Published in final edited form as:

Exp Mol Pathol. 2012 December ; 93(3): 378–385. doi:10.1016/j.yexmp.2012.09.020.

Modulation of immune responses following solid organ transplantation by microRNA

Nayan J. Sarma, PhD¹, Venkataswarup Tiriveedhi, MD, PhD¹, Sabarinathan Ramachandran, PhD¹, Jeffrey Crippin, MD³, William Chapman, MD¹, and T. Mohanakumar, PhD^{1,2}

¹Department of Surgery, Washington University School of Medicine, St. Louis, MO

²Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO

³Department of Medicine, Washington University School of Medicine, St. Louis, MO

Abstract

Organ transplantation, an accepted treatment for end stage organ failure, is often complicated by allograft rejection and disease recurrence. In this review we will discuss the potential role of microRNAs in allograft immunity especially leading to rejection of the transplanted organ. microRNAs (miRNAs), originally identified in *C. elegans*, are short non-coding 21–24 nucleotide sequences that bind to its complementary sequences in functional messenger RNAs and inhibits post-translational processes through RNA duplex formation resulting in gene silencing (Lau et al., 2001). Gene specific translational silencing by miRNAs regulates pathways for immune responses such as development of innate immunity, inflammation, T-cell and B-cell differentiation and signaling that are implicated in various stages of allograft rejection. miRNAs also play a role in development of post-transplant complications like fibrosis, cirrhosis, carcinogenesis often leading to graft loss and poor patient outcome. Recent advancements in the methods for detecting and quantifying miRNA in tissue biopsies, as well as in serum and urine samples, has led to identification of specific miRNA signatures in patients with allograft rejection and have been utilized to predict allograft status and survival. Therefore, miRNAs play a significant role in post-transplant events including allograft rejection, disease recurrence and tumor development impacting patient outcome.

Introduction

Organ transplantation is the choice for treatment of end stage organ failure. The major challenge faced by transplant recipients is allograft rejection and disease recurrence. microRNAs (miRNAs) are short non-coding 21–24 nucleotide sequences that inhibits protein synthesis by targeting messenger RNAs in a sequence specific manner (Lau et al., 2001). Gene specific translational silencing by miRNAs regulates pathways for various inflammatory responses, immune cells differentiation and signaling, development of

© 2012 Elsevier Inc. All rights reserved.

Manuscript correspondence: T. Mohanakumar, Ph.D., Washington University School of Medicine, Department of Surgery, Campus Box 8109-3328 CSRB, 660 S. Euclid Avenue, St. Louis, MO 63110, kumart@wustl.edu, 1-314-362-8463 (office), 1-314-747-1560 (fax).

Conflict of Interest: The authors declare that there are no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

immunity and molecular pathways for allograft rejection (Harris et al., 2010; Lodish et al., 2008). miRNAs have been shown to influence Toll-like Receptor Signaling, inflammatory gene expression, T-cell and B-cell differentiation, lineage specificity and development of post-transplant complications like fibrosis, cirrhosis, carcinogenesis and graft loss. Genome-wide gene expression analysis using microarrays have led to identification of specific miRNA signatures in patients with allograft rejection and utilized to predict allograft status and patient outcome. Significant advancements have been made in methods for detecting and quantify miRNA in tissue biopsies as well as in serum and urine samples obtained from patients (Chaudhuri and Chatterjee, 2007). Artificial modulation of miRNAs can be achieved by delivering short RNA sequences that mimics mature miRNAs or chemically engineered miRNA antagonists (antagomirs) that inhibits miRNA activity in cells (Krutzfeldt et al., 2005; Stenvang and Kauppinen, 2008).

All of the above clearly demonstrates that miRNAs have a tremendous potential to be used as biomarkers for detection of post-transplant events including allograft rejection, disease recurrence and tumor development leading to patient outcome. The ability to detect circulating miRNAs also provides an effective means for non-invasive detection and diagnosis in patients with high risk of graft loss. Development of miRNAs mimics, that functions as mature miRNAs and antagomirs that inhibits specific miRNAs have also potential in use of miRNAs in development of therapeutics. In this review we will discuss biogenesis and detection methods of miRNAs in tissue samples and in circulation and their role in modulation of various immune responses following solid organ transplantation.

miRNA: its biogenesis and their mechanism of action

In recent years non-coding RNAs that includes catalytic, structural or regulatory RNAs have been shown to regulate expression of genes involved in various cellular processes (Eddy, 2001). miRNAs are short nucleotide sequences (21–24 nucleotides) originally identified in *C. elegans* (Lau et al., 2001). These short RNA sequences bind to its complementary sequences in functional messenger RNAs and inhibits post-translational processes through RNA duplex formation resulting in gene silencing (Lai, 2002; Lee et al., 1993) thereby play vital role in cellular mechanisms including cell cycle progression, signaling, metabolism, immune regulation, apoptosis and various diseases. To date several hundred of miRNAs have been identified and are conserved in wide range of organisms from nematodes to humans (Grosshans and Filipowicz, 2008; Lee et al., 2007).

miRNA genes are transcribed in the nucleus by RNA polymerase II as several kilobases long nascent primary miRNAs and processed to form precursor miRNAs (~70 base pairs) (Cai et al., 2004; Lee et al., 2003). The primary miRNAs are cleaved by Drosha, a double stranded RNA binding RNase III-like enzyme in the nucleus to generate precursor miRNAs with a stem-loop structure (Lee et al., 2002). This precursor miRNA is exported to the cytoplasm by the RanGTP dependent double stranded RNA binding nuclear export factor Exportin-5 (Bohnsack et al., 2004; Yi et al., 2003). In the cytoplasm another RNase III-like endonuclease Dicer cleaves the precursor miRNA stem loop to generate 21–25 nucleotide long mature double stranded miRNA duplex (Hutvagner et al., 2001; Tijsterman and Plasterk, 2004). For target specific translational repression one of the miRNA strands is degraded by ATP-dependent RNA helicase generating the single stranded miRNA which is incorporated into the RNA-induced silencing complex (RISC) (Schwarz et al., 2003). The single stranded miRNA binds to the 3' untranslated region of messenger RNA to repress translation into proteins (Filipowicz et al., 2008). Thus, biogenesis of miRNA involves coordination of multiple regulatory complexes that confers specific and effective silencing of their target genes. A schematic representation of miRNA biogenesis is illustrated in figure 1.

miRNA detection and methods for functional analysis

miRNAs can be successfully detected and analyzed in different type of cells, tissues, organ biopsies, urine, serum and peripheral blood. One of the earlier methods employed for detection of miRNAs in small organs such as zebrafish embryos and early stage mouse embryos was *in-situ* hybridization. In this method, thin tissue sections were prepared and hybridized with heat resistant modified oligonucleotides called locked nucleic acids (LNAs) (Braasch and Corey, 2001). Tissue specific expression of more than a hundred miRNAs, which are conserved between different vertebrates were detected from intact embryos and tissue sections using digoxigenin-labeled LNA probes (Tuddenham et al., 2006; Wienholds et al., 2005). Northern blot analysis has also been widely employed to detect both the mature and precursor miRNAs and has been modified to increase sensitivity by using LNAs (Lagos-Quintana et al., 2001; Valoczi et al., 2004). The mechanisms for miRNA processing and biogenesis was determined using RNase protection assay, where an *in-vitro* synthesized labeled RNA probe was hybridized to its complementary sequences (Lee et al., 2002). Primer extension technique was used to demonstrate processing of primary miRNA to precursor miRNA by the nuclear RNase III Drosha enzyme (Lee et al., 2003). The ability of the hairpin ribozymes to perform fluorophore-labeled RNA cleavage has also been utilized to quantitatively detect selected miRNAs from a RNA pool (Hartig et al., 2004). Other advanced methods for miRNA detection includes direct quantitative analysis of multiple miRNAs (Dodgson et al., 2012), capillary electrophoresis with laser-induced fluorescence (Chang et al., 2008) and protein-facilitated affinity capillary electrophoresis (Khan et al., 2011).

High throughput Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Quantitative Real Time RT-PCR has been widely used to determine the expression of miRNA. Schmittgen et al for the first time used gene specific primers and reverse transcriptase enzyme to convert miRNA precursors to cDNA and quantified using PCR (Schmittgen et al., 2004). This technology has been further modified for development of high throughput analysis of genomewide expression pattern of miRNAs using microarray analysis platforms (Krichevsky et al., 2003; Liu et al., 2004). Circulating miRNAs were also successfully detected in peripheral blood and urine and has been used as biomarker for diseases (Mitchell et al., 2008; Scian et al., 2011).

Various databases and computational programs have been developed for known miRNAs and prediction of their potential targets (Chaudhuri and Chatterjee, 2007; Sethupathy et al., 2006; Watanabe et al., 2007). The miRBase database (<http://www.mirbase.org>) provides an online repository and integrated interfaces for comprehensive miRNA sequences, annotations and predicted gene targets in wide range of organisms (Griffiths-Jones et al., 2006). Another reliable resource is available at <http://targetscan.org/> for mammalian miRNA target prediction (Lewis et al., 2005; Lewis et al., 2003). Advancement of the miRNA detection methods and target analysis have played a vital role in elucidating molecular pathways for various cellular mechanisms involved both physiologically and in diseases.

Role of miRNAs in inflammation and innate immunity

miRNAs are known to regulate expression of genes involved in various cellular mechanisms including inflammatory responses. The Toll-like Receptors (TLRs) are transmembrane proteins that recognize structurally conserved molecules derived from pathogens during an infection and activate the innate immunity cascade (Medzhitov et al., 1997). Recognition of the pathogenic ligands by the TLRs initiates a wide spectrum of inflammatory and immune responses including phagocytosis and production of cytokines. Expression of miRNA-147, miRNA-146a/b, miRNA-132 and miRNA-155 were induced by bacterial

Lipopolysaccharides (LPS) and miRNA-146a/b down regulated expression of IL-1 Receptor-Associated Kinase 1 (IRAK1) and TNF receptor-associated factor 6 which are components of the TLR4 signaling cascade (Kopp and Medzhitov, 1999; Liu et al., 2009; Taganov et al., 2006). NF Kappa B (NFKB) regulated miRNA-146 expression to establish a control of TLR-mediated cytokine signaling through a negative feedback regulation loop (Taganov et al., 2006). Similarly, LPS induced expression of miRNA-21 in a NFKB dependent manner that led to down regulation of the pro-inflammatory protein programmed cell death 4 that resulted in a negative feedback regulation of NFKB and increase in anti-inflammatory Interleukin-10 (IL10) production (Sheedy et al., 2010). LPS also induced miRNA-210 and miRNA-125a in macrophages. miRNA-210 inhibited NFKB to establish a negative feedback loop for LPS-induced excessive pro-inflammatory responses by down regulating expression of IL-6 and TNF- α (Qi et al., 2012). LPS stimulation in macrophages down regulated expression of miRNA-34a, that directly targeted NOTCH1 and NFKB, to regulate the production of inflammatory cytokines like TNF- α and IL-6 (Jiang et al., 2012).

Studies have shown that TLR signaling also regulated expression of several miRNAs involved in inflammatory responses (O'Neill et al., 2011). Kaposi's sarcoma-associated herpesvirus miRNA-K9 targeted TLR signaling components such as IRAK1 and Myeloid Differentiation Primary Response Protein 88 (MYD88) to reduce NFKB mediated inflammatory cytokine expression (Abend et al., 2012). miRNA-200b and miRNA-200c also decreased expression of MYD88 as well as NFKB activity to modulate TLR4 signaling cascade (Wendlandt et al., 2012). This miRNA mediated silencing of MYD88 resulted in impairment of LPS-induced expression of the pro-inflammatory cytokines like IL-6, CXCL9 and TNF- α (Wendlandt et al., 2012). LPS induction resulted in up regulation of several miRNAs including miRNA-155 that targeted TGF-beta Activated kinase 1/MAP3K7 Binding protein 2 (TAB2) to modulate TLR/Interleukin-1 inflammatory pathway in dendritic cells (Ceppi et al., 2009). LPS induced expression of v-Akt murine thymoma viral oncogene homolog 1 up regulated miRNA-let7 that targeted TLR4 and down regulated miR-155 that targeted Suppressor of Cytokine Signaling 1 (SOCS1), both proteins shown to be involved in TLR signaling (Androulidaki et al., 2009). Studies have shown that miRNA-let7 down regulated expression of IL6 (Iliopoulos et al., 2009), miRNA-125b targeted TNF α (Tili et al., 2007) and miRNA-16 targeted LPS-induced production of IL-8, IL-6, and IL-1 α by modulating NFKB (Zhou et al., 2012). Transactivation of the IL-8 gene by miRNA-16 was achieved by targeting silencing mediator for retinoid and thyroid hormone receptor that altered NFKB activity upon LPS induction (Zhou et al., 2012). We have found that miRNA-449a is down regulated in Hepatitis C Virus (HCV) patients compared to non-HCV patients and controls. Molecular pathways analyses have shown that expression of inflammatory genes including Chitinase-3-like protein 1 (CHI3L1) and Chemokine (C-C motif) ligand 2 (CCL2) were regulated by miRNA-449a following HCV infection (Submitted for publication). miRNA-449a targets NOTCH1 for post-transcriptional silencing which regulates functions of DNA binding transcription factor NFKB/P65 to modulate CHI3L1 expression. Similarly, miRNA-449a also regulates components of the IL-6 receptor pathway to modulate expression of CCL2 in response to HCV infection (our unpublished data). It is evident from these studies that miRNAs both positively (miRNA-155) and negatively (miRNA-146, miRNA-21) regulate important events in the inflammatory cascades.

Modulation of miRNAs following organ transplantation

The major challenge faced by the transplant recipients is allograft rejection and recurrence of diseases including tumor development. Various cellular and immunological mechanisms regulate the process of organ rejection and there are emerging evidence indicating that miRNAs play a vital role in these processes.

a) Role for miRNAs in liver transplantation

HCV infection and its complications, such as fibrosis, cirrhosis, hepatic failure and hepatocellular carcinoma (HCC), are major health problems in the United States and worldwide (Alter et al., 1999; Seeff, 2002). Liver transplantation is the most effective treatment option for patients with end stage liver disease. However, HCV recurrence following liver transplantation results in development of cirrhosis in 20–30% of patients within the first five years following the transplant (Gane, 2008; Rodriguez-Luna and Douglas, 2004). HCC commonly occurs on a background of cirrhosis and is detected in about 90% of HCC cases (Okuda, 1992). Although surgical resection and liver transplantation are common treatment options for liver cancer, recurrence or metastasis is very frequent and the survival rate is 55% to 65% in 5 years post-transplantation (Thuluvath et al., 2009).

miRNAs play a significant role in HCV infection, fibrosis, cirrhosis and development of HCC in the graft post-liver transplant. Role of miRNAs in the development of liver fibrosis has been an area of high clinical significance (He et al., 2012b; Jiang et al., 2010). Expression levels of miRNA-199a, miRNA-200a, and miRNA-200b were shown to have positive correlation with degree of hepatic fibrosis in both human and mouse samples (Murakami et al., 2011). miRNA-146a regulated TGF β mediated induction of hepatic stellate cells which are known to play a major role in the development of hepatic fibrosis (He et al., 2012a). miRNA-122, abundantly expressed in livers, binds HCV RNA to facilitate replication and protects the HCV viral genome from degradation by the host mRNA decay mechanisms (Jopling et al., 2005; Shimakami et al., 2012). Similarly miRNA-199 and miRNA-let7B target HCV RNA and regulate its replication (Cheng et al., 2012; Murakami et al., 2009). A genome-wide microarray analysis identified 86 miRNAs that were up regulated and 82 miRNAs that were down regulated in HCC patients who had developed HCC recurrence compared to non-recurrent patients post-liver transplant (Han et al., 2012b). In these patients, significant up regulation in expression was observed for miRNA-147, miRNA-21, miRNA-155, miRNA-10b, miRNA-338 and miRNA-597 and down regulation was noted for miRNA-19a, miRNA-886-5p, miRNA-126, miRNA-223, miRNA-24, miRNA-203, miRNA-106a, miRNA-27a, miRNA-205, miRNA-17, miRNA-122 and miRNA-20a (Han et al., 2012b). miRNA-155, a positive regulator of inflammatory responses, was up regulated by HCV infection as well as elevated in tumor tissues obtained from patients with HCC recurrence post-liver transplant suggesting that HCV induced up regulation of miRNA-155 also induced HCC (Han et al., 2012a; Zhang et al., 2012). Using a genome-wide microarray analysis we have identified modulation of 45 miRNAs (23 up regulated and 22 down regulated) in the liver of HCV patients compared to controls (submitted for publication). miRNA-200c, miRNA-31, miRNA-597 were up regulated and miRNA-592, miRNA-128b, miRNA-107 were noted to be down regulated in HCV infected livers compared to the controls. We have also identified significant modulation of miRNAs in HCC patients. miRNA-183 and miRNA-96 were up regulated and miRNA-449a and miRNA-323 were down regulated in HCC patients compared to the controls (unpublished data). These studies suggest the miRNA expression pattern can be used to assess the risk of HCC development and predict the survival of the patient following liver transplantation for HCC (Barry et al., 2012; Han et al., 2012b; Villanueva et al., 2010). Expression of valosin containing protein/p97, which has been correlated with increased incidence of HCC recurrence, was found to be directly targeted by miRNA-129-5p thereby regulating the tumor progression (Liu et al., 2012). Expression of miRNA-203 was down regulated in patients with post-liver transplant HCC recurrence compared to patients with non-recurrence and can be of importance in predicting patient outcome (Chen et al., 2012a). miRNA-15b, miRNA-17-5p, miRNA-92, miRNA-20a, miRNA-18a, miRNA-34c and miRNA-361 were also found to be associated with HCC recurrence and miRNA-15b showed

most significant negative correlation with recurrence (Chung et al., 2010). Taken together, these miRNAs influence various molecular pathways involved in allograft rejection and recurrence of the diseases including viral infections, fibrosis and development of hepatocarcinogenesis.

b) miRNAs in lung transplantation

Chronic allograft rejection clinically diagnosed as Bronchiolitis Obliterans Syndrome (BOS) has remained a major setback following lung transplantation. BOS results in progressive airflow obstruction affecting up to 65% of patients who survive 5 years post-surgery, regardless of the transplant procedure (Boehler and Estenne, 2000; Boehler and Estenne, 2003). Another major problem following lung transplantation is bronchogenic carcinoma which results from high dose of immunosuppression and previous history of obstructive pulmonary diseases or idiopathic pulmonary fibrosis, and smoking (Minai et al., 2008; Raviv et al., 2011; Robbins and Arcasoy, 2011). Although no definitive studies are currently available towards elucidating the role of miRNAs in development of BOS post-lung transplantation, several recent advancements have been made on use of miRNAs as diagnostic tools for development of lung cancer in transplant recipients.

miRNAs are implicated in different stages of development of lung cancers and the ability to detect differential modulation of miRNAs has the potential to improve patient outcome following lung transplantation. Our preliminary studies have identified miRNA-144, implicated in the TGF- β signaling cascade, being up regulated in biopsies from BOS positive lung transplant recipients compared to the controls suggesting that this miRNA may play a role in the fibrosis and occlusion of small airways seen in BOS following human lung transplantation (our unpublished data). Expression level of miRNA-1254 and miRNA-574-5p was significantly up regulated in the early-stage non-small cell lung cancer (NSCLC) in serum samples obtained from patients compared to the controls (Foss et al., 2011). Differences in expression of these two miRNAs were detected several months before clinical diagnosis of NSCLC with 82% sensitivity and 77% specificity and thus can be useful as minimally invasive biomarkers (Foss et al., 2011). Differential expression levels of miRNA-152, miRNA-20a, miRNA-199a-5p, miRNA-24, miRNA-222, miRNA-221, miRNA-145, miRNA-25, miR-223 and miRNA-320 and their correlation with progressive stages of NSCLC were detected 33 months before clinical diagnosis in serum samples obtained from patients (Chen et al., 2012b). miRNA-126 and miRNA-183 were down regulated in serum samples obtained NSCLC patients compared to the controls and can serve as potential biomarkers (Lin et al., 2012). Expression levels of these miRNAs showed significant differences between the stage I/II patients and stage IV patients, but not between the controls and stage I/II patients suggesting their potential as excellent biomarkers to distinguish between different stages of cancer progression (Lin et al., 2012). miRNA-146b, miRNA-221, miRNA-let-7a, miRNA-155, miRNA-17-5p, miRNA-27a and miRNA-106a were down regulated and miRNA-29c was up regulated in the serum of NSCLC patients (Heegaard et al., 2012). Based on these results it is evident that miRNAs play an important role in development of cancers post-lung transplantation and determination of their expression levels can be of tremendous importance in improving patient outcome.

c) miRNAs in kidney transplantation

Viral infections, renal fibrosis, recurrence of the original disease and graft rejections are common problems following kidney transplantation. Modulation of miRNAs has also been demonstrated in kidney allograft rejections. In one study, miRNA-let-7c, miRNA-10a, miRNA-10b, miRNA-125a, miRNA-200a, miRNA-30a-3p, miRNA-30b, miRNA-30c, miRNA-30e-3p and miRNA-32 were shown to be down regulated in biopsies obtained from patients with acute rejection compared to normal allograft biopsies (Anglicheau et al., 2009).

In the same biopsies miRNA-142-5p, miRNA-142-3p, miRNA-155, miRNA-223, miRNA-146b, miRNA-146a, and miRNA-342 were up regulated. A similar miRNA expression analysis in patients with acute rejection of renal allografts miRNA-324-23p, miRNA-611, miRNA-330, miRNA-524, miRNA-17-3p, miRNA-483, miRNA-663, miRNA-516-5p, miRNA-326, miRNA-197 and miRNA-346 were found to be down regulated while miRNA-658, miRNA-125a, miRNA-320, miRNA-381, miRNA-628, miRNA-602, miRNA-629 and miRNA-125a were found to be up regulated compared to normal controls (Sui et al., 2008). Differential regulation of 56 miRNAs including miRNA-142-3p, miRNA-204, miRNA-107, miRNA-211, miRNA-32, miRNA-142-3p, miRNA-204 and miRNA-211 was observed for chronic allograft dysfunction patients with interstitial fibrosis and tubular atrophy (Scian et al., 2011). Studies in mouse models of renal transplantation have also showed up regulation of miRNA-21 and down regulation of miRNA-29 by SMAD3/TGF β pathways which result in development of renal fibrosis (Qin et al., 2011; Zhong et al., 2011). In mice, miRNA-192, induced by TGF- β 1, increased collagen synthesis by targeting the E-box repressors Zeb1/2 and inhibition of miRNA-192 significantly increased Zeb1/2 and decreased Collagen, TGF- β , and Fibronectin expression and reduced development of renal fibrosis (Putta et al., 2012). Thus, modulation of miRNAs post-kidney transplantation has significant impact in development of renal fibrosis and allograft rejection and carcinoma.

miRNA as biomarkers for allograft rejection

miRNAs have been implicated in regulation of inflammation, innate immunity and signaling mechanisms involved in allograft rejections. Recent advancements in detection methods for circulating miRNAs present in the serum have become a powerful tool in performing minimally invasive diagnosis of post-transplant events. Differential expression of miRNAs in the circulation and in organ biopsies has been shown to correlate positively with the allograft status.

a) miRNAs in tissue biopsies predict allograft status

miRNA-122 and miRNA-148a are associated with liver injury and acute rejection following liver transplantation and can be used as effective biomarkers for tissue injury (Farid et al., 2012). miRNA expression patterns has been shown to predict outcomes of HCC patients following liver transplantation (Han et al., 2012b). Using the genome-wide microarray analysis, we have identified several miRNAs (>50) differentially regulated in HCV induced HCC patients compared to HCV infected non-HCC patients. Expression levels of miRNA-587, miRNA-198, miRNA-449a and miRNA-323 were found to be down regulated and miRNA-183 and miRNA-96 levels were up regulated compared to normal livers (our unpublished data). Differential expression levels of these miRNAs showed positive correlation with occurrence of HCC in liver transplant recipients.

It has been shown that during kidney transplant rejection 17 miRNA were significantly modulated. Expression of 10 miRNAs were up regulated and 7 were down regulated in biopsies obtained from patients with acute rejection compared to normal allograft biopsies (Anglicheau et al., 2009). Another study has shown that 20 miRNAs were differentially expressed (12 down regulated and 8 up regulated) in patients with acute renal allograft rejection compared to normal controls (Sui et al., 2008). Modulation of miRNAs was also observed in animal models of renal ischemia reperfusion injury which was significantly associated with morbidity and mortality following renal transplantation (Godwin et al., 2010; Shapiro et al., 2011).

Modulation of miRNA also been reported in acute rejections following small bowel transplantations and it has been suggested that miRNAs play a significant role in cellular

infiltration during rejection (Asaoka et al., 2012). In murine cardiac transplantation, during allograft rejections miRNA-182 was shown to be significantly up regulated in both peripheral blood mononuclear cells and plasma (Wei et al., 2012). Other studies in heart transplant models demonstrated differential regulation of 82 miRNAs compared to controls (Wang et al., 2010). In an islet transplant rat model, 26 miRNAs were differentially regulated in an NF κ B dependent manner and these miRNAs potentially targeted expression of 238 genes involved in various cellular pathways (Bravo-Egana et al., 2012). Determination of miRNA expression levels in various tissues obtained from patients has serious implications in determination of the status of the renal allograft and possibility for long-term survival.

b) Circulating miRNAs

Biomarker analysis in tissue samples or biopsies obtained from patients is the most favored choice for diagnosis of various complications post-transplant. However, this is often met with the challenges associated with obtaining tissue samples and availability of normal controls for comparison. The alternate approach is utilization of body fluids such as the serum from peripheral blood, urine or saliva obtained from patient or controls for diagnosis. These samples can be easily obtained and can be used for variety of diagnostics and biomarker assays that can also accurately detect immune response to any allograft and its status (Hartono et al., 2010; Heidt et al., 2011). miRNAs circulating in the extracellular fluids are extremely stable and protected from RNAase mediated degradation. Advancement in the detection techniques and stability of these circulating miRNAs have high potential to be used as biomarkers for allograft rejection and subsequent diseases (Etheridge et al., 2011).

Serum hepatocytes derived miRNAs were shown to be potential biomarkers of hepatic injury and acute rejection post-liver transplantation (Farid et al., 2012). Increased expression of miRNA-155, miRNA-122, miRNA-125b and miRNA-146a was detected in serum samples obtained from HCV patients and can indicate inflammation induced hepatocyte damage (Bala et al., 2012). miRNA-21, up regulated in both HCV infection and HCC, was also detected in serum samples obtained from patients and can be used as a biomarker for necroinflammation (Bihrer et al., 2011). Circulating levels of miRNA-122, miRNA-34a and miRNA-16 were shown to be significantly higher in HCV patients and determination of their levels in patients can be used as non-invasive biomarkers for diagnosis (Cermelli et al., 2011).

miRNAs isolated from serum have also been used as effective biomarkers to detect NSCLC months prior to clinical diagnosis in lung cancer patients (Chen et al., 2012b; Foss et al., 2011; Heegaard et al., 2012; Lin et al., 2012). Circulating miRNA-182 level was found to be elevated in both peripheral blood mononuclear cells and plasma in a cardiac graft rejection model indicating its potential as a biomarker for predicting allograft status (Wei et al., 2012). In renal transplant patients with acute rejection, miRNAs are successfully detected in urine samples. Expression analysis of miRNAs isolated from urine samples showed that miRNA-10b and miRNA-210 were down regulated and miRNA-10a was up regulated in renal transplant patients with T-cell mediated acute rejection compared to the controls (Lorenzen et al., 2011b). Differential expression of 56 miRNAs was detected in urine samples obtained from patients with chronic allograft dysfunction with interstitial fibrosis and tubular atrophy following transplantation (Scian et al., 2011). In acute kidney injury patients, circulating levels of miRNA-16 and miRNA-320 were down regulated and miRNA-210 was up regulated compared with healthy controls, suggesting its potential use as a novel biomarker for kidney diseases (Lorenzen et al., 2011a). In renal cell carcinoma patients, levels of circulating miRNA-451 was decreased and miRNA-378 was increased in serum compared to healthy controls (Redova et al., 2012). Therefore, monitoring levels of circulating miRNAs in patients can predict the status of the kidney allograft and patient

outcome. Thus, circulating miRNAs can prove to be very important non-invasive diagnostic tool in predicting immune responses to the transplanted livers and lungs, its survival and patient outcome.

Putative mechanisms by which miRNAs regulate allograft status

Following transplantation, the organ induces an immune response because of mismatches in cell surface proteins, most important being the major histocompatibility complexes/human leukocyte antigens. miRNAs play an important role in immune regulation, differentiation of immune cells and the lineage commitments following an immune response and therefore is likely to be involved in transplant rejection. miRNAs has been shown to modulate intracellular communication between antigen presenting cells (APC) such as dendritic cells (DC), macrophages and B-cells and T-cells.

a) Regulation of monocytes and dendritic cells by miRNAs

After an organ transplant, APCs such as DC present both in the host as well as the allograft are activated and initiates the process of graft rejection by presenting alloantigens to the T-cells (Afzali et al., 2008). Interestingly, DC play a dual role in both activating the graft rejection process as well as induction of graft tolerance (Morelli and Thomson, 2007). Differential expression of miRNAs plays an important role in differentiation of monocytes into immature DC and mature DC at different stages (Lu et al., 2011; Tserel et al., 2011). Down regulation of miRNA-7-5p, miRNA-20a and miRNA-106a, that targets AML1 were shown to be associated with the monocytic differentiation process (Fontana et al., 2007). miRNA-21 was up regulated in macrophages and monocytes in response to inflammation and negatively regulated IL-3 regulated IL-12p35 expression (Lu et al., 2009b). Increased expression of miRNA -155 and decreased expression of miRNA-221 regulated DC maturity by targeting the cell cycle inhibitor p27kip1 (Lu et al., 2011). Increased levels of miRNA-511, that positively regulates TLR-4 signaling, and miRNA-99b that potentially targets SMAD7 were also shown to impact DC differentiation (Tserel et al., 2011). miRNA-155 was shown to participate in DC maturation by directly targeting PU.1, a protein involved in expression of DC-specific intercellular adhesion molecules (Martinez-Nunez et al., 2009). In DC miRNA-155 was found to be also part of a negative feedback loop that targeted TLR/interleukin-1 (TLR/IL-1) inflammatory pathway and signal transduction molecule TAK1-binding protein TAB2 (Ceppi et al., 2009). In animal models of small intestine transplantation, TLR4 expression was shown to be up regulated and implicated in development of allograft rejection (Krams et al., 2010). These studies suggest that miRNAs influence the pathways for DC phenotype and differentiation and mediates their function in development of graft rejection as well as graft tolerance.

b) Regulation of B-cells by miRNAs

B-cells play a wide range of roles in mediating allograft rejection starting from antibody secretion, serving as APCs leading to activation of antigen-specific T- cells and production of cytokines. miRNAs play a pivotal role in mediating these B-cell responses. Modulation of a large number of miRNAs regulates every step in differentiation of mature B-cells (Zhang et al., 2009). Studies have shown that expression of miRNA-181 increased production of B-lymphoid lineage cells both *in-vivo* and *in-vitro* and expression of miRNA-150 impaired formation of mature B-cells (Chen et al., 2004; Zhou et al., 2007). miRNA-155, that targets PU.1 for silencing, was found to be required by B-cells for high-affinity antibody production and immunoglobulin class switching (Vigorito et al., 2007). Expression of miRNA-23a cluster, a downstream target of PU.1, decreased in B-lymphopoiesis and increased myelopoiesis (Kong et al., 2010). miRNA-17-92 cluster was shown to regulate B-cell proliferation, development and immunoglobulin rearrangement by targeting Bim and PTEN

(Xiao et al., 2008). Elevated expression of miRNA-17-92 in mice resulted in development of autoimmunity (Xiao et al., 2008). Thus miRNAs control the regulatory pathways for B-cell differentiation and immunoglobulin class switching leading to development of immune response to allografts.

c) Regulation of T-cells by miRNAs

T-cell mediated allograft rejection is effected by the CD8+ cytotoxic T-lymphocytes (CTLs) and CD4+ T-helper (Th) cells. Like B-cells, T-cells also originate from hematopoietic stem cells in the bone marrow and miRNAs play a significant role in each stage of T-cell development (Wu et al., 2007). miRNA-181 was shown to be highly abundant in thymocytes (CD4+CD8+) prior to differentiation into CTLs and Th cells and targeted CD69, Bcl-2, and the T-cell receptors which are involved in positive selection (Neilson et al., 2007). Expression of miRNA-150 was down regulated in naïve T-cells and gradually increased with progression of T-cell maturation (Ghisi et al., 2011). miRNA-214 was shown to promote activation of T-cells after stimulation by targeting the negative regulator PTEN (Jindra et al., 2010). miRNA-155 was up regulated in patients with acute graft-versus-host disease and blocking miRNA-155 using anti-miRNA-155 decreased the disease severity and prolonged survival in a allogeneic hematopoietic stem cell transplant mouse model (Ranganathan et al., 2012). miRNA-155 was also shown to participate in T-cell responses by regulating Th1/Th2 cell differentiation and inhibition of miRNA-155 in T-cells resulted in impairment of IL-2 and IFN- γ production and cells differentiate into Th2 cells (Rodriguez et al., 2007). This resulted in increased production of IL-4, IL-5 and IL-10 by the Th2 cells. On the other hand, increased expression of miRNA-155 resulted in increased production of IL-2 and IFN- γ and shift in differentiation towards Th1 cells (Banerjee et al., 2010). IL-2 was shown to induce expression of miRNA-182 that targeted Foxo1 for silencing, a transcription factor expressed in the resting stage that suppresses proliferation of Th cells, thereby promoting switching of T-cells from a resting state to clonal expansion upon stimulation (Stittrich et al., 2010). Mice expressing miRNA-17-92 developed lymphoproliferative disease, autoimmunity and premature death possibly by suppressing expression of its targets PTEN and Bim (Xiao et al., 2008).

Regulatory T-cells (Tregs) also control immune responses, facilitate maintenance of peripheral tolerance to self-antigens and play an important role in inhibition of transplant rejection. Mice lacking Dicer, an RNase III-like endonuclease that cleaves precursor miRNAs into mature miRNA, developed fatal autoimmunity similar to a Foxp3 knock out phenotype suggesting their role in Treg regulation (Liston et al., 2008; Zhou et al., 2008). Dicer deficient T-cells were shown to be unstable, lacked expression of Foxp3 and exhibited altered expression of several Treg associated genes such as Neuropilin 1, glucocorticoid-induced tumor necrosis factor receptor, and CTL antigen 4 (Zhou et al., 2008). Foxp3 regulated elevated levels of miRNA-155 and its deficiency in Tregs led to increased expression of SOCS1 and decreased activation of Signal Transducer and Activator of Transcription 5 (Stat5) (Lu et al., 2009a). Expression of miRNA-146a was elevated in Tregs and its deficiency resulted in increased production of pro-inflammatory IFN γ , impaired their function and ability to preserve immunological tolerance leading to autoimmune diseases (Lu et al., 2010). Antibody mediated blocking of IFN γ prevented these autoimmune diseases in mice carrying miRNA-146a deficient Tregs and can be attributed to increased expression of miRNA-146a target Stat1 and decreased expression of SOCS1, a negative regulator of Stat1 phosphorylation in the IFN γ receptor signaling pathway (Lu et al., 2010).

Taken together, it is evident that regulation by miRNAs are essential for each stages of T-cell differentiation, maturity, lineage commitments and development of allograft rejection as well as Treg mediated preservation of self-tolerance to the transplanted organ.

Therapeutic implications of miRNAs in organ transplantation

miRNA regulation has been shown to impact major signaling pathways including onset of allograft rejections. Significant progress has been made in understanding miRNA regulatory pathways implicated in immune responses and therefore development of therapeutics based on miRNAs are feasible. Advancement of high throughput techniques such as microarray analysis enables determination of the miRNA signatures in both donor and recipients that in turn may provide biomarkers for allograft rejections. Analysis of either or both circulating and tissue specific miRNA levels can be extremely important in future diagnostic or prognostic evaluation of allograft rejection. Organ rejection also involves coordination of various cellular pathways including graft infiltration by components of innate and adaptive immune system and inflammation. Since miRNAs play an essential role in execution of these signaling cascades it is reasonable to predict that miRNAs can be a viable therapeutic target. The expression levels of miRNAs can be modulated through various methods that can regulate expression of the target genes, thereby inhibiting favorable pathways for organ rejection. The expression levels of specific miRNAs can be increased by introducing synthetic single stranded RNAs mimics, that exhibits gene targeting activity by mimicking mature endogenous miRNA (Chorn et al., 2012). These miRNA mimics were shown to be incorporated into the RISC complex and significantly down regulated target gene expression (Guennewig et al., 2012). Significant progresses have also been made in development of chemically engineered miRNA antagonists (antagomirs) that inhibits its activity. Antagonists specific for miRNA-16, miRNA-122, miRNA-192 and miRNA-194 exhibited specific, efficient and long-lasting silencing of corresponding miRNA levels in various organs including liver, lung, kidney and heart (Krutzfeldt et al., 2005). An increased level of miRNA-21 augmented the extent of interstitial fibrosis and cardiac hypertrophy through the ERK-MAP kinase signaling pathway and silencing of miRNA-21 activity by specific miRNA antagonists reduced the extent of fibrosis (Thum et al., 2008). These miRNA mimics and antagonists can be directly administered or expressed in cells through several gene transfection methods (Krutzfeldt et al., 2005; Scherr et al., 2007; Surdziel et al., 2010). (Qu et al., 2012)

Modulation of miRNA expression levels for therapeutic applications has tremendous potential. Although several methods for delivery of miRNAs mimics of antagomirs exist, their clinical use has not been successfully utilized to prevent allograft rejections. However, expression analysis of miRNAs in various clinical samples in organ transplant recipients provides effective disease specific expression signatures, biomarkers for diagnosis and prognosis of patient outcomes.

Summary

miRNAs, the short nucleic acid sequences, play an important role in various cellular processes including development of immune reactions towards a transplanted organ by specifically inhibiting expression of their target genes. As shown in figure 1, miRNAs are involved in various immunological processes including development of innate immunity, inflammation, T-cell and B-cell mediated immune responses and development of autoimmunity. Most importantly miRNA-155, miRNA-146 and miRNA-181 have been shown to modulate several important immunological responses. Significant advancements have been made in development of methods towards detection of miRNAs in both tissue and serum samples in transplant patients. Modulation of miRNA expression levels in response to an immunological response towards a transplanted organ has positive correlation with development of rejection following transplantation. Recent development in the microarray technology has led to identification of genome-wide changes in the miRNA and their target genes in patient samples. Modulation of a huge numbers of miRNAs has been observed with

patients with allograft rejection or recurrence. These miRNA signatures have tremendous potential to be utilized as biomarkers for organ rejection and predict patient outcome. Although it is in the early stage of development, administration of miRNA mimics and antagomirs have future application as miRNA based therapeutics in the clinical setting.

Acknowledgments

Funding source: NIH HL056643-15A1, NIH HL092514, and Barnes Jewish Children's Foundation (TM). The authors would like to thank Billie Glasscock for her assistance in submitting this manuscript.

References

- Abend JR, et al. KSHV microRNAs target two components of the TLR/IL-1R signaling cascade, IRAK1 and MYD88, to reduce inflammatory cytokine expression. *J Virol*. 2012
- Afzali B, et al. Pathways of major histocompatibility complex allorecognition. *Curr Opin Organ Transplant*. 2008; 13:438–44. [PubMed: 18685342]
- Alter MJ, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med*. 1999; 341:556–62. [PubMed: 10451460]
- Androulidaki A, et al. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. *Immunity*. 2009; 31:220–31. [PubMed: 19699171]
- Anglicheau D, et al. MicroRNA expression profiles predictive of human renal allograft status. *Proc Natl Acad Sci U S A*. 2009; 106:5330–5. [PubMed: 19289845]
- Asaoka T, et al. MicroRNA signature of intestinal acute cellular rejection in formalin-fixed paraffin-embedded mucosal biopsies. *Am J Transplant*. 2012; 12:458–68. [PubMed: 22026534]
- Bala S, et al. Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. *J Transl Med*. 2012; 10:151. [PubMed: 22846613]
- Banerjee A, et al. Micro-RNA-155 inhibits IFN-gamma signaling in CD4+ T cells. *Eur J Immunol*. 2010; 40:225–31. [PubMed: 19877012]
- Barry CT, et al. Micro RNA expression profiles as adjunctive data to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. *Am J Transplant*. 2012; 12:428–37. [PubMed: 22008552]
- Bihrer V, et al. Serum microRNA-21 as marker for necroinflammation in hepatitis C patients with and without hepatocellular carcinoma. *PLoS One*. 2011; 6:e26971. [PubMed: 22066022]
- Boehler A, Estenne M. Obliterative bronchiolitis after lung transplantation. *Curr Opin Pulm Med*. 2000; 6:133–9. [PubMed: 10741773]
- Boehler A, Estenne M. Post-transplant bronchiolitis obliterans. *Eur Respir J*. 2003; 22:1007–18. [PubMed: 14680094]
- Bohnsack MT, et al. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA*. 2004; 10:185–91. [PubMed: 14730017]
- Braasch DA, Corey DR. Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem Biol*. 2001; 8:1–7. [PubMed: 11182314]
- Bravo-Egana V, et al. Inflammation-Mediated Regulation of MicroRNA Expression in Transplanted Pancreatic Islets. *J Transplant*. 2012; 723614. [PubMed: 22655170]
- Cai X, et al. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA*. 2004; 10:1957–66. [PubMed: 15525708]
- Ceppi M, et al. MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. *Proc Natl Acad Sci U S A*. 2009; 106:2735–40. [PubMed: 19193853]
- Cermelli S, et al. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One*. 2011; 6:e23937. [PubMed: 21886843]
- Chang PL, et al. Analysis of BART7 microRNA from Epstein-Barr virus-infected nasopharyngeal carcinoma cells by capillary electrophoresis. *Anal Chem*. 2008; 80:8554–60. [PubMed: 18922020]

- Chaudhuri K, Chatterjee R. MicroRNA detection and target prediction: integration of computational and experimental approaches. *DNA Cell Biol.* 2007; 26:321–37. [PubMed: 17504028]
- Chen CZ, et al. MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 2004; 303:83–6. [PubMed: 14657504]
- Chen HY, et al. miR-203 expression predicts outcome after liver transplantation for hepatocellular carcinoma in cirrhotic liver. *Med Oncol.* 2012a; 29:1859–65. [PubMed: 21786180]
- Chen X, et al. Identification of ten serum microRNAs from a genome-wide serum microRNA expression profile as novel noninvasive biomarkers for nonsmall cell lung cancer diagnosis. *Int J Cancer.* 2012b; 130:1620–8. [PubMed: 21557218]
- Cheng JC, et al. Let-7b is a novel regulator of hepatitis C virus replication. *Cell Mol Life Sci.* 2012; 69:2621–33. [PubMed: 22391672]
- Chorn G, et al. Single-stranded microRNA mimics. *RNA.* 2012
- Chung GE, et al. High expression of microRNA-15b predicts a low risk of tumor recurrence following curative resection of hepatocellular carcinoma. *Oncol Rep.* 2010; 23:113–9. [PubMed: 19956871]
- Dodgson BJ, et al. Detection of a Thousand Copies of miRNA without Enrichment or Modification. *Anal Chem.* 2012; 84:5470–4. [PubMed: 22697284]
- Eddy SR. Non-coding RNA genes and the modern RNA world. *Nat Rev Genet.* 2001; 2:919–29. [PubMed: 11733745]
- Etheridge A, et al. Extracellular microRNA: a new source of biomarkers. *Mutat Res.* 2011; 717:85–90. [PubMed: 21402084]
- Farid WR, et al. Hepatocyte-derived microRNAs as serum biomarkers of hepatic injury and rejection after liver transplantation. *Liver Transpl.* 2012; 18:290–7. [PubMed: 21932376]
- Filipowicz W, et al. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet.* 2008; 9:102–14. [PubMed: 18197166]
- Fontana L, et al. MicroRNAs 17-5p-20a-106a control monocytopoiesis through AML1 targeting and M-CSF receptor upregulation. *Nat Cell Biol.* 2007; 9:775–87. [PubMed: 17589498]
- Foss KM, et al. miR-1254 and miR-574-5p: serum-based microRNA biomarkers for early-stage non-small cell lung cancer. *J Thorac Oncol.* 2011; 6:482–8. [PubMed: 21258252]
- Gane EJ. The natural history of recurrent hepatitis C and what influences this. *Liver Transpl.* 2008; 14(Suppl 2):S36–44. [PubMed: 18825724]
- Ghisi M, et al. Modulation of microRNA expression in human T-cell development: targeting of NOTCH3 by miR-150. *Blood.* 2011; 117:7053–62. [PubMed: 21551231]
- Godwin JG, et al. Identification of a microRNA signature of renal ischemia reperfusion injury. *Proc Natl Acad Sci U S A.* 2010; 107:14339–44. [PubMed: 20651252]
- Griffiths-Jones S, et al. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res.* 2006; 34:D140–4. [PubMed: 16381832]
- Grosshans H, Filipowicz W. Molecular biology: the expanding world of small RNAs. *Nature.* 2008; 451:414–6. [PubMed: 18216846]
- Guennewig B, et al. Properties of N(4)-methylated cytidines in miRNA mimics. *Nucleic Acid Ther.* 2012; 22:109–16. [PubMed: 22373008]
- Han ZB, et al. Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. *J Cancer Res Clin Oncol.* 2012a; 138:153–61. [PubMed: 22071603]
- Han ZB, et al. Identification of recurrence-related microRNAs in hepatocellular carcinoma following liver transplantation. *Mol Oncol.* 2012b; 6:445–57. [PubMed: 22552153]
- Harris A, et al. MicroRNAs as immune regulators: implications for transplantation. *Am J Transplant.* 2010; 10:713–9. [PubMed: 20199506]
- Hartig JS, et al. Sequence-specific detection of MicroRNAs by signal-amplifying ribozymes. *J Am Chem Soc.* 2004; 126:722–3. [PubMed: 14733539]
- Hartono C, et al. Noninvasive diagnosis of acute rejection of renal allografts. *Curr Opin Organ Transplant.* 2010; 15:35–41. [PubMed: 19935064]
- He Y, et al. MicroRNA-146a modulates TGF-beta1-induced hepatic stellate cell proliferation by targeting SMAD4. *Cell Signal.* 2012a; 24:1923–30. [PubMed: 22735812]

- He Y, et al. The potential of microRNAs in liver fibrosis. *Cell Signal*. 2012b
- Heegaard NH, et al. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. *Int J Cancer*. 2012; 130:1378–86. [PubMed: 21544802]
- Heidt S, et al. Peripheral blood sampling for the detection of allograft rejection: biomarker identification and validation. *Transplantation*. 2011; 92:1–9. [PubMed: 21494177]
- Hutvagner G, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science*. 2001; 293:834–8. [PubMed: 11452083]
- Iliopoulos D, et al. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*. 2009; 139:693–706. [PubMed: 19878981]
- Jiang P, et al. MiR-34a inhibits lipopolysaccharide-induced inflammatory response through targeting Notch1 in murine macrophages. *Exp Cell Res*. 2012; 318:1175–84. [PubMed: 22483937]
- Jiang X, et al. MicroRNAs and the regulation of fibrosis. *FEBS J*. 2010; 277:2015–21. [PubMed: 20412055]
- Jindra PT, et al. Costimulation-dependent expression of microRNA-214 increases the ability of T cells to proliferate by targeting Pten. *J Immunol*. 2010; 185:990–7. [PubMed: 20548023]
- Jopling CL, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science*. 2005; 309:1577–81. [PubMed: 16141076]
- Khan N, et al. Quantitative analysis of microRNA in blood serum with protein-facilitated affinity capillary electrophoresis. *Anal Chem*. 2011; 83:6196–201. [PubMed: 21714529]
- Kong KY, et al. MIR-23A microRNA cluster inhibits B-cell development. *Exp Hematol*. 2010; 38:629–640 e1. [PubMed: 20399246]
- Kopp EB, Medzhitov R. The Toll-receptor family and control of innate immunity. *Curr Opin Immunol*. 1999; 11:13–8. [PubMed: 10047546]
- Krams SM, et al. Toll-like receptor 4 contributes to small intestine allograft rejection. *Transplantation*. 2010; 90:1272–7. [PubMed: 21197709]
- Krichevsky AM, et al. A microRNA array reveals extensive regulation of microRNAs during brain development. *RNA*. 2003; 9:1274–81. [PubMed: 13130141]
- Krutzfeldt J, et al. Silencing of microRNAs in vivo with ‘antagomirs’. *Nature*. 2005; 438:685–9. [PubMed: 16258535]
- Lagos-Quintana M, et al. Identification of novel genes coding for small expressed RNAs. *Science*. 2001; 294:853–8. [PubMed: 11679670]
- Lai EC. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet*. 2002; 30:363–4. [PubMed: 11896390]
- Lau NC, et al. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science*. 2001; 294:858–62. [PubMed: 11679671]
- Lee CT, et al. Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. *DNA Cell Biol*. 2007; 26:209–18. [PubMed: 17465887]
- Lee RC, et al. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993; 75:843–54. [PubMed: 8252621]
- Lee Y, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003; 425:415–9. [PubMed: 14508493]
- Lee Y, et al. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J*. 2002; 21:4663–70. [PubMed: 12198168]
- Lewis BP, et al. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005; 120:15–20. [PubMed: 15652477]
- Lewis BP, et al. Prediction of mammalian microRNA targets. *Cell*. 2003; 115:787–98. [PubMed: 14697198]
- Lin Q, et al. A cluster of specified microRNAs in peripheral blood as biomarkers for metastatic non-small-cell lung cancer by stem-loop RT-PCR. *J Cancer Res Clin Oncol*. 2012; 138:85–93. [PubMed: 22009180]
- Liston A, et al. Dicer-dependent microRNA pathway safeguards regulatory T cell function. *J Exp Med*. 2008; 205:1993–2004. [PubMed: 18725526]

- Liu CG, et al. An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. *Proc Natl Acad Sci U S A*. 2004; 101:9740–4. [PubMed: 15210942]
- Liu G, et al. miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. *Proc Natl Acad Sci U S A*. 2009; 106:15819–24. [PubMed: 19721002]
- Liu Y, et al. VCP/p97, down-regulated by microRNA-129-5p, could regulate the progression of hepatocellular carcinoma. *PLoS One*. 2012; 7:e35800. [PubMed: 22536440]
- Lodish HF, et al. Micromanagement of the immune system by microRNAs. *Nat Rev Immunol*. 2008; 8:120–30. [PubMed: 18204468]
- Lorenzen JM, et al. Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol*. 2011a; 6:1540–6. [PubMed: 21700819]
- Lorenzen JM, et al. Urinary miR-210 as a mediator of acute T-cell mediated rejection in renal allograft recipients. *Am J Transplant*. 2011b; 11:2221–7. [PubMed: 21812927]
- Lu C, et al. miR-221 and miR-155 regulate human dendritic cell development, apoptosis, and IL-12 production through targeting of p27kip1, KPC1, and SOCS-1. *Blood*. 2011; 117:4293–303. [PubMed: 21355095]
- Lu LF, et al. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. *Cell*. 2010; 142:914–29. [PubMed: 20850013]
- Lu LF, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity*. 2009a; 30:80–91. [PubMed: 19144316]
- Lu TX, et al. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. *J Immunol*. 2009b; 182:4994–5002. [PubMed: 19342679]
- Martinez-Nunez RT, et al. MicroRNA-155 modulates the pathogen binding ability of dendritic cells (DCs) by down-regulation of DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN). *J Biol Chem*. 2009; 284:16334–42. [PubMed: 19386588]
- Medzhitov R, et al. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*. 1997; 388:394–7. [PubMed: 9237759]
- Minai OA, et al. Bronchogenic carcinoma after lung transplantation: characteristics and outcomes. *J Thorac Oncol*. 2008; 3:1404–9. [PubMed: 19057264]
- Mitchell PS, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008; 105:10513–8. [PubMed: 18663219]
- Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol*. 2007; 7:610–21. [PubMed: 17627284]
- Murakami Y, et al. Regulation of the hepatitis C virus genome replication by miR-199a. *J Hepatol*. 2009; 50:453–60. [PubMed: 19144437]
- Murakami Y, et al. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. *PLoS One*. 2011; 6:e16081. [PubMed: 21283674]
- Neilson JR, et al. Dynamic regulation of miRNA expression in ordered stages of cellular development. *Genes Dev*. 2007; 21:578–89. [PubMed: 17344418]
- O'Neill LA, et al. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol*. 2011; 11:163–75. [PubMed: 21331081]
- Okuda K. Hepatocellular carcinoma: recent progress. *Hepatology*. 1992; 15:948–63. [PubMed: 1314774]
- Putta S, et al. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. *J Am Soc Nephrol*. 2012; 23:458–69. [PubMed: 22223877]
- Qi J, et al. microRNA-210 negatively regulates LPS-induced production of proinflammatory cytokines by targeting NF-kappaB1 in murine macrophages. *FEBS Lett*. 2012; 586:1201–7. [PubMed: 22575656]
- Qin W, et al. TGF-beta/Smad3 signaling promotes renal fibrosis by inhibiting miR-29. *J Am Soc Nephrol*. 2011; 22:1462–74. [PubMed: 21784902]
- Qu B, et al. A Novel Vector-Based Method for Exclusive Overexpression of Star-Form MicroRNAs. *PLoS One*. 2012; 7:e41504. [PubMed: 22829954]

- Ranganathan P, et al. Regulation of acute graft-versus-host disease by microRNA-155. *Blood*. 2012; 119:4786–97. [PubMed: 22408260]
- Raviv Y, et al. Lung cancer in lung transplant recipients: experience of a tertiary hospital and literature review. *Lung Cancer*. 2011; 74:280–3. [PubMed: 21529983]
- Redova M, et al. Circulating miR-378 and miR-451 in serum are potential biomarkers for renal cell carcinoma. *J Transl Med*. 2012; 10:55. [PubMed: 22440013]
- Robbins HY, Arcasoy SM. Malignancies following lung transplantation. *Clin Chest Med*. 2011; 32:343–55. [PubMed: 21511094]
- Rodriguez-Luna H, Douglas DD. Natural history of hepatitis C following liver transplantation. *Curr Opin Infect Dis*. 2004; 17:363–71. [PubMed: 15241083]
- Rodriguez A, et al. Requirement of bic/microRNA-155 for normal immune function. *Science*. 2007; 316:608–11. [PubMed: 17463290]
- Scherr M, et al. Lentivirus-mediated antagomir expression for specific inhibition of miRNA function. *Nucleic Acids Res*. 2007; 35:e149. [PubMed: 18025036]
- Schmittgen TD, et al. A high-throughput method to monitor the expression of microRNA precursors. *Nucleic Acids Res*. 2004; 32:e43. [PubMed: 14985473]
- Schwarz DS, et al. Asymmetry in the assembly of the RNAi enzyme complex. *Cell*. 2003; 115:199–208. [PubMed: 14567917]
- Scian MJ, et al. MicroRNA profiles in allograft tissues and paired urines associate with chronic allograft dysfunction with IF/TA. *Am J Transplant*. 2011; 11:2110–22. [PubMed: 21794090]
- Seeff LB. Natural history of chronic hepatitis C. *Hepatology*. 2002; 36:S35–46. [PubMed: 12407575]
- Sethupathy P, et al. A guide through present computational approaches for the identification of mammalian microRNA targets. *Nat Methods*. 2006; 3:881–6. [PubMed: 17060911]
- Shapiro MD, et al. MicroRNA expression data reveals a signature of kidney damage following ischemia reperfusion injury. *PLoS One*. 2011; 6:e23011. [PubMed: 21887224]
- Sheedy FJ, et al. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nat Immunol*. 2010; 11:141–7. [PubMed: 19946272]
- Shimakami T, et al. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. *Proc Natl Acad Sci U S A*. 2012; 109:941–6. [PubMed: 22215596]
- Stenvang J, Kauppinen S. MicroRNAs as targets for antisense-based therapeutics. *Expert Opin Biol Ther*. 2008; 8:59–81. [PubMed: 18081537]
- Stittrich AB, et al. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. *Nat Immunol*. 2010; 11:1057–62. [PubMed: 20935646]
- Sui W, et al. Microarray analysis of MicroRNA expression in acute rejection after renal transplantation. *Transpl Immunol*. 2008; 19:81–5. [PubMed: 18346642]
- Surdziel E, et al. Lentivirus-mediated antagomir expression. *Methods Mol Biol*. 2010; 667:237–48. [PubMed: 20827538]
- Taganov KD, et al. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A*. 2006; 103:12481–6. [PubMed: 16885212]
- Thuluvath PJ, et al. Survival after liver transplantation for hepatocellular carcinoma in the model for end-stage liver disease and pre-model for end-stage liver disease eras and the independent impact of hepatitis C virus. *Liver Transpl*. 2009; 15:754–62. [PubMed: 19562709]
- Thum T, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature*. 2008; 456:980–4. [PubMed: 19043405]
- Tijsterman M, Plasterk RH. Dicers at RISC; the mechanism of RNAi. *Cell*. 2004; 117:1–3. [PubMed: 15066275]
- Tili E, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol*. 2007; 179:5082–9. [PubMed: 17911593]
- Tserel L, et al. MicroRNA expression profiles of human blood monocyte-derived dendritic cells and macrophages reveal miR-511 as putative positive regulator of Toll-like receptor 4. *J Biol Chem*. 2011; 286:26487–95. [PubMed: 21646346]

- Tuddenham L, et al. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett.* 2006; 580:4214–7. [PubMed: 16828749]
- Valoczi A, et al. Sensitive and specific detection of microRNAs by northern blot analysis using LNA-modified oligonucleotide probes. *Nucleic Acids Res.* 2004; 32:e175. [PubMed: 15598818]
- Vigorito E, et al. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity.* 2007; 27:847–59. [PubMed: 18055230]
- Villanueva A, et al. New strategies in hepatocellular carcinoma: genomic prognostic markers. *Clin Cancer Res.* 2010; 16:4688–94. [PubMed: 20713493]
- Wang J, et al. miRNA changes in the reverse remodeling heart of rats. *Zhonghua Xin Xue Guan Bing Za Zhi.* 2010; 38:745–50. [PubMed: 21055145]
- Watanabe Y, et al. Computational methods for microRNA target prediction. *Methods Enzymol.* 2007; 427:65–86. [PubMed: 17720479]
- Wei L, et al. Differential expression of microRNAs during allograft rejection. *Am J Transplant.* 2012; 12:1113–23. [PubMed: 22300508]
- Wendlandt EB, et al. The role of MicroRNAs miR-200b and miR-200c in TLR4 signaling and NF-kappaB activation. *Innate Immun.* 2012
- Wienholds E, et al. MicroRNA expression in zebrafish embryonic development. *Science.* 2005; 309:310–1. [PubMed: 15919954]
- Wu H, et al. miRNA profiling of naive, effector and memory CD8 T cells. *PLoS One.* 2007; 2:e1020. [PubMed: 17925868]
- Xiao C, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol.* 2008; 9:405–14. [PubMed: 18327259]
- Yi R, et al. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* 2003; 17:3011–6. [PubMed: 14681208]
- Zhang J, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. *Blood.* 2009; 113:4586–94. [PubMed: 19202128]
- Zhang Y, et al. Hepatitis C Virus-induced upregulation of miR-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology.* 2012
- Zhong X, et al. Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *J Am Soc Nephrol.* 2011; 22:1668–81. [PubMed: 21852586]
- Zhou B, et al. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. *Proc Natl Acad Sci U S A.* 2007; 104:7080–5. [PubMed: 17438277]
- Zhou R, et al. miR-16 targets transcriptional corepressor SMRT and modulates NF-kappaB-regulated transactivation of interleukin-8 gene. *PLoS One.* 2012; 7:e30772. [PubMed: 22292036]
- Zhou X, et al. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. *J Exp Med.* 2008; 205:1983–91. [PubMed: 18725525]

Figure 1. Schematic representation of miRNA biogenesis and their role in modulation of immunological pathways involved in allograft rejection