Experimental and Molecular Pathology 93 (2012) 378–385

Contents lists available at SciVerse ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Modulation of immune responses following solid organ transplantation by microRNA

Nayan J. Sarma [a], Venkataswarup Tiriveedhi [a], Sabarinathan Ramachandran [a], Jeffrey Crippin [c], William Chapman [a], T. Mohanakumar [a,b,*]

[a] Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA
[b] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA
[c] Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA

ARTICLE INFO

Article history:
Received 25 September 2012
Available online 1 October 2012

Keywords:
microRNA
Transplantation
Chronic rejection
Inflammation
Gene regulation
T-cell
B-cell
Biomarkers
Therapeutics

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 complicacies 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 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 complicacies 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

* Corresponding author at: Washington University School of Medicine, Department of Surgery, Campus Box 8109-3328 CSRB, 660 S. Euclid Avenue, St. Louis, MO 63110, USA. Fax: +1 314 747 1560.
E-mail address: kumart@wustl.edu (T. Mohanakumar).

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http://dx.doi.org/10.1016/j.yexmp.2012.09.020

post-translational processes through RNA duplex formation resulting in gene silencing (Lai, 2002; Lee et al., 1993) thereby playing a 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 indentified 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 Fig. 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 were 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 fluorophor-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., 2003, 2005). 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.

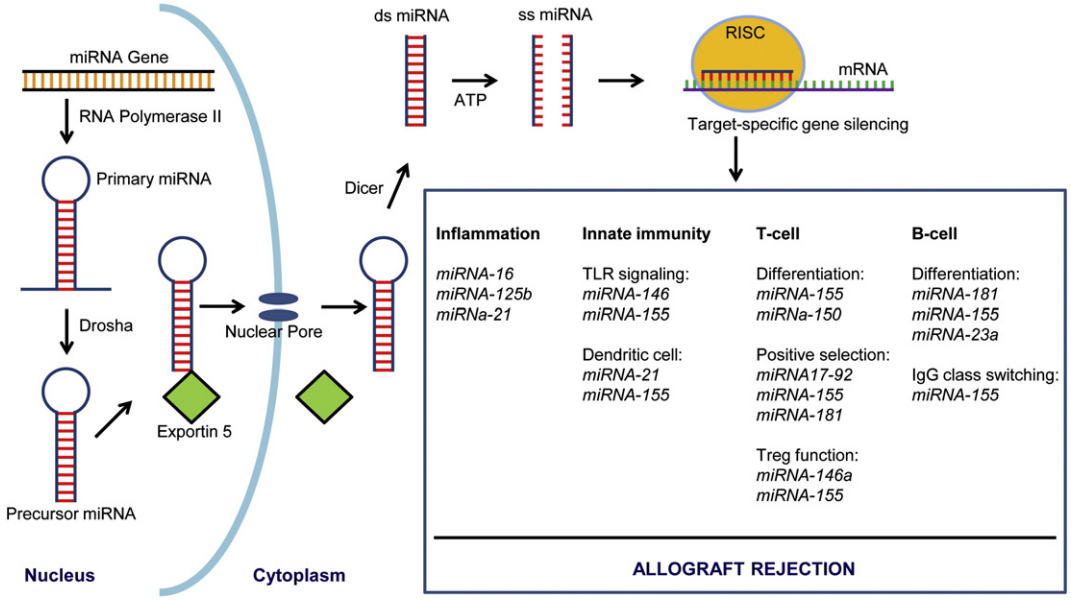

Fig. 1. Schematic representation of miRNA biogenesis and their role in modulation of immunological pathways involved in allograft rejection.