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Review

# The Assessment of microRNA Role in Kidney Transplantation

Aslı Özkızılcık Koçyiğit[1,2], Mustafa Soyöz[1,2], Tülay Kılıçaslan Ayna[1,2]

[1]Department of Medical Biology and Genetics, İzmir Katip Çelebi University School of Health Sciences, İzmir, Turkey

[2]Tissue Typing Laboratory, İzmir Tepecik Training and Research Hospital, İzmir, Turkey

**ABSTRACT**

miRNAs are short, single stranded, noncoding RNA molecules with approximately 22 nucleotides. They play crucial roles in post-transcriptional regulation. To date, more than 21,000 mature miRNAs have been discovered from 193 species. miRNAs are important in renal physiology. Their expressions may be specific to different organs and tissues, or the same miRNAs may be expressed in different organs. With this discovery, it has been reported that tissue-specific miRNAs can be used as biomarkers for allograft rejection and ischemia reperfusion injury following renal transplantations. Although allograft biopsy is accepted as a gold standard, scientists have been developing novel approaches to prevent the associated risks, such as bleeding and infection, following biopsy. Recently, several attempts have been made in terms of the use of urine as a noninvasive material instead of biopsy samples. However, no procedure is available for routine testing. Therefore, further investigations are warranted.

**Keywords:** Kidney, rejection, graft, microRNAs

## INTRODUCTION

MicroRNA (miRNA) is a short (~22 nucleotides), noncoding, and single-stranded RNA molecule that plays a significant role during posttranscriptional regulation in various physiological processes ranging from development to oncogenesis. It was first discovered in 1993 from *Caenorhabditis elegans* as "miRNA lin-4." More than 21,000 mature miRNAs from a total of 193 species have been identified till date. miRNAs play critical roles in cell proliferation, apoptosis, lipid metabolism, neuronal process, hematopoietic differentiation, and immunity (1, 2). However, its role in the regulation of plant and animal gene expression was unknown until a decade ago. Previous studies found that this molecule acts as a repressor for a number of genes (2, 3).

The first miRNA database included only 218 miRNA loci. However, the number increased to >21,264 in plants and animals owing to the developments in miRNA expression profiling techniques (3). According to recent study, 2588 mature miRNAs belonging to Homo sapiens are found in the miRBase database (http://www.mirbase.org/cgi-bin/browse.pl?org=hsa, 26.12.2016).

miRNAs are synthesized from primary miRNAs (pri-miRNAs) in animals. This synthesis occurs in two steps, and two RNase III-type proteins, namely "Drosha" and "Dicer," play valuable roles in the nucleus and cytoplasm during this process, respectively. Pri-miRNA is transcribed from miRNA genes through RNA polymerase. This pri-miRNA is spliced to precursor miRNA (pre-miRNA) by Drosha in the nucleus. Thereafter, pre-miRNA is transported to the cytoplasm with the help of transport proteins. In the cytoplasm, it is spliced by Dicer protein, which is loaded on Argonaut (Ago) proteins (generating effector RNA-induced silencing complex (RISC)), and mature miRNA is generated (2).

In miRNA expression studies, there are some miRNAs that have been reported to be expressed, particularly, in adult kidneys (miR-215, miR-146a, and miR-886). Additionally, it has been shown that some of the other miRNAs, such as miR-192, miR-194, and miR-21 have been expressed in the kidneys and other organs. miRNAs play a number of crucial roles in the physiology of the kidneys. Unusual miRNA splicing in the ureteric bud leads to enormous cell proliferation and apoptosis. Nevertheless, ciliogenesis is destroyed on the ureteric bud epithelium, and, as a result, renal cysts are produced. In addition, miRNAs have critical roles in podocyte homeostasis. They have unique roles in a number of processes, including blood pressure control, body fluid, and electrolyte homeostasis (4).

In this review, the effects of miRNAs on immune response to allograft after kidney transplantation were evaluated.

**ORCID IDs of the authors:** A.Ö.K. 0000-0002-0015-7070; T.K.A. 0000-0001-7993-978X; M.S. 0000-0001-5159-6463.

**Corresponding Author:** Aslı Özkızılcık Koçyiğit **E-mail:** asli.ozkizilcik@gmail.com

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## CLINICAL AND RESEARCH CONSEQUENCES

**miRNA Synthesis in Humans**

All animal miRNAs are first processed in the nucleus followed by their transcription by RNA polymerase II. The transcript is called pri-miRNA. After the hairpin structure of pri-miRNA is cleaved, the released hairpin structure (60-70 nucleotides) is called pre-miRNA. A protein "Drosha" and its cofactor protein DGCR8 (DiGeorge syndrome critical region on the eighth gene) are involved in the process during this step. Pri-miRNAs contain 33 base-paired (bp) stem, loop, and single-stranded segments. DGCR8 protein interacts with these single-stranded segments and leads to Drosha to cut pri-miRNA (Figure 1) (2, 5).

Pre-miRNAs are transported to the cytoplasm for further processing. Transport occurs through nuclear pore complexes (large channels) with the help of RanGTP-dependent nuclear transport receptor exportin-5 (EXP5) protein. It was reported that transport begins when EXP5 recognizes a double-stranded RNA loop structure (14 bp) followed by binding of Ran protein (GTP-bound cofactor). Then, pre-miRNA-EXP5 complex is transported to the cytoplasm, and pre-miRNA is released by GTP hydrolysis (Figure 1) (2, 6).

In the cytoplasm, pre-miRNA is processed by Dicer protein (endonuclease cytoplasmic RNase III enzyme), and mature miRNA is produced. Dicer protein cuts miRNA (approximately 22 nucleotides), and this miRNA molecule combines with Ago protein complex. Generally, one of the strands of miRNA is destroyed, whereas the other strand with Ago protein complex becomes a mature miRNA, which is called RISC (Figure 1) (2, 3, 6).

After mature miRNAs are produced, they are ready to be transported to the regions where they function. For instance, if a miRNA plays a role in the nucleus (i.e., miR-29b), then it is transported back into the nucleus (6). On the other hand, if its specific function is in the cell of another distant tissue, it is carried through plasma-derived vesicles, such as exosome and microparticles in the bloodstream. Previous studies on miRNA transport showed that they are carried by exosomes. However, subsequent studies reported that microparticles also play roles in cell communication, which is based on miRNAs (7). In addition, various studies have revealed that high-density lipoprotein (HDL) and low-density lipoprotein (LDL) help miRNA transport in plasma (7, 8). It was indicated that some miRNAs are carried by exosome, HDL, and LDL, whereas others are transported by only one of them (7). miRNAs may be found in peripheral blood cells (miR-142-5p, miR-155, and miR-223), plasma (miR16, miR-210, miR21, miR-155, and vb), and/or urine (miR-10a, miR-10b, and miR-210) (9).

**Detection of miRNA Expression Profiles**

Detection of miRNA expression profiles may help to identify miRNAs. miRNAs are well preserved in plasma, serum, urine, and formalin-fixed tissue blocks and can be measured more sensitively than proteins. Thus, they may be used as a biomarker in various molecular diagnosis applications, such as cancer, cardiovascular, and autoimmune diseases (10). They can be isolated from cells, tissues, and body fluids (plasma, serum, and urine), and their expressions can be analyzed by RNA sequencing, quantitative real-time polymerase chain reaction, or microarray applications (Figure 2). miRNA profiling studies may provide benefits in the discovery of novel miRNAs, analysis of miRNA-mRNA, and miRNA-protein associations.

**Role of miRNA in Solid Organ Transplantations**

The alterations in miRNA expressions in allograft or serum of the recipient during acute or chronic rejection episodes have been studied after the findings of the role of miRNA in immune cell development and activities. In addition, its diagnostic and prognostic biomarker features have been investigated to estimate the status of allograft and to develop individual-specific immunosuppressive drug strategy (11).

Figure 1. miRNA synthesis steps

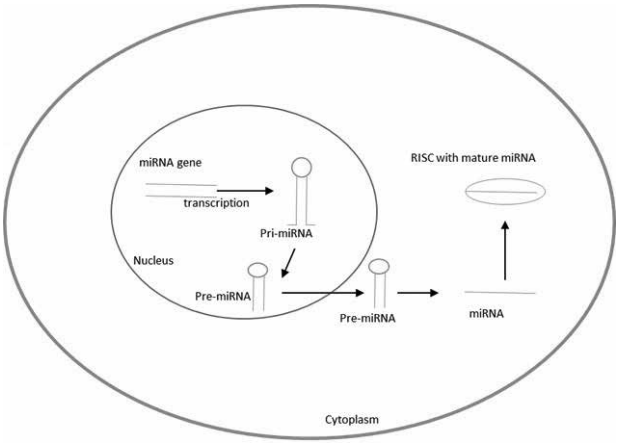
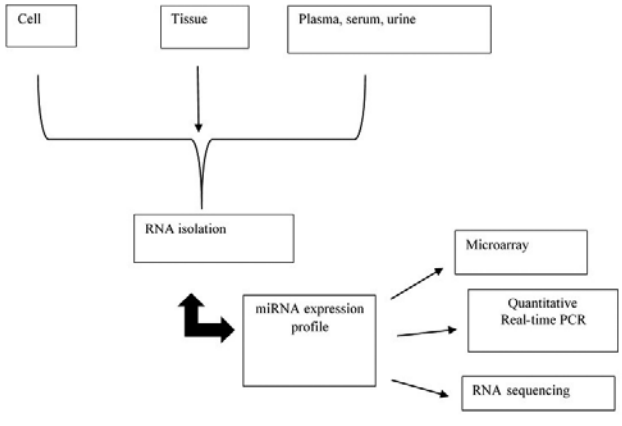



Figure 2. miRNA profile study pathway. RNA, ribonucleic acid; PCR, polymerase chain reaction



Dendritic cells, which have significant roles in transplant immunity, present alloantigens to T lymphocytes and trigger immune rejection. In addition, they function as regulators of immune reactions. miRNAs regulating dendritic cell activation were discovered in a previous study. For example, it was observed that miR-155 expression increases during the maturation of these cells. Moreover, it was shown that dendritic cells fail in antigen presenting and cannot activate T cells effectively owing to costimulatory function in miR-155 deficiency (11, 12).

The most frequently observed activity in allograft rejection is T cell-mediated reaction. It was reported that seven miRNAs are most commonly expressed in CD8+ T cell subgroup, and it was assumed that these miRNAs may regulate the genes that direct cytotoxic T cell functions. miRNA expression profile is also altered during CD4+ T cell activation. It was presented that miR-181a regulates T cell activation by altering T cell receptor signal strength and cut-off value. miR-155 also functions in T cell response by playing a crucial role in the regulation of T helper 1/2 cell differentiation. T-regulator cells can control a number of immune responses and may function in the prevention of rejection. It was observed that there are miRNAs (i.e., miR-155) playing crucial roles in T-regulator cell activation (11).

B cells are also important defenders in graft rejection. It was reported that graft rejection is associated with, particularly, CD20+ B cells; however, the mechanism has not been clearly elucidated yet (13). miR-155 is the well-studied miRNA in B cell response, and it was assumed that germinal center development, immunoglobulin class switching, and antibody production may be affected by miR-155 (11).

Major histocompatibility complex class I-related gene A (MICA) and gene B (MICB) are ligands for the receptors on natural killer and CD8+ T cell surfaces. MICA and MICB are expressed by the cells under stress because of infection or inflammation. It was reported that miRNAs that downregulate MICA and MICB expressions are found, and these miRNAs play roles in MIC-related rejections (11).

Researchers have been studying miRNAs as rejection-related biomarkers as well as therapeutic targets in various solid organ transplantations (e.g., heart, small intestine, and kidneys). Allograft quality is important for graft survival in organ transplants. There have been a number of studies associated with graft quality, ischemia-reperfusion injury, and miRNA expressions. It was reported that alterations in miRNA expression might be used for ischemia-reperfusion injury. The results revealed that there are lymphocyte-independent alterations in miRNA expression profiles of ischemia-reperfusion injury (14, 15).

Scian et al. (16) assessed miRNA expressions related to chronic allograft dysfunction in urine samples in parallel to allograft tissues. Genes showed different expression levels of miR-142-3p, miR-204, miR-107, miR-211, and miR-32 in tissue and urine. These findings suggest that miRNAs can be used as a noninvasive marker for chronic allograft dysfunction.

**Role of miRNA in Kidney Transplantation**

Kidney transplantation is the only treatment for end-stage renal failure. Human leukocyte antigen compatibility is critical for transplantation, and the recipients with more mismatches may produce de novo antibodies against donor graft because of alloimmunizations (pregnancy, blood transfusion, and previous transplants) and result in graft rejection (17). There are various methods to detect the antibodies during/after rejection, such as complement-dependent cytotoxicity crossmatch and solid-phase assays (e.g., flow cytometric crossmatch, Luminex crossmatch, and panel reactive antibody screening) (18, 19). However, early prediction of rejections is very important to prevent graft failure.

Ischemia-reperfusion injury is an inevitable result of kidney transplantation. Ischemia results in ATP deficiency, ion gradient failure, cell swelling, and increase in toxic products because of nutrition and oxygen failure. Ischemia-reperfusion injury is also associated with acute and chronic rejections (20). Godwin et al. (21) reported that miR-21, miR-20a, miR-146a, miR-199a-3p, miR-214, miR-192, miR-187, miR-805, and miR-194 are expressed differently in ischemia-reperfusion injury; therefore, they could be used as biomarkers of this injury. Currently, miRNAs, which can be used in ischemia-reperfusion injury, are not used in routine methods. Research is ongoing to address this issue.

miRNAs play important roles in immune processes, including T and B cell differentiation, cytokine production, T cell and Toll-like receptor signalization, and antigen processing and presenting. In studies on acute rejection, it was reported that there are differently expressed miRNAs (let-7c, miR-10a, miR-10b, miR-125a, miR-200a, miR-30a-3p, miR-30b, miR30c, miR30e-3p, and miR-32) in biopsy samples. Some of these miRNAs were upregulated, whereas others were downregulated (22).

It was also highlighted that miRNAs that are expressed differently in renal biopsy samples in chronic allograft dysfunction (chronic rejection) are miR-142-3p, miR-32, miR-204, miR-107, and miR-211 (16).

Two parameters have been analyzed in serum to determine renal graft status after kidney transplantation. One of these parameters is serum creatinine, which determines glomerular filtration rate, whereas the other one is proteinuria, which identifies if there is an injury in glomerular filtration barrier. However, to identify these parameters, the injury should progress gradually. Under this condition, biopsy, which is the golden standard method, is used; however, this method has approximately 3% risk for patients. Thus, recent studies have been performed to analyze miRNAs as biomarkers in a noninvasive sample "urine" (23). Maluf et al. (24) assessed miRNA expressions in 191 samples.

They found that 22 of 1733 mature miRNAs, which were tested by microarray analyses, were expressed differently between the groups. They reported that some of these miRNAs might be used as biomarkers because they could be detected at an early stage after kidney transplantation before the histological progression of allograft injury.

Discovery of miRNAs was a milestone in various areas, such as system biology, immunology, and cellular biology. miRNAs play crucial roles as regulators in various cellular functions ranging from cell development to apoptosis.

miRNAs have also been analyzed in solid organ transplantations. It has been shown that miRNAs may be associated with allograft rejection, ischemia-reperfusion injury, and fibrosis after kidney and other solid organ transplantations. Some miRNAs may be expressed in tissues of several organs, such as the kidneys, liver, and lung. However, the important issue in miRNA studies is its tissue and/or body fluid specificity. In kidney transplantations, the specificity of miRNAs to kidney tissue is crucial, and with the discovery of the specific miRNAs, the idea of using miRNA as a biomarker has emerged owing to their stability and resistant structure. Currently, biopsy samples are collected to analyze tissue-specific miRNAs; however, this method has some risks, such as bleeding and infection. Therefore, miRNAs have been studied as noninvasive biomarkers. Further studies have suggested that urine may be used as a noninvasive material to assess graft rejections at an early stage before kidney injury. There are studies indicating thatmiRNA expressions are compatible in urine. The studies are usually performed by evaluating all patient groups. However, results may not be representative of all patients when we consider that their lifestyles, diseases, and habits are different from each other. Thus, the evaluation of miRNA profiles individually may be useful particularly in studies dealing with transplantation.

## CONCLUSION

The role of miRNAs in kidney transplantations is discussed in the current study. Although there have been several studies conducted regarding this subject, the effective mechanisms of miRNA and their role in cell communication have not been completely understood yet. Thus, the use of miRNAs as biomarkers is not yet included in the routine diagnosis. Further investigations with broad range should be performed.

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