MicroRNAs as Potential Graft Rejection or Tolerance Biomarkers

Subjects: Transplantation

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Allograft rejection is a widespread complication in allograft recipients with chronic kidney disease. Undertreatment of subclinical and clinical rejection and later post-transplant problems are caused by an imperfect understanding of the mechanisms at play and a lack of adequate diagnostic tools. Many different biomarkers have been analyzed and proposed to detect and monitor these crucial events in transplant outcomes. In this sense, microRNAs may help diagnose rejection or tolerance and indicate appropriate treatment, especially in patients with chronic allograft rejection. As key epigenetic regulators of physiological homeostasis, microRNAs have therapeutic potential and may indicate allograft tolerance or rejection. However, more evidence and clinical validation are indispensable before microRNAs are ready for clinical prime time.

allograft rejection biomarker chronic kidney disease microRNAs tolerance

1. Background

The standard method for identifying organ rejection is typically the kidney allograft biopsy [1]. However, this procedure is invasive and can be unclear or inconclusive in some cases. Recent advancements have led to the discovery of successful kidney allograft rejection biomarkers, some of which are now available for routine clinical use [2][3][4]. An intriguing aspect of organ transplantation evolution and outcome involves monitoring the expression of key immune molecules that play a role in the biological response against donor allografts [5][6]. MicroRNA (miRNA) is one such regulatory element that can determine, modulate, or influence the expression of these molecules [7]. Previous research has implicated miRNAs in transplant outcomes [8][9][10][11][12], and this study explores their role in both pre- and post-transplant monitoring.

2. MicroRNA Molecules and Biological Functions

RNA molecules transcribed from DNA include different types, such as RNA messenger (mRNA) or miRNAs. miRNAs, a class of non-coding RNAs, play a crucial role in post-transcriptional regulation, affecting processes such as cell cycle, differentiation, proliferation, and cell death.

The study of miRNAs, which are non-coding endogenous RNA molecules with regulatory and modulatory roles that prevent target mRNAs from being translated and have a length of 20–24 nucleotides, is ongoing [11]. Different

studies estimate that these molecules could regulate approximately 60% of the human transcriptome [9][10][13]. Cell cycle, differentiation, proliferation, and cell death are only a few of the critical biological processes that their post-transcriptional repression of significant and determinant mRNA targets regulate. The development of high-throughput sequencing technology [13] and predictive computational and bioinformatics tools [14] has considerably expanded the research of miRNAs, including their putative regulatory targets and biological functions.

In brief and functionally, miRNAs bind to the complementary mRNA's 3′-UTR region, inhibiting protein translation or promoting mRNA degradation [11]. Most miRNA sequences are found in non-coding RNA molecules' introns or exons. However, some miRNAs (known as mirtrons) come from introns in pre-mRNA. As previously reported, different miRNA localizations determine canonical or non-canonical pathways [15].

A crucial implication is that one miRNA can target hundreds of mRNAs, and vice versa; several miRNAs can target one mRNA [16]. Consequently, genetic expression is fine-tuned via a complex network of miRNAs and mRNAs. The fact that patterns of miRNA expression appear to be tissue-specific and substantially conserved across species emphasizes the significance of these molecules for evolution [17][18].

Dysregulation of particular miRNAs is published in and may enhance or direct oncological, inflammatory, autoimmune, metabolic, or neurodegenerative diseases [19][20][21][22]. In addition to being essential intracellular regulators of gene expression, miRNAs can have paracrine and endocrine effects after being actively taken up by cells [23][24]. miRNAs have recently been identified as interorgan circulating communication mediators [25][26][27].

3. MicroRNAs as Circulating Biomarkers in Health and Disease

As previously indicated, microRNAs can be present in biofluids, packaged inside extracellular vesicles (exosomes and microvesicles), or bound to lipoproteins and ribonucleoproteins. Previous studies have shown that active cellular transport routes mediate the selective secretion and absorption of miRNAs, which determines the composition of circulating miRNAs. However, the varied pool of extracellular miRNAs is also influenced by the passive leakage of miRNAs from damaged or dead cells [28][29][30][31][32][33].

Circulating miRNAs are great candidates for significant clinical biomarkers due to some characteristics of miRNAs. As carriers shield them from endogenous RNases, microRNAs are stable in peripheral blood and can be successfully detected in samples kept for a considerable amount of time. Also, miRNAs are believed to resist repeated freeze–thaw cycles [34][35][36][37].

Another critical point is that circulating miRNAs represent tissue-specific expression levels, as reported [38][39][40][41][42].

miRNAs have been identified as a novel class of very sensitive circulating biomarkers for numerous metabolic and age-related disorders, as evidenced by the advancement of this field's research over the past ten years [31].

Licensed diagnostic miRNA panels are available for cardiovascular illness, Alzheimer's disease, thyroid, pancreatic, and breast cancer [43].

Even some of these panels have the backing of well-known insurance companies. For instance, osteomiRTM, a panel of 19 plasma miRNAs, has been suggested as a risk predictor for osteoporotic fractures in postmenopausal women independent of bone mineral density (BMD) [44]. Several pools of miRNAs have also been proposed as biomarker illnesses.

It is also necessary to mention the difficulties encountered when analyzing circulating miRNAs. Preanalytical errors are to blame for most errors in a clinical chemistry lab. Controlling the preanalytical variability of biospecimens is crucial since it can considerably impact subsequent studies [45].

With more time spent on the bench before processing, there is a greater chance of hemolysis or blood cell leakage, which could change miRNA expression. To reduce the variability of miRNAs, it is crucial to standardize the collection, processing, transport, and storage processes. Variability in analytical results should also be taken into account. Different normalization techniques and the heterogeneity of the commercially available tests reduce reproducibility and lead to ambiguous results. [46][47][48][49].

4. MicroRNA Molecules and Their Role in Transplantation

The role of miRNAs in kidney transplantation begins, particularly in knowing that prior to transplantation, they can play a crucial role in nephrological pathologies that give rise to the poor function of the healthy kidney, which can lead to needing a transplant. In this way, miRNAs seem to play a role in an extensive catalog of urinary tract pathologies that cover various tumors, infectious pathologies, systemic autoimmune pathologies with renal involvement, and isolated renal pathologies related to the immune system, which can cause native kidney damage and that could presumably reappear after kidney transplantation. miRNAs appear to regulate all these processes, pathologies, evolution, and results. It is known and inferred that different changes in miRNA expression could modify or modulate kidney transplantation's short- and long-term evolution. The vast majority of studies on kidney allograft transplantation that direct or analyze the role of miRNAs or mRNA detection and expression have been conducted with blood samples (isolating cells as peripheral blood leukocytes or separating plasma or serum) or urine samples, and a small percentage of studies have analyzed the expression of intra-graft miRNAs comparing their eventual correlation with the levels of the rest of the mRNA [5][6][8][50]. RNA quantification indirectly measures the gene expression levels in a person's blood, cells, fluids, or tissues.

In this sense, the analysis transcriptomics can help discover expression profiles that allow not only to stratify patients according to immunological risk to rejection or tolerance but also to differentiate the type of rejection when this occurs, providing valuable information to the clinician concerning modifying the treatment of a particular patient [51].

On the other hand, these molecules could also have an essential function in cold ischemia-reperfusion injury, as previously reported [52][53]. Additionally, since their discovery, research on miRNA expression profiles has shown promise for predicting kidney graft status in a variety of clinical contexts, including acute or delayed graft function, rejection, liver and heart transplant, hematological transplantation, or bone fragility in chronic kidney disease [6][22][54][55][56][57][58][59][60].

In this sense and with more detail in these facts, a research group examined urinary exosomal microRNAs to identify novel biomarkers of rejection [54]. Candidate microRNAs were selected using NanoString-based urinary exosomal microRNA profiling, meta-analysis of a web-based, public microRNA database, and a literature review. They identified 29 urinary exosomal microRNAs as candidate biomarkers of acute rejection, of which seven were differentially expressed (DE) in recipients with acute rejection. A three-microRNA acute rejection signature, composed of hsa-miR-21-5p, hsa-miR-31-5p, and hsa-miR-4532, could discriminate recipients suffering acute rejection. Other authors also provide a summary of information about the therapeutic relevance of aberrant miRNA expression profiles in hematologic cancer patients and their relationships with various antagomiRs, mimetics, and circular RNAs (circRNAs), as well as with diagnosis, prognosis, and therapy response monitoring [55]

In a separate investigation, kidney transplant recipients who developed acute rejection episodes had higher expression levels of six specific miRNAs (miR-191-5p, miR-223-3p, miR-346, miR-423-5p, miR-574-3p, and miR-181d), while miR-150-5p expression had decreased. The following analysis revealed a potential connection between miR-150-5p and transcription factor MBD6, suggesting that the modification of this interaction may be responsible for the beginning or development of acute rejection episodes [8].

In this way, miR-150 plays a crucial role in immune regulatory processes such as B and T lymphocyte proliferation, activation, and apoptosis. It is also explicitly expressed in lymph nodes, the spleen, and mature B and T cells [56][61]. One of the most researched miRNAs, it is involved in innate and adaptive immune responses and is crucial for the pathogenesis of various malignancies [60][61]. By controlling mTOR expression, miRNA-150 is also involved in Treg cell development and has been considered a lymphocyte activation biomarker [62][63].

5. MicroRNA Molecules in B Cell, Humoral Rejection, and DSA Production

Firstly, transcriptomics studies in tolerant recipients have shown increased expression of important genes related to B lymphocytes, such as CD79B or CD20 [64]. Other studies have accordingly shown that the decreased expression of CD79B, CD20, and TCL1A is associated with patients suffering acute rejection episodes [65] and the increased expression of cytokines such as CXCL9 and CXCL10, produced by B lymphocytes after their interaction with T lymphocytes, is present in rejection processes in various types of solid organ transplantation [66].

Numerous B lymphocyte subpopulations, including germline naive B lymphocytes, have had their expression of miRNAs examined, demonstrating that these molecules may be crucial for effector activities and regulatory

networks governing cell growth [50][56]. In this manner, different miRNAs have been implicated in B cell function and antibody production.

Firstly, miR-150-defective animals have been shown to secrete more antibodies in response to antigenic stimulation [67]. Secondly, the abnormalities in miR-155 lead to an isotype change deficiency and poor plasma cell development [68].

Thirdly, another study by other authors revealed that B cells from tolerant kidney transplant recipients overexpressed the miR-142-3p gene [69], pointing to a potential mechanism involving the TGF-signaling pathway.

On the other hand, research on miRNA expression in B lymphocytes, including naive and germinal center B lymphocytes, has demonstrated that these molecules can be crucial in the regulatory pathways of cell growth and effector functions [70][71].

Finally, other critical studies in kidney transplantation concerning miRNA expression changes in clinical AMR complications in humans are the following: let-7c, miR-28, miR-30d, miR-99b, miR-125a, miR-195, miR-374b, miR-484, miR-501, and miR-520c up-regulation, and miR-29b and miR-885 down-regulation have been associated with AMR [64].

6. MicroRNA Molecules in Viral and Bacterial Infection in Transplantation

Kidney transplant recipients exhibit a series of infectious complications post-transplant that can be pretty common and have to do with urinary complications.

For example, in the case of bacterial infections, scholars have gastrointestinal tract infections caused mainly by uropathogenic *Escherichia coli* associated with the immunosuppressant cyclosporine. This will activate the cells that are a preferential site of adhesion and translocation for this specific pathogen, inducing the inhibition of the lipopolysaccharide-induced activation of the cells and down-regulating the expression of TLR4 through the miRNA let-7i.

In this way, ebvmiR-BART2-5p was detected in more children with IM and chronic high viral loads than in children who resolved the EBV infection. The same trend was observed among the EBV miRNAs expressed in the plasma and viral load. Several ebv-miRs were detected, including ebv-miRBART7-3p, ebv-miR-15, ebv-miR-9-3p, ebv-miR-11-3p, ebv-miR-1-3p, and ebv-miR-3-3p only in children with IM and with chronically high viral loads. Lytic ebvmiRs-BHRF1-2-3p and ebv-miR-1-1 (indicators of active viral replication) were only detected in children with MI [72]. Therefore, EBV-specific miRNA expression could represent a marker for monitoring the phase of infection in pediatric and EBV-infected kidney recipients.

Another vital point in viral infections in kidney transplantation is the Bk polyomavirus infection, which is usually a common asymptomatic infection in healthy people. However, in transplant patients with the continued use of immunosuppressants, which turns them into immunocompromised individuals, it can lead to nephropathy associated with polyomavirus and produce serious complications depending on its evolution [73].

It is known that the BK polyomavirus encodes two mature miRNAs, bkv-miR-B1-3p and bkv miR-B1-5p, which appear to regulate the life cycle of the virus itself [73] and appear to be involved in modulating and controlling viral replication, allowing the virus to evade the patient's immune response.

7. microRNAs in Therapeutic Approaches in Transplantation

Finally, validating miRNAs differentially expressed (DE) in rejection, complications, or transplant outcomes using additional mRNA microarray data from the Gene Expression Omnibus, as reported in earlier publications [8][74][75], may also be of actual interest.

Future research will be required to fully comprehend miRNAs' role in graft rejection and how they might interact with other expression proteins [76].

The miRNA target databases primarily contain theoretical interactions that have not been verified by experiments, which makes it exceedingly challenging to make an accurate interpretation. Validating these intriguing connections using the various sample types in each instance is also required. Despite these significant limitations, statistically sound assessments and analyses with positive implications for diagnosis have been published.

Considering this, emphasizing the significance of miRNA dysregulation is a frequent finding in these fundamental and clinical processes, particularly in tumoral processes that lead to cancer. It is crucial to talk about how other RNA transcripts, such as circRNAs and lncRNAs, can act as sponges for miRNAs in controlling many processes and diseases.

In this way, artificial miRNAs (miRNA mimetic molecules) have been created to try to increase the expression of a specific beneficial miRNA or introduce short hairpin duplexes, similar to the pre-miRNA, into a target cell or an appropriate tissue. Apart from local injection into tissues, systemic administration can allow its development [77][78][79].

Thus, antisense oligonucleotides (ASOs) that seek to block miRNAs, specifically anti-miRNAs, can be developed [80].

miRNA sponges have also been artificially designed to inhibit several miRNAs with several binding sites and help sequester a family of miRNAs. Similarly, miRNA masks and erasers have also been designed to mask the miRNA binding site on its target (mRNA) or to use only two copies of the antisense sequence. Other gene-specific miRNA

mimicking and miRNA masking antisense procedures and protocols have also been designed as eventual therapeutic targets [81][82].

How to ensure that all these inhibition, modulation, and regulation procedures for the activity of miRNAs specifically reach the specific organ or tissue is still a matter of further research and development.

To this end, extracellular vesicles are potentially being analyzed to administer, conduct, and deposit miRNAs or their inhibitors in the specific anatomical site scholars want to regulate [81].

These vesicles, depending on the cell of origin that produces them, participate in improving complement activation or secreting complement inhibitors and preventing cell lysis, affect pro-coagulation and pro-thrombosis, promote endothelial survival and angiogenesis, and can induce rejection and/or autoimmunity with pro-coagulant and pro-inflammatory effects [82]. However, they can also promote immune tolerance [83].

In summary, the genesis and function of miRNAs are detailed, explaining their transcription, processing, and regulation mechanisms. Mature miRNAs can circulate extracellularly, especially in vesicles such as exosomes, with potential implications as biomarkers for pathological conditions. The stability of circulating miRNAs and their resistance to freeze–thaw cycles are highlighted, making them promising candidates as clinical biomarkers. The importance of standardizing collection, processing, transportation, and storage processes is emphasized for accurate analysis.

In conclusion, miRNAs can be significant regulators in biological processes, valuable biomarkers in health and disease, and crucial players in the context of transplants. Although the field holds promise, the need for further research, standardization, and validation is highlighted to fully exploit the diagnostic and therapeutic implications of miRNAs in clinical practice.

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