MicroRNomics: a newly emerging approach for disease biology

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Zhang C. MicroRNomics: a newly emerging approach for disease biology. *Physiol Genomics* 33: 139–147, 2008. First published February 26, 2008; doi:10.1152/physiolgenomics.00034.2008.—Genomic evidence reveals that gene expression in humans is precisely controlled in cellular, tissue-type, temporal, and condition-specific manners. Completely understanding the regulatory mechanisms of gene expression is therefore one of the most important issues in genomic medicine. Surprisingly, recent analyses of the human and animal genomes have demonstrated that the majority of RNA transcripts are relatively small, noncoding RNAs (sncRNAs), rather than large, protein coding message RNAs (mRNAs). Moreover, these sncRNAs may represent a novel important layer of regulation for gene expression. The most important breakthrough in this new area is the discovery of microRNAs (miRNAs). miRNAs comprise a novel class of endogenous, small, noncoding RNAs that negatively regulate gene expression via degradation or translational inhibition of their target mRNAs. As a group, miRNAs may directly regulate ~30% of the genes in the human genome. In keeping with the nomenclature of RNomics, which is to study sncRNAs on the genomic scale, “microRNomics” is coined here to describe a novel subdiscipline of genomics that studies the identification, expression, biogenesis, structure, regulation of expression, targets, and biological functions of miRNAs on the genomic scale. A growing body of exciting evidence suggests that miRNAs are important regulators of cell differentiation, proliferation/growth, mobility, and apoptosis. These miRNAs therefore play important roles in development and physiology. Consequently, dysregulation of miRNA function may lead to human diseases such as cancer, cardiovascular disease, liver disease, immune dysfunction, and metabolic disorders. microRNomics may be a newly emerging approach for human disease biology.

microRNAs; genomics; gene expression; cancer; cardiovascular disease
tion in the 1980s with Zaug and Cech discovering the enzymatic activity of RNA (126a), this recent discovery of regulatory small RNAs may represent the second RNA revolution (65).

miRNAs and siRNAs have similar sizes (18–23 nt) and share the similar mechanisms of gene expression regulation. However, their biogenesis and origins are different (30, 60). siRNAs are produced from long, double-stranded (bimolecular) RNAs, or long hairpins, often of exogenous origin, and usually target sequences at the same locus or elsewhere in the genome for destruction (gene silencing). In contrast, miRNAs are endogenous. They are encoded within the genome and come from endogenous short hairpin precursors and usually target sequences at other loci. Therefore, miRNAs may be more important because they are endogenous regulators of gene expression.

miRNAs comprise a novel class of endogenous, small, noncoding RNAs that control gene expression by directing their target mRNAs for degradation and/or translational repression (3, 42, 89). The first miRNA, lin-4, was discovered in 1993 (71, 121). The lack of homology with other species at that time led lin-4 to be considered a genetic oddity that was restricted to Caenorhabditis elegans. The situation did not change until the second miRNA (let-7) was discovered in 2000 (68) and, along with its target lin-4, was found to be conserved in many species. Since then, an increasing number of miRNAs have been identified in mammals (70, 72). Over 700 miRNAs have been identified and sequenced in humans, and the estimated number of miRNA genes is as high as 1,000 in the human genome (8). As a group, miRNAs may directly regulate at least 30% of the genes in the human genome, based on in silico predictions (8, 70, 72).

Consistent with the nomenclature of RNomics, which is to study sncRNAs on the genomic scale (43, 51), “microRNomics” is coined here to describe a novel subdiscipline of genomics that studies the identification, expression, biogenesis, structure, expression regulation, targets, and biological functions of miRNAs at on the genomic scale (44). Recently, there has been an explosion in miRNA research because of the important roles of these noncoding RNAs in diverse biological processes. A growing body of exciting evidence suggests that miRNAs are important regulators for cell differentiation, proliferation/growth, mobility, and apoptosis (4, 34, 53, 58, 96). Therefore, these miRNAs play important roles in development, physiology, and pathophysiology (Fig. 1). Consequently, dysregulation of miRNA function may lead to human diseases such as cancer, cardiovascular disease, liver disease, immune dysfunction, and metabolic disorders (4, 12, 34, 53, 58, 96, 114). The purpose of this review article is to summarize the progress and to provide a perspective of microRNomics in disease biology.

**BIOSYNTHESIS OF miRNAs AND THEIR ROLE IN GENE REGULATION**

Mature miRNAs are noncoding, single-stranded RNAs of ~22 nucleotides and constitute a novel class of gene regulators. miRNAs are initially transcribed by RNA polymerase II or III (Pol II or Pol III, respectively) in the nucleus, to form large pri-miRNA transcripts, which are usually several kilobases long and are capped (MGpppG) and polyadenylated (14, 62). The pri-miRNAs are processed in the nucleus by the RNase III enzyme Drospha and the dsRNA binding protein Pasha (also known as DGCR8), into ~70-nucleotide pre-miRNAs, which fold into stem-loop hairpin structures. RAN-GTP and exportin 5 transport the pre-miRNA into the cytoplasm. Subsequently, another RNase III enzyme, Dicer, processes the pre-miRNA to generate a transient ~18–24 nucleotide duplex. The duplex is loaded into the miRNA associated multiprotein RNA-induced silencing complex, which includes the Argonaute proteins. One strand of the miRNA is preferentially retained in this complex and becomes the mature miRNA; the opposite strand, known as the passenger strand or miRNA*, is eliminated from the complex. In addition to this pathway for miRNA biogenesis, some intronic miRNA precursors are able to bypass Drosha processing to produce miRNAs by Dicer, possibly representing an alternative novel pathway for miRNA biogenesis (61, 100).

The mature miRNA binds to complementary sites in the mRNA target to negatively regulate target gene expression in one of two ways. The mechanism of subsequent target gene suppression depends on the degree of complementarity between the miRNA and its target, in addition to other criteria that have yet to be defined. miRNAs that bind to mRNA targets with imperfect complementarity block target gene expression via translational silencing. In contrast, miRNAs that bind to their mRNA targets with perfect complementarity induce target mRNA cleavage (Fig. 1) (32, 52, 127, 128). However, the above opinion may not be completely correct. Recent studies suggest that even imperfect base-pairing of miRNA with its target mRNA can lead to a decreased abundance of the mRNA (5, 74).

**MAJOR APPROACHES IN MicroRNomics**

Microarray analysis of miRNAs on the genome scale is the most powerful method in microRNomics to determine the expression signature of cells, tissues, and organs within an organism under different conditions (7, 32, 107). Currently, there are 5,234 miRNAs that have been sequenced and added into the miRBase Sequence Database. Accordingly, microarray chips containing these updated miRNA probes for a specific organism are commercially available. For example, current human microarray probes include 711

**Fig. 1. Biological function of microRNAs and their mechanisms.**

mRNA gene → mature miRNA → miRNA:target mRNA

gene expression → cell differentiation

development, physiology; disease

cell proliferation/growth

cell mobility

cell apoptosis
human miRNAs, whereas 568 mouse miRNAs and 348 rat
miRNAs are included in mouse microarray chips and rat
microarray chips, respectively.

Computational approaches can be used in microRNomics to
identify miRNAs and their target prediction (22, 44, 67, 119,
129). Computational methods to identify miRNA are based on
the following three observations. First, miRNAs generally
derive from precursor transcripts of 70–100 nucleotides with
extended stem-loop structure. Second, miRNAs are usually
highly conserved between the genomes of related species.
Third, miRNAs display a characteristic pattern of evolution-
ary divergence. Lai has successfully identified Drosophila
microRNAs using this computational approach (69). The
more important application of the computational approach
in microRNomics is to predict miRNAs’ mRNA targets. To
bridge the bioinformatics void in the miRNA database with the
in cyto and in vivo biology of an organism, a number of
computer programs have been developed for prediction of
miRNA targets (9, 45, 50, 80). The common criteria used for
target prediction by these computer programs are as follows:
1) the degree of base complementarity between the miRNA
and mRNA with special focus on identifying a perfect or
near-perfect complementarity between a target miRNA and the
miRNA in the “seed” region (i.e., nucleotides 2–8 of the
miRNA); 2) the calculated thermodynamic stability of the pre-
dicted miRNA/mRNA complex; 3) the degree of conservation of
orthologous target sites in the 3′-untranslated region (UTR) of
different species.

The integrative analysis of miRNA expression with comparat-
itive genomics, transcriptionomics, or proteomics is another im-
portant approach to study miRNA on the genome scale. Com-
parative genomics has been intensively used to discover a wide
range of functional elements, including protein-coding genes,
RNAs, and various classes of regulatory elements or motifs
(83). Recent studies suggest that comparative genomics pro-
vides an opportunity to discover functional miRNAs system-
atically, making use of their conservation across multiple
species (69, 94, 104). miRNAs is able to control a large-scale
gen expression by directing their target mRNAs for degrada-
tion. Paired expression profiles of miRNAs and genome-wide
miRNA expression (transcriptomic) approach is therefore a
useful method to identify functional miRNA target relationship
(49). Furthermore, the combination of miRNA research with
proteome has been proven to be an important approach for
miRNA study, because miRNAs control protein levels as their
final step for the gene expression regulation (35, 113). This
approach is particularly important because miRNAs control
some protein expression by mRNA translational silencing, but
not by mRNA degradation.

The data obtained from miRNA microarray and computa-
tional analyses should be verified experimentally by Northern
blot and/or real-time quantitative reverse transcriptase poly-
merase chain reaction (qRT-PCR) (22, 24, 56). In addition, in
situ hybridization is a good method to detect and localize
miRNAs in both paraffin-embedded and frozen tissue sections
(85, 103, 112). To verify the predicted target, a widely used
method is to make a plasmid construct, which encodes a
reporter, such as firefly luciferase, with a 3′-UTR containing
the predicted miRNA target and transfect the reporter plasmid
into a cell expressing the cognate miRNA. If the target and
miRNA interact, a decrease in luciferase activity should be
measured (1, 131). As a control, a similar reporter construct
with a mutated target sequence is tested. The advantage of this
method is that it is able to verify whether or not the miRNA is
a direct target under this special condition in a cell. However,
as one miRNA may have multiple mRNA targets, the tested
mRNA needs to be verified as the major target in native
experimental cells. Alternatively, one can inhibit the endoge-
nous miRNA by introducing an antisense oligonucleotide to
the cells and thus relieve miRNA-mediated repression of the
target mRNA with increased expression as a result. Further-
more, an miRNA-targeted gene expression should be verified
by qRT-PCR and Western blot at both mRNA and protein
levels.

To verify the biological function of a specific miRNA, the
following gain-of-function and loss-of-function approaches
should be applied both in vitro in cultured cells and in vivo in
mammals (56, 115). Virus-mediated miRNA gene transfer is the
first choice for the gain-of-function experiments. In addi-
tion, transfer of its pre-mRNA into the cultured cells in vitro
or tissues in vivo under some conditions is also suitable for
the gain-of-function approach. For the loss-of-function exper-
iments, antisense-based miRNA inhibitors or their modified
forms are broadly used both in vitro and in vivo (56). However,
efficacy is a big pitfall for these inhibitors in vivo. Thus,
miRNA knockout mice, especially conditional knockout mice,
should be the most powerful loss-of-function approach.

CELLULAR FUNCTIONS OF miRNAs

miRNAs regulate the expression of over 10,000 genes in a
cell. It is therefore not surprising that miRNAs are involved in
the regulation of almost all major cellular functions, such as
cell differentiation, proliferation/growth, mobility, and apopto-
sis (Fig. 1). These cellular effects of miRNAs are demonstrated
in many different cells, such as cancer cells and cardiovascular
cells (4, 34, 53, 58, 96). Thus, we have summarized these
cellular effects here using cardiovascular cells as examples.

The role of miR-1 in cardiomyocyte differentiation was
discovered in 2005 (130). It was found that the miR-1 gene is
a direct transcriptional target of several muscle differentiation
regulators, including serum response factors, myogenic differ-
entiation factor D, and the myocyte enhancing factor 2 (130).
Correspondingly, excess miR-1 in the developing heart leads to
a decreased pool of proliferating ventricular cardiomyocytes,
suggesting that miR-1 genes modulate the effects of critical
cardiac regulatory proteins to control the balance between
differentiation and proliferation during cardiogenesis.

The role of miRNAs in cardiac myocyte growth has been
documented in three recent studies (24, 101, 115). Overexpression
of miR-23a, miR-23b, miR-24, miR-195, or miR-214
via adenovirus-mediated gene transfer induced hypertrophic
growth of cultured cardiomyocytes, whereas overexpression of
miR-150 or miR-181b caused a reduction in cardiomyocyte
cell size (115). We have recently shown that miRNAs are
aberrantly expressed in cultured neonatal hypertrophic cardio-
myocytes that are stimulated by angiotensin II or phenyleph-
rine (24). Modulating an aberrantly upregulated miRNA such
as miR-21, via antisense-mediated knockdown, has a signifi-
cant negative effect on cardiomyocyte hypertrophy in vitro
(24). In contrast, overexpression of an aberrantly downregu-
lated miRNA such as miR-1, via adenovirus-mediated gene
transfer, is sufficient to prevent hypertrophic growth of cardiac myocytes (101). The cellular effects of miRNAs on the heart have been further confirmed both in vitro and in vivo (20, 111, 116).

In our recent study of the potential roles of miRNAs in vascular smooth muscle cell (VSMC) proliferation and apoptosis (56), we found that depletion of miR-21, an miRNA that is upregulated in proliferative VSMCs, results in decreased cell proliferation and increased cell apoptosis in a dose-dependent manner in cultured rat aortic VSMCs. The results suggest that miR-21 has a proproliferative and antiapoptotic effect on VSMCs.

The effects of miR-221 and miR-222 on vascular endothelial cell migration (mobility) were initially determined by tube formation and wound healing assays (92). The results suggest that the influence of miR-221 and miR-222 on endothelial cell migration occurs, at least in part, through their target c-kit. The effects of some other miRNAs, such as let-7, on human endothelial cell migration were also demonstrated in two recent reports (66, 106).

**MicroRNomics IN CANCER**

Cancer is a complex disease involving a variety of changes in gene expression that result in abnormal cell growth, migration, and apoptosis (11). As those genes and cellular functions are regulated by miRNAs, cancer became the first popular miRNA research area (4, 34, 53, 58, 96). During the past few years, expression signatures of miRNAs in many human cancers have been identified (12). Experimental approaches have confirmed that some miRNAs are tumor suppressors [tumor suppressor miRNAs (TS-miRs)] (23, 29) and some other miRNAs are oncogenes (oncomiRs) (25, 40). Thus, miRNAs may play important roles in cancer development, progression, prognosis, diagnosis, and evaluation of treatment response (12, 29) (Table 1).

Expression profiles have recently been generated by microarray analysis in multiple cancer types including chronic lymphocytic leukemia (CLL) (17, 18, 90), breast (55, 79), colon (6, 31, 82), lung (47, 125), pancreatic endocrine (99), pancreatic adenocarcinoma (13), prostate (79), stomach (118), and glioblastomas (21, 26). These microRNomic approaches reveal that a large number of miRNAs are aberrantly expressed in diverse cancers. The majority of these dysregulated miRNAs are targeted at either oncogenes or tumor-suppressing genes. As tissue and cell-specific expression is an important feature for miRNAs, these bioinformatic measurements of expression profiles are useful to identify and diagnose human cancers. It is well known that some miRNAs are critical regulators for cell differentiation, and identification of these key miRNAs’ expression signatures could be an alternative way to evaluate cancer progression and prognosis. Moreover, recent findings in preclinical studies suggest that miRNA expression in cancer cells is sensitive to drug treatments (81). It is therefore possible to use miRNA expression profiles as a novel clinical method to monitor the treatment responses.

The one important weakness for these expression profiles is that the number of known and predicted human miRNAs is consistently increasing. Most of these early expression experiments only contain part of them (<200 miRNAs). Thus, these expression profiles may not reflect the correct complete signatures. When the 1,000 human miRNA sequences are totally verified, reperforming these expression profiles should be warranted.

An increasing number of cancer-related miRNAs have been identified recently by microRNomics approaches. Indeed, some of miRNAs are expressed at much lower levels in tumors and most of them are oncomiRs. In contrast, some miRNA are overexpressed in tumors and most of them are confirmed as TS-miRs. Among these oncomiRs and TS-miRs, the following four groups are well studied and well documented.

The first study demonstrating direct involvement of miRNAs in cancer was the linking of miR-15a and miR-16-1 with human CLL (17, 18). It is well established that a 30 kb region of chromosome 13 (13q14) is a critical locus responsible for CLL (104). However, this region does not have any protein coding genes, but it has been recognized that two miRNAs, miR-15a, and miR-16-1, exist at this locus (16). Expression of these miRNAs was found to be diminished or completely ablated in >65% of CLL cases examined (17, 18). Although their mRNA targets are not completely elucidated, they appear

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Expression in Diseases</th>
<th>Functions</th>
<th>Potential Targets</th>
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<tbody>
<tr>
<td>miR-15a and miR-16-1</td>
<td>downregulation in chronic lymphocytic leukemia</td>
<td>tumor suppressor</td>
<td>Bcl-2</td>
</tr>
<tr>
<td>miR-17-92 cluster</td>
<td>upregulation in a wide range of tumors such as breast, colon, lung, prostate, and pancreatic endocrine</td>
<td>oncogene</td>
<td>E2F1, Tsp1, CTGF</td>
</tr>
<tr>
<td>Let 7 family</td>
<td>dowregulation in lung and colon cancers</td>
<td>tumor suppressor</td>
<td>Ras, PRDM1</td>
</tr>
<tr>
<td>miR-155</td>
<td>dowregulation in breast cancer</td>
<td>oncogene</td>
<td>MYC (?)</td>
</tr>
<tr>
<td>miR-125a and miR-125b</td>
<td>dowregulation in breast cancer</td>
<td>tumor suppressor</td>
<td>ERBB2, ERBB3</td>
</tr>
<tr>
<td>miR-21</td>
<td>upregulation in many tumors such as breast cancer and glioblastomas</td>
<td>oncogene</td>
<td>PTEN, Bcl-2 (?), PDCD4</td>
</tr>
<tr>
<td>miR-143 and miR-145</td>
<td>upregulation in heart with hypertrophy and vessel with neointimal formation</td>
<td>induces cardiac hypertrophy and</td>
<td>PTEN, Bcl-2 (?), PDCD4</td>
</tr>
<tr>
<td>miR-195</td>
<td>dowregulation in colorectal cancer, breast cancer and B-cell malignities</td>
<td>neointimal lesion formation</td>
<td>ERK5</td>
</tr>
<tr>
<td>miR-208</td>
<td>upregulation in heart with hypertrophy</td>
<td>induces cardiac hypertrophy</td>
<td>N/A</td>
</tr>
<tr>
<td>miR-133</td>
<td>dowregulation in heart with hypertrophy</td>
<td>inhibits cardiac hypertrophy</td>
<td>TLR7AP1</td>
</tr>
<tr>
<td>miR-1</td>
<td>downregulation in heart with hypertrophy</td>
<td>inhibits cardiac hypertrophy</td>
<td>RhoA, Cdc42, Nelf-A, WHSC2</td>
</tr>
<tr>
<td>miR-133</td>
<td>upregulation in ischemic heart tissue</td>
<td>arrhythmogenesis</td>
<td>RasGAP, Cdk9, fibronectin, and Rheb</td>
</tr>
<tr>
<td>miR-133</td>
<td>upregulation in diabetic heart</td>
<td>arrhythmogenesis</td>
<td>GJA1, KCNJ2</td>
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miR, micro-RNA.
to mediate their effects largely by downregulating the anti-apoptotic protein, BCL-2. This protein is often expressed at very high levels in CLL and is thought to be important for the survival of the malignant cells. Thus, the decreased expression of miR-15a and miR-16-1 results in the elevated levels of BCL-2 (17, 18, 27). Moreover, expression of these miRNAs is capable of inducing apoptosis in leukemia cell lines. The evidence suggests miR-15a and miR-16-1 may be important targets for CCL treatment.

The second group of miRNAs that are well documented in cancer is the miR-17-92 cluster that is frequently upregulated in lymphomas. This cluster consists of the seven following individual miRNAs: miR-17-5p, 17-3p, 18, 19a, 19b1, 20, and 92. All of these miRs are encoded from a frequently amplified locus at 13q31.3 (47, 88). It was shown that the miR-17-92 cluster, but not the individual miRNAs, can enhance tumorigenesis by inhibiting apoptosis in tumors (47). Further studies in human cell lines showed that transcription of the miR-17-92 cluster was directly regulated by c-Myc and that the individual miRs-17-5p and miR-20 regulate the translation of E2F1, a transcription factor with both proapoptotic and proproliferative activity (48). Thus, coexpression of c-Myc and miR-17 is believed to fine tune E2F1 activity so that proliferation is enhanced and apoptosis is inhibited (87). In addition to its confirmed role in lymphoma development, this miRNA cluster may also have broad significance in tumor biology, as members of this cluster are overexpressed in a wide range of tumors such as breast, colon, lung, prostate, and pancreatic endocrine (46).

Another group of cancer-related miRNAs that are extensively studied are miR-155 (37, 63, 64), the let-7 family (57), miR-125a and miR-125b (102), miR-21 (21), miR-143, and miR-145 (2). miR-155 is overexpressed in many tumors including B-cell lymphomas, Burkitt lymphoma, Hodgkin’s lymphoma, and breast, lung, colon, and thyroid cancers. Although the molecular mechanisms involved in miR-155-mediated procarcinogenesis are not clear, the interaction between miR-155 and the oncogene MYC seems to be one of the mechanisms. Intriguingly, mice overexpressing miR-155 under control of the Eμ enhancer are able to develop B-cell malignancy rapidly (28). Let-7 family members of miRNAs are also downregulated in lung and colon cancer cells. It was observed that low Let-7 expression correlated with a shortened postoperative survival in lung cancer patients who had undergone potentially curative operative procedures. miR-125a and miR-125b, whose expression is frequently lost or reduced in breast cancer, have been reported to regulate the important oncogenes ERBB2 and ERBB3. miR-21 is found to be overexpressed in many human tumors, such as breast cancers and glioblastomas, and has been confirmed as an oncomiRNA via its antiapoptosis effect. miR-143 and miR-145 are often downregulated in colorectal and breast cancers as well as B-cell malignancies, and there may be cancer related miRNAs in other human cancers.

MicroRNomics IN CARDIOVASCULAR DISEASE

Cardiac hypertrophy is a common pathological response to a number of cardiovascular diseases such as hypertension, ischemic heart disease, valvular diseases, and endocrine disorders. Cardiac hypertrophy often leads to heart failure in humans and is a major determinant of mortality and morbidity in cardiovascular diseases. miRNAs are important regulators for the differentiation and growth of cardiac cells, and it is therefore reasonable to hypothesize that miRNAs play important roles in cardiac hypertrophy and heart failure.

Almost simultaneously, three independent groups (including the current author) reported dramatic results in the miRNA expression signature of mouse hearts that were made hypertrophic by either aortic binding or expression of activated calcineurin (24, 101, 115) (Table 1). It should be noted that miRNAs are aberrantly expressed in hypertrophic hearts in both animal models, and these results were confirmed by in vitro studies of cardiac myocytes with hypertrophy (24, 101, 111, 115). Furthermore, overexpression of some miRNAs that are upregulated in hypertrophic hearts induces cardiac myocyte hypertrophy, whereas overexpression of some miRNAs that are downregulated in hypertrophic hearts prevents cardiac myocyte hypertrophy. On the other hand, inhibition of miR-21, an miRNA that is upregulated in the hypertrophic animal and human hearts, inhibits hypertrophic hearts in vitro (24). The role of miR-21 was further confirmed by another group (111). In vivo, overexpression of miR-195, a miRNA that is upregulated in hypertrophic hearts, is sufficient to induce cardiac hypertrophy (115), while a gene mutation or “decoy” approach has confirmed the role of miR-208 and miR-133 in cardiomyocyte hypertrophy (20, 114). Taken together, these findings demonstrate that multiple miRNAs are involved in cardiac hypertrophy and that modulating one aberrantly expressed miRNA is sufficient to modulate the hypertrophy. However, the molecular mechanisms responsible for individual miRNA-mediated effects on cardiac hypertrophy are unclear.

More recently, the roles of miRNAs in human cardiac hypertrophy and heart failure have been elucidated in several clinical studies (76, 115, 126). Northern blot analysis of the hypertrophy-regulated miRNAs in idiopathic, end-stage, failing human hearts shows that the expression of miR-24, miR-125b, miR-195, miR-199a, and miR-214 is significantly increased compared with control hearts (115). Forty-three out of 87 detected miRNAs are aberrantly expressed in hearts with ischemic cardiomyopathy, dilated cardiomyopathy, or aortic stenosis (87), indicating that miRNAs are indeed involved in the pathophysiology of human cardiac hypertrophy and heart failure.

Neointimal lesion formation is a common pathological lesion found in diverse cardiovascular diseases such as atherosclerosis, coronary heart diseases, postangioplasty restenosis, and transplantation arteriopathy. Using microarray analysis and a well-established neointimal formation model, we determined the miRNA expression profile in the vascular wall with neointimal lesion formation (24). Compared with normal, uninjured arteries, microarray analysis demonstrated that aberrant miRNA expression is a characteristic of vascular walls after angioplasty. Those miRNAs that are highly expressed in the rat carotid artery and are more than onefold upregulated or 50% downregulated after angioplasty were further verified by qRT-PCR and/or Northern blot analysis (24). Modulating an aberrantly overexpressed miRNA, miR-21, via antisense-mediated knockdown has a significantly negative effect on neointimal lesion formation in rat artery after angioplasty (Fig. 2). These results indicate that miRNAs are important regulators in the development of proliferative vascular diseases (Table 1).

Cardiac arrhythmias in the setting of ischemic heart disease remain a serious health problem because of their sudden and
unpredictable nature and their potentially grave consequences. In a rat model of myocardial infarction and in human heart with coronary heart disease, the muscle-specific miRNA, miR-1, was significantly upregulated in ischemic heart tissue (126). To further determine the role of miR-1 in arrhythmogenesis, both gain-of-function and loss-of-function approaches were applied to enhance or inhibit miR-1 expression in the infarcted myocardium. The results show that injection of mature miR-1 exacerbates arrhythmogenesis, whereas elimination of miR-1 by an antisense inhibitor suppresses arrhythmias. The results indicate that miR-1 has proarrhythmic, as well as arrhythmogenic effects (126). Silencing the genes for the ion channels GJA1 and KCNJ2 verified that these proteins are important players in the miR-1-mediated arrhythmogenic effect (126) (Table 1).

miR-133 expression is upregulated (123) in diabetic rabbit heart. The ether-a-go-go-related gene (ERG), a long QT syndrome gene encoding a key K⁺ channel (IK₆) in cardiac cells, was confirmed to be a target for miR-133 (123). Delivery of exogenous miR-133 into the rabbit myocytes and cell lines produces posttranscriptional repression of ERG, thereby downregulating ERG protein levels without altering its transcription level, subsequently causing substantial depression ofIK₆, an effect that is abrogated by the miR-133 antisense inhibitor (123). Thus, depression ofIK₆ via repression of ERG bymiR-133 may contribute to the slowing of myocyte repolarization and, thereby, QT prolongation and the associated arrhythmias in diabetic hearts (Table 1).

In cardiac cells, KCNQ1 assembles with KCNE1 and forms a channel complex constituting the slow delayed rectifier currentIK₆. Expression of KCNQ1 and KCNE1 is regionally heterogeneous and changes with pathological states of the heart; however, the molecular mechanisms responsible for these changes are unclear. Recently, one study has characterized KCNQ1 and KCNE1 as targets of the muscle-specific miRNAs, miR-133 and miR-1, respectively (124). The heterogeneous expression of miR-1 and miR-133 offers an explanation for the well-recognized regional differences in expression ofKCNQ1 and KCNE1 and for the disparity between the levels of their mRNA and protein in each region (124).

HCN2 and HCN4 are two important cardiac pacemaker channel proteins that control rhythmic activity of the heart. One recent study has demonstrated that HCN2 mRNA is a target of miR-1 and miR-133 and that HCN4 mRNA is a target of miR-1 (122). To explore the possibility of using miRNAs in a gene-specific manner, the authors of this study developed two new therapeutic approaches, which were the gene-specific miRNA mimic and miRNA-masking antisense approaches. Their results demonstrate that gene-specific miRNA mimics, which are 22-nt RNAs designed to target the 3’-UTRs of HCN2 and HCN4, are efficient in abrogating the expression and function of HCN2 and HCN4. Meanwhile, the microRNA-masking antisense, based on the miR-1 and miR-133 target sites in the 3’-UTRs of HCN2 and HCN4, markedly enhances HCN2 and HCN4 expression and function. Thus, these two therapeutic approaches based on the principles of action of miRNAs could provide novel gene therapy strategies for cardiac arrhythmias (122).

miRNAs are also involved in the regulation of insulin release and cholesterol metabolism. Dysfunction of these miRNAs might be related to some metabolic disorders. For example, miR-375 was shown to directly regulate insulin secretion from pancreatic islet cells (93). Upregulation of miR-375 led to an enhanced inhibition of insulin release. In contrast, the miR-375 inhibitor enhanced insulin secretion via blocking the effect of the miRNA (93). miR-122 is an important miRNA in liver. Antisense targeting this miRNA revealed that inhibition of miR-122 resulted in decreased levels of cholesterol in the plasma and improved liver function in obese mice (39). Recent studies demonstrated that some miRNAs such as miR-155 (98, 117), miR-146, and miR-181a were able to regulate T and B cell function (73, 110). Thus, miRNAs are also implicated in immune function regulation, and dysregulation of these miRNAs may be related to some immune and inflammatory disorders (84, 86, 109).

CLOSING REMARKS AND PERSPECTIVE

Investigating the role of miRNAs in disease biology is a new frontier in biomedical research. Although the newly coined term microRNomics has increasingly been used in personal communications to describe this new subdiscipline of genomics, there is no formal nomenclature reported previous to this review article. While the field of miRNAs is at an early stage, the study of their roles in human disease has a history of less than 3 yr; increasing evidence has revealed that miRNAs may play important roles in human disease development, progression, prognosis, diagnosis, and evaluation of treatment response. Moreover, miRNAs may represent a novel new therapeutic target in diverse human diseases.

As mentioned earlier, although miRNA expression profiles in some humans have been determined recently, the probe content of these miRNA microarray chips only contain parts of the entire human miRNA repertoire. As all the 1,000 human miRNAs are eventually identified and sequenced, using microarray chips containing all the human miRNAs for expression signatures in diverse human diseases will be critical to identify the key miRNAs responsible for a particular disease state. We predict that more and more disease expression profiles of miRNAs will be presented in the next several years. Based on these novel microRNomics data, the key miRNAs for a specific disease and their miRNA targets will be further verified by experimental approaches.

An important avenue for future research is the development of therapies based on miRNAs. A promising approach is to...
target disease-related miRNAs using anti-miRNA oligos (miRNA inhibitors) to knock down overexpressed miRNAs or their mature or precursor form, to increase downregulated miRNAs. In animals and cultured cells, these oligos are proving to have promising therapeutic effects. However, until now, no studies in humans have been performed in vivo. One challenge of these treatments is the delivery method to transfer the miRNAs into the desired tissues. Given that these oligos cannot discriminate between healthy and diseased cells, side effects of these treatments remain a concern. Nevertheless, with a deeper understanding of the pharmacology of these oligos, the molecular mechanisms of miRNA actions, and the development of new delivery technologies, these small molecules may well fulfill their promise as valuable novel therapeutics.

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REFERENCES

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