, anti-CXCR5, anti-HLA-G, and anti-CTLA-4	The proportion of CD4+CXCR5+CXCR3+CCR6-exosomes was significantly higher in ABMR group than in non-ABMR group, and CTLA-4 expression of CD4+CXCR5+exosomes was significantly lower in ABMR group than in non-ABMR group. Neither group had significantly different HLA-G expression.Tfh cell-derived exosomes in ABMR patients enhanced B cell proliferation and differentiation by 87.52% and 110.2%, respectively, compared to non-ABMR patients.
	Wang, 2019[30]	9 KTx Rs from DCD donors	Serum samples miRNA expression profiling with high-throughput sequencing	Three co-expressed miRNAs, hsa-miR-33a-5p_R-1, 98-5p, and 151a-5p, were highly elevated in DGF.
	Carreras-Planella, 2020[30]	5 calcineurin inhibitors toxicity 5 interstitial fibrosis and tubular atrophy 7 with normal kidney function	Urine samples LC-MS/MS	Compared to IFTA and normal kidney function, urinary EVs with calcineurin inhibitor toxicity elevated several citolinker proteins, including UPK1A, UPK1B, UPK2, and UPK3A.
	Saejong, 2020[30]	14 Stable KTx recipients 15 with IF/TA I 17 with IF/TA II 6 with IF/TA III	Serum and kidney allograft biopsy (protocol biopsies) samples Western blot for sEV proteins including anti-CD9, anti-CD63 Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Real-time PCR miR-21, miR-142-3p, miR-221, RNU-44 (house-keeping miR for renal tissue), and cel-39.	In renal histology samples with high fibrosis score (Banff classification), miR-21, miR-142-3p, and miR-221 expression was higher than in samples with lower scores (n = 17/group). Expression of miRs in plasma exosomes or whole plasma of post-KT patients with varying degrees of IF/TA (grade I (5-25%) (n=15), grade II (26-50%) (n=15), grade III (≥ 50%) (n=6), and stable graft function (n=15) was not distinct. Exosomes expressed more miR-21

				than total plasma in IF/TA grade II and III compared to grade I.
	Freitas, 2020[30]	23 KTx Rs (1st KTx)	Urine samples miRNAs' expression	KTxRs with overexpressed urinary exo-miRs (miR-146b, miR-155, and miR-200a) had a negative correlation with TAC dosage. Positive correlation between miR-200a and proteinuria.
	Carreras-Planella, 2020[30]	7 KTx Rs with normal kidney function, 5 with IF/TA, 6 with TCMR 5 with calcineurin inhibitors toxicity 41 in KTx Rs-validation cohort	Urine samples LC-MS/MS	Distinct KTx Rs groups express distinct proteins in urine EVs. Patients with chronic interstitial and tubular lesions (ci and ct mean > 2 according to Banff criteria) express vitronectin (VTN) differently.
	Takada, 2020[30]	20 KTx with normal histology 19 with IF/TA 17 with calcineurin inhibitors toxicity 22 with chronic active ABMR	Urine and kidney biopsy samples Exosomes-Western blot for SYT17 Biopsies -immunohistochemistry with anti-SYT17, anti-STAT3 pY705, anti-phospho NFκB p65 Ser276 antibodies	SYT17 protein was not found in whole-urine samples, but it was found in urinary exosome fractions and enriched in urine from chronic active AMR patients compared to healthy volunteers and persons with normal histology KTx.The chronic active ABMR group expressed SYT17 protein more robustly than other KTx groups, mostly in kidney tubular cells but not in endothelial cells (glomeruli) or epithelial cells.
	Chen, 2020[30]	27 healthy controls 58 KTx Rs	Plasma samples the expression of miRNAs	In the training set, exosomal miR-21, miR-210, and miR-4639 correlated negatively with eGFR and were chosen for investigation. In the validation set, miR-21, miR-210, and miR-4639 effectively differentiated between chronic allograft failure (eGFR < 60 mL/min/1.73 m2) and normal graft function.
	El Fekih, 2021[30]	175 KTx Rs	Urine and kidney allograft biopsy (for cause biopsies) samples RT-PCR and Real-time PCR for gene expression analysis	An exosomal mRNA signature differentiated biopsy samples from all-cause rejection and no rejection, while a gene signature distinguished TCMR from ABMR.

	Woud, 2022 [37]	8 Ktx Rs from deceased donors	Perfusate samples EVs examined with nanoparticle tracking analysis and imaging flow cytometry (IFCM).	Nanoparticle tracking analysis showed <400 nm nanoparticles released into the perfusate during NMP. Using IFCM, tetraspanin protein profiles of released nanoparticles revealed that out of CFSE$^+$ EVs, 75% were CD81$^+$, 16% were CD9$^+$, and 8% were CD63$^+$. Significant associations were found between EV subset concentrations, crude donor features, NMP viability parameters, cold ischemia time, donor age, and renal flow. We found that NMP releases separate EV subgroups from discarded expanded-criteria donor kidney transplants. EVs may be novel kidney graft quality indicators because these subcategories correspond with well-established transplant outcome variables.
	Woud, 2022 [38]	36 HLA-A3 mismatched donor (HLA-A3 +) and kidney transplant recipients (KTRs; HLA-A3-). .	Plasma and kidney biopsy samples Imaging Flow Cytometry (IFCM)-based methodology to detect/characterize circulating tissue-specific EV subsets in the clinical setting of kidney transplantation.	Donor and KTR samples had similar total CD9 + EV concentrations before transplantation. EVs specific to tissues were identified as CD9$^+$ HLA-A3$^+$. In HLA-A3-PPP, serial dilution tests revealed that single CD9 + HLA-A3$^+$ EVs were detected at ~1% over the recipient's 'self-signal'. Post-transplantation, CD9 + HLA-A3 + EVs were elevated in stable allograft function individuals but not in dysfunctional ones. We found that our calibrated IFCM-based technique can directly detect tissue-specific EV subsets in clinical samples. We feel this EV technique is relevant in many therapeutic settings.

AR acute rejection, ABMR antibody mediated rejection, BAL broncho-alveolar lavage, BKV BK virus, BOS bronchiolitis obliterans syndrome, CAV coronary artery vasculopathy, DGF delayed graft function, DSA donor specific antibodies, ESKD end-stage kidney disease, HTx heart transplant, IF/TA interstitial fibrosis and tubular atrophy, ITx islet transplantation, KTx kidney transplant, KPTx kidney/pancreas transplant, LC-MS/MS liquid chromatography-tandem mass spectrometry, LTx lung transplant, MHC major

histocompatibility complex, nano-UPLC-MS/MS nano-ultra performance liquid chromatography-tandem mass spectrometry, OLTx orthotopic liver transplantation, PGD primary graft dysfunction, Rs recipients, RVI respiratory viral infection, Sags selfantigens, TCMR T-cell mediated rejection, TG transplant glomerulopathy, TCMAR T-cell mediated acute rejection, wo without. This table was modified from [30].

9.5 CONCLUSION

Both physiological and pathological events in cells are the result of cellular communication. For many years, the scientific world thought that communication between cells occurred through cell-to-cell contact or by taking molecules secreted from the donor cell into the target cell. However, the discovery of EVs in recent years has brought a new perspective to scientists. Today, it is thought that the membrane and cytoplasmic contents of EVs can change the fate of the target cell. Additionally, these vesicles can be used to transport engineering materials. Studies on EVs will continue to increase in the future.

9.6 REFERENCES

1. Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, and Baran J. The Methods of Choice for Extracellular Vesicles (EVs) Characterization. International Journal of Molecular Sciences 2017; 18(6): 1153. https://doi.org/10.3390%2Fijms18061153
2. L. A. Hargett and N. N. Bauer, "On the Origin of Microparticles: From 'Platelet Dust' to Mediators of Intercellular Communication," Pulm Circ, vol. 3, no. 2, pp. 329–340, Apr. 2013, https://doi.org/10.4103%2F2045-8932.114760
3. D. Sacks et al., "Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke," International Journal of Stroke, vol. 13, no. 6, pp. 612–632, Aug. 2018, https://doi.org/10.1177/1747493018778713
4. E. I. Buzas, "The roles of extracellular vesicles in the immune system," Nature Reviews Immunology 2022 23:4, vol. 23, no. 4, pp. 236–250, Aug. 2022, https://doi.org/10.1038/s41577-022-00763-8

5. K. W. Witwer and C. Théry, "Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature," J Extracell Vesicles, vol. 8, no. 1, p. 1648167, Dec. 2019, https://doi.org/10.1080/20013078.2019.1648167

6. B. György et al., "Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles," Cellular and Molecular Life Sciences 2011 68:16, vol. 68, no. 16, pp. 2667–2688, May 2011, https://doi.org/10.1007%2Fs00018-011-0689-3

7. A. Piccin, W. G. Murphy, and O. P. Smith, "Circulating microparticles: pathophysiology and clinical implications," Blood Rev, vol. 21, no. 3, pp. 157–171, May 2007, http://doi.org/10.1016/j.blre.2006.09.001

8. E. Cocucci, G. Racchetti, and J. Meldolesi, "Shedding microvesicles: artefacts no more," Trends Cell Biol, vol. 19, no. 2, pp. 43–51, Feb. 2009, https://doi.org/10.1016/j.tcb.2008.11.003

9. C. Théry, M. Ostrowski, and E. Segura, "Membrane vesicles as conveyors of immune responses," Nature Reviews Immunology 2009 9:8, vol. 9, no. 8, pp. 581–593, Jun. 2009, https://doi.org/10.1038/nri2567

10. M. Baj-Krzyworzeka et al., "Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes," Cancer Immunology, Immunotherapy, vol. 55, no. 7, pp. 808–818, Nov. 2006, http://doi.org/10.1007/s00262-005-0075-9

11. K. Trajkovic et al., "Ceramide triggers budding of exosome vesicles into multivesicular endosomes," Science (1979), vol. 319, no. 5867, pp. 1244–1247, Feb. 2008, https://doi.org/10.1126/science.1153124

12. M. Colombo, G. Raposo, and C. Théry, "Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles," Annu Rev Cell Dev Biol, vol. 30, pp. 255–289, 2014, https://doi.org/10.1146/annurev-cellbio-101512-122326

13. D. M. Pegtel and S. J. Gould, "Exosomes," https://doi.org/10.1146/annurev-biochem-013118-111902, vol. 88, pp. 487–514, Jun. 2019, https://doi.org/10.1146/annurev-biochem-013118-111902J. Ratajczak, M. Wysoczynski, F. Hayek, A. Janowska-Wieczorek, and M. Z. Ratajczak, "Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication," Leu-

kemia 2006 20:9, vol. 20, no. 9, pp. 1487–1495, Jul. 2006, https://doi.org/10.1038/sj.leu.2404296

14. J. S. Bonifacino, "Vesicular transport earns a Nobel," Trends Cell Biol, vol. 24, no. 1, pp. 3–5, Jan. 2014, https://doi.org/10.1016/j.tcb.2013.11.001
15. J. Ashcroft, P. Leighton, T. R. Elliott, S. A. Hosgood, M. L. Nicholson, and V. Kosmoliaptsis, "Extracellular vesicles in kidney transplantation: a state-of-the-art review," Kidney Int, vol. 101, no. 3, pp. 485–497, Mar. 2022, https://doi.org/10.3390/ijms24065102
16. J. H. Y. Siu, V. Surendrakumar, J. A. Richards, and G. J. Pettigrew, "T cell allorecognition pathways in solid organ transplantation," Front Immunol, vol. 9, no. NOV, p. 419060, Nov. 2018, https://doi.org/10.3389/fimmu.2018.02548
17. S. Zhao, L. Zhang, S. Xiang, Y. Hu, Z. Wu, and J. Shen, "Gnawing Between Cells and Cells in the Immune System: Friend or Foe? A Review of Trogocytosis," Front Immunol, vol. 13, p. 791006, Feb. 2022, https://doi.org/10.3389/fimmu.2022.79100
18. R. Chakraborty, S. Belian, and C. Zurzolo, "Hijacking intercellular trafficking for the spread of protein aggregates in neurodegenerative diseases: a focus on tunneling nanotubes (TNTs)," Extracell Vesicles Circ Nucl Acids, vol. 4, no. 1, p. null, 2023, https://doi.org/10.3389/fphys.2023.1214210
19. S. Bansal, M. Sharma, R. Ranjithkumar, and T. Mohanakumar, "The role of exosomes in allograft immunity," Cell Immunol, vol. 331, pp. 85–92, Sep. 2018, https://doi.org/10.1016/j.cellimm.2018.06.003
20. G. Benichou, M. Wang, K. Ahrens, and J. C. Madsen, "Extracellular vesicles in allograft rejection and tolerance," Cell Immunol, vol. 349, p. 104063, Mar. 2020, https://doi.org/10.3389%2Ffimmu.2021.682948
21. J. A. Frelinger, J. E. Niederhuber, C. S. David, and D. C. Shreffler, "EVIDENCE FOR THE EXPRESSION OF Ia (H-2-ASSOCIATED) ANTIGENS ON THYMUS-DERIVED LYMPHOCYTES," Journal of Experimental Medicine, vol. 140, no. 5, pp. 1273–1284, Nov. 1974, https://doi.org/10.1084/jem.140.5.1273
22. B. P. Dolan, K. D. Gibbs, and S. Ostrand-Rosenberg, "Dendritic Cells Cross-Dressed with Peptide MHC Class I Complexes Prime CD8+ T Cells," The Journal of Immunology, vol. 177, no. 9, pp. 6018–6024, Nov. 2006, https://doi.org/10.4049/jimmunol.177.9.6018

23. L. A. Smyth, R. I. Lechler, and G. Lombardi, "Continuous Acquisition of MHC: Peptide Complexes by Recipient Cells Contributes to the Generation of Anti-Graft CD8+ T Cell Immunity," American Journal of Transplantation, vol. 17, no. 1, pp. 60–68, Jan. 2017, https://doi.org/10.1111/ajt.13996
24. J. Marino et al., "Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation," Sci Immunol, vol. 1, no. 1, Jul. 2016, https://doi.org/10.1126%2Fsciimmunol.aaf8759
25. Q. Liu et al., "Donor dendritic cell-derived exosomes promote allograft-targeting immune response," J Clin Invest, vol. 126, no. 8, pp. 2805–2820, Aug. 2016, https://doi.org/10.1172/jci84577
26. F. Zeng and A. E. Morelli, "Extracellular vesicle-mediated MHC cross-dressing in immune homeostasis, transplantation, infectious diseases, and cancer," Semin Immunopathol, vol. 40, no. 5, pp. 477–490, Sep. 2018, https://doi.org/10.3390%2Fcells10081959
27. A. Agarwal et al., "Regulatory T cell-derived exosomes: Possible therapeutic and diagnostic tools in transplantation," Front Immunol, vol. 5, no. NOV, p. 110048, Nov. 2014, https://doi.org/10.3389/fimmu.2014.00555
28. M. Quaglia, G. Merlotti, G. Guglielmetti, G. Castellano, and V. Cantaluppi, "Recent Advances on Biomarkers of Early and Late Kidney Graft Dysfunction," International Journal of Molecular Sciences 2020, Vol. 21, Page 5404, vol. 21, no. 15, p. 5404, Jul. 2020, https://doi.org/10.3390/ijms21155404
29. J. E. Gołębiewska, A. Wardowska, M. Pietrowska, A. Wojakowska, and A. Dębska-ślizień, "Small Extracellular Vesicles in Transplant Rejection," Cells 2021, Vol. 10, Page 2989, vol. 10, no. 11, p. 2989, Nov. 2021, https://doi.org/10.3390/cells10112989
30. S. Bansal et al., "Circulating exosomes induced by respiratory viral infections in lung transplant recipients activate cellular stress, innate immune pathways and epithelial to mesenchymal transition," Transpl Immunol, vol. 69, p. 101480, Dec. 2021, https://doi.org/10.1016/j.trim.2021.101480
31. Y. Itabashi et al., "Decline in Club Cell Secretory Proteins, Exosomes Induction and Immune Responses to Lung Self-Antigens, Kα1 Tubulin and Collagen V, Leading to Chronic Rejection after Human

Lung Transplantation," Transplantation, vol. 105, no. 6, pp. 1337–1346, Jun. 2021, https://doi.org/10.1097%2FTP.0000000000003428

32. R. Ravichandran et al., "A decline in club cell secretory proteins in lung transplantation is associated with release of natural killer cells exosomes leading to chronic rejection," The Journal of Heart and Lung Transplantation, vol. 40, no. 12, pp. 1517–1528, Dec. 2021, https://doi.org/10.1016/j.healun.2021.08.016
33. S. Bansal et al., "Restrictive allograft syndrome vs bronchiolitis obliterans syndrome: Immunological and molecular characterization of circulating exosomes," The Journal of Heart and Lung Transplantation, vol. 41, no. 1, pp. 24–33, Jan. 2022, https://doi.org/10.1016/j.healun.2021.09.001
34. A. Habertheuer et al., "Donor extracellular vesicle trafficking via the pleural space represents a novel pathway for allorecognition after lung transplantation," American Journal of Transplantation, vol. 22, no. 7, pp. 1909–1918, Jul. 2022, https://doi.org/10.1111/ajt.17023
35. W. Wang et al., "Serum extracellular vesicle MicroRNAs as candidate biomarkers for acute rejection in patients subjected to liver transplant," Front Genet, vol. 13, p. 1015049, Oct. 2022, https://doi.org/10.3389%2Ffgene.2022.1015049
36. W. W. Woud et al., "Extracellular Vesicles Released during Normothermic Machine Perfusion Are Associated with Human Donor Kidney Characteristics," Transplantation, vol. 106, no. 12, pp. 2360–2369, Dec. 2022, https://doi.org/10.1097/TP.0000000000004215
37. W. W. Woud, D. A. Hesselink, M. J. Hoogduijn, C. C. Baan, and K. Boer, "Direct detection of circulating donor-derived extracellular vesicles in kidney transplant recipients," Scientific Reports 2022 12:1, vol. 12, no. 1, pp. 1–11, Dec. 2022, https://doi.org/10.1038/s41598-022-26580-6

THE FUNCTION OF WNT SIGNALING IN KIDNEY TRANSPLANTATION

Melek PEHLIVAN[1]
[1]İzmir Katip Celebi University, Vocational School of Health Services, Department of medical Laboratory Techniques, Izmir, Turkey

10.1 INTRODUCTION

The Wnt signaling system is a signaling pathway that is conserved throughout evolution and has a role in both development and disease-related activities. It is involved in biological processes such as embryogenesis, organogenesis, regeneration, immune cell regulation and stem cell development [1]. However, abnormal regulation of the pathway causes an inappropriately activated cell growth process, leading to various diseases and cancer [2]. The incorrect regulation of Wnt signaling in disease and cancer processes is exceedingly complex and remains poorly understood, despite the fact that the primary components of the Wnt pathway have been exhaustively characterized. Wnt genes consist of an extensive protein ligand family that engage in interactions with numerous plasma membrane receptors and co-receptors. These interconnections, which involve ten Frizzled (Fzd) receptors and nineteen Wnt ligands that bind to an extensive number of co-receptors in mammals, represent a highly intricate association among external ligands and the receptors on the cell surface [3].

The Wnt signaling pathway is classified into noncanonical and canonical pathways. The two non-canonical Wnt routes are Wnt/PCP (planar cell polarity) and Wnt/$Ca2^{+}$ pathways. The Wnt/β-catenin pathway is called canonical Wnt pathway (Figure 1) [1]. Wnt signaling pathways are modulated by many modulators responsible for regulating ligand-receptor interactions outside the cell or the cytosolic or nuclear components of the pathway within the cell [4]. The Wnt signaling pathway is activated by secreted lipid-modified proteins. The Wnt signaling cascade initiates with the production of Wnt ligand in the cell that releases it. Wnt ligand is produced and presented through the action of two transmembrane proteins called Wntless (WLS) and Porcupine (PORCN). PORCN is an acyltransferase enzyme that is widely preserved and is responsible for adding a palmitoleic acid group to Wnt proteins within the endoplasmic reticulum and is responsible for the lipid modification required for Wnt activity [5]. The WLS gene is responsible for encoding a transmembrane protein that is found in the Golgi apparatus, is reused between the plasma membrane and the Golgi apparatus, and is responsible for transporting the Wnts that are lipid-modified from the ER to the surface of the cell [6].

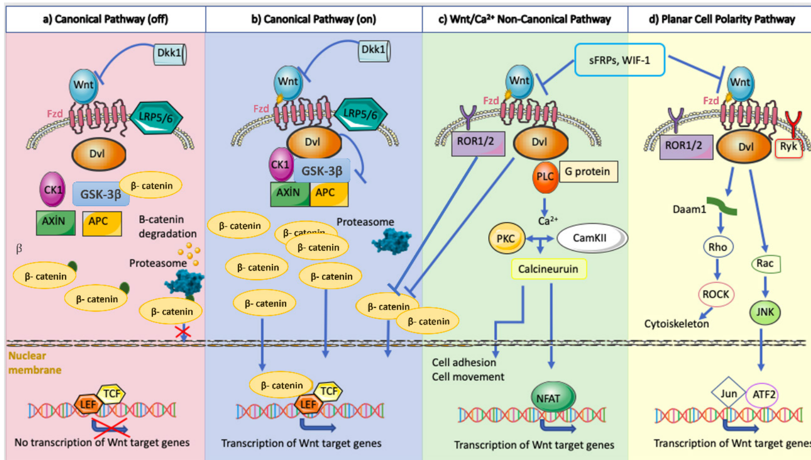


Figure 1: Wnt Signaling pathway

10.2 CANONICAL β-CATENIN/WNT SIGNALING PATHWAY

In the classical pathway, Wnt signaling is activated when Wnt ligand, secreted from a cell, binds to LRP5 or LRP6, members of the Fzd family receptor and Low-density lipoprotein receptor-related protein family, in the recipient cell. Wnt coreceptors are facilitated by LRP5/6, which are found on the plasma membrane. Several phosphorylation sites found in the intracellular region of LRP5/6 are essential for the start of Wnt/β-catenin signaling [1]. Binding of the Wnt ligand to the Fzd receptor, and its coreceptor LRP5/6 leads to phosphorylation and endocytosis of LRP5/6, recruitment of Disheveled (Dvl) present in the cytoplasm to the membrane, and subsequent molecular changes such as oligomerization of Dvl. Activated Dvl causes the interaction of Fzd and LRP6 and promotes the phosphorylation of LRP6. In addition to binding to Fzd, Dvl's DEP domain also modifies its conformation, which activates the Wnt/Fzd signalosome and permits dimerization. Furthermore, GSK-3β and AXIN are accumula-

ted on the plasma membrane by activated Dvl, which inhibits their activities [1,7]. The modifications result in the formation of the Wnt signalosome, where the breakdown complex binds with receptors and Dvl. Following that, Dvl1 is enlisted, resulting in the phosphorylation of GSK3β rather than β-catenin, leading to the disassociation of the destruction complex including APC, β-catenin, CK1α, AXIN, and GSK3β. Thus, β-catenin is unable to be transported to the proteasome for destruction and builds up in the cytoplasm and moves to the nucleus at the same time. β-catenin enters the nucleus and interacts with TCF/LEF, as well as co-activators such as BCL9, CBP, PYGO, and P300 to activate the expression of genes targeted by Wnt [8].

When the Wnt signaling is absent, the pathway is maintained in an inactive state. β-catenin undergoes progressive phosphorylation, starting with CK1α phosphorylating residue S45, followed by GSK3β phosphorylating residues c. The proteins APC and AXIN that combine with kinases to create the β-catenin destruction complex are necessary for this type of phosphorylation. Because it has interaction sites for other parts of DC, such as the RGS domain (N-terminal side) and the DIX domain (C-terminal side), AXIN is able to interact with the APC protein (9). β-catenin is phosphorylated by AXIN-bound GSK3β with the aid of CK1α and APC. Receptor complexes are created when the Wnt ligand joins forces with LRP/Fzd, and DVL binds to AXIN and Fzd molecules on the cell membrane. GSK3β and CK1α-mediated phosphorylation of β-catenin undergoes proteasomal degradation via Skp1- Cullin-F box (SCF) E3 ubiquitin ligases and beta transducing-repeat containing protein (β-TrCP) [1,10].

10.3 NON-CANONICAL WNT SIGNALING PATHWAY

The non-canonical PCP pathway, which acts independently of β-catenin, plays a role invertebrate development and regulates coordinated, properly polarized cellular behavior in cells. Non-canonical Wnt ligands engage in interactions with the PCP pathway's receptor Fzd and co-receptors, which include receptor tyrosine kinase-like orphan receptor (ROR)/protein tyrosine kinase 7 (PTK7)/ receptor tyrosine kinase-associated tyrosine kinase (RYK). Rho/Rho Associated Coiled Coil Kinase, mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) are all activated as a result of these interactions, which also regulate the small GTPase molecules Rac, Cdc42, and Rho [11]. The primary phenotype linked to the activation of the PCP pathway are alterations in cell motility and polarization, as RhoA and Rac1 help regulate cytoskeletal dynamics. It has been demonstrated that WNT mediated JNK activation can be regulated by the PCP pathway via RAC1 [12].

In the Wnt/Ca2+ pathway, through the attachment of Wnt molecule to Fzd and the interaction of Fzd and G proteins, raises intracellular Ca2+. Nuclear factor of activated T cells (NFAT), calcium/calmodulin-dependent protein kinase type II (CaMKII), and protein kinase C (PKC) are all activated by released Ca2+. This route governs the processes of cellular locomotion, cellular determination, and cellular translocation. The Wnt/Ca2+ pathway is involved in neurodegenerative diseases, infection, dorsal/ventral modeling, gastrulation and cardiac development, and cancer processes [13,14]. Wnt is an important signal transduction pathway in both physiological and pathological processes. In past few years, interest in the Wnt signaling pathway has increased in the field of renal failure and transplantation. Below, literature information about the significanceof the Wnt pathway in kidney physiology, various kidney diseases and its role in kidney transplantation is given.

10.4 RELATIONSHIP BETWEEN KIDNEY AND WNT SIGNALING PATHWAY

Kidneys are the organ responsible for removing toxins and metabolic waste from the human body. In addition to this basic function, it also play a crucial role in filtration and reabsorption, regulation of water-electrolyte balance, stabilization of blood pressure, erythropoiesis and bone development processes. Kidney development in mammals matures through the embryonic kidney, pronephros, mesonephros and metanephros stages. Many signal transmission pathways play an active role in these stages. Of these, the Wnt signaling has been shown to have an essential role in various step of kidney development, including nephron formation, formation of renal tubules, and differentiation of kidney cells [15]. Wnt signaling is required for the specification and differentiation of various cell types in the nephron, including podocytes, distal tubule cells and proximal tubule cells. [16]. For example, Wnt11, Wnt9b, Wnt7b, Wnt6, Wnt5b, Wnt4, and Wnt2b have been shown to be expressed during kidney ontogenesis. While Wnt11, Wnt9b, Wnt7b, and Wnt6 expression have been detected the branching ureteric bud in the early stages of organogenesis, Wnt4 and Wnt2b play a role in kidney mesenchymal cells [15].

When kidneys undergo ischemic or toxic damage, they create a natural response by activating stem/progenitor cells for nephron regeneration. The fact that canonical Wnt/β-catenin signaling is involved in processes such as progenitor cell and stem cell differentiation and renewal during kidney development and maturation shows that the Wnt signaling is a crucial regulator [17].

Wnt/β-catenin signaling, according to the size and duration of its activation, not only promotes repair/regeneration

in the kidneys, but also has a role in the process of kidney diseases. Wnt/β-catenin signaling causes acute kidney injury, proteinuric kidney diseases by taking part in the podocyte damage and oxidative stress process, polycystic kidney diseases by triggering cyst growth, kidney fibrosis and chronic kidney disease by taking part in the epithelial-mesenchymal transition (EMT) process and promoting matrix accumulation[18].

Acute kidney injury (AKI) is the quick loss of kidney function that can occur as a result of a variety of causes, such as dehydration, low blood pressure, infection or drug toxicity. AKI can lead to the accumulation of waste products and fluid in the body. This condition can cause various symptoms and can be life-threatening if left untreated[15]. Wnt/β-catenin signaling has dual roles that also facilitate renal patient progression to chronic kidney injury (CKD) after AKI. In the case of AKI, the proper activation of Wnt/β-catenin has a beneficial effect on the kidneys, resulting in decreased kidney damage and faster restoration of kidney function and structure. This perspective is supported by numerous data indicating that the Wnt/β-catenin pathway functions as a survival signal, safeguarding renal tubular epithelial cells from undergoing apoptosis both in laboratory settings (in vitro) and in living organisms (in vivo)[14]. While temporary stimulation of Wnt/β-catenin pathway triggers the healing and restoration of kidney tissue following injury, prolonged or unregulated Wnt/β-catenin signaling causes development of many kidney ailments, including cystic kidney diseases, podocyte damage, and kidney fibrosis[18]. Podocytes, which are specialized to protect the integrity of the kidney's glomerular filtration barrier, can cause dysfunction, proteinuria and kidney damage. It has been shown that inhibition of the Wnt pathway preserves podocyte integrity by regulating the transcription factor

Wilms tumor protein and improves proteinuric kidney disease[19]. In in vivo studies, high levels of mortality, increased serum creatinine levels and morphological damage were observed after acute kidney injury in proximal tubule-specific β-catenin deficiency[20]. It has been found that when β-catenin is activated via Wnt 4 at different intervals after AKI, it improves kidney damage[21]. The increase in Wnt 4 in the proximal tubules after AKI causes an increase in the expression of Cyclin A and Cyclin D1, supporting tubular cell proliferation and repair[16]. However, in animal models of AKI, it has been shown that abnormal activation of the Wnt signaling pathway promotes inflammation and damage in kidney tissue, and inhibition of abnormal activation has a protective effect against kidney damage[18]. Persistent Wnt/β-catenin activation leads to the development to chronic kidney disease (CKD), which exhibits excessive extracellular matrix deposition and interstitial myofibroblast activation. CKD is a long term condition that occurs when the kidneys become damaged and can no longer function properly. When various forms of CKD were examined, it was observed that many Wnt ligands were induced simultaneously in different kidney cells. In animal models of CKD, excessive activation of the Wnt signaling pathway has been demonstrated to stimulate inflammation and fibrosis in kidney tissue[22]. It has been shown that the expression of Dkk1, one of the regulators of the Wnt signaling pathway, is a biological biomarker of fibrotic CKD and is also an essential factor in the development of fibrosis in kidney[23]. However, it is also known that a Wnt antagonist causes reduced renal fibrosis after unilateral ureteral obstruction[24]. It has been reported that increased expression of Wnt9a in CKD causes renal tubular cell senescence, which stops cell growth and causes the cells to lose their epithelial properties[25].

In renal tubules, blocking tubule derived Wnt secretion has also been shown to inhibit myofibroblast activation and reduce renal fibrosis after injury[26]. Another study found that Wnt5A protein, which activates the Wnt signaling pathway, was upregulated in human kidney tissues with CKD and that inhibiting Wnt5A had a protective effect against renal fibrosis[27]. Thus, Wnt/β-catenin signaling is thought to exert a dual effect of ameliorating or enhancing kidney damage and fibrotic lesions in CKD models.

While the Wnt signaling pathway exerts its effects on the kidneys, they work in cooperation with many signaling pathways. For example, the Wnt/β-catenin signaling pathway regulates the expression of matrix metalloproteinase 7 (MMP-7), Transient receptor potential Canonical type 6, Snail1, Plasminogen activator inhibitor-1, Twist, and renin-angiotensin system components in epithelial cells, fibroblasts, and macrophages to promote renal interstitial fibrosis[28]. Uterine sensitization-related gene-1 (USAG-1) is a regulator of the standard Wnt signaling pathway. It has been found to be extensively expressed in the kidneys and has been shown to protect renal tubules from drug toxicity and ischemia-reperfusion injuries. This protective effect is achieved through the regulation of Wnt signaling and other related pathways. This finding was uncovered in a research study that examined the functions of USAG-1[29]. In a different study, a Wnt agonist used after ischemic reperfusion injury was shown to reduce inflammation and oxidative stress[17].

Functioning as a tumor suppressor in many types of cancer, Klotho has a crucial role in regulating mineral metabolism and is altered as a result of renal failure. Some of the mechanisms responsible for changes in Klotho levels are related to the Wnt signaling pathway. Klotho's external domain sup-

presses Wnt signaling by attaching to several Wnt ligands. It has been observed that the interaction between Klotho and Wnt signaling has a protective role against renal damage, CKD, polycystic kidney disease, progression of EMT following kidney transplantation, and renal allograft fibrosis[30]. However, it has been shown that Klotho has the ability to inhibit Wnt/β-catenin signaling, thus suppressing peritoneal fibrosis[31]. In vitro and in vivo studies have reported that in cases where Klotho is silenced, it supports the development of EMT and chronic allograft dysfunction via Wnt signaling[32].

The Wnt signaling pathway is a prominent mechanism that is triggered throughout the process of cell and tissue regeneration. Nevertheless, the activation of this pathway must be carefully regulated and coordinated with numerous other signals, including inflammatory factors and growth factors [33]. The impact of stimulation and dysfunction of Wnt pathways on T cell responses remains uncertain. T cells are involved in adaptive immunological responses mediated by CD8+ and CD4+ T cells. It has been shown that the expression of TCF-1 is high at the end of overstimulation of Wnt/β-catenin signaling in immature memory CD8+T and CD8+T cells, and that the expression of TCF-1 decreases when immature CD8 + T cells become effector CD8+T cells[34]. Another study demonstrated that the activation of conventional Wnt signaling, through the use of inhibitors targeting Wnt3a, GSK3β or, β-catenin facilitates the formation of CD8+ T cell memory. This is achieved by inhibiting the differentiation of CD8+ T cells and inhibiting the development of effector T cells[35]. It has been stated that TCF-1 and LEF-1 have a role in the transformation of naive CD4+T cells into Tfh cells at the early stage of cell differentiation, and that the Wnt/β-catenin signaling pathway has crucial roles in the control of CD4+T cell biology. In addition

to the affects of the Wnt signaling pathway on T cells, it has been suggested that innate inflammatory cells and infiltrated T cells may also secrete Wnt proteins that affect the kidney tissue[33]. Canonical Wnt signals, generated by dendritic cells or antigen-presenting cells (APCs), are believed to have a role in several T cell-mediated processes. These signals directly influence T cells, stimulating the formation of pro-inflammatory Th17 cells while suppressing the growth and activity of Treg cells.

10.5 WNT SIGNALING PATHWAY IN TRANSPLANTATION

Organ transplantation is the process of removing an organ from living bodies or cadavers and transplanting it to a recipient to replace a damaged or missing organ. Transplantation is the only chance for survival for people with end-stage disease. Organs such as heart, lung, kidney, liver, pancreas, intestine and stomach can be transplanted [36]. The success of organ transplantation depends on whether the body accepts the transplanted organ or does not show an immune response against the transplanted organ. Organ rejection is the most important risk observed after transplantation and is classified in three ways. Chronic rejection, acute rejection and hyperacute rejection. Hyperacute rejection; It is a type of rejection that develops shortly after transplantation due to pre-existing donor-specific antibodies. Acute rejection refers to the rejection that takes place within the initial 6 months following transplantation, caused by T cells' adaptive immune responses. On the other hand, chronic rejection refers to the rejection that happens after 6 months, resulting from immunological mechanisms and various other circumstances [37]. One of the reasons for unsuccessful organ transplantation is T cell-mediated immune responses against the graft tissue. The intricate mechanism involved in eliciting these reactions involves the

control of T cell responses, antigen-presenting cells, cytokines, regulatory cells, and major histocompatibility complex molecules (MHC) on donated organs. The Wnt signaling pathway is involved in the physiological processes of stem cells and the maturation of undeveloped T lymphocytes in the thymus. By controlling the ratio of T regulatory 17/ T helper cells and influencing the production of memory and effector cytotoxic CD8 T cell responses, recent study indicates that the Wnt pathway is vital in regulating T cell immunological responses. There is a hypothesis that Wnt pathways might impact the result of the immune response during the transplantation process[33].

Kidney transplantation is the most common type of organ transplantation in the world and in our country, and its transplantation success rate is higher than other organs, although immunological complications may occur after transplantation. The Wnt signaling pathway is also related with acute and chronic rejections mediated by T cell, as it can affect T lymphocyte responses. At the same time, the Wnt signaling pathway is very essential in terms of regenerating the tissue function of the transplanted organ.

Wnt signaling is thought to be involved a role in graft survival or rejection after kidney transplantation. Their active role in kidney development from the embryogenesis stage, damage repair, and the development of kidney diseases suggests that this pathway will be active in the same processes after transplantation. Despite the small number of studies conducted in this field, it has been showed that Wnt signaling have a role in the development of fibrosis, a frequent problem that can arise following kidney transplantation. Fibrosis is the process of scarring in the transplanted kidney that can lead to decline in kidney function and eventual transplant failure. According to studies, Wnt signaling overstimulation may en-

courage fibrosis in transplanted kidneys, whereas Wnt signaling inhibition may prevent fibrosis.

Research employing the rat kidney transplant model revealed that TGFβ and Wnt signaling target molecules (Nos2, Cd44, FN1, and MMP7) were expressed at higher levels and were linked to the advancement of kidney damage developed. It was also stated that the main players of the Wnt/Ca2+ signaling pathway (NFAT, Wnt7a, Wnt6, CaMKII, protein kinase C (PKC)) changed significantly with the progression of chronic injury. The production of collagen I and collagen III is linked to the gradual harm and scarring in the kidneys, and it is enhanced by the phosphorylation of CaMKII [38,39]. The increase observed in the gene expression of chloride intracellular channel 1 (Clic1), which serves as a detector of oxidative stress in endothelial cells, in samples taken from patients who underwent kidney transplantation, is regulated by Wnt signaling [39]. In kidney transplantation, kidney transplant dysfunction occurs as antibody- and T-cell-mediated damage to endothelial cells causes microvascular injury (MVI) lesions of glomerulitis and peritubular capillaritis. The Wnt signaling pathway is associated with microvascular injury and therefore plays a role in a successful kidney transplantation process [40]. It was shown that the ratio of β-catenin/Foxo1 to β-catenin/TCF was positively correlated with EGFR in biopsies taken in the early period of kidney transplant patients, and was negatively correlated with inflammation, wound healing, and fibrosis in biopsies taken one year after transplantation [41]. It has also been suggested that increased β-catenin expression levels in transplant patients after transplantation may have a protective function for kidney survival [42].

10.6 CONCLUSION

The evolutionarily conserved Wnt signaling system is crucial for the growth, damage repair, and tissue remodeling processes of organs. There are data indicating that it plays as a protective role in the survival after organ transplantation, but its excessive activation is also effective in organ rejection processes. This is exciting in terms of modulating Wnt levels, regulating the immune response during the transplantation process and perhaps help generate desired cell types.

10.7 REFERENCES

1. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022 Jan 3;7(1):3. https://doi.org/10.1038/s41392-021-00762-6
2. Galluzzi L, Spranger S, Fuchs E, López-Soto A. WNT Signaling in Cancer Immunosurveillance. Trends Cell Biol. 2019 Jan;29(1):44–65. https://doi.org/10.1016/j.tcb.2018.08.005
3. Haseeb, Pirzada, Ain, Choi. Wnt Signaling in the Regulation of Immune Cell and Cancer Therapeutics. Cells. 2019 Nov 3;8(11):1380. https://doi.org/10.3390/cells8111380
4. Yu F, Yu C, Li F, Zuo Y, Wang Y, Yao L, et al. Wnt/β-catenin signaling in cancers and targeted therapies. Signal Transduct Target Ther. 2021 Aug 30;6(1):307. https://doi.org/10.1038/s41392-021-00701-5
5. Galli LM, Zebarjadi N, Li L, Lingappa VR, Burrus LW. Divergent effects of Porcupine and Wntless on WNT1 trafficking, secretion, and signaling. Exp Cell Res. 2016 Sep;347(1):171–83. https://doi.org/10.1016/j.yexcr.2016.07.028
6. Mohamed R, Kennedy C, Willmore WG. Responses of Porcupine and Wntless proteins to oxidative, hypoxic and endoplasmic reticulum stresses. Cell Signal. 2021 Sep; 85:110047. https://doi.org/10.1016/j.cellsig.2021.110047
7. Ren Q, Chen J, Liu Y. LRP5 and LRP6 in Wnt Signaling: Similarity and Divergence. Front Cell Dev Biol. 2021 May 6;9. https://doi.org/10.3389/fcell.2021.670960

8. Rim EY, Clevers H, Nusse R. The Wnt Pathway: From Signaling Mechanisms to Synthetic Modulators. Annu Rev Biochem. 2022 Jun 21;91(1):571–98. https://doi.org/10.1146/annurev-biochem-040320-103615
9. Wang Z, Li Z, Ji H. Direct targeting of β-catenin in the Wnt signaling pathway: Current progress and perspectives. Med Res Rev. 2021 Jul 21;41(4):2109–29. https://doi.org/10.1002/med.21787
10. Schaefer KN, Pronobis MI, Williams CE, Zhang S, Bauer L, Goldfarb D, et al. Wnt regulation: exploring Axin-Disheveled interactions and defining mechanisms by which the SCF E3 ubiquitin ligase is recruited to the destruction complex. Fehon R, editor. Mol Biol Cell. 2020 May 1;31(10):992–1014. https://doi.org/10.1091/mbc.E19-11-0647
11. Yang Y, Mlodzik M. Wnt-Frizzled/Planar Cell Polarity Signaling: Cellular Orientation by Facing the Wind (Wnt). Annu Rev Cell Dev Biol. 2015 Nov 13;31(1):623–46. https://doi.org/10.1146/annurev-cellbio-100814-125315
12. Akoumianakis I, Polkinghorne M, Antoniades C. Non-canonical WNT signalling in cardiovascular disease: mechanisms and therapeutic implications. Nat Rev Cardiol. 2022 Dec 13;19(12):783–97. https://doi.org/10.1038/s41569-022-00718-5
13. Patel S, Alam A, Pant R, Chattopadhyay S. Wnt Signaling and Its Significance Within the Tumor Microenvironment: Novel Therapeutic Insights. Front Immunol. 2019 Dec 16;10. https://doi.org/10.3389/fimmu.2019.02872
14. Zhou D, Tan RJ, Fu H, Liu Y. Wnt/β-catenin signaling in kidney injury and repair: a double-edged sword. Lab Investig. 2016 Feb 21;96(2):156–67. https://doi.org/10.1038/labinvest.2015.153
15. Pulkkinen K, Murugan S, Vainio S. Wnt signaling in kidney development and disease. Organogenesis. 2008 Apr 27;4(2):55–9. https://doi.org/10.4161/org.4.2.5849
16. Meng P, Zhu M, Ling X, Zhou L. Wnt signaling in kidney: the initiator or terminator? J Mol Med. 2020 Nov 17;98(11):1511–23. https://doi.org/10.1007/s00109-020-01978-9
17. Kuncewitch M, Yang WL, Corbo L, Khader A, Nicastro J, Coppa GF, et al. WNT Agonist Decreases Tissue Damage and Improves Renal Function After Ischemia-Reperfusion. Shock. 2015 Mar;43(3):268–

75. https://doi.org/10.1097/SHK.0000000000000293

18. Schunk SJ, Floege J, Fliser D, Speer T. WNT–β-catenin signalling — a versatile player in kidney injury and repair. Nat Rev Nephrol. 2021 Mar 28;17(3):172–84. https://doi.org/10.1038/s41581-020-00343-w
19. Zhou L, Liu Y. Wnt/β-catenin signalling and podocyte dysfunction in proteinuric kidney disease. Nat Rev Nephrol. 2015 Sep 9;11(9):535–45. https://doi.org/10.1038/nrneph.2015.88
20. Zhou D, Li Y, Lin L, Zhou L, Igarashi P, Liu Y. Tubule-specific ablation of endogenous β-catenin aggravates acute kidney injury in mice. Kidney Int. 2012 Sep;82(5):537–47. https://doi.org/10.1038/ki.2012.173
21. Terada Y, Tanaka H, Okado T, Shimamura H, Inoshita S, Kuwahara M, et al. Expression and Function of the Developmental Gene Wnt-4 during Experimental Acute Renal Failure in Rats. J Am Soc Nephrol. 2003 May;14(5):1223–33. https://jasn.asnjournals.org/lookup/doi/10.1097/01.ASN.0000060577.94532.06
22. Huang R, Fu P, Ma L. Kidney fibrosis: from mechanisms to therapeutic medicines. Signal Transduct Target Ther. 2023 Mar 17;8(1):129. https://doi.org/10.1038/s41392-023-01379-7
23. Hsu YC, Chang CC, Hsieh CC, Huang YT, Shih YH, Chang HC, et al. Dickkopf-1 Acts as a Profibrotic Mediator in Progressive Chronic Kidney Disease. Int J Mol Sci. 2023 Apr 21;24(8):7679. https://doi.org/10.3390/ijms24087679
24. Surendran K, Schiavi S, Hruska KA. Wnt-Dependent β-Catenin Signaling Is Activated after Unilateral Ureteral Obstruction, and Recombinant Secreted Frizzled-Related Protein 4 Alters the Progression of Renal Fibrosis. J Am Soc Nephrol. 2005 Aug;16(8):2373–84. https://doi.org/10.1681/ASN.2004110949
25. Luo C, Zhou S, Zhou Z, Liu Y, Yang L, Liu J, et al. Wnt9a Promotes Renal Fibrosis by Accelerating Cellular Senescence in Tubular Epithelial Cells. J Am Soc Nephrol. 2018 Apr;29(4):1238–56. https://doi.org/10.1681/ASN.2017050574
26. Zhou D, Fu H, Zhang L, Zhang K, Min Y, Xiao L, et al. Tubule-Derived Wnts Are Required for Fibroblast Activation and Kidney Fibrosis. J Am Soc Nephrol. 2017 Aug;28(8):2322–36. https://journals.lww.com/00001751-201708000-00011

27. Feng Y, Liang Y, Zhu X, Wang M, Gui Y, Lu Q, et al. The signaling protein Wnt5a promotes TGFβ1-mediated macrophage polarization and kidney fibrosis by inducing the transcriptional regulators Yap/Taz. J Biol Chem. 2018 Dec;293(50):19290–302. https://doi.org/10.1074/jbc.RA118.005457
28. Li SS, Sun Q, Hua MR, Suo P, Chen JR, Yu XY, et al. Targeting the Wnt/β-Catenin Signaling Pathway as a Potential Therapeutic Strategy in Renal Tubulointerstitial Fibrosis. Front Pharmacol. 2021 Aug 16;12. https://doi.org/10.3389/fphar.2021.719880
29. Lu L, Zhu J, Zhang Y, Wang Y, Zhang S, Xia A. Febuxostat inhibits TGF-β1-induced epithelial-mesenchymal transition via downregulation of USAG-1 expression in Madin-Darby canine kidney cells in vitro. Mol Med Rep. 2019 Jan 2; https://doi.org/10.3892/mmr.2019.9806
30. Muñoz-Castañeda JR, Rodelo-Haad C, Pendon-Ruiz de Mier MV, Martin-Malo A, Santamaria R, Rodriguez M. Klotho/FGF23 and Wnt Signaling as Important Players in the Comorbidities Associated with Chronic Kidney Disease. Toxins (Basel). 2020 Mar 16;12(3):185. https://doi.org/10.3390/toxins12030185
31. Kadoya H, Satoh M, Nishi Y, Kondo M, Wada Y, Sogawa Y, et al. Klotho is a novel therapeutic target in peritoneal fibrosis via Wnt signaling inhibition. Nephrol Dial Transplant. 2020 May 1;35(5):773–81. https://doi.org/10.1093/ndt/gfz298
32. Li X, Lu P, Shao XF, Jiang T, Liu F, Li G. Klotho Regulates Epithelial-to-Mesenchymal Transition In Vitro via Wnt/β-Catenin Pathway and Attenuates Chronic Allograft Dysfunction in a Rat Renal Transplant Model. Ann Transplant. 2021 Feb 22;26. https://doi.org/10.12659/AOT.930066
33. Staal FJT, Arens R. Wnt Signaling as Master Regulator of T-Lymphocyte Responses. Transplantation. 2016 Dec;100(12):2584–92. https://doi.org/10.1097/TP.0000000000001393
34. Li X, Xiang Y, Li F, Yin C, Li B, Ke X. WNT/β-Catenin Signaling Pathway Regulating T Cell-Inflammation in the Tumor Microenvironment. Front Immunol. 2019 Sep 26;10. https://doi.org/10.3389/fimmu.2019.02293
35. Gattinoni L, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, et al. Wnt signaling arrests effector T cell differentiation and genera-

tes CD8+ memory stem cells. Nat Med. 2009 Jul 14;15(7):808–13. https://doi.org/10.1038/nm.1982

36. Bezinover D, Saner F. Organ transplantation in the modern era. BMC Anesthesiol. 2019 Dec 4;19(1):32. https://doi.org/10.1186/s12871-019-0704-z
37. Watson CJE, Dark JH. Organ transplantation: historical perspective and current practice. Br J Anaesth. 2012 Jan;108: i29–42. https://doi.org/10.1093/bja/aer384
38. von Toerne C, Schmidt C, Adams J, Kiss E, Bedke J, Porubsky S, et al. Wnt Pathway Regulation in Chronic Renal Allograft Damage. Am J Transplant. 2009 Oct;9(10):2223–39. https://doi.org/10.1111/j.1600-6143.2009.02762.x
39. Wang H, Zhang R, Wu X, Chen Y, Ji W, Wang J, et al. The Wnt Signaling Pathway in Diabetic Nephropathy. Front Cell Dev Biol. 2022 Jan 4;9. https://doi.org/10.3389/fcell.2021.701547
40. Seifert ME, Gaut JP, Guo B, Jain S, Malone AF, Geraghty F, et al. WNT pathway signaling is associated with microvascular injury and predicts kidney transplant failure. Am J Transplant. 2019 Oct;19(10):2833–45. https://doi.org/10.1111/ajt.15372
41. YANG Y, Rao P, Zhao J, Yu H, Chen T, Cao Q, et al. SUN-129 The significance of β-catenin/Foxo in kidney transplantation. Kidney Int Reports. 2019 Jul;4(7):S212. https://doi.org/10.1016/j.ekir.2019.05.527
42. Yüksel O, Pehlivan M, Çöven HİK, Çerçi B, Soyöz M, Tatar E, et al. The changes in the expression levels of β-catenin gene in pre- and post- Kidney Transplants. Transpl Immunol. 2021 Dec; 69:101471. https://doi.org/10.1016/j.trim.2021.101471

IMMUNE CHECKPOINT INHIBITORS (ICI) TREATMENTS IN SOLID ORGAN TRANSPLANTATION PATIENTS WITH CANCER

Yüksel KUCUKZEYBEK[1], Zeynep Gülsüm GUC[1]

[1]İzmir Katip Çelebi University Medical Oncology, Izmir, Turkey

[1]Medical Oncology at Izmir Ataturk Training and Research Hospital, Izmir, Turkey

11.1 INTRODUCTION

Cancer is a complex and heterogeneous disease with diverse symptoms, prognosis, and outcomes in each patient. Cancer cells can grow and migrate to other regions in the body, reduce the extracellular matrix (ECM), and survive in the blood and new tissue environments. The treatment modality for each case is chosen according to cancer type, development, and phase. Treatment options include chemotherapy, surgery, radiation therapy, and immunotherapy [1].

The body's defense mechanism, the immune system, can recognize and neutralize foreign organisms and molecules, hence suppress spontaneous tumor development or progression. While traditional anticancer therapies directly target the tumor cells, the goal of immunotherapy is to identify cancer cells and trigger immune cells to fight and destroy tumor cells. This allows to treat the different types of cancer with the same agent. The success of immunotherapy has led to a

more detailed evaluation of cancer. Presently, it is not only the cancer cells that are targeted and destroyed, but the cancer's immune environment is also taken into account.

In the United States alone, about 30,000 solid organ transplants are done every year. Thanks to the development of immunosuppressive therapies, life expectancy and graft survival rates of solid organ recipients have been improving over the years. Nevertheless, the risk of carcinogenesis is significantly high in transplant recipients. Despite mostly presenting at advanced stages, these patients can often respond to immunotherapy. As available evidence largely comes from individual case reports, case series, or small early-phase trials, the safety and efficacy of immunotherapies. In this backdrop, this section aims to discuss the data on immune checkpoint inhibitors (ICIs) and their future use in solid organ transplantation (SOT) patients.

11.2 CANCER AND THE IMMUNE SYSTEM

The immune system can recognize what is foreign in the organism, record it in its memory, and rapidly respond to this foreign antigen at the next encounter. The cells of the immune system are produced through hematopoiesis and originate from hematopoietic stem cells (HSCs). The lymphoid and myeloid cells that form in this process include specialized cells related to both adaptive and natural immunity. T, B, and NK lymphocytes are formed from lymphoid precursor cells, and neutrophil leukocytes, dendritic cells, and macrophages are formed from myeloid precursor cells.

11.3 IMMUNOTOLERANCE AND IMMUNOSURVEILLANCE

Immunotolerance is a state of unresponsiveness of an organism to its own antigens in a healthy immune system. It

can occur naturally or be externally induced. Immunotolerance develops in the primary organs, the bone marrow where immune cells are formed, and the thymus where the immune cells mature, or in the peripheral tissues and the lymph nodes where mature T and B lymphocytes reside. If this is disrupted, the person's own immune system attacks their own cells and leads to autoimmune diseases, allergies, or organ transplant rejection. Immunosurveillance is the immune system's recognition and destruction of cancerous or precancerous cells through specific antigens or molecules with increased synthesis. Two other ways in which the immune system prevents cancer formation are the elimination of viruses to prevent the development of tumors in which viruses are etiologically involved and the elimination of pathogens to shorten the inflammatory process that accelerates tumor formation.

11.3.1 Immunoediting

Immunoediting has become the preferred term for broadly defining the immune system's role in cancer development and prevention. This three-phase process is often referred to as the "3 Es" of tumor immunology. The phases that make up this process are similar to the elimination phase concept of immunosurveillance. Equilibrium is a quiescent state of tumor cells during which they cannot be destroyed by the immune system. Escape is the uncontrolled proliferation and growth of the tumor cells that cannot be blocked during the equilibrium phase and escape the immune system.

11.3.2 Immune Checkpoint Inhibitors

ICIs have been extensively researched as a class of immunotherapy. These receptors are expressed in both peripheral tissues and immune cells and have crucial roles in preventing autoimmune reactions by maintaining self-tolerance and

modulating immune response. Understanding the relationship between immunosurveillance and tumor proliferation is essential for advancing immunotherapy.

Co-inhibitory molecules such as PD-L1/2 (programmed cell death ligand proteins 1/2), LAG-3 (lymphocyte activation gene 3), and PD-1 (programmed cell death protein 1) are pivotal in the regulation of cancer. Cancer cells can evade the immune response when the T-cells are suppressed once the T-cells bind to the cancerous cells or the APCs (antigen-presenting cells) via PD-1/PD-L1 or PD-L2 association. Monoclonal antibodies such as anti-PD-1 antibodies may attach to the molecules on T-cells called PD-1and prevent its association with PD-L1/2, thus disrupting their interaction and repair their suppressive impact on tumors. Other monoclonal antibodies such as anti-PD-L1 antibodies achieve a similar outcome; they prevent the interaction of PD-1 with the T-cells by attaching to PD-L1 on cancerous cells and APCs. ICIs also target the specific molecules on active and regulatory T cells (Treg) called CTLA-4 (cytotoxic T-lymphocyte-associated protein 4, also known as CD152). This molecule is one of CD28/B7 immunoglobulin superfamily participant. It suppresses the activation of T-cell. One MoA of CTLA-4 comes from its higher affinity for B7-1 and B7-2 (CD80/86) compared to CD28 on APCs involved in co-stimulatory signaling. CTLA-4 binds to B7 (CD80/CD86) on APCs to inhibit T-cell activation. Anti-CTLA-4 antibody reactivates the T-cells by inhibiting CTLA-4 from binding to B7, and thereby allowing CD28 to bind to B7. A second mechanism involves negative signaling which occurs at low surface expression levels using the cytoplasmic tail of CTLA-4. When LAG-3, produced by Tregs, is inhibited, depleted T-cells can regain their effector function and immunity responses. Currently, it is often utilized with anti-PD-1 antibodies in clinical practice.

11.3.3 Anti-PD-1 Monoclonal Antibodies

Pembrolizumab: acts by binding to the PD-1 molecules of T-cells. It associates with stimulatory molecules, suppressing immune cells and thereby the immune system. Blocking both stimulatory molecules (PD-L1 and PD-L2) from binding to PD-1 and helping to restore T-cell response, provides the immune system activation. As a monoclonal antibody (mAB), anti-PD-1 has led to significant gains in survival rates in many cancer types. It is effective in lung cancer, malignant melanoma, lymphoma and esophagus, stomach, colon, bladder, kidney, cervix, and endometrial cancers. The use of the presence and the degree of PD-1 expression as a biomarker is still not fully recognized. A first has been achieved in cancer treatment with its use in tumors with high microsatellite instability (MSI-H) regardless of the organ and the region (tumor agnostic).

Nivolumab: is a PD-1-blocking mAB successfully used in several types of cancer, especially in non-small cell lung cancers, melanoma, renal cell cancers, head and neck cancers, and lymphoma. It is also effective in the treatment of MSI-H tumors regardless of anatomical localization.

Dostarlimab: is another PD-1-blocking Mab that binds to PD-1 with high affinity. It was recently (2021) approved in the European Union and the United States and is successfully used to treat endometrial cancers.

11.3.4 Anti-PDL-1 Monoclonal Antibodies

Atezolizumab: is an mAB used in immunotherapy. It binds to PD-L1, a molecule in cancer cells that inhibits the immune response. Among others, it is utilized for the advanced small-cell lung cancer (SCLC) and urothelial cancer therapies.

Avelumab: is in IgG1 type and binds to PD-L1 molecules on tumor-infiltrating or cancerous cells and thereby blocks PD-L1 from interacting with the B7.1 receptors and PD-1 on APCs and T-cells. By inhibiting the pathway, it disrupts immune checkpoint (IC) mechanisms to overcome immune escape strategies and amplify the immune response of the T-cell. This, in turn, leads to T-cell activation and proliferation. What sets Avelumab apart from other PD-L1/PD-1 IC-blocking antibodies is its ability to induce antibody-dependent cellular cytotoxicity. This additional feature contributes to its effectiveness in combatting cancer. It is indicated for the treatment of metastatic urothelial carcinoma, renal cell carcinoma, and Merkel cell carcinoma.

Durvalumab: is a human immunoglobulin G1 kappa mAB (IgG1k) that hinders PD-L1 from interacting with PD-1. It has been approved and shown to have impact on non-small cell lung cancer (NSCLC) at stage III and bladder cancer.

Cemiplimab: is a mAB that binds to PD-1 to block its pathway to PD-L1. The FDA has approved (September 2018) cemiplimab for use in locally advanced cSCC or metastatic cutaneous squamous cell carcinoma (metastatic cSCC) patients not suitable for repairing radiation or surgery therapies.

11.3.5 Anti-CTLA-4 Monoclonal Antibodies

Ipilimumab: is the first CTLA-4-targeted drug to be discovered. It was seen to be effective and used in melanoma treatment, but presently is not used alone due to its high number of side effects and the fact that anti-PD-1 targeted drugs are more effective; it is used in combination with other immunotherapy drugs.

Tremelimumab: is a fully human mAB, which by binding CTLA-4, nullifies the binding of CTLA-4 to CD80/CD86. It is used in mesothelioma and hepatocellular carcinoma but is still under investigation for other cancer types.

Studies on other ICs are ongoing. Among these, T-cell immunoglobulin (TIM), T-cell immunoglobulin and ITIM domain (TIGIT), V-domain immunoglobulin suppressor of T-cell activation (VISTA), LAG-3, and OX40 stand out as targets with positive results.

While in 2011, only 1.5% of cancer patients in the United States were suitable for ICIs, by 2022 ICIs were indicated in more than 40% of the cancer cases. According to the 2020 global statistics, the number of new cancer cases was 19.3 million. Adjusting this rate, it can be estimated that approximately 7.72 million individuals annually are eligible to derive benefits from ICIs, with the potential for ICIs to benefit around 2.4 million people each year.

11.4 CANCER AND ICI AFTER TRANSPLANTATION

Today, SOT is accepted as a practical and definitive option in the treatment of patients with organ dysfunction. It offers life-saving treatments for diseases that cause significant deterioration in the patient's quality of life (QoL) or for those that are considered terminal. Whilst kidney, liver, lung, and heart transplantations are the most commonly reported SOTs, inflammations, cardiovascular events, and malignancies are reported as predominant reason of mortality. Survival rates among transplant recipients have advanced, thanks to state-of-the-art surgical techniques and immunosuppressants, this advancement has led to an increase in the number of patients developing cancer after transplantation. According to the data published by UNOS (The United Network for Organ

Sharing) and OPTN (The Organ Procurement and Transplantation Network), the incidence of deaths from post-transplant malignancies 5-10 years after transplantation is 21.5% in heart, 18.7% in liver, and 14.5% in kidney transplant patients. The occurrence of malignancies in organ transplantation can vary based on factors associated with the donor and recipient, including the transplanted organ, pre-existing malignancy in the donor or the recipient, and the nature of the immunosuppressive therapy such as type, intensity, or duration.

Malignancies after organ transplant are deemed to arise via three mechanisms: de novo, transmission from the donor, and recurrence of a pre-existing malignancy. Kaposi's sarcomas, non-melanoma skin cancers, anogenital and lung cancers, and post-transplant lymphoproliferative disorders are considered to increase recently. However, malignant melanoma and other malignancies in kidney transplant patients are usually linked to the donor. Cholangio- and hepatocellular carcinomas have a predilection for liver transplant recipients. The development of these malignancies, a significant contributing factor to carcinogenesis, is often linked to immune systems that are imbalanced or altered by chronic immunosuppression.

The primary pathogenic mechanisms considered to be oncogenic are compromised immune surveillance of neoplastic cells, weak immune response to cancer-causing viruses (most notably the human herpes virus and the Epstein-Barr virus) and the direct carcinogenic effects of immunosuppressive agents.

Since basic imaging can be guiding for timely diagnosis, it is key in the screening, monitoring, and extended monitoring of individuals with malignancies.

A major factor contributing to carcinogenesis in transplant patients is the impairment of innate immunity which is caused by long-term use of immunosuppressives. The immunosuppressive drugs used in SOT recipients and their risks of malignancy are given in the Table (Table 1) [36].

Table 1: The immunosuppressive drugs used in SOT recipients and their risks of malignancy

Type of medication	Risk
Antimetabolites Azathioprine Mycophenolate mofetil	While azathioprine has a direct carcinogenic effect, the use of mycophenolate mofetil is associated with a reduced risk of cancer development.
Calcineurin inhibitors Cyclosporine Tacrolimus	Direct pro-oncogenic effect and increased cancer risk with increasing dose
Corticosteroids Methylprednisolone Prednisone	Direct pro-oncogenic effect on lymphoid cells
Biological agents Lymphocyte-eliminating antibodies Anti-thymocyte globulin Belatacept Rituximab Interleukin-2 receptor blockers Basiliximab Daclizumab	Anti-thymocyte globulin, or belatacept, is associated with an increased risk Early Epstein-Barr virus-positive PTLD Rituximab protects against PTLD No pro-oncogenic potential
mTOR inhibitors Sirolimus Everolimus	Direct antitumor activity that reduces the incidence of malignancy

11.4.1 How safe are ICIs for cancer patients undergoing organ transplantation?

Brunet et al. were the first to report about the IC molecule, naming it CTLA-4 in 1987. The first ICI that the FDA approved is ipilimumab in 2011 after being studied in long-term trials. Nevertheless, the earliest real-world data on the use of immunotherapy in transplant patients is from 2014. Lipson et al. reported their experience with ipilimumab in

two metastatic malignant melanoma patients with histories of kidney transplantation. As of 2014, data on the use of ICI in an increasing number of patients and after different organ transplant procedures have accumulated. Standard clinical trials in oncology often exclude patients with organ impairment or transplant recipients. As the number of reported cases of ICI use in transplant recipients increases, clinical studies have begun to be designed on this subject.

SOT is a treatment modality that increases the survival and QoL of patients with end-stage organ failure. Organ transplantations have been increasingly performed across Turkey after the first kidney transplantation in 1975. Today it has become the major modality in end-stage kidney failure cases. According to the data of the Ministry of Health, 63,359 patients have received organ transplants in Turkey since 2008. Of these, 43,867 received kidney transplants, 18,230 liver transplants, 821 heart transplants, 325 lung transplants, and 66 received pancreas transplants. While kidney and liver transplants remain the most common among all organ transplants, the most common malignancies in these two patient groups are cSCC and melanoma.

11.4.2 Mechanisms and Risk Factors of Rejection Stimulated by ICI

The release of donor antigens from donor cells triggers an immune response against the alloantigen in the transplant patients. Immunosuppressive agents, including steroids, mammalian target of rapamycin (mTOR) inhibitors, mycophenolate mofetil (MMF), and calcineurin inhibitors (CNIs), play a pivotal role in T-cell inhibition and immune tolerance regulation. The dosing of immunosuppressive therapies is typically decreased after transplantation to avoid excessive immune suppression.

The adjustment is made to restore sufficient tumor immunity, depending on the type and severity of the cancer. It is important to note that ICIs have the potential to disrupt immunologic tolerance, potentially leading to acute rejection, the occurrence of which varies based on the transplanted organ type.

The efficacy of mTOR inhibitors has been shown in preventing and treating cancer. In their multicenter study, Murakami et al. reported graft lifespan without rejection and total graft lifespan to be longer in kidney transplant recipients with cSCC who were treated with mTOR inhibitors compared to those who were not treated with mTOR inhibitors. Reports also suggest that mTOR inhibitors may be beneficially used to reduce rejection and control cancer.

The presence of previous rejection significantly increased the possibility of failure with ICI therapy, and the study additionally demonstrated that utilizing a minimum of a single extra immunosuppressant in along with steroids decreased the rejection possibility. Immunologic tolerance may build after a certain time in post-transplant patients, typically necessitating fewer immunosuppressants.

The measurement of IFI27 gene expression through biopsy may serve as a potential biomarker to differentiate ICI-mediated allograft rejection. Histopathologically, it is difficult to discriminate between ICI-related acute allograft rejection and other forms of acute tubulointerstitial nephritis or T-cell-related rejection. Adam et al. reported higher levels of the expression for the interferon alpha-induced transcript IFI27 in patients who had rejection related to T cell after ICI utilization in transplanted kidney compared to acute interstitial nephritis. The expression level of IFI27 could be used as a potential biomarker to detect ICI-related allograft rejection.

Ipilimumab blocks the CTLA-4 binding region, hindering the T-cells suppression. By interacting with the PD-1 binding area on cancer cells, pembrolizumab and nivolumab prevent PD-L1 from inhibiting T-cells. Immunosuppressants used in transplant patients modulate immunological tolerance and curb T-cell stimulation.

Immunosuppressive drugs are essential for maintaining graft function in organ transplant recipients, with extensive research on the molecular mechanisms involved in graft rejection. Donor cells release donor antigens in patients undergoing organ transplantation, triggering an immune response to alloantigens. Normally, PD-1 and CTLA-4 act to modulate T-cell transformation and to inhibit the excessive activation of T-cells against foreign molecules. The suppression of T cells is inhibited by Ipilimumab via CTLA-4 binding site blockadge. Nivolumab and pembrolizumab interact with the binding region of PD-1 and hinder its ligand on cancer cells from negatively regulating the T-cells. Immunosuppressants used in transplant patients regulate immunological tolerance by suppressing T-cell activation. Anticancer therapy may also trigger immunosuppression in patients with a history of organ transplantation and subsequent diagnosis of cancer. The dosing of the immunosuppressants used concurrently with cancer therapy in transplant patients is reduced to regain both tumor immunity and avoid excessive immunosuppression. Presently there are no guidelines on ICIs in transplant patients. It is assumed that there are factors that can affect both the safety and treatment methods in this patient group.

These factors include:

i. Lower activity of the CTLA-4 pathway, despite being associated with a reduced possibility of rejection,

ii. Sequential application of different ICIs instead of combination therapy,

iii. Length of time after transplantation,

iv. Potency of the immunosuppressive agents used,

v. Immunogenicity of the transplanted organ graft.

Everolimus, an mTOR inhibitor with established efficacy, is indicated for treating patients with breast cancer, renal cell carcinoma, and neuroendocrine tumors (NET). As mentioned above, Murakami et al. reported that both overall and rejection-free graft survival times were longer in their cSCC patients who received mTOR inhibitor therapies. The benefits of mTOR inhibitors for reducing rejection and controlling cancer are also suggested in other reports.

It is generally believed that long-term transplant recipients develop a certain degree of immunological tolerance and often require less immunosuppression. Therefore, the length of the time after transplantation is considered one of the factors determining the risk of rejection in ICI use. According to d'Izarny-Gargas et al., the rejection possibility had significantly decreased with ICIs in patients after eight years of transplantation. The authors further reported that risk rejection had decreased when at least one immunosuppressant was added to the steroid treatment, and the likelihood of rejection while on ICI treatment had significantly increased in the presence of previous rejection.

11.5 USE OF ICI IN COMMON ORGAN TRANSPLANTS

11.5.1 Kidney Transplantation

This section will first discuss the mechanisms of rejection and risk factors related to ICI use in transplant patients, and further, some of the examples of ICI use as reported in the literature. The most commonly performed solid organ transplant is kidney transplant, with reported survival times exceeding 15 years. Therefore, the most data on cancer and ICI use in transplant patients come from kidney transplant cases., however, cancer rates are rising among these patients in association with prolonged immunosuppression. The availability of hemodialysis as a backup option encourages clinicians to use ICIs, given their proven efficacy in certain types of cancers. Among all SOT recipients, the highest number of cases utilizing ICI has been shown in renal transplant patients, even though ICI utilization increases graft rejection risk. The most reported cancers are cSCC and melanoma. Cases of renal cell carcinoma and NSCLC have also been noticed.

The largest information in the literature on the utilization of ICIs in kidney transplant patients comes from a retrospective efficacy and safety analysis by Murakami et al. Among their cohort of 69 patients including 23 cSCC and 14 melanoma patients, the authors found an increased response rate with the use of ICI in cancer treatment, while their retrospective analysis showed a survival benefit in cSCC. Nevertheless, 29 (42%) had experienced rejection, and of these 29 patients, 19 (66%) developed allograft failure and required dialysis.

One of the reports in the literature is about a case of kidney re-transplant in a patient who experienced severe allograft rejection and lost a kidney two months after pembrolizumab treatment for cSCC. In terms of cancer, the patient

had a complete response (CR) in the ninth month of pembrolizumab treatment and the treatment was discontinued.

In kidney transplant patients using ICIs, biopsy-proven rejections are mostly seen to be T-cell-mediated, while antibody-mediated rejections are less common. As the current reports in the literature lack the necessary data, risk factors, and immune modulation strategies will be better understood as prospective trials are conducted. There are ongoing clinical trials that include kidney transplant patients.

One of these trials, a multi-center, open-label, two-tier study conducted in Australia, is investigating the Nivolumab safety in kidney transplant patients with advanced malignancy. In this phase I trial, participants are given intravenous nivolumab (3 mg/kg) per two weeks, and this therapy will be continued for up to two years if there are clinical benefits.

Another study is investigating the effect of tacrolimus, ipilimumab, and nivolumab in kidney transplant recipients. A prospective phase I trial conducted in the USA, this study compares the three ICIs in patients who have received a kidney transplant and have malignancies that cannot be removed surgically or have spread to other parts of the body.

A stage I/II, two-cohort, and open-labelled clinical trial was designed to assess the efficacy and safety of cemiplimab for treating severe cSCC in patients who underwent allogeneic kidney transplants or hematopoietic stem cell. In the study, kidney transplant recipients are given cemiplimab in combination with prednisone and sirolimus or everolimus to inhibit the rejection.

11.5.2 Liver Transplantation

The number of patients awaiting transplantation due to HCV is decreasing, thanks to the advances in antiviral agents. Diagnoses of liver diseases associated with alcohol and other/undetermined causes (often steatohepatitis) now constitute the leading indications for liver transplantation. One aspect particularly worth noting is ICIs function in hepatocellular carcinoma (HCC) treatment both pre- and post-transplantation.

CheckMate 040, a trial studying the safety and efficacy of nivolumab indicated an objective response rate (ORR) of 15% (CR, 6%; PR, 9%) in severe HCC patients and a history of sorafenib treatment. Overall survival (OS) was 15% (PR 9%, CR 6%). For both first-line and second-line treatments, the median overall survival was 28.6 months and 15.6 months, respectively. The FDA has approved nivolumab as an adjunctive medication for individuals who do not respond to sorafenib therapy.

Pembrolizumab was approved after nivolumab based on the results of the KEYNOTE-224 study. The response rate for pembrolizumab in this patient group was noticed as 17% (PR 16%, CR 1%,)).

Treatments with combined molecular-targeted therapies and ICIs are gaining widespread acceptance among the general population for HCC treatment. Since the FDA's clearance, options for combined therapies in HCC have grown of combined ipilimumab-nivolumab therapy in 2019. Treatment with combined atezolizumab and bevacizumab was shown to extend progression-free survival (PFS) and OS in unresectable HCC patients compared to sorafenib. The conclusions of the HIMALAYA study indicated significantly improved OS in unresectable HCC patients treated with combined durvalumab

and tremelimumab compared to sorafenib. All these studies relate to the treatment processes of patients diagnosed with HCC.

As in kidney transplant recipients, ICI treatment has been predominantly documented in patients who received liver transplants with cutaneous squamous cell carcinoma and melanoma.

A total of 42 liver transplant cases using ICI have been reported in the literature. Of these 22 were post-transplant and 20 were pre-transplant patients. Six patients (27.3%) responded to the therapy, while post-transplant rejection was shown in five patients (22.7%). Of the patients who received ICI before the transplant, five (25%) experienced early acute rejection after transplantation, while six patients (30%) responded completely or partially to treatment. The innate tolerogenicity of liver transplants may be the cause of the observed decreased rates of acute rejection with ICI treatment in liver transplant patients compared to renal transplant patients. The current therapeutic studies' outcomes will provide us with the much-awaited data on rejection and treatment responses. There are two ongoing studies in this regard.

A prospective study conducted in China aims to evaluate the security and efficiency of the PD-1 inhibitor JS001 in liver transplant patients who were diagnosed with recurrent or metastatic HCC post-transplant and administered with specific treatment or sorafenib. Individuals expressing PD-L1 in their grafts are excluded after confirmation biopsy.

Another ongoing study with liver transplant recipients is conducted in the USA to investigate the preoperative effects of bevacizumab and atezolizumab in patients who has resectable liver cancer. This phase II trial will be the inaugural

worldwide investigation to evaluate the benefit and security of bevacizumab and atezolizumab as a bridge treatment to liver transplantation for recipients exceeding the Milan criteria. More clinical trials are warranted, as the total amount of patients rises, a more secure and efficient approach is needed for utilizing ICIS in those who receive transplants.

11.6 THE UTILIZATION OF ICIs IN RARE TRANSPLANTION CASES

11.6.1 Lung Transplantation

It is one of the most challenging solid organ transplants. The results of lung transplantation, which was first done approximately 20 years after the first renal allograft transplantation, have shown better survival rates, and lung transplantation is now accepted as one of the standard treatment options in end-stage lung diseases.

The lungs are among the most immunogenic SOTs and recipients need higher doses of immune suppressor therapies. The number of cancer cases increases as survival times increase, and there are potential ICI candidates among post-lung transplant patients.

Presently, in the literature, there are three lung transplant cases reported to receive immunotherapy. While none of the patients had acute rejection, immune-related pneumonitis was seen after two cycles of cemiplimab in a cSCC patient reported by Tsung et al. The patient was treated and discharged with a complete cSCC response. Of the other two cases, as reported by Daud et al., one developed acute graft dysfunction after being treated with ipilimumab for melanoma and one individual passed away within a year of receiving pembrolizumab medication due to persistent lung allograft

malfunction. ICIs are predicted to pose higher allograft rejection and/or dysfunction risks in lung transplant recipients but are anticipated to provide therapeutic benefits for the cancer.

11.6.2 Heart Transplantation

A rare but fatal side effect of ICI is myocarditis. Even though myocarditis in patients using ICI has been reported at a rate of 0.09%, the mortality rate attributed to ICI-related myocarditis ranges from 36% to 67%.

There are a limited number of studies investigating the effects of ICIs on heart transplant recipients. ICI treatments have been reported for NSCLC, cSCC, and melanoma in patients who had heart transplantation. Grant et al. reported two patients who underwent orthotopic heart transplantation and received ICI treatment for melanoma. One of these patients had metastatic malignant melanoma and was given ipilimumab followed by pembrolizumab, while the other was given trametinib followed by pembrolizumab. Cardiac allograft rejection was not seen in either of the patients. Another case report described a patient diagnosed with cSCC in the 16th year of heart transplantation who received nivolumab treatment due to progression after cytotoxic chemotherapy and died of cardiac allograft rejection. Despite the numerous data on cardiac toxicity associated with the use of immunotherapy and its management, clinical studies examining ICIs in individuals who have undergone heart transplantation are scarce.

11.6.3 CAR T-cell

The recent regulation of Chimeric Antigen Receptors (CARs) on T-cells, especially in cancer cell therapy such as

B-cell lymphomas (CD20-CD19), has shown great promise. This development has also paved the way for the potential use of Treg cells. Moreover, some studies have indicated that CAR expression in Treg cells could potentially be used in the treatment of Xeno-Graft Versus Host Disease (xeno-GVHD), and allograft rejection. In a human skin xenograft transplantation incidence, adoptive CAR Treg cells were more successful than polyclonal Treg cells in alleviating alloimmune-mediated skin damage caused by the transfer of allogeneic peripheral blood mononuclear cells (PBMCs). Designed not to be limited by the Major Histocompatibility Complex (MHC), CAR-Treg cells especially hold the advantage of widespread application in transplantation and autoimmunity. Data regarding the treatment of solid organ tumors with CAR T-cells is rather limited. More data is anticipated on cancer patients undergoing organ transplantation as positive results are obtained.

11.7 CONCLUSION

Individuals with kidney, liver, heart, or lung transplants carry a higher risk of cancer than the general population. As selected transplant recipients may clinically benefit from ICI therapies, the use of these agents should be permitted at the discretion of the clinician after transplantation. With the rising frequency of cancer diagnoses, especially among liver and kidney transplant recipients, ICI treatment in these patients remains a challenge. ICI therapy should be accompanied by a carefully selected immunosuppressant therapy. Although not yet adequately investigated, mTOR inhibitors could offer several benefits. The recipients, who could profit from ICI therapies are increasingly better understood, changing according to the duration after transplantation and the optimization of immunosuppressive therapies. Ongoing potential trials aim to enhance our understanding of rejection risk factors, identify

noninvasive biomarkers for monitoring rejection, and pinpoint therapeutic targets.

11.8 REFERENCES

1. Dobosz P., Dzieciątkowski T. The intriguing history of cancer immunotherapy. Frontiers in Immunology 2019; 10: 2965. https://doi.org/10.3389/fimmu.2019.02965

2. Blomberg OS., Spagnuolo L., de Visser KE. Immune regulation of metastasis: mechanistic insights and therapeutic opportunities. Disease Models and Mechanisms 2018; 11 (10): 1-12. https://doi.org/10.1242/dmm.036236

3. Weintraub K. Drug development: releasing the brakes. Nature 2013; 504 (7480): 6-8. https://doi.org/10.1038/504S6a

4. Finn OJ. Human tumor antigens, immunosurveillance, and cancer vaccines. Immunologic Research 2006; 36 (1-3): 73-82. https://doi.org/10.1385/IR:36:1:73

5. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nature Communications 2020; 11 (1): 3801. https://doi.org/10.1038/s41467-020-17670-y

6. Organ procurement and transplantation network. https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#.

7. Kittai AS., Oldham H., Cetnar J., Taylor M. Immune checkpoint inhibitors in organ transplant patients. Journal of Immunotherapy 2017; 40 (7): 277e81. https://doi.org/10.1097/CJI.0000000000000180

8. Kim R., Emi M., Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology 2007; 121 (1): 1-14. https://doi.org/10.1111/j.1365-2567.2007.02587.x

9. Nakamura Y. Biomarkers for immune checkpoint inhibitor mediated tumor response and adverse events. Frontiers in Medicine 2019; 6: 119. https://doi.org/10.3389/fmed.2019.00119

10. Li L., He ZY., Wei XW., Wei YQ. Recent advances of biomaterials in biotherapy. Regenerative Biomaterials 2016; 3 (2): 99-105. https://doi.org/10.1093/rb/rbw007

11. Chowdhury F., Dunn S., Mitchell S., Mellows T., Ashton-Key M. et al. PD-L1 and CD8+PD1+ lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy.

OncoImmunology 2015; 4 (10): e1029701. https://doi.org/10.1080/2162402X.2015.1029701

12. Gibney GT., Weiner LM., Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. The Lancet Oncology 2016; 17 (12): e542-e551. https://doi.org/10.1016/S1470-2045(16)30406-5
13. Murakami N., Motwani S., Riella LV. Renal complications of immune checkpoint blockade. Current Problems in Cancer 2017; 41 (2): 100-10. https://doi.org/10.1016/j.currproblcancer.2016.12.004
14. Lichtenegger FS., Rothe M., Schnorfeil FM., Deiser K., Krupka C. et al. Targeting LAG-3 and PD-1 to enhance T cell activation by antigen-presenting cells. Frontiers in Immunology 2018; 9: 385. https://doi.org/10.3389/fimmu.2018.00385
15. Fu j., Wang F., Dong LH., Zhang j., Deng CL., et al. Preclinical evaluation of the efficacy, pharmacokinetics and immunogenicity of JS-001, a programmed cell death protein-1 (PD-1) monoclonal antibody. Acta Pharmacologica Sinica 2017; 38 (5): 710-8. https://doi.org/10.1038/aps.2016.161
16. Chang L, Chang M, Chang HM, Chang F. Microsatellite instability: a predictive biomarker for cancer immunotherapy. Applied Immunohistochemistry & Molecular Morphology 2018; 26 (2): e15-e21. https://doi.org/10.1097/PAI.0000000000000575
17. Gomes F, Serra-Bellver P, Lorigan P. The role of nivolumab in melanoma. Future Oncology 2018; 14 (13): 1241-52. https://doi.org/10.2217/fon-2017-0484
18. Chen R., Tao Y., Xu X., Shan L., Jiang H., et al. The efficacy and safety of nivolumab, pembrolizumab, and atezolizumab in treatment of advanced non-small cell lung cancer. Discovery Medicine 2018; 26 (143): 155-66.
19. "FDA grants accelerated approval to dostarlimab-gxly for dMMR endometrial cancer." U.S. Food and Drug Administration (FDA) (Press release). 22 April 2021. Archived from the original on 22 April 2021. Retrieved 22 April 2021. Public Domain This article incorporates text from this source, which is in the public domain.
20. Inman BA., Longo TA., Ramalingam S., Harrison Mr. Atezolizumab: a PDL1-blocking antibody for bladder cancer. Clinical Cancer Research 2017; 23 (8): 1886-90. https://doi.org/10.1158/1078-0432.CCR-16-1417

21. Calles A., Aguado G., Sandoval c., Álvarez R. The role of immunotherapy in small cell lung cancer. Clinical and Translational Oncology 2019; 21 (8) :961-76. https://doi.org/10.1007/s12094-018-02011-9
22. Gaiser MR., Bongiorno M., Brownell I. PD-L1 inhibition with avelumab for metastatic Merkel cell carcinoma. Expert Review of Clinical Pharmacology 2018; 11 (4): 345-59 https://doi.org/10.1080/17512433.2018.1445966
23. Rodriguez-Vida A., Bellmunt J. Avelumab for the treatment of urothelial cancer. Expert Review of Anticancer Therapy. 2018; 18 (5): 421-9. https://doi.org/10.1080/14737140.2018.1448271
24. Alvarez-Argote J., Dasanu CA. Durvalumab in cancer medicine: a comprehensive review. Expert Opinion on Biological Therapy 2019; 19 (9): 927-35. https://doi.org/10.1080/14712598.2019.1635115
25. U.S. Food and Drug Administration. 28 September 2018. Retrieved 7 August 2020. Public Domain This article incorporates text from this source, which is in the public domain.
26. Specenier P. Ipilimumab in melanoma. Expert Review of Anticancer Therapy 2016; 16 (8): 811-26. https://doi.org/10.1080/14737140.2016.1211936
27. Gao X., McDermott DF. Ipilimumab in combination with nivolumab for the treatment of renal cell carcinoma. Expert Opinion on Biological Therapy 2018; 18 (9): 947-57. https://doi.org/10.1080/14712598.2018.1513485
28. Fumet JD., Limagne E., Thibaudin M., Truntzer C., Bertaut A. et al. Precision medicine phase II study evaluating the efficacy of a double immunotherapy by durvalumab and tremelimumab combined with olaparib in patients with solid cancers and carriers of homologous recombination repair genes mutation in response or stable after olaparib treatment. BMC Cancer 2020; 20 (1): 748. https://doi.org/10.1186/s12885-020-07253-x
29. Comin-Anduix B., Escuin-Ordinas H., Ibarrondo FJ. Tremelimumab: research and clinical development. OncoTargets and Therapy 2016; 9:1767-76. https://doi.org/10.2147/OTT.S65802
30. Andrews LP., Yano H., Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nature Immunology 2019; 20 (11): 1425-34. https://doi.org/10.1038/s41590-019-0512-0

31. Haslam A., Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. JAMA Network Open 2019; 2 (5): e192535. https://doi.10.1001/jamanetworkopen.2019.2535
32. Sung H., Ferlay J., Siegel RL., Laversanne M., Soerjomataram I. et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 2021; 71 (3): 209-49. https://doi.org/10.3322/caac.21660
33. Chapman JR., Webster AC., Wong G. Cancer in the transplant recipient. Cold Spring Harbor Perspectives in Medicine 2013; 3 (7): a015677. https://doi.10.1101/cshperspect.a015677
34. Campistol JM., Cuervas-Mons V., Manito N., Almenar L., Arias M. et al. New concepts and best practices for management of pre- and post-transplantation cancer. Transplantation Reviews 2012; 26 (4): 261-79. https://doi.org/10.1016/j.trre.2012.07.001
35. Gutierrez-Dalmau A., Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients. Drugs 2007; 67 (8): 1167-98. https://doi.org/10.2165/00003495-200767080-00006
36. Brunet JF., Denizot F., Luciani MF., Roux-Dosseto M., Suzan M. et al. A new member of the immunoglobulin superfamily--CTLA-4. Nature 1987; 328 (6127): 267-70.
37. Lipson EJ., Bagnasco SM., Moore J Jr., Jang S., Patel MJ. et al. Tumor regression and allograft rejection after administration of anti-PD-1. The New England Journal of Medicine 2016; 374 (9): 896-8. https://doi.10.1056/NEJMc1509268
38. LaRosa C., Glah C., Baluarte HJ., Meyers KE. Solid-organ transplantation in childhood: transitioning to adult health care. Pediatrics 2011; 127 (4): 742-53. https://doi.org/10.1542/peds.2010-1232
39. Haberal M. Historical evolution of kidney and liver transplantation in Turkey. Transplant Proceedings. 1995; 27 (5): 2771-4.
40. De Bruyn P., Van Gestel D., Ost P., Kruse V., Brochez L., et al. Immune checkpoint blockade for organ transplant patients with advanced cancer: how far can we go? Current Opinion in Oncology 2019; 31 (2): 54-64. https://doi.10.1097/CCO.0000000000000505

41. Xie J., Wang X., Proud CG. mTOR inhibitors in cancer therapy. F1000Research 2016; 5 (F1000 Faculty Rev): 2078. https://doi.org/10.12688/f1000research.9207
42. Murakami N., Mulvaney P., Danesh M., Abudayyeh A., Diab A. et al. A multi-center study on safety and efficacy of immune checkpoint inhibitors in cancer patients with kidney transplant. Kidney International 2021; 100 (1): 196-205. https://doi.org/10.1016/j.kint.2020.12.015
43. Barnett R., Barta VS., Jhaveri KD. Preserved renal-allograft function and the PD-1 pathway inhibitor nivolumab. The New England Journal of Medicine 2017; 376 (2): 191-2. https://doi.10.1056/NEJMc1614298
44. Adam BA., Murakami N., Reid G., Du K., Jasim R., et al. Gene expression profiling in kidney transplants with immune checkpoint inhibitor-associated adverse events. Clinical Journal of the American Society of Nephrology 2021; 16 (9): 1376-86. https://doi.10.2215/CJN.00920121
45. d'Izarny-Gargas T., Durrbach A., Zaidan M. Efficacy and tolerance of immune checkpoint inhibitors in transplant patients with cancer: a systematic review. American Journal of Transplantation 2020; 20(9): 2457-65. https://doi.org/10.1111/ajt.15811
46. Saberianfar S., Nguyen LS., Manouchehri A., Lebrun-Vignes B., Moslehi JJ. et al. Solid organ transplant rejection associated with immune-checkpoint inhibitors. Annals of Oncology 2020; 31 (4): 543-4. https://doi.10.1016/j.annonc.2020.01.012
47. Nguyen LS., Ortuno S., Lebrun-Vignes B., Johnson DB., Moslehi JJ. et al. Transplant rejections associated with immune checkpoint inhibitors: a pharmacovigilance study and systematic literature review. European Journal of Cancer 2021; 148: 36-47. https://doi.org/10.1016/j.ejca.2021.01.038
48. Australian New Zealand Clinical Trials Registry (ANZCTR). Trial registered on ANZCTR [Internet]. Camperdown: ANZCTR [cited 2023 August 18]. Available from:
49. ClinicalTrials.gov. Home [Internet]. Bethesda, MA: ClinicalTrials.gov [cited 2023 August 18]. Available from: https://clinicaltrials.gov/study/NCT03816332?tab=results

50. ClinicalTrials.gov. Home [Internet]. Bethesda, MA: ClinicalTrials.gov [cited 2023 August 18]. Available from: https://clinicaltrials.gov/study/NCT04339062?tab=results
51. Yau T., Kang YK., Kim TY., El-Khoueiry AB., Santoro A., et al. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical in JAMA Oncology 2021 Jan 1; 7 (1): 140. https://doi.org/10.1001/jamaoncol.2020.6961
52. Verset G., Borbath I., Karwal M., Verslype C., Van Vlierberghe H. et al. Pembrolizumab Monotherapy for Previously Untreated Advanced Hepatocellular Carcinoma: Data from the Open-Label, Phase II KEYNOTE-224 Trial. Clinical Cancer Research 2022;28(12):2547-2554. https://doi.org/10.1158/1078-0432.CCR-21-3807
53. ClinicalTrials.gov. Home [Internet]. Bethesda, MA: ClinicalTrials.gov [cited 2023 August 18]. Available from: https://clinicaltrials.gov/ct2/home .
54. M.D. Anderson Cancer Center. Atezolizumab and bevacizumab before surgery for the treatment of resectable liver cancer. In: ClinicalTrials.gov [Internet]. Bethesda, MD: U.S. National Library of Medicine; 2022 [cited 2022 August 13]. Available from: https://clinicaltrials.gov/ct2/show/NCT04721132
55. Weill D., Benden C., Corris PA., Dark JH., Davis RD. et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation 2015; 34: 1-15. https://doi.org/10.1016/j.healun.2014.06.014
56. Chambers DC., Perch M., Zuckermann A., Cherikh WS., Harhay MO. et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-eighth adult lung transplantation report - 2021; focus on recipient characteristics. The Journal of Heart and Lung Transplantation 2021; 40 (10): 1060-72. https://doi.org/10.1016/j.healun.2021.07.021

57. Ivulich S., Westall G., Dooley M., Snell G. The evolution of lung transplant immunosuppression. Drugs 2018; 78 (19): 965-82. https://doi.org/10.1007/s40265-018-0930-6
58. Tsung I., Worden FP., Fontana RJ. A pilot study of checkpoint inhibitors in solid organ transplant recipients with metastatic cutaneous squamous cell carcinoma. The Oncologist 2021; 26 (2): 133-8. https://doi.org/10.1002/onco.13539
59. Daud A., Mehra MR., Siu A., Johnson MR., Perch M. et al. Immune checkpoint inhibitors in heart or lung transplantation: early results from a registry initiative. The Journal of Heart and Lung Transplantation 2020; 39 (6): 604-6. https://doi.org/10.1016/j.healun.2020.02.015
60. Ganatra S., Neilan TG. Immune Checkpoint Inhibitor-Associated Myocarditis. The Oncologist. 2018; 23 (8): 879-86. https://doi.org/10.1634/theoncologist.2018-0130
61. Moslehi JJ., Salem JE., A Sosman J., Lebrun-Vignes B., Johnson DB. Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis. The Lancet 2018; 391 (10124): 933. https://doi.org/10.1016/S0140-6736(18)30533-6
62. Grant MJ., DeVito N., Salama AKS. Checkpoint inhibitor use in two heart transplant patients with metastatic melanoma and review of high-risk populations. Melanoma Manag. 2018 Oct 26; 5 (4): MMT.
63. Noyan F., Zimmermann K., Hardtke-Wolenski M., Knoefel A., Schulde E. et al. Prevention of allograft rejection by use of regulatory T cells with an MHC-specific chimeric antigen receptor. American Journal of Transplantation 2017; 17 (4): 917-30. https://doi.org/10.1111/ajt.14175
64. Di Ianni M., Falzetti F., Carotti A., Terenzi A., Castellino F. et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood, The Journal of the American Society of Hematology 2011; 117 (14): 3921-8. https://doi.org/10.1182/blood-2010-10-311894

SECTION 2
MESENCHYMAL STEM CELL

MESENCHYMAL STEM CELLS: SOURCES, REGENERATION POTENTIAL AND USAGE IN CELL THERAPY

Burcu CERCI ALKAC[1,2]

[1]*Medical Biology Department, Izmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey*

[2]*Cell, Tissue, Organ Transplantation Application and Research Center, University of Izmir Katip Celebi, Izmir, Turkey*

12.1 INTRODUCTION

Stem cells offer limitless promise in sophisticated tissue engineering and cell treatments due to their ability to regenerate and differentiate into many cell lineages that may give therapeutic solutions for a variety of disorders [1]. High proliferation capacity and ability not to change physiology when undergoing many passages, easily collecting in large numbers, and differentiation capacity to a wide range of cell phenotypes make stem cells advantageous to use in various medicinal applications [2,3,4]. Stem cells can be used for the treatment of many disorders as congenital anomalies, tissue loss, and organ failure [5,6,7]. In the last 20 years, scientists target the use of stem cells in organ transplantation [8,9].

Stem cells can be obtained mostly from various sources such as cord blood, peripheral blood, bone marrow, spleen, thymus, and dental pulp. The choice of stem cell source is determined according to the frequency of the stem cells in

tissue and the ease of obtaining stem cells [10]. Stem cells can be classified into five according to differentiation capacity as totipotent, pluripotent, multipotent, unipotent, and oligopotent. Totipotent stem cells are able to form all cell types and have unlimited capacity (Figure 1). Pluripotent cells can be divided into four as adult progenitor cells, embryonic germ cells, embryonic carcinoma cells and embryonic stem cells. On the other hand, multipotent stem cells have a limited differentiation potential belonging to their location in the body. However, some studies showed that adult stem cells can differentiate into cells of different tissues [11]. Unipotent stem cells produce a limited number of cell types and mainly they repair damaged tissues while the differentiation capacity of oligopotent stem cells are restricted [12].

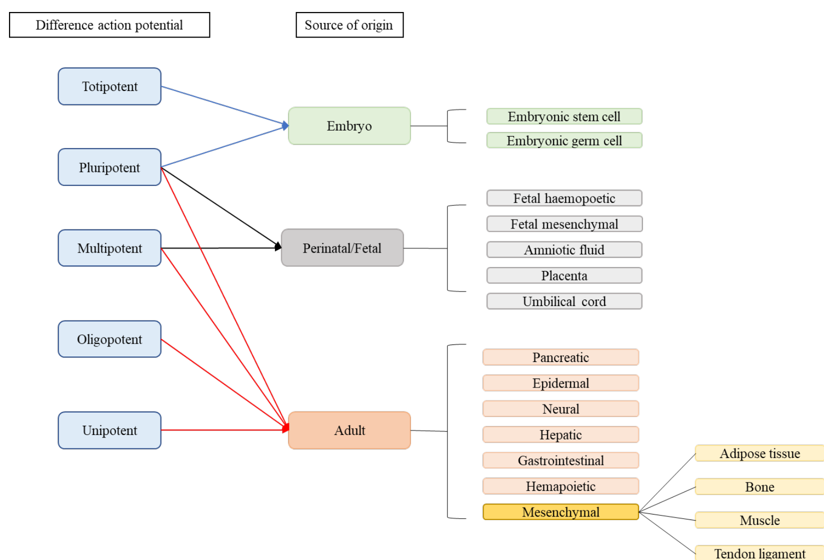


Figure 1: Categories of stem cells, origins, and types of mesenchymal stem cells

The isolation of Mesenchymal stem cells (MSCs) can be performed from many tissues, including bone, skin, skeletal muscle, adipose tissue, peripheral blood, dental pulp, peri-

odontal ligament, and tumors. However, harvesting of stem cells is a difficult process despite there are many sources of MSCs. [13]. Therefore, choice of the mesenchymal stem cell source belongs to the aim. Low immunogenetic potential makes MSCs appropriate source for stem cell therapy. In this section, we will discuss sources of the mesenchymal stem cells, differentiation and regeneration potential and usage in cell therapy.

12.2 SOURCES OF MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) are spindle-shaped cells with self-renewal abilities and are identified as safe cell sources for stem cell therapy. By using cell culture techniques, they can be isolated from various sources and grow by attaching to the tissue culture dishes. They express specific surface markers CD73, CD90, and CD105 [14]. And the absence of hematopoietic markers such as Cd45, CD34, CD14, CD19, and CD3. MSCs give rise to osteoblasts, adipocytes, and chondrocytes [15]. The major and most studied sources of mesenchymal stem cells are bone marrow, umbilical cord, and adipose tissue. Moreover, obtaining MSCs from dental pulps, peripheral blood, skin, and placenta is also a target of recent studies. The restrictions on isolating MSCs are mainly invasive isolation procedures and the availability of the sources. Therefore, selecting the cell source and considering the possible negative effects of collecting cells from donors are important [16].

Even the umbilical cord and placenta have a better proliferative capacity, the most used tissue for isolation of MSCs is bone marrow. Obtained bone marrow MSCs can be scaled up to a commercial scale [17]. However, obtaining MSCs from BM is an invasive process in which the cells are collected from iliac crest of pelvic bone and requires anesthesia. This process

can cause infection, bleeding, or pain. UC-MSCs specified a high proliferation rate and low expression of p53, p16, and p21. Whole umbilical cord or its compartments can be used for the isolation of MSCs. The easily accessibility of the UC makes it a good source for MSCs [18]. Placental MSCs showed higher therapeutic effects when compared with BM in animal models. Difference expression levels of cell surface antigens, proliferation and differentiation capacity can be compared between the sources of MSCs [16]. For example, Bernardo et al. determined a higher chondrogenic differentiation potential of BM-MSCs than fetal and PL MSCs. [19]. The advantage of using perinatal tissues for MSC source is the discard of the perinatal tissues after delivery [20]. Moreover, it was evaluated that perinatal MSCs are affected by environmental factors less than adult MSCs which can cause GVHD therefore they are optimal sources for the MSC based therapy [21]. Placental MSCs share common features as high self-renewal capacity, high proliferation rate, and, multipotency while showing different morphologies and different roles in cellular therapy. Amniotic MSCs have a high potential to differentiate into cardiomyocytes while umbilical cord MSCs can differentiate osteogenic phenotypes [22,23]. Recent studies showed chorionic MSCs differentiated into neuronal cells, on the other hand, decidual MSCs have immunomodulatory effects [24,25]. It was evaluated that based on the cellular therapy the origin of the placental MSCs must be chosen well.

MSCs of adipose tissue isolated after liposuction or lipectomy and from fat tissue located in the abdomen, femoral or gluteal sections. MSCs obtained from the latter two sections have higher differentiation capacity than abdominal cells. Moreover, the harvesting depth of the adipose tissue affects the differentiation capacity of the MSCs. Interestingly, adipose

tissue-derived MSCs obtained from pregnant women have higher proliferation capacities [26]. When compared to BM MSCs, collection of adipose-derived MSCs is less invasive and become one of the ideal sources of MSCs [18]. Dental MSCs can be isolated from various dental tissues such as dental pulp, papilla, periodontal ligament, gingiva, dental follicle, tooth, and alveolar bone, which show typical MSC features and can be obtained in a safe, effective, and easy way. They can be differentiated into ectodermal and endodermal lineages, moreover, they have immunomodulatory properties by secreting cytokines [15].

12.3 DIFFERENTIATION POTENTIAL OF MESENCHYMAL STEM CELLS

The huge differentiation capacity of the mesenchymal stem cells is one of the main and most important features of the MSCs and this feature mainly depends on the source of MSC (Figure 2). While BM-MSCs has superior capacity to differentiate into osteoblasts and chondrocytes, UC-MSCs have many biological advantages to differentiate into many cell types than adults MSCs as adipose tissue MSCs. It was evaluated that MSC types should be chosen according to the purpose [27].

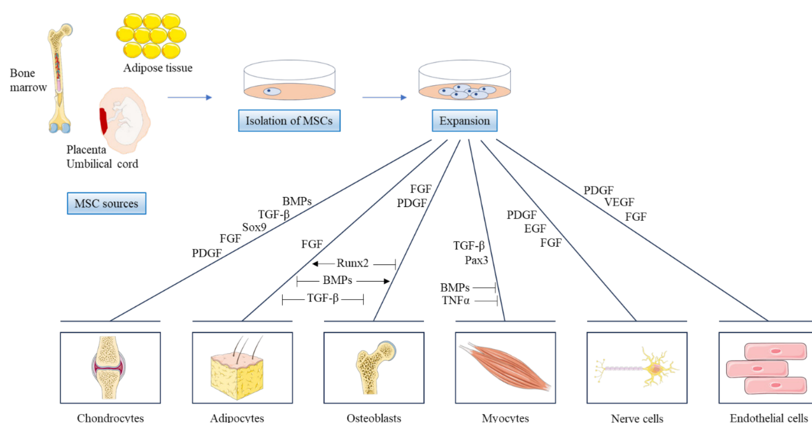


Figure 2: Differentiation of Mesenchymal Stem Cells from Different Sources and molecules

12.3.1 Osteogenic and chondrogenic differentiation

The first osteogenic differentiation of MSCs identified in 1970s by Friedenstein et al. [28]. In vitro studies showed MSCs can differentiated into osteoblasts during a one-month period and after second week of the differentiation expression of the osteoblast markers (as alkaline phosphatase (ALP) can be detected [29]. Extracellular matrix is an important indicator of the osteogenic differentiation [30]. The comparison of placental MSCs in osteogenic differentiation capacity showed that AM-MSCs and UC-MSCs can be optimal sources for bone repairment and osteogenic therapies [31]. Inflammatory factors stimulate migration and differentiation of MSCs in acute infections, however in chronic inflammation, the ability of differentiation can change. Therefore, it is worthy to evaluate that the acute and chronic inflammations have different effects on MSC differentiation and the regulatory mechanisms of these differentiation can be variable [32]. Developments in the tissue engineering reveals scaffolds to be used in bone repair and it was showed that bone-marrow derived MSCs could be use

as a more prevalent in vitro model [33]. On the other hand, osteogenic differentiation capacity depends on replicative senescence and origin of tissue [34]. It has been stated that not only tissue repair related pathways, but also immune system is effective in osteogenic differentiation. This is due to the intertwined interactions of the skeletal system and the immune system [35].

TGF-beta family especially beta1,2 and 3 is important in chondrogenesis of MSCs and bone morphogenetic proteins (BMPs) as well. After stimulation by these growth factors and proteins, collagen type II and proteoglycans produced, and cartilage formation started. When adipose derived MSCs compared to bone marrow derived MSCs, adipose tissue MSCs lack of TGF-beta receptor and BMP-2, 4 and 6 expression has lower levels than BM-MSCs. Therefor it was evaluated that if you are working with adipose tissue MSCs, it is necessary to supply BMP-6 and TGF-beta for chondrogenic differentiation [36]. Moreover, it was showed that canonical Wnt signaling influences MSC osteogenesis [37]. Signal transduction pathways as MAPK, Smads are induce chondrogenic differentiation of MSCs. Due to these pathways, extracellular matrix proteins produced and cartilage formation started. A transcription factor in Sox9 is important in bone marrow derived MSC chondrogenesis [38]. Many other transcription factors determined in chondrogenic differentiation, and it is obvious that these factors take place in MSCs from different sources.

12.3.2 Neuronal differentiation

During development of a fetus, neuronal stem cells generate neurons, however they have limited ability for the regeneration. On the other hand, adult mesodermal stem cells have a potential to generate neuronal and glial cells due to

inducers. It was already known that these adult mesenchymal stem cells can be used in tissue repair by differentiation into, adipocytes, osteoblasts, chondrocytes, cardiomyocytes, hepatocytes, and neuronal cells. In neurodegenerative disorders adult MSCs become an option for the treatment and clinical trials showed that alterations in cellular signaling and metabolic events observed[39].

Ikegame et al indicated that AD-MSCs have a higher neuronal differentiation potential than BM-MSCs in an animal model[40]. In an in vitro co-culture system, BM-MSCs and spleen and thymus MSCs have the similar capacities of the differentiation into glial cells [41]. Stem cell survival mainly based on the growth factors as Epidermal Growth Factors (EGF), Fibroblast Growth Factor, basic (bFGF), Platelet-derived Growth Factor (PDGF). Moreover, hedgehog signaling pathway regulate the neuronal development. It is obvious that many growth factors, signaling pathways or receptor/ligand interactions effect the neuronal regeneration of the MSCs. Therefore, it is not just a mechanism controlled by a few signaling pathways of genes, rather than it consists of a more complex and dynamic interactions of the many proteins[39].

12.3.3 Adipogenic differentiation

Adipogenic differentiation of MSCs are important in the treatment of soft tissue defects, damaged organs, and repair of adipose tissue[42]. Adipogenesis is the movement of the lipid particles from intracellular vesicles and MSCs have a feature to differentiate into adipose tissue. As the other differentiation processes, several transcription factors play role in adipogenesis. Peroxisome proliferation activated receptor proteins are a group of these transcription factors and regulates gene expression which is responsible for adipogenesis[43]. Early B

cell factor EBF-1 have important role both in adipogenesis and osteogenesis[44]. Zinc finger proteins and transcription factors mainly induce proliferation and differentiation of the cells. However, it was also showed that GATA-2, a zinc finger transcription factor, plays role in adipogenic differentiation of MSCs[45]. Several key molecules including peroxisome proliferator-activated receptor-gamma (PPAR-γ), transforming growth factor-beta (TGF-β), bone morphogenic protein (BMP), and Wnt signaling pathway proteins could control the adipogenic differentiation. It was also reported that adipogenic/osteogenic differentiation balance have a critical role in differentiation of MSCs into bone tissue [46]. Among these molecules PPAR-γ is the most important one during adipocyte formation and is the master regulator of adipogenesis [47]. Genetic manipulation of PPAR-γ demonstrated that induced adipocyte formation and increased bone mass have been developed in PPAR—γ+ mices[48]. Last years, among 2D studies, 3D culture systems and different matrix have place in adipogenesis of MSCs. Studies showed that low-viscosity gelation solutions could be useful for adipogenic differentiation of MSCs.[49]. With the development in bioengineered matrixes give us more efficient regeneration potential of MSCs into adipose tissues.

12.3.4 Myogenic differentiation

Myogenic differentiation can be divided in to three as differentiation into skeletal muscle cells, cardiac cells, and smooth muscle cells. The differentiation potential of the MSCs depend on the transcription factors that regulate myogenic differentiation as paired bow 3 (Pax3), MyoD and myogenin. Pax3 is main protein of the myogenic differentiation, and its over expression inhibits adipogenic, osteogenic and chondrogenic differentiation while induce myogenesis [50]. Increasing levels of signaling factors as insulin-like-growth factor (IGF-II) pro-

motes to myogenic differentiation[51]. TNF-alpha has regulatory factor in myogenesis that inhibits MyoD expression. Moreover TNF-alpha have inhibitor effect on myocyte differentiation of MSCs via NF-KB pathway[52].

MSC coculture by cardiomyocytes promotes to the cardiogenic differentiation of these cells. Zinc finger transcription factors also play role in cardiomyogenic transformation of the MSCs. GATA4 is one of the zinc finger transcription factors which increasing levels lead to the differentiation to cardiomyocytes[53]. It was reported that overexpression of the myocardin gene causes induction of expression of cardiomyogenic markers on MSCs[54]. Several researches showed that adenovectors which carry transcription factors that is required for MSC cardiogenic differentiation increase differentiation capacity of MSCs into cardiomyocytes. In vitro and in vivo studies revealed that transfection of MSCs with adenoviruses can develop the differentiation potential of the MSCs [55,56]. TGF-beta was shown the most effective protein on smooth muscle cell differentiation of MSCs which inhibits proliferation of MSCs and induces muscle cell differentiation. GATA6 is important in smooth muscle cell differentiation and activates the expression of smooth muscle cell marker genes[57]. There is still need studies to investigate the role of proteins and genes in myogenic differentiation of MSCs.

12.3.5 Endothelial cell differentiation

Asahara et al defined endothelial progenitor cells which is mainly originated from bone marrow developed into endothelial cells [58]. Endothelial cells important in lesion repairs and angiogenesis. Therefore, strategies for this regeneration can solve several clinical problems. Studies showed that interaction between MSCs and endothelial cells can lead to the

formation of microvessel like structures[60]. Not only direct interactions but also paracrine signaling plays role in this differentiation process [61]. VEGF is an important factor in endothelial differentiation of MSCs[62].

Khaki et al. indicated that VEGF-A is very effective in differentiation of MSCs to ECs especially when they transfected with VEGF-A expressing plasmids[63]. Oswald at al showed the first-time differentiation capacity of the bone marrow derived MSCs into endothelial cells in vitro[64]. Wang et al developed a method that leads differentiation of MSCs into ECs. They used bone marrow derived MSCs which growth into a medium contains VEGF, basic fibroblast growth factor (bFGF), insulin like growth factor (IGF), epidermal growth factor (EGF), ascorbic acid and heparin[65].

The differentiation potential of MSCs depends on several transcription factors, growth factors and proteins. They have multi-lineage differentiation potential and therefore they can be used in several therapeutic processes as tissue regeneration, inherited disorders, chronic inflammations. Understanding of the MSC differentiation mechanisms and molecules that promotes this regenerative potential carry us to a new therapeutic world.

12.4 MSC USAGE IN CELL THERAPY

Migration ability toward secreted cytokines, chemokines and growth factors, tissue repair and regeneration capacity, immunomodulation ability, anti-inflammatory effects, anti-apoptotic activities by inhibiting programmed cell death, neovascularization capacity and antimicrobial effects makes MSCs valuable in employing them for several therapies (Figure 3). MSCs can be applied on local/topical as a cell-spray, intraperitoneal or muscular injection, intravenous or intra-ar-

terial and, bioengineered molecules as scaffolds. These routes could be chosen according to the therapy and disease [66]. Embryonic stem cells (ESCs) induced pluripotent stem cells (iPSC) and adult stem cells (mesenchymal stem cells) are mainly used for the therapies. Even ESCs have pluripotency, the major limitation of use of ESCs is ethical concerns [67]. iPSCs have great potential in cell therapy however tumorigenic potential, genetic mutations, epigenetic abnormalities are major disadvantages of usage of iPSCs in cellular therapy. Adult stem cells originated from several sources reported as more advantageous than ESCs and iPSCs [68].

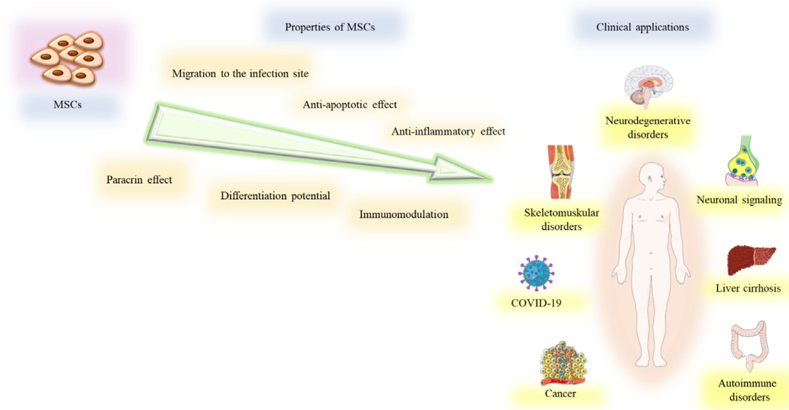


Figure 3: Properties and clinical usage of MSCs

MSC based cell therapy is clinically studied mainly in cardiovascular diseases, neurodegenerative diseases, bone diseases, cancers, liver diseases, kidney diseases, autoimmune diseases. The regeneration and differentiation mechanisms of transplanted MSCs are still unknown however, paracrine signaling have effect on differentiation process[69]. In Parkinson disease differentiation of MSCs into dopaminergic neuron cells have been reported in rat models[70]. In MS, a degenerative inflammatory disease of the central nervous system, MSCs was indicated to prevent inflammation in animal models[71].

Graft versus host disease (GVHD) is one of the problems in hematopoietic stem cell transplantation and in a case report that has been published that the researchers overcome this problem by using MSCs. After then usage of MSCs in transplantation generated interest [72]. Studies showed that MSCs can modulate immune system that is responsible for the tissue rejection, and it was reported in murine models that by using MSCs rejection of the tissue and organs in skin, cornea, kidney, liver transplantations can be prevented [73-76].

As seen in transplantation studies, MSCs regulate immune response therefore they can be used in autoimmune disorders. In autoimmune diseases, body's immune cells reacted to own cells which causes several problems. Intravenous injection of allogenic MSCs reduces mucosal inflammation in ulcerative colitis [77]. Moreover beta-cell function preservation was reported by BMMSCs in Type 2 diabetes mellitus [78]. Allogenic MSC therapy could have a therapeutic potential in autoimmune arthritis [79]. The damage of Lupus and Crohn disease on kidneys could be suppress by using autologous and allogeneic MSCs [80,81].

Immunomodulatory effect of MSCs lead to prevent inflammation symptoms of bone degeneration. One of the most common bone degenerative disorders is osteoarthritis. All medications are used for symptom and pain control, therefore MSC based studies focused on osteoarthritis long term cure [82]. Different phase reports (Phase 1,2,3) were published included BM-MSCs, ADMSCs or UC-MSCs [83-86]. The first report of usage of MSCs in liver cirrhosis was published in 2004 and, demonstrated the reduction of liver fibrosis by BM-MSCs infusion in murine models [87]. Cirrhosis is an end stage hepatic disease caused by hepatitis viruses, alcohol and several liver diseases. In many patients, organ transplantation could be the

last choice for the treatment of the end stage liver disease. Several preclinical studies evaluated the BM-MSCs can inhibit inflammatory responses and ameliorate cirrhosis [88,89,90]. In several phases 1,2,3 studies have been published with different MSCs sources and different causatives of liver failure or cirrhosis. However further clinical studies needed to be ensured the usage of MSCs effectively in liver failures [91-99].

Pulmonary diseases can be divided into two as non-infectious and infectious, and both of which can be ameliorated by using MSCs. Asthma, one of the most common non-infectious pulmonary diseases, causes alveolar epithelial damage and MSCs have been thought to repair these damages. In infectious pulmonary diseases, as acute respiratory distress syndrome (ARDS), it was determined that MSC cell therapy could be useful to overcome host immune response [100-101]. After COVID-19 pandemic spread all over the world and started to cause irreversible damages in alveolar cells, MSC therapy studies gave rise to treat several SARS-CoV-2 caused disorders. This RNA virus pathogenesis starts by recognizing of angiotensin-converting enzyme-2 (ACE2) receptor by its spike protein and therefore all the ACE-2 positive cells could be infected by this virus [102]. In general, ACE2 receptor is distributed on alveoles, capillary epithelium and therefore the most damaged are of the bodies were lungs. Moreover, bone marrow, lymph nodes, immune cells, spleen can also be affected by this virus infection [103]. Not only by the receptor ligand interactions, but also cytokine storm caused by this virus results in pulmonary edema, ARDS, acute cardiac injury and death [104]. MSC therapy thought to prevent cytokine storm by inhibits the effect of immune cells [105]. Intravenous MSC injection could recover the alveolar epithelial damage and can cure lung disfunction [106]. However, preparation of

MSCs restricts the usage of them in clinical level and at this points stem cell banks can be the best opportunity to cure acute diseases caused by COVID-19 or any other disorder[107]. On the shelf MSC cells could be a good solution to reach this therapy choice in clinic and could be used in combination with conventional therapies.

12.5 CONCLUSION

MSCs can be applied in several disorders and diseases among their features as huge proliferation and multilineage differentiation capacity, easily isolation techniques, compatibility of manufacturing, and paracrine effects. The most studied MSCs are derived from UC, BM and AD and researchers trying to optimize the usage of biomaterials for MSCs from different origins. Every type of MSC can be activated or inhibited by several proteins and studies focused on these molecules as well as miRNA or epigenetic effects on MSC production. Clinical trials study on different MSCs types, doses, and combined therapies with conventional treatments. A better relationship between pre-clinical and clinical studies are mandatory to enhance the MSC therapy. This chapter focused on the place of the MSCs among stem cells, the origins, differentiation capacities and therapeutically usefulness in several diseases. Further studies required to ensure the potential of the regenerative MSC therapy.

12.6 REFERENCES

1. Polak JM, Bishop AE. Stem cells and tissue engineering: past, present, and future. Annals of the New York Academy of Sciences 2006; 1068(1): 352-366. https://doi.org/10.1196/annals.1346.001
2. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Reviews and Reports 2011; 7: 269-291. https://doi.org/10.1007/s12015-010-9193-7
3. Fitzsimmons RE, Mazurek MS, Soos A, Simmons CA. Mesenchymal stromal/stem cells in regenerative medicine and tissue engineering. Stem cells international 2018; 1-16. https://doi.org/10.1155/2018/8031718
4. Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. Journal of Cellular and molecular medicine 2004; 8(3): 301-316. https://doi.org/10.1111/j.1582-4934.2004.tb00320.x
5. Velasquillo C, Madrazo-Ibarra A, Gutiérrez-Gómez C, Pérez-Dosal MR, Melgarejo-Ramírez Y et al. Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases. Plastic and Aesthetic Research 2020; 7: 31. http://dx.doi.org/10.20517/2347-9264.2020.30
6. Varderidou-Minasian S, Lorenowicz MJ. Mesenchymal stromal/stem cell-derived extracellular vesicles in tissue repair: challenges and opportunities. Theranostics 2020; 10(13): 5979. https://doi.org/10.7150/thno.40122
7. Marofi F, Vahedi G, Hasanzadeh A, Salarinasab S, Arzhanga P et al. Mesenchymal stem cells as the game-changing tools in the treatment of various organs disorders: Mirage or reality? Journal of Cellular Physiology 2019; 234(2): 1268-1288. https://doi.org/10.1002/jcp.27152
8. Zhao L, Chen S, Yang P, Cao H, Li L. The role of mesenchymal stem cells in hematopoietic stem cell transplantation: prevention and treatment of graft-versus-host disease. Stem cell research & therapy 2019;10(1): 1-13. https://doi.org/10.1186/s13287-019-1287-9

9. Edgar L, Pu T, Porter B, Aziz JM, La Pointe C et al. Regenerative medicine, organ bioengineering, and transplantation. Journal of British Surgery 2020; 107(7): 793-800. https://doi.org/10.1002/bjs.11686

10. Khan WS, Hardingham TE. Mesenchymal stem cells, sources of cells and differentiation potential. Journal of Stem Cells 2012; 7(2): 75.

11. Bongso A, Richards M. History and perspective of stem cell research. Best practice & research Clinical obstetrics & gynaecology 2004; 18(6): 827-842. https://doi.org/10.1016/j.bpobgyn.2004.09.002

12. Barky AR, Ali Ehab MM, Mohamed TM. Stem cells, classifications and their clinical applications. Am J Pharmacol Ther, 2017; 1(1): 001-007.

13. Si Z, Wang X, Sun C, Kang Y, Xu J et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. Biomedicine & Pharmacotherapy 2019; 114: 108765. https://doi.org/10.1155/2016/6737345

14. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell proliferation *2020; 53*(1): e12712. https://doi.org/10.1111/cpr.12712

15. Lu Gan, Ying Liu, Dixin Cui, Yue Pan, Liwei Zheng et al. Dental Tissue-Derived Human Mesenchymal Stem Cells and Their Potential in Therapeutic Application. Stem Cells International 2020; 1-17. https://doi.org/10.1155/2020/8864572.

16. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem cell research & therapy 2021;12(1): 1-30. https://doi.org/10.1186/s13287-021-02265-1

17. Rodríguez-Fuentes DE, Fernández-Garza LE, Samia-Meza JA, Barrera-Barrera SA, Caplan AI et al. Mesenchymal stem cells current clinical applications: a systematic review. Archives of medical research 2021; 52(1): 93-101. https://doi.org/10.1016/j.arcmed.2020.08.006

18. Naji A, Eitoku M, Favier B, Deschaseaux F, Rouas-Freiss N et al. Biological functions of mesenchymal stem cells and clinical implications. Cellular and Molecular Life Sciences 2019*;* 76: 3323-3348. https://doi.org/10.1007/s00018-019-03125-1

19. Bernardo M, Emons J, Karperien M, Nauta A, Willemze R et al. Human mesenchymal stem cells derived from bone marrow display a better chondrogenic differentiation compared with other sources. Connect Tissue Res 2007; 48:132–40. https://doi.org/10.1080/03008200701228464

20. Brown C, McKee C, Bakshi S, Walker K, Hakman E et al. Mesenchymal stem cells: Cell therapy and regeneration potential. Journal of tissue engineering and regenerative medicine 2019; 13(9): 1738-1755. https://doi.org/10.1002/term.2914

21. Avercenc-Léger L, Guerci P, Virion JM, Cauchois G, Hupont S et al. Umbilical cord-derived mesenchymal stromal cells: Predictive obstetric factors for cell proliferation and chondrogenic differentiation. Stem Cell Research & Therapy 2017: 8(1); 1-13. https://doi.org/10.1186/s13287-017-0609-z.

22. Markmee R, Aungsuchawan S, Tancharoen W, Narakornsak S, Pothacharoen P. Differentiation of cardiomyocyte-like cells from human amniotic fluid mesenchymal stem cells by combined induction with human platelet lysate and 5-azacytidine. Heliyon. 2020;16:6(9):e04844. https://doi.org/10.1016/j.heliyon.2020.e04844.

23. Hendrijantini N, Hartono P. Phenotype Characteristics and Osteogenic Differentiation Potential of Human Mesenchymal Stem Cells Derived from Amnion Membrane (HAMSCs) and Umbilical Cord (HUC-MSCs). Acta Inform Med. 2019;27(2):72-77. https://doi.org/10.5455/aim.2019.27.72-77.

24. Miao Z, Sun H, Xue Y. Isolation and Characterization of Human Chorionic Membranes Mesenchymal Stem Cells and Their Neural Differentiation. Tissue Eng Regen Med. 2017;14(2):143-151. https://doi.org/10.1007/s13770-017-0025-6.

25. Kim SH, Jung J, Cho KJ, Choi JH, Lee HS et al. Immunomodulatory Effects of Placenta-derived Mesenchymal Stem Cells on T Cells by Regulation of FoxP3 Expression. Int J Stem Cells. 2018;11(2):196-204. https://doi.org/10.15283/ijsc18031.

26. Yang HJ, Kim KJ, Kim MK, Lee SJ, Ryu YH et al. The Stem Cell Potential and Multipotency of Human Adipose Tissue-Derived Stem Cells Vary by Cell Donor and Are Different from Those of Other Types of Stem Cells. Cells Tissues Organs 2015; 199 (5-6): 373–383. https://doi.org/10.1159/000369969.

27. Han Y, Li X, Zhang Y, Han Y, Chang F et al. Mesenchymal stem cells for regenerative medicine. Cells 2019; 8(8): 886. https://doi.org/10.3390/cells8080886

28. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970; 3: 393–403. https://doi.org/10.1111/j.1365-2184.1970.tb00347.x

29. Quarles LD, Yohay DA, Lever LW, Caton R, Wenstrup RJ. Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: an in vitro model of osteoblast development. J Bone Miner Res Off J Am Soc Bone Miner Res. 1992; 7: 683–92. http://dx.doi.org/10.1002/jbmr.5650070613

30. Hanna H, Mir LM, Andre FM. In vitro osteoblastic differentiation of mesenchymal stem cells generates cell layers with distinct properties. Stem Cell Res Ther 2018; 9: 203 https://doi.org/10.1186/s13287-018-0942-x.

31. Shen C, Yang C, Xu S, Zhao H et al. Comparison of osteogenic differentiation capacity in mesenchymal stem cells derived from human amniotic membrane (AM), umbilical cord (UC), chorionic membrane (CM), and decidua (DC). Cell Biosci 2019;9: 17. https://doi.org/10.1186/s13578-019-0281-3.

32. Gu F, Zhang K, Li J, Xie X, Wen Q et al. Changes of Migration, Immunoregulation and Osteogenic Differentiation of Mesenchymal Stem Cells in Different Stages of Inflammation. Int J Med Sci 2022; 19(1): 25-33. https://doi.org/10.7150/ijms.58428.

33. Mazzoni E, Mazziotta C, Iaquinta MR, Lanzillotti C, Fortini F et al. Enhanced osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by a hybrid hydroxylapatite/collagen scaffold. Frontiers in Cell and Developmental Biology 2021; 8: 610570. https://doi.org/10.3389/fcell.2020.610570

34. Grotheer V, Skrynecki N, Oezel L, Windolf J, Grassmann J. Osteogenic differentiation of human mesenchymal stromal cells and fibroblasts differs depending on tissue origin and replicative senescence. Sci Rep 2021; 11: 11968. https://doi.org/10.1038/s41598-021-91501-y.

35. Ye F, Li J, Xu P, Xie Z, Zheng G et al. Osteogenic differentiation of mesenchymal stem cells promotes c-Jun-dependent secretion

of interleukin 8 and mediates the migration and differentiation of CD4+ T cells. Stem Cell Research & Therapy 2022; 13(1): 1-18. https://doi.org/10.1186/s13287-022-02735-0

36. Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A et al. 2007. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFβ receptor and BMP profile and is overcome by BMP-6. J Cell Physiol 2007; 211: 682–691. https://doi.org/10.1002/jcp.20977
37. Tuli R, Tuli S, Nandi S, Huang X, Manner PA et al. Transforming growth factor-beta-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. J Biol Chem 2003; 278: 41227–41236. https://doi.org/10.1074/jbc.m305312200
38. Tsuchiya H, Kitoh H, Sugiura F, Ishiguro N. Chondrogenesis enhanced by overexpression of sox9 gene in mouse bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun 2003; 301: 338–343. https://doi.org/10.1016/s0006-291x(02)03026-7
39. George S, Hamblin MR, Abrahamse H. Differentiation of Mesenchymal Stem Cells to Neuroglia: in the Context of Cell Signalling. Stem Cell Rev and Rep 2019; 15: 814–826. https://doi.org/10.1007/s12015-019-09917-z.
40. Ikegame Y, Yamashita K, Hayashi SI, Mizuno H, Tawada M et al. Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 2011; 13(6): 675-685. https://doi.org/10.3109/14653249.2010.549122
41. Krampera M, Marconi S, Pasini A, Galiè M, Rigotti G et al. Induction of neural-like differentiation in human mesenchymal stem cells derived from bone marrow, fat, spleen and thymus. Bone 2007; 40(2): 382-390. https://doi.org/10.1016/j.bone.2006.09.006
42. Zhong Y, Li X, Wang F, Wang S, Wang X et al.Emerging potential of exosomes on adipogenic differentiation of mesenchymal stem cells. Frontiers in Cell and Developmental Biology 2021; 9: 649552. https://doi.org/10.3389/fcell.2021.649552
43. Bionaz M, Monaco E, Wheeler MB.Transcription adaptation during in vitro adipogenesis and osteogenesis of porcine mesenchymal stem cells: dynamics of pathways, biological processes,

up-stream regulators, and gene networks. PloS one 2015; 10(9): e0137644. https://doi.org/10.1371/journal.pone.0137644

44. Hesslein DG, Fretz JA, Xi Y, Nelson T, Zhou S et aş. Ebf1-dependent control of the osteoblast and adipocyte lineages. Bone 2009; 44(4): 537-546. https://doi.org/10.1016%2Fj.bone.2008.11.021

45. Okitsu Y, Takahashi S, Minegishi N, Kameoka J, Kaku M et al. Regulation of adipocyte differentiation of bone marrow stromal cells by transcription factor GATA-2. Biochemical and biophysical research communications 2007; 364(2): 383-387. https://doi.org/10.1016/j.bbrc.2007.10.031

46. Cen S, Li J, Cai Z, Pan Y, Sun Z et al. TRAF4 acts as a fate checkpoint to regulate the adipogenic differentiation of MSCs by activating PKM2. EBioMedicine 2020;54: 1-21 https://doi.org/10.1016/j.ebiom.2020.102722

47. James AW. Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. Scientifica 2013;1-18. https://doi.org/10.1155/2013/684736

48. Tzameli I., Fang H, Ollero M, Shi H, Hamm JK et al. Regulated production of a peroxisome proliferator-activated receptor-γ ligand during an early phase of adipocyte differentiation in 3T3-L1 adipocytes. Journal of Biological Chemistry 2004; 279(34): 36093-36102. https://doi.org/10.1074/jbc.m405346200

49. Lee K, Chen Y, Yoshitomi T, Kawazoe N, Yang Y et al. Osteogenic and adipogenic differentiation of mesenchymal stem cells in gelatin solutions of different viscosities. Advanced Healthcare Materials 2020; 9(23): 2000617. https://doi.org/10.1002/adhm.202000617

50. Gang EJ, Bosnakovski D, Simsek T, To K, Perlingeiro RC. Pax3 activation promotes the differentiation of mesenchymal stem cells toward the myogenic lineage. Experimental cell research 2008, 314(8), 1721-1733. https://doi.org/10.1016/j.yexcr.2008.02.016

51. Almalki SG, Agrawal DK. Key transcription factors in the differentiation of mesenchymal stem cells. Differentiation 2016; 92(1-2): 41-51. https://doi.org/10.1016%2Fj.diff.2016.02.005

52. Sitcheran R, Cogswell PC, Baldwin AS. NF-κB mediates inhibition of mesenchymal cell differentiation through a posttranscriptional gene silencing mechanism. Genes & development 2003; 17(19): 2368-2373. https://doi.org/10.1101/gad.1114503

53. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circulation research 2010; 107(7): 913-922. https://doi.org/10.1161/circresaha.110.222703
54. van Tuyn J, Knaän-Shanzer S, van de Watering MJ, de Graaf M, van der Laarse A et al. Activation of cardiac and smooth muscle-specific genes in primary human cells after forced expression of human myocardin. Cardiovascular research 2005; 67(2):245-255. https://doi.org/10.1016/j.cardiores.2005.04.013
55. Suresh SC, Selvaraju V, Thirunavukkarasu M, Goldman JW, Husain A et al. Thioredoxin-1 (Trx1) engineered mesenchymal stem cell therapy increased pro-angiogenic factors, reduced fibrosis and improved heart function in the infarcted rat myocardium. International journal of cardiology 2015; 201: 517-528. https://doi.org/10.1016/j.ijcard.2015.08.117
56. Ding R, Jiang X, Ha Y, Wang Z, Guo J et al. Activation of Notch1 signalling promotes multi-lineage differentiation of c-KitPOS/NKX2. 5POS bone marrow stem cells: implication in stem cell translational medicine. Stem cell research & therapy 2015; 6(1): 1-15. https://doi.org/10.1186%2Fs13287-015-0085-2
57. Deaton RA, Su C, Valencia TG, Grant SR. Transforming growth factor-β1-induced expression of smooth muscle marker genes involves activation of PKN and p38 MAPK. Journal of Biological Chemistry 2005, 280(35), 31172-31181. https://doi.org/10.1074/jbc.m504774200
58. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964–967. https://doi.org/10.1126/science.275.5302.964
59. Geng YJ, Yang YJ, Casscells SW, Willerson JT. Vascular stem cells: A new concept in the pathogenesis of atherosclerosis and interventions for coronary heart disease. Future Cardiol 2006; 2: 585–592. https://doi.org/10.2217/14796678.2.5.585
60. Li H, Daculsi R, Grellier M, Bareille R, Bourget C et al. The role of vascular actors in two dimensional dialogue of human bone marrow stromal cell and endothelial cell for inducing self-assembled network. PLoS One 2011; 6:e167672011. https://doi.org/10.1371/journal.pone.0016767

61. Mayer H, Bertram H, Lindenmaier W, Korff T, Weber H et al. Vascular endothelial growth factor (VEGF-A) expression in human mesenchymal stem cells: Autocrine and paracrine role on osteoblastic and endothelial differentiation. J Cell Biochem 2005; 95: 827–839. https://doi.org/10.1002/jcb.20462
62. Ge Q, Zhang H, Hou J, Wan L, Cheng W et al. VEGF secreted by mesenchymal stem cells mediates the differentiation of endothelial progenitor cells into endothelial cells via paracrine mechanisms. Molecular Medicine Reports 2018; 17 (1): 1667-1675. https://doi.org/10.3892%2Fmmr.2017.8059
63. Khaki M, Salmanian AH, Abtahi H, Ganji A, Mosayebi G. Mesenchymal Stem Cells Differentiate to Endothelial Cells Using Recombinant Vascular Endothelial Growth Factor -A. Rep Biochem Mol Biol. 2018;6(2):144-150.
64. Oswald J, Boxberger S, Jørgensen B, Feldmann S, Ehninger G et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem cells 2004; 22(3): 377-384. https://doi.org/10.1634/stemcells.22-3-377
65. Wang C, Li Y, Yang M, Zou Y, Liu H et al. Efficient differentiation of bone marrow mesenchymal stem cells into endothelial cells in vitro. European Journal of Vascular and Endovascular Surgery 2018; 55(2): 257-265. https://doi.org/10.1016/j.ejvs.2017.10.012
66. Saeedi P, Halabian R, Imani Fooladi AA. A revealing review of mesenchymal stem cells therapy, clinical perspectives and modification strategies. Stem Cell Investig. 2019; 25(6):34. https://doi.org/10.21037/sci.2019.08.11.
67. de Wert G, Mummery C. Human embryonic stem cells: Research, ethics and policy. Human Reproduction 2003;18: 672–682 https://doi.org/10.1093/humrep/deg143
68. Jovic D, Yu Y, Wang D, Wang K, Li H et al. A brief overview of global trends in MSC-based cell therapy. Stem Cell Reviews and Reports, 2022; *18*(5): 1525-1545. https://doi.org/10.1007/s12015-022-10369-1
69. Lee S, Choi E, Cha MJ, Hwang KC. Cell adhesion and long-term survival of transplanted mesenchymal stem cells: A prerequisite for cell therapy. Oxidative Medicine and Cellular Longevity 2015; 632902 https://doi.org/10.1155/2015/632902

70. Wang Y, Yang J, Li H, Wang X, Zhu L et al. Hypoxia promotes dopaminergic differentiation of mesenchymal stem cells and shows benefits for transplantation in a rat model of Parkinson's disease. PLoS ONE 2013; 8(1): e54296. https://doi.org/10.1371/journal.pone.0054296
71. Uccelli A, Laroni A, Brundin L, Clanet M, Fernandez O et al. MEsenchymal StEm cells for Multiple Sclerosis (MESEMS): A randomized, double blind, cross-over phase I/II clinical trial with autologous mesenchymal stem cells for the therapy of multiple sclerosis. Trials 2019; 20(1): 1-13. https://doi.org/10.1186/s13063-019-3346-z
72. Dahlke MH, Hoogduijn M, Eggenhofer E, Popp FC, Renner P et al. Toward MSC in solid organ transplantation: 2008 position paper of the MISOT study group. Transplantation 2009; 88(5): 614-619. https://doi.org/10.1097/tp.0b013e3181b4425a
73. Zhang Y, Zhao D, Tian C, Li F, Li X et al. Stro-1-positive human mesenchymal stem cells prolong skin graft survival in mice. Transplant Proc. 2013; 45: 726-729. http://dx.doi.org/10.1016/j.transproceed.2012.06.086
74. Niu J, Yue W, Song Y, Zhang Y, Qi X et al. Prevention of acute liver allograft rejection by IL-10-engineered mesenchymal stem cells. Clinical & Experimental Immunology 2014;176(3): 473-484. https://doi.org/10.1111/cei.12283
75. Cao Z, Zhang G, Wang F, Liu H, Liu L et al. Protective effects of mesenchymal stem cells with CXCR4 up-regulation in a rat renal transplantation model. PLoS One. 2013; 8: e82949. https://doi.org/10.1371/journal.pone.0082949
76. Jia Z, Jiao C, Zhao S, Li X, Ren X et al. Immunomodulatory effects of mesenchymal stem cells in a rat corneal allograft rejection model. Exp Eye Res 2012;102:44-49. https://doi.org/10.1016/j.exer.2012.06.008
77. Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. Clin Gastroenterol Hepatol. 2014; 12: 64-71. https://doi.org/10.1016/j.cgh.2013.06.021

78. Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K. Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes 2015; 64: 587-592. https://doi.org/10.2337/db14-0656

79. Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum. 2007;56(4):1175-86. https://doi.org/10.1002/art.22511

80. Sun L, Wang D, Liang J, Zhang H, Feng X et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum 2010; 62: 2467–75. https://doi.org/10.1002/art.27548

81. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulizing Crohn's disease. Gut 2011; 60: 788–98. https://doi.org/10.1136/gut.2010.214841

82. Mobasheri A, Kalamegam G, Musumeci G, Batt ME. Chondrocyte, and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopedic conditions. Maturitas 2014; 78: 188–198. https://doi.org/10.1016/j.maturitas.2014.04.017

83. Emadedin M, Ghorbanı LM, Fazeli R, Mohseni F, Moghadasali R et al. Long-term follow-up of intra-articular injection of autologous mesenchymal stem cells in patients with knee, ankle, or hip osteoarthritis. Arch Iran Med. 2015;18:336-344.

84. Qiao Z, Tang J, Yue B, Wang J, Zhang J et al. Human adipose-derived mesenchymal progenitor cells plus microfracture and hyaluronic acid for cartilage repair: a phase IIa trial. Regen Med. 2020; 15: 1193-1214. https://doi.org/10.2217/rme-2019-0068

85. Matas J, Orrego M, Amenabar D, Infante C, Tapia-Limonchi R et al. Umbilical cord-derived mesenchymal stromal cells (MSCs) for knee osteoarthritis: repeated MSC dosing is superior to a single MSC dose and to hyaluronic acid in a controlled randomized phase I/II trial. Stem Cells Translational Medicine. 2019; 8: 215-224. https://doi.org/10.1002/sctm.18-0053

86. Weiss JN. Comparative Effectiveness of Arthroscopy and Non-arthroscopy Using Mesenchymal Stem Cell Therapy (MSCs) and Conditioned Medium for Osteoarthritis (OA). In: Orthopedic Stem Cell Surgery. Springer, Cham. 2021; 255-258. https://doi.org/10.1007/978-3-030-73299-8_50

87. Fang B, Shi M, Liao L, Yang S, Liu Y et al. Systemic infusion of FLK1(+) mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice. Transplantation. 2004; 78: 83-88. https://doi.org/10.1097/01.tp.0000128326.95294.14
88. Wang S, Lee JS, Hyun J, Kim J, Kim SU et al. Tumor necrosis factor-inducible gene 6 promotes liver regeneration in mice with acute liver injury. Stem Cell Res Ther. 2015; 6: 20. https://doi.org/10.1186%2Fs13287-015-0019-z
89. Mardpour S, Hassani SN, Mardpour S, Sayahpour F, Vosough M et al. Extracellular vesicles derived from human embryonic stem cell-MSCs ameliorate cirrhosis in thioacetamide-induced chronic liver injury. J Cell Physiol. 2018; 233: 9330-9344. https://doi.org/10.1002/jcp.26413
90. Milosavljevic N, Gazdic M, Simovic Markovic B, Arsenijevic A et al. Mesenchymal stem cells attenuate liver fibrosis by suppressing Th17 cells—an experimental study. Transpl Int. 2018; 31:102-115. https://doi.org/10.1111/tri.13023
91. Li T, Xia M, Gao Y, Chen Y, Xu, Y. Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. Expert opinion on biological therapy 2015; 15(9): 1293-1306. https://doi.org/10.1517/14712598.2015.1051528
92. Wang L, Han Q, Chen H, Wang, K, Shan GL et al. Allogeneic bone marrow mesenchymal stem cell transplantation in patients with UDCA-resistant primary biliary cirrhosis. Stem cells and development 2014; 23(20): 2482-2489. https://doi.org/10.1089/scd.2013.0500
93. Zhang Z, Lin H, Shi M, Xu R, Fu J et al. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. Journal of gastroenterology and hepatology 2012; 27: 112-120. https://doi.org/10.1111/j.1440-1746.2011.07024.x
94. Kantarcıoğlu M, Demirci H, Avcu F, Karslıoğlu Y, Babayiğit MA et al. Efficacy of autologous mesenchymal stem cell transplantation in patients with liver cirrhosis. Turk J Gastroenterol 2015; 26(3): 244-250. https://doi.org/10.5152/tjg.2015.0074
95. Can A, Celikkan FT, Cinar O. Umbilical cord mesenchymal stromal cell transplantations: a systemic analysis of clinical trials.

Cytotherapy 2017; 19(12); 1351-1382. https://doi.org/10.1016/j.jcyt.2017.08.004

96. Pampalone M, Corrao S, Amico G, Vitale G, Alduino R et al. Human Amnion-Derived Mesenchymal Stromal Cells in Cirrhotic Patients with Refractory Ascites: A Possible Anti-Inflammatory Therapy for Preventing Spontaneous Bacterial Peritonitis. Stem Cell Reviews and Reports 2021;17: 981-998. https://doi.org/10.1007%2Fs12015-020-10104-8

97. Shi M, Li YY, Xu RN, Meng FP, Yu SJ et al. Mesenchymal stem cell therapy in decompensated liver cirrhosis: a long-term follow-up analysis of the randomized controlled clinical trial. Hepatology international 2021; 15: 1431-1441. https://doi.org/10.1007/s12072-021-10199-2

98. Fang X, Liu L, Dong J, Zhang J, Song H et al. A study about immunomodulatory effect and efficacy and prognosis of human umbilical cord mesenchymal stem cells in patients with chronic hepatitis B-induced decompensated liver cirrhosis. Journal of gastroenterology and hepatology 2018; 33(4): 774-780. https://doi.org/10.1111/jgh.14081

99. Li YH, Xu Y, Wu HM, Yang J, Yang LH et al. Umbilical cord-derived mesenchymal stem cell transplantation in hepatitis B virus related acute-on-chronic liver failure treated with plasma exchange and entecavir: a 24-month prospective study. Stem Cell Reviews and Reports 2016; 12: 645-653. https://doi.org/10.1007/s12015-016-9683-3

100. Baer PC, Sann J, Duecker RP, Ullrich E, Geiger H et al. Tracking of infused mesenchymal stem cells in injured pulmonary tissue in Atm-deficient mice. Cells 2020; 9(6), 1444. https://doi.org/10.3390/cells9061444

101. Wang LT, Liu KJ, Sytwu HK, Yen ML, Yen BL. Advances in mesenchymal stem cell therapy for immune and inflammatory diseases: Use of cell-free products and human pluripotent stem cell-derived mesenchymal stem cells. Stem cells translational medicine 2021; 10(9): 1288-1303. https://doi.org/10.1002/sctm.21-0021

102. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity 2020; 109: 102433. https://doi.org/10.1016/j.jaut.2020.102433

103. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. Journal of Pathology 2004; 203(2): 631–637. https://doi.org/10.1002/path.1570

104. Metcalfe, S. M. Mesenchymal stem cells and management of COVID-19 pneumonia. Medicine in drug discovery 2020; 5: 100019. https://doi.org/10.1016/j.medidd.2020.100019

105. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The lancet 2020; 395(10229):1033-1034. https://doi.org/10.1016/s0140-6736(20)30628-0

106. Leng Z, Zhu R, Hou W, Feng Y, Yang Y et al. Transplantation of ACE2- Mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging and Disease 2020; 11(2): 216 https://doi.org/10.14336%2FAD.2020.0228

107. Golchin A, Seyedjafari E, Ardeshirylajimi,A. Mesenchymal Stem Cell Therapy for COVID-19: Present or Future. Stem Cell Rev and Rep 2020; 16: 427–433 https://doi.org/10.1007/s12015-020-09973-w

MESENCHYMAL STEM CELL AND IMMUNE SYSTEM

Tülay KILICASLAN AYNA[1,2,3], Ibrahim PIRIM[1,2,3]

[1,2,3]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[1,2,3]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[1,2,3]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

13.1 INTRODUCTION

Mesenchymal stem cells (MSCs) have immune regulation capabilities and are currently under investigation as potential therapies for different immunological illnesses. These cells are mature, multipotent cells that can maintain themselves and differentiate into specific cells like osteoblasts, chondrocytes, and adipocytes [1]. It possesses a robust capacity to engage with cells of the adaptive and innate immune systems, via physical contact or by means of the molecules they secrete. Based on their efficacy, stem cells are divided into three basic classes: fetal stem cells (FSCs), adult stem cells (ASCs), and embryonic stem cells (ESCs). The interior cell mass of human embryo blastocysts yields undifferentiated cells known as ESCs. ESCs possess the exceptional capacity to undergo diversification into the three fundamental embryonic regions, namely endoderm, mesoderm, and ectoderm. Nevertheless, the utilization of ESCs in medical settings for human embryos is restricted due to their pronounced propensity to generate

teratomas and the ethical concerns surrounding their elimination. Conversely, stem cells obtained from fetal and adult tissue are becoming increasingly popular due to little ethical concerns. Extra-embryonic tissues, such as Wharton's jelly, cord blood, placenta, amniotic membrane, and amniotic fluid may be utilized to extract fetal stem cells. Tooth pulp, adipose tissue, bone marrow, and other tissues are common sources of adult stem cells (ASCs), which are multipotent cells. These cells can form colonies, renew themselves, and change and transform into many types of tissues. Stem cells derived from fetal and adult tissues can undergo differentiation into other cell variants and are commonly referred to as MSCs [2]. MSCs can transfrom to cells that come from the mesoderm, but they also have the capability to transdifferentiate into cells from the ectoderm or endoderm lineages [3]. Although the precise markers of mesenchymal stem cells (MSCs) differ based on their origin, the surface antigens CD105, CD73, CD90, and Stem cell antigen-1 (Sca-1) are widely used to identify MSCs. They do not have CD34, CD45, CD11b, MHC II, and CD31 [4]. Stem cell research has increased expectations for the therapies.

MSCs are generating enthusiasm in the repair therapy area and immune dysfunction-related disorders due to their isolatability, robust self-renewal capabilities, and varied differentiation capacities. Furthermore, it is devoid of any potential difficulties associated with the utilization of MSCs, iPSCs, and ESCs. The researchers have been thoroughly researched for MSCs' function in regenerating tissue and their ability to regulate immune-related conditions. Even though they aren't as strong as pluripotent stem cells (iPSCs) or induced embryonic stem cells (ESCs), they can replace damaged tissues directly through differentiation. Multiple studies have documented the

effective stimulation of tissue regrowth, such as in the kidney, heart, liver, and pancreas, by utilizing MSCs. Significantly, mesenchymal stem cells (MSCs) exert control over tissue regeneration and diverse immunological diseases by means of their immune regulatory characteristics. MSCs play a crucial role in immune control by their direct interaction with cells involved in both the innate and adaptive immune systems, as well as the release of soluble substances and extracellular vesicles (EVs) from MSCs. MSCs maintain homeostasis of the immune response and modulate inflammatory profiles, hence facilitating the effective management of several disorders associated with immune cells [5].

13.2 IMMUNE SYSTEM CELLS AND MESENCHYMAL STEM CELLS

13.2.1 Adaptive immune cells

13.2.1.1 T Cells

These cells are frequently present in all tissues throughout our body. Hematopoietic stem cell-derived pioneer T cells migrate to the thymus via the bloodstream. Mature T cells are generated following both positive and negative selection. Two signals are required for inducing the T cells. The signals being referred to are T cell receptor signaling and costimulatory signals. Upon activation, CD4+ T cells have the ability to undergo differentiation into many subsets, including regulatory T cell (Treg) sub types, Th17, Th9, Th2, or T-helper 1 (Th1). The specific subset that a CD4+ T cell differentiates into is determined by the intensity of stimulation it receives and the cytokine environment it is exposed to. Additionally, a variety of infections stimulate and accelerate the transformation of CD8+ T cells into cytotoxic T lymphocytes, which expel diseased cells by releasing cytokines, performs, and granzymes. A

vital part of the adaptive immune system that defends from illnesses, autoimmune diseases, and malignancies is T cell-mediated immunity [6].

The communication between MSCs and T cells and has been well investigated. MSCs have been discovered to strongly suppress the growth of T cells in various experimental scenarios. In vitro, mesenchymal stem cells (MSCs) generated from human bone marrow (BM) substantially suppressed the growth of T lymphocytes. MSCs inhibit T cells by secreting hepatocyte growth factor (HGF) and transforming growth factor-β (TGF-β), which lowers cyclin D levels and increases p27kip1 expression. Within T cells, it leads to a halt in cell division during the G1 phase [7,8].

Active T cells can undergo programmed cell death when exposed to MSCs; this process is connected to the Fas/Fas ligand-dependent pathway and the alteration of tryptophan to kynurenine [9,10].

MSCs can not only impact T cell growth and death, but also modify the process by which T cells become activated and differentiated. Multiple pieces of evidence suggest that MSCs inhibit the release of interferon (IFN)-γ and IL-17, while promoting the production of IL-10 by T cells. This is achieved by opposing the development of Th17 and Th1 cells, hence facilitating the development of Treg cells. MSCs exert an indirect inhibitory influence on the activation pathway of effector T-cells via regulating dendritic cells (DC) and natural killer (NK) cells [11]. Given the excellent mitigation of many inflammatory disorders with MSC transplantation, these findings can be extrapolated to multiple in vivo models. The illnesses include systemic lupus erythematosus (SLE), experimental autoimmune uveitis, graft versus host disease (GVHD), transplant arteriosc-

lerosis, arthritis and experimental autoimmune encephalomyelitis (EAE)_[4,12].

Remarkably, mesenchymal stem cells (MSCs) lack the ability to inhibit T cells unless they are first activated with particular inflaming cytokines, such as IL-1β, IFN-γ and tumor necrosis factor (TNF-α) [11,13]. Upon being stimulated by these inflammatory cytokines, MSCs upregulate the expression of cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS). This upregulation of expression regulates the immunological responses of the suppressive prostaglandin E2 (PGE2) and nitric oxide (NO) molecules. Furthermore, these MSCs secrete proteins such ICAM-1, VCAM-1, CXCR3 ligands and CCR5 ligands. It generates a wide range of chemokines and adhesion molecules. The chemokines have a crucial role in attracting lymphocytes to surrounding damaged regions, hence assuring their optimal suppressive activity [14,15,16].

The induction of soluble immune regulatory molecules and adhesion molecules is crucial for the efficient suppression of T-cells. Blocking one of them significantly counteracts the inhibitory impacts of MSCs [17,18].

Nevertheless, the immunosuppressive capacity of MSCs is not consistently attainable, as conflicting research has demonstrated that MSCs may fail to suppress or even amplify T cell responses in different circumstances. MSCs' potential to modulate immunity relies on the particular kinds and degrees of infectious signals to which they are subjected. The study examined MSCs' capacity to regulate the immune response by exploring how varying levels of IFN-γ and TNF impact their capabilities. The study found that when proinflammatory cytokines were present at low levels, murine MSCs produced an inadequate amount of NO. Conversely, when proinflam-

matory cytokines were present at high levels, murine MSCs produced a satisfactory amount of NO. The documented effects of this substance include inhibition of T lymphocytes. The capacity for flexibility observed in mice MSCs can likewise be applied to human MSCs [19]. The immunosuppressive effect of MSCs is influenced by specific chemicals, which vary depending on the species. Important molecules in mice are iNOS, but in humans they are indoleamine 2,3-dioxygenase (IDO).

In murine models of graft-versus-host disease (GVHD) and experimental arthritis, mesenchymal stem cells (MSCs) that were treated with iNos-/- (inducible nitric oxide synthase) or an iNOS inhibitor were unable to suppress T cells, resulting in a lack of therapeutic effects.

IDO effectively suppressed immunological responses by reducing the levels of tryptophan and facilitating the buildup of tryptophan metabolites. Human MSC-derived IDO exhibits immunosuppressive effects in numerous animals, much like iNOS. Human mesenchymal stem cells (MSCs) also release substantial quantities of soluble human leukocyte antigen-G5 (HLA-G5) to facilitate their immune inhibition capabilities. However, there are certain chemicals that both murine and human MSCs have in common when it comes to facilitating T-cell immunosuppression. PGE2, a molecule of significant importance, has been extensively studied and its role has been emphasized in numerous research papers. MSCs produce significant quantities of PGE2, which is linked to increased effectiveness in relieving collagen-induced arthritis and reducing the mixed lymphocyte reaction [4,20].

13.2.1.2 B cells

B cells serve as effector cells, which is another characteristic feature of the adaptive immune system. These cells, like

other blood cells, are developed from hematopoietic stem cells and undergo differentiation into B cells at various stages. After identifying particular antigens through their B cell receptors, responsive B cells will multiply and transform into memory cells and active antibody-secreting cells, which play a role in defending against and preserving immunity to foreign infections. In contrast to conventional B2 cells, which are found in many locations, the peritoneum and pleural cavities of mice contain larger concentrations of B1 cells. These cells efficiently react to natural immunological signals and contribute to the eradication of infections and supplying the host with long-term protection. Regulatory B cells, also known as Bregs, are a distinct group of B cells that secrete IL-10 and possess immune regulating properties in many experimental paradigms [4,21]. Both human and mouse MSCs can prevent the growth, specialization, and stimulation of B cells. It was shown that when MSC is present, B cells had cell cycle termination, which inhibited the production of plasma cells, altered their ability to release immunoglobulin, and diminished their chemotactic characteristics. The inhibitory function relies heavily on soluble components. C-C Motif Chemokine Ligand 2 (CCL2) is a chemokine derived from MSCs that has been processed by metalloproteinase. It functions by preventing the expression of Paired Box 5 (PAX5) by inhibiting the activation of Signal Transducer and Activator of Transcription 3 (STAT3). This expression of PAX5 results in the suppression of immunoglobulin synthesis. CCL2 is responsible for mediating these effects. It is considered as one of the factors involved in this process [4,22].

The work conducted by Crawford et al. demonstrated that IL-1 receptor antagonist (IL-1Rα) produced from MSCs effectively regulates both B cell differentiation and the course of

arthritis [4,23]. Cell-cell interaction plays a vital role in inhibiting the growth, specialization, and generation of antibodies in B cells. This process is linked to the PD-1/PD-L1 pathway. Furthermore, other researches have emphasized the regulation of many signaling pathways, including B-lymphocyte-induced maturation protein 1 (Blimp1), extracellular response kinase 1/2, Akt, and p38. MSCs have been found to influence B cell responses by inducing the production of Bregs. In humans, Bregs are characterized by being CD19+ CD24high CD38high, whereas in mice, they are characterized by being CD19+ CD-1dhigh CD5+. The cells secrete substantial quantities of IL-10, resulting in the suppression of immunological responses [39]. Indeed, the activation of Bregs by MSCs has demonstrated efficacy in the treatment of many diseases in mice models, including GVHD, SLE (Systemic Lupus Erythematosus), and EAE [2,4,24].

Similar to T cells, the activation of MSCs by inflammation increases their ability to suppress B cells. The activation of the suppressor role of MSCs relies on strong and resilient IFN-γ stimulation. Furthermore, an adequate number of inflammatory signals, specifically signals originating from the bacteria *Mycoplasma arginini*, significantly enhance the capacity of MSCs to inhibit B cell antibody production. Conversely, mesenchymal stem cells (MSCs) that receive inadequate inflammatory signals, such as those obtained from lupus-like mice or individuals with systemic lupus erythematosus (SLE), are unable to effectively limit the proliferation and differentiation of B cells. In fact, they may even contribute to an increase in the number of B cells that secrete antibodies.

Hence, it is comprehensible that multiple contradictory outcomes were noted, as certain researchers have shown that the inclusion of MSCs can amplify antibody production,

differentiation, activation, and proliferation of B cells. While it has been proposed that these discrepancies could be attributed to variations in MSC source, MSC-to-B cell ratio, B cell quality, and stimulation, it is important to also take into account the adaptability of MSCs in response to varying levels of inflammatory signals [4,24].

13.2.2 Native Immune Cells and Mesenchymal Stem Cells

13.2.2.1 Naturel killer cells (NK cells)

Natural killer cells, also known as NK cells, are a subset of cytotoxic lymphocytes that play a crucial role in the innate immune response. They migrate in the bloodstream as fully-grown cells after beginning life as primitive cells inside the bone marrow. They exhibit prompt responsiveness to cells infected with viruses, manifest their impact within 3 days of infection, and react to the development of tumors. T cells have a crucial function in the rejection of transplanted tissue and serve a major role in controlling the destruction of cells in response to the human leukocyte antigen molecule. Examining the fundamental consequences of the relationship between MSCs and NK cells is essential because of the growing popularity of MSCs in the medical management of GvHD. The proportion of MSC immune system cells is also crucial in the role of MSC-mediated NK cells [4,26].

These cells function by the process of activation and inhibition on the cell surface, facilitated by receptors that transmit signals to the cell. NK cells typically possess regulatory capabilities and are capable of secreting cytokines and chemokines that regulate the immunological response of the host. IL-12 is the primary inflaming agent that reacts to invasive infections and functions via its receptors with a strong affinity.

Related cells including DCs, macrophages, and monocytes secrete it. Furthermore, NK cells secrete mainly IFN-γ with the IL-12 stimulation. NK cells stimulate the production of IFN-γ, which enhances the expression of IL-12 and DCs through a feedback loop. The MSCs developed in bone marrow directly impede the growth, secretion of signaling molecules, and, in certain instances, NK cells' capability to destroy target cells. Interactions between MSCs and NK cells are intricating and mostly influenced by NK cell stimulation and the microenvironment. Essentially, MSCs inhibit the INF-γ, IL-15, and, IL-2 secretion, while without affecting the NK cells' cytotoxicity upon isolation. Moreover, upon activation, NK cells inhibit NK-mediated cytotoxicity when they encounter MSCs through cell-cell interaction and the release of IDO, PGE-2, TGFβ1, and HLA-G5. According to other studies, MSCs that had obtained a license were subjected to IFN-γ. This exposure may have been caused by an increase in the expression of Major histocompatibility complex-I (MHC-I) on the cell surface and a decrease in the expression of UL16 binding protein 3 (ULBP-3). As a result, there was an increase in cell proliferation driven by NK cells. It was asserted that they were shielded from being slain. These factors, including elevated synthesis of both IDO and PGE-2, provide several ways to decrease the NK response to MSCs [2,4,27,28].

MSCs have been employed in clinical trials with the objective of enhancing the attachment of hematopoietic stem cells and mitigating or managing acute GvHD. Furthermore, the administration of MSCs has a beneficial impact on GvHD. Nevertheless, it can also impede the activation of NK cells. Within this specific framework, investigations conducted in a laboratory setting have examined the outcomes resulting from the association between NK cells and MSCs. Indeed,

studies have demonstrated that the interaction between NK cells and MSCs can have significant impacts on the functionality of both types of cells. Although MSCs can hinder the growth and activity of newly obtained peripheral blood NK cells, they also impede the growth of NK cells subjected to cytokines like IL-2 and IL-15. Nevertheless, there is a lack of evidence indicating the occurrence of apoptosis or cellular demise in this specific inhibition of NK cell multiplication. The inhibition seen was dependent on the dosage and was detected when the ratio of NK cells to MSCs ranged from 1:1 to 1:1. The decline occurs when the NK/MSC ratio reaches 10:1 or above. The suppressive impact is facilitated by soluble substances generated from MSCs. The inhibitory effect has been demonstrated using transwell tests, in which it occurs even without direct cell contact [4,24].

Furthermore, MSC not only hinder the growth of NK cells, but also diminish their ability to destroy target cells. Specifically, the cytotoxicity mediated by NK cells is a significant method for eliminating tumor and virus-infected cells. Additionally, it has been demonstrated that this process serves as a quality control measure in the production of fully functional dendritic cells by excluding those that do not exhibit sufficient quantities of HLA class I molecules. The induction of NK-cytotoxicity in in vitro tests was significantly influenced by MSC. Curiously, in contrast to the situation with NK cell growth, the prevention of cytotoxicity stimulation seems to necessitate direct interaction between cells. MSCs prevent MHC-class I- and I+ target cells (undeveloped dendritic cells and different tumor cell lines) from being destroyed. Typically, when cytokines stimulate NK cells, it results in the creation of new or heightened levels of activating receptors on the cell surface. These receptors include NKp44, NKp30, and NKG2D. These molecules, inclu-

ding DNAM-1 and NKp46, as well as other coreceptors, play a crucial role in activating NK cells and triggering their ability to carry out duties such as killing cells and producing cytokines [4,24,29].

MSCs have been documented to suppress the production of NKG2D, NKp30, and NKp44, as well as other crucial substances such as CD132 (IL-2Rγ chain) and coreceptor 2B4. Specifically, a decrease in CD132 expression reduces the sensitivity of NK cells to cytokine stimulation. Hence, it likely signifies a further mechanism implicated in the suppressive impact. As previously stated, the generation of cytokines is the primary function of NK cells. NK cells, when activated by triggering signals, have the ability to generate several cytokines, such as IFN-γ, TNF-α, and IL-10. The cytokine production from NK cells cultivated in combination with MSCs decreases regardless of the activating signal (which may consist of cytokines such as I, IL-12, IL-15 and L-18) or association with tumor cells [2,24,30]. Under transwell conditions, there was also inhibition seen, specifically in the context of NK cell growth, indicating the involvement of soluble mediators.

IDO, soluble HLA-G5 (sHLA-G5) and PGE2 play a crucial role in suppressing the growth and activity of cytokine-induced NK cells. When the synthesis of PGE2 was blocked, there was a notable improvement in the expansion and toxic effect of natural killer cells, indicating the function of PGE2 during the inhibitory effect. In addition, it has been observed that MSCs secrete sHLA-G5 molecules that suppress the cytotoxicity of NK cells. Spaggiari et al discovered that inhibiting IDO activity had a substantial effect in restoring the proliferation of NK cells. Nevertheless, the cytotoxicity is mitigated by the administration of NS-398, an inhibitor of PGE2 synthesis. Remarkably, the combination of both inhibitors resulted in a ne-

ar-total recovery of both toxicity in NK/MSC cocultures and NK cell growth. The observations suggest that PGE2 and IDO play a crucial role in the inhibitory effect of MSCs on NK cells and may work together in a synergistic manner. Some soluble chemicals can also inhibit the proliferation or cytolytic activity of NK cells. More precisely, the process of IDO breakdown resulting in a decrease in tryptophan levels may have a more significant effect on cell growth, while PGE2 may mainly impede with cells' capacity to produce cytokines to destroy other cells. MSC continuously create PGE2, however when exposed to TNF-α or IFN-γ, IDO is freshly expressed. During interactions between NK cells and MSCs, it is plausible that NK cells rapidly secrete TNF-α or IFN-γ, leading to a subsequent augmentation in the production of PGE2 and the synthesis of IDO. It is widely accepted that IFN-γ functions in initiating the immune modulating function of MSCs. Meisel et al. initially documented that the expression of IDO was stimulated by IFN-γ. as defined in references [2,4,31]. Furthermore, Krampera et al. observed that the inhibition of IFN produced by NK cells resulted in a partial recovery of NK cell proliferation in NK/MSC cocultures. Aggarwal et al. showed that the production of PGE2 was enhanced when MSCs were exposed to IFN-γ. Moreover, IFN-γ may enhance the production of sHLA-G5. An important discovery regarding the association between MSC and NK cells is that these cells have the ability to eliminate both self-derived and donor derived MSC [2,24,32].

MSCs exhibit reduced or moderate amounts of HLA-class I molecules. Alloreactive cytolytic T cells are unable to detect them because of this. The absence of costimulatory receptors in MSCs is believed to decrease alloreactivity. Clinical experiments have utilized allogeneic HLA mismatched MSCs due of this characteristic. Nevertheless, HLA class I molecules are

produced by MSCs at low levels, providing them open to NK cell eradication. At this point, MSCs produce other substances that act as activating ligands for NK receptors, such as NKp30 ligands including Nectin-2 (ligands for DNAM-1), MICA (ligands for NKG2D), ULBP1-4, and PVR, as well as other ligands that have not yet been found [2,33]. Due to their phenotypic character, MSCs are vulnerable to NK cell-induced cell death, regardless of whether they are derived from the same individual or a different individual. It is crucial to emphasize that the elimination of MSC can only occur when NK cells are stimulated by cytokines like IL-15 and IL-2, which increase the activity of NKG2D and NKp30 activating receptors. Conversely, when NK cells are newly obtained and in a state of rest, they lack the ability to eliminate MSC, even when the ratio of NK cells to MSC is high. The killing of MSC is facilitated by both an elevation in intracellular calcium leading to the release of perforin, and the binding of FasL and TRAIL and to their respective receptors Fas DR5, and DR4. These receptors are expressed by adult and fetal MSC, as indicated by previous studies [2, 24, 34]. Pro-inflammatory cytokines, such as IFN, are exposed to cells during infection or in the broader context of inflammatory reactions, which enhances the expression of HLA classes I and II on the surface of MSC cells. The way that suppressor NK receptors and HLA class I interact may affect how MSCs opened are to destruction by NK cells. The classical HLA class I molecules' allotypic determinants are specifically targeted by deadly Ig-like receptors, while the non-classical HLA-E molecules are preferentially targeted by NKG2A receptors. Effective NK cell driven MSC death can be restored by disrupting inhibitory connections with monoclonal antibodies [2,24,35].

13.2.2.2 Dendritic cells (DC)

In the absorption, preparation, delivery, and presentation of many antigens, DCs have critical roles. These cells are commonly considered the most efficient antigen-presenting cells (APCs) in the human immune system. Due to their specific role in presenting antigens, these cells are essential in directing the adaptive immune system's responses. There is increasing data that suggests that MSCs have strong inhibitory impacts on DCs. MSCs and the culture supernatants were found to impede DC activation, inhibit DCs' ability to take up substances from their environment (endocytosis), and decrease DCs' production of IL-12 during an in vitro experiment. Furthermore, MSCs and culture supernatants impeded the maturation of DCs and diminished their capability to stimulate alloreactive T cells [4]. In 2005, Aggarwal and Pittenger initially documented that MSCs developed in bone marrow suppressed the release of tumor necrosis factor (TNF-α) by CD1c+ myeloid dendritic cells (DC), while simultaneously enhancing the synthesis of interleukin-10 (IL-10) by BDCA-4+ plasmacytoid DC. It has been demonstrated that MSCs indirectly restrict T cells by stimulating regulatory APCs that possess T-cell inhibitory capabilities. MSCs may disrupt the normal process of differentiating precursor cells into DCs, leading to decreased DC function. Indeed, it was noted that the presence of MSC led to an increase in CD1a expression and did not cause a decrease in CD14 expression in monocytes that were stimulated by granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4. As a result, the creation of DCs was hindered. In addition, lipopolysaccharides (LPS), which promote the maturation of dendritic cells (DCs), also exhibit the costimulatory molecules CD80 and CD86, as well as low amounts of the DC maturation marker CD83 [24,36].

Cell loss did not occur with the suppression of DC differentiation in these experiments. Jiang et al. concluded that the cell vitality remained unaffected by MSC, as reported in their study [37]. Crucially, the suppression caused by MSC can be undone. Therefore, when monocyte-derived cells were cultivated together with MSC and then transferred to a new culture dish with fresh cytokines, they displayed the characteristics of DC, namely, they no longer had the CD14 marker, but instead expressed CD83 and CD1a. Nevertheless, the combined cultivation tests were performed under transwell culture circumstances.

A separate investigation involved the co-cultivation of MSCs and monocytes. Upon isolating MSC from monocyte cultures after a period of 2 days, the cells exhibited a decrease in CD14 expression. However, it did not exhibit CD1a expression. This suggests that the limitation may not be totally reversed and that initial stimulation with MSC is probably necessary for the suppressive function. Besides monocytes, MSCs can also suppress the formation of dendritic cells from CD34+ progenitors. MSCs have been demonstrated to hinder the transformation of dermal/interstitial DCs from CD34+ cells obtained from umbilical cord blood. While having no effect on the development of CD14-CD1a+ Langerhans cells, this blockage prevents intermediate precursors (CD14-CD1a+) from transitioning to CD14+CD1a- cells. Furthermore, the CD14+CD1a- fraction exhibited reduced expression of CD40, CD83, CD86, and CD80 following LPS activation [38].

Conversely, a third group of researchers stated that MSCs had the ability to impede the process of specialization in both Langerhans and interstitial/dermal cells. Furthermore, they revealed that MSCs can suppress the growth of DC precursors by decreasing their proliferation rate by a factor of three.

The variation in outcomes can be partly attributed to the utilization of distinct DC precursors, such as adult bone marrow and cord blood derived CD34+ cells, as well as disparities in the experimental conditions. A further issue is if MSCs can affect not just the initial stages of DC development but also the shift from adolescent DCs to fully developed DCs. Divergent findings have been acquired by several groups about this matter. Jiang et al. observed that MSC have a limited ability to inhibit the development of DCs from monocyte triggered by lipopolysaccharide (LPS). In the mixed lymphocyte response, the resultant cells showed reduced levels of IFN-induction, IL-12 production, and allogeneic T-cell growth activation in comparison to control competent DC. However, Spaggiari et al. demonstrated that MSCs did not impede the maturation of DCs caused by LPS. During these investigations, DC that were in the process of completing their differentiation and were exposed to MSC displayed a typical appearance and were discovered to be more efficient in stimulating the mixed lymphocyte reaction compared to fully mature DC generated using conventional methods. Aldinucci et al. found that immature DC could not develop functional defensive interactions with lymphocytes when subjected to LPS in an environment of MSC. This was despite the fact that the DC showed signs of maturity and had a normal production profile of IL-12 and IL-10.

IL-6 and macrophage-CSF, which are soluble substances, have been demonstrated to have a role in the ability of MSCs to prevent the monocyte transformation into DCs. Anti-macrophage-CSF neutralizing antibodies and anti-IL-6 usage resulted in the depletion of CD14 during the formation of dendritic cells. Nevertheless, the expression of CD1a was not regained. PGE2, a distinct product of MSCs that is recognized as a crucial mediator of the suppressive impact on various

immune cells, has been demonstrated to strongly impede the development of DCs. In fact, compared to monocytes alone, the supernatants of co-cultures of monocytes and MSCs had much higher amounts of PGE2. Additionally, the inhibitory effect was successfully counteracted by selectively suppressing cyclooxygenase-2 activity and PGE2 synthesis. Both the DC morphology and activity were restored, confirming this. This remarkable result was obtained despite the coculture supernatants containing higher levels of IL-6. Thus, it indicates that PGE2, rather than IL-6, is primarily implicated in the inhibitory impact.

Indeed, studies employing CD34+ cells generated from bone marrow as DC precursors have revealed a significant role for cell-to-cell contacts, indicating to researchers that distinct mechanisms might be accountable for disrupting distinct differentiation pathways [24].

13.2.2.3 Monocyte and macrophage

There is a limited amount of research on how MSC affects the maturation of macrophages that promote inflammation. Nevertheless, all of these investigations demonstrated that MSCs hinder the development of the pro-inflammatory M1 phenotype while facilitating M2 polarization, which possesses anti-inflammatory characteristics.

Kim and Hematti initially documented that MSCs derived from human bone marrow have the ability to facilitate the development of alternatively activated macrophages. These macrophages have an M2 macrophage cytokine characteristic and are distinguished by their elevated CD206 production. This profile includes heightened production of IL-10 and reduced production of IL-12 and TNF-.

M2 macrophages can become polarized in vitro by human gum-derived MSC, as demonstrated by Zhang et al. [41]. Using a model of skin healing in mice that involved removing tissue, the researchers found that regularly injecting gum-derived MSCs from humans resulted in the MSCs being present at the wound site together with host macrophages. Furthermore, macrophages were oriented in the direction of the M2 characteristics by the MSCs. Therefore, mesenchymal stem cells produced from the gums can decrease inflammation in the immediate area by inhibiting the entry of immune cells, raising the IL-10 expression and reducing the synthesis of IL-6 and TNF-α. In a separate investigation conducted by Cultler et al., it was demonstrated that umbilical cord-derived mesenchymal stem cells (UC-MSC) may inhibit the T cell growth in cell cultures when exposed to alloantigens [42]. Significantly, in these investigations, monocytes that were separated from co-cultures of PBMC/UC-MSC showed an elevated CD206 production, decreased amount of HLA-DR on their surface, and a diminished capacity to induce the immune response of alloreactive T-cells in mixed lymphocyte reaction assays. The immunosuppressive effect of UC-MSC was diminished when monocytes were eliminated from PBMC cultures. Therefore, we propose that these cells may serve as a crucial mediator in the inhibition of T-cell growth caused by UC-MSCs. François et al. emphasized the significant contribution of MSC-conditioned monocytes. Research has verified that the removal of monocytes from PBMC triggered by anti-CD3/CD28 antibodies causes a reduction in the amount of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells (Treg) stimulated by the presence of bone marrow derived MSCs [43]. In these investigations, it was observed that CD206 and IL-10 are produced as M2 indicators by monocytes in the presence of MSC, compared to monocytes cultured without MSC. Various soluble substances have

been demonstrated to have a significant role in the M2 polarization effect produced by MSC on macrophages. Zhang et al. showed that GM-CSF and IL-6 have activity in inducing the M2 phenotype by gingival-derived MSCs, using particular neutralizing antibodies. Regarding UC-MSC, Cutler et al observed a higher production of PGE2 in co-cultures of PBMC-UC-MSC compared to UC-MSC cultures by themselves.

Furthermore, the application of indomethacin (the PGE2 suppresor) to UC-MSC before treatment partially counteracted their capacity to regulate the characteristics and activities of monocytes. The study conducted by Francois et al. showed that IDO activity had an important role in the development of CD14+CD206+ immunosuppressive macrophages that produce IL-10. Nevertheless, it is important to consider that the bone marrow MSCs utilized in the tests had undergone prior "activation" with IFN- and TNF-. Human MSCs are not known to naturally express IDO mRNA. However, their expression and activity can be induced by inflammatory cytokines including IFN- and TNF-. Thus, it is plausible that IDO could play a role if MSCs have been subjected to an inflaming environment [24].

13.2.2.4 Neutrophils

Neutrophils, a type of white blood cell with many nuclei, are acknowledged as vital contributors to the process of acute inflammation. These cells are plentiful in the circulatory system and can be transported to the areas of injury within a couple of minutes. Infections are eradicated by neutrophils via many techniques including phagocytosis, production of chemicals that kill bacteria, and the formation of neutrophil extracellular traps. The positive benefits of MSCs on neutrophils were initially documented in 2008.

MSCs from human bone marrow in good condition were able to successfully prevent inactive or IL-8-stimulated neutrophils from dying by programmed cell death._This inhibition was mainly facilitated by the release of IL-6 and remained effective even at very low ratios of MSCs to neutrophils. In the same way, MSCs that were treated beforehand with the TLR3 stimulator Poly (I:C) demonstrated strong abilities to prevent cell death in neutrophils. This effect was mainly achieved through the joint action of GM-CSF, IFN-β, and IL-6. MSCs can also recruit neutrophils in laboratory settings by releasing MIF and IL-8. The veracity of these data was confirmed by other in vivo investigations.

Neutrophils have been observed to be efficiently attracted by MSCs that have been activated with LPS and injected under the skin. Furthermore, TNF-α-stimulated or MSCs generated from gastric cancer effectively attracted neutrophils to the tumor, hence facilitating the spread of the tumor and the formation of new blood vessels. It is hypothesized that MSCs may aid in preserving the reservoir of neutrophils in the bone marrow and assist in resolving infection and inflammation by promoting the movement of neutrophils to sites of inflammation. Nevertheless, there are also conflicting results. In a vasculitis instance of a mouse, it has been discovered that MSCs block the activation of neutrophils, prevent the creation of neutrophil extracellular traps, and reduce the excessive release of tissue-damaging proteases. As a result, this reduces uncontrolled inflammation and mitigates tissue damage. The therapeutic impact of MSCs in this model was achieved through the continuous express of superoxide dismutase-3.

MSCs from various sources effectively restricted neutrophil recruitment in an alternate neutrophil type infiltration stimulated by endothelial cells that are induced by cytokines. In

addition, it has been demonstrated that extracellular vesicles produced from MSCs can also hinder the entry of neutrophils into the lung in a lung damage example induced by endotoxin. The reasons behind these inconsistencies and the necessity for additional research in this domain are evident [24].

13.2.2.5 Mast cells

These cells are widely regarded as the primary cells responsible for carrying out allergic reactions. They may also play a role in inflammatory illnesses, where non-allergic stimuli stimulate them and exacerbate autoimmunity, according to numerous evidence. The degranulation, inflammatory cytokine release, and chemotaxis capacities of mast cells were inhibited when they were co-cultured with bone marrow derived MSCs. This phenomenon occurs as a result of the increased expression of COX2 in MSCs. This observation was validated in live organisms, since the introduction of MSCs effectively suppressed the release of granules from skin mast cells and peritoneal cavity of mice.

MSC inoculation prevented mast cell recruitment and breakdown in an animal instance of atopic dermatitis, a process made possible by MSC production of TGF-β1 and PGE2. MSCs also inhibited the invasion of mast cells and the production of new inflammatory cytokines in a mouse model of touch hypersensitivity through the production of PGE2. Remarkably, mesenchymal stem cells (MSCs) able to triggered by IgE-stimulated mast cells, resulting in the release of hematopoietic growth factors and thymic stromal lymphopoietin. The regulation of lineage commitment and proliferation of CD34+ progenitor cells are controlled. Furthermore, mast cells exert an influence on MSCs by stimulating their growth and buildup, while impeding their specialization by the platelet-derived

growth factor stimulation. This mechanism potentially contributes to the enhancement of the heart regeneration process [24].

13.3 IMMUNE MOLECULES AND MSCs

In order to engage in tissue healing, MSCs need to be in close proximity to different stromal and immune cells. The mechanism by which MSCs facilitate tissue healing is intricate. MSC-derived immunoregulatory factors have a crucial function in this context. MSCs have been documented to secrete various growth factors and chemicals that modulate the immune system. They investigate how the release of anti-inflammatory chemicals at tissue injury sites is affected by the inflammatory microenvironment and find that MSC-mediated inhibition occurs in the milieu around MSCs.

The immunological response triggers the production of inflammatory substances, which activate the inhibitory ability of MSCs. The onset of tolerance by the immune system coincides with the growth of the fetus during gestation, emphasizing the critical function of fetal-derived MSCs. Several cells involved in both innate and adaptive immunity are suppressed by these multifunctional cells, including B cells, DCs, macrophages, as well as diverse effector cells such as CD4+ T cells, NK cells, CD8+ T cells, Tregs and natural killer T (NKT) cells.

13.3.1 Indoleamine-2,3-dioxygenase (IDO)

IDO is an enzyme found in the cytoplasm of mammals. It plays a crucial role in breaking down tryptophan through the kynurenine degradation pathways. IDO is composed of two helices with a heme group positioned between them. It is a crucial amino acid that facilitates the decomposition of tryptophan through kynurenine. The immunomodulatory impact triggered by cells expressing IDO is initiated by a decrease in

the nearby level of tryptophan or its derivative. Research involving placental cells has demonstrated their ability to inhibit the destruction of fetal cells by maternal T cells during pregnancy. The synthesis of IDO in placental cells is responsible for this protective effect. Unlike earlier partially genetically dissimilar transplants, the developing embryo during pregnancy releases paternal markers that do not induce rejection by the mother's immune system. Dendritic cells can produce IDO, which in turn can trigger an immune response that promotes tolerance. Su et al proposed that MSCs do not possess an inherent capacity to produce IDO, but instead develop this capability after being stimulated by the inflaming cytokines TNF-α, IL-1β, and IFN-γ. IDO's involvement in the regulation of immune response by MSCs has been recently shown to decrease different groups of NK and T cells.

13.3.2 Human leukocyte antigen-G (HLA-G)

A well-known antigen from class I of the major histocompatibility complex is HLA-G. The gene responsible for expressing it is located on chromosome 6p21. This differentiates itself from conventional HLA class I molecules due to its limited occurrence in particular tissues and a limited diversity in the coding area. HLA-G can exist in seven different protein variants, which are produced by a specific transcript. There exist four distinct kinds of proteins that are attached to the cell membrane, specifically referred to as HLA-G1, HLA-G2, HLA-G3, and HLA-G4. Furthermore, there exist three distinct forms of aqueous proteins, specifically HLA-G5, HLA-G6, and HLA-G7.

The molecule exerts its immunomodulatory effects by communicating with distinct receptors, namely KIR2DL4 (CD158d), LILRB2 (ILT4/CD85d), and LILRB1

(ILT2/CD85j), that are released in varying patterns by immune cells. HLA-G can bind to CD8 independently of TCR interaction, resulting in elevated synthesis and release of FasL. This can lead to apoptosis triggered by activated $CD8^{+}$ T cells and NK cells. HLA-G has a vital role in facilitating tolerance throughout both pregnancy and transplantation. Leukemia inhibitory factor (LIF) and IL-10 may increase the production of HLA-G in MSCs. The production of HLA-G in immune cells has been discovered to be regulated by molecules including IFN-β and glucocorticoids. HLA-G has been studied in the context of allogeneic solid organ transplantation. It has been strongly linked to a decrease in the occurrence of immunological rejection in kidney and liver transplants from a different donor.

13.3.3 Prostaglandin E2 (PGE-2)

These are chemical compounds derived from arachidonic acid (AA) through the action of cyclooxygenase (COX), which includes both inducible cyclooxygenase (COX2) and constitutively active cyclooxygenase (COX1) as well as PG synthases. PGE-2 can be generated by different cells, such as infiltrating inflammatory cells, fibroblast, and epithelial being the primary producers of PGE-2 during an immune response. All cell types in the body have the ability to manufacture PGE-2, however during an immune response, invading inflammatory cells, fibroblast, and epithelial cells, are the main producers of PGE-2. The extensive role of PGE-2 is indicated by the presence of PGE2 receptors (EP1–EP4) in a variety of cell types. Although it exhibits relative stability in controlled laboratory circumstances, the presence of albumin accelerates its degradation. Conversely, PGE-2 exhibits a high rate of movement within an organism. Moreover, it is rapidly removed from tissues and the circulatory system. This attribute of PGE-2 is

likely to function during the development of disorders connected to the immune system and indicates a potential area of interest for immune modulation. It is crucial to note that the influence of PGE-2 on the immunoregulation mediated by MSCs usually takes place when it is paired with other drugs that depress the immune system. The combination of PGE-2 and IDO was found to alter the proliferation of T cells, NK cell capability to destroy cells, and the production of cytokines in human MSCs.

13.3.4 Inducible nitric oxide synthase (iNOS)

iNOS is an enzyme that facilitates the synthesis of nitric oxide (NO) within living organisms. Nitric oxide, when present in high amounts, exhibits potent immunosuppressive properties. The calcium level in the cell is essential for all cellular activities. Nevertheless, similar to other NO isoforms, its functionality relies on the interaction with calmodulin (CaM). The presence of elevated levels of NO has been observed to impede immunological responses, however the specific mechanisms behind this effect remain largely unknown. Furthermore, during the initiation process, cytokines such as IFN-γ and TNF-α, either itself or in conjunction, trigger the production of NO. This exerts a substantial influence on both immunological responses. As an illustration, NO specifically targets DCs, which play a vital role in developing robust immune responses. It was discovered that the inhibition of GM-CSF prevents the maturation of rat lung DCs. Likewise, NO hinders the activity of TNF-α and hampers the process of DC development in humans. Substantial quantities of adhesion molecules and chemokines are produced by MSCs. Lymphocytes aggregate nearby mesenchymal stem cells. The abundant release of NO by MSCs is believed to inhibit the activity of immune cells.

13.3.5 Interleukin 10 (IL-10)

Both lymphoid and myeloid cells can produce IL-10. While it possesses beneficial immunosuppressive properties, it also exhibits certain immunostimulatory effects. IL-10 exerts its influence on monocytes, macrophages, and T cells, resulting in the suppression of inflammatory reactions. Therefore, it controls the proliferation and specialization of NK cells, T cells, B cells, and other immune system cells. Thus, it impacts the body's inflammatory reactions. It can suppress the synthesis of IL-10, IFN- γ, IL-2, IL-12, and TNF- α. Additionally, it will decrease the expression of HLA class I. While IL-10 is thought to have a role in the suppressive effects of MSCs, there is currently no evidence showing that MSCs directly produce IL-10. Conversely, it has been discovered that antigen-presenting cells, such as monocytes or dendritic cells and the interaction between MSCs can induce the production of IL-10.

13.3.6 Other mediators

Upon inflammatory activation by heme oxygenase-1 (HO-1), leukemia inhibitory factor (LIF), MSCs or other adult stem/progenitor cells, and inhibitory surface protein programmed death ligand 1 (PD-L1) are among the substances that are present. Galectins and TGF-β molecules are synthesized. Nevertheless, additional research is needed to explore the specific ways in which MSCs suppress the immune system and the molecular mechanisms that underlie this process [2,4].

13.4 EXTRACELLULAR VESICLES (EVs) AND MSC VESICLES

MSCs possess potent paracrine activities, which serve as the primary mechanism underlying their therapeutic actions. The paracrine impact can be partially elucidated by the re-

lease of microvesicles (MVs) or exosomes from mesenchymal stem cells, which leads to the transfer of mRNA, microRNA, and proteins in a horizontal manner. EVs, which are either exosomes released from the endosomal compartment or vesicles shed from the cell membrane, are regarded as a crucial feature of the intercellular milieu. EVs are essential for facilitating cell-to-cell contact by serving as carriers for transferring information. EVs imitate the behavior of mesenchymal stem cells in many experimental scenarios by preventing programmed cell death and promoting cell growth. In addition, they maintain control over the microenvironment with the growth factors, cytokines, and chemical compounds.

These stem cells are multipotent and possess strong immunosuppressive and regenerative capabilities, making them highly valuable for regenerative therapy. Nevertheless, it is imperative to establish common norms or procedures for MSCs. Simultaneously, it is equally crucial to seek out safer and more efficient implementation strategies. Hence, conducting research focused on exosomes generated from MSCs as an alternative holds significant relevance in the present day. MCSs release a variety of EVs, including as MVs with a diameter of 0.1-2 mm and exosomes with a diameter of 30-150 nm. Between the targeted cells and MCSs, these EVs may function as paracrine agents. Exosomes are the extracellular vesicles most frequently utilized in these investigations.

The discovery of MSC exosomes was initially made by Lai et al. Furthermore, the isolated exosomes reduced the size of injured cardiac tissue in an animal model of myocardial ischemia/reperfusion (I/R) injury. Exosomes are therefore thought to be unique molecules that improve the repair of tissues.

MSC exosomes are extensively utilized in clinical studies for cellular therapy, specifically for immunomodulation and regenerative medicine. Mesenchymal stem cell exosomes consist of a variety of bioactive substances, including mRNA. These bioactive molecules, such as proteins or bioactive lipids, have strong therapeutic potential for tissue healing. They achieve this by supporting the body's own stem cells, improving regenerative properties, preventing cell death, promoting blood vessel growth, and modulating the immune system.

The paracrine effect, a crucial characteristic of MSCs in the field of regenerative medicine, was initially documented over two decades ago by Haynesworth et al. MSCs have been observed to produce and release a substantial quantity of growth factors, chemokines, and cytokines, which can have a substantial impact on the neighboring cells. Subsequent reports have indicated that these undisclosed elements enhance the growth of new arteries, safeguard against lack of blood flow and tissue injury, stimulate novel blood vessel generation, and enhance the process of blood vessel formation. An additional illustration of the paracrine impact of MSCs on immunity is evidenced by their immune regulating characteristics. MSCs may suppress several functions of T-lymphocytes, whereas DCs can alter NKs' and T cells' cytokine output, causing a more resilient and anti-inflammation profile to emerge.

While MSC-based therapies offer some benefits compared to ESC and FSC therapies, they can potentially give rise to issues related to cell viability, regenerative capacity, immunological rejection, and tumor formation. To circumvent these issues, exosomes can be employed as a cell-free therapeutic approach. The limited abundance of proteins on exosome membrane greatly reduces the probability of immunological failure, even when administered allogeneically. Furthermore,

exosomes lack the ability to undergo cell division, thereby eliminating any potential for the development of tumors. Thus, MSC-exosomes possess a more secure architecture compared to MSCs for clinical purposes. Adult MSCs possess crucial immunosuppressive and immunomodulatory characteristics, rendering them very appropriate for many regenerative applications and organ transplantation. Consequently, it can significantly impede the activities of natural immune effector cells, specifically targeting MSCs, B cells, T cells, macrophages, neutrophils, NK cells, mast cells, and DCs. Additionally, it may also aid in preventing graft loss [44,45].

EVs play a crucial role in intercellular communication. Although exosomes are typically emphasized in this process, certain papers may refer to the topic using the broader term EV, encompassing all types of extracellular vesicles. Occasionally, these articles may particularly discuss exosomes and MVs in detail. Various expressions are employed in this section, contingent upon the cited source. Examination of exosomes' mobility and roles in studies has revealed that endosomes produce them, kept in multivesicular endosomes (MVEs), and discharged by exocytosis. Exosomes are either secreted into the extracellular space through the fusion of multivesicular bodies (MVBs) with the cell plasma membrane, or they are broken down by MVBs and lysosomes. The exosome communicates with target cells through three potential mechanisms. Membrane fusion facilitates the transmission of membrane receptors, proteins, and microRNAs (miRNAs) to recipient cells. Furthermore, several entities have the ability to transmit signals through protein molecules that bind to receptors on the surface of receiving cells, such as heat shock proteins found in the exosome membrane. Exosomes, which contain miRNAs and proteins, may be delivered to target

cells through endocytosis. The transfer of exosome content to target cells occurs through the activation of receptors and/ or membrane fusion. Exosomes have the ability to activate target cells by attaching protein ligands to receptors on recipient cells through the process of receptor binding. Upon endocytosis, exosomes transfer their cargo of proteins, mRNA, or miRNA into the target cells. While the process of MV production may vary, they share common features in terms of content and communication with the target cell. MVs have the ability to directly activate target cells by binding to receptors on their surface. MVs have the ability to transport receptors or proteins from one cell to another [46].

One advantage of using exosomes is that isolating them from MCSs is simpler compared to isolating MCSs. Additionally, the time required for production and the cost associated with producing exosomes derived from MSCs are reduced compared to those of the parent cells. Exosome therapy is considered safer than MSC-based therapies due to the absence of proliferative capacity in exosomes and the lack of significant numbers of markers that can be recognized as antigens by the host body, leading to a decrease in immunological rejection. Moreover, exosome-based regeneration does not include any worries about cell survival, and it exhibits long-term structural stability even when stored at a temperature of 20°C. This makes exosomes an ideal option for cell-free therapy. Although there is limited research directly comparing MSC-EV treatments to MSC treatments, the prevailing consensus suggests that MSCs are primarily instrumental while MSC-EV is more inclined to impact areas of damage. Utilizing MSC-EV instead of MSCs offers several benefits, including enhanced safety, reduced immunogenicity, and the capacity to surpass biological obstacles. MSC-EV usage mitigates the occurrence

of difficulties arising from the development of abnormal tumors formed from stem cells and the the immune system failure. The benefits of MSC-EV and the increasing evidence of their therapeutic potential are driving the advancement of EV as viable treatment choices. Exosomes released by MSCs via paracrine signaling possess similar properties to MSCs, while additionally offering the benefits of precise delivery, minimal immune response, and enhanced regenerative potential [47]. MSC exosomes possess a superior immunological benefit due to their elevated amounts of miRNA in comparison to MSCs. Additionally, it stimulates inflammatory priming by enhancing the expression of miRNA 155 and miR 146. Other investigations have found that the proportion of regulatory T cells to effector T cells rises when the IL-10 cytokine of exosomes generated from MSCs increases. Furthermore, research has demonstrated that exosomes produced from MSCs have the ability to enhance the process of CD4+ T cell differentiation towards Tregs, while simultaneously reducing the activation of NK cells. The regulatory impacts of MSCs are regulated by suppresive factors such as IL-10, iNOS, IDO, PGE2, and TGF-β1. Immunosuppressive substances have been proposed by researchers to be contained within extracellular vesicles, particularly exosomes. Chen et al. found that MSC-mediated exosomes have an immunological impact on peripheral blood mononuclear cells, targeting T cells. In addition, it enhances the release of cytokines that are anti-inflammatory such as TGF-β1, while suppressing the production of pro-inflammatory cytokines such as IL-1β and TNF-α. This cytokine profile replicates the immunomodulatory impact of MSC. Zang et al. found that exosomes produced from MSCs stimulate the development of CD4+ T cells into regulatory T cells [48,49].

Multiple research involving exosomes produced from MSCs have demonstrated their capacity to modulate the function of various immune cells, such as T cells, B cells, NK cells, and macrophages. Research has indicated that the healing impact of exosomes is facilitated through the transportation of mRNA. Several of these factors are involved in the control of gene expression, cell division, and immunological response, and they contribute to the stimulation of tissue regeneration. Studies show that exosomes produced from MSCs are as effective as MSCs in treating many illnesses, without the potential hazards for cell transplantation. Placenta is a more abundant source of MSCs compared to other substances such as adipose tissue or bone marrow. During pregnancy, the placenta acts as an immune regulatory organ, meaning it is a 'tolerated allograft'. As a result, this tissue may serve as a superior source of allogeneic stem cells. This difficulty has been exemplified in the xenotransplantation of this particular tissue. As a consequence, there was a reduced ability to provoke an immune response in animals with a fully functional immune system. In addition, placental cells do not possess MHC class II antigens, which have a role in the rejection of transplanted tissues. The placenta is considered an immune-privileged tissue for isolating MSCs due to various factors, including those mentioned and others. In a study involving humans with cutaneous graft-versus-host disease, exosome-enriched EVs were obtained from MSCs generated from the placenta. These exosome enriched EVs were then delivered to the patients. Consequently, the patients' skin exhibited reduced hyperpigmentation. Furthermore, there was a reduction in the occurrence and intensity of ulcers, wounds, and keratotic and atrophic lesions, and there was evidence of improved wound healing. In addition, a noticeable enhancement in skin texture and moisture levels was noticed following the therapy. Exoso-

mes, due to their significantly smaller particle size compared to their parent cells, are capable of traversing capillaries without causing obstruction. Hence, intravenous delivery of MSC-derived exosomes proves to be more efficacious compared to the parent cells [50]. The study discovered that when exosomes obtained from immature DCs were coupled with Tregs, they were able to establish immunological tolerance and enhance the survival of a rat liver allograft model [51]. A separate investigation involving BM-MSC exosomes revealed that their administration resulted in an augmentation of the differentiation process of CD4 + T cells into Treg cells. In order to promote the formation of Treg cells, which in turn improve immunological tolerance during kidney donation, BM-MSCs produce exosomes that modify the maturation of CD4+ T cells. Research has demonstrated that exosomes produced by BM-MSCs stimulate immature DCs to release IL-10 [50,52].

GvHD is a common complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), where donor T and B lymphocytes from the transplanted graft identify and attack antigens in the recipient's body, occurring in approximately 50% of cases. Researchers observed a significant inhibition of Th17 cell activity and a reduction in pathological damage by infusing MSC-mediated exosomes into a mouse GvHD model. Exosomes from MSCs extended the lifespan of mice with chronic GvHD and reduced the severity of both pathological and symptomatic symptoms. In a GvHD model created by infusing mononuclear cells from human peripheral blood into treated mice, it was discovered that exosomes produced by MSC cells might reduce signs and enhance lifespan. Exosomes generated from MSCs stimulated the generation of Tregs both in living organisms and in laboratory conditions through pathways involving APCs. Mesenchymal stem cells are being increasingly utilized as an

alternate strategy for treating GVHD, owing to their ability to modulate the immune system and regulate the immunological response [53,54,55].

Cold ischemia-reperfusion has long been a worry in transplantation due to the potential damage it might cause to grafts. A study was conducted to induce ischemia reperfusion injury in a rat kidney model by introducing MSC-mediated exosomes, and the outcomes were observed. Perfusion resulted in a notable decrease in indicators of kidney damage in kidneys that were treated with exosomes delivered by MSCs. In addition to BM-MSC-mediated exosomes, other studies have documented the tumoricidal capabilities of exosomes derived from human UC-MSC [56,57,58]. Experiments have demonstrated that exosomes produced from UC-MSCs, when given after a heart attack, can enhance the number of myofibroblasts in the damaged area, encourage the transformation of fibroblasts into inflammatory myofibroblasts, and decrease inflammation in laboratory settings [59].

Given the numerous benefits of MSC-mediated exosomes, significant advancements in the medical domain are undoubtedly feasible in the future. Exosomes, biomolecules utilized for therapeutic drug delivery, exhibit comparable effects to synthetic nanoscale carriers (e.g. liposomes, nanoparticles) currently employed in drug transfer. However, exosomes possess biological mechanisms and activities depending on cells. Exosomes possess inherent integration with living organisms, exhibit enhanced chemical durability, facilitate long-range intercellular communication, and possess innate capacity for intercellular fusion and transport. Numerous investigations suggest that exosomes can preferentially interact with cells and particular tissues, including the ability to traverse dense tissue barriers like the blood-brain barrier [60,61,62,63].

13.5 CONCLUSION

While MSCs-derived exosomes show promise as a potential alternative to MSCs in the field of regeneration therapy, before they can be utilized in medical settings, a number of issues need to be addressed. Currently, exosomes are extracted by categorizing them according to their vesicle size. In order to ensure consistent treatment effectiveness for heart reconstruction and healing, it is necessary to employ more precise isolation techniques if the varying sizes of vesicles in exosomes are indicative of various components. Furthermore, exosomes are sequestered within MVEs prior to their secretion into the extracellular matrix. Prior to release, it may be beneficial to separate exosomes formed from MSCs from MVBs in order to obtain purified exosomes. This is because exosomes obtained from culture media and blood through MSC-mediated processes may contain contaminants from other cell types. Furthermore, there is currently a lack of a clearly defined technique for analyzing individual exosomes. An examination of the cargo contents of an individual exosome, as opposed to the total exosome population, will yield a more comprehensive comprehension of the specific mechanisms that underlie exosome-based therapies. An in-depth analysis of payloads from an individual exosome, as opposed to the total exosome population, will yield a more comprehensive comprehension of the specific mechanisms that underlie exosome-based therapies [60,61,64].

13.6 REFERENCES

1. Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12(5):383–396. https://doi.org/10.1038/nri3209
2. Rawat S, Gupta S, Mohanty S. Mesenchymal Stem Cells Modulate the Immune System in Developing Therapeutic Interventions. Tyagi RK, Bisen PS (editors). Immune Response Activation and Immunomodulation. IntechOpen, 2019. https://doi.org/10.5772/intechopen.80772
3. Davies JE, Walker JT, Keating A. Wharton's Jelly: The Rich, but Enigmatic, Source of Mesenchymal Stromal Cells Stem Cells. Transl Med. 2017 Jul;6(7):1620-1630. https://doi.org/10.1002/sctm.16-0492
4. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci, 2017 Jul;74(13):2345-2360. https://doi.org/10.1007/s00018-017-2473-5
5. Wang Y, Chen X, Cao W, Shi Y (2014) Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 15(11):1009–1016. https://doi.org/10.1038/ni.3002
6. Kumar BV, Connors TJ, Farber DL. Human T Cell Development, Localization and Function throughout Life Me 48, ISSUE 2, P202-213. https://doi.org/10.1016/j.immuni.2018.01.007
7. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002 May 15;99(10):3838-43. https://doi.org/10.1182/blood.v99.10.3838
8. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood. 2005 Apr 1;105(7):2821-7. https://doi.org/10.1182/blood-2004-09-3696
9. Plumas J, Chaperot L, Richard M-J, Molens J-P, Bensa J-C, et al. Mesenchymal stem cells induce apoptosis of activated T cells. Leukemia,2005 Sep;19(9):1597-604. https://doi.org/10.1038/sj.leu.2403871

10. Akiyama K, Chen C, Wang D, Xu X, Qu C, et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis, Cell Stem Cell 2012 May 4;10(5):544-55. https://doi.org/10.1016/j.stem.2012.03.007
11. Polchert D, Sobinsky J, Douglas Gw, Kidd M, Moadsiri A, et al. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease, Eur J Immunol. 2008 Jun;38(6):1745-55. https://doi.org/10.1002/eji.200738129
12. Consentius C, Akyuz L, Schmidt-Lucke JA, Tschope C, Pinzur L, et al. (2015) Mesenchymal stromal cells prevent allostimulation in vivo and control checkpoints of Th1 priming: migration of human DC to lymph nodes and NK cell activation. Stem Cells 33(10):3087–3099. https://doi.org/10.1002/stem.2104
13. Lopez-Santalla M, Mancheno-Corvo P, Menta R, Lopez-Belmonte J, DelaRosa O, et al. (2015) Human adipose-derived mesenchymal stem cells modulate experimental autoimmune arthritis by modifying early adaptive T cell responses. Stem Cells 33(12):3493–3503. https://doi.org/10.1002/stem.2113
14. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, et al. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. J Immunol, 2010 Mar 1;184(5):2321-8. https://doi.org/10.4049/jimmunol.0902023
15. Ren G, Su J, Zhang L, Zhao X, Ling W, et al. (2009) Species variation in the mechanisms of mesenchymal stem cellmediated immunosuppression. Stem Cells 27(8):1954–1962. https://doi.org/10.1002/stem.118S
16. Ma S, Xie N, Li W, Yuan B, Shi Y, et al. Immunobiology of mesenchymal stem cells Cell Death Differ, 2014 Feb;21(2):216-25. https://doi.org/10.1038/cdd.2013.158
17. Crop MJ, Baan CC, Korevaar SS, Ijzermans JN, Pescatori M, et al. (2010) Inflammatory conditions affect gene expression and function of human adipose tissue-derived mesenchymal stem cells. Clin Exp Immunol 162(3):474–486. https://doi.org/10.1111/j.1365-2249.2010.04256.x
18. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted

action of chemokines and nitric oxide. Cell Stem Cell, 2008 Feb 7;2(2):141-50. https://doi.org/10.1016/j.stem.2007.11.014

19. Li W, Ren G, Huang Y, Su J, Han Y, et al. Mesenchymal stem cells: a double-edged sword in regulating immune responses. Cell Death Differ, 2012 Sep;19(9):1505-13. https://doi.org/10.1038/cdd.2012.26
20. Su J, Chen X, Huang Y, Li W, Li J, et al. Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. Cell Death Differ, 2014 Mar;21(3):388-96. https://doi.org/10.1038/cdd.2013.149
21. Yang M, Sun L, Wang S, Ko K-H, Xu H, et al. Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells, J Immunol, 2010 Apr 1;184(7):3321-5. https://doi.org/10.4049/jimmunol.0902551
22. Che N, Li X, Zhou S, Liu R, Shi D, et al. Umbilical cord mesenchymal stem cells suppress B-cell proliferation and differentiation, Cell Immunol, 2012;274(1-2):46-53. https://doi.org/10.1016/j.cellimm.2012.02.004
23. Luz-Crawford P, Djouad F, Toupet K, Bony C, Franquesa M, et al. Mesenchymal Stem Cell-Derived Interleukin 1 Receptor Antagonist Promotes Macrophage Polarization and Inhibits B Cell Differentiation, Stem Cells, 2016 Feb;34(2):483-92. https://doi.org/10.1002/stem.2254
24. Spaggiari GM, Moretta L. Cellular and molecular interactions of mesenchymal stem cells in innate immunity, Immunol Cell Biol, 2013 Jan;91(1):27-31. https://doi.org/10.1038/icb.2012.62
25. Che N, Li X, Zhang L, Liu R, Chen H, et al. Impaired B cell inhibition by lupus bone marrow mesenchymal stem cells is caused by reduced CCL2 expression, J Immunol, 2014 Nov 15;193(10):5306-14. https://doi.org/10.4049/jimmunol.1400036
26. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study, Clinical Trial Lancet, 2008 May 10;371(9624):1579-86. https://doi.org/10.1016/S0140-6736(08)60690-X
27. Noone C, Kihm A, English K, O'Dea S, Mahon BP. IFN-γ stimulated human umbilical-tissue-derived cells potently suppress NK

activation and resist NK-mediated cytotoxicity in vitro, Stem Cells Dev, 2013 Nov 15;22(22):3003-14. https://doi.org/10.1089/scd.2013.0028

28. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation, Blood,2006 Feb 15;107(4):1484-90. https://doi.org/10.1182/blood-2005-07-2775
29. Huang Y, Wu Q, Tam PKH. Immunomodulatory Mechanisms of Mesenchymal Stem Cells and Their Potential Clinical Applications. Int J Mol Sci. 2022 Sep 2;23(17):10023. https://doi.org/10.3390/ijms231710023
30. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells, Stem Cells, 2006 Jan;24(1):74-85. https://doi.org/10.1634/stemcells.2004-0359
31. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, et al. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation, Blood, 2004 Jun 15;103(12):4619-21. https://doi.org/10.1182/blood-2003-11-3909
32. Banas RA, Trumpower C, Bentlejewski C, Marshall V, Sing G, et al. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells, Hum Immunol, 2008 Jun;69(6):321-8. https://doi.org/10.1016/j.humimm.2008.04.007
33. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, et al. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2, Blood, 2008 Feb 1;111(3):1327-33. https://doi.org/10.1182/blood-2007-02-074997
34. Götherström C, Lundqvist A, Duprez IR, Childs R, Berg L, et al. Fetal and adult multipotent mesenchymal stromal cells are killed by different pathways, Cytotherapy, 2011 Mar;13(3):269-78. https://doi.org/10.3109/14653249.2010.523077
35. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells, Exp Hematol, 2003 Oct;31(10):890-6. https://doi.org/10.1016/s0301-472x(03)00110-3

36. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2, Blood, 2009 Jun 25;113(26):6576-83. https://doi.org/10.1182/blood-2009-02-203943
37. Jiang X-X, Zhang Y, Liu B, Zhang S-X, Wu Y, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells, Blood, 2005 May 15;105(10):4120-6. https://doi.org/10.1182/blood-2004-02-0586
38. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells, J Immunol, 2006 Aug 15;177(4):2080-7. https://doi.org/10.4049/jimmunol.177.4.2080
39. Li Y-P, Paczesny S, Lauret E, Poirault S, Bordigoni P, et al. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway, J Immunol, 2008 Feb 1;180(3):1598-608. https://doi.org/10.4049/jimmunol.180.3.1598
40. Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages, Exp Hematol, 2009 Dec;37(12):1445-53. https://doi.org/10.1016/j.exphem.2009.09.004
41. Zhang Q-Z, Su W-R, Shi S-H, Wilder-Smith P, Xiang AP, et al. Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing, Stem Cells, 2010 Oct;28(10):1856-68. https://doi.org/10.1002/stem.503
42. Cutler AJ, Limbani V, Girdlestone J, Navarrete CV. Umbilical cord-derived mesenchymal stromal cells modulate monocyte function to suppress T cell proliferation, J Immunol, 2010 Dec 1;185(11):6617-23. https://doi.org/10.4049/jimmunol.1002239
43. François M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation, Mol Ther, 2012 Jan;20(1):187-95. https://doi.org/10.1038/mt.2011.189

44. Pegtel DM, Gould SJ. Exosomes, Annu Rev Biochem. 2019 Jun 20;88:487-514. https://doi.org/10.1146/annurev-biochem-013118-111902

45. Tang Y, Zhou Y, Li H-J. Advances in mesenchymal stem cell exosomes: a review. Stem Cell Res Ther. 2021 Jan 19;12(1):71. https://doi.org/10.1186/s13287-021-02138-7

46. Wei W, Ao Q, Wang X, Cao Y, Liu Y, et al. Mesenchymal Stem Cell-Derived Exosomes: A Promising Biological Tool in Nanomedicine, Front Pharmacol, 2021 Jan 25;11:590470. https://doi.org/10.3389/fphar.2020.590470

47. Xunian Z, Kalluri R. Biology and therapeutic potential of mesenchymal stem cell-derived exosomes. Cancer Sci. 2020 Sep;111(9):3100-3110. https://doi.org/10.1111/cas.14563

48. Wang D, Wang X, Song Y, Si M, Sun Y, et al. Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts, Cell Death Dis, 2022 Apr 20;13(4):380. https://doi.org/10.1038/s41419-022-04825-6

49. Nail HM, Chiu C-C, Leung C-H, Ahmed MMM, Wang H-MD. Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments J Biomed Sci. 2023; 30: 69. https://doi.org/10.1186/s12929-023-00964-w

50. Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard C. Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke. 2018 Aug;13(6):612-632. https://doi.org/10.1177/1747493018778713

51. Pang X-L, Wang Z-G, Liu L, Feng Y-H, Wang J-X, et al. Immature dendritic cells derived exosomes promotes immune tolerance by regulating T cell differentiation in renal transplantation, Aging,2019 Oct 26;11(20):8911-8924. https://doi.org/10.18632/aging.102346

52. Negi N, Griffin MD. Effects of mesenchymal stromal cells on regulatory T cells: Current understanding and clinical relevance. Stem Cells. 2020 May; 38(5): 596–605. https://doi.org/10.1002/stem.3151

53. Kordelas L, Rebmann V, Ludwig A-K, Radtke S, Ruesing J, et al. MSC-derived exosomes: a novel tool to treat therapy-refrac-

tory graft-versus-host disease. Leukemia, 2014 Apr;28(4):970-3. https://doi.org/10.1038/leu.2014.41

54. Tekeli S, Arısu Naghavi E, Gökçe B, Sır G, Yiğittürk G, et al. Kök hücreler; mezenkimal kök hücreler ve güncel klinik uygulamaları, FNG & Bilim Tıp Transplantasyon Dergisi 2016;1(2):72-83. https://doi.org/10.5606/fng.transplantasyon.2016.013
55. Saidu NEB, Bonini C, Dickinson A, Grce M, Inngjerdingen M, et al. New Approaches for the Treatment of Chronic Graft-Versus-Host Disease: Current Status and Future Directions.Front Immunol. 2020. https://doi.org/10.3389/fimmu.2020.578314
56. Lotfy A, AboQuella NM, Wang H. Mesenchymal stromal/stem cell (MSC)-derived exosomes in clinical trials, Stem Cell Res Ther. 2023; 14: 66. Published online 2023 Apr 7. https://doi.org/10.1186/s13287-023-03287-7
57. Lim SW, Kim KW, Kim BM, Shin YJ, Luo K, et al. Alleviation of renal ischemia/reperfusion injury by exosomes from induced pluripotent stem cell-derived mesenchymal stem cells Korean J Intern Med. 2022 Mar; 37(2): 411–424. https://doi.org/10.3904/kjim.2020.438
58. Zheng Q, Zhang S, Guo W-Z, Li X-K. The Unique Immunomodulatory Properties of MSC-Derived Exosomes in Organ Transplantation, Front Immunol. 2021; 12: 659621. https://doi.org/10.3389/fimmu.2021.659621
59. Liu J, Liang X, Li M, Lin F, Ma X, et al. Intramyocardial injected human umbilical cord-derived mesenchymal stem cells (HucMSCs) contribute to the recovery of cardiac function and the migration of CD4+ T cells into the infarcted heart via CCL5/CCR5 signaling, Stem Cell Res Ther. 2022; 13: 247. 2022 Jun 11. https://doi.org/10.1186/s13287-022-02914-z
60. Norouzi-Barough L, Shirian S, Gorji A, Sadeghi M. Therapeutic potential of mesenchymal stem cell-derived exosomes as a cell-free therapy approach for the treatment of skin, bone, and cartilage defects. Connect Tissue Res.2022 Mar;63(2):83-96. https://doi.org/10.1080/03008207.2021.1887855
61. Panda B, Sharma Y, Gupta S, Mohanty S. Mesenchymal Stem Cell-Derived Exosomes as an Emerging Paradigm for Regenerative Therapy and Nano-Medicine: A Comprehensive Review. Life 2021, 11(8), 784. https://doi.org/10.3390/life11080784

62. Hade MD, Suire CN, Suo Z. Mesenchymal Stem Cell-Derived Exosomes: Applications in Regenerative Medicine. Cells 2021 Aug 1;10(8):1959. https://doi.org/10.3390/cells10081959

63. Sanmartin MC, Borzone FR, Giorello MB, Yannarelli G, Chasseing NA. Mesenchymal Stromal Cell-Derived Extracellular Vesicles as Biological Carriers for Drug Delivery in Cancer Therapy, Front Bioeng Biotechnol. 2022; 10: 882545. https://doi.org/10.3389/fbioe.2022.882545

64. Fuloria S, Subramaniyan V, Dahiya R, Dahiya, S Sudhakar K, et al. Mesenchymal Stem Cell-Derived Extracellular Vesicles: Regenerative Potential and Challenges Biology (Basel) 2021 Mar; 10(3): 172. https://doi.org/10.3390/biology10030172

KEY TRANSCRIPTION FACTORS IN MESENCHYMAL STEM CELL DIFFERENTIATION

Mustafa SOYOZ[1,2,3,4], Seyma Nur CELIK[2]

[1]Director of Izmir Katip Çelebi University Vocational School of Health Services, Izmir, Turkey

[2]Medical Biology Department, İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[3]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

[4]Izmir Tepecik Training and Research Hospital Tissue Typing Laboratory, Izmir, Turkey

14.1 INTRODUCTION

Mesenchymal stem cell is a adult stem cells, which can differentiate into may cell types, such as muscle, cartilage, bone, and fat are known as mesenchymal stem cell. MSC isolations from fatty tissue, testicles, umbilical cord blood, placenta, bone marrow, pancreas, liver, spleen, amniotic fluid, dental pulp, menstrual blood, and many tissues and organs have recently been carried [1,2,3]. Their easy isolation, high migration capacity, rapid reproduction, and ability to escape an allergenic response after transplantation make them prominent players in regenerative medicine [4]. These cells are distinguished by the structure of their retinas and their capacity for in vitro differentiation into adipocytes osteocytes, and chondrocytes. A singular indicator does not differentiate MSCs from

cells that possess comparable fibroblastic features. When cells are separated, they are distinguished by their ability to express some of the multiple surface markers and some not to express them. MSCs generally represent surface molecules like CD105, CD90, CD29, CD73, and CD44, while they do not have endothelial and hematopoietic surface molecules like CD45, CD34, CD31, CD14, and CD11 [5,6]. MSCs can be diverge into many cell types that make up tissues. The differentiation process of MSCs takes place through complex cellular paths, including transcription factors and the organization of gene expressions. Transcription factors, extracellular matrix molecules, cytokines, growth factors, and regulate the transformation of MSCs into mature cell types [7]. For MSCs to establish a distinction, particular inducers are necessary, such as certain growth factors. Transcription factors may influence the maturation of MSCs into various cell types. The differentiation of a particular cell type can be induced through the stimulation of transcription factors, which subsequently triggers the expression of the genes accountable for advancement. This chapter will discuss the attributes and operations of transcription factors, containing PPARγ, MYOD, RUNX2, NANOG and, SOX9 which play critical roles in the differentiation process [8,9].

14.2 MESENCHYMAL DIFFERENTIATION

14.2.1 Adipogenic Differentiation

For MSCs to differentiate into adipocytes, either the blockage or stimulation of specific transcription factors is necessary. An essential transcription factor in the differentiation ofMSCs from adipocytes is PPARγ (the adipogenic-specific peroxisome proliferation-activated receptor γ). It is known that adipogenic transformation of MSCs induces an increase in the expression of PPARγ; blocking it inhibits adipogenesis.

The isoforms PPARγ2 and PPArγ1 have vital functions in the process of development of MSCs to adipocytes. The attachment of PPARγ to various substrates triggers the PPARγ stimulation and suppression [10]. One of these ligands, TAZ, has been to act as a common suppressor of PPARγ, thus blocking adipogenic differentiation. Another study showed that PPARγ2 on its own or in conjunction with PRDM16 or CEBPB supports adipogenic transformation with 90% effectiveness. It is also known that the transcriptional inhibitor, which interacts with myocyte amplifier factor-2, functions significantly in impeding the adipogenic transformation of MSCs by means of its relationship with PPARγ2 and subsequent inhibition of this transcription factor's activity [7].

EBF-1, also known as the early B cell factor, is a crucial component of transcription process that has a significant impact on cellular activity and transformation. It has an important function in supporting the transformation of MSCs to osteocytes and adipocytes. Gene expression analyses have shown that PPARγ2 and EBF-1 induce two separate gene sets, both of which are associated with adipogenic transformation[11].

As a constituent of the zinc finger transcription factor family, GATA-2 regulates the growth and development of diverse cell strains and hematopoietic stem cells. GATA-2 also regulates adipogenic differentiation by preserving hematopoietic. The suppression of GATA-2 increases MSC transformation to adipocytes, while its stimulation tends to stop adipogenic differentiations. In a same way, the silencing of the forkhead transcription factor (Foxa1) enhances the MSC transformation to adipocytes, and PPARγ, the key transcription factors in Adipogenesis, increases C/EBPα expression [12]. It was also found that HOXC8 exhibited decreased expression

levels throughout the transformation process of MSCs into adipocytes. Conversely, overexpression of HOXC8 in MSCs hindered the their development to adipocytes [13].

The most widely studied transcription factors have been demonstrated in several studies where a group of translation factors, including PPARγ1, PPARg2 and EBF-1, play important controlling functions in the adipogenic transformation of MSCs. In addition, several additional transcription factors have important functions in the transformation of MSCs to adipocytes. These include Oct4, Sox2, COUP-II, Dermo-1, Twist-1, PRDM16, and CEBPB. But there are also transcription factors, such as HOXC8, Foxa1, and GATA2, which have a suppressive influence on the MSC transformation to adipocytes [7].

14.2.2 Osteogenic Differentiation

Hormones and transcription factors cooperate at the molecular level to regulate the development of MSCs into osteocytes. Osterix and CBFA-1/Runx2 are the primary transcription factors that are crucial in the transformation of MSCs into osteocytes. The transformation of MSCs towards osteocytes can be stimulated in a single-layer culture environment by incubating them in a differentiating environment containing combinations of vitamin D3, bone morphogenetic proteins (BMP), transforming growth factor-beta (TGF-β), ascorbic acid-2-phosphates, dexamethasone, and β-glycerophosphate[7,14].

Several transcription factors like β-catenine, osterix, and Runt-related transcription factor 2 (Runx2) control transformation to osteocytes. The Runx gene contains an area for DNA binding (runt area) and heterodimer forms of the Runx protein. Various studies have shown that Runx2 is necessary for osteogenic transformation. The Runx2 transcription factor

plays a crucial role in controlling bone development and the transformation of MSCs into bone cells. It leads to MSC transformation to pre-osteoblast and prevents transformation to adipocytes and chondrocytes. The symmetrical division of Runx2 mRNA to new cells with post-mitotic symmetric division has been found to support osteogenic genealogy and the preservation of the osteoblast phenotype. Overexpression of the homeobox protein Hox-B7 (HOXB7) has also been shown to affect mRNA synthesis of main transcription factor Runx2 and promote osteogenic transformation. HOXB7 has been found to increase osteogenic transformation by upward controlling Runx2[15]. During bone development Hoxa2 is increased, whereas Hoxa9 is downregulated[13].

Runx2 synthesis is controlled by various signal paths, like the Notch, BMP, and Wnt signalling routes. BMP interacts with BMPR (the BMMP receptor) and stimulates the intra-cellular Smad that can be trasnported to the nucleus and act like a transcription factor. Osteogenic differentiation has also been deteriorated by the deletion of BMP ligands. BMP9 has also been found to support Smad8, 5 and 1 activation and transformation to osteocytes in MSCs. Transformation to osteocytes needs Smad-Runx2 association. Mutations occurring in the C-end region of Runx2 interfere with the Smad-Runx2 activity, leading to the inhibition of osteogenic development. The transcription factor TWIST functions being an afterwards effector of hypoxia-induced factor-1α (HIF-1α) and inhibits the production of Runx2 in MSCs. This inhibition leads to the control of development of osteocytes. Over-expression of HIF-1α increases cell survival, proliferation, and the pro-angiogenic gene synthesis. However, the production of osteogenic indicators, containing Runx2 and BMP-2, is also reduced, suggesting that overexpression of HIF-1α has no effect on the osteogenetic transformation of MSCs[16].

Osterix is a transcription factor belonging to Sp1 family. There are three zinc finger motifs on it. It is known that in the absence of osterix, intramembranous or endochondral ossification is known to prevent the production of bone trabeculae and cortical bone. The lack of expression of osterix in Runx2/Cbfa1 null mice suggests that osterix functions downstream of Runx2. The study determined that Osterix is essential for guiding MSCs towards osteoblasts, therefore playing a crucial role in the process of bone development [17]. Furthermore, stimulating the Wnt route in MSCs inhibits PPAR-γ and leads to the production of Osterix [18].

β-catenin is crucial in the process of MSC development into osteoblasts. β-catenin is necessary for osteogenic development and its absence prevents this process, hence enabling MSCs to eventually transform into chondrocytes. The biological function of β-catenin is controlled by the Wnt route [19]. Wnt molecules attach to the LRP5 co-receptor and Frizzled receptor, causing β-catenin to build up in the cytosol. Afterward, it enters the nucleus and connects with the LEF/TCF. β-catenin, a transcription factor, forms a bond with LEF/TCF and initiates the process of transcribing the resulting gene [20].

CBF-1α transcription factor is crucial in the process of osteogenic maturation of MSCs. Hypoxia in MSCs derived from bone marrow leads to a gradual upregulation of CBF-1α production, hence enhancing the transformation capacity of MSCs into osteocytes [21]. The Notch signaling pathway controls CBF-1α, facilitating the generation of the Notch intracellular domain (NICD). This process results in the stimulation of CBF-1α within the nucleus [7]. Transgenic mice that have an excessive amount of NICD experience the development of osteosclerotic bone. In contrast, the absence of Notch signaling leads to osteoporosis associated with aging. TNF-α has

been discovered to stimulate the process of osteogenic development in MSCs generated from the umbilical cord. NF-kB route mediates this effect [22,23].

Numerous investigations have shown that different transcription factors can either promote or inhibit the osteogenic development of MSCs. During bone development, the transcription factor DLX5, which is a member of the homeoprotein family, is produced. Through the action of SOX2, it has been demonstrated that DLX5 overexpression inhibits MSCs' ability to differentiate terminally into osteocytes in vitro. The Wnt signaling pathway is crucial to the stimulation of MSC osteogenic development via the winged helix/forkhead family transcription factor Foxc2. As a transcriptional regulator involved in the homeostasis, progression, repair, growth, and advancement of various malignancies, YAP (yes-associated protein) is a co-stimulator directed in collaboration with the TEAD family of transcription factors. Research has discovered that the process of osteogenic development is improved by the influence of YAP.

Osterix and CBFA-1/Runx2 are transcription factors that play crucial regulatory functions in the osteogenic development of MSCs. Several other transcription factors, such as β-catenin, BMP9, HOXB7, HOXA2, YAP, CBF-1α, FOXC2, and TNF-α have been documented and investigated for their functional involvement in the transformation of MSCs into osteocytes. TWIST and HIF-1α hinder the development of MSCs into osteocytes by directly or indirectly interacting with Runx2, so exerting an inhibitory effect [7].

14.2.3 Chondrogenic Differentiation

Although only a few genetic factors have been identified that regulate chondrogenesis of MSCs, transcription factors

are crucial in controlling the transcription of collagen type II, IX, X, and XI, cartilage, and aggrecan attachment proteins, that are recognized as indicators for chondrocytes. A supplemented environment containing TGF-β1, ascorbic acid, L-glutamine, proline, selenous acid, transferrin sodium pyruvate, phosphate, linoleic acid, bovine serum albumin, and dexamethasone induces chondrogenic development of MSCs in vitro. During the process of transformation, the physical appearance of MSCs undergoes a transformation from a fibroblast-like structure to a spherical shape [24,25]

Sox9 (SRY-related high mobility group-box gene 9) is the primary transcription factor responsible for the transformation of MSCs into chondrocytes. It mediates the expression of important genes involved in the process of chondrogenesis. The protein binds to the promoter of the collagen type 9 gene and creates complexes with various other proteins to activate the gene's production [26,27]. Specifically focusing on Sox9 by either overexpressing or inhibiting mRNA-145 has been demonstrated to lower or raise the mRNA amount of the indicator genes of chondrocytes such as aggrecan, collagen types 11, 9, and 2. The overexpression of miR-574-3p suppresses the activity of Sox9 and the process of chondrogenic transformation in MSCs. Additionally, a significant enhancement of chondrogenic differentiation was observed in MSCs transfected with an interaction of Sox9, Sox6, and Sox5. In addition, TNF-α was also shown to increase Sox9 gene transcription. Adenovirus-mediated expression of Sox9 and BMP2 in mouse embryonic MSCs has also been shown to significantly improve the process of chondrogenic transformation in a laboratory setting [28,29]

It is known that the increase in FOXO3A expression is crucial in the transformation of MSCs to chondrocytes, especially

in this process, down-regulation of miR-29a is also known to contribute to the process. Hoxa2 has been shown to be downregulated while undergoing chondrogenic differentiation of MSCs, and its overexpression results in the inhibition of transformation of MSCs to the chondrocytes. Furthermore, there have been reports indicating that the genes HOXD13 and HOXD9 are increased in expression during the process of chondrogenic transformation of MSCs. Additionally, it has been observed that blocking the activity of the genes HOXD13, HOXD11, and HOXD10 hinders the transformation of MSCs into chondrocytes [13,30]

Another transcription factor, zinc finger protein 145 (ZNF145), is involved in the process of MSC transformation into chondrocytes. Suppression of ZNF145 limits the process of MSCs transforming into chondrocytes, while its excessive production promotes chondrogenesis and enhances the levels of Sox9 [31]. Studies have demonstrated that Smads act as controllers of the process of chondrogenic transformation in MSCs. TGF-β1 controls the activity of Smad 2 and 3 during the initial phases of chondrogenesis. Smad3 forms a bond with Sox9, which consequently hinders the process of chondrogenic development. YAP, previously identified as a controller of the process by which MSCs develop into bone cells, has also been discovered to hinder the transformation of MSCs into cartilage cells. STAT3 has been demonstrated to have a crucial function in the determination of MSCs to differentiate into chondrogenic generations by activating the STAT3 pathway through IL-6. Increased levels of Wnt11 promote the activation of genes that regulate the formation of cartilage, and when combined with TGF-β, it enhances the process of cartilage formation in MSCs [32,33].

In many studies investigating chondrogenic differentiation of MSCs, it has been found to have distinct master regulators, including ZNF145 and Sox9. Additional transcription factors, including as Wnt11, STAT3, HOXD13, FOXO3A, HOXD11, HOXD10, and HOXD9 have been documented to have significant functions in the process of MSC transformation into chondrocytes. Nevertheless, it is established that Hoxa2, Smad3, and YAP hinder the process of MSC transformation into chondrocytes by directly or indirectly interacting with Sox9 [7].

14.2.4 Myogenic Differentiation

14.2.4.1 Skeletal Muscle Cells

The biological dedication of MSCs to skeletal muscle cell development relies on the essential suppression or stimulation of specific transcription factors. The myogenic transformation of MSCs is initiated by the stimulation of certain myogenic transcription factors, such as myogenin, Myf-5, MyoD, and paired box 3 (Pax3). The transcription factors induce the development of the sclerotome and dermo-myotome. Multiple studies have documented that MSCs can transform into skeletal muscle cells when exposed to a substance called 5-azacytidine, which removes methyl groups from DNA. In addition, the process of myogenic transformation has been proven through the co-cultivation of MSCs with neonatal cardiomyocytes, neonatal fibroblasts, and skeletal myocytes [34].

Cells undergo migration toward the dorsomedial end of the dermomyotome during the transcription of Pax3 to establish the myotome and induce myogenic transformation. Pax7 and Pax3 belong to the paired box family of transcription factors. These entities are recognized as important controllers of myogenic development because they have a role in

the early formation of striated muscle during the growth and repair of skeletal muscle. Previous studies have shown that increasing the production of Pax3 in MSCs stimulates the development of muscle cells while inhibiting the formation of cartilage, bone, and fat, cells in MSCs [35].

Myogenin, MyoD, and Myf-5 are the transcription factors belonging to the helix-loop-helix family and have crucial roles in the regulation of myogenic transformation. However, Myf-5 and MyoD are not produced in MSCs obtained from the same source; therefore, Myf-5 and MyoD specify distinct muscle cell lines in MSCs derived from various sources [7]. MyoD increase was shown to supress Twist-1 by miR-206 stimulation, leading to increased muscle cell transformation. It has also been shown that human pluripotent stem cells overexpressed with MyoD1 using a vector undergo myogenic differentiation. TAZ, which is recognized as a regulator of the transformation of MSCs into bone and fat cells, was also observed to increase the process of muscle cell development mediated by MyoD[36]. Signaling variables can stimulate or hinder myogenic development. IGF-II can stimulate myogenic transformation by binding to insulin-like growth factor receptor-1, which in turn activates coregulators that are crucial cofactors for MyoD. TNF-α plays a controlling function in the process of myogenic transformation of MSCs. It inhibits the production of the MyoD that is essential for the growth of muscle cells. Studies have demonstrated that it can impede the process of myogenic transformation by activating NF-κB and TNF-α, as well as reducing the IGF-1 signaling pathway. Smad3, a member of the Smad family that is controlled by the TGF-β receptor, inhibits the process of myogenic transformation in conjunction with myogenic transcription factors. There are reports indicating that TGF-β-stimulated Smad3 specifically inhibits the controlling function of myogenin and MyoD [37,38].

It has been reported that some transcription factors such as MyoD, Myf-5, Pax7, and Pax3 have primary function in the transformation of skeletal muscle from MSC. Others, such as Myogenin, TAZ and IGF-II, are said to play functions in transformation into skeletal muscle. On the contrary, TNF-α was reported to have an suppressive effect on trasnformation in MSC by the NF-κB pathway [7].

14.2.4.2 Cardiomyocytes

Numerous transcription factors have been discovered at a molecular scale as controllers of the transformation of mesenchymal stem cells into cardiomyocytes. GATA4, a member of the GATA zinc finger transcription factor family, has been demonstrated to control the growth and development of several cell kinds. MSCs that have been genetically modified with GATA4 exhibit a greater level of GATA4 production compared to non-modified MSCs when undergoing the process of differentiating into cardiomyocytes. This indicates that the excessive presence of GATA4 enhances the cardiomyocytic transformation ability of MSCs. Studies have revealed that when myocytes are present, MSCs can transform into cardiomyocytes, exhibiting a greater level of GATA4 transcription compared to immature MSCs. Studies have shown that the process of MSCs transforming into cardiomyocytes is facilitated by the increased expression of GATA4 and Nkx2.5. Furthermore, they are essential for the transformation of MSCs into cardiomyocytes. Introducing the Wnt11 gene enhances the expression of GATA4, which allows MSCs to undergo transformation into cardiomyocytes [39,40].

The cardiomyogenic transcription factor and smooth muscle cell known as myocardin has two or more important serum response factor (SRF) binding sites. Experiments condu-

cted outside of a living organism have shown that increasing the amount of the myocardin gene in MSCs leads to the activation of multiple indicators associated with the maturation of heart muscle cells. However, it is important to note that this increase does not lead to full maturation of heart muscle cells, indicating that these genes are involved in the early stages of maturation. Animal investigations have shown that Thioredoxin-1 (Trx1), which acts as a growth factor regulator transcription factor, and antioxidant, can greatly improve the transformation of MSCs into cardiomyocytes. Research has showed that genetically engineered MSCs, using an adenovector that increases the Notch1 intracellular domain (NICD), have enhanced conversion capacity, allowing them to transform to cardiomyocytes [41,42,43].

NKX2.5 and GATA4 are well studied transcription factors that play crucial regulatory functions in the transformation of MSCs into cardiomyocytes. Notch1, Wnt11, Trx1, and Myocardin are additional transcription factors that have been examined and researched for their activity during transformation of MSCs into osteocytes [7].

The process of myogenic transformation was investigated using the co-cultivation of MSCs with neonatal cardiomyocytes, neonatal fibroblasts, and skeletal myocytes. It has been documented that MSCs possess the capability to transform into cardiomyocytes during a treatment period of 2-3 weeks with 5-azacytidine. This therapy occurs in a medium enriched with 10% FBS and containing low-glucose DMEM [13].

14.2.4.3 Smooth Muscle Cells

TGF-β is the most efficient stimulant for transforming mesenchymal stem cells into vascular smooth muscle cells. The expression of alpha smooth muscle actin (α-SMA), smooth

muscle myosin heavy chain (SMMHC), and calponin genes is increased by it. Furthermore, it has been demonstrated that TGF-β1 suppresses MSC growth and stimulates the development of avascular smooth muscle, and that the treatment of 5-azacitidine with amphotericin B causes MSCs to transform into myoblasts [44].

There are several transcription factors commonly reported in smooth muscle cell differentiation, including serum response factor (SRF), GATA6, and myocardin. MEK inhibitor application to MSCs has been shown to upregulate the production of smooth muscle cell indicators and induce myocardin transcription. In addition, sphingosylphosphylcholine (SPC) was found to induce the transformation of MSCs into smooth muscle cells via a RhoA/Rho kinase-dependent pathway. SPC has been shown to upregulate the transcription of myocardium-associated transcription factor. TGF-β activates SRF and GATA6 during smooth muscle transformation. This stimulation increases the transcription of smooth muscle indicator genes calponin, SMMHC, SM22-α, and α-SMA in MSCs. PPARγ has been shown to suppress the transformation of MSCs into myofibroblasts that have many characteristics of smooth muscle cells [45,46,47]. Additionally, there have been reports indicating that the introduction of PPARγ-siRNA into MSCs and the application of TGF-β led to an augmentation in the production of alpha smooth muscle actin (α-SMA). The presence of the laminin variant LM-521 in TGF-β-containing medium has been reported to promote the transformation of MSCs into smooth muscle cells throughout the process of differentiation. In addition, it was shown that olfactomedin 2 (Olfm2) plays a significant role in the transformation of MSCs into smooth muscle cells, triggered by TGF-β. Olfm2 was observed to be increased throughout this transformation process.

Suppression of Olfm2 led to a decrease in the production of smooth muscle cell indicators, while elevated levels of Olfm2 enhanced the transcription of these markers [7]. Olfm2 forms a bond with serum response factor (SRF) and enhances the association between CARG box and SRF, resulting in elevated production of smooth muscle cell indicators.

This section focuses on the primary transcription factors, GATA6 and SRF, that have been identified as key contributors to the transformation of MSCs into smooth muscle cells. The upregulation of Olfm2 and administration of an optimal dosage of TGF-β were initiated to enhance the production of smooth muscle cell indicators throughout the course of differentiation. Furthermore, research has demonstrated that the production of PPARγ has a suppressive impact on the process of MSC transformation into smooth muscle cells.

14.2.5 Endotelial Cell Differentiation

Using MSCs to regenerate the endothelial layer can be considered as an option. Determination of cellular controllers that stimulate MSC transformation into endothelial cells (ECs) has also been reported in several studies as important for the future of MSC therapy. A recent study showed that increase in Sox18 production in MSCs stimulated markers of EC transformation [48]. Thus, Sox18 has been found to be a critical regulator of the transformation of MSCs into endothelial cells, potentially offer a novel clinical use of MSC therapy in the treatment of cardiovascular disorders. Following the transformation of MSCs into endothelial cells, the production of HOXB3 and HOXA7 was observed to increase, whereas the production of HOXB13 and HOXA3 was observed to decrease dramatically. Additionally, there have been reports indicating that HOXB5 enhances the production of vascu-

lar endothelial growth factor receptor-2 (VEGFR-II), a crucial component in the process of converting MSCs into endothelial cells [7,13]. A separate study examining the impact of αNotch signaling on the transformation of MSCs into endothelial cells and the development of formations resembling capillaries in both laboratory settings and living organisms demonstrated that the suppression of Notch1 by shRNA caused the production of capillary-like formations. Notch1 knockdown resulted in a considerable decrease in the production of biomarkers unique to endothelial cells. In addition, MSCs that were administered with VEGF-A and angiotensin type II (ATII) demonstrated an increased production of distinct indicators seen in endothelial cells, in comparison to MSCs that were administered with VEGF-A alone.

MSCs are highly suitable for tissue regeneration and differentiation of cells investigations, making them a top choice across other cell types. They are an appealing cell supply for transplantation due to their ability to be extracted from various tissues. MSCs possess the capacity to transform into several cell types, including cardiomyocytes endothelial cells, smooth muscle cells, osteocytes, chondrocytes, and adipocytes. Furthermore, they can transform into neurons and hepatocytes, among other cell lines [8,49,50]. There are distinct protein production patterns linked to each of these cell types. Nevertheless, comprehending the processes that drive the transformation of MSCs into separate lineages poses numerous hurdles. These problems involve the determination of transcription and signaling elements, as well as the interaction between signaling ways that induce the ability of MSCs to renew themselves and transform into certain cell lineages. The ability of MSCs to transform into a certain mesenchymal line depends on the activation or inhibition of genes that are

specific to that line. In the process of transformation, the activation or inhibition of transcription factors takes place via distinct signaling paths or via contact with additional transcription elements that serve as coregulators.

The maintenance of transformation of MSC into each of the cell lines indicated above is regulated by unique transcription factors that vary depending on the cell line. Runx2 is a crucial transcription element, which has a significant impact on the process of osteogenic development. It promotes the maturation of osteoblasts while preventing the development of adipocytes and chondrocytes. Runx2 production is controlled by various signaling routes, such as Notch, BMP, and Wnt pathways. Sox9 drives chondrogenic transformation. Nevertheless, the presence of Nkx3.2 is necessary for the activation of Sox9 and the inhibition of bone development [26]. In addition, Sox9 can directly engage with and restrain the activity of Runx2. This association has the potential to influence the durability and ultimate destiny of MSCs in terms of their ability to specialize into chondrocytes or osteocytes. The process of adipogenic transformation is primarily regulated by PPARγ, which collaborates with many transcription factors to enhance the production of adipogenic biomarkers. The transformation of MSCs into osteocytes or adipocytes is controlled by the association of many transcription factors, such as the crucial transcription factors PPARγ and Runx2. The determination of MSCs to transform into osteocytes or adipocytes is also controlled by many signaling pathways, such as IGF signaling, BMP, Wnt, NELL-1, and Hedgehog.

MSCs have the capacity to transform into fibroblast-like cells in a suitable in vitro setting, which contains 50 mg/ml ascorbic acid and 100 ng/ml connective tissue growth factor (CTGF), according to studies examining the mechanism and

function of transcription factors. Additionally, research has demonstrated that CTGF can induce the transformation of MSCs into fibroblasts. Furthermore, once the cells have differentiated, they no longer have the capacity to transform into additional kinds of cells. Treatment with CTGF was also observed to decrease the production of MSC indicators STRO-1 and CD44, while increasing the transcription of fibroblast-specific protein 1 (FSP-1), an indicator exclusive to fibroblasts. It has been demonstrated that fibroblast-like cells derived from MSCs are capable of synthesizing extracellular matrix proteins. The fibroblastic transformation of MSCs has significant significance in the field of tissue engineering, particularly in the context of ligament and tendon repair. Further research is necessary to determine the communication routes, critical signaling elements, and transcription factors that have a function in MSC transformation to the fibroblasts [51].

Several methodologies have been experimented to integrate transcription factor genes into MSCs for application in regenerative medicine. The transfection methods exhibited low efficacy and elevated morbidity when transferring the DNA plasmid to MSCs. In contrast, viral transduction techniques offer the chance to transmit the DNA plasmid with a high level of effectiveness and very little harm. Nevertheless, the presence of safety issues about viral transduction prompts talks on this matter. Multiple studies have demonstrated that the transfer of transcription factors to MSCs enhances and sustains their ability to transform into the desired cell line.

Identification of specific signaling molecules, receptors, and transcription factors throughout the transformation is important in understanding the connection between extracellular and intracellular signaling routes. It is important to reveal the connection between many factors during a differentiation.

We now know that there is almost no mechanism involving a single factor in cellular processes. Besides these factors, the cellular microenvironment plays an important role. Therefore, in vitro studies are limited to fully understand the mechanism. To better understand the mechanisms of action of the transcription factors described here, in vivo studies are needed in which microenvironments appropriate to the MSC source are taken into account.

14.3 ALTERNATIVE SPLAYS IN MESENCHYMAL STEM CELL DIFFERENTIATION

Identifying the precise molecular processes that control the development of MSCs in living organisms is crucial for correctly foreseeing the results of MSC-based cell treatment in a clinical setting. The equilibrium between extrinsic signaling pathways and the collective or separate effects of hormones, growth factors, and cytokines regulates the induction or inhibition of transcription factors that dictate the destiny of MSCs and the development of progenitor cells into fully specialized cell types. We have mentioned these transcription factors in detail above. Alternative splicing (AS) mechanisms, which we will talk about below, have received less attention than studies on these transcription factors, but they have a significant place in the control of these factors. Gene expression studies conducted in the early stages of differentiation into MSC have found an inconsistency between the transcription factors involved in this process and the expression of the genes they regulate. The findings indicate that other processes that are regulating transcription, such as post-transcriptional control, may be necessary. Furthermore, recent research has demonstrated that pre-messenger RNAs (pre-mRNAs) that produce important transcription factors, which are necessary for the first determination of cell lines in mesenchymal stem cell (MSC)

transformation, experience alternative splicing (AS). This process results in the production of numerous protein variants with varied functions. The pre-mRNAs of the adipogenic and osteogenic transcription factors PPARγ and RUNX2 undergo alternative splicing (AS), resulting in the production of numerous mRNA variants. These mRNA variants subsequently give rise to protein variants that possess controlling function [34].

Girardot and colleagues showed that Sox9, the key regulatory molecule for chondrogenesis, controls the modificaiton of many genes during chondrogenic differentiation without an impact on their expression [52]. Researchers discovered that this control takes place by means of direct interaction between pre-mRNAs and Sox9, as well as other RNA-binding proteins (RBPs), rather than via changing the amounts of target RBPs and indirectly influencing alternative splicing. Furthermore, it has been discovered that over 33% of the regulators governing neural and ES (Embryonic Stem Cell)-specific AS are transcription factors. This implies that many transcriptional factors may have multiple roles during both neural and ES transformation. An intriguing finding from the study is that regulatory transcription factors govern AS and dictate the cell's destiny during development by directly attaching to the target pre-mRNA. The rate of transcriptional extension has a well-established relationship with pre-mRNA splicing. It not only affects AS, but also influences the overall transcriptional rate in the AS [53].

14.3.1 Alternative Splays and Regulation Mechanisms

Altenative splicing is one of the significant control processes of gene transcription. It enables cells to produce multiple mRNA and protein isoforms from a precursor mRNA to increase their functional capacity. AS occurs in nearly all human

genes, and AS varies widely among human tissues. This indicates that there may be a relationship between tissue-specific structure and function and AS-regulated gene expression [54]. The development of RNA sequencing (RNA-Seq) methods are important for general biological roles of AS in gene expression.

The process of enzymatically cutting introns from the precursor mRNA and recombining exons is defined as splicing. Introns always start with GT bases and end with AG bases. However, these bases alone are not sufficient to recognize the intron. The third and important intron sequences are those defined as the "branch region" present at the end of the intron, a maximum of 40 nucleotides away from the AG end. Basically, splicing takes place in 3 steps;

• Cleavage at the exon-intron border in the 5′ direction (donor region)

• G nucleotide in the donor region approaches an A nucleotide in the branch region and a lasso is formed.

• Cleavage at the exon-intron border in the 3′ direction (acceptor region) and recombination of exons

Many small nuclear RNA (sn-RNA) complexes take part in splicing. The spliceosome, the biggest macromolecular complex in the cell, catalyzes this apparently straightforward chemical reaction. This huge mechanism is comprised of around 300 protein components and RNA [55]. In AS, splicing in the precursor mRNA occurs in various ways. For example, various mature mRNAs are formed from the precursor mRNA as a result of alternative selection of the 5′ or 3′ splicing site or alternative splicing by skipping one, two or sometimes more exons. Therefore, they encode various related proteins. The

control of AS is governed by the interplay between cis-acting elements and trans-acting factors, particularly RNA-binding proteins (RBPs). Trans-acting factors engage with distinct constituents of the spliceosome while it is being formed on the newly synthesized mRNA. The additional splicing elements mentioned are different types of cis splicing enhancers found in the precursor mRNA. These include intronic splicing silencers (ISPs), intronic splicing enhancers, exonic splicing silencers, and exonic splicing enhancers. The small nuclear ribonucleoprotein (snRNP) complexes are responsible for identifying and acknowledging efficient sequence elements. The production of these RNA-binding proteins (RBPs) is specific to certain tissues and stages of development. The level of transcription of key RBPs in each cell influences the proportion of variants, which in turn regulates if a specific exon is present in the mRNA [35].

14.3.2 Direction Of Stem Cells with AS

In studies showing that AS has a significant function in the pluripotency-differentiation axis in ESCs, a different short FGF4 isoform, FGF4si, was found to attenuate FGF4 activity with a new fibroblast growth factor 4 (FGF4) and thus induce ESC [56]. Transcription factor FOXP1, Tcf3, RNA binding motif protein 24 (RBM24), and Sal4paired box 6 (PAX6) different isoforms formed by AS have also been found to play a role in the embryonic process. Recent studies have shown that RNA binding domain protein 4 (RBM4) controls AS of PKM (Pyruvate kinase M) pre-mRNA, thus promoting neural differentiation of hMSCs [57,58].

Genome-wide RNA-seq analyzes showed that a specific AS process exists in ESCs and a general AS alteration is seen in transformed ESCs. In contrast, the overall AS system in transformed cells has been shown to revert to the pluripotent

state throughout the reprogramming of the stem cell. Although these findings do not exactly show that AS plays a key role in differentiation, they do suggest that it may contribute to the general gene expression profile. Moreover, the identification of diverse AS processes in MSCs derived from old and young donors indicates that AS is a prevalent factor in modulating the transcriptome profile throughout transformation, as well as in different cell and tissue types. The modulation of MSC development by AS has been acknowledged as an extra process that regulates the exact identification of maturation and cell destiny the into a specific cell type. pre-mRNAs of crucial transcription factors, which play a role in determining the destiny of cells by regulating the transcription of target genes downstream, undergo AS, resulting in the emergence of alternative variants. The variants have distinct functions in the process of MSC development through diverse molecular pathways [35].

14.3.3. Control Of Osteogenic Transformation by AS

Stimulation of RUNX2 is required during the initial osteogenic period and is subsequently suppressed during osteocyte development. Four different RUNX2 mRNA isoforms have been found in studies suggesting that AS may have a function in regulating osteogenic transformation; Δ5Δ7, Δ7, RUNX2Δ5, RUNX2, and WT. Two of these variants were generated through the exclusion of exon 5, resulting in RUNX2 variants that do not possess nuclear placement and so lose their ability to carry out transcriptional functions. Conversely, there is a third mRNA variant called RUNX2Δ7 that produces a variant of RUNX2 with a deletion in the area near the activation site at the end of the protein. The RUNX2Δ7 protein variant is found in the nucleus and has comparable binding capacity and transcriptional function for the osteocalcin (OC)

gene as the wild-type (WT) RUNX2. The patterns of transcription of the two variants (WT and RUNX2Δ7) vary between osteocytes and chondrocytes [59]. Chondrocytes produced from hMSCs have approximately similar levels of transcription for the variants. In contrast, osteocytes produced from hMSCs exclusively have the WT variant. The amount of WT to RUNX2Δ7 is a crucial factor in determining the cell destiny of hMSCs. The WT/RUNX2Δ7 ratio exhibits variation in many types of osteoblast-like cells, such as hMSC-differentiated osteocytes, osteosarcomas, and primary osteoblasts obtained from human donors. This suggests that the ratio of these two variants could serve as a possible indicator for osteocytic malignancies [35].

A further significant discovery is that OSX (Sp7) is an obvious target gene of RUNX2 that plays a role in guiding the development of pre-osteoblasts into mature osteoblasts and enhancing bone formation by regulating the production of various osteogenic genes, such as BGLAP, BSP-1, COL1A, and SPARC [60]. 3' alternative splicing of exon 3 causes two mRNA variants encoding peptides of 431 (Sp7L) and 413 (Sp7S) amino acids in length. These variants are differentially produced in numerous tissue and cell types, particularly between chondrocytes and adult and fetal osteoblasts [61].

The variants of vascular endothelial growth factor A (VEGF-A), a versatile growth factor that has been demonstrated to enhance the process of MSC transformation into bone cells by stimulating mineralization inside the cells themselves and boosting the formation of new blood vessels in the surrounding tissue, have been shown to be regulated in different conditions. It is also known that AS and some isoforms of VEGF-A, which are regulated especially under mechanical stress, regulate osteogenic differentiation [62].

14.3.4 Regulation Of Chondrogenic Differentiation by AS

The results demonstrated in alternative splicing studies generally come from mouse models. One of these genes is Sox9, which plays a crucial role in controlling the development of cartilage. It has been demonstrated that Sox9 acts as a stimulator of genes involved in cartilage formation by connecting with a specific protein called p54nrb in an isolated area of the cell nucleus. In the interchromatin area, there is a paraspeckle, which is a ribonucleoprotein structure. It is believed to serve as a storage site for a certain group of mRNAs, functioning as a control system for gene transcription. The work explores the potential role of Sox9 in directly controlling alternative splicing. The data suggest that Sox9 may possess two distinct functions: transcriptional and splicing [63].

A variant of TATA-binding protein-associated factor 4 (TAF4) has been identified as an additional control system connected with AS. This variant of TAF4 inhibits the growth of MSCs and enhances the transformation of hMSCs into chondrocytes, as reported by Park et al. in 2020. It has been demonstrated that TAF4 pre-mRNA generates four distinct TAF4 mRNA variants. According to reports, there is a direct correlation between the potential for transformation and the transcript level of TAF4ΔTAFH in hMSCs. Additionally, overexpressing TAF4ΔTAFH in hMSCs inhibits the growth of MSCs and specifically enhances chondrogenic transformation.

14.3.5 Regulation Of Adipogenic Differentiation by AS

Studies examining the variety of 3′-UTR configurations of recently formed transcripts that are attached to polysomes throughout the process of adipogenesis. have found that changes in 3′-UTR elongation or shortening after the initiation

of adipogenesis are controlled by different mechanisms. These changes in the 3′-UTR were found to affect the expression levels and intracellular localization of molecules that have a key role in adipogenesis[64].

Recently, an innate PPARγ variant with significant negative properties has been identified that regulates the function of the key transcription factor PPARγ in the process of adipogenesis. Skipping exon 5 in AS results in a shorter isoform, PPARγΔ5, that lacks the entire ligand-binding domain. Studies have demonstrated that it is capable of fighting with WT PPARγ by engaging with an undiscovered coreceptor, resulting in a decrease in adipogenic transformation of hMSCs. Notably, there is a significant correlation between the PPARγΔ5/PPARγ ratio in human adipose tissues and the BMI index. This indicates that the amount of the PPARγΔ5 variant may be associated with adipose tissue malfunction.

LPIN1 was first identified as a protein linked to illnesses involving the breakdown of adipose tissue and is primarily found in adipose tissues. During the process of adipocyte development from pre-adipocytes to mature adipocytes, two distinct forms of LPIN1 pre-mRNA, namely LPIN1a and LPIN1β, have been found [31]. Although it is not clear whether LPIN1 variants control adipocyte transformation, it is considered that they may have a function in regulating adipocyte transformation[65].

14.4 REFERENCES

1. in 't Anker PS, Noort WA, Scherjon SA, Kleijburg-van der Keur C, Kruisselbrink AB et al. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica. 2003 Aug;88(8):845-52. https://doi.org/10.3324/%x.
2. Sabatini, F., Petecchia, L., Tavian, M., Jodon de Villeroché, V., Rossi, G. A., et al. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. *Laboratory investigation; a journal of technical methods and pathology*. 2005;*85*(8),962–971. https://doi.org/10.1038/labinvest.3700300
3. Pierdomenico, L., Bonsi, L., Calvitti, M., Rondelli, D., Arpinati, M., et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental-pulp. *Transplantation*, 2005;*80*(6),836–842. https://doi.org/10.1097/01.tp.0000173794.72151.88
4. Yu, W. H., Li, F. G., Chen, X. Y., Li, J. T. et al. PPARγ suppression inhibits adipogenesis but does not promote osteogenesis of human mesenchymal stem cells. *The international journal of biochemistry & cell biology*. 2012;*44*(2), 377–384. https://doi.org/10.1016/j.biocel.2011.11.013
5. Suva, D., Garavaglia, G., Menetrey, J., Chapuis, B., Hoffmeyer, P., et al. Non-hematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. *Journal of cellular physiology*, 2004;*198*(1),110–118. https://doi.org/10.1002/jcp.10396
6. Lodie, T. A., Blickarz, C. E., Devarakonda, T. J., He, C., Dash, A. B., et al. Systematic analysis of reportedly distinct populations of multipotent bone marrow-derived stem cells reveals a lack of distinction. *Tissue engineering*, 2002;*8*(5), 739–751. https://doi.org/10.1089/10763270260424105
7. Almalki SG, Agrawal DK. Key transcription factors in the differentiation of mesenchymal stem cells. Differentiation. 2016 Jul-Aug;92(1-2):41-51. https://doi.org/10.1016/j.diff.2016.02.005
8. Yuan, H., Liu, H., Tian, R., Li, J., & Zhao, Z. Regulation of mesenchymal stem cell differentiation and insulin secretion by differen-

tial expression of Pdx-1. *Molecular biology reports*, 2012;*39*(7), 7777–7783. https://doi.org/10.1007/s11033-012-1619-7

9. Morganstein DL, Wu P, Mane MR, Fisk NM, White R, et al. Human fetal mesenchymal stem cells differentiate into brown and white adipocytes: a role for ERRalpha in human UCP1 expression. Cell Res. 2010 Apr;20(4):434-44. https://doi.org/10.1038%2Fcr.2010.11
10. Bionaz, M., Monaco, E., & Wheeler, M. B. Transcription Adaptation during In Vitro Adipogenesis and Osteogenesis of Porcine Mesenchymal Stem Cells: Dynamics of Pathways, Biological Processes, Up-Stream Regulators, and Gene Networks. *PloS one*, 2015;*10*(9), e0137644. https://doi.org/10.1371/journal.pone.0137644
11. Hesslein DG, Fretz JA, Xi Y, Nelson T, Zhou S, Lorenzo JA, Schatz DG, Horowitz MC. Ebf1-dependent control of the osteoblast and adipocyte lineages. Bone. 2009 Apr;44(4):537-46. https://doi.org/10.1016%2Fj.bone.2008.11.021
12. Fujimori, K., & Amano, F. Forkhead transcription factor Foxa1 is a novel target gene of C/EBPβ and suppresses the early phase of adipogenesis. *Gene*, 2011;*473*(2), 150–156. https://doi.org/10.1016/j.gene.2010.12.002
13. Seifert A, Werheid DF, Knapp SM, Tobiasch E. Role of Hox genes in stem cell differentiation. World J Stem Cells. 2015 Apr 26;7(3):583-95. https://doi.org/10.4252%2Fwjsc.v7.i3.583
14. Komori T. Regulation of osteoblast differentiation by transcription factors. *Journal of cellular biochemistry*, 2006;*99*(5), 1233–1239. https://doi.org/10.1002/jcb.20958
15. Gao, R. T., Zhan, L. P., Meng, C., Zhang, N., et al. Homeobox B7 promotes the osteogenic differentiation potential of mesenchymal stem cells by activating RUNX2 and transcript of BSP. *International journal of clinical and experimental medicine*, 2015;*8*(7), 10459–10470.
16. Lampert, F. M., Kütscher, C., Stark, G. B., & Finkenzeller, G. Overexpression of Hif-1α in Mesenchymal Stem Cells Affects Cell-Autonomous Angiogenic and Osteogenic Parameters. *Journal of cellular biochemistry*, 2016;*117*(3), 760–768. https://doi.org/10.1002/jcb.25361
17. Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J. et al. The novel zinc finger-containing transcription factor osterix is required for

osteoblast differentiation and bone formation. *Cell*, 2002;*108*(1), 17–29. https://doi.org/10.1016/s0092-8674(01)00622-5

18. Kang, S., Bennett, C. N., Gerin, I., Rapp, L. A., et al. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. *The Journal of biological chemistry*, 2007;*282*(19), 14515–14524. https://doi.org/10.1074/jbc.M700030200
19. Day, T. F., Guo, X., Garrett-Beal, L., & Yang, Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Developmental cell*, 2005;*8*(5), 739–750. https://doi.org/10.1016/j.devcel.2005.03.016
20. Qiu, W., Andersen, T. E., Bollerslev, J., Mandrup, S., Abdallah, B. M., et al. Patients with high bone mass phenotype exhibit enhanced osteoblast differentiation and inhibition of adipogenesis of human mesenchymal stem cells. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, 2007;*22*(11), 1720–1731. https://doi.org/10.1359/jbmr.070721
21. Huang, J., Deng, F., Wang, L., Xiang, X. R., et al. Hypoxia induces osteogenesis-related activities and expression of core binding factor α1 in mesenchymal stem cells. *The Tohoku journal of experimental medicine*, 2011;*224*(1), 7–12. https://doi.org/10.1620/tjem.224.7
22. Marupanthorn, K., Tantrawatpan, C., Tantikanlayaporn, D., Kheolamai, P., & Manochantr, S. The Effects of TNF-α on Osteogenic Differentiation of Umbilical Cord Derived Mesenchymal Stem Cells. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*, 2015;*98 Suppl 3*, S34–S40.
23. Choi, C. K., Xu, Y. J., Wang, B., Zhu, M., Zhang, L., et al. Substrate Coupling Strength of Integrin-Binding Ligands Modulates Adhesion, Spreading, and Differentiation of Human Mesenchymal Stem Cells. *Nano letters*, 2015;*15*(10), 6592–6600. https://doi.org/10.1021/acs.nanolett.5b02323
24. Sekiya, I., Tsuji, K., Koopman, P., Watanabe, H., Yamada, Y., et al. SOX9 enhances aggrecan gene promoter/enhancer activity and is up-regulated by retinoic acid in a cartilage-derived

cell line, TC6. *The Journal of biological chemistry*, 2000;*275*(15), 10738–10744. https://doi.org/10.1074/jbc.275.15.10738

25. Zhang, P., Jimenez, S. A., & Stokes, D. G. Regulation of human COL9A1 gene expression. Activation of the proximal promoter region by SOX9. *The Journal of biological chemistry*, 2003;*278*(1), 117–123. https://doi.org/10.1074/jbc.M208049200
26. Augello, A., & De Bari, C. The regulation of differentiation in mesenchymal stem cells. *Human gene therapy*, 2010;*21*(10), 1226–1238. https://doi.org/10.1089/hum.2010.173
27. Wang, Z. H., Li, X. L., He, X. J., Wu, et al. Delivery of the Sox9 gene promotes chondrogenic differentiation of human umbilical cord blood-derived mesenchymal stem cells in an in vitro model. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas*, 2014;*47*(4), 279–286. https://doi.org/10.1590/1414-431X20133539
28. Guérit, D., Philipot, D., Chuchana, P., Toupet, K., et al. Sox9-regulated miRNA-574-3p inhibits chondrogenic differentiation of mesenchymal stem cells. *PloS one*, 2013;*8*(4), e62582. https://doi.org/10.1371/journal.pone.0062582
29. Jagielski M, Wolf J, Marzahn U, Völker A, Lemke M, et al. The influence of IL-10 and TNFα on chondrogenesis of human mesenchymal stromal cells in three-dimensional cultures. Int J Mol Sci. 2014 Sep 9;15(9):15821-44. https://doi.org/10.3390%2Fijms150915821
30. David Guérit, Jean-Marc Brondello, Paul Chuchana, Didier Philipot, et al.. FOXO3A Regulation by miRNA-29a Controls Chondrogenic Differentiation of Mesenchymal Stem Cells and Cartilage Formation. Stem Cells and Development, 2014, 23 (11), pp.1195 - 1205. http://doi.org/10.1089/scd.2013.0463
31. Wei, F., Wang, T., Liu, J., Du, Y., & Ma, A. The subpopulation of mesenchymal stem cells that differentiate toward cardiomyocytes is cardiac progenitor cells. *Experimental cell research*, 2011;*317*(18), 2661–2670. https://doi.org/10.1016/j.yexcr.2011.08.011
32. Kondo, M., Yamaoka, K., Sakata, K., Sonomoto, K., Lin, L., et al. Contribution of the Interleukin-6/STAT-3 Signaling Pathway to Chondrogenic Differentiation of Human Mesenchymal Stem Cells. *Arthritis & rheumatology (Hoboken, N.J.)*, 2015;*67*(5), 1250–1260. https://doi.org/10.1002/art.39036

33. Liu, S., Zhang, E., Yang, M., & Lu, L. Overexpression of Wnt11 promotes chondrogenic differentiation of bone marrow-derived mesenchymal stem cells in synergism with TGF-β. *Molecular and cellular biochemistry*, 2014;*390*(1-2), 123–131. https://doi.org/10.1007/s11010-014-1963-0

34. Park JW, Fu S, Huang B, Xu RH. Alternative splicing in mesenchymal stem cell differentiation. Stem Cells. 2020 Oct 1;38(10):1229-1240. https://doi.org/10.1002%2Fstem.3248

35. Charytonowicz, E., Matushansky, I., Castillo-Martin, M., et al. Alternate PAX3 and PAX7 C-terminal isoforms in myogenic differentiation and sarcomagenesis. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico*, 2011;*13*(3), 194–203. https://doi.org/10.1007/s12094-011-0640-y

36. Koutalianos, D., Koutsoulidou, A., Mastroyiannopoulos, N. P., Furling, D., & Phylactou, L. A. MyoD transcription factor induces myogenesis by inhibiting Twist-1 through miR-206. *Journal of cell science*, 2015;*128*(19), 3631–3645. https://doi.org/10.1242/jcs.172288

37. Zhao, Q., Yang, S. T., Wang, J. J., Zhou, J., Xing, S. S., et al. TNF alpha inhibits myogenic differentiation of C2C12 cells through NF-κB activation and impairment of IGF-1 signaling pathway. *Biochemical and biophysical research communications*, 2015;*458*(4), 790–795. https://doi.org/10.1016/j.bbrc.2015.02.026

38. Liu, D., Kang, J. S., & Derynck, R. TGF-beta-activated Smad3 represses MEF2-dependent transcription in myogenic differentiation. *The EMBO journal*, 2004;*23*(7), 1557–1566. https://doi.org/10.1038/sj.emboj.7600179

39. Li, H., Zuo, S., He, Z., Yang, Y., Pasha, Z., et al. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. *American journal of physiology. Heart and circulatory physiology*, 2010;*299*(6), H1772–H1781. https://doi.org/10.1152/ajpheart.00557.2010

40. He Z, Li H, Zuo S, Pasha Z, Wang Y, Yang Y, et al. Transduction of Wnt11 promotes mesenchymal stem cell transdifferentiation into cardiac phenotypes. Stem Cells Dev. 2011 Oct;20(10):1771-8. https://doi.org/10.1089%2Fscd.2010.0380

41. Grauss, R. W., van Tuyn, J., Steendijk, P., Winter, E. M., Pijnappels, D. A., et al. Forced myocardin expression enhances the therapeutic

effect of human mesenchymal stem cells after transplantation in ischemic mouse hearts. *Stem cells (Dayton, Ohio)*, 2008;*26*(4), 1083–1093. https://doi.org/10.1634/stemcells.2007-0523

42. Suresh, S. C., Selvaraju, V., Thirunavukkarasu, M., Goldman, J. et al. Thioredoxin-1 (Trx1) engineered mesenchymal stem cell therapy increased pro-angiogenic factors, reduced fibrosis and improved heart function in the infarcted rat myocardium. *International journal of cardiology*, 2015;*201*, 517–528. https://doi.org/10.1016/j.ijcard.2015.08.117
43. Ding R, Jiang X, Ha Y, Wang Z, Guo J, et al. Activation of Notch1 signalling promotes multi-lineage differentiation of c-Kit(POS)/NKX2.5(POS) bone marrow stem cells: implication in stem cell translational medicine. Stem Cell Res Ther. 2015 May 9;6(1):91. https://doi.org/10.1186%2Fs13287-015-0085-2
44. Chamberlain, G., Fox, J., Ashton, B., & Middleton, J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem cells (Dayton, Ohio)*, 2007;*25*(11), 2739–2749. https://doi.org/10.1634/stemcells.2007-0197
45. Tamama K, Sen CK, Wells A. Differentiation of bone marrow mesenchymal stem cells into the smooth muscle lineage by blocking ERK/MAPK signaling pathway. Stem Cells Dev. 2008 Oct;17(5):897-908. https://doi.org/10.1089%2Fscd.2007.0155
46. Jeon, E. S., Park, W. S., Lee, M. J., Kim, Y. M., et al. A Rho kinase/myocardin-related transcription factor-A-dependent mechanism underlies the sphingosylphosphorylcholine-induced differentiation of mesenchymal stem cells into contractile smooth muscle cells. *Circulation research*, 2008;*103*(6), 635–642. https://doi.org/10.1161/CIRCRESAHA.108.180885
47. Donati C, Marseglia G, Magi A, Serratì S, Cencetti F, et al. Sphingosine 1-phosphate induces differentiation of mesoangioblasts towards smooth muscle. A role for GATA6. PLoS One. 2011;6(5):e20389. https://doi.org/10.1371%2Fjournal.pone.0020389
48. Ikhapoh, I. A., Pelham, C. J., & Agrawal, D. K. Synergistic effect of angiotensin II on vascular endothelial growth factor-A-mediated differentiation of bone marrow-derived mesenchymal stem cells into endothelial cells. *Stem cell research & therapy*, 2015;*6*(1), 4. https://doi.org/10.1186/scrt538

49. Taléns-Visconti, R., Bonora, A., Jover, R., Mirabet, V., et al. Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. *World journal of gastroenterology*, 2006;*12*(36), 5834–5845. https://doi.org/10.3748/wjg.v12.i36.5834

50. Pacary, E., Legros, H., Valable, S., Duchatelle, P., Lecocq, M., et al. Synergistic effects of CoCl(2) and ROCK inhibition on mesenchymal stem cell differentiation into neuron-like cells. *Journal of cell science*, 2006;*119*(Pt 13), 2667–2678. https://doi.org/10.1242/jcs.03004

51. Hu R, Ling W, Xu W, Han D. Fibroblast-like cells differentiated from adipose-derived mesenchymal stem cells for vocal fold wound healing. PLoS One. 2014 Mar 24;9(3):e92676. https://doi.org/10.1371%2Fjournal.pone.0092676

52. Girardot, M., Bayet, E., Maurin, J., Fort, P., Roux, P., & Raynaud, P. SOX9 has distinct regulatory roles in alternative splicing and transcription. *Nucleic acids research*, 2018;*46*(17), 9106–9118. https://doi.org/10.1093/nar/gky553

53. Han, H., Braunschweig, U., Gonatopoulos-Pournatzis, T., Weatheritt, R. J., et al. Multilayered Control of Alternative Splicing Regulatory Networks by Transcription Factors. *Molecular cell*, 2017;*65*(3), 539–553.e7. https://doi.org/10.1016/j.molcel.2017.01.011

54. Pan, Q., Shai, O., Lee, L. J., Frey, B. J., et al. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nature genetics*, 2008;*40*(12), 1413–1415. https://doi.org/10.1038/ng.259

55. Zhang, X., Yan, C., Hang, J., Finci, L. I., et al. An Atomic Structure of the Human Spliceosome. *Cell*, 2017;*169*(5), 918–929.e14. https://doi.org/10.1016/j.cell.2017.04.033

56. Mayshar, Y., Rom, E., Chumakov, I., Kronman, A., Yayon, A., et al. Fibroblast growth factor 4 and its novel splice isoform have opposing effects on the maintenance of human embryonic stem cell self-renewal. *Stem cells (Dayton, Ohio)*, 2008;*26*(3), 767–774. https://doi.org/10.1634/stemcells.2007-1037

57. Zhang, T., Lin, Y., Liu, J., Zhang, Z. G., Fu, W., et al. Rbm24 Regulates Alternative Splicing Switch in Embryonic Stem Cell Cardiac Lineage Differentiation. *Stem cells (Dayton, Ohio)*, 2016;*34*(7), 1776–1789. https://doi.org/10.1002/stem.2366

58. Park, J. W., Yang, J., & Xu, R. H. PAX6 Alternative Splicing and Corneal Development. *Stem cells and development*, 2018;*27*(6), 367–377. https://doi.org/10.1089/scd.2017.0283

59. Makita, N., Suzuki, M., Asami, S., Takahata, R., et al. Two of four alternatively spliced isoforms of RUNX2 control osteocalcin gene expression in human osteoblast cells. *Gene*, 2008;*413*(1-2), 8–17. https://doi.org/10.1016/j.gene.2007.12.025

60. Sinha, K. M., & Zhou, X. Genetic and molecular control of osterix in skeletal formation. *Journal of cellular biochemistry*, 2013;*114*(5), 975–984. https://doi.org/10.1002/jcb.24439

61. Johnson, C., Primorac, D., McKinstry, M., McNeil, J., et al. Tracking COL1A1 RNA in osteogenesis imperfecta. splice-defective transcripts initiate transport from the gene but are retained within the SC35 domain. *The Journal of cell biology*, 2000;*150*(3), 417–432. https://doi.org/10.1083/jcb.150.3.417

62. Mayer, H., Bertram, H., Lindenmaier, W., Korff, T., Weber, H., et al. Vascular endothelial growth factor (VEGF-A) expression in human mesenchymal stem cells: autocrine and paracrine role on osteoblastic and endothelial differentiation. *Journal of cellular biochemistry*, 2005;*95*(4), 827–839. https://doi.org/10.1002/jcb.20462

63. Hata, K., Nishimura, R., Muramatsu, S., Matsuda, A., Matsubara, T., et al. Paraspeckle protein p54nrb links Sox9-mediated transcription with RNA processing during chondrogenesis in mice. *The Journal of clinical investigation*, 2008;*118*(9), 3098–3108. https://doi.org/10.1172/JCI31373

64. Spangenberg, L., Shigunov, P., Abud, A. P., Cofré, A. R., et al. Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes. *Stem cell research*, 2013;*11*(2), 902–912. https://doi.org/10.1016/j.scr.2013.06.002

65. Péterfy, M., Phan, J., & Reue, K. Alternatively spliced lipin isoforms exhibit distinct expression pattern, subcellular localization, and role in adipogenesis. *The Journal of biological chemistry*, 2005;*280*(38), 32883–32889. https://doi.org/10.1074/jbc.M503885200

EPIGENETIC CHANGES IN MESENCHYMAL STEM CELLS DIFFERANTIATION

Melek PEHLIVAN[1]
[1]*Izmir Katip Celebi University, Vocational School of Health Services, Department of Medical Laboratory Techniques*

15.1 INTRODUCTION

Multipotent mature stem cells with the capacity for self-rejuvenation are designated as mesenchymal stem cells (MSCs). They are derived from the mesoderm during the embryonic stage and can be found in the bone marrow, adipose tissue, spinal cord, placenta, lungs, liver, and skin among other tissues[1]. These multipotent cells, characterised by the expression of stem cell/progenitor cell markers, have the ability to transform into a variety of cell types, including osteocyte, adipose, chondrosite, hepatosite, enocyte or myogenic strains[2,3]. The process of differentiation of MSCs into specific cell types is regulated by the complex interaction of signalling pathways and transcription factors. The signall pathways involved in these processes include Notch, transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP), MAPK/p38, Hedgehog, Wnt/β-Catenin, and fibroblast growth factors.[4]. When the differentiation process begins, a series of transcription factors are activated that regulate the expression of specific genes necessary for differentiating. For example, the differentiation of MSCs to osteoblasts is regulated by the activation of transcription factors such as RUNX Family Transcription

Factor 2 (RUNX2) and Osterix, which activate genes involved in bone formation. Similarly, the differentiation of MSCs to adipots is controlled by transcription elements like Peroxisome proliferator-activated receptor γ (PPARγ), which activates genes that play a role in adipogenesis[5]. During the phases of both differentiation and self-renewal of MSCs, the opening and closing of different set of genes at the transcriptional level is achieved not only through transcriptal regulators, but also through their interaction with epigenetic regulators. So, in addition to the fact that gene expression is regulated by transcription factors, epigenetic changes also have a crucial function in MSC differentiation.

The process of modifying a phenotype by altering the transcription of mRNAs with no effect on the DNA region is known as epigenetic modification. These changes can persist throughout cell division, as the cell can remain throughout its lifetime, and can be passed on over generations in a particular cell descent[6]. In order to preserve cell and niche homeostasis, epigenetic modifications take place in cells in response to external environmental stimuli and cellular intrinsic heredity. Numerous processes, such as histone modifications, DNA methylation, chromatin reorganization, and gene changes via non-coding RNA, can lead to epigenetic alteration (Figure 1)[1].

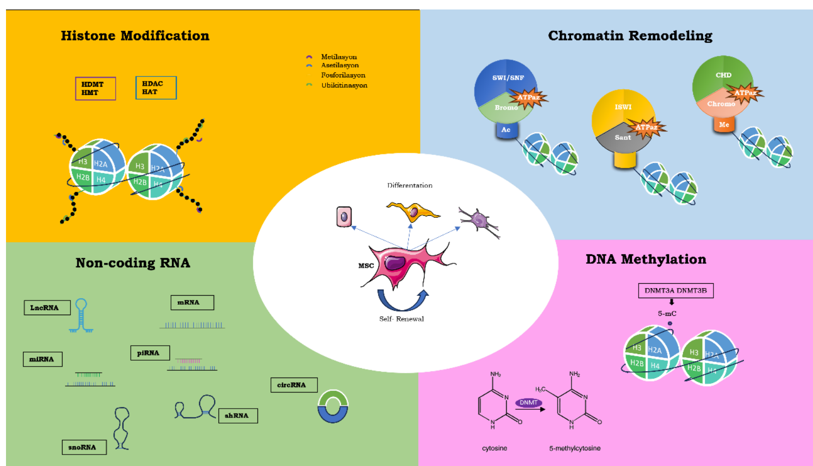


Figure 1: Epigenetic mechanisms that play a role in optimal stem cell differentiation

15.2 EPIGENETIC MECHANISMS

15.2.1 DNA methylation

This mechanism can suppress gene expression by preventing transcription factors from physically binding to DNA. DNA methylation mainly occurs in the islands of cytosine phosphate guanin (CpG), which is located next to the cytosine guanine. A methyl group is added from S-adenosylmethionine to the cytosine residues in the DNA by means of DNA-methyl transferases [7]. DNA methylation models are created during embryonic development and can be altered throughout life by environmental factors. Variations in DNA methylation models can control gene accessibility and impact transcription factors' capacity to bind to particular DNA regions. For example, when the cytosines in the promoter area are methylated, the methyl-CpG binding proteins physically block the transcription factors from attaching to them due to their increased affinity, which prevents the gene from being expressed. Furthermore, methyl-CpG binding proteins convert

chromatin into a closed structure by histone deacetylase activity, involving additional proteins that binds together and form a complex. Therefore, the transcription of the gene is suppressed as transcription factors do not reach the promoter region. It is widely recognized that promoters with low or often methylated CpG content control how DNA methylation affects gene expression, which is dependent on CpG density.[6,8].

Research has indicated that variations in DNA methylation models, a type of epigenetic mechanism, can impact MSC function and differentiation capacity. MSCs from different tissue origins, such as adipose tissue, muscle and bone marrow, are known to share similar methylation patterns across the genome. The promoter regions of specific genes that involved in osteogenesis, adipogenesis and chondrogenesis have been shown to be hypomethylation during MSC differentiation[9,10]. Decreased levels of DNA methylation can lead to high osteogenic differentiation capacity in MSCs derived from bone tissue, and impaired osteogenous differentiating capacity for MSC from fatty tissue.

The methylation pattern of the CpG regions is preserved and modified by DNA methyletransferases (DNMTs). DNMT1 functions to copy the DNA methylation model during DNA replication from the parent's DNA chain to the newly synthesized chain[11]. The deacetylation of DNMT1 through Sirtuin protein 6 (SIRT6), which has a crucial function in cell transformation, prevents DNA hypermethylation in the NOTCH1 and NOTCH2 genes, leading to their transcriptional upward regulation, thereby promoting osteogenic differentiation of MSCs derived from adiposis[12]. In vitro studies, when MSCs were treated with 5aza-2′-deoxycidine (5azadC), a DNMT inhibitor, before they were cultivated in the osteogenic environment,

DLX5, Runt-related transcription factor 2 (RUNX2), COL1a1, Osterix (OSX) and Osteocalcin (OCN) have been shown to significantly promote osteogenetic differentiation due to the decrease in the methylation of genomic DNA, along with a rise in the transcription of osteogenic genes[10,13]. The differentiation potential of MSCs is also regulated through the methylation of promoters of other gene-specific genes. Stemness genes such as octameral binding transcription factor (OCT4) and NANOG have been shown to be hypomethylated in non-differentiated stem cells directly through DNMT1, but methylated at a high rate during differentiation[10,14,15].

DNMT3a and DNMT 3b are known de novo as DNMT and have been shown to regulate the osteogenic, adipogenic and chondrogenic functions of MSCs by altering the expression of associated genes. Interestingly, during the in vitro chondrogenic differentiation of MSCs, a significant increase in DNMT3A and DN MT3B has been observed. Over-expression of DNMT3A increases gene expression of type II collagen, significantly increasing chondrogenesis in MSCs, while 5-AzaC or siRNA and loss of function of DN MT3A have been observed to inhibit chondrogenic differentiation of MSC. Therefore, DNA methylation also plays an important role in regulating chondrogenic differentiation in MSCs[16].

15.2.2 Histone modifications

Proteins called histones aid in the packing of DNA into a compact structure known as chromatin. A post-translational alteration of one of the side chains of an amino acid in a histone protein is known as a histone modification. Histone modifications such as acetylation, methylation and phosphorylation can change the physical feature of chromatin and control the accessibility of DNA to transcription factors, depending

on the amount and type of modification [6]. These changes can occur individually, in sequence or in combination. Of these alterations, histone acetylases (HAT) and deacetylases (HDAC) regulate the acetylation of histones, which is linked to gene activation. Histone acetylation is the process by which HATs facilitate lysine acetylation, eliminate the positive load from the lysine, and encourage DNA relaxing from histones. HDACs inhibit the addition of acetyl group to histone by catalyzing the removal of the acetyl group. Histone methylation from histone modifications can activate or suppress gene expression depending on the modified specific histone residues. Histone demethylases (HDMs) and histone methyltransferases (HMTs) regulate the histone methylation, which happens in the N-terminals of H3 and H4 subunits of arginine or lysine residues. Histone phosphorylation usually occurs in areas associated with centromeric function, chromosome concentration, and transcriptional activation[8].

During MSC differentiation, histone changes such as acetylation, methylation and phosphorylation are crucial in controlling gene expression. These modifications can cause changes in the structure and accessibility of chromatin, affecting the binding capability of transcription factors to specific DNA sequences. Some histone modifications may support the differentiation of MSCs to certain cell types, while others may prevent differentiations. Together with histone modification, which plays a role in differentiating osteoblasts from MSCs, DNA methylation has been shown to regulate the expression of several genes associated with MSC's osteogenic potential, as well as the regulation of different stages of osteoblastic differentiation[10].

Treatment studies with HDAC inhibitors show that HDAC activity is essential forming the self-renewal, multiple potential,

and differentiation potential of MSCs[17]. A decrease in the levels of histone acetylation in the osteogenic differentiation of MSCs is observed, and this reduction is associated with the concentration of chromatin in certain regions to restrict the transcription of genes that are involved in cellular differentiation and the potential for differentiating to other cell strains. In addition, many of the genes that activated transcription during differentiation are more densely present in acetylated histone regions[18]. Studies have some positive effects on adipogenic differentiation under certain conditions of BMPs, which are thought to inhibit adipogenous differentiation. Furthermore, it has been demonstrated that the BMP signal pathway controls the acetylation of H3K9 to promote osteogenic differentiation[19]. Valproic acid-induced HDAC suppression in MSCs derived from fatty tissue or spinal cord has been shown to block adipogenic and chondrogenic differentiation; on the other hand, in MSCs derived from adipose tissue and bone marrow, valproic acid has been observed to induce osteogenic differantiation by histone hyperacetylation. Shen and Ark. In their study using CHIP-seq techniques, they observed a decrease in H3K4 methylation during osteogenic transformations of normal osteoblasts and ROS17/2.8 osteosarcoma cells, and an increase in the H3C9 acetylation [20,21]. Overall, in vitro studies have shown that treatment of histone hyperacetylation through HDAC inhibitors supports osteogenic differentiation in MSCs, and that the increase in histone acetylation through HAT modules the actions of the osteogenetic main regulator RUNX2[10]. HDAC6 hypoacetylation in the RUNX2 promoter has also been shown to decrease osteogenesis in aging mice BMSCs, while decreased methylation levels at the RUNX2 promoter H3K9me2, and induces osteogenic differentiation of the BMSC[16].

H3K4 and H3K36 histone methylation is a positive osteogenic factor in MSC differentiation, while H3K36 and H3K9 histone Methylations are defined as a negative osteogenetic factor. HDMTs and HMTs regulate the addition of methyl groups to histone in the residues of arginine and lysine in these histone straps. The two main HMT family genes have counter-functions, such as silencing or enabling its activation. Histone methylation is catalyzed by the Polycomb Repressor Complex 2 (PRC2), which is mostly composed of the Enhancer of Zeste 2 (EZH2)[16]. The inactivation of EZH2 by the major kinase CDK1, which controls the transition of the cell cycle from G2 phase to M, causes a decrease in the methylation of histone H3 (H3K27) in the RUNX2 promoter in lysine 27, thereby inducing osteogenic differentiation. Consequently, CDK1 and EZH2 are play a key role for MSC osteogenic differentiation[10].

Histone demethylases (HDMTs) are enzymes that remove methyl groups from residues of certain lysine in histone tails. Some histone demethylases have been observed to be significantly up-regulated following osteogenic induction. In vivo or in vitro studies, inhibition of HDMTs has been found to block osteogenic differentiation while inducing adipogenic differentiation[22].

15.2.3 Chromatin remodeling

Re-modelling the nucleosomes, which are the building blocks of chromatin, modifies the accessibility of DNA to transcription factors. This process is known as chromatin remodeling. Chromatin re-modelling occurs with the separation of genomic DNA from the nucleosome, and repositioning of the DNA circuit on the nuclear mebrane without altering the total number of DNA histone connections. Gene expression is regu-

lated through a variety of protein complexes that can move, remove or re-shape nucleosomes[8].

ATP-dependent chromatin changes may determine the level of transcription of a specific gene as one of the main factors affecting the state of chromatin. ATPase and ATP-dependent chromatin remodeling complexes, composed of multiple subunits, form various chromatin remodeling complexes with different functions as a result of different combinations of subunits. ATP-dependent chromatin remodeling complexes are essential for MSC self-renewal and multilineage differantiation. SWI/SNF, ISWI, KKH, INO80/SWR are known to have played a role in MSC differentiation[23]. SWI/SNF family members have been shown to have a role in the osteogenesis process of BRG1, BAF47, BAF200, BAF180, BRD7 and have an effect on the adipogenesis. In environments that promote adiposite cell differentiation, MSCs have been observed to accelerate the acquisition of mature phenotypes associated with the activation of differential markers by over-expression of BRG1 from ATP-dependent reconstruction complexes[24]. In a different study, the lower regulation of BRG1 increases methylation in the promoter of the NANOG core transcription factor through DNMT1 and Rb, and transcriptionally suppresses the NANOG. The over-expression of BRG1 induces the BRG1-expression in the NANOG promoter, so that with the involvement of the HDACs the chromatin gets stuck[23]. A participant in the SWI/SNF complex, the polybromous BAF is a re-modeler of an ATP-dependent chromate that plays a role in multi-cellular development. The polybromous BAF (PBAF) component Pbrm1 plays a role in regulating differentiation of mesenchymal stromal cell osteolineage by integrating PBAF-bound chromatin reconstitution and BMP/TGF-β signaling[25].

Observations of degraded mineral accumulations in MSCs where the INO80 chromatin re-modelling complex was silenced, in osteogenic induction conditions, showed a reduction in bone formation in implantable mice with INO80[26]. Exogenous induction of CHD7 from ATP-dependent chromatin re-modelling enzymes has been shown to induce osteogenic differentiation of MSC[27]. However, SWI/SNF has been shown to co-operate with miRNAs to participate in adipogenic differentiation. verexpression of miRNA378 contributes to the development of adipose tissue by accumulating triglycerides and activating lipogenic genes like GLUT4 and PPARγ2[23,28].

15.2.4 Non-coding RNA gene regulation

Small non-encoded RNAs (sncRNAs), non-long encoded rna (lncRNA), micro RNA (miRNA) and circular Rnas (circRNAs) can control gene transcription by interacting with the target mRNA, inhibiting their translations or promoting their degradation. Non-coding RNAs bind to the chromatin and alter its structure, which controls the expression of genes as well.

miRNAs, which are non-coding RNAs, bind to target genes' 3'-unconverted regions, such as those of transcription factors, receptors, and kinases, to control the translation of proteins or the stability of messenger RNA. miRNAs can be modified with a number of epigenetic processes, including histone modifications and DNA methylation; they participate in epigenetic processes by influencing important epigenic modification enzymes, like DNMTs and HDACs. miRNAs have a function in controlling MSC differentiation. Targeting the RUNX2 gene of miR-204 and mir-211 has been demonstrated in studies to control the osteogenic and adipogenic differentiation of MSCs[5]. MiR-30e, on the other hand, has been

found to directly modulate the signal pathway of the protein 6/β-catenin/TCF associated with the canonical Wnt/LDL receptor by suppressing osteoblast differentiation. miR-124 has been shown to be a negative controller of bone cell development and bone creation in living organisms by focusing on the transcription factors DIX2, DIX3 and, Dlx5 which play a role in osteoblast differentiations[29]. miR-20a targets Crim1, Bambi and, PPARγ, which are negative controller of BMP pathway, and jointly regulates the osteogenesis of hMSCs, promoting osteogenic transformation of human mesenchymal stem cells[30]. In vitro studies have shown that upward regulation of miR-143/145 can lead to differentiation of MSCs into flat muscle cells (SMCs)[31].

The ERK-MAPK signal path supports adipogenesis and osteogenesis through phosphorylation of transcription factors associated with MSC differentiation and is the only signal path that is active in all three lineages (adipogenic, osteogenous and chondrogenic) during MSC differentiation. By controlling the expression of SPRY2, a recognized regulator of the receptor tyrosine kinase (RTK) signal line during human mesenchymal stem cell development, MiR-21 has been demonstrated to modulate the ERK-MAPK signal pathway[32]. A different study demonstrated that miR-21 uses the TGF-β/Smad signal pathway to promote adipogenesis during MSC development[33]. miR-22 is effective on osteoblast differentiation and adipogenic differentiations. The excess expression of miR-22 suppresses HDAC by suppressing adipogenic differentiation of human adiposis stem cells (hADSCs)[34].

The transcripts longer than 200 nt that have no or very little ability to encode proteins are known as long non-coding RNAs, or lncRNAs. Through a variety of mechanisms, lncRNAs can suppress transcriptional levels of gene expression.

lncRNAs are involved in epigenetic processes as modular scaffold of DNA methylation and histone modification complexes. There is evidence to show that long noncoding RNAs (lncRNAs) are more influential than microRNAs in regulating the osteoblast differentiation of MSCs, and that they are a pioneer in MSC differentiation[4]. The transcriptome microtissue analysis showed that lncRNAs are expressed differently in adipose derivative stem cell (ADSCs), optimum human bone marrow stem cells (BMSCs), and osteogenically transformed and non-differentiated MSCs, and have control functions in the transformation mechanism[35]. For example, MODR, MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), and H19 lncRNAs have been shown to promote osteogenic differentiation, Hotair (HOX transcript antisense RNA), DANCR (differentiation antagonizing non-protein-coding RNA) and MEG3 (maternally expressed 3), HoxA-AS3 (HoXA cluster antisense RNA 3), MIAT (myocardial infarction- associated transcript), POIR, MIR31HG (MIR31 host gene), and AK028326 lnc RNAs have been demonstrated to inhibit the process of osteogenetic differentiation. In a different study, the down-regulation of ANCR by lncRNA promoted osteogenic differentiation via controlling the expression of RUNX2 and targeting histone methyltransferase EZH2[36]. It has been demonstrated that lncRNA HIF1a-AS1 promotes addition of acetyl groups to the histone proteins, which increases the osteogenic formation of BMSCs and raises HOXD10 expression[37].

However, some lncRNAs are known to be pioneers in regulating the expression of miRNA genes. H19 has a function in control of TGF-β1/Smad3/HDAC4/5 via miR-675, thus regulating the osteogenic differentiation of human MSCs.[38]. The lower regulation of HOTAIR led to a decrease in the addition of methyl groups to the miR-17-5p promoter, and the

reduction in the transcription of SMAD7, resulting in increased miR17-5p expression, and induced osteogenic differentiation[4]. In chondrogenic differentiation, ZBED3-AS1 (ZBED3 antisense RNA1), lncRNA HIT and DANCR (differentiation antagonizing non-protein-coding RNA) have been found to be useful[21].

15.3 CONCLUSION

In general, epigenetic restructuring has a crucial function in regulating the behavior of MSCs and increasing their therapeutic potential. Epigenetic processed such as non-coding RNAs, histone modifications, and DNA methylation can regulate the expression of key genes involved in the regulation and differentiation of MSC function. Understanding these mechanisms is crucial for the potential use of MSCs. Overall, while epigenetic mechanisms are still being explored to increase the therapeutic potential of MSCs, there is promising evidence that they may be useful in increasing the safety and effectiveness of MSC-based treatments.

15.4 REFERENCE

1. Wang R, Wang Y, Zhu L, Liu Y, Li W. Epigenetic Regulation in Mesenchymal Stem Cell Aging and Differentiation and Osteoporosis. Stem Cells Int. 2020 Sep 10; 2020:1–17. https://doi.org/10.1155/2020/8836258

2. Dos Santos A, Balayan A, Funderburgh ML, Ngo J, Funderburgh JL, Deng SX. Differentiation Capacity of Human Mesenchymal Stem Cells into Keratocyte Lineage. Investig Opthalmology Vis Sci. 2019 Jul 16;60(8):3013. https://doi.org/10.1167/iovs.19-27008

3. Teven CM, Liu X, Hu N, Tang N, Kim SH, Huang E, et al. Epigenetic Regulation of Mesenchymal Stem Cells: A Focus on Osteogenic and Adipogenic Differentiation. Stem Cells Int. 2011; 2011:1–18. https://doi.org/10.4061/2011/201371

4. Yang Q, Jia L, Li X, Guo R, Huang Y, Zheng Y, et al. Long Noncoding RNAs: New Players in the Osteogenic Differentiation of Bone Marrow- and Adipose-Derived Mesenchymal Stem Cells. Stem Cell Rev Reports. 2018 Jun 20;14(3):297–308. https://doi.org/10.1007/s12015-018-9801-5

5. Huang J, Zhao L, Xing L, Chen D. MicroRNA-204 Regulates Runx2 Protein Expression and Mesenchymal Progenitor Cell Differentiation. Stem Cells. 2010 Feb 1;28(2):357–64. https://doi.org/10.1002/stem.288

6. Stephens KE, Miaskowski CA, Levine JD, Pullinger CR, Aouizerat BE. Epigenetic Regulation and Measurement of Epigenetic Changes. Biol Res Nurs. 2013 Oct 3;15(4):373–81. https://doi.org/10.1177/1099800412444785

7. Ozkul Y, Galderisi U. The Impact of Epigenetics on Mesenchymal Stem Cell Biology. J Cell Physiol. 2016 Nov;231(11):2393–401. https://doi.org/10.1002/jcp.25371

8. Wu YL, Lin ZJ, Li CC, Lin X, Shan SK, Guo B, et al. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. Signal Transduct Target Ther. 2023 Mar 2;8(1):98. https://doi.org/10.1038/s41392-023-01333-7

9. Sørensen AL, Jacobsen BM, Reiner AH, Andersen IS, Collas P. Promoter DNA Methylation Patterns of Differentiated Cells Are Largely Programmed at the Progenitor Stage. Heldin CH, editor. Mol Biol Cell. 2010 Jun https://doi.org/10.1091/mbc.e10-01-0018

10. Perez-Campo F, Riancho J. Epigenetic Mechanisms Regulating Mesenchymal Stem Cell Differentiation. Curr Genomics. 2015 Sep 7;16(6):368–83. http://dx.doi.org/10.2174/1389202916666150817202559

11. Moore LD, Le T, Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacology. 2013 Jan 11;38(1):23–38. https://doi.org/10.1038/npp.2012.112

12. Jia B, Chen J, Wang Q, Sun X, Han J, Guastaldi F, et al. SIRT6 Promotes Osteogenic Differentiation of Adipose-Derived Mesenchymal Stem Cells Through Antagonizing DNMT1. Front Cell Dev Biol. 2021 Jun 22;9. https://doi.org/10.3389/fcell.2021.648627

13. Zhou GS, Zhang XL, Wu JP, Zhang RP, Xiang LX, Dai LC, et al. 5-Azacytidine facilitates osteogenic gene expression and differentiation of mesenchymal stem cells by alteration in DNA methylation. Cytotechnology. 2009 Jul 26;60(1–3):11–22. https://doi.org/10.1007/s10616-009-9203-2

14. Fouse SD, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, et al. Promoter CpG Methylation Contributes to ES Cell Gene Regulation in Parallel with Oct4/Nanog, PcG Complex, and Histone H3 K4/K27 Trimethylation. Cell Stem Cell. 2008 Feb;2(2):160–9. https://doi.org/10.1016/j.stem.2007.12.011

15. Tsai CC, Su PF, Huang YF, Yew TL, Hung SC. Oct4 and Nanog Directly Regulate Dnmt1 to Maintain Self-Renewal and Undifferentiated State in Mesenchymal Stem Cells. Mol Cell. 2012 Jul;47(2):169–82. https://doi.org/10.1016/j.molcel.2012.06.020

16. Wang X, Yu F, Ye L. Epigenetic control of mesenchymal stem cells orchestrates bone regeneration. Front Endocrinol (Lausanne). 2023 Mar 6;14. https://doi.org/10.3389/fendo.2023.1126787

17. Franzen J, Wagner W, Fernandez-Rebollo E. Epigenetic Modifications upon Senescence of Mesenchymal Stem Cells. Curr Stem Cell Reports. 2016 Sep 1;2(3):248–54. https://doi.org/10.1007/s40778-016-0051-7

18. Håkelien AM, Bryne JC, Harstad KG, Lorenz S, Paulsen J, Sun J, et al. The Regulatory Landscape of Osteogenic Differentiation. Stem Cells. 2014 Oct 1;32(10):2780–93. https://doi.org/10.1002/stem.1759

19. Zhang P, Liu Y, Jin C, Zhang M, Lv L, Zhang X, et al. Histone H3K9 Acetyltransferase PCAF Is Essential for Osteogenic Differentiation Through Bone Morphogenetic Protein Signaling and May Be Involved in Osteoporosis. Stem Cells. 2016 Sep 1;34(9):2332–41. https://doi.org/10.1002/stem.2424
20. Shen J, Hovhannisyan H, Lian JB, Montecino MA, Stein GS, Stein JL, et al. Transcriptional Induction of the Osteocalcin Gene During Osteoblast Differentiation Involves Acetylation of Histones H3 and H4. Mol Endocrinol. 2003 Apr 1;17(4):743–56. https://doi.org/10.1210/me.2002-0122
21. Peng S, Cao L, He S, Zhong Y, Ma H, Zhang Y, et al. An Overview of Long Noncoding RNAs Involved in Bone Regeneration from Mesenchymal Stem Cells. Stem Cells Int. 2018; 2018:1–11. https://doi.org/10.1155/2018/8273648
22. Yang X, Wang G, Wang Y, Zhou J, Yuan H, Li X, et al. Histone demethylase KDM7A reciprocally regulates adipogenic and osteogenic differentiation via regulation of C/EBPα and canonical Wnt signalling. J Cell Mol Med. 2019 Mar 7;23(3):2149–62. https://doi.org/10.1111/jcmm.14126
23. Du W, Guo D, Du W. ATP-Dependent Chromatin Remodeling Complex in the Lineage Specification of Mesenchymal Stem Cells. Stem Cells Int. 2020 Sep 9;2020:1–10. https://doi.org/10.1155/2020/8839703
24. Napolitano MA, Cipollaro M, Cascino A, Melone MAB, Giordano A, Galderisi U. Brg1 chromatin remodeling factor is involved in cell growth arrest, apoptosis and senescence of rat mesenchymal stem cells. J Cell Sci. 2007 Aug 15;120(16):2904–11. https://doi.org/10.1242/jcs.004002
25. Sinha S, Biswas M, Chatterjee SS, Kumar S, Sengupta A. Pbrm1 Steers Mesenchymal Stromal Cell Osteolineage Differentiation by Integrating PBAF-Dependent Chromatin Remodeling and BMP/TGF-β Signaling. Cell Rep [Internet]. 2020 Apr;31(4):107570. https://doi.org/10.1016/j.celrep.2020.107570
26. Zhou C, Zou J, Zou S, Li X. INO80 is Required for Osteogenic Differentiation of Human Mesenchymal Stem Cells. Sci Rep. 2016 Nov 2;6(1):35924. https://doi.org/10.1038/srep35924

27. Chen Y, Wang M, Chen D, Wang J, Kang N. Chromatin remodeling enzyme CHD7 is necessary for osteogenesis of human mesenchymal stem cells. Biochem Biophys Res Commun. 2016 Sep;478(4):1588–93. https://doi.org/10.1016/j.bbrc.2016.08.161
28. Ortega FJ, Moreno-Navarrete JM, Pardo G, Sabater M, Hummel M, Ferrer A, et al. MiRNA Expression Profile of Human Subcutaneous Adipose and during Adipocyte Differentiation. Wang Y, editor. PLoS One. 2010 Feb 2;5(2):e9022. https://doi.org/10.1371/journal.pone.0009022
29. Qadir AS, Um S, Lee H, Baek K, Seo BM, Lee G, et al. miR-124 Negatively Regulates Osteogenic Differentiation and In vivo Bone Formation of Mesenchymal Stem Cells. J Cell Biochem. 2015 May;116(5):730–42. https://doi.org/10.1002/jcb.25026
30. Zhang J fang, Fu W ming, He M liang, Xie W dong, Lv Q, Wan G, et al. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol. 2011 Sep 5;8(5):829–38. https://doi.org/10.4161/rna.8.5.16043
31. Yeh YT, Wei J, Thorossian S, Nguyen K, Hoffman C, del Álamo JC, et al. MiR-145 mediates cell morphology-regulated mesenchymal stem cell differentiation to smooth muscle cells. Biomaterials. 2019 Jun; 204:59–69. https://doi.org/10.1016/j.biomaterials.2019.03.003
32. Mei Y, Bian C, Li J, Du Z, Zhou H, Yang Z, et al. miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. J Cell Biochem. 2013 Jun;114(6):1374–84. https://doi.org/10.1002/jcb.24479
33. Kim YJ, Hwang SJ, Bae YC, Jung JS. MiR-21 Regulates Adipogenic Differentiation through the Modulation of TGF-β Signaling in Mesenchymal Stem Cells Derived from Human Adipose Tissue. Stem Cells. 2009 Dec 1;27(12):3093–102. https://doi.org/10.1002/stem.235
34. Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, et al. Upregulation of miR-22 Promotes Osteogenic Differentiation and Inhibits Adipogenic Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells by Repressing HDAC6 Protein Expression. Stem Cells Dev. 2012 Sep;21(13):2531–40. https://doi.org/10.1089/scd.2012.0014

35. Zhang W, Dong R, Diao S, Du J, Fan Z, Wang F. Differential long noncoding RNA/mRNA expression profiling and functional network analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. Stem Cell Res Ther. 2017 Dec 7;8(1):30. https://doi.org/10.1186/s13287-017-0485-6
36. Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. Biochem Biophys Res Commun. 2013 Mar;432(4):612–7. https://doi.org/10.1016/j.bbrc.2013.02.036
37. XU Y, WANG S, TANG C, CHEN W. Upregulation of long non-coding RNA HIF 1α-anti-sense 1 induced by transforming growth factor-β-mediated targeting of sirtuin 1 promotes osteoblastic differentiation of human bone marrow stromal cells. Mol Med Rep. 2015 Nov;12(5):7233–8. https://doi.org/10.3892/mmr.2015.4415
38. Huang Y, Zheng Y, Jia L, Li W. Long Noncoding RNA H19 Promotes Osteoblast Differentiation Via TGF-β1/Smad3/HDAC Signaling Pathway by Deriving miR-675. Stem Cells. 2015 Dec 1;33(12):3481–92. https://doi.org/10.1002/stem.2225

EXTRACELLULAR MATRIX DYNAMICS IN THE DEVELOPMENT OF MESENCHYMAL STEM CELL

Aslı OZKIZILCIK KOCYIGIT[1,2]
[1]Medical Biology Department,
İzmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

[2]Cell, Tissue, Organ Transplantation Application and Research Center, University of İzmir Katip Çelebi, Izmir, Turkey

16.1 INTRODUCTION

Mesenchymal stem cells (MSCs) are the multipotent cells that renew themselves and differentiate to any type of the cell in the body (multipotency). They are mainly present in bone marrow, but they can be isolated from umbilical cord, lung, fat, blood, placenta, liver, and skin tissues. These cells can be identified according to their positive and negative markers [1]. These markers are significant especially for their accurate identification.

MSCs can be induced for in vitro osteogenesis, adipogenesis, and chondrogenesis. The environmental dynamics such as extracellular matrix (ECM) have a crucial role in the differentiation of the stem cells. They create the optimal dynamic niche for their differentiation and proliferation providing other components such as collagen, fibronectin, etc. [1]. ECM is an active complex which includes various macromolecules allowing signal transduction for the development and diffe-

rentiation of the stem cells. ECM allows stem cell adhesion to the niche and signal transduction between the cells and their microenvironment, acts as a growth factor source, determines the cell behavior, regulates tissue rigidity, and determines the future of the cells [2].

In recent years, MSCs have had a potential for their usage in cellular therapies and regenerative medicine due to their abundance and easy isolation procedures from various tissues as well as their self-renewal and mutlipotency. Understanding of their relationship between their dynamic microenvironment may further allow the simulation of stem cell microenvironment in vitro conditions. It may also be used for the development of novel products for cellular therapy.

16.2 MSC SELF- RENEWAL AND DIFFERENTIATION

The multipotent MSCs can differentiate into various cell lines such as cartilage, bone, muscle, and adipose cells. They are significant supporters of tissue repair and homeostasis. Their destiny to develop into the other cell lineages and to proliferate depends on the regulation of major genes and related signal transductions [3]. MSCs collaborate with microenvironmental elements in vivo. Physical characteristics such as cell shape, external mechanical pressures, and ECM are all involved in stem cell fate determination. It is critical to recognize that environmental signals in the niche do influence stem cell activity. The extracellular matrix (ECM) is one of the most critical specialized components. A plethora of studies have provided insights into how stem cells perceive signals from the ECM and respond to these signals at the molecular level, which ultimately controls their fate. Because stem cells can modify the niche in response to signals from it, relationships with it are bidirectional [1].

16.3 EXTRACELLULAR MATRIX IN MSC MICROENVIRONMENT

ECM is non-cellular part of the tissues and organs consisting of more than 200 glycoproteins and 300 proteins. These binding proteins are detected by cell membrane integrin receptors, which bind to the matrix and attract focal adhesion proteins and cytoskeletal components to establish permanent adhesions capable of transmitting and transducing force [4]. All of the cells in our body are connected to ECM. The content and quantity of the ECM vary from tissue to tissues[5]. In fetal heart tissue, for example, fibronectin is the most predominant ECM component. During development, the percentage of fibronectin declines, and type I collagen remains the most prevalent ECM element in adult heart tissue [4]. Although ECMs are available in a variety of forms, the two most common forms are basement membrane and interstitial ECM. Basement membranes are highly specific, flat laminar ECMs composed primarily of core proteins structured into sheet-like connections of associated ECM components such as collagen IV, laminins, and proteoglycans (for example, perlecan). Basement membranes are found beneath epithelia and surround most metazoan organs. Collagens and non-collagenous proteins (e.g., fibronectin, elastin, laminin, and tenascin) of interstitial ECM assist to the fibrous connections of ECMs, whereas proteoglycans and water serve to their extracellular spaces [5].

One of the primary constituents of ECM are glycosaminoglycans (GAGs), the majority of which connect with core proteins to create proteoglycans, as well as collagen and non-collagenous proteins. The number and location of these elements will dictate the physicochemical properties of a particular ECM, i.e. collagen fibers have high tensile strength, whereas GAGs have the negative charges that attracts po-

sitive ions and draws water through osmosis [1]. In addition, GAGs are also significant inducers and modifiers of several signaling pathways, including SMAD, WNT, Mitogen-Activated Protein Kinases (MAPK), PI3K/Akt, Receptor Activator of NF-B Ligand/osteoprotegerin, and various inflammatory pathways [6]. Fibrillar fibronectin (Fn) is another initial ECM that a variety of cells effectively build after damage, either by taking plasma Fn or by generating their own. It is essential for wound repair, as well as the reconstruction of tissues in touch with implants and early embryonic development [7]. Fn can be classified as plasma (pFn) and cellular fibronectin(cFn). cFN may be important in MSC development and differentiation [8].

ECM has turnover capability to maintain homeostasis. This turnover is controlled by various proteinases. The most common proteinases are Matrix Metalloproteinases (MMPs). MMPs affect cytokines, growth factors, cell receptors, chemokines as well as ECM. This may lead to deactivation of signaling molecule, development of an antagonistic role, or enhanced activity. MMPs are also required for tissue signaling and ECM dynamics [1].

16.4 ECM ROLE IN MSC DEVELOPMENT AND DIFFERENTIATION

Mechanical pressure on the ECM plays a role in MSC differentiation. Mechanical pressure on the ECM plays a role in MSC differentiation. On rigid surfaces, transforming growth factor-beta (TGF-β) stimulates MSC development into a smooth muscle lineage. MSC maturation into osteogenic, adipogenic and chondrogenic lineages is aided by soft matrix. However, matrix flexibility may not be limited to a single lineage. Other biochemical factors such as TGF-β are essential to define a distinct differentiation pathway. The rigidity of the ECM is re-

gulated by matrix turnover. This turnover is influenced by the proper ratio of tissue inhibitors of metalloproteinases (TIMPs) and MMPs [9].

16.4.1 In Adipose Tissue

Fibronectin, collagen type I, V, and VI are abundantly secreted in pre-adipocytes. In a similar way, ECM is mostly constituted of laminin complexes, collagen I, IV, V, VI, and fibronectin in mature adipose tissue. All collagen types are abundant in this tissue, but only type II collagen is little expressed. In previous studies, it was reported that MMPs also have direct roles in adipogenesis. The expression levels of some MMPs (MMP-2, MMP-3, MMP-7, MMP10, MMP-11, MMP-12, and MMP-13) was found to be high in mature adipocytes, whereas the others were low expressed (MMP-16, TIMP3, and TIMP4) [1]. In addition, fibronectin plays a crucial role in adipocyte development. The ECM is abundant in fibronectin in early stages, whereas cortical actin components are more in mature adipocytes (Figure 1) [10].

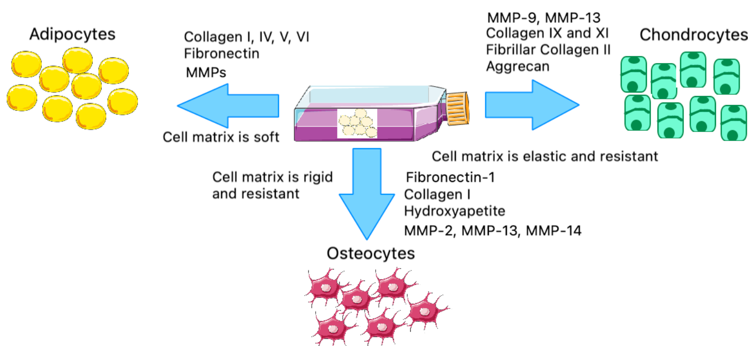


Figure 1: ECM rigidity and matrix components during differentiation of MSCs

16.4.2 In Chondrogenic Tissue

Due to its ECM structure, the articular cartilage is elastic and resistant to the environmental conditions. The ECM contains mostly collagen XI, fibrillary kollagen II, and collagen IX. Aggrecan is also abundant providing the resistance. This ECM also allows the cell-ECM interaction via integrins on the cell surface. The chondrocytic development route of MSCs begins with high levels of collagen I and fibronectin synthesis. Then, collagen type XI, IIb, and IX are expressed by these cells (Figure 1). During this process, the roles of MMPs are also crucial. MMP-9 and MMP-13 triggers the cellular condensation and contributes to the differentiation. However, MMP-2 increases the expression of fibronectin and integrins leading to the inhibition of cellular condensation [1]. Dexamethasone, β-glycerolphosphate, and ascorbic acid are used for in vitro osteogenic differentiation of MSCs.

16.4.3 In Osteogenic Tissue

Collagen and hydroxyapatite are the main components of ECM in osteogenic tissue. During the development and differentiation, there is an active interaction between ECM and osteocytes. This also provides mechanical induction for the differentiation and development [11]. MMP-2, MMP-13, and MMP-14 are important for the proper development and function of bone [1]. During the development, osteoblasts produce fibronectin-1 and collagen type I contributing to the osteoblast mineralization. Fibronectin-1 was also found to be an effective regulator for in vitro osteogenic differentiation (Figure 1) [12].

16.5 CONCLUSION

Especially the rigidity of ECM physically affects the MSC development and differentiation. For instance, hydrogel is a scaffold that contains a single ECM molecule such as collagen [13]. Thus, the scaffold material is crucial for the in vitro development and differentiation of MSCs. In addition, decellularized ECM of in vitro MSC cultures can be used to maintain in vitro MSC cultures themselves [12]. miRNAs should also be considered during the cultivation, because they regulate functions of the components in ECM such as MMPs [14].

16.6 REFERENCES

1. Assis-Ribas T, Forni MF, Winnischofer SMB, Sogayar MC, and Trombetta-Lima M. Extracellular matrix dynamics during mesenchymal stem cells differentiation. Developmental Biology 2018; 437 (2) :63–74. https://doi.org/10.1016/J.YDBIO.2018.03.002.
2. Gattazzo F, Urciuolo A, and Bonaldo P. Extracellular matrix: A dynamic microenvironment for stem cell niche. Biochimica et Biophysica Acta - General Subjects 2014; 1840 (8): 2506–2519. https://doi.org/10.1016/j.bbagen.2014.01.010.
3. Benayahu D. Mesenchymal stem cells for biotechnology. Biomaterials Translation 2022; 3 (1): 17–23. https://doi.org/10.12336/biomatertransl.2022.01.003.
4. Stanton AE, Tong X, and Yang F. Extracellular matrix type modulates mechanotransduction of stem cells. Acta Biomaterialia 2019; 96: 310–320. https://doi.org/10.1016/J.ACTBIO.2019.06.048.
5. Cruz Walma DA and Yamada KM. The extracellular matrix in development. Development (Cambridge) 2020; 147 (10): dev175596. https://doi.org/10.1242/DEV.175596/224217.
6. Bains AK, Wu LB, Riviere J, Rother S, Magno V, et al. Bone marrow mesenchymal stromal cell-derived extracellular matrix displays altered glycosaminoglycan structure and impaired functionality in Myelodysplastic Syndromes. Frontiers in Oncology 2022; (12): 961473. https://doi.org/10.3389/FONC.2022.961473/BIBTEX.

7. Li B, Moshfegh C, Lin Z, Albuschies J, and Vogel V. Mesenchymal Stem Cells Exploit Extracellular Matrix as Mechanotransducer. Scientific Reports 2013; 3 (1): 1–8. https://doi.org/10.1038/srep02425.

8. Dalton CJ and Lemmon CA. Fibronectin: Molecular Structure, Fibrillar Structure and Mechanochemical Signaling. Cells 2021; 10 (9): 2443. https://doi.org/10.3390/CELLS10092443.

9. Prathipati P, Nandi SS, and Mishra PK. Stem Cell-Derived Exosomes, Autophagy, Extracellular Matrix Turnover, and miRNAs in Cardiac Regeneration during Stem Cell Therapy. Stem Cell Reviews and Reports 2016; 13 (1): 79–91. https://doi.org/10.1007/S12015-016-9696-Y.

10. Uetaki M. Onishi N, Oki Y, Shimizu T, Sugihara E, et al. Regulatory roles of fibronectin and integrin α5 in reorganization of the actin cytoskeleton and completion of adipogenesis. Molecular Biology of The Cell 2022; 33 (9): 78–79. https://doi.org/10.1091/MBC.E21-12-0609/ASSET/IMAGES/LARGE/MBC-33-AR78-G006.JPEG.

11. Qin L, Liu W, Cao H, and Xiao G. Molecular mechanosensors in osteocytes. Bone Research 2020; 8 (1): 1–24, Jun. 2020, https://doi.org/10.1038/s41413-020-0099-y.

12. Yang C, Wang C, Zhou J, Liang Q, He F, et al. Fibronectin 1 activates WNT/β-catenin signaling to induce osteogenic differentiation via integrin β1 interaction. Laboratory Investigation 2020; 100 (12): 1494–1502. https://doi.org/10.1038/s41374-020-0451-2.

13. Zhou C, Zhou L, Liu J, Xu L, Xu Z, et al. Kidney extracellular matrix hydrogel enhances therapeutic potential of adipose-derived mesenchymal stem cells for renal ischemia reperfusion injury. Acta Biomaterialia 2020; 115: 250–263. https://doi.org/10.1016/J.ACTBIO.2020.07.056.

14. Prathipati P, Nandi SS, and Mishra PK. Stem Cell-Derived Exosomes, Autophagy, Extracellular Matrix Turnover, and miRNAs in Cardiac Regeneration during Stem Cell Therapy. Stem Cell Reviews and Reports 2016; 13 (1): 79–91. https://doi.org/10.1007/S12015-016-9696-Y.

POTENTIAL APPLICATIONS OF MESENCHYMAL STEM CELLS IN KIDNEY DISEASES

Belde Kasap Demir
Department of Child Health and Diseases, Department of Pediatric Nephrology and Pediatric Rheumatology, Izmir Katip Çelebi University Faculty of Medicine, Izmir, Turkey

17.1 INTRODUCTION

Regenerative cell-based therapies are considered as encouraging approaches for many disorders as well as kidney diseases. Stem cells have been demonstrated to have therapeutic potential in healing of renal injury, protecting kidney function and structure. Stem cells are undifferentiated, fibroblast-like cells that can regenerate and, in the right conditions, develop into a variety of specialized cell types [1].

Stem cells can be classified into groups with regard to their origins. These are [2,3,4]:

a. Embryonic stem cells,

b. Fetal stem cells,

c. Adult stem cells

d. Induced pluripotent stem cells

Stem cells can be classified according to their differentiation potentials as well:

17.1.1 Totipotent stem cells

Totipotent stem cells can differentiate into any organ in the body, including placenta or umblical cord[1]. The zygote and the blastomers of the early embryo are considered as totipotent[1].

17.1.2 Pluripotent stem cells

Pluripotent stem cells can form any of the three germ lineages of the human body including endoderm, mesoderm and ectoderm, except for placenta or umblical cord[1]. They are found on the inner surface of the blastocyst. The two primary types of pluripotent stem cells are induced Pluripotent Stem Cells (iPSCs) and Embryonic Stem Cells (ESCs)[1].

17.1.3 Multipotent stem cells

The potential to develop into a variety of restricted cell types is possessed by multipotent stem cells. Hematopoietic stem cells and mesenchymal stem cells are the most well-known examples for multipotent stem cells. For instance, MSCs can differentiate into cells of mesenchymal cell lineages such as osteocytes, chondrocytes, adipocytes, and so on [1,5].

17.1.4 Unipotent stem cells

Unipotent stem cells have the lowest potency and they can differentiate into only one cell type [1,2]. Renal stem/progenitor cells, for instance, can only develop into particular kidney cells [1]. Mesenchymal stem cells (MSC) are undifferentiated multipotent adult cells that are characterized by a potential of clonogenicity, high proliferation, self-renewal, multidirectional differentiation into cells of mesodermal origin, regenerating organs with certain lesions and by inducing paracrine effects [6,7,8]. MSCs interact with resident cells and

secrete soluble substances to regulate tissue healing in addition to their progenitor characteristics. At present, the cells under the most extensive investigations in experimental and clinical studies are MSCs [9]. The kidney diseases that those studies are conducted on are acute kidney injury (AKI), ischemic nephropathy, renovascular diseases, chronic kidney diseases (CKD), diabetic nephropathy, lupus nephritis and kidney transplantation [10].

ISCT (International Society for Cellular Therapy) suggested minimal criteria for MSCs to standardize the isolation of MSCs from humans in 2006 as follows [7,11]:

1. Adherence to plastic under standard culture conditions

2. Expression of CD73, CD90 and CD105 surface molecules,

3. Being negative for CD45, CD34, CD14 or CD11b, CD79alpha, CD19 or HLA-DR

4. Capacity for differentiating into osteoblasts, adipocytes and chondroblasts *in vitro*.

MSCs enhance proliferation, decrease renal inflammation, endoplasmic reticulum stress and apoptosis via secreting proangiogenic and trophic factors, suppression of proinflammatory, fibrotic and apoptotic cytokines and through paracrine mechanisms[12,13]. In summary, MSCs have anti-fibrotic, pro-angiogenic, anti-inflammatory, pro-repair, anti-apoptotic, and immunomodulatory effects (Figure 1).

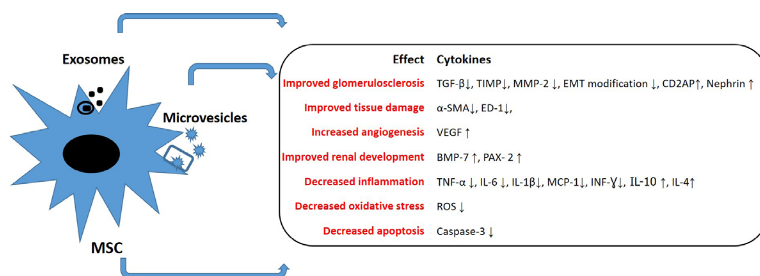


Figure 1: Both mesenchymal stem cells and their membrane-bound vesicles (extracellular vesicles and exosomes) have anti-inflammatory, anti-fibrotic, anti-apoptotic, pro-angiogenic, pro-repair, proliferative and anti-oxidative effects through cytokines.

As MSCs lack major histocompatibility complex-II expression, MSCs were thought to allow for transplantation without the use of immunosuppression. However, accumulating studies over the years have suggested that these cells are not as privileged as they were thought to be [12]. Although they can be transplanted as allogenic or autologous as well with different advantages and disadvantages (Table 1), more studies are needed to determine whether allogeneic or autologous MSCs are more desirable in a transplantation setting, and autologous MSCs administration appears as the safest policy. In addition, whether these cells should be obtained from BM or AT should also be decided.

Table 1: Advantages and disadvantages of autologous and allogenic MSC transplantation

	Advantages	Disadvantages
Autologous	1. Low immunogenicity 2. No risk of infection 3. More ethical	4. Takes long to prepare (long *in vitro* culture cycle)
Allogeneic	5. May be produced from healthy young donors in large quantities	6. Higher immunogenicity 7. Shorter cell survival

17.2 CHARACTERISTICS OF MSC TREATMENT

17.2.1 Source

They were originally identified in the bone marrow (BM) stroma by Friedenstein and his colleagues [15]. Later on, as bone marrow procurement is an invasive procedure and the amount, differentiation capacity and lifespan of bone marrow-derived mesenchymal stem cells decrease with the age of the donor [16], many other sources that MSCs may be obtained from were defined (Table 2). Among them, umbilical cord, amniotic fluid or placenta derived cells are difficult to obtain. On the other hand, adipose-tissue (AT) derived stem cells are much more accessible as the collection of fat tissue is less invasive, and yields higher cell numbers in comparison with bone marrow (BM) (Table 3).

Table 2: Special characteristics of MSCs.

Sources of MSCs:	Cells that MSCs can transform into:	Transplantation route of MSCs:
Bone marrow Adipose tissue Peripheral blood Umbilical cord Amniotic fluid Placenta Urine Dental pulp Testis Lungs Skeletal muscles	Osteoblasts Myoblasts Cardiomyocytes Renal parenchymal cells	Intravenous Arterial Intraperitoneal Local injections

Table 3: Properties of MSCs from different sources [Modified from Ref[14]].

	Immune activity	Proliferative potential
Bone marrow	+	+
Adipose tissue	++	++
Umblical cord	+++	+++
Placenta	+++	+++

17.2.2 Potency

MSCs may be transformed into different cells (Table 1). MSCs have the ability to develop into mesangial cells, endothelial cells, tubular epithelial cells and podocytes in the kidneys[8].

17.2.3 Route of delivery

MSCs may be transplanted by different routes (Table 1). Intravenous administration provides easy access and has been widely used in clinical trials[17]. On the other hand, MSCs obtained intravenously have the ability to proliferate into many organs such as the kidneys, lung, skin, liver or thymus, with engraftment levels varying from 2.7% to 0.1[18]. In other words, in kidney disease models, following systemic injection, most of the cells entrap in the lung and other organs, and a very small amount of them home in injured kidneys[19]. Renal arterial injection of MSCs resulted in retention rates of 10 to 15%[12, 20,21]. Furthermore, injection to the abdominal aorta resulted in occlusion in the vasculature in case the cell sizes do not fit as seen in injection of human MSCs to rodents [22]. Direct renal arterial injection of MSCs has been shown to be more effective than intravenous injection in stimulating renal regeneration. On the other hand, local injection of MSCs into the renal parenchyma plays a positive role in renal repair[23,24], but this route is a challenging method to translate into clinical practice[8,12].

17.2.6 Dose

The optimal dose of MSCs is often empirical in experimental models of CKD, with doses ranging from 0.5×10^6 to 10×10^6[20,26]. Although the doses are different, MSCs were reported to be safe and beneficial in most of the studies. Nevertheless,

the higher the doses are used, the more efficient results can be achieved[12], thus increasing doses is strongly recommended in clinical trials.

17.2.5 Timing

Prophylactic MSC injections such as MSC treatment before kidney transplantation revealed no benefit compared to the control group in previous studies. This has been explained by the lack of microenvironment like ischemia or inflammation needed to react by the MSCs[26].

17.2.6 Homing

Previously MSCs were thought to be able to localize to the injured parts of the body. However, this ability is limited in MSCs and they need mediators like adhesion molecules, chemokine receptors or cytokines to precisely localize to the damaged tissues[8]. CD44 and hyaluronic acid expression, CXCR4 and CXCR7 secretion and preconditioning with insulin-like growth factor-1 resulting in upregulation of the CXCR4 expression have been shown to mediate MSCs to injured kidneys[12]. As infused MSCs respond well to homing signals, it is promising that preconditioning of MSCs may enhance their homing to injured tissues.

17.2.7 Engraftment

Some studies suggest that MSCs may engraft the impaired tissue and may differentiate into tubular cells or mesangial cells in kidney, however it was also demonstrated in drug-induced acute kidney injury and ischemia-reperfusion-injury models that renal engraftment is not essential to attain beneficial effects on kidney as paracrine effects of MSCs are more important than integration into target cells[20,21,27-30].

17.2.8 Safety profile

The adverse events after treatment with MSCs are similar to the control groups in most of the animal models and clinical studies [26]. Although no severe adverse reactions or tumorigenicity have been defined for MSC transplantation as seen in embryonic stem cells, none of the MSCs on market has ever been approved by FDA (US Food and Drug Administration).

17.2.9 Proliferation / Thriving

MSCs have the ability to induce the proliferation of renal glomerular and tubular cells. They cause tubular cells to proliferate and undergo apoptosis by secreting proangiogenic and trophic substances, respectively [13, 20]. It has been shown that MSCs reduce apoptosis via declining the expression of caspase-3 [31].

17.3 EXTRACELLULAR VESİCLES (EVs)

To avoid the immunologic disadvantages of MSC transplantation and enhance the paracrine mechanisms, cell-free therapies have been emerged. For that purpose, extracellular vesicles (EVs) have been used. EVs are membrane-bound, nanoscale vesicles made up of microvesicles (100-1000 nm) and exosomes (30-150 nm). They contain proteins, lipids, carbohydrates, and nucleid acids (DNA, miRNA, RNA). EVs have anti-inflammatory, pro-angiogenic, anti-fibrotic and anti-apoptotic, anti-oxidative effects and proliferative just like MSCs (Figure 1). Because EV injections are highly stable, permeability, immunogenicity, and cytotoxicity-free, they could be a safe substitute for cell-based therapy[32].

MSC-derived EV administration has therapeutic efficacy, which is equivalent to MSC[33] and successfully avoids the

drawbacks of MSC-based treatment[32]. Their small size endows them to avoid the pulmonary entrapment and to penetrate deeply[34]. Unsettled difficulties about EVs are isolation and purification problems and insufficient tracking tools to follow injected EVs[32]. The optimal origin, dose, or route is not determined yet for EVs as well.

17.4 STUDIES ON KIDNEY DISORDERS CONDUCTED WITH MSC OR MSC DERIVED EVS

17.4.1 AKI

Acute kidney injury (AKI) is defined as a rapid increase in serum creatinine concentration with or without decreased urine output[35]. The underlying pathogenic components are diminished renal tubular injury, renal perfusion, tubulointerstitial inflammation and decreased glomerular filtration rate [36]. AKI can result from many different etiologies such as sepsis, nephrotoxic drugs and major surgery. Drug-induced nephrotoxicity or ischemia/reperfusion injury (IRI) models are used for preclinical AKI studies.

The drug-induced nephropathy may either be caused by direct toxicity to the tubular cells, which results in acute tubular necrosis or may be caused by immune-mediated injury, which results in interstitial nephritis[36]. Well known nephrotoxic drugs frequently used in the clinical setting are cisplatin, aminoglycosides amphotericin B, antiviral agents (cidofovir, adefovir or tenofovir) and radiocontrast agents. Nephrotoxicity mostly occurs in the proximal renal tubules[14]. Cisplatin-induced AKI is a common animal model to study drug-induced nephrotoxicity. In drug-induced AKI, cytoskeletal structure of the proximal tubular epithelial cells is lost and necrosis and apoptosis occurs[14,37,38]. The histological features of drug-induced AKI are luminal dilation, cytoplasmic simplification, and increased

cytoplasmic eosinophilia. Due to the inflammation induced by necrosis and oxidative stress, tubular damage accelerates.

In IRI induced AKI models, kidneys are exposed to renal hypoxia, hemodynamic changes, endothelial and tubular cell injury. These leads to oxidative stress and inflammatory response, which causes cell damage and apoptosis. Ischemia/reperfusion injury models represent surgical procedures like kidney transplantation or partial nephrectomy and clinical problems including hypovolemic shock, dehydration, hypotension and acute tubular necrosis.

There are subtle differences in the immune processes behind ischemia reperfusion damage- and drug-induced acute kidney injury (AKI). More research is needed to improve the immunomodulatory effects of MSCs in both drug-induced and IRI-induced AKI.

It has been demonstrated that favorable effects observed after MSCs administration in experimental AKI models are mainly endorsed to the secretion of the EVs and releasing bioactive cytokines and trophic factors acting in a paracrine fashion[37].

17.4.2 Experimental Studies on MSC

There are many experimental studies about MSC treatment in AKI models. The most recent meta-analysis[36] based on 50 studies showed that MSC therapy may improve the kidney and renal functions of rats with AKI. In any route or dose, the results were significantly better in rats treated with MSC compared to the placebo groups. However, intravenous route was more successful than that of intrarenal or arterial MSC transplantation, and higher doses were more effective than that of low-dose transplantation[36].

17.5 Clinical Trials on MSCs

Although there are 7 more clinical trials registered on ClinicalTrial.gov to evaluate MSC treatment in AKI, only two of them have been completed yet (Table 4) [35,39,40]. Both of the studies were on patients with ischemic AKI after cardiac surgery. Both of the studies demonstrated safety of MSC administration. The first study included 16 cases that received intra-aortic single dose of BM-derived MSC during surgery and resulted in a reduction of AKI to 0% vs. 20% in the control group [39]. The second study was a multicenter study including 27 centers conducted on 67 cases receiving intra-arterial BM- derived MSC 48 hours after surgery and 68 control cases. The study demonstrated that MSC treatment showed no difference for renal recovery or mortality [40].

Table 4: Clinical trials to evaluate MSC treatment in AKI.

Author [ref] (clinical trial registration number)	N	MSC origin	Route	Outcome
Tögel FE & Westenfelder C[39] (NCT00733876)	16	BM	Intra-aortic	safe and well tolerated with renal recovery
Swaminathan M [40] (NCT01602328)	67	BM	Intra-arterial	safe and well tolerated but not different from the control group

MSC: Mesangial stem cells, AKI: acute kidney injury, BM: Bone marrow

17.5.1 Studies with EVs

Many preclinical experiments collected in recent two reviews/meta-analyses conducted on rodents with only IRI and with all forms of AKI revealed that stem cell or progenitor cell derived ECVs may improve IRI[32] and renal functions, inflammatory response and cellular apoptosis in rodent AKI models[41], respectively.

17.5.2 Renovascular diseases

17.5.2.1 Experimental studies on MSCs

A recent meta-analysis of sixteen preclinical studies showed that MSC therapy could result in higher levels of IL-10, microvascular density, RBF, GFR, and Scr and lower levels of Scr, MCP-1, PRA, TNF-α and IFN-γ. Renal fibrosis may get better in the interim following MSC treatment[41].

17.5.2.2 Clinical Trials on MSCs

There are only 3 clinical studies in cases with AKI (Table 5). Although MSC therapy cannot be recommended for AKI in clinical care yet, the cell-based therapy is becoming more attractive for physicians[42].

Table 5: Clinical trials on MSC treatment in AKI

Authors [ref]	Year of publication	N	MSC origin	Route	Outcomes
Kim SR [43]	2021	13	AT	Intra-arterial (renal artery)	p21+ urinary exosomes were elevated compared to control group, and only slightly improved by MSC, whereas p16+ exosomes remained unchanged
Abumoawad A [44]	2020	19	AT	Intra-arterial (renal artery)	Mean RBF and GFR significantly increased, hypoxia, renal vein inflammatory cytokines, and angiogenic biomarkers, mean systolic blood pressure significantly decreased after MSC infusion
Saad A [45]	2017	14	AT	Intra-arterial (renal artery)	Cortical perfusion and RBF both in the intervened and contralateral kidney increased, and renal hypoxia decreased after MSC infusion

MSC: Mesenchymal stem cell, AT: adipose tissue, RBF: renal blood flow, GFR: glomerular filtration rate.

17.5.2.3 Studies with EVs

There are few studies on EV treatment in animal models of AKI (Table 6).

Table 6: Animal studies on EV treatment in AKI models

Authors [ref]	Year of publication	MSC origin	Animal	Route	Outcome
Hong S.[46]	2023	AT	pig	Intra-renal	Autologous EVs attenuate cardiac injury in experimental RVD more effectively than their parent MSCs,
Ferguson CM.[47]	2021	AT	pig	Intra-renal	MSC-derived EVs elicit a better preservation of the stenotic kidney microvasculature and greater attenuation of renal injury and fibrosis compared to PTRA
Ishiy CSRA[48]	2020	AT	rat	IV (tail vein)	EVs produced beneficial results but with lower efficacy than MSCs.
Zhang L[49]	2020	AT	pig	Intra-renal	Improved stenotic-kidney GFR and RBF, and decreased renal release of MCP-1 and IL-6, normalized cardiac diastolic function, attenuated LV remodeling, cellular senescence and inflammation, and improved myocardial oxygenation and capillary density
Eirin A [50]	2018	AT	pig	Intra-renal	restored the renal microcirculation and in turn hemodynamics

AT: adipose tissue, EV: extracellular vesicle, RVD: renovascular disease, MSC: mesenchymal stem cell, PTRA: percutaneous transluminal renal angioplasty, GFR: glomerular filtration rate, RBF: renal blood flow, MCP1: monocyte chemoattractant protein-1, IL-6: interleukin-6, LV: left ventricle

17.5.3 CKD

CKD is a progressive and irreversible loss of kidney function, which is a rising health problem worldwide. It is characterized by fibrosis that might lead to end stage renal disease. One prevalent route to end-stage renal disease (ESRD) is by chronic glomerular and tubulointerstitial fibrosis, which is frequently linked to apoptosis, oxidative damage, and microvascular rarefaction.

17.5.3.1 Experimental studies on MSCs

There are numerous experimental studies about MSC treatment in CKD. A meta-analysis including studies conducted on rats, mice and pig animal models revealed that cell-based therapies improve renal function and structure[51].

17.5.3.2 Clinical Trials on MSCs

Endothelial progenitor cells (EPCs) and MSCs have similar reparative functions in chronic kidney disease. However, although there are several clinical studies on the use of EPCs both in adults and children with CKD and/or dialysis, studies with MSCs are limited[52]. More studies in patients with CKD are needed to assess the efficacy of cell-based regenerative therapy, particularly of MSCs.

17.5.3.3 Studies with EVs

A meta-analysis including mostly MSC derived ECVs for experimental CKD models including 26 rodent or porcine models has determined that EVs improved glomerulosclerosis and interstitial fibrosis and slowed down renal damage in a dose and time dependent manner[53]. Experimental CKD models were induced by unilateral ureteral obstruction, hypertension, diabetes, toxic-CKD or 5/6 nephrectomy.

17.5.4 Diabetic Nephropathy

17.5.4.1 Experimental studies on MSCs

In a recent review including 40 preclinical studies on 992 rodents about treatment with MSC, non-MSC, umblical cord/amniotic fluid cells and cell-derived products in diabetic kidney disease resulted in improvement in kidney functions and reduced kidney injury[54]. In a previous meta-analysis on 32 preclinical studies mainly including rat models and only 1 monkey model and 1 clinical study established that MSC may provide glycemic control, and reduce Scr, BUN and urine protein excretion[55].

17.5.4.2 Clinical Trials on MSCs

There are clinical trials with identification numbers planned to assess the safety and efficacy of MSCs in patients with diabetic kidney disease[56]. However, although some of them have been completed, none of them have been published yet. The completed studies just can only demonstrate that allogenic MSCs are safe. To use MSCs in clinical practice, the safety and efficacy profiles should be determined.

17.5.4.3 Studies with EVs

Mesenchymal stem cell derived exosomes enhance diabetic kidney diseases in rodents and podocytes[57].

17.5.5 Lupus Nephritis

Systemic lupus erythematosus is the prototype of the autoimmune diseases. It may involve several organs, but most decisive for the prognosis is the kidney involvement. Unfortunately, kidney involvement may lead to CKD. MSC administration is a widely used treatment option for both experimental and clinical lupus nephritis. SLE is hypothesized as a potenti-

ally MSC-mediated disease and different from those of other diseases, allogeneic rather than autologous MSC transplantation may be preferred as a more beneficial treatment of choice for patients with SLE[58].

17.5.5.1 Experimental studies on MSCs

There are numerous studies conducted on mouse models of SLE most of which were found efficient and summarized in Table 7 (Table 7).

Table 7: Experimental studies on MSCs in lupus nephritis

Authors [Ref]	Year of publication	MSC origin	Animal	Outcomes
Hoseinzadeh A[59]	2023	BM	mouse	Beneficial with a time and microenvironment dependent manner
Chun S[60]	2022	BM	mouse	Determines the safe doses for MSC
Matsuda S[61]	2022	AT	mouse	Only if enhanced with LWMH
Bukulmez H[62]	2021	BM	mouse	10-fold higher survival rates
Liu J[63]	2019	PL	mouse	Ameliorates renal injury and inflammation
Zhang Z[64]	2019	UC	mouse	Prevents podocyte injury via anti-inflammatory process
Mai S[65]	2018	UC	mouse	Hydroxychloroquine unexpectedly decreased the therapeutic effects of MSCs
Yang X[66]	2018	BM	mouse	MSC treatment may relieve lupus nephritis
Choi EW[67]	2016	AT	mouse	Treatment efficacy based on miRNA expression
Jang E[68]	2016	BM	mouse	Decreased levels of autoantibodies and proteinuria
Thiel A[69]	2015	Emb	mouse	Co-culture of MSCs with LPS-stimulated lymphocytes enhance their immune effects
Li Y[70]	2014	BM	mouse	Genetically transferred OXR1 enhanced the effects of BMCs.

Li Y[71]	2013	BM	mouse	Kallikrein transduced MSCs modulate inflammation and oxidative stress
Ma X[72]	2013	BM	mouse	MSCs inhibit lupus nephritis by suppressing B-cell activity
Choi EW[73]	2012	AT	mouse	MSCs ameliorate serological, immunological and histological findings and better results may be observed in an earlier stage.
Chang JW[74]	2011	UC	mouse	MSCs ameliorate lupus nephritis via immunosuppression rather than direct engraftment and differentiation.
Gu Z[75]	2010	UC	mouse	MSCs ameliorate lupus nephritis by inhibiting MCP-1 and HMGB-1 production and engraftment in kidneys.
Youd M[76]	2010	BM	mouse	MSCs are not beneficial in lupus.

BM: bone marrow, MSC: mesenchymal stem cell, AT: adipose tissue, LWMH: low-molecular weight heparin, PL: placental, UC: umbilical cord, MSC: mesenchymal stem cells, Emb: Embryological, LPS: lipopolysaccharide, MCP-1: monocyte chemoattractant protein-1, HMGB-1: high mobility group box 1.

17.5.5.2 Clinical Trials on MSCs

A meta-analysis including 8 clinical studies on 213 patients with lupus nephritis, MSC improved disease activity, complement levels and urinary protein excretion [77]. The most recent meta-analysis on MSC treatment including 12 studies on 586 cases also concluded that MSC improves the disease activity and kidney function with a favorable safety profile [78].

17.5.5.3 Studies on EVs

There are only few studies conducted on treatment of lupus nephritis treated with EVs (Table 8).

Table 8: Studies on EVs in lupus nephritis

Authors [Ref]	Year of publication	MSC origin	Outcomes
Zhang M[79]	2022	BM	Anti-inflammatory and anti-apoptotic effects
Sun W[80]	2022	UC	Anti-inflammatory and immunomodulatory effects

EV: BM: bone marrow, UC: umbilical cord

17.5.6 Kidney Transplantation

Mesenchymal stem cells increase regulatory T (Treg) cells, which in turn induce kidney transplant tolerance. Exosomes of bone marrow mesenchymal stem cells (BMMSC-Ex) stimulate the differentiation of Treg cells.

17.5.6.1 Experimental studies on MSCs

A few studies on the topic is summarized in Table 9 (Table 9).

Table 9: Experimental studies on MSCs in kidney transplantation

Authors [Ref]	Year of publication	MSC origin	Outcome
Luo Y[81]	2023	PER	Periost derived MSCs exerted stronger immunoregulation provided with Treg differentiation by inhibiting the mTOR pathway.
Xie HC[82]	2023	BM	MSCs prevent renal transplant rejection by facilitating a proliferation-inducing ligand phosphorylation to induce IL-10^{+} B-regulatory cells
Wang ZG[83]	2021	BM	EVs improved acute rejection by transferring long non-coding RNAs to dendritic cells.
Casiraghi F[84]	2019	BM	Tx of MSCs on the day of Renal tx is safe and complement is crucial for MSC recruitment into the kidney allograft.

MSC: mesenchymal stem cells, PER: periostal, BM: bone marrow, Treg: regulatory T cells, EVs: Extracellular vesicles

17.5.6.2 Clinical Trials

In a metaanalysis of 4 studies published in 2021, MSCs were used as induction therapy in 197 kidney transplant patients and it was safe and provided less CNI exposure and less infection in the first post-transplant year [85]. There are many unpublished but registered studies exist about MSC treatment in kidney transplantation (Table 10).

Table 10: Clinical trials on MSC treatment in kidney transplantation

Authors [Ref]	Year of publication	n	MSC origin	Route	Clinical Benefit
Wei Y[86]	2021	23	BM	IV	MSCs can delay the deterioration of allograft function, probably by decreasing DSA level and reducing DSA-induced injury in recipients with cABMR
Dreyer GJ[87]	2020	10	BM	IV	HLA selected allogeneic MSCs 6 months after transplantation is safe and feasible.
Erpicum P[88]	2018	10	BM	IV	Safe and efficaous. Improved early allograft function.
Sun et al[89]	2018	21	UC	IV	Safe and efficaous.
Pan et al[90]	2016	16	BM	IV	MSCs permits the use of lower dosages of nephrotoxic calcineurin inhibitors following renal transplantation.
Reinders M[91]	2013	6	BM	IV	Safe and efficaous in tx recipients with subclinical rejection. Findings are suggestive of systemic immunosuppression.

Peng Y[92]	2013	12	BM	1st dose: intraarterial during tx 2nd dose: IV	MSC may help reduce immunosuppressive treatment.
Perico N[93]	2013	2	BM	IV	MSC treatment resulted in increased number of Tregs and control of CD8 T cell functions.
Lee H et al.[94]	2013	7	BM	Intraosseous	Treatment could not prevent acute rejection in almost half of the cases and mixt chimerism could not be determined.
Vanikar AV et al.[95]	2012	916	AT	ND	MSC May help minimize immunosuppressive reduction and patient and graft survival at the end of 4 years.
Tan J et al.[96]	2012	159	BM	IV	autologous MSCs compared with anti-IL-2 receptor antibody induction therapy resulted in lower incidence of acute rejection, decreased risk of opportunistic infection, and better estimated renal function at 1 year

BM: bone marrow, IV: intravenous, MSC: mesenchymal stem cell, DSA: donor specific

IS: immunosuppression, ND: not determined, UC: umbilical cord, AT: adipose tissue

17.5.6.3 Studies with EVs:

Studies on EV treatment in kidney transplantation has been summarized in Table 11 (Table 11)

Table 11: Studies on EV treatment in kidney transplantation

Authors	Year of publication	MSC origin	Outcome
Wu X[97]	2022	BM	EVs Treg promoted cell differentiation and induced immune tolerance via long non-coding RNAs
Kubat GB[98]	2021	BM	Mitocondria isolated from MSCs protect against IRI in kidney tx.
Wang ZG[99]	2021	BM	EVs improved acute rejection by transferring long non-coding RNAs to dendritic cells.

MSC: mesenchymal stem cells, BM: bone marrow, EVs: extracellular vesicles, Treg: regulatory T cells, IRI: ischemia-reperfusion injury.

17.6 CONCLUSION

Preclinical studies have established a safe and efficacious profile for MSC treatment in kidney diseases and transplantation, however, the number of clinical studies are limited with conflicting results and unknown long-term effects. Lately, EVs are emerging as efficacious alternatives to MSCs. They are at least as effective and have much fewer side effects.

Although much progress has been made, there are still many difficulties for translation to clinical studies. The fact that the kidneys have a high structural complexity and it is hard to differentiate the injected MSCs to the targeted cells is already challenging in itself. Timing is another hard issue to overcome. For instance, as preparing MSCs ready for injection would take time, renal injury should be detected as soon as possible in AKI and efficient biomarkers are needed to establish that. In addition, the appropriate amount and frequency of dosing, the optimal route of delivery or timing of MSC injections for optimal survival, homing and engraftment, the best option for ensuring thriving or tracking of injected cells are not determined yet.

Nevertheless, despite these obstacles, it is obvious that MSCs treatment will have a great future as the treatment of choice in kidney diseases. Changing cellular microenvironment, preconditioning or gene modifications are promising initiatives to improve homing capacity and paracrine ability of MSCs. For better clinical response, the patients most likely to benefit from MSC treatment should be carefully identified as well as the optimal dose and route.

17.7 REFERENCES

1. Liu D, Cheng F, Pan S, Liu Z. Stem cells: a potential treatment option for kidney diseases. Stem Cell Research & Therapy 2020; 11 (1): 249. https://doi.org/10.1186/s13287-020-01751-2
2. Kaya E, Şenol Ö, Mir S. Renal Stem Cells. Turkish Nephrology Dialysis Transplantation 2017; 26 (2): 140-146.
3. Kumar R, Sharma A, Pattnaik AK, Varadwaj PK. Stem cells: An overview with respect to cardiovascular and renal disease. Journal of Natural Science, Biology and Medicine 2010;1 (1): 43-52. https://doi.org/10.4103%2F0976-9668.71674
4. Alvarez CV, Garcia-Lavandeira M, Garcia-Rendueles ME, Diaz-Rodriguez E, Garcia-Rendueles AR, et al. Defining stem cell types: understanding the therapeutic potential of ESCs, ASCs, and iPS cells. Journal of Molecular Endocrinology 2012; 49(2): R89-111. https://doi.org/10.1530/jme-12-0072
5. Fleig SV, Humphreys BD. Rationale of mesenchymal stem cell therapy in kidney injury. Nephron Clinical Practice 2014; 127 (1-4): 75-80. https://doi.org/10.1159/000363680
6. Saidi RF, Moghadasali R, Shekarchian S. Utilization of Mesenchymal Stem Cells in Kidney Transplantation: From Bench to Bedside. Iranian Journal of Kidney Diseases 2019; 13 (4): 213-224. PMID: 31422386.
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8 (4): 315-7. https://doi.org/10.1080/14653240600855905

8. Chen F, Chen N, Xia C, Wang H, Shao L, et al. Mesenchymal Stem Cell Therapy in Kidney Diseases: Potential and Challenges. Cell Transplantation 2023; 32: 9636897231164251. https://doi.org/10.1177/09636897231164251

9. Bochon B, Kozubska M, Surygała G, Witkowska A, Kuźniewicz R, et al. Mesenchymal Stem Cells-Potential Applications in Kidney Diseases. International Journal of Molecular Sciences 2019; 20 (10): 2462. https://doi.org/10.3390%2Fijms20102462

10. Rota C, Morigi M, Imberti B. Stem Cell Therapies in Kidney Diseases: Progress and Challenges. International Journal of Molecular Sciences 2019; 20 (11): 2790. https://doi.org/10.3390%2Fijms20112790

11. Sávio-Silva C, Soinski-Sousa PE, Balby-Rocha MTA, Lira ÁO, Rangel ÉB. Mesenchymal stem cell therapy in acute kidney injury (AKI): review and perspectives. Revista da Associacao Medica Brasileira (1992). 2020; 66 Suppl 1(Suppl 1): s45-s54. https://doi.org/10.1590/1806-9282.66.s1.45

12. Eirin A, Lerman LO. Mesenchymal stem cell treatment for chronic renal failure. Stem Cell Research & Therapy 2014; 5 (4):83. https://doi.org/10.1186/scrt472

13. Bi B, Schmitt R, Israilova M, Nishio H, Cantley LG. Stromal cells protect against acute tubular injury via an endocrine effect. Journal of American Society of Nephrology 2007; 18: 2486-2496. https://doi.org/10.1681/asn.2007020140

14. Han Q, Wang X, Ding X, He J, Cai G, Zhu H. Immunomodulatory Effects of Mesenchymal Stem Cells on Drug-Induced Acute Kidney Injury. Frontiers in Immunology 2021; 12: 683003. https://doi.org/10.3389/fimmu.2021.683003

15. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968; 6 (2): 230-47. PMID: 5654088.

16. Steenhard BM, Isom KS, Cazcarro P, Dunmore JH, Godwin AR, St John PL, Abrahamson DR. Integration of embryonic stem cells in metanephric kidney organ culture. Journal of American Society of Nephrology 2005; 16 (6): 1623-31. https://doi.org/10.1681/asn.2004070584

17. Zhang Y, Wang C, Bai Z, Li P. Umbilical Cord Mesenchymal Stem Cell Exosomes Alleviate the Progression of Kidney Failure by Modulating Inflammatory Responses and Oxidative Stress in an Ischemia-Reperfusion Mice Model. Journal of Biomedical Nanotechnology 2021; 17 (9): 1874-1881. https://doi.org/10.1166/jbn.2021.3155

18. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 2003; 101 (8): 2999-3001. https://doi.org/10.1182/blood-2002-06-1830

19. Xiang E, Han B, Zhang Q, Rao W, Wang Z, Chang C, Zhang Y, Tu C, Li C, Wu D. Human umbilical cord-derived mesenchymal stem cells prevent the progression of early diabetic nephropathy through inhibiting inflammation and fibrosis. Stem Cell Research & Therapy 2020; 11 (1): 336. https://doi.org/10.1186/s13287-020-01852-y

20. Eirin A, Zhu XY, Krier JD, Tang H, Jordan KL, et al. Adipose tissue-derived mesenchymal stem cells improve revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. Stem Cells 2012; 30 (5):1030-1041. https://doi.org/10.1002/stem.1047

21. Ebrahimi B, Eirin A, Li Z, Zhu XY, Zhang X, et al. Mesenchymal stem cells improve medullary inflammation and fibrosis after revascularization of swine atherosclerotic renal artery stenosis. PLoS One 2013;8 (7): e67474. https://doi.org/10.1371%2Fjournal.pone.0067474

22. Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, et al. Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy. Microvascular Research 2009; 77 (3): 370-376. https://doi.org/10.1016/j.mvr.2009.02.001

23. Cavaglieri RC, Martini D, Sogayar MC, Noronha IL. Mesenchymal stem cells delivered at the subcapsule of the kidney ameliorate renal disease in the rat remnant kidney model. Transplantation Proceedings 2009; 41 (3): 947-51. https://doi.org/10.1016/j.transproceed.2009.01.072

24. Alfarano C, Roubeix C, Chaaya R, Ceccaldi C, Calise D, et al. Intraparenchymal injection of bone marrow mesenchymal stem

cells reduces kidney fibrosis after ischemia-reperfusion in cyclosporine-immunosuppressed rats. Cell Transplantation 2012; 21 (9): 2009-19. https://doi.org/10.3727/096368912x640448

25. Ezquer FE, Ezquer ME, Parrau DB, Carpio D, Yañez AJ, Conget PA. Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice. Biology of Blood and Marrow Transplantation 2008; 14 (6): 631-640. https://doi.org/10.1016/j.bbmt.2008.01.006
26. Savio-Silva, English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunology & Cell Biology 2013; 91:19-26. https://doi.org/10.1038/icb.2012.56
27. Yokoo T, Fukui A, Ohashi T, Miyazaki Y, Utsunomiya Y, et al. Xenobiotic kidney organogenesis from human mesenchymal stem cells using a growing rodent embryo. Journal of American Society of Nephrology 2006; 17 (4): 1026-34. https://doi.org/10.1681/asn.2005101043 .
28. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. International Journal of Molecular Medicine 2004; 14 (6): 1035-41. https://doi.org/10.3892/ijmm.14.6.1035
29. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure Journal of American Society of Nephrology 2004; 15 (7): 1794-1804. https://doi.org/10.1097/01.asn.0000128974.07460.34
30. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. American Journal of Physiology Renal Physiology 2005; 289 (1): F31-42. https://doi.org/10.1152/ajprenal.00007.2005
31. Zhu XY, Urbieta-Caceres V, Krier JD, Textor SC, Lerman A, Lerman LO. Mesenchymal stem cells and endothelial progenitor cells decrease renal injury in experimental swine renal artery stenosis through different mechanisms. Stem Cells 2013; 31 (1): 117-125. https://doi.org/10.1002/stem.1263

32. Li XQ, Liu JF, Liu H, Meng Y. Extracellular vesicles for ischemia/reperfusion injury-induced acute kidney injury: a systematic review and meta-analysis of data from animal models. Systematic Reviews 2022; 11 (1): 197. https://doi.org/10.1186/s13643-022-02003-5
33. Harrell CR, Fellabaum C, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem Cell-Derived Secretome. Cells 2019; 8 (5): 467. https://doi.org/10.3390/cells8050467
34. Farzamfar S, Hasanpour A, Nazeri N, Razavi H, Salehi M, et al. Extracellular micro/nanovesicles rescue kidney from ischemia-reperfusion injury. Journal of Cellular Physiology 2019; 234 (8): 12290-12300. https://doi.org/10.1002/jcp.27998
35. Liu Y, Holmes C. Tissue Regeneration Capacity of Extracellular Vesicles Isolated From Bone Marrow-Derived and Adipose-Derived Mesenchymal Stromal/Stem Cells. Frontiers in Cell and Developmental Biology 2021; 9: 648098. https://doi.org/10.3389/fcell.2021.648098
36. Wanyan P, Wang X, Li N, Huang Y, She Y, Zhang L. Mesenchymal stem cells therapy for acute kidney injury: A systematic review with meta-analysis based on rat model. Frontiers in Pharmacology. 2023; 14: 1099056. https://doi.org/10.3389%2Ffphar.2023.1099056
37. Zhou J, An C, Jin X, Hu Z, Safirstein RL, Wang Y. TAK1 deficiency attenuates cisplatin-induced acute kidney injury. American Journal of Physiology Renal Physiology 2020; 318 (1): F209-F215. https://doi.org/10.1152%2Fajprenal.00516.2019
38. Ozkok A, Edelstein CL. Pathophysiology of cisplatin-induced acute kidney injury. BioMed Research International 2014; 2014: 967826. https://doi.org/10.1155/2014/967826
39. Tögel FE, Westenfelder C. Kidney protection and regeneration following acute injury: Progress through stem cell therapy. American Journal of Kidney Diseases 2012; 60 (6): 1012-22. https://doi.org/10.1053/j.ajkd.2012.08.034
40. Swaminathan M, Stafford-Smith M, Chertow GM, Warnock DG, Paragamian V, et al. Allogeneic Mesenchymal Stem Cells for Treatment of AKI after Cardiac Surgery. Journal of Ameri-

can Society of Nephrology 2018; 29 (1): 260-267. https://doi.org/10.1681/asn.2016101150 .

41. Liu C, Wang J, Hu J, Fu B, Mao Z, et al. Extracellular vesicles for acute kidney injury in preclinical rodent models: a meta-analysis. Stem Cell Research & Therapy 2020; 11 (1): 11. https://doi.org/10.1186/s13287-019-1530-4
42. Wang HS, Yi MY, Wu X, Liu Q, Deng YH, et al. Effects of mesenchymal stem cells in renovascular disease of preclinical and clinical studies: a systematic review and meta-analysis. Scientific Reports 2022; 12 (1): 18080. https://doi.org/10.1038/s41598-022-23059-2
43. Kim SR, Zou X, Tang H, Puranik AS, Abumoawad AM, et al. Increased cellular senescence in the murine and human stenotic kidney: Effect of mesenchymal stem cells. Journal of Cellular Physiology 2021; 236 (2): 1332-1344. https://doi.org/10.1002/jcp.29940
44. Abumoawad A, Saad A, Ferguson CM, Eirin A, Herrmann SM, et al. In a Phase 1a escalating clinical trial, autologous mesenchymal stem cell infusion for renovascular disease increases blood flow and the glomerular filtration rate while reducing inflammatory biomarkers and blood pressure. Kidney International 2020; 97 (4): 793-804. https://doi.org/10.1016/j.kint.2019.11.022
45. Saad A, Dietz AB, Herrmann SMS, Hickson LJ, Glockner JF, McKusick MA, Misra S, Bjarnason H, Armstrong AS, Gastineau DA, Lerman LO, Textor SC. Autologous Mesenchymal Stem Cells Increase Cortical Perfusion in Renovascular Disease. Journal of American Society of Nephrology 2017; 28 (9): 2777-2785. https://doi.org/10.1681/asn.2017020151
46. Hong S, Zhu XY, Jiang Y, Zhang L, Tang H, et al. Autologous Extracellular Vesicles Attenuate Cardiac Injury in Experimental Atherosclerotic Renovascular Disease More Effectively Than Their Parent Mesenchymal Stem/Stromal Cells. Stem Cell Reviews and Reports 2023; 19 (3): 700-712. https://doi.org/10.1007/s12015-022-10473-2
47. Ferguson CM, Farahani RA, Zhu XY, Tang H, Jordan KL, et al. Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles Elicit Better Preservation of the Intra-Renal Microvasculature Than Renal Revascularization in Pigs with Renovascular Disease. Cells 2021; 10 (4): 763. https://doi.org/10.3390/cells10040763

48. Ishiy CSRA, Ormanji MS, Maquigussa E, Ribeiro RS, da Silva Novaes A, Boim MA. Comparison of the Effects of Mesenchymal Stem Cells with Their Extracellular Vesicles on the Treatment of Kidney Damage Induced by Chronic Renal Artery Stenosis. Stem Cells International 2020; 2020: 8814574. https://doi.org/10.1155/2020/8814574
49. Zhang L, Zhu XY, Zhao Y, Eirin A, Liu L, et al. Selective intrarenal delivery of mesenchymal stem cell-derived extracellular vesicles attenuates myocardial injury in experimental metabolic renovascular disease. Basic Research in Cardiology 2020; 115 (2):16. https://doi.org/10.1007/s00395-019-0772-8
50. Eirin A, Zhu XY, Jonnada S, Lerman A, van Wijnen AJ, Lerman LO. Mesenchymal Stem Cell-Derived Extracellular Vesicles Improve the Renal Microvasculature in Metabolic Renovascular Disease in Swine. Cell Transplantation 2018; 27 (7): 1080-1095. https://doi.org/10.1177/0963689718780942
51. Papazova DA, Oosterhuis NR, Gremmels H, van Koppen A, Joles JA, Verhaar MC. Cell-based therapies for experimental chronic kidney disease: a systematic review and meta-analysis. Disease Models & Mechanisms 2015; 8 (3): 281-93. https://doi.org/10.1242/dmm.017699
52. Hickson LJ, Eirin A, Lerman LO. Challenges and opportunities for stem cell therapy in patients with chronic kidney disease. Kidney International. 2016 Apr;89(4):767-78. https://doi.org/10.1016/j.kint.2015.11.023
53. Nowak N, Yamanouchi M, Satake E. The Nephroprotective Properties of Extracellular Vesicles in Experimental Models of Chronic Kidney Disease: a Systematic Review. Stem Cell Reviews and Reports 2022; 18 (3): 902-932. https://doi.org/10.1007/s12015-021-10189-9
54. Hickson LJ, Abedalqader T, Ben-Bernard G, Mondy JM, Bian X, et al. A systematic review and meta-analysis of cell-based interventions in experimental diabetic kidney disease. Stem Cells Translational Medicine 2021; 10 (9):1304-1319. https://doi.org/10.1002/sctm.19-0419
55. Lin W, Li HY, Yang Q, Chen G, Lin S, Liao C, Zhou T. Administration of mesenchymal stem cells in diabetic kidney disease: a systematic review and meta-analysis. Stem Cell Research & Therapy 2021; 12 (1): 43. https://doi.org/10.1186/s13287-020-02108-5

56. Xu N, Liu J, Li X. Therapeutic role of mesenchymal stem cells (MSCs) in diabetic kidney disease (DKD). Endocrinology Journal. 2022; 69 (10): 1159-1172. https://doi.org/10.1507/endocrj.ej22-0123
57. Wang Y, Liu J, Wang H, Lv S, Liu Q, et al. Mesenchymal Stem Cell-Derived Exosomes Ameliorate Diabetic Kidney Disease Through the NLRP3 Signaling Pathway. Stem Cells 2023; 41 (4): 368-383. https://doi.org/10.1093/stmcls/sxad010
58. Fathollahi A, Gabalou NB, Aslani S. Mesenchymal stem cell transplantation in systemic lupus erythematous, a mesenchymal stem cell disorder. Lupus; 27 (7): 1053-1064. https://doi.org/10.1177/0961203318768889
59. Hoseinzadeh A, Rezaieyazdi Z, Mahmoudi M, Tavakol Afshari J, Lavi Arab F, et al. Dysregulated balance in Th17/Treg axis of Pristane-induced lupus mouse model, are mesenchymal stem cells therapeutic? Intenational Immunopharmacology 2023; 117: 109699. https://doi.org/10.1016/j.intimp.2023.109699
60. Chun S, Choi CB, Kim MS, Nam JY, Lee TY, et al. Safety and tolerability of bone marrow-derived mesenchymal stem cells in lupus animal models and a phase I clinical trial in humans. Lupus 2022; 31 (10): 1245-1253. https://doi.org/10.3389/fimmu.2021.792739
61. Bukulmez H, Horkayne-Szakaly I, Bilgin A, Baker TP, Caplan AI, Jones OY. Intrarenal injection of mesenchymal stem cell for treatment of lupus nephritis in mice - a pilot study. Lupus 2021; 30 (1): 52-60. https://doi.org/10.1177/0961203320968897
62. Liu J, Lu X, Lou Y, Cai Y, Cui W, et al. Xenogeneic Transplantation of Human Placenta-Derived Mesenchymal Stem Cells Alleviates Renal Injury and Reduces Inflammation in a Mouse Model of Lupus Nephritis. Biomed Research International 2019; 2019: 9370919. https://doi.org/10.1155/2019/9370919
63. Zhang Z, Niu L, Tang X, Feng R, Yao G, et al . Mesenchymal stem cells prevent podocyte injury in lupus-prone B6.MRL-Fas-lpr mice via polarizing macrophage into an anti-inflammatory phenotype. Nephrology Dialysis Transplantation 2019; 34 (4): 597-605. https://doi.org/10.1093/ndt/gfy195
64. Mai S, Zou L, Tian X, Liao X, Luan Y, et al. Double-Edged Effect of Hydroxychloroquine on Human Umbilical Cord-Derived Mesenchymal Stem Cells Treating Lupus Nephritis in MRL/lpr

Mice. Molecular Pharmaceutics 2018; 15 (5): 1800-1813. https://doi.org/10.1021/acs.molpharmaceut.7b01146

65. Yang X, Yang J, Li X, Ma W, Zou H. Bone marrow-derived mesenchymal stem cells inhibit T follicular helper cell in lupus-prone mice. Lupus 2018; 27 (1): 49-59. https://doi.org/10.1177/0961203317711013
66. Choi EW, Shin IS, Park SY, Park JH, Kim JS, et al. Reversal of serologic, immunologic, and histologic dysfunction in mice with systemic lupus erythematosus by long-term serial adipose tissue-derived mesenchymal stem cell transplantation. Arthritis Rheumatology 2012; 64 (1): 243-53. https://doi.org/10.1002/art.33313
67. Jang E, Jeong M, Kim S, Jang K, Kang BK, Lee DY, Bae SC, Kim KS, Youn J. Infusion of Human Bone Marrow-Derived Mesenchymal Stem Cells Alleviates Autoimmune Nephritis in a Lupus Model by Suppressing Follicular Helper T-Cell Development. Cell Transplantation 2016; 25 (1): 1-15. https://doi.org/10.3727/096368915x688173
68. Thiel A, Yavanian G, Nastke MD, Morales P, Kouris NA, et al. Human embryonic stem cell-derived mesenchymal cells preserve kidney function and extend lifespan in NZB/W F1 mouse model of lupus nephritis. Scientific Reports 2015; 5: 17685. https://doi.org/10.1038/srep17685
69. Li Y, Li W, Liu C, Yan M, Raman I, Du Y, et al. Delivering Oxidation Resistance-1 (OXR1) to Mouse Kidney by Genetic Modified Mesenchymal Stem Cells Exhibited Enhanced Protection against Nephrotoxic Serum Induced Renal Injury and Lupus Nephritis. Journal of Stem Cell Research & Therapeutics. 2014 Sep 10; 4 (9): 231. https://doi.org/10.4172%2F2157-7633.1000231
70. Li Y, Raman I, Du Y, Yan M, Min S, et al. Kallikrein transduced mesenchymal stem cells protect against anti-GBM disease and lupus nephritis by ameliorating inflammation and oxidative stress. PLoS One 2013; 8 (7): e67790. https://doi.org/10.1371%2Fjournal.pone.0067790
71. Ma X, Che N, Gu Z, Huang J, Wang D, et al. Allogenic mesenchymal stem cell transplantation ameliorates nephritis in lupus mice via inhibition of B-cell activation. Cell Transplantation 2013; 22 (12): 2279-90. https://doi.org/10.3727/096368912x658692
72. Choi EW, Shin IS, Park SY, Park JH, Kim JS, et al. Reversal of serologic, immunologic, and histologic dysfunction in mice with

systemic lupus erythematosus by long-term serial adipose tissue-derived mesenchymal stem cell transplantation. Arthritis Rheumatology 2012; 64 (1): 243-53. https://doi.org/10.1002/art.33313

73. Chang JW, Hung SP, Wu HH, Wu WM, Yang AH, et al. Therapeutic effects of umbilical cord blood-derived mesenchymal stem cell transplantation in experimental lupus nephritis. Cell Transplantation 2011; 20 (2): 245-57. https://doi.org/10.3727/096368910x520056
74. Gu Z, Akiyama K, Ma X, Zhang H, Feng X, et al. Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in MRL/lpr mice. Lupus; 19 (13): 1502-14. https://doi.org/10.1177/0961203310373782
75. Youd M, Blickarz C, Woodworth L, Touzjian T, Edling A, et al. Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clinical & Experimental Immunology. 2010; 161 (1): 176-86. https://doi.org/10.1111/j.1365-2249.2010.04158.x
76. Liu S, Guo YL, Yang JY, Wang W, Xu J. Efficacy of mesenchymal stem cells on systemic lupus erythematosus:a meta-analysis]. Beijing Da Xue Xue Bao Yi Xue Ban. 2018; 50(6): 1014-1021. https://doi.org/10.19723/j.issn.1671-167X.2018.06.013
77. Xia Y, Ye H, Li K, Shi B, Sun X, Wu J. Efficacy of Mesenchymal Stem Cell Therapy on Lupus Nephritis and Renal Function in Systemic Lupus Erythematosus: A Meta-Analysis. Clinical and Investigative Medicine 2023; 46 (1): E24-35. https://doi.org/10.25011/cim.v46i1.39561
78. Zhang M, Johnson-Stephenson TK, Wang W, Wang Y, Li J, et al. Mesenchymal stem cell-derived exosome-educated macrophages alleviate systemic lupus erythematosus by promoting efferocytosis and recruitment of IL-17^{+} regulatory T cell. Stem Cell Research & Therapy 2022; 13 (1): 484. https://doi.org/10.1186/s13287-022-03174-7
79. Sun W, Yan S, Yang C, Yang J, Wang H, et al. Mesenchymal Stem Cells-derived Exosomes Ameliorate Lupus by Inducing M2 Macrophage Polarization and Regulatory T Cell Expansion in MRL/lpr Mice. Immunological Investigations 2022; 51 (6): 1785-1803. https://doi.org/10.1080/08820139.2022.2055478

80. Luo Y, Zhang K, Wu J, Zeng H, Chen J, et al. Periosteum-derived mesenchymal stem cell alleviates renal fibrosis through mTOR-mediated Treg differentiation. Renal Failure 2023; 45 (1): 2212079. https://doi.org/10.1080%2F0886022X.2023.2212079

81. Xie HC, Wang ZG, Feng YH, Wang JX, Liu L, et al. Bone marrow mesenchymal stem cells repress renal transplant immune rejection by facilitating the APRIL phosphorylation to induce regulation B cell production. Physiological Genomics 2023; 55 (2): 90-100. https://doi.org/10.1152/physiolgenomics.00103.2022

82. Wang ZG, Xu HE, Cheng FM, Zhang J, Feng YH, Liu DH, Shang WJ, Feng GW. Donor BMSC-derived small extracellular vesicles relieve acute rejection post-renal allograft through transmitting Loc108349490 to dendritic cells. Aging Cell 2021; 20 (10): e13461. https://doi.org/10.1111%2Facel.13461

83. Casiraghi F, Todeschini M, Azzollini N, Cravedi P, Cassis P, et al. Effect of Timing and Complement Receptor Antagonism on Intragraft Recruitment and Protolerogenic Effects of Mesenchymal Stromal Cells in Murine Kidney Transplantation. Transplantation 2019; 103 (6): 1121-1130. https://doi.org/10.1097/tp.0000000000002611

84. Zhao L, Hu C, Han F, Chen D, Cheng J, et al. Induction therapy with mesenchymal stromal cells in kidney transplantation: a meta-analysis. Stem Cell Research & Therapy 2021; 12 (1): 158. https://doi.org/10.1186/s13287-021-02219-7

85. Wei Y, Chen X, Zhang H, Su Q, Peng Y, et al. Efficacy and Safety of Bone Marrow-Derived Mesenchymal Stem Cells for Chronic Antibody-Mediated Rejection After Kidney Transplantation- A Single-Arm, Two-Dosing-Regimen, Phase I/II Study. Frontiers in Immunology 2021; 12: 662441. https://doi.org/10.3389/fimmu.2021.662441

86. Dreyer GJ, Groeneweg KE, Heidt S, Roelen DL, van Pel M, et al. Human leukocyte antigen selected allogeneic mesenchymal stromal cell therapy in renal transplantation: The Neptune study, a phase I single-center study. American Journal of Transplantation 2020; 20 (10): 2905-2915. https://doi.org/10.1111/ajt.15910

87. Erpicum P, Weekers L, Detry O, Bonvoisin C, Delbouille MH, et al. Infusion of third-party mesenchymal stromal cells after kidney transplantation: a phase I-II, open-label, clinical study. Kid-

ney International 2019; 95 (3): 693-707. https://doi.org/10.1016/j.kint.2018.08.046

88. Sun Q, Huang Z, Han F, Zhao M, Cao R, et al. Allogeneic mesenchymal stem cells as induction therapy are safe and feasible in renal allografts: pilot results of a multicenter randomized controlled trial. Journal of Translational Medicine 2018; 16 (1): 52. https://doi.org/10.1186/s12967-018-1422-x
89. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, et al. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. Oncotarget 2016; 7 (11): 12089-101. https://doi.org/10.18632/oncotarget.7725
90. Reinders ME, de Fijter JW, Roelofs H, Bajema IM, de Vries DK, et al. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. Stem Cells Translational Medicine 2013; 2 (2): 107-11. https://doi.org/10.5966/sctm.2012-0114
91. Peng Y, Ke M, Xu L, Liu L, Chen X, et al. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. Transplantation 2013; 95 (1): 161-8. https://doi.org/10.1097/tp.0b013e3182754c53
92. Perico N, Casiraghi F, Gotti E, Introna M, Todeschini M, et al. Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. Transplantation International 2013; 26 (9): 867-878. https://doi.org/10.1111/tri.12132
93. Lee H, Park JB, Lee S, Baek S, Kim H, Kim SJ. Intra-osseous injection of donor mesenchymal stem cell (MSC) into the bone marrow in living donor kidney transplantation; a pilot study. Journal of Translational Medicine 2013; 11: 96. https://doi.org/10.1186/1479-5876-11-96
94. Vanikar AV, Trivedi HL. Stem cell transplantation in living donor renal transplantation for minimization of immunosuppression. Transplantation 2012; 94 (8): 845-850. https://doi.org/10.1097/tp.0b013e3182664000

95. Tan J, Wu W, Xu X, Liao L, Zheng F, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. JAMA 2012 Mar 21; 307 (11): 1169-1177. https://doi.org/10.1001/jama.2012.316
96. Wu X, Wang Z, Wang J, Tian X, Cao G, Gu Y, Shao F, Yan T. Exosomes Secreted by Mesenchymal Stem Cells Induce Immune Tolerance to Mouse Kidney Transplantation via Transporting LncRNA DANCR. Inflammation 2022; 45 (1): 460-475. https://doi.org/10.1007/s10753-021-01561-5
97. Kubat GB, Ozler M, Ulger O, Ekinci O, Atalay O, et al. The effects of mesenchymal stem cell mitochondrial transplantation on doxorubicin-mediated nephrotoxicity in rats. Journal of Biochemical and Molecular Toxicology 2021; 35 (1): e22612. https://doi.org/10.1002/jbt.22612
98. Wang ZG, Xu HE, Cheng FM, Zhang J, Feng YH, et al. Donor BMSC-derived small extracellular vesicles relieve acute rejection post-renal allograft through transmitting Loc108349490 to dendritic cells. Aging Cell 2021; 20 (10): e13461. https://doi.org/10.1111/acel.13461

MESENCHYMAL STEM CELL AND CRISPR TECHNOLOGY

Hüseyin YILMAZ[1]
[1]Izmir Katip Çelebi University Faculty of Medicine, Department of Medical Pharmacology, Izmir, Turkey

18.1 INTRODUCTION

Discovery of molecular components in the CRISPR system, making history as the first time two women have been awarded the Nobel Prize in Chemistry [1]. Emmanuelle Charpentier and Jennifer Doudna awarded by the Nobel Prize in Chemistry in 2020. Firstly, CRISPR was discovered in the DNA sequences of *E. Coli* bacteria by Ishino et al. from Osaka University, Japan in 1987 [2]. However, it was Charpentier and Doudna's pioneering work that unlocked its full potential. Although the significance of CRISPR in the bacterial cell was unknown at the time of its discovery, sequencing DNA fragments took months back then. The CRISPR system's biological function remains unclear. Nevertheless, early studies have demonstrated a high degree of polymorphism in CRISPR loci among different strains of the same pathogenic bacterial species. Therefore, scientists have proposed utilizing this information to genotype bacterial strains of *M. Tuberculosis* and later *S. Pyogenes*. This approach has successfully identified bacterial strains in clinical conditions [3,4].

In 1995, Francisco Mojica made a significant discovery. He identified similar nucleic acid sequences in the archae-

al genome of *H. Mediterranei* [5]. This discovery shed light on the CRISPR loci and their functional importance, leading to further research. Mojica confidently identified similarities between elements in archaea and DNA repeats previously found in bacterial genomes, providing compelling evidence for their functional role. The atypical loci contain foreign DNA fragments and play an integral role in the immune systems of both archaea and bacteria [6]. In 1995, different two centers independently reached to similar point. They unequivocally demonstrated that viral DNA, separated by short palindromic repeats, serves as a library for potentially harmful genetic information [7,8]. The system was initially hypothesised to work through RNA modification. However, Marraffini and Sontheimer's publication demonstrated experimentally for the first time that the prokaryotic immune system targets foreign DNA. Therefore, this system could be a potential tool for genomic editing in the laboratory[9]. Later studies have shown that certain CRISPR systems work directly with RNA molecules. This allows for the selective deactivation of specific transcripts within the cell[10, 11, 12].

18.2 GENERAL MECHANISM

CRISPR/Cas mechanisms are a diverse microbial immune system used by most of the archaeas (90%) and some part of eubacterias (40%) to protect themselves from viruses and plasmids. These systemsallow the cell to distinguish 'foreign' DNA from its own. CRISPRs are composed of CRISPR loci consist of highly conserved short repeated sequences, separated by short spacer sequences of similar size (Figure 1). It is important to note that CRISPR loci should be written in a consistent manner throughout the document. The sizes of CRISPR repeats and spacers range from 23 to 47 base pairs and 21 to 72 base pairs, respectively. The bacterial genome may

contain more than one CRISPR locus. Spacer sequences are diverse and hypervariable, even among closely related strains. These unique sequences come from viral or plasmid DNA and when new spacers are added, they allow for recognition and destruction of new matching viral or plasmid genomes [13].

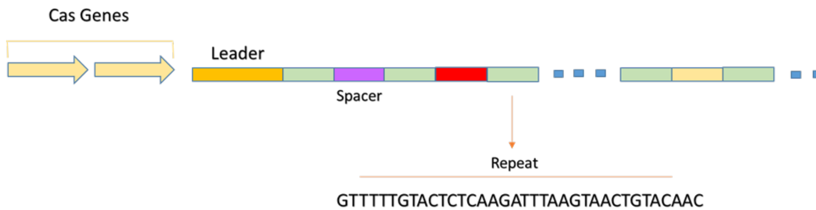


Figure 1: CRISPR-CAS gene locus

CRISPR loci are associated with a conserved sequence called 'Leader', which is located upstream of the CRISPR in the direction of transcription [14]. To function, CRISPR requires a set of CRISPR-associated (Cas) genes, typically found adjacent to the CRISPR and encoding Cas genes encode proteins with diverse functions, including nucleases, helicases, and polymerases, that are required for the immune response. The CRISPR immune system relies on the cooperation of multiple Cas proteins [15]. Kumar provides an analogy for the CRISPR mechanism: imagine the Earth is under attack by an alien species for which no earthly weapon is effective. In this scenario, someone collects parts of the aliens and develops a new weapon unique to them, storing some parts for future reference. Similarly, the CRISPR mechanism collects and stores genetic material for future use. When a bacterium is attacked by a bacteriophage, it lacks a defence mechanism. To address this, a small Cas protein takes a piece of the bacteriophage's DNA and integrates it into the CRISPR site. From there, a crRNA (CRISPR RNA) is synthesized. The complementary sequence involved in bacteriophage invasion is recognized

and cleaved by proteins, including others [13].

The general mechanism of CRISPR takes place in three distinct stages (Figure2):

i. Adaptation

ii. Expression and maturation (Biogenesis)

iii. Intervention (Targeting)[16]

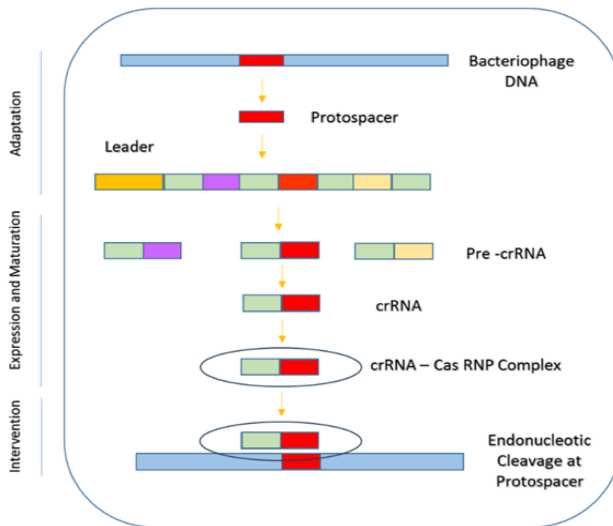


Figure 2: General Mechanism of CRISPR-CAS System

18.2.1 Adaptation

The adaptation process is almost identical for the different CRISPR classes, but biogenesis and targeting differ between classes [13]. Cas proteins confidently bind to double-stranded DNA upon encountering the protospacer adjacent motif during the adaptation step. This results in the formation of two double-stranded breaks at that site. The protospacer, which is released as a fragment, is then incorporated into the DNA.

The CRISPR sequence confidently acquires spacers, mostly after the leader sequence in the proximal repeat units, becoming a Spacer. This process is also confidently known as Spacer acquisition or incorporation. Spacers can be acquired through pure acquisition or differential acquisition pathways. In the pure acquisition pathway, the pathogen acquires spacers from foreign DNA it has encountered before. In the differential acquisition pathway, the CRISPR-Cas system recognizes and integrates foreign DNA that the organism has not previously encountered, requiring the effector proteins Cas1 and Cas2.

The pathogen has already attacked in the primed acquisition pathway. It is crucial to understand that various CRISPR-Cas systems have distinct adaptations. For instance, in the type I-F system of *P. Aeruginosa*, where Cas1 and Cas2 are absent, supplementary machinery is necessary to acquire spacers. Similarly, Cas4 is required for adaptation in type I-B systems. In type II-A systems, Cas9 integrates new spacers using Cas1, Cas2, and csn2. Cas9 and csn2 proteins have significant functions in integration of spacer. Cas9 identifies the PAM site in the protospacer, while in type III-B systems, Cas1 is coupled to reverse transcriptase also recruits both RNA and DNA. In type I-E systems, the frequency of spacer acquisition increases with the presence of the interference complex. Following spacer acquisition, crRNA processing and maturation occur [17].

18.2.2 Expression and Maturation Phase (Biogenesis)

The intermediate crRNAs undergo further modification through the trimming of the 3′ tag using nucleases, resulting in the formation of mature crRNA. The mature crRNA comprises a complete spacer region at the 5′ end and a repetitive

region at the 3′ end, which takes the form of a hairpin like shape and stays bound to the Cas6 protein via the 3′ end. The other parts are bounded via the 5′ spacer and utilized for choose of target [18].

For class II systems, the biogenesis process involves two phase of catalysis made by RNase III: one for repeats and another for spacers. The Cas9 RNA duplex is stabilized by tracrRNA, a non-protein-coding RNA that interacts with the repeat structural unit of the pre-crRNA transcript. Host dsRNA-specific RNase III specifically recognizes and cleaves the RNA duplex, resulting in the intermediate crRNA, which is then processed by nucleases. Type II systems feature a mature crRNA without the 5′ nucleotide tag and with a 20 nucleotide repeat sequence element. Notably, the type II system of Neisseria meningitidis deviates from the dual RNA mechanism [17].

18.2.3 Intervention Phase

Interference is the last step in adaptive CRISPR immune system. Here, CRISPR effector recognises the target DNA (or RNA) via attach mature crRNA (function as a guide RNA) and cleaves it using effector nucleases. Class-I systems need CASCADE (CRISPR-associated complex for antiviral defence) to function. Class-II systems need just a single complex for target interference. No new content has been added. Class II systems need just a single complex to interfere with the target. Type I, II and V systems detect PAM sites and have different target interference requirements. Type III systems use a 5′ tag to distinguish the self and non-self to prevent self-targeting. The mechanism of interference's type I systems involves the CASCADE complex and the Cas3 effector. The language has been made more concise and unambiguous, and sentences have been restructured for better coherence

and sequential logic. Grammar, spelling and punctuation have been checked and corrected. The protein is recognised by the crRNA-directed CASCADE complex, which recognises the PAM site on the target DNA via the Cse1 large subunit [17].

The PAM site recognition triggers DNA unwinding and the subsequent bounding of the CASCADE complex to the target. The DNA, crRNA, and proto-spacer region then form the R-ring shape, inducing structural changes in the CASCADE complex. The Cas3 protein causes a cleavage in the target by unwinding the RNA-DNA duplex in the 3′-5′ direction and cutting the DNA with a nuclease. In type II interference, the tracrRNA: crRNA duplex directs Cas9 to the target for a double-stranded DNA cut. Type III systems involve identifying the The Cas10-Csm (type III-A and type III-D), Cas10-Cmr (type III-B and III-C), and Cas7 (Csm3 and Cmr4) proteins cause a cleavage in the target RNA and ssDNA using RNA-activated DNases. The Csm/Csr complex binds to the complementary site in the genome with precision and accuracy, demonstrating the remarkable capabilities of these proteins[17].

18.3 CURRENT USE OF CRISPR-CAS 9 TECHNOLOGY

DNA restriction enzymes were discovered in the 1970s during bacteriophage research. They cut DNA by recognising specific sequences, revolutionising molecular biology. CRISPR systems also cut specific DNA sequences but can adapt to a wider range than restriction enzymes. DNA and RNA molecules can be programmed for specific targets. Although complex, they offer more flexibility than specialized DNA sequences that rely on restriction enzymes to target specific sequences[19]. CRISPR loci were first discovered for typing bacterial isolates, such as Mycobacterium and Yersinia[20]. Later, these loci became widely used for genotype analysis of industrially

important bacteria and determining the common origin of bacteria with the same CRISPR loci. The CRISPR adaptive immunity system was discovered thereafter. The CRISPR system was used in Streptococcus thermophilus bacteria in the dairy industry to produce more efficient, long-lived, and phage-resistant mutants[21]. It not only contributes to viral resistance but also has the potential to protect bacterial species against the insertion and replication of unwanted genetic material, such as plasmids carrying antibiotic resistance genes or disease-causing capabilities. This feature allows for the control and direction of the spread of genetic material that carries undesirable characteristics of microorganisms[22]. Emmanuelle Charpentier and Jennifer Doudna led a research team that developed a genome editing concept using the CRISPR Type II system of the bacterium Streptococcus pyogenes. They combined crRNA (CRISPR RNA) and tracrRNA (Transactivating CRISPR RNA) in this study.

The process involved combining molecules to create a single RNA molecule known as sgRNA (Single guide RNA). This was then combined with the Cas9 nuclease enzyme to form a complex. The sgRNA-Cas9 complex was then targeted to specific genomic regions based on standard Watson-Crick base pairing rules and used to perform the cutting process. This study was a breakthrough in genomic manipulation as it allowed for precise recognition and editing of specific genetic regions[23]. The CRISPR-Cas9 system is now the most popular DNA editing tool due to its effective design for targeting specific regions. It has been widely used for performing gene editing studies have been conducted on various organisms, including mammals. This has made it an important tool for understanding genetic structures and making precise genetic changes.

Wu and his team are one of the pioneering researchers using the CRISPR system to treat cataract, a genetic disease, in mouse embryos. In their work, they specifically targeted cataract disease caused by mutations in the Crygc gene. This genetic disease is based on mutations that cause a loss of function that leads to vision problems. In their research, they injected mouse embryos with Cas9 mRNA, which enables the production of Cas9 nuclease, and an sgRNA that corrects the Crygc allele with the targeted mutation. In this way, they ensured that the wild-type (normal) allele was used instead of the allele with the targeted mutation. This study demonstrated the successful use of CRISPR technology at the embryonic stage in mice for the potential treatment of genetic diseases [24]. A study by Yin and colleagues used the in vivo CRISPR-Cas9 genome editing system to successfully treat Tyrosinaemia Type 1 disease in mice in the postnatal period. Inherited Tyrosinaemia Type 1 is a genetic disease which mechanism of this disease is deficiency of the enzyme FAH (fumarylacetoacetate hydrolase), which causes to the accumulation of cytotoxic metabolites and death of liver cells. In the study, a vector containing Cas9 nuclease and a specific sgRNA was used. This vector was delivered together with a mould DNA molecule via hydrodynamic tail vein injection into the liver of mice. This delivery of the vector led to correction of the genetic defect in the mutant fumarylacetoacetate hydrolase allele and stabilisation of the protein. As a result, toxicity in liver cells was reduced, and weight loss of the mice was noticeably reduced. This study demonstrated that CRISPR-Cas9 technology is a promising approach in the treatment of genetic diseases [25]. Cas9 has the potential to treat viral infections. It has been shown to effectively target and inactivate the genetic material of viruses such as HIV and Hepatitis B, reducing their infection capacity and controlling disease spread.

This is a significant development that may pave the way for new and effective strategies for treating viral diseases have been developed[26, 27, 28].

An ex vivo study used gene editing based on the CRISPR-Cas9 system to combat human immunodeficiency virus (HIV) infection. Through the cycle of virus, HIV-1 integrates into the genome of target cells, resulting in viral infection. During this step, HIV infection is transcriptionally silent, causes a latent infection. Latent viruses are often found in long-lived cells and can persist even with strong antiretroviral drugs. Researchers are currently testing two approaches using genome editing technologies to combat latent HIV infection [29]. The initial approach aims to eliminate HIV DNA by targeting the viral genome sequence with nucleases. The Liao team conducted a study on targeting the highly conserved repeat regions in HIV-1 in infected CD4+ T cells using the CRISPR-Cas9 system. This approach aims to disrupt the virus genome by expressing the CRISPR-Cas9 vector targeted to the repeat regions in the virus genome of the relevant cell line. The production decreased and the latently replicating viruses were eliminated. Furthermore, researchers have demonstrated that HIV reservoir cells, obtained from pluripotent stem cells in which CRISPR-Cas9 vectors targeted HIV-1 repeats, are also resistant to new HIV infections [26]. The second approach used gene editing to modify the chemokine receptor 5 (CCR5) gene, which is a coreceptor required for HIV-1 T-cell infection. Mandal and colleagues used CRISPR/Cas9 system to impair the function of receptor in CD34+ haematopoietic stem cells and progenitor cells (HSPC). The authors used CRISPR/Cas9 vectors to target CCR5 and demonstrated a 30% efficiency in knocking it down in HSPCs. They also noted that the CCR5 knockdown HSPC clones maintained full multi-cell line

potential after xenotransplantation in mice and had only a small number of side target mutations [27]. Lin and colleagues demonstrated that the CRISPR-Cas9 gene editing method can be used to treat hepatitis B virus (HBV) infection, which can cause liver cirrhosis and cancer in many patients. While antiviral therapies have been developed for HBV, they are not always effective. In patients with chronic HBV, these therapies often do not achieve to eliminate the HBVfrom the liver due to the stability of the covalently closed circular DNA (cccDNA) responsible for viral replication. A 2014 study modelled HBV infection in mouse liver by introducing an HBV expression vector via the tail. The CRISPR-Cas9 system was injected into mice with modelled HBV infection to target the HBV sequence. This achieved disruption of cccDNA and a subsequent decrease hepatitis B surface antigen in serum [28].

18.4 CONCLUSION

The discovery of molecular components in the CRISPR system made history as the first time two women have been awarded the Nobel Prize in Chemistry. Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry in 2020. CRISPR was first discovered in the DNA sequences of E. Coli bacteria by Ishino et al. from Osaka University, Japan in 1987. Although the significance of CRISPR in the bacterial cell was unknown at the time of its discovery, Charpentier and Doudna's pioneering work that unlocked its full potential.

Although CRISPR-Cas systems are primarily known for their interference with foreign genetic material, they also play a role in other cellular processes, such as virulence regulation, genome evolution, and DNA repair. However, the mechanisms behind these alternative functions are still poorly understood and require further research.

In the coming period, research and applications in the field of gene editing will continue to grow. This process will intersect with technology areas such as machine learning, live cell observation, and sequencing. The combination of biogenetics and engineering disciplines will further enrich and enhance CRISPR tools, providing solutions to current challenges and enabling a wider range of applications. CRISPR technology has a wide range of applications, from basic science to applied research. The future innovation of CRISPR technology will be shaped by scientific curiosity and the desire to benefit society, as was the case in the beginning of CRISPR gene editing technology.

18.5 REFERENCES

1. Gostimskaya I. CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry. Biokhimiia, 2022;87(8), 777–788. https://doi.org/10.1134/S0006297922080090
2. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. Journal of bacteriology, 1987;169(12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987
3. Groenen, P. M., Bunschoten, A. E., van Soolingen, D., & van Embden, J. D. Nature of DNA polymorphism in the direct repeat cluster of Mycobacterium tuberculosis; application for strain differentiation by a novel typing method. Molecular microbiology, 1993;10(5), 1057–1065. https://doi.org/10.1111/j.1365-2958.1993.tb00976.x
4. Hoe, N., Nakashima, K., Grigsby, D., Pan, X., Dou, S. J., et al. Rapid molecular genetic subtyping of serotype M1 group A Streptococcus strains. Emerging infectious diseases, 1999;5(2), 254–263. https://doi.org/10.3201/eid0502.990210
5. Mojica, F. J., Juez, G., & Rodríguez-Valera, F. Transcription at different salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites. Molecular microbiology, 1993;9(3),

613–621. https://doi.org/10.1111/j.1365-2958.1993.tb01721.x

6. Mojica, F. J., Díez-Villaseñor, C., García-Martínez, J., & Soria, E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. Journal of molecular evolution, 2005;60(2), 174–182. https://doi.org/10.1007/s00239-004-0046-3
7. Bolotin, A., Quinquis, B., Sorokin, A., & Ehrlich, S. D. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology (Reading, England), 2005;151(Pt 8), 2551–2561. https://doi.org/10.1099/mic.0.28048-0
8. Pourcel, C., Salvignol, G., & Vergnaud, G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology (Reading, England), 2005;151(Pt 3), 653–663. https://doi.org/10.1099/mic.0.27437-0
9. Marraffini, L. A., & Sontheimer, E. J. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science (New York, N.Y.), 2008;322(5909), 1843–1845. https://doi.org/10.1126/science.1165771
10. Shmakov, S., Abudayyeh, O. O., Makarova, K. S., Wolf, Y. I., Gootenberg, J. S., et al. Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. Molecular cell, 2015;60(3), 385–397. https://doi.org/10.1016/j.molcel.2015.10.008
11. Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., et al. Diversity and evolution of class 2 CRISPR-Cas systems. Nature reviews. Microbiology, 2017;15(3), 169–182. https://doi.org/10.1038/nrmicro.2016.184
12. Abudayyeh, O. O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M.,et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science (New York, N.Y.), 2016;353(6299), aaf5573. https://doi.org/10.1126/science.aaf5573
13. Kumar, Pranav. (2021). Biophysics and Molecular Biology 4th edition
14. Jansen, R., Embden, J. D., Gaastra, W., & Schouls, L. M. Identification of genes that are associated with DNA repeats in prokar-

yotes. Molecular microbiology, 2002;43(6), 1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x

15. Marraffini, L. A., & Sontheimer, E. J. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nature reviews. Genetics, 2010;11(3), 181–190. https://doi.org/10.1038/nrg2749
16. Amitai, G., & Sorek, R. CRISPR-Cas adaptation: insights into the mechanism of action. Nature reviews. Microbiology, 2016;14(2), 67–76. https://doi.org/10.1038/nrmicro.2015.14
17. Bhatia, S., Pooja, & Yadav, S. K. CRISPR-Cas for genome editing: Classification, mechanism, designing and applications. International journal of biological macromolecules, 2023;238, 124054. https://doi.org/10.1016/j.ijbiomac.2023.124054
18. Hille, F., & Charpentier, E. CRISPR-Cas: biology, mechanisms and relevance. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 2016;371(1707), 20150496. https://doi.org/10.1098/rstb.2015.0496
19. Kelly, T.J., Smith, H.O., "A restriction enzyme from Hemophilus influenzae", II. Journal of Molecular Biology 51, 393-409, 1970.
20. Pourcel, C., Salvignol, G., & Vergnaud, G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology (Reading, England), 2005;151(Pt 3), 653–663. https://doi.org/10.1099/mic.0.27437-0
21. Barrangou, R., & Horvath, P. CRISPR: new horizons in phage resistance and strain identification. Annual review of food science and technology, 2012;3, 143–162. https://doi.org/10.1146/annurev-food-022811-101134
22. Barrangou R, Marraffini LA. CRISPR-Cas systems: Prokaryotes upgrade to adaptive immunity. Mol Cell. 2014 Apr 24;54(2):234-44. https://doi.org/10.1016/j.molcel.2014.03.011
23. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.), 2012;337(6096), 816–821. https://doi.org/10.1126/science.1225829
24. Wu, Y., Liang, D., Wang, Y., Bai, M., Tang, W., et al. Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell stem cell, 2013;13(6), 659–662. https://doi.org/10.1016/j.stem.2013.10.016

25. Yin, H., Xue, W., Chen, S., Bogorad, R. L., Benedetti, E., et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nature biotechnology, 2014;32(6), 551–553. https://doi.org/10.1038/nbt.2884
26. Liao, H. K., Gu, Y., Diaz, A., Marlett, J., Takahashi, Y.,et al. Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nature communications, 2015;6, 6413. https://doi.org/10.1038/ncomms7413
27. Mandal, P. K., Ferreira, L. M., Collins, R., Meissner, T. B., Boutwell, C. L., et al. Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. Cell stem cell, 2014;15(5), 643–652. https://doi.org/10.1016/j.stem.2014.10.004
28. Lin, S. R., Yang, H. C., Kuo, Y. T., Liu, C. J., Yang, T. Y., et al. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. Molecular therapy. Nucleic acids, 2014;3(8), e186. https://doi.org/10.1038/mtna.2014.38
29. Rath, D., Amlinger, L., Rath, A., & Lundgren, M. The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie, 2015;117, 119–128. https://doi.org/10.1016/j.biochi.2015.03.025
30. Gün Gök, Z. & Çağdaş Tunalı, B. Biology, Mechanism and Applications of CRISPR-Cas Immune System. International Journal of Engineering Research and Development, 2016;8 (2), 11-21. https://doi.org/10.29137/umagd.346148
31. Wang, J. Y., & Doudna, J. A. CRISPR technology: A decade of genome editing is only the beginning. Science (New York, N.Y.), 2023;379(6629), eadd8643. https://doi.org/10.1126/science.add8643

BRIEF INTRADUCTION ON ADSCS, WJMSCS, SSCS AND STEMNESS PROPERTIES, STEM CELL SENESCENCE AND REGENERATIVE POTENTIAL

Margherita Maioli[1,2], Giuseppe Garroni[1], Renzo Pala[1]

[1]Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy

[2]Center for Developmental Biology and Reprogramming-CEDEBIOR, Department of Biomedical Sciences, University of Sassari,Viale San Pietro 43/B, 07100 Sassari, Italy

Stem cells are unspecialized cells capable of unlimited division giving rise simultaneously to a stem cell (a characteristic known as self-renewal) and a daughter cell or transit progenitor cell, with limited proliferative capability, committed to highly specialized cell populations losing their stemness property [1,2] Stem cells are classified according to their differentiation potential and origin [3,4]. There are different stages of specialization: totipotent, pluripotent, multipotent, oligopotent and unipotent, each being associated to a decreasing capability of differentiation, which means that a unipotent stem cell has a reduced differentiation capacity as compared to a pluripotent one[5]. Totipotent stem cells are capable of differentiating into any cell of the body. Totipotency has the highest potential for differentiation and allows cells to form both embryonic and extra-embryonic structures. An example of a totipotent cell is the zygote. This cell can later develop into one of the three germ layers or form the placenta.

Approximately 4 days after fertilization, the inner cell mass of the blastocyst becomes pluripotent. This structure is the source of pluripotent cells [5]. Pluripotent stem cells are the progenitors of cells belonging to all germ layers but not extraembryonic structures, as the placenta. Multipotent stem cells have a lower capability of differentiation than pluripotent stem cells and can only differentiate into specific cell lineages. An example are hematopoietic stem cells, which can develop into different types of blood cells. After differentiation, a hematopoietic stem cell becomes an oligopotent cell. Its differentiation capabilities are therefore limited to cells of the same lineage. Unipotent stem cells are characterized by a reduced capability of differentiation, being able to form only one cell type, also dividing repeatedly. This feature makes them a promising candidate for therapeutic use in regenerative medicine (5). Based on their origin, cells are subdivided into embryonic stem cells and adult stem cells. The last are able to differentiate into the specific cell population of the tissue in which they reside responsible for cell turnover to balance the loss of cells that occurs physiologically or in case of damage during the normal life of an organism [4,6,7]. Adult stem cells or Adult mesenchymal stem cells (MSCs) are multipotent stem cells that are able to differentiate into all the cell types that make up the tissue from which they are derived; they are usually in a dormant state and, if necessary, are recruited and begin to divide and differentiate [7,8,9,10]. MSCs can be obtained from different tissues as dental pulp, placenta, adipose tissue, skin and Wharton's jelly and can be used in regenerative medicine[11,12,13,14,15].

ADSCs (adipose-derived stem cells) resident in the adipose tissue, can be isolated by different methods. Stem cells isolated from adipose tissue represents an excellent source

of mesenchymal stem cells. The main advantage of ADSCs, as compared to other sources, is that they can be harvested during surgical procedures with noninvasive techniques [16,11,13]. These cells, found in the stromal vascular fraction (SVF) of adipose tissue, play a central role during adipogenesis as they can differentiate to generate mature adipocytes [17]. ADSCs exhibit several superficial markers typical of mesenchymal stem cells as CD9, CD10, CD13, CD29, CD44, CD49d, CD49e, CD54, CD55, CD73, CD90, CD105, CD106, CD146, CD166, and STRO-1 ((stromal precursor antigen-1) and are negative for the hematopoietic cell lineage markers CD14, CD19 (B4), CD34, CD45, CD16, CD56, CD61, CD62E, CD104 and CD106 and are also negative for the endothelial cell (EC) markers CD31 and CD144 [18]. Another source of mesenchymal stem cells is the skin. Skin stem cells on the basal layer, are mesenchymal stem cells (MSCs) responsible for keratinocyte differentiation and enrolled to restore tissue homeostasis after injury [15,19]. Stem cells in the skin play an important role in enabling tissue homeostasis, as they replacing elements that are continuously lost during the course of tissue turnover or after damage [20]. There are different groups of skin stem cells responsible for the maintenance and repair of the different part of the skin, including the interfollicular epidermis, hair follicles, and sebaceous glands [19]. Skin stem cells, as well as those identified in other tissues, represent an important resource for regenerative medicine studies, in fact they show a mesenchymal phenotype and are able to differentiate toward a specific lineage, as osteogenic and adipogenic, under stimulation. The cells show positivity for all mesenchymal surface markers (CD73, CD90, CD105) and are negative for CD31 and CD45 [15]. Another important source of mesenchymal stem cells is Wharton's jelly, a gelatinous tissue, primary connective tissue, found in the umbilical cord, containing mesenchymal

stromal cells (MSCs), first described in 1656 by Thomas Wharton [21,22].

Stem cells derived from WJ-MSCs are an excellent source of multipotent stem cells, easily obtained from waste materials. Stem cells derived from WJ-MSCs are an excellent source of multipotent stem cells, easily obtained from waste materials. These cells show a young phenotype and great plasticity. They are able to differentiate into osteogenic and adipogenic lineages when exposed to specific stimuli. Moreover, the WJ cell population expresses MSCs, specific features, showing plastic adhesion and expression of CD90, CD73 and CD105 [23,24,25,14]. The pluripotency capability of stem cells can be assessed by gene expression evaluation of the stemness markers SOX2, OCT4 and Nanog. These factors are highly expressed in embryonic stem cells and are known for their ability to influence the regulation of cell fate. Aberration in the expression of Sox2, Oct4 and Nanog can affect cell proliferation and proper differentiation, which can lead to morphological abnormalities [26]. p53 being a protein with a suppressive function regulates the expression of Sox2, Oct4 and Nanog and helps in maintaining stem cells in an undifferentiated state. Long-term in vitro culture along with the influence of the tissue-specific environment may influence the expression of these genes. Observations on the dynamics of these changes may help determine the best strategy in MSC production for potential use in cell therapy [27].

Senescence is a cellular response characterized by a stable cell cycle arrest that limits the proliferative potential of cells. To date, 4 types of senescence have been distinguished: replicative senescence, oncogene-induced senescence, stress-induced premature senescence, and developmental senescence [28]. An important point of regenerative medicine is the loss

of the regenerative potential of stem cells during aging, which is related to cellular senescence and ROS production [29]. The molecular control of stem cell fate and senescence is regulated mainly by two different pathways, telomerase-independent and telomerase-dependent. In the telomerase-independent senescence pathway, epigenetic events, as histone modification, have been implicated. It follows that genes affecting chromatin remodeling and regulation of gene expression may be directly involved in decisions influencing stem cell fate, including self-renewal and senescence [30]. Telomerase is a specialized ribonucleoprotein composed of telomerase reverse transcriptase (TERT), an intrinsic template RNA (TR) and several associated proteins. Its main function is to stabilize telomeres, which shorten along with each cycle of cell division, thereby protecting chromosomes from end-to-end recombination and fusion. Telomerase is expressed in highly proliferating cells throughout the developing embryo, whereas it is reduced with cell differentiation [31].

Studies on tumor cells and engineered somatic cells overexpressing telomerase have shown that this enzyme can confer an immortal phenotype. Therefore, the development of a tool able to reverse the molecular mechanisms underlying stem cell senescence in vitro could pave the way for accessible stem cell expansion strategies, improving the outcomes of cell therapy efforts [32]. Within this context, it was recently shown that Bmi1, a member of the Polycomb and Trithorax family repressor group, is an essential factor for self-renewal of adult murine hematopoietic stem cells and neuronal stem cells, acting as a repressor of senescence. The pluripotency transcription factors Nanog, Oct4, Sox2 and cMyc, along with polycomb repressive complexes, have also been identified to regulate pluripotency and differentiation of stem cells [33]. The

regenerative capability of MSCs is not only related to the source of the tissue (adipose tissue, bone marrow, placental fetal membranes, amniotic fluid), but also to the age of the tissue donor, as stem cells also undergo cellular senescence[34].

REFERENCES

1. Zakrzewski W., Dobrzyński M., Szymonowicz M., Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Feb 26;10(1):68. https://doi.org/10.1186/s13287-019-1165-5

2. Biehl JK., Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs. 2009 Mar-Apr;24(2):98-103; quiz 104-5. https://doi.org/10.1097/jcn.0b013e318197a6a5

3. Zakrzewski W., Dobrzyński M., Szymonowicz M., Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Feb 26;10(1):68. https://doi.org/10.1186/s13287-019-1165-5

4. Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. Respiration. 2013;85(1):3-10. https://doi.org/10.1159/000345615

5. Sobhani A., Khanlarkhani N., Baazm M., Mohammadzadeh F., Najafi A. et al. Multipotent Stem Cell and Current Application. - Acta Med Iran 2017; 55(1):6-23;

6. Santoro A., Vlachou T., Carminati M., Pelicci PG., Mapelli M. Molecular mechanisms of asymmetric divisions in mammary stem cells. EMBO Rep. 2016;17(12):1700-1720. https://doi.org/10.15252/embr.201643021

7. Caplan AI. Adult Mesenchymal Stem Cells: When, Where, and How. Stem Cells Int. 2015; 2015:628767. https://doi.org/10.1155/2015/628767

8. Ullah I., Subbarao RB., Rho GJ. Human mesenchymal stem cells - current trends and future prospective. Biosci Rep. 2015 Apr 28;35(2):e00191. https://doi.org/10.1042/bsr20150025 .

9. Porada CD., Zanjani ED., Almeida-Porad G. Adult mesenchymal stem cells: a pluripotent population with multiple applications. Curr Stem Cell Res Ther. 2006 Sep;1(3):365-9. https://doi.org/10.2174/157488806778226821

10. Poliwoda S., Noor N., Downs E., Schaaf A., Cantwell A. et al. Stem cells: a comprehensive review of origins and emerging clinical roles in medical practice. Orthop Rev (Pavia). 2022 Aug 25;14(3):37498. https://doi.org/10.52965/001c.37498
11. Bianchi F., Maioli M., Leonardi E., Olivi E., Pasquinelli G. et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant. 2013;22(11):2063-77. https://doi.org/10.3727/096368912x657855 .
12. Maioli M., Basoli V., Santaniello S., Cruciani S., Delitala AP. et al. Osteogenesis from Dental Pulp Derived Stem Cells: A Novel Conditioned Medium Including Melatonin within a Mixture of Hyaluronic, Butyric, and Retinoic Acids. Stem Cells Int. 2016; 2016:2056416. https://doi.org/10.1155%2F2016%2F2056416
13. Maioli M., Rinaldi S., Santaniello S., Castagna A., Pigliaru G. et al. Radioelectric asymmetric conveyed fields and human adipose-derived stem cells obtained with a nonenzymatic method and device: a novel approach to multipotency. Cell Transplant. 2014;23(12):1489-500. https://doi.org/10.3727/096368913x672037
14. Balzano F., Bellu E., Basoli V., Dei Giudici S., Santaniello S. et al. Lessons from human umbilical cord: gender differences in stem cells from Wharton's jelly. Eur J Obstet Gynecol Reprod Biol. 2019 Mar; 234:143-148. https://doi.org/10.1016/j.ejogrb.2018.12.028
15. Bellu E., Garroni G., Balzano F., Satta R., Montesu MA. et al. Isolating stem cells from skin: designing a novel highly efficient non-enzymatic approach. Physiol Res. 2019 Dec 30;68(Suppl 4):S385-S388. http://doi.org/10.33549/physiolres.934373
16. Frese L., Dijkman PE., Hoerstrup SP. Adipose Tissue-Derived Stem Cells in Regenerative Medicine. Transfus Med Hemother. 2016 Jul;43(4):268-274. https://doi.org/10.1159/000448180
17. Balwierz A., Czech U., Polus A., Filipkowski RK., Mioduszewska B. et al. Human adipose tissue stromal vascular fraction cells differentiate depending on distinct types of media. Cell Prolif. 2008 Jun;41(3):441-59. https://doi.org/10.1111/j.1365-2184.2008.00531.x
18. Huang SJ., Fu RH., Shyu WC., Liu SP., Jong GP. et alAdipose-derived stem cells: isolation, characterization, and differentiation potential. Cell Transplant. 2013;22(4):701-9. https://doi.org/10.3727/096368912x655127

19. Díaz-García D., Filipová A., Garza-Veloz I., Martinez-Fierro ML. A Beginner's Introduction to Skin Stem Cells and Wound Healing. Int J Mol Sci. 2021 Oct 13;22(20):11030. https://doi.org/10.3390%2Fijms222011030
20. Blanpain C., Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol. 2009 Mar;10(3):207-17. https://doi.org/10.1038/nrm2636
21. Kim D.-W., Staples M., Shinozuka K., Pantcheva P., Kang S.-D., et al. Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications. Int. J. Mol. Sci. 2013; 14:11692. https://doi.org/10.3390/ijms140611692
22. Stefańska K., Ożegowska K., Hutchings G., Popis M., Moncrieff L, et al. Human Wharton's Jelly-Cellular Specificity, Stemness Potency, Animal Models, and Current Application in Human Clinical Trials. J Clin Med. 2020 Apr 12;9(4):1102. https://doi.org/10.3390/jcm9041102
23. Pala R., Cruciani S., Manca A., Garroni G., El Faqir MA. et al. Mesenchymal Stem Cell Behavior under Microgravity: From Stress Response to a Premature Senescence. Int J Mol Sci. 2023 Apr 24;24(9):7753. https://doi.org/10.3390/ijms24097753
24. Ansari AS., Yazid MD., Sainik NQAV., Razali RA., Saim AB. et al. Osteogenic Induction of Wharton's Jelly-Derived Mesenchymal Stem Cell for Bone Regeneration: A Systematic Review. Stem Cells Int. 2018 Nov 11;2018:2406462. https://doi.org/10.1155%2F2018%2F2406462
25. Chen MY., Lie PC., Li ZL., Wei X. Endothelial differentiation of Wharton's jelly-derived mesenchymal stem cells in comparison with bone marrow-derived mesenchymal stem cells. Exp Hematol. 2009 May;37(5):629-40. https://doi.org/10.1016/j.exphem.2009.02.003
26. Kozlowska U., Krawczenko A., Futoma K., Jurek T., Rorat M. et al. Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. World J Stem Cells. 2019 Jun 26;11(6):347-374. https://doi.org/10.4252%2Fwjsc.v11.i6.347

27. Maioli M., Contini G., Santaniello S., Bandiera P., Pigliaru G. et al. Amniotic fluid stem cells morph into a cardiovascular lineage: analysis of a chemically induced cardiac and vascular commitment. Drug Des Devel Ther. 2013 Sep 27;7:1063-73. https://doi.org/10.2147/DDDT.S44706

28. Zhou X, Hong Y, Zhang H, Li X. Mesenchymal Stem Cell Senescence and Rejuvenation: Current Status and Challenges. Front Cell Dev Biol. 2020 Jun 3; 8:364. https://doi.org/10.3389%2Ffcell.2020.00364

29. Rinaldi S., Maioli M., Pigliaru G., Castagna A., Santaniello S. Stem cell senescence. Effects of REAC technology on telomerase-independent and telomerase-dependent pathways. Sci Rep. 2014 Sep 16;4:6373. https://doi.org/10.1038/srep06373

30. Roy NS., Chandler-Militello D., Lu G, Wang S., Goldman SA. Retrovirally mediated telomerase immortalization of neural progenitor cells. Nat Protoc. 2007;2(11):2815-25. https://doi.org/10.1038/nprot.2007.402

31. Zimmermann S., Martens UM. Telomeres, senescence, and hematopoietic stem cells. Cell Tissue Res. 2008 Jan;331(1):79-90. https://doi.org/10.1007/s00441-007-0469-4

32. Isenmann S., Cakouros D,. Zannettino A., Shi S., Gronthos S. hTERT transcription is repressed by Cbfa1 in human mesenchymal stem cell populations. J Bone Miner Res. 2007 Jun;22(6):897-906. https://doi.org/10.1359/jbmr.070308

33. Rinaldi S., Maioli M., Pigliaru G., Castagna A., Santaniello S. et al. Stem cell senescence. Effects of REAC technology on telomerase-independent and telomerase-dependent pathways. Sci Rep. 2014 Sep 16; 4:6373. https://doi.org/10.1038/srep06373

34. Wagner W., Bork S., Horn P., Krunic D., Walenda T. et al. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One. 2009 Jun 9;4(6):e5846. https://doi.org/10.1371/journal.pone.0005846

METHODS FOR THEIR ISOLATION, CHARACTERIZATION AND DIFFERENTIATION TOWARD DIFFERENT PHENOTYPES

Margherita Maioli[1,2], Giuseppe Garroni[1], Renzo Pala[1]

[1]Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy

[2]Center for Developmental Biology and Reprogramming-CEDEBIOR, Department of Biomedical Sciences, University of Sassari,Viale San Pietro 43/B, 07100 Sassari, Italy

Stem cells can be isolated from different tissues, covering different isolation method procedures. The procedures of isolating adipose tissue components converge in the standard methods, based on enzymatic digestion. These methods are designed to separate the two easily recognized fractions, mature adipocytes and the stromal vascular fraction (SVF). However, it is well known that SVF comprises preadipocytes, endothelial cells, pericytes, fibroblasts, adipose tissue-derived stem cells (ADSCs), and hematopoietic stem cells. After collagenase digestion, mature high-fat adipocytes are separated as a floating layer. All cells remaining after removal of mature adipocytes constitute the SVF. The adipose tissue is washed repeatedly with sterile phosphate-buffered saline (DPBS) containing 200 U/mL penicillin and 0.1 mg/mL streptomycin to remove blood cells. The tissue is dissociated into small fragments with a scalpel, followed by enzymatic digestion

with 0.1% type I collagenase for 60 minutes at 37°C in Hanks' balanced salt solution under continuous gentle agitation. Enzyme is neutralized with 10% fetal bovine serum (FBS) and filtered (70 μm cell filter), the samples are then centrifuged at 600× g for 10 min to separate distinct cellular fractions. The supernatant obtained was composed of mature adipocytes and the pellet fraction was made up of the SVF components, in which ADSCs were presumably present. Adipocytes were transferred to basal medium (BM), Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 200 mm l -glutamine, and 200 U/mL penicillin—0.1 mg/mL streptomycin. Cells are resuspended in basic medium (BM), plated in 12 cm2 culture flasks and transferred to a 37°C incubator with 5% CO2 and saturated humidity [1].

Skin biopsies are usually used for isolation. The biopsies are reduced into small fragments of approx 2 mm x 4 mm in a Petri dish with the help of a sterile scalpel. The tissue fragments were placed on the bottom of a 35-mm culture plate, three to five pieces per well with specific culture medium (SC-medium): DMEM supplemented with 15 percent fetal bovine serum (FBS), 0.1 mM β-mercaptoethanol, 1 percent L-glutamine, 1 percent nonessential amino acids, 200 U/m3 folic acid, 200 U/ml penicillin and 0.1 mg/ml streptomycin. The cultures are transferred to 37 °C at 5 % CO^2 [2].

During isolation, a mixed population of cells is obtained, so a positive selection of stem cells is made using a primary monoclonal antibody (usually for mesenchymal stem/stromal cells anti cKit), then the cell suspensions are magnetically labeled in columns with a secondary antibody directly conjugated to MicroBeads [1]. Flow cytometry analysis is used for characterization, which is capable of evaluating mesenchymal markers. The sample is fixed with 1% formaldehyde for 10 min

at room temperature, the cells are permeabilized using permeabilization buffer for 30 min at 4 °C. After a washing step, cells are incubated with primary antibodies directed against CD 34, CD114, CD73, CD90, CD105, CD45 and CD31 (all at 1 μg/106 cells) for 1 h at 4 °C and with 1 μg of fluorescein isothiocyanate-conjugated secondary antibody (FITC) for 1 h at 4 °C in the dark. After washing, cells are analyzed on a flow cytometer by collecting 10,000 [1].

Stem cell differentiation is controlled by the activation of a specific transcriptional program, involving several transcriptional and epigenetic factors, including miRNAs. For example, for adipogenic differentiation, there are several adipocyte-specific genes that are activated during adipogenesis [6]. Fatty acid binding protein (FABP), also known as aP2, is a lipid-binding protein that acts as an adipokine in regulating systemic metabolism. Lipoprotein lipase (LPL) is the main enzyme involved in the uptake of fatty acids from lipoproteins and de novo lipogenesis. Acyl-CoA thioesterase 2 (ACOT2) also plays a role as an auxiliary enzyme supporting efficient fat burning through a thermogenic mechanism. For example, white adipose tissue (WAT) stores energy as fat storage and produces adipokines and prostaglandins while brown adipose tissue (BAT) dissipates heat through fat metabolism and high mitochondrial activity. Brown, as well as the similar kind of adipose tissue related to adulthood, the so called beige, show strong mitochondrial activity. In particular, beige adipose tissue result from de novo differentiation of adipocytes by stem and progenitor cells or transdifferentiation of white adipocytes following a process called "browning." [4].

Several signaling pathways and epigenetic factors contribute to the regulation of MSC differentiation into specific phenotypes. It is widely demonstrated that many bioactive mo-

lecules regulate adipogenic differentiation and the expression of key transcription factors, as for example vitamin D, which is well known for its ability to counteract adipogenesis and induce osteogenesis [5]. Within this context, the combination of vitamin D and metformin has also been shown to counteract adipogenic differentiation of ADSCs by modulating vitamin D metabolism and the expression of specific epigenetic factors[6].

REFERENCES

1. Basoli V., Santaniello S., Cruciani S., Ginesu GC., Cossu ML. et al. Melatonin and Vitamin D Interfere with the Adipogenic Fate of Adipose-Derived Stem Cells. Int J Mol Sci. 2017 May 5;18(5):981. https://doi.org/10.3390%2Fijms18050981
2. Bellu E., Garroni G., Balzano F., Satta R., Montesu MA. et al. Isolating stem cells from skin: designing a novel highly efficient non-enzymatic approach. 2019 Dec 30;68:S385-S388. http://dx.doi.org/10.33549/physiolres.934373
3. Cruciani S., Garroni G., Pala R., Barcessat ARP., Facchin F. et al. Melatonin finely tunes proliferation and senescence in hematopoietic stem cells. Eur J Cell Biol. 2022 Jun-Aug;101(3):151251. https://doi.org/10.1016/j.ejcb.2022.151251
4. Cruciani S., Garroni G., Pala R., Coradduzza D., Cossu ML. et al. Metformin and vitamin D modulate adipose-derived stem cell differentiation towards the beige phenotype. Adipocyte. 2022 Dec;11(1):356-365. https://doi.org/10.1080/21623945.2022.2085417
5. Makki K., Froguel P., Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. 2013 Dec 22; 2013:139239. https://doi.org/10.1155/2013/139239
6. Cruciani S., Garroni G., Pala R., Cossu ML., Ginesu GC. et al. Metformin and Vitamin D Modulate Inflammation and Autophagy during Adipose-Derived Stem Cell Differentiation. 2021 Jun 22;22(13):6686. https://doi.org/10.3390%2Fijms22136686