MicroRNAs as master regulators of immune responses in transplant recipients

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Purpose of review
MicroRNAs (miRNAs) have emerged as highly evolutionarily conserved moieties that have very selective gene-regulatory functions. miRNAs are being researched for their use as potential biomarkers for diagnostics, routine prognostics as well as selective therapeutics in cancer, infectious diseases, autoimmune disorders and transplantation. This review summarizes how immune regulation by miRNAs affects the outcome of transplantation.

Recent findings
Many miRNAs have been identified as selective markers for specific disease states and transplant conditions in the past two decades. In this review, we will discuss the recent advances and some seminal discoveries in miRNA research and their role as immune regulators in transplantation. Lastly, we will highlight the ongoing clinical trials for miRNA-based therapeutics for clinical applications and present our opinion on the future of miRNA-based diagnostics and therapeutics.

Summary
miRNA-based diagnosis is a fast-moving field with new miRNA signatures being identified each day. Recent advances have also been successful at taking a few of these miRNAs to clinical trials for therapeutic interventions.

Keywords
biomarker, graft rejection, immunomodulatory, miRNAs, organ transplantation, tolerance

INTRODUCTION

miRNAs were discovered about two decades ago, in the *Caenorhabditis elegans* developmental pathway, as small temporal noncoding RNAs, which could bind to 3′ UTR of a coding messenger RNA and modulate its translation [1,2]. They were initially believed to be present exclusively in *C. elegans*. Findings in the early 2000s identified miRNAs present in other systems including human cells [3–5]. Since then, about 1881 human miRNAs have been deposited into miRBase v21.

BIOGENESIS AND MECHANISM OF ACTION

miRNAs are endogenously expressed as short noncoding RNAs with regulatory functions in a myriad of gene pathways. miRNAs, which are synthesized in a highly regulated manner, bind to complementary target protein-encoding mRNAs in a RNA-dependent gene silencing process leading to translational repression or mRNA degradation (RNA interference or RNAi) [6]. The location of miRNAs is mostly intergenic generally within introns of coding or noncoding genes and sometimes within exons of noncoding genes [7]. miRNAs are transcriptionally regulated in a 'protein coding-like manner,' with Oct4, Sox2, Nanog and Tcf3 being important transcriptional regulators [8] (Fig. 1 [9–18]). Many fundamental biological processes are regulated by miRNAs including cell differentiation, proliferation, maturation and cellular homeostasis; indeed, miRNAs regulate about 60% of the human genome [19–25]. Expression of miRNAs is altered in many disease conditions. miRNAs are interesting and important moieties because they can alter entire gene pathways rather than a single gene [26].

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KEY POINTS

- miRNAs have emerged as important small noncoding RNAs with immunomodulatory functions that regulate gene expression at posttranscriptional level.
- miRNAs regulate many important genes and pathways in both the innate and adaptive immune systems.
- Many miRNAs have been identified as potential biomarkers for graft function in both solid organ transplantation and hematopoietic stem cell transplantation for diagnosis and prognosis of patient outcomes.
- Synthetic RNA mimics and antagomirs used to modulate endogenous miRNA can be the next frontier in RNA-based clinical strategies for therapeutics.

MICRORNAS IN B CELL DEVELOPMENT AND FUNCTION

miRNAs are essential for B cell development as shown in studies by Koralov *et al*. Mice with B cell-specific deletion of the endoribonuclease Dicer fail to have pro-B stage to pre-B transition [27]. Removal of Dicer in antigen-activated B cells results in significantly decreased antibody responses and prevents the formation of both germinal center B cells, and long-lived plasma cell memory B cells [28]. Overexpression of the miR-17∼92 cluster (including miR-17, miR-19a, miR-19b, miR-20a, and miR-92) in transgenic mice leads to lymphoproliferative and autoimmune phenotypes because of reduced phosphatase and tensin homolog (PTEN) and bcl-2 interacting protein (BIM) expression [29]. The expression of c-Myb (important in B1 cell development) is controlled by miR-150 [30,31]. Constitutive expression of miR-34a targets the expression of Foxp1, leading to arrest of the pro-B to pre-B cell transition [31]. Another miRNA, miR146a, controls the expression of Irak1 and Traf6 in splenic B cells and has been implicated in B1 cell development [32,33]. miR-221 plays a role in the retention of the early B lineage cells in bone marrow [34]. miR-155 plays an important role in both germinal center B cells and B cell memory responses [35,36] and regulates a transcription factor PU.1. Overexpression of PU.1 in B cells leads to reduced numbers of IgG1-switched cells and thus to reduced generation of high-affinity antibodies

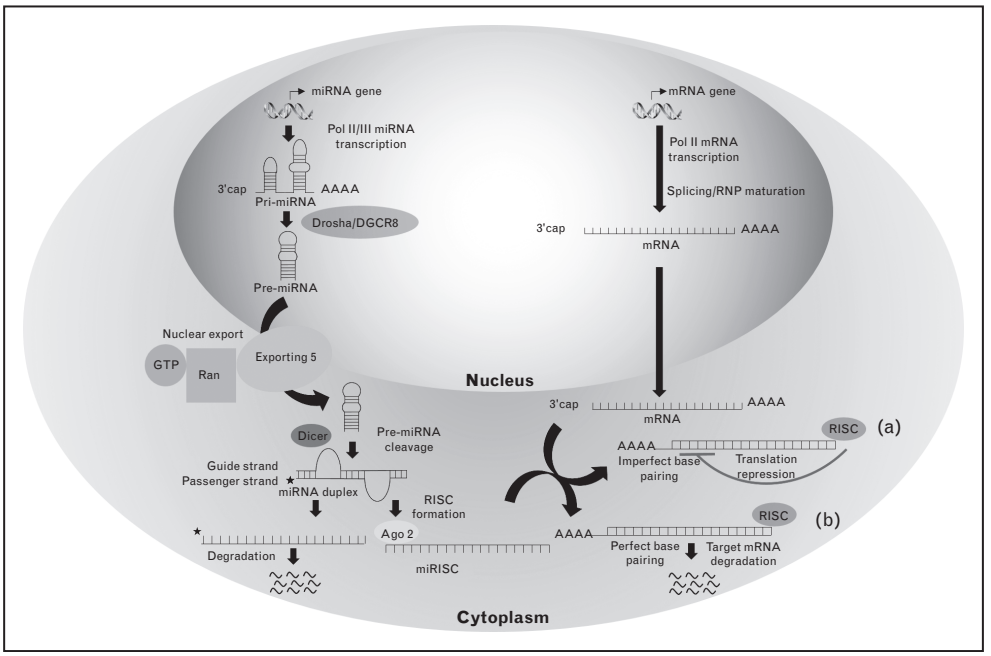

FIGURE 1. Schematic for microRNA (miRNA) biogenesis and function. MicroRNA coding regions form primary miRNAs (pri-miRNA), which are processed in the nucleus into 70-nucleotide precursor miRNAs (pre-miRNA) by the Microprocessor complex consisting of the RNase II enzyme Drosha and its co-factor DiGeorge syndrome critical region gene 8 (DGCR8) [9]. Exportin 5 Ran-GTP complex transports the pre-miRNAs to the cytoplasm, which are then processed by RNase III Dicer-Argonaute protein complex. Dicer cleaves the pre-miRNAs to generate a mature miRNA–miRNA* duplex (22 nucleotides) [10,11]. miRNA 'guide' strand binds with Ago protein and forms miRISC (miRNA-associated multiprotein RNA-induced silencing complex) [12]. miRISC binds the target mRNA at the complementary miRNA seed sequence [13,14]. Complementarity of miRNA–mRNA at the seed sequence determines the target repression (a) or degradation (b), with perfect complementarity mostly leading to mRNA target degradation [15]. Recently, miRNAs have also been discovered to target long noncoding RNAs (lncRNAs), antisense RNAs and competing endogenous RNAs [16–18].

against T cell-dependent antigen [37]. miR-155 also regulates the Aid (activation-induced cytidine deaminase) gene, thus regulating class-switch recombination and somatic hypermutation [38,39]. Immunoglobulin class-switching recombination is also regulated by miR-181b and miR-210 [40,41▪].

MICRORNAS IN T CELL DEVELOPMENT AND FUNCTION

T cell development and function are similarly regulated by miRNAs. Profiling studies have shown that miRNAs are differentially expressed in functionally naïve, effector or memory $CD8^+$ T cell populations [42]. Conditional Dicer deletion in the mouse T cell lineage results in a significant decrease in mature $CD8^+$ T cells and some reduction in $CD4^+$ T cells. These $CD4^+$ T cells were predisposed to the Th1 lineage because of a deficiency in repression of interferon (IFN)-γ and also had diminished proliferation and increased apoptosis [43]. T cell differentiation has been shown to be regulated by miRNAs. The loss of miR-155 enhances Th2 development but is negatively correlated to Th1 or Th17 differentiation [35,44]. Th17 differentiation is regulated by miR-326 [45]. miR-142-3p has been shown to be involved in regulation of T lymphocytes by controlling leukocyte activation [46]. Another class of T cells, T follicular helper (Tfh) cells is also modulated by miRNAs. The plasticity of Tfh cells is regulated by miR-10a which directly inhibits the expression of Bcl-6, an important transcription factor for Tfh differentiation [47,48]. Conversely, the miR-17∼92 cluster induces Tfh differentiation by suppressing the expression of RORα and PHLPP2 (phosphatases that inhibit inducible T-cell co-stimulator (ICOS)-mediated phosphatidylinositol kinases (PIK)-signaling pathways) [49▪,50]. High-level expression of the miR-17∼92 cluster in lymphocytes leads to lymphoproliferative disease and autoimmunity because of increased $CD4^+$ and elevated interleukin-10 and IFN-γ levels. These activated $CD4^+$ T cells were CD28-costimulation-independent for proliferation and survival. The miR-17∼92 cluster, when overexpressed, negatively regulates BIM and PTEN in mice, proteins important in maintenance of central and peripheral tolerance [29]. miR-181 regulates T cell receptor (TCR) responses to peptide antigens, with decreased expression in immature T cells leading to decrease in sensitivity and altered positive and negative selection. Multiple cytoplasmic phosphatases such as SHP2 that regulate TCR signaling are also controlled by miR-181 [51]. Recently, the miR-23a cluster has been shown to significantly reduce IFN-γ secretion and cytotoxic activity of antigen-specific $CD8^+$ T cells [52▪].

miRNAs also control Treg cell generation, stability and function. Dicer knockout mice develop fatal autoimmunity [53]. T cells lacking Dicer are unstable and do not express several Treg-associated genes such as Foxp3, CTLA-4, neurophilin-1 and glucocorticoid-induced tumor necrosis factor receptor [54]. It has been shown that Foxp3, the canonical Treg transcription factor, binds to the promoter of miR-155 gene, *bic*. miR-155 and miR-146a also influence Treg homeostasis and its suppressor activity by modulating SOCS1 and Stat1, respectively [55,56]. Recent reports show that miRNA-containing exosomes released by Tregs transfer miR-Let-7d to Th1 cells suppressing Th1 cell proliferation and IFN-γ secretion (both *in vitro* and *in vivo*) [57▪▪]. Tregs are one of the most important regulatory cells in the immune system that play a major role in maintenance of peripheral tolerance to self-antigens and thus have an important role in transplant rejection.

MICRORNAS IN INNATE IMMUNITY: MONOCYTES, MACROPHAGES, DENDRITIC CELLS, NK AND NKT

miRNAs have been shown to regulate components of the innate immune system including dendritic cells, macrophages, natural killer (NK) cells and natural killer T (NKT) cells. For example, the miR-17∼92 cluster regulates monocyte differentiation by targeting Runx1 [58]. Differential expression of miRNAs has important functions in the transformation of monocytes into immature and mature dendritic cells at specific stages of differentiation [59,60]. The microRNAs miR-7-5p, miR-20a and miR-106a also modulate monocytic differentiation [58]. miR-21 increases in expression in monocytes and macrophages during inflammation and influences interleukin-12p35 expression [61]. In virus-infected macrophages, inflammation is suppressed by miR-146a that targets IRAK2 and modulates RIG-I-dependent type 1 interferon production [62]. Dendritic cell maturation is regulated by a number of miRNAs, directly or indirectly. For example, both miR-221 and miR-155 regulate dendritic cell apoptosis and miR-155 regulates interleukin-12p70 production by targeting suppressor of cytokine signaling 1 (SOCS-1) [49▪]. Furthermore, miR-511 and miR-99b have been shown to play a role in dendritic cell maturation by targeting TLR signaling and SMAD7, respectively [60]. TLRs have also been shown to induce miRNA-155, miRNA-146a and miRNA-21 [63–65]. In mouse macrophages, TLRs induce miR-155, which in turn represses negative regulators of TLR signaling such as SHIP1 and SOCS1 [66,67]. miR-155 also regulates

dendritic cell maturation by targeting an important protein PU.1 involved in the expression of dendritic cell-specific intercellular adhesion molecules [68]. Similarly, miR-146 is also induced by the TLR4 ligand lipopolysaccharide (LPS), TLR2 ligand Pm3CSK4 (synthetic triacylated lipoprotein) and TLR5 ligand flagellin [63]. Interestingly, TLR4 expression has been shown to be upregulated in animal models of intestine transplantation and also implicated in allograft rejection [69].

Dicer-deficient mice show a defect in iNKT cell development and function [70]. miRNA-150 has been shown to play a role in the maturation of iNKT cells in the thymus and periphery. Further, miR-150 knockout mice show significantly increased IFN-γ production and upregulation of the miR-150 target c-myb, which is a major transcription factor that primes immature thymocytes into the iNKT lineage [71]. Targeted deletion of miR-150 leads to a defect in the generation of mature NK cells and a gain-of-function transgene promotes an increase of mature NK cells, which are also more responsive to activation. On the contrary, however, miR-150 overexpression leads to a significant reduction of iNKT cells in the thymus and peripheral lymphoid organs [72]. Recent studies show that miR-181-deficient mice have severe defects in NKT cell maturation in the thymus and periphery. miR-181 has also been shown to modulate the phosphatase PTEN expression to control PI3K signaling, an important stimulus for anabolic metabolism in immune cells [73▪▪]. Overexpression of miR-155, in bone marrow and thymus, has been correlated with iNKT cell developmental defects in the thymus. iNKT cells overexpressing miR-155 exhibit defects in cytokine production by targeting Inducible T cell kinase (Itk) [74].

ROLE OF MICRORNAS AS IMMUNE MODULATORS IN TRANSPLANTATION

Based on the importance of miRNAs in regulating immune functions, recent studies suggest that some miRNAs are critical in promoting outcomes after both solid organ and hematopoietic stem cell transplantation [75–78].

Genome-wide analysis of miRNA–mRNA interactions has identified a unique expression pattern of miRNAs and mRNAs following the allostimulation of T cells in an allogenic bone marrow transplantation model. It has been shown that miR-155 regulates T cell proliferation in cardiac allograft rejection by targeting glycogen synthase kinase 3 β [79].

Increased expression of miR-142-3p was reported specifically in the B cells of transplant recipients that are 'operational tolerant' of their grafts suggesting a role for B cells and miR-142-3p in tolerance [80].

Hepatic plasmacytoid dendritic cells (pDC) have been associated with promoting tolerance in several models and recent studies demonstrate that miR-181a, which is highly expressed in hepatic plasmacytoid dendritic cells, may have a critical role in the 'tolerogenic' capacity of liver pDC. Whereas miR-181a pDC significantly prolonged allograft survival, pDC from miR-181a$^{-/-}$ mice abrogated the graft prolongation suggesting miR-181a plays a critical role in the 'tolerogenic' capacity of liver pDC [81].

Thus, miRNAs have been shown to critically modulate the immune system components vis-à-vis transplantation [79–87]. A summary of recent studies of miRNA-mediated immune modulation in transplantation is described in Table 1 [79–87].

Table 1. Summary of microRNAs and their impact on immune system in transplantation

miRNA	Transplant	Target/function	References
miR-181a	Bone marrow	Increased expression in HSC increases B cell lineage	[79]
miR-155	Cardiac allograft	Target GSK3β/T cell proliferation/regulates allograft rejection	[80]
miR-181a	Cardiac allograft	Increased expression in hepatic pDC/increased in tolerance	[81]
miR-146a	Liver	Regulates TGF-β mediated induction of hepatic stellate cells/hepatic fibrosis	[82]
miR-144	Lung	TGF-β signaling pathway/role in fibrosis and occlusion of airways in bronchiolitis obliterans syndrome in lung transplantation	[83]
miR-29	Renal	TGF-β/Smad3/renal fibrosis	[84]
miR-21	Renal	Smad3 upregulation/renal fibrosis	[85]
miR-192	Renal	Induced by TGF-β, increases collagen synthesis by targeting E-box repressors Zeb1/2/Renal fibrosis	[86]
miR-142-3p	Renal	Increased expression in B cells/operational tolerance	[87]

GSK, glycogen synthase kinase; pDC, plasmacytoid dendritic cells; HSC, hematopoietic stem cell; TGF, transforming growth factor.

Table 2. miRNAs (host or viral) implicated in posttransplant complications because of viral infections

Transplant	Virus	miRNA	Effect	References
PTLD	EBV	miR-194	Targets interleukin-10 and modulates apoptosis of EBV$^+$ B cell lymphoma lines	[88]
Liver	HCV	miR-194, miR-21	Regulate HCV receptor expression	[90]
Liver	HCV	miR-146a, miR-19a, miR-20a, and miR-let7e, miR-19a and miR-20a	Regulate genes which control fibrogenic and angiogenic pathways	[91]
Liver	HCV	miR-449a	Regulate expression of YKL40, NOTCH signaling pathway	[92]
Liver	HCV	9-microRNA signature (miR-155, miR-34a, miR-222, miR-23b, miR-361, miR-455, miR-30b, miR-30c and miR-27b)	Biomarkers to identify early post-liver transplantation patients at high risk of severe HCV recurrence during long-term follow-up	[93▪]
Renal	BK	bkv-miR-B1-5p	Diagnosis of active viral replication	[94]

EBV, Epstein–Barr virus; HCV, hepatitis C virus; miRNA, microRNA; PTLD, posttransplant lymphoproliferative disorder; TGF, transforming growth factor.

MICRORNAS IN VIRUS-MEDIATED POSTTRANSPLANT INFECTIONS

Complications, including viral infections, are an unfortunate consequence of transplantation, generally as a result of immunosuppressive drugs that target T cells. Host cells produce miRNAs in response to viral infections, which have a regulatory role on the immune components elicited during infection. Viruses can modulate the gene expression of host immune machinery. A recent study found that Epstein–Barr virus (EBV) suppresses the host miRNA-194 in EBV$^+$ B cell lymphoma lines from patients with posttransplant lymphoproliferative disorder. Suppression of miR-194 resulted in upregulation of its target interleukin 10 and modulation of apoptosis [88].

Latent membrane protein 1 (LMP1) has been shown to activate the Akt pathway and upregulate Mcl-1 through miR-155, thereby inducing cell survival signals, whereas inhibition of miR-155 resulted in a significant decrease in the survival of EBV$^+$ cells when treated with Rituximab [89].

Viruses also employ miRNAs that can modulate both host and viral gene expression. The importance of the role of miRNAs in virus-induced transplantation complications has recently become apparent (see summary in Table 2), although the mechanisms remain unknown [88,90–92,93▪,94].

MICRORNAS AS BIOMARKERS

It has been shown that miRNAs are highly stable in human blood and in paraffin-embedded tissues suggesting that miRNAs could be good markers for identifying disease conditions [95,96]. Circulating miRNAs detectable in serum, plasma, urine and other body fluids remain stable, mostly in lipid and lipoprotein complexes like exosomes and microvesicles [97–100]. Early studies examined hundreds of samples and demonstrated that miRNA profiles could distinguish the developmental origin of a tumor [101]. In transplantation, urinary miR-210 has been suggested as a biomarker of acute rejection in renal transplants [102]. Other studies indicated an association of miR-142-5p and chronic antibody-mediated rejection [103▪]. However, only a handful of these potential miRNA-based biomarkers have been translated to commercially available diagnostics. One such example is a panel of 64 miRNA biomarkers (miRview-mets2) to identify the origin of metastatic cancers by Rosetta Genomics, (Israel) [104].

MICRORNAS AS THERAPEUTICS

miRNAs are very promising therapeutic moieties because they can target and fine-tune entire gene pathways, in contrast to the selective protein-based inhibitors that have only single-target functionality. To date, however, only one miRNA drug has reached clinical trials. SPC3649, an antagomir of miR-122 developed by Santaris Pharma, (Denmark) for chronic hepatitis C is currently in Phase II clinical trials [105▪▪]. In spite of miRNAs being very stable molecules, the main deterrent in making them effective therapeutics is creating complex delivery vehicles for them. Several chemical modifications such as Lock–nucleic acid (LNA) have been designed to make miRNA delivery more stable [106,107].

CONCLUSION

In the two decades since miRNAs were discovered, there has been substantial research focused on miRNA-based gene regulatory mechanisms. However, the specific roles that each miRNA has in the regulation of the immune system are still being discovered. Newly identified roles of miRNAs in transplantation and their potential use as biomarkers for predicting graft function and as

therapeutics for gene and pathway-specific regulation for induction of allograft tolerance have sparked a robust interest in miRNA-based clinical strategies. However, there still are a number of limitations regarding technical methodology for studying miRNAs, and the elucidation and follow-up of clinical outcomes of miR-based research. Nonetheless, we foresee a big effort toward miRNA-based diagnostics and therapeutics in the near future, mainly driven by the need for more specific treatment and diagnostic modalities for a number of disease conditions including posttransplant complications, immune malignancies, infectious diseases and autoimmune disorders. Greater collaboration between academic and industry research is required to reach our ambitious goals for translating miRNA research to the clinic.

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Conflicts of interest

There are no conflicts of interest.

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