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TRANSLATING MicroRNAs TO THE CLINIC

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CHAPTER 1

MicroRNAs: Mirrors of Health and Disease

T.H. Tran, M.A. Montano

Key Concepts

This chapter gives examples of tissue and cell type-specific knockdown of components of the microRNA machinery and their deleterious effects. It discusses the genetic organization of microRNAs and how that architecture is designed to regulate multiple targets within regulatory pathways, and their role in adaptive regulation in response to changes in the microenvironment. It looks at the role of MicroRNA networks in specific tissue and organ diseases, including lung, heart, and skeletal muscle, as well as in modulating cancers in those tissues. Newer research is introduced linking shifts in microRNA expression with biological aging and diseases associated with aging. The potential role for MicroRNAs as therapeutic tools to modulate target RNA sequences implicated in disease states is explored. Finally, the utility of MicroRNAs as circulating biomarkers for disease risk and severity, as well as in the therapeutic response to candidate drug interventions is discussed.

1. INTRODUCTION

Since their discovery as a novel class of small noncoding RNAs capable of regulating protein translation and/or messenger RNA (mRNA) stability, microRNAs (miRNAs) have been implicated as key regulators in the homeostasis of multiple biological systems that include organs such as heart, lung, and skeletal muscle, as well as modulation of the pathobiology processes such as cancer and aging. Experimental loss of key regulators of miRNA biogenesis has revealed the crucial role for the control of posttranscriptional gene expression. The genetic organization, the variability of their loci, and the tissue specificity of their expression further illustrate an adaptive capacity of the miRNA machinery to modify and fine-tune the transcriptome and translated protein targets in response to physiologic environmental cues, as well as their vulnerability to becoming co-opted in diseases such as cancer, fibrosis, sepsis, aging, and autoimmune disease. Current efforts to leverage knowledge of the miRNA regulatory system to

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diagnose, track, and attenuate disease progression, represent a major new research opportunity, and challenge in this rapidly growing area of translational medicine.

2. KNOCKING OUT MICRORNA: SMALL RNAs WITH BIG EFFECTS

As our understanding of miRNA biogenesis and downstream regulatory activities unfold, so too does our appreciation for the extent and scope of influence these small RNAs have on multiple biological processes across human health and disease. The endonuclease Dicer is an obligatory first step in the RNA interference pathway and formation of the RNA-inducing silencing complex (RISC). Without RISC formation, argonaute-mediated degradation of target mRNA is lost [1,2]. Therefore, Dicer knockdown, in effect, disables the processing of miRNA from precursors of miRNA, ie, premiRNA. This can have deleterious effects. In murine models of cancer, global gene-disruption of Dicer can enhance tumor susceptibility [3]. Tissue- and cell type–specific knockdown of Dicer can also lead to deleterious effects. For example, cardiomyocyte-specific ablation of Dicer can result in cardiomyopathy [4]. Kidney podocyte-specific knockdown of Dicer can lead to systemic proteinuria and glomeruli abnormalities in mice [5]. Moreover, conditional knockdown of Dicer in the kidney impairs its normal development, further emphasizing the normal and dysregulatory potential in miRNA processing. Dicer deletion in skeletal muscle can result in increased myocyte apoptosis, skeletal muscle hypoplasia, and eventually perinatal death [6]. Hepatocytes obtained from Dicer-null mice exhibit abnormal lipid buildup and eventual renal steatosis [7]. Liver-specific Dicer deletion at 3 weeks leads to progressive hepatic steatosis [8]. This phenotype, however, has not been universally observed. While unlikely to change the overall conclusion that miRNAs clearly play a significant role in regulating multiple pathways, the subtleties or disparities in observation of distinct phenotypes in these conditional knockdowns may hinge upon the buildup of precursor miRNAs that activate other RNA surveillance pathways, such as RNA editing, which would then introduce an additional variable [9,10]. Comparative transcriptome profiling with high throughput RNA sequencing will likely provide more insight into the global regulatory profile of these knockdowns.

3. GENETIC ORGANIZATION, VARIATION IN MICRORNAs, AND TISSUE-SPECIFIC EXPRESSION OF MICRORNAs

MicroRNAs (miRNAs) undergo multiple processing events to reach their functional 21–23 ribonucleotide RNA sequence. Canonical miRNAs are generated from protein-coding transcriptional units; whereas, other miRNAs (ie, noncanonical miRNAs) are generated from nonprotein-coding transcriptional units. In both cases, the miRNAs can be located either within intronic or exonic regions. A noteworthy mechanistic distinction in canonical versus noncanonical miRNAs is that canonical intronic miRNAs are Drosha dependent and are thus processed cotranscriptionally with protein-coding transcripts in the nucleus. The premiRNA then enters the miRNA pathway, whereas the rest of the transcript undergoes premRNA splicing to produce mature mRNA which will then direct protein synthesis. Noncanonical intronic small RNAs (also called mirtrons) can derive from small introns that resemble premiRNAs, and bypass the Drosha-processing step [11]. miRNAs tend to be organized in a related cluster and also tend to target multiple mRNA transcripts within common cellular response pathways (eg, proliferation, apoptosis). This organizational thematic provides miR clusters with a capacity for coordinate regulation of multiple steps within a pathway, providing an opportunity for complex and adaptive regulatory control of entire pathways. An interesting class of miRNAs is myomiRs—so-called because they are coded within myosin heavy chain (MYH) genes. myomiRs are transcribed in the same precursor mRNA as the parental MYH gene [4]. Of special note is the myomiR-499, which despite the absence of a parent mRNA, is one of the most highly expressed miRNAs in heart tissue. In an apparently novel evolutionary phenomenon, alternative splicing in the heart uncouples production of mature miR-499 from expression of parent MYH7b mRNA, meaning that the mRNA has perhaps evolved into a nonfunctional host mRNA for its intronic miR (ie, miR-499).

Comparative studies evaluating the organizational structure of the mammalian genome have identified a wealth of chromosomal insertion–deletions, copy number variants, and single nucleotide polymorphisms (SNPs) that, depending on the environmental context, contribute to the genetic variation that can underlay phenotypic diversity. This diversity is evident in nearly every aspect of human health and disease that has been investigated. Perhaps not surprisingly, there is now a growing recognition that variation in miRNAs and their target genes also contribute to this phenotypic variability. Several

solid and hematologic malignancies can be linked to miRNAs located at amplified, deleted, or translocated chromosomal regions in the mammalian genome [12]. Variation in gene expression and regulation is likely influenced by genetic variants in *cis*- and *trans*-acting SNPs (also known as expression quantitative trait loci) [13]. An interesting observation of miRNA binding is their ability to recognize binding site polymorphism (miRSNPs) in transcribed functional genes. For example, miR-24 appears to be deregulated in human colorectal tumor through a target site polymorphism in the dihydrofolate reductase gene. In another example, a polymorphism within the myostatin gene creates a target site for miR-1 and miR-206, which are highly expressed in skeletal muscle. The binding of these miRs to the polymorphism in myostatin causes translational inhibition of myostatin transcripts and can phenocopy the observed muscle hypertrophy that is observed with genetic knockouts of the myostatin gene [14]. Given the significant differences in gene expression and genetic variation across human populations, analysis of the role of miRNAs in contributing to population differences in gene expression is likely to provide substantial insights in population based health disparities and physical functionalities [13]. Indeed, comparative genomic studies indicate that the target mRNA sequences for miRNAs: untranslated regions (UTRs) on mRNAs often display sequence diversity. This may suggest adaptive evolution of coexpressed miRNAs and cognate mRNAs with these UTR variants. Depending on whether the dampening of protein output is beneficial, inconsequential, or harmful, the UTR sites may be selectively conserved, neutral, or avoided during miRNA:mRNA coevolution [1].

Studies evaluating the tissue-specific expression of miRNAs illustrate a "cross-regulation" feature of miRNAs that contributes to cell fate specification by repressing alternate cell fates to facilitate commitment to one cell fate and to maintain stability of a differentiated phenotype [15]. For example the myomiRs miR-1, miR-133, miR-206, miR-208, miR-486, and miR-499 are enriched in skeletal muscle and play crucial roles in the development, growth, and maintenance of skeletal muscle [16]. Notably, miR-133 prevents osteogenic cell lineage differentiation by repressing Runx2, a factor essential for bone formation [15]. MiR-7, miR-24, miR-128, miR-134, miR-219, and others are highly expressed in the mammalian brain and regulate neurite outgrowth, neuronal differentiation, and dendritic spine size [17]. Regionally enriched miRNAs in specific tissues can exert specialized function. For example, miR-7 and miR-24 are highly expressed in the hypothalamus and fine-tuning expression of Fos and oxytocin which play vital roles in the control of water, lactation, and parturition [18].

4. MICRORNA REGULATORY COMPLEXITY: FEEDBACK LOOPS AND ENVIRONMENTAL SENSING

Perhaps one of the most salient and unexpected features of miRNA is the role for miRNA in amplifying or tempering cell signaling. The modulatory capacity provides an adaptive tool that allows the initiation of cellular signaling to be calibrated to accommodate cues from the microenvironment, as well as to potentially buffer signaling. This buffering function has been proposed to function as a network stabilizing effect in the context of dynamic and interlocking feedback loops [19]. In the lung, miR-21 promotes transforming growth factor (TGF-β) amplification and fibrosis in a feed-forward loop by relieving the repression of the inhibitor Smad 7 through targeting the mRNA [20]. Interestingly, TGF-β both inhibits miR let-7 and upregulates miR-21. These two miRNAs appear to be functionally opposed in lung tissue from human subjects with idiopathic pulmonary lungs (let-7 acts as a negative regulator and miR-21 as a positive regulator) that in effect can balance the fibrotic phenotype. In the context of sepsis, monocyte expression of miR-146 and miR-155 are rapidly upregulated during endotoxin/LPS exposure, representing an innate response regulatory role for these miRNAs [11,13]. MiR-146a appears to function as a negative feedback regulator by targeting IL-1 receptor-associated kinase 1 (IRAK-1) and TNF receptor-associated factor 6 (TRAF6). A reciprocal negative feedback is achieved through MAP kinase phosphatase-1 (MKP-1)-mediated suppression of miR-155, which in turn targets suppressor of cytokine signaling-1. Thus, MKP-1 appears to function as a derepressor of miR-155-mediated suppression to modulate LPS response in monocyte/macrophages. In the context of skeletal muscle, C2C12, a skeletal muscle precursor cell line, miR-1 targets mRNAs for the insulin growth factor-1 (IGF-1) and the IGF-1 receptor. In a familiar theme of regulatory loops, IGF-1 reciprocally regulates miR-1 via the transcription factor Foxo3a, apparently through an enhancer-binding element within the miR-1 promoter. Interestingly, skeletal muscle response to endurance or resistance exercise stimuli has been linked to changes in miRNA levels [21,22]. However, such changes are depending on the age of the subjects, the mode of exercise, and the duration of exercise. For example, when anabolic response is dichotomized into so-called "low responders" versus "high responders" to resistance exercise-induced muscle hypertrophy, the former, but not the latter, exhibit a decrease in miR-26a, miR-29, and miR-378. Since miR-29 has been implicated as positive regulator of myogenesis, its reduction in

"low responders" may contribute to the attenuated response in muscle hypertrophy. Interestingly, physical inactivity such as prolonged bed rest (10 days) leads to muscle atrophy and a reduction in the levels miR-206, miR23a, and several members of let-7 family of miRNAs [23]. These miRNAs contribute to a wide array of functions in that influence muscle function and maintenance, insulin response, growth and atrophy, cell cycle, differentiation, and glucose homeostasis. Other anabolic modulators such as estrogen and androgen also alter miRNA levels [11].

5. ORGAN DISEASES AND MICRORNAs

A potentially important and therapeutically exploitable distinction between disease and healthy states in tissue homeostasis may reside in the regulatory miRNA networks that reflect healthy and disease states. There is a growing recognition that miRNA networks are often associated with tissue dysfunction and are likely to be a key source of altered gene expression that underlays and distinguishes healthy versus disease states. A better understanding of the key perturbations that lead to these alternative regulatory network states may help to inform therapeutics. A reduction in Dicer expression has been implicated in cardiac disease. In skeletal muscle, a reduction of miR-29 expression has been associated with decreased muscle regeneration and Duchenne's muscular dystrophy, but also chronic kidney disease, pointing to the complexity of miRNA control networks [21]. To add to this complexity, miR-29 expression increases with age and this induction may inhibit muscle regeneration by affecting overall protein translation via modulating IGF-1 and p85α. In the context of lung biology, profiling studies of bronchial airway epithelia reveal an array of changes, wherein multiple miRNAs were downregulated in smokers that could be correlated with mRNA target expression in vivo [24]. In the kidney, miR-155 is differentially expressed in different tissue compartments (ie, kidney cortex versus kidney medulla regions) [5]. In individuals with trisomy 21, the chromosomal disorder also known as Down's syndrome, fibroblasts display higher levels of miR-155. In kidney, the expression of miR-192 is dysregulated in diabetic nephropathy, with a suggestive role in mediating TGF-β-induced collagen expression. In that study, deletion of the inhibitory Smad 7 promoted miR-192 expression, in a model for obstructive kidney disease. In diabetes, mRNA profiles are altered based on the observation that there is an apparent discordance between proteomic profiles and their respective mRNA levels, suggesting a potential role for miRNAs [7]. Interestingly, insulin-secreting cells exposed