MicroRNAs and Their Potential

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Contents

Abstract With the advent of microRNA (miRNA) we are compelled to revise our understanding of the mechanisms underlying gene regulation during health and disease. A miRNA is approximately 21 ribonucleotides long, genetically encoded, with a potential to recognize multiple mRNA targets guided by sequence complementarity and RNA-binding proteins. This class of molecules is functionally versatile, with the capacity to specifically inhibit translation initiation or elongation, as well as induce mRNA degradation, through predominantly targeting the 3′-untranslated regions of mRNA. Early on it was realized that the levels of individual miRNA varied under different developmental, biological, or pathological conditions, thus implicating these molecules in normal and pathological cellular attributes. In this chapter, we will discuss how the functions of miRNA relate to our existing knowledge on post-transcriptional regulation of gene expression that is the underlying mechanism of many diseases, including cardiac hypertrophy and failure, and their potential as biomarkers and therapeutic targets in diseases.

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1 The Importance of Being miRNA

In computer terms, a macro is a single command that is programmed to replace multiple separate commands that perform a series of actions, thus simplifying, expediting, and minimizing error. Similarly, the cell employs a single miRNA to regulate post-transcriptional expression of an array of genes that are involved in a particular cellular function. This mechanism will ensure synchronization of the regulatory effects and promptness of the response. Moreover, post-transcriptional regulation of multiple genes via a single miRNA circumvents the need for transcriptional regulation of individual genes and is, thus, potentially faster and more energy efficient. This is what Beyer et al. (2004) refer to as "translation on demand," not overlooking the fact that regulating RNA with miRNA is unhindered by the need for a translation product. Thus, it becomes clear that the functions of miRNA complement perfectly our current knowledge of post-transcriptional regulation of expression.

2 Post-Transcriptional Regulatory Mechanisms During Cardiac Hypertrophy

Compensatory cardiac hypertrophy is characterized by a change in the gene expression pattern that recapitulates the neonatal profile (Johnatty et al. 2000). This switch is triggered by regulation of transcriptional and post-transcriptional functions (Fig. 1). Transcription is regulated by selective accessibility of the promoter to the initiation complex and upstream enhancers and/or repressors. Some of the regulators that have been shown to play a role during cardiac hypertrophy include the histone variant, H2A.z (Chen et al. 2006), histone acetylases (Dai and Markham 2001; Gusterson et al. 2003), and deacetylase (Chang et al. 2004; Zhang et al. 2002). Once access to the promoter is granted the availability and/or activity of RNA polymerase II and transcriptional regulators, such as GATA4 (Molkentin et al. 1998), SRF (Zhang et al. 2001), and NKx2-5 (Chen et al. 1996), among others, will determine the level of mRNA produced. But it is well established that translation of mRNA is also a tightly controlled function. It is regulated by: targeted mRNA localization, mRNA stability (half-life), the rate of translation initiation, and, finally, when translation is completed, the rate of protein degradation will determine the extent of the functional outcome. MiRNA are a newly discovered class of post-transcriptional regulators that can inhibit translation and/or induce mRNA degradation (Fig. 2).

3 Post-Transcriptional Regulation by miRNA

Transcription of a gene does not connote automatic translation of the transcript. In other words, we cannot use relative mRNA levels as predictive measures of relative protein levels of a given gene (Gygi et al. 1999), which suggest post-transcriptional

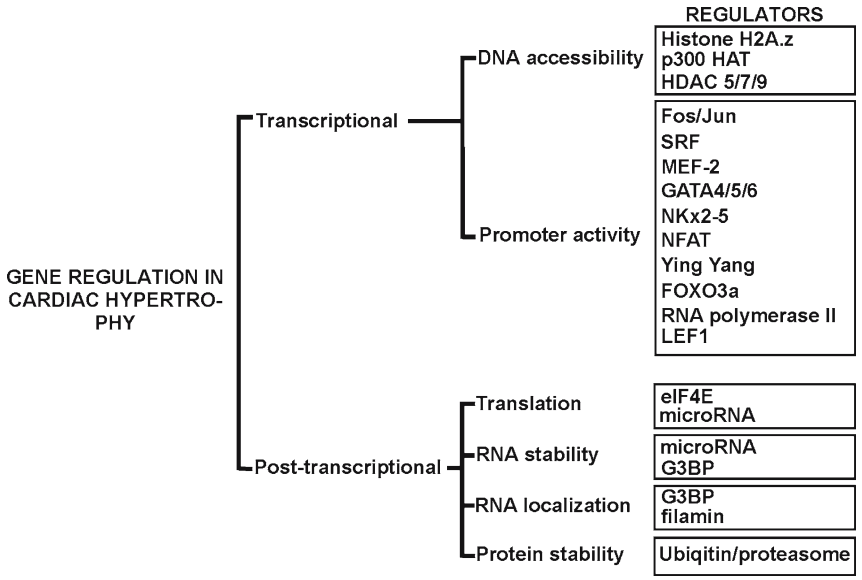

Fig. 1 A schematic showing gene expression regulatory mechanisms involved in cardiac hypertrophy. Gene expression is regulated by transcriptional and post-transcriptional mechanisms. Transcription is restricted by factors that regulate genomic DNA accessibility, while the activity of a promoter is regulated by the availability and activity of various transcription factors, some of which are highlighted in the diagram. Post-transcriptional mechanisms involve regulation of translation initiation and elongation, protein stability, and mRNA stability and localization. MicroRNA plays a role in both translation initiation and elongation, as well as, mRNA stability

regulation of gene expression. Post-transcriptional regulation refers to events that limit the availability or accessibility of mRNA for translation. The study by Beyer et al. (2004) confirms weak or no correlation between mRNA and protein abundance for the whole cell. Similar results were obtained when the calculations were repeated on separate cellular compartments. But, interestingly, a more positive correlation emerged when they grouped functionally related genes. From what we know, an individual miRNA (or a miRNA family) also has the potential to post-transcriptionally regulate a set of specific genes involved in a given cellular function. For example, we observed that miR-1 targets an array of growth-related genes that have been previously implicated in the development of cardiac hypertrophy, which include Ras GTPase-activating protein, cyclin-dependent kinase 9, endothelin, fibronectin, Ras homologue enriched in brain, eukaryotic initiation factor 4E, JunD, quaking, insulin-like growth factor, and Rap1 (Table 1). Concordantly, upon induction of cardiac hypertrophic growth, miR-1 is down-regulated, allowing for the up-regulation of these targets (Sayed et al. 2007). Similarly, when John at al. (2004) analyzed individual miRNA for gene ontology terms they observed that some favored certain terms. For example, miR-208 targets were biased toward “transcription factor,” while miR-105 had a preponderance of “small GTPase mediated signal transduction.” To the best of our knowledge, there is no other class of molecules identified to

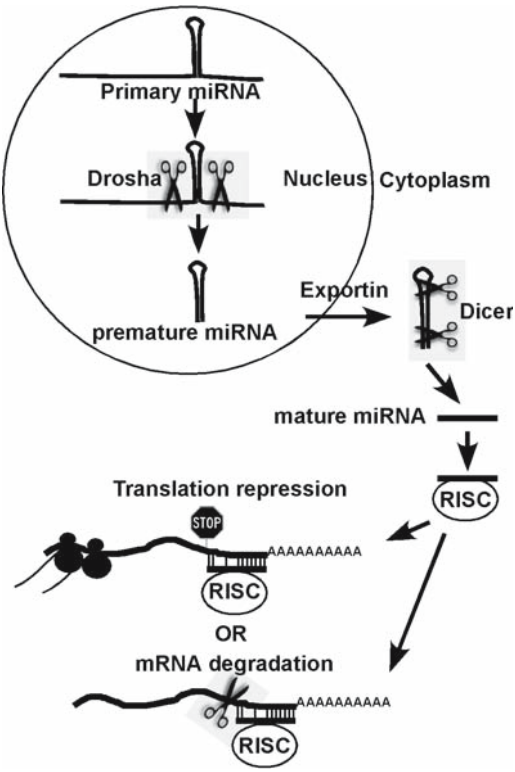

Fig. 2 A diagram showing the processing of microRNA and its fate. MicroRNA are expressed as part of a much longer transcript that matures via a two-step process, one in the nucleus and the second in the cytoplasm, by the enzymes Drosha and Dicer, respectively. Guided by sequence complimentarity and the RISC complex, the mature microRNA targets mRNA and has the potential to induce translation inhibition or mRNA degradation

date that has the potential to fulfill this function as globally and specifically as observed by miRNA. This supports the idea that miRNA are major post-transcriptional regulators.

Transcriptional regulation is the main first step in determining the availability of mRNA, but it immediately follows that the mRNA's half-life will dictate its temporal availability for translation of the open reading frame. Wang et al. (2002) have reported that in yeast the half-lives of mRNA ranged from 3 to 90 min. There was no correlation between the half-life and ribosome density (translational activity), but the half-lives of proteins that form a physical complex were very similar. For example, the 4 core histones have a decay rate of $t_{1/2} = 7 \pm 2$ min, while 131 ribosomal protein mRNA have a $t_{1/2} = 22 \pm 6$ min. Similarly, decay rates of mRNA of functionally related genes were also comparable. In general, genes related to metabolism had longer half-lives than those enrolled in regulatory functions, such

Table 1 Growth-related "TargetscanS" predicted miR-1 targets and their relevant functions in cardiac hypertrophy

TargetscanS predicted miR-1 target genes	Hypertrophy-related functions
Ras GTPase activating protein, RasGAP	Induces RNA polymerase II activity and hypertrophy (Abdellatif et al. 1998)
RasGAP SH3-binding protein-2, G3BP2	A downstream effector of RasGAP necessary for mediating its hypertrophic effects (Lypowy et al. 2005)
Cyclin-dependent kinase 9, Cdk9	An RNA polymerase II kinase involved in mRNA elongation and is necessary for hypertrophy (Sano et al. 2002).
Fibronectin	An extracellular substrate that increases during, and promotes, hypertrophy (Samuel et al. 1991)
Ras homologue enriched in brain, Rheb	An upstream regulator of the mTOR pathway necessary for hypertrophy (Iwata et al. 2005; Nakamura et al. 2005)
Hepatocyte growth factor receptor, MET	A growth factor that enhances angiogenesis and inhibits fibrosis and remodeling in the heart (Iwata et al. 2005; Nakamura et al. 2005)
Endothelin	A growth factor that increases during, and promotes, hypertrophy (Iwata et al. 2005; Nakamura et al. 2005)
Insulin-like growth factor, IGF1	A growth factor that increases during, and promotes, hypertrophy (Ito et al. 1993)
Eukaryotic initiation factor 4E, eIF4E	A factor necessary for initiation of protein synthesis during cardiac hypertrophy (Wada et al. 1996)
JunD	A transcription factor involved in cardiac hypertrophy (Ricci et al. 2005)
Rap1A and Rap1B	A Ras family members involved in cytoskeletal organization and increased during hypertrophy (Johnatty et al. 2000)
Quaking	An RNA-binding protein increased during hypertrophy (Johnatty et al. 2000)
TATA binding protein, TBP	A factor necessary for RNA polymerase II-mediated transcription (Killeen et al. 1992)
Connexin 43	A gap junction protein increased during compensatory hypertrophy (Formigli et al. 2003)

as initiation of translation. These data reconcile well with the fact that mRNA and protein abundance correlate best within the same functional group of genes. Although mammalian miRNAs are commonly known for inhibiting translation versus inducing mRNA degradation, there is substantial evidence to support the latter as well. Farh et al. (2005) showed that predicted mRNA targets of tissue-specific miRNA were lower in the corresponding tissue than they were in others. More convincingly, those targets were, initially, preferentially high in those tissues, before declining, upon the inclining of the targeting miRNA, although they were not completely eliminated. This suggested that miRNA are involved in both mRNA decay, as well as, translational repression. In support, Lim et al. (2005) showed that by expressing the muscle-specific miR-1 or the brain-specific miR-124 in HeLa cells the mRNA expression pattern shifted towards the corresponding tissue.

The mechanism of mRNA degradation is similar to that employed by siRNA, where the endonuclease "Dicer" mediates mRNA cleavage. Alternatively, miRNA may induce deadenylation, which induces mRNA degradation (Wu et al. 2006).

It is well established that, in metazoans, miRNA inhibits mRNA translation. One study showed that miRNA requires both the Cap and poly(A) tail structures to inhibit translation initiation, but the mechanism remains unknown (Humphreys et al. 2005). It was previously shown that both of these structures act in synergy to enhance translation. This may be explained by the fact that eukaryotic initiation factors 4G (eIF4G), a subunit of the 5′cap-binding initiation factor, binds the polyA-binding protein (PABP) and promotes circularization of the mRNA molecule, a structure that is translationally superior to the linear form (Sachs 2000). Thus, one can envision a mechanism whereby the 3′UTR-binding miRISC complex may interfere with the formation of this structure and compromise translation. Alternatively, in an intact circular mRNA, the miRISC complex may come into close proximity to the translation initiation complex eIF4F and physically or biochemically hinder its function. But in case the mRNA is already engaged in translation, miRNA also has the capacity to inhibit post-translation initiation steps (Olsen and Ambros 1999; Seggerson et al. 2002). So, what dictates whether a miRNA will induce mRNA degradation versus translation inhibition? We believe that this will be dictated by duration of exposure of a gene to its targeting miRNA. Chronic high levels of a miRNA in a given tissue will eventually eliminate its target mRNA.

Description of post-transcriptional regulation is incomplete without discussing mRNA localization. The cytoskeletal framework provides mRNA with an anchor, as a mean for targeted localization within the cell. By using in situ hybridization and electron microscopy Bassell et al. (1994) were able to visualize mRNA that was mainly localized to microfilaments (72%), and partially to vimentin filaments and microtubules in human fibroblasts. Both the poly(A) mRNA and the polysomes colocalized to the actin crosslinking proteins, filamin, α-actinin, and actin-binding elongation factor 1α. This feature of mRNA is mediated by the 3′ untranslated region (3′UTR). More than a decade ago, Gottlieb (1990) suggested that mRNA localization evolved by inducing degradation of all un-localized mRNA. In concordance, there is a correlation between translation repression and degradation of some transcripts (Cooperstock and Lipshitz 1997). Interestingly, one example of a molecule that is involved in this process is a non-coding RNA molecule, Yellow Crescent (YC), found in the ascidian eggs (Swalla and Jeffery 1996). Swalla and Jeffery (1996) showed that YC is complimentary to the 3′UTR of proliferating cell nuclear antigen (PCNA), but the two do not exist in the same compartment. This suggested that their association results in degradation of PCNA. This brings to our attention the untapped potential of miRNA to specifically repress un-localized transcripts, earmark them for localization, or maybe aid in regulating the targeting process. Since miRNA have the capacity to both inhibit translation and induce degradation of the mRNA, it is plausible that it exercises both effects on the same transcript in a temporal and spatial manner. For example, if a translationally repressed target does not reach its destination promptly, it must face degradation. More will be revealed when we know if, when, and where miRNA is localized.

4 Transcriptional and Post-Transcriptional Regulation of miRNA

Before discussing the changes of miRNA levels in disease and their potential as diagnostic and therapeutic targets, it is necessary to briefly describe how they themselves are regulated. MiRNA were first discovered by means of large scale cloning and sequencing of the mature forms (Lagos-Quintana et al. 2001; Lau et al. 2001; Lee and Ambros 2001). This group of miRNA was then used as a "training-set" for computer software to generate algorithms for computational prediction of further miRNA (reviewed in (Bentwich 2005). The data from that information was then further examined in an attempt to delineate the transcriptional units of those genes (Rodriguez et al. 2004). Out of 232 mammalian miRNA analyzed, approximately 40% existed within introns of coding RNA, 10% within introns of non-coding RNA (ncRNA), and 13% within exons of ncRNA. In addition, a small portion of miRNA exists within exons (3′UTR) of coding RNA and antisense strands of overlapping genes. The remaining miRNA are intergenic, with as yet undefined transcriptional units. MiRNA contained within host genes are expected to be expressed from the same transcriptional unit, as was indeed confirmed for a subset (Rodriguez et al. 2004). As for intergenic miRNA, Jegga et al. (2007) and Zhou et al. (2007) have developed searchable databases for prediction of miRNA core promoters. From these studies. it was predicted that those genes have features common to RNA polymerase II-dependent promoters, as well as unique sequence motifs. Besides that, there are only a handful of studies that examine the transcriptional regulation of individual miRNA promoters. Examples include regulation of mouse miR-1 promoter by serum response factor, myoD, and muscle enhancer factor 2 (Zhao et al. 2005) and *Drosophila* miR-1 by twist and muscle enhancer factor 2 (Sokol and Ambros 2005) among others (Dews et al. 2006; O'Donnell et al. 2005). Also, recently a fair number of miRNA present within areas of Alu repeats were found to be transcribed from RNA-polymerase III-dependent promoters (Borchert et al. 2006). In addition, it was suggested that miRNA found within CpG islands may be regulated by methylation (Weber et al. 2007).

The dogma is that the primary miRNA transcripts are processed in the nucleus into the premature form by Drosha, which is then exported to the cytoplasm for further processing by Dicer (Lee et al. 2002). But, as with other RNA species, miRNA transcripts are not exempt from post-transcriptional regulation. This was convincingly demonstrated by Thomson et al. (2006), where they compared the mature versus the primary miRNA profile for normal and cancer tissues and found no correlation. We recently found that hypoxia induces down-regulation of miR-199a through specifically inhibiting the processing of the stem-loop precursor of the miRNA (unpublished data). It thus appears that mature miRNA abundance is regulated at the levels of Drosha and/or Dicer processing. This may be a consequence of regulation of the activity or availability of those enzymes or the targeted localization of the primary or premature miRNA. Thus, for diagnostic or prognostic purposes, we must rely on measurements of the mature form.

5 The Mechanism of miRNA-Target Recognition

To evaluate the significance of a change in the levels of a certain miRNA during health or disease, we have to understand how it selects and targets specific mRNA. Very few miRNA targets have been identified or validated experimentally. But there are multiple searchable databases that have computationally predicted miRNA targets in several species, using various algorithms, which continue to be modified as more targets are being validated experimentally. At present, these databases are vital in guiding us in experimentally validating miRNA targets. The miRanda software uses parameters that include: (1) binding energy between miRNA and its target; (2) asymmetric 5′-3′ base-pairing, with more weight on the 5′ end, which can, to some degree, be compensated for by stronger complementation with the target at the 3′ end, also allowing G:U wobble pairing; and (3) the most important criterion is evolutionary conservation of the sequence and its position in 3′UTRs of human, mouse, and rat genes (John et al. 2004). A second database, TargetScanS, uses somewhat different parameters, which includes: (1) exact Watson–Crick (W–C) pairing of nucleotides 2–7 of the miRNA to the target, with no weight given to pairing of its 3′ end; (2) a conserved adenosine at the first position and/or a W–C match at position 8; (3) disregarding the binding energy; and (4) using the human, mouse, rat, dog, and chicken 3′UTR, for determining target site conservation (Lewis et al. 2005). A third searchable database, PicTar, looks for: (1) a perfect ~7 nucleotide seed at position 1–7 or 2–8, but mismatches are allowed if the free binding energy does not increase (Krek et al. 2005); (2) it takes into account the binding energy of the entire miRNA-mRNA duplex; and (3) it includes the genomes of chimpanzee, pufferfish, and zebrafish, in addition to all those listed above, for determining conservation, which further reduces false positive predictions. It should be noted, though, that non-conserved miRNA target sites respond equally well to inhibition by miRNA (Farh et al. 2005). This first suggested that other factors such as secondary structures, RNA binding protein, and other pairing features must contribute to the in vivo specificity of a miRNA. But, upon a closer examination, the investigators established that those messages appeared to have specifically evolved to avoided co-localization with the corresponding miRNA. Those genes were dubbed “antitargets.” While the three databases overlap in many predicted targets they diverge in others. Thus, it might be beneficial to search all the databases for potential targets of a miRNA of interest, for experimental validation. But one has to bear in mind that not all predicted targets are genuine, as they may be subject to spatial and temporal restrictions. In addition, binding to the targeting site might be modulated by 3′UTR *cis*-acting sequences or transacting factors. Moreover, a single 3′UTR may be targeted by multiple miRNA. Thus, the level of an mRNA or its translation product is the governed by the combinatorial effect of its targeting miRNA.

While computational analysis predicted the structural basis for miRNA : target pairing, Brennecke et al. (2005) experimentally validated some of the parameters applied above. Essentially, they confirmed that nucleotides 2–8 from the 5′ end of the miRNA are the most critical in establishing base pairing with the target. This

criterion could be relaxed to 2–5 nucleotides, under conditions where the nucleotides in the 3′ end had strong complementarity with the target. Also, the position of the base pairing rather than the pairing energy determined the functional efficiency of the miRNA.

It is worth noting that another dimension is added to the mechanism of miRNA targeting by introducing selective miRNA editing. This process converts adenosine to inosine, catalyzed by adenine deaminase in double stranded RNA substrates (reviewed in (Maas and Rich 2000). Luciano et al. (2004) were the first to notice that premature miR-22 is edited at multiple sites. It is estimated that 6% of human miRNA are edited (Blow et al. 2006). Importantly, some of the edited sites are within the "seed" region and result in reassignment of targets (Kawahara et al. 2007).

6 miRNA in Development and Disease

The first miRNA was discovered in 1993 when Lee et al. (1993) found that lin-4, which downregulates the levels of lin-14 during the development of *C. elegans*, expresses a small non-coding 22 nucleotide RNA. Not until 7 years later did Reinhart et al. (2000) report the discovery of the second miRNA, let-7, which induces downregulation of lin-41 protein. This was shortly followed by identification of a multitude of human and fly miRNA that were highly conserved (Lagos-Quintana et al. 2001). Thence, it was noticed that in each of the heart, brain, and liver, there is a distinct tissue-specific miRNA that predominates, which suggested that miRNAs must be involved in tissue specification or differentiation (Lagos-Quintana et al. 2002). MiRNA also play a role in stem cell division (reviewed in Shcherbata et al. 2006). To date, more than 4,000 miRNA sequences have been identified in a wide range of species. These are annotated and catalogued in a searchable web-based data registry by Welcome Trust Sanger Institute (Griffiths-Jones 2004; Griffiths-Jones et al. 2006).

MiR-1 has a dominant expression pattern and function in the heart. First identified as a muscle-specific miRNA (Lagos-Quintana et al. 2002), its expression is detected as early as embryonic day (E) 8.5 in the mouse heart, and increases with the progression of differentiation (Zhao et al. 2005). In *Drosophila,* a DmiR-1^{KO} mutant dies as a small second in-star larvae, 2–7 days after hatching, from apparent paralysis (Sokol and Ambros 2005). Similarly, disrupting miR-1-2 in a homozygous mouse model results in ventricular septal defects and lethality between E15.5 to immediate postnatal (Zhao et al. 2007). Recently, several reports have also implicated miR-1 in development of cardiac hypertrophy (Care et al. 2007, Sayed et al. 2007) and arrhythmias (Yang et al. 2007). While other miRNA are also involved in the progression of hypertrophy, including miR-133 (Care et al. 2007), miR-21 (Cheng et al. 2007), miR-208 (van Rooij et al. 2007), and miR-195 (van Rooij et al. 2006), the emphasis remains on miR-1, which is singularly downregulated within 24 h of triggering hypertrophy. Notably, adult cardiac hypertrophy starts out as a compensatory growth that is characterized by recapitulating the neonatal heart gene

expression profile (Johnatty et al. 2000). Concordantly, our results confirm similar changes in miRNA patterns in adult hypertrophied versus sham and neonatal versus adult hearts (unpublished data). Thus, we expected that subsequent progress into heart failure would coincide with a deviation of the miRNA signature from this pattern. To test this idea, we searched for miRNA that were deregulated during the period of transition from hypertrophy to failure in a mouse model. While the results showed an overall continuum of the hypertrophy signature, only one miRNA, miR-451, was sharply upregulated at the onset of contractile dysfunction. Such a molecule would serve as an ideal therapeutic target.

Because of their major roles as oncogenes and tumor suppressors, a group of miRNAs have been dubbed "oncomirs" (Esquela-Kerscher and Slack 2006). In cancer, the discovery of miRNA is offering answers to previously unresolved questions. For example, after years of futile attempts to identify the gene(s) in the deleted 13q14 region that participate in the pathogenesis of chronic lymphocytic leukemia (CLL), Calin et al. (2002) discovered that miR-15 and miR-16 are located within this sequence and that both are downregulated in ~68% of cases. These miRNA molecules are now considered tumor suppressors, one of their targets being Bcl2, the level of which reciprocally correlates with that of miR-15/16 in CCL (Cimmino et al. 2005). Calin et al. (2004b) have then gone on to show that 52.5% of miRNA are located in previously established cancer-associated regions. In contrast, over-expression of miRNA is also associated with cancer. For example, miR-155 is upregulated in B cell lymphoma (Eis et al. 2005). Notably, tissue-specific over-expression of miR-155 in a transgenic mouse model proved it sufficient for induction of lymphoblastic leukemia (Costinean et al. 2006). Overall, unique miRNA expression profiles have been detected in chronic lymphocytic leukemia (Calin et al. 2004a), breast (Iorio et al. 2005), and lung cancers (Yanaihara et al. 2006).

Although we have learned much about the miRNA signatures in cardiac hypertrophy and cancer we have very little knowledge about the targets and functions of individual miRNA deregulated during these diseases. This is indeed quite a challenging task. But until we learn more about function, we cannot use miRNA successfully as therapeutic targets. In an attempt to gain some insight, we examined the differences between the miRNA expression patterns that emerged from studies of various diseases. Accordingly, we tabulated some of the miRNA that were emphasized in cardiac hypertrophy, different cancers, hypoxia, and atherosclerosis (Table 2). We searched for trends, such as an association between certain miRNA and known disease mechanisms. For example, it was immediately noticed that miR-21 is upregulated in all cancer forms, as well as cardiac hypertrophy and atherosclerosis, but not hypoxia. Unlike cancer or atherosclerosis, cardiac hypertrophy is a process of cellular growth in the absence of cell cycling. Thus, by deduction, we may predict that miR-21 may be related to a growth process common to both hypertrophy and proliferation. On the other hand, we could also infer that miR-21 does not play a direct role in the cell cycle or hypoxia. In contrast, miR-15/16 cluster is deleted in chronic lymphocytic leukemia (CLL), but remains high and unchanged in the heart during hypertrophy, which later shows a further increase in miR-15b in

Table 2 Select tissue-specific and disease-related microRNA

Tissue-specific	Cardiac hypertrophy	Cancer	Hypoxia	Athero-sclerosis
miR-1, heart and skeletal muscle (Lagos-Quintana et al. 2002)	miR-1 ↓ (Sayed et al. 2007)	miR-107 ↑, H&N (Tran et al. 2007), colon, and stomach (Volinia et al. 2006), pancreas (Volinia et al. 2006; Bloomston et al. 2007)	miR-107 ↑ (Kulshreshtha et al. 2007)	miR-21 ↑ (Ji et al. 2007)
miR-122, liver (Lagos-Quintana et al. 2002)	miR-107 ↑ (Sayed et al. 2007)	miR-15/16 ↓, CLL (Calin et al. 2002; Fulci et al. 2007)	miR-210 ↑ (Kulshreshtha et al. 2007)	
miR-124, brain (Lagos-Quintana et al. 2002)	miR-133 ↓ (Care et al. 2007)	miR-150 ↑, lung (Yanaihara et al. 2006), CLL (Fulci et al. 2007)	miR-213 ↑ (Kulshreshtha et al. 2007)	
miR-133, heart and skeletal (Lagos-Quintana et al. 2002)	miR-15a/b ↑ (Sayed et al. 2007)	miR-122 ↓, liver (Kutay et al. 2006)	miR-23a ↑ (Kulshreshtha et al. 2007)	
miR-208, heart	miR-150 ↓ (Sayed et al. 2007; Cheng et al. 2007)	miR-125b ↑, H&N (Tran et al. 2007), pancreas (Lee et al. 2006)	miR-24-1 ↑ (Kulshreshtha et al. 2007)	
miR-375, Pancreatic islet (Poy et al. 2004)	miR-155 ↓ (Sayed et al. 2007)	miR-143↓, colon (Michael et al. 2003), pancreas (Bloomston et al. 2007)	miR-26b ↑ (Kulshreshtha et al. 2007)	
	miR-185 ↓ (Sayed et al. 2007; Cheng et al. 2007)	mi-145 ↓, colon (Michael et al. 2003)		
	miR-195 ↑ (van Rooij et al. 2006)	miR-155 ↑, B cell lymphoma (Eis et al. 2005), breast and colon (Volinia et al. 2006), lung (Yanaihara et al. 2006), pancreas (Bloomston et al. 2007), CLL (Fulci et al. 2007)		
	miR-199a/b/a* ↑ (Sayed et al. 2007)	miR-199a ↑, lung (Yanaihara et al. 2006), pancreas (Volinia et al. 2006; Bloomston et al. 2007), prostate (Volinia et al. 2006)		
	miR-208 ↑ (van Rooij et al. 2006)	miR-205 ↑, H&N (Tran et al. 2007), pancreas (Bloomston et al. 2007), lung (Yanaihara et al. 2006)		

(continued)

Table 2 (continued)

Tissue-specific	Cardiac hypertrophy	Cancer	Hypoxia	Athero-sclerosis
	miR-21 ↑ (Sayed et al. 2007; Cheng et al. 2007)	miR-21 ↑, H&N (Tran et al. 2007), breast, colon, lung, prostate, and stomach (Volinia et al. 2006), pancreas (Bloomston et al. 2007), CLL (Fulci et al. 2007), liver (Kutay et al. 2006)		
	miR-214 ↑ (Sayed et al. 2007; Cheng et al. 2007)	miR-210 ↑, pancreas (Bloomston et al. 2007)		
	miR-221 ↑ (Sayed et al. 2007)	miR-213 ↑, pancreas (Bloomston et al. 2007),		
	miR-222 ↑ (Sayed et al. 2007)	miR-214 ↑, pancreas, prostate, stomach, lung (Volinia et al. 2006)		
	miR-23a ↑ (Sayed et al. 2007)	miR-221 ↑, H&N (Tran et al. 2007), colon and smotach (Volinia et al. 2006), pancreas (Bloomston et al. 2007)		
	miR-23b ↑ (Sayed et al. 2007)	miR-222 ↑, pancreas (Bloomston et al. 2007)		
	miR-27a ↑ (Sayed et al. 2007; Cheng et al. 2007)	miR-23a ↑, pancreas (Bloomston et al. 2007), liver (Kutay et al. 2006)		
	miR-27b ↑ (Sayed et al. 2007; Cheng et al. 2007)	miR-23b ↑, H&N (Tran et al. 2007), pancreas (Bloomston et al. 2007), liver (Kutay et al. 2006)		
	miR-29a ↓ (Sayed et al. 2007; Cheng et al. 2007)	miR-27a ↑, H&N (Tran et al. 2007)		
		miR-29a ↑, H&N (Tran et al. 2007)		

Select miRNA were compiled from various reports in an attempt to highlight some of the differences and similarities in disease patterns, as well as, the involvement of tissue specific miRNA in pathogenesis.

Abbreviations: H&N, head and neck; CLL, chronic lymphocytic leukemia; ↑, upregulation; ↓, downregulation

late hypertrophy and failure. This agrees with a role for the miRNA cluster in cell cycling, as indeed has been established recently. Another trend involves downregulation of tissue-specific miRNA (miR-1, miR-133, miR-122) in disease states. Overall, such comparisons may also prove useful when evaluating miRNA therapeutic agents and their side effects.

Besides cardiovascular diseases and cancer very little is known about miRNA in other disorders. But there is little doubt that it will soon prove that miRNA have a role in almost every aspect of a cell's function, as seen in its emerging role in regulating metabolism and the lifespan (reviewed in Boehm and Slack (2006).

7 The Diagnostic and Prognostic Potential of miRNA

Within the past 5 years we have seen the rise and fall of cDNA microarrays as diagnostic and prognostic measures in disease (Ioannidis 2005; Michiels et al. 2005; Reis-Filho et al. 2006). While much work is still being done in an attempt to optimize and standardize the methodology, there will remain inconsistencies inherent to the characteristics of mRNA itself: having multiple isoforms and splice variants, and a notoriously unstable nature.

So what does miRNA offer us in the way of a diagnostic and prognostic tool that mRNA does not? While a single mRNA generally dictates the translation of a single protein, a single miRNA molecule has the capacity to regulate the translation of an array of genes governing a certain function (John et al. 2004; Sayed et al. 2007). The significance of which is underscored upon recalling that the underlying apparatus, generally regulating normal cellular functions and pathologies, consist of a consortium of genes. Those genes frequently exhibit deliberate structural and functional redundancy, to ensure a fail-safe operation. More importantly, changes in a gene's mRNA levels can be counterbalanced by post-transcriptional regulation. Thus, we must come to the realization that changes in the levels of mRNA are not necessarily predictive of functional outcome. Using the counter argument, it is expected that miRNA levels are more tightly regulated and less variable, as a single miRNA has the capacity to regulate a cellular function. Moreover, mature miRNA are 18–25 nt long, end products that have developed beyond post-transcriptional regulation and are inherently very stable. Thus, it is expected that miRNA provide us with a more superior predictive parameter in diseases. When Lu et al. (2005) put this idea to the test, they found that miRNA profiling was highly accurate in predicting the differentiation state of tumors and in classification of poorly differentiated tumors, a fact that they could not determine by mRNA profiling. But clearly, more studies are needed to further validate the predictive powers of miRNA in cancer.

We also hope that miRNA might aid in the diagnosis, prognosis, and treatment of heart diseases, which remain the leading cause of death in the United States. Currently, diagnosis and prognosis of heart failure relies mainly on clinical signs and symptoms, aided with only a handful of circulating biomarkers

(Macabasco-O'Connell and Miller 2006). Unfortunately, there "isn't a single patient characteristic that can be used to reliably predict a patient's outcome" (Frankel et al. 2006). This is probably due to the fact that the utility of more direct biomarkers is hindered by the impracticality of routine cardiac biopsies. Alternatively, miRNA profiling of other secondarily affected tissues, in particular the liver, might provide a promising substitute for establishing miRNA biomarkers in heart failure. Our initial results look promising, showing differential regulation of miRNA in liver during heart failure in a mouse model.

8 miRNA as Therapeutic Targets

MiRNA are characterized by targeting a functional group of genes, versus a single mRNA, which renders them a potentially powerful therapeutic target. As an example, it proved more effective to simultaneously, rather than individually, inhibit the cell cycle-related miR-16 targets (Linsley et al. 2007). Thus, it becomes clear that before therapeutically targeting a miRNA, we require knowledge of its gene targets, function, and tissue distribution. Since the discovery of mammalian miRNA is still in its early phase, we have very little information in that regard. In the mean time, however, Krutzfeldt et al. (2005) developed a method for antagonizing miRNA function in vivo. The design involves synthesis of a miRNA-specific, anti-sense, cholesteryl-conjugated, 2′-*O*-methyl oligoribonucleotide, which was dubbed "antagomir." This non-hydrolysable oligoribonucleotide is cell permeable and inhibits the miRNA by inducing its elimination. Hence, it offers a long-lived effect and circumvents the need for a cell delivery vehicle. This approach, though, is not suitable for replenishing depleted miRNA. That is because it would require delivery of the premature miRNA in its native form, so as to allow for proper processing and incorporation into the RISC complex. Thus, other known delivery methods employed in gene therapy, including viral vectors and liposomes, will have to serve as substitutes in this case.

9 Concluding Remarks

As pointed out in the above discussion, we remain in dire need of new biomarkers and therapeutic targets for the two leading causes of death in the United States, cardiovascular diseases and cancer. MiRNA offer us that prospect. But in order to maximally exploit their potential, we must continue to dissect their function in great detail. It is still necessary to identify the specific mode of transcriptional and post-transcriptional regulation of individual miRNA, their cellular localization, targets, and overall function, in parallel with continuing to devise modes for in vivo delivery of miRNA and anti-miRNA.

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