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Review

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

IT IS CLEAR THAT GENE EXPRESSION in the human is precisely controlled in a cell, tissue, time, and condition-specific manner. Large-scale microarray data suggest that different cells, tissues, and organ systems within an organism (including humans) have different gene expression profiles, although they have the same genome. Moreover, these gene expression signatures are sensitive to changes in condition, such as development, diseases, environment changes, and therapeutic drugs (10, 36, 75, 94). Therefore, completely understanding the regulatory mechanisms of gene expression is one of the most important issues in genomic medicine (36, 59, 75, 97). Any important breakthroughs in this research area will have the potential to give rise to impacts on modern clinical medicine in diagnosis and therapy, because most of human diseases are multigene (multifactor) diseases, in which the expression of multiple genes is changed directly or indirectly (15, 19, 41, 91).

Since the discovery of the DNA double-helix structure by Watson and Crick (120) in 1953, the standard pathway of information flow in a cell from DNA to message RNA (mRNA) to protein has been the dominant theme in molecular biology. However, recent analyses of the human and animal genomes have demonstrated that the majority of RNA transcripts are not protein coding RNAs (mRNAs), but noncoding RNAs (ncRNAs) (77, 105, 108). Indeed, large-scale complementary DNA sequencing and genome tiling array studies have shown that ~50% of genomic DNA in humans is transcribed into RNA transcripts, of which 2% is translated into proteins and the remaining 98% is ncRNAs (38, 77, 78, 105, 108). In general, the sizes of the majority of ncRNA species vary from 18 nt to 500 nt, well below the size of the majority of mRNA species, and are therefore termed small ncRNAs (sncRNAs). The term ncRNA is commonly employed for RNA that does not encode a protein, but this does not mean that such RNAs do not contain information or have function (38, 77, 78, 105, 108). For example, ribosomal RNAs and transfer RNAs, which make up a large proportion of RNA based on amount, are two known sncRNAs that provide help for protein expression. Quite recently, two novel classes of sncRNAs were discovered: microRNAs (miRNAs) and small interfering RNAs (siRNAs) (38, 78, 108). Both have strong regulatory effects on mRNA translation and represent a novel important layer for gene expression (38, 78, 108). Analogous to the first RNA revolu-

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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-41*, 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.

BIOGENESIS 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 Drosha 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

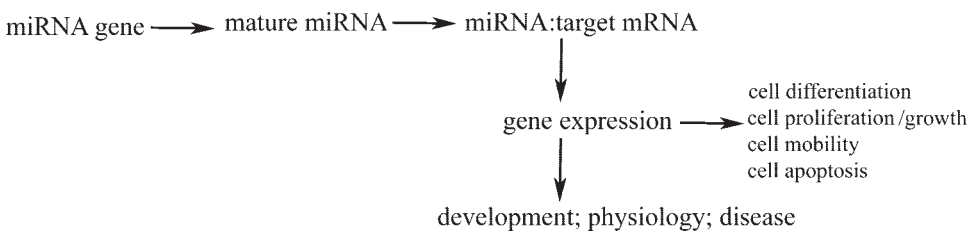

Fig. 1. Biological function of microRNAs and their mechanisms.

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 evolutionary 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 mRNA 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 mRNA and the miRNA in the "seed" region (i.e., nucleotides 2–8 of the miRNA); *2*) the calculated thermodynamic stability of the predicted 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 comparative genomics, transcriptomics, or proteomics is another important approach to study miRNA on the genome scale. Comparative 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 provides an opportunity to discover functional miRNAs systematically, making use of their conservation across multiple species (69, 94, 104). miRNAs is able to control a large-scale gene expression by directing their target mRNAs for degradation. Paired expression profiles of miRNAs and genome-wide mRNA 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 computational analyses should be verified experimentally by Northern blot and/or real-time quantitative reverse transcriptase polymerase 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 mRNA 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 endogenous 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. Furthermore, 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 addition, 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 experiments, 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 apoptosis (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 differentiation 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 cardiomyocytes that are stimulated by angiotensin II or phenylephrine (24). Modulating an aberrantly upregulated miRNA such as miR-21, via antisense-mediated knockdown, has a significant negative effect on cardiomyocyte hypertrophy in vitro (24). In contrast, overexpression of an aberrantly downregulated 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 1. *miRNAs in disease biology determined by microRNomic approach*

miRNAs	Expression in Diseases	Functions	Potential Targets
miR-15a and miR-16-1	downregulation in chronic lymphocytic leukemia	tumor suppressor	BcL-2
miR-17-92 cluster	upregulation in a wide range of tumors such as breast, colon, lung, prostate, and pancreatic endocrine	oncogene	E2F1, Tsp1, CTGF
Let 7 family	dowregulation in lung and colon cancers	tumor suppressor	Ras, PRDM1
miR-155	upregulation in lymphomas and breast cancer	oncogene	MYC (?)
miR-125a and miR-125b	dowregulation in breast cancer	tumor suppressor	ERBB2, ERBB3
miR-21	upregulation in many tumors such as breast cancer and glioblastomas	oncogene	PTEN, Bcl-2 (?), PDCD4
miR-21	upregulation in heart with hypertrophy and vessel with neointimal formation	induces cardiac hypertrophy and neointimal lesion formation	PTEN, Bcl-2 (?), PDCD4
miR-143 and miR-145	dowregulation in colorectal cancer, breast cancer and B-cell maligancies	tumor suppressor	ERK5
miR-195	upregulation in heart with hypertrophy	induces cardiac hypertrophy	N/A
miR-208	upregulation in heart with hypertrophy	induces cardiac hypertrophy	THRAP1
miR-133	downregulation in heart with hypertrophy	inhibits cardiac hypertrophy	RhoA,Cdc42,Nelf-A/WHSC2
miR-1	downregulation in heart with hypertrophy	inhibits cardiac hypertrophy	RasGAP, Cdk9, fibronectin, and Rheb
miR-1	upregulation in ischemic heart tissue	arrhythmogenesis	GJA1,KCNJ2
miR-133	upregulation in diabetic heart	arrhythmogenesis	ERG

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

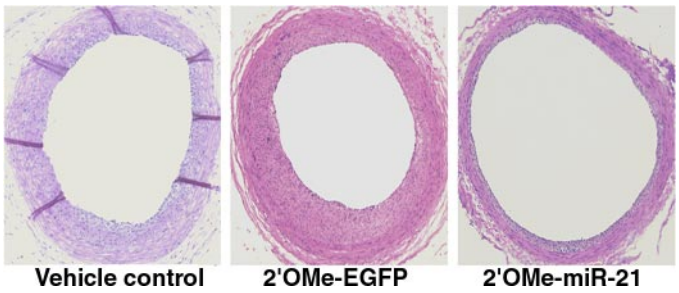

Fig. 2. Downregulation of miR-21 decreases neointimal lesion formation in rat carotid artery after angioplasty. Representative hematoxylin-eosin-stained photomicrographs of rat carotid arteries from vehicle-, miR-21 antisense oligonucleotide (2′OMe-miR-21)-, and control oligonucleotide (2′OMe-EGFP)-treated groups at 14 days after angioplasty. Reproduced with permission from *Cir Res* (56).

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 (I_{Kr}) 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 transcript level, subsequently causing substantial depression of I_{Kr}, an effect that is abrogated by the miR-133 antisense inhibitor (123). Thus, depression of I_{Kr} via repression of ERG by miR-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 current I_{Ks}. 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 of KCNQ1 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 enhance 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).

MicroRNomics IN OTHER DISEASES OR DISORDERS

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 mRNA 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

1. **Adams BD, Furneaux H, White BA.** The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. *Mol Endocrinol* 21: 1132–1147, 2007.
2. **Akao Y, Nakagawa Y, Naoe T.** MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* 16: 845–850, 2006.
3. **Ambros V.** MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 113: 673–676, 2003.
4. **Ambros V.** The functions of animal microRNAs. *Nature* 43: 350–355, 2004.
5. **Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE.** Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell* 122: 553–563, 2005.
6. **Bandrés E, Cubedo E, Agirre X, Malumbres R, Zárate R, Ramirez N, Abajo A, Navarro A, Moreno I, Monzó M, García-Foncillas J.** Identification by real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Mol Cancer* 5: 29–32, 2006.
7. **Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, Einav U, Gilad S, Hurban P, Karov Y, Lobenhofer EK, Sharon E, Shiboleth YM, Shtutman M, Bentwich Z, Einat P.** MicroRNA expression detected by oligonucleotide microarrays: system establishment and expression profiling in human tissues. *Genome Res* 14: 2486–2494, 2004.
8. **Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z.** Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet* 37: 766–770, 2005.
9. **Betel D, Wilson M, Gabow A, Marks DS, Sander C.** The microRNA.org resource: targets and expression. *Nucleic Acids Res* 36: D149–D153, 2008.
10. **Birchler JA, Veitia RA.** The gene balance hypothesis: from classical genetics to modern genomics. *Plant Cell* 19: 395–402, 2007.
11. **Blagosklonny MV.** Molecular theory of cancer. *Cancer Biol Ther* 4: 621–627, 2005.
12. **Blenkiron C, Miska EA.** miRNAs in cancer: approaches, aetiology, diagnostics and therapy. *Hum Mol Genet* 16: R106–R113, 2007.
13. **Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM.** MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 297: 1901–1908, 2007.
14. **Borchert GM, Lanier W, Davidson BL.** RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol* 13: 1097–1101, 2006.
15. **Burke W.** Genomics as a probe for disease biology. *N Engl J Med* 349: 969–974, 2003.
16. **Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM.** Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 99: 15524–15529, 2002.
17. **Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM.** A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 353: 1793–1801, 2005.
18. **Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, Croce CM.** MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci USA* 101: 11755–11760, 2004.
19. **Cambien F, Tiret L.** Genetics of cardiovascular diseases: from single mutations to the whole genome. *Circulation* 116: 1714–1724, 2007.
20. **Carè A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Høydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G.** MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13: 613–618, 2007.
21. **Chan JA, Krichevsky AM, Kosik KS.** MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 65: 6029–6033, 2005.
22. **Chaudhuri K, Chatterjee R.** MicroRNA detection and target prediction: integration of computational and experimental approaches. *DNA Cell Biol* 26: 321–337, 2007.
23. **Chen CZ.** MicroRNAs as oncogenes and tumor suppressors. *N Engl J Med* 353: 1768–1771, 2005.
24. **Cheng Y, Ji R, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C.** MicroRNAs are aberrantly expressed in hypertrophic heart: do they play a role in cardiac hypertrophy? *Am J Pathol* 170: 1831–1840, 2007.
25. **Cho WC.** OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer* 6: 60–69, 2007.
26. **Ciafrè SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG.** Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun* 334: 1351–1358, 2005.
27. **Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM.** miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA* 102: 13944–13949, 2005.
28. **Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM.** Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA* 103: 7024–7029, 2006.
29. **Cowland JB, Hother C, Grønbaek K.** MicroRNAs and cancer. *APMIS* 115: 1090–1106, 2007.
30. **Cullen BR.** Derivation and function of small interfering RNAs and microRNAs. *Virus Res* 102: 3–9, 2004.
31. **Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LA Jr, Sjoblom T, Barad O, Bentwich Z, Szafranska AE, Labourier E, Raymond CK, Roberts BS, Juhl H, Kinzler KW, Vogelstein B, Velculescu VE.** The colorectal microRNAome. *Proc Natl Acad Sci USA* 103: 3687–3692, 2006.
32. **Davison TS, Johnson CD, Andruss BF.** Analyzing micro-RNA expression using microarrays. *Methods Enzymol* 411: 14–34, 2006.
33. **Doench JG, Peterson CP, Sharp PA.** siRNAs can function as miRNAs. *Genes Dev* 17: 438–442, 2003.
34. **Du T, Zamore PD.** Beginning to understand microRNA function. *Cell Res* 17: 661–663, 2007.
35. **Duchaine TF, Wohlschlegel JA, Kennedy S, Bei Y, Conte D Jr, Pang K, Brownell DR, Harding S, Mitani S, Ruvkun G, Yates 3rd JR, Mello CC.** Functional proteomics reveals the biochemical niche of *C. elegans* DCR-1 in multiple small-RNA-mediated pathways. *Cell* 124: 343–354, 2006.
36. **Eckhardt F, Beck S, Gut IG, Berlin K.** Future potential of the Human Epigenome Project. *Expert Rev Mol Diagn* 4: 609–618, 2004.

37. **Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE.** Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci USA* 102: 3627–3632, 2005.
38. **Erdmann VA, Barciszewska MZ, Hochberg A, de Groot N, Barciszewski J.** Regulatory RNAs. *Cell Mol Life Sci* 58: 960–977, 2001.
39. **Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP.** miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 3: 87–98, 2006.
40. **Esquela-Kerscher A, Slack FJ.** Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 6: 259–269, 2006.
41. **Evans J, Khoury MJ.** Evidence based medicine meets genomic medicine. *Genet Med* 9: 799–800, 2007.
42. **Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge CB, Bartel DP.** The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science* 310: 1817–1821, 2005.
43. **Filipowicz W.** Imprinted expression of small nucleolar RNAs in brain: time for RNomics. *Proc Natl Acad Sci USA* 97: 14035–14037, 2000.
44. **Ghosh Z, Chakrabarti J, Mallick B.** miRNomics-the bioinformatics of microRNA genes. *Biochem Biophys Res Commun* 363: 6–11, 2007.
45. **Griffiths-Jones S.** miRBase: the microRNA sequence database. *Methods Mol Biol* 342: 129–138, 2006.
46. **Hagan JP, Croce CM.** MicroRNAs in carcinogenesis. *Cytogenet Genome Res* 118: 252–259, 2007.
47. **Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T.** A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 65: 9628–9632, 2005.
48. **He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A.** The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci USA* 102: 19075–19080, 2005.
49. **Huang JC, Babak T, Corson TW, Chua G, Khan S, Gallie BL, Hughes TR, Blencowe BJ, Frey BJ, Morris QD.** Using expression profiling data to identify human microRNA targets. *Nat Methods* 4: 1045–1049, 2007.
50. **Huang TH, Fan B, Rothschild MF, Hu ZL, Li K, Zhao SH.** MiRFinder: an improved approach and software implementation for genome-wide fast microRNA precursor scans. *BMC Bioinformatics* 8: 341–342, 2007.
51. **Hüttenhofer A, Brosius J, Bachellerie JP.** RNomics: identification and function of small, non-messenger RNAs. *Curr Opin Chem Biol* 6: 835–843, 2002.
52. **Hutvágner G, Zamore PD.** A microRNA in a multiple-turnover RNAi enzyme complex. *Science* 297: 2056–2060, 2002.
53. **Hwang HW, Mendell JT.** MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 94: 776–780, 2006.
54. **Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, Allen PD, Golub TR, Pieske B, Pu WT.** Altered microRNA expression in human heart disease. *Physiol Genomics* 31: 367–373, 2007.
55. **Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM.** MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 65: 7065–7070, 2005.
56. **Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C.** MicroRNA expression signature and antisense-mediated depletion reveal an essential role of microRNA in vascular neointimal lesion formation. *Circ Res* 100: 1579–1588, 2007.
57. **Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ.** RAS is regulated by the let-7 microRNA family. *Cell* 120: 635–647, 2005.
58. **Jovanovic M, Hengartner MO.** miRNAs and apoptosis: RNAs to die for. *Oncogene* 25: 6176–6187, 2006.
59. **Jura J, Wegrzyn P, Jura J, Koj A.** Regulatory mechanisms of gene expression: complexity with elements of deterministic chaos. *Acta Biochim Pol* 53: 1–10, 2006.
60. **Kim VN.** Small RNAs: classification, biogenesis, function. *Mol Cells* 19: 1–15, 2005.
61. **Kim YK, Kim VN.** Processing of intronic microRNAs. *EMBO J* 26: 775–783, 2007.
62. **Kim VN.** MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 6: 376–385, 2005.
63. **Kluiver J, Haralambieva E, de Jong D, Blokzijl T, Jacobs S, Kroesen BJ, Poppema S, van den Berg A.** Lack of BIC and microRNA miR-155 expression in primary cases of Burkitt lymphoma. *Genes Chromosomes Cancer* 45: 147–153, 2006.
64. **Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, Kroesen BJ, van den Berg A.** BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J Pathol* 207: 243–249, 2005.
65. **Kong Y, Han JH.** MicroRNA: biological and computational perspective. *Genomics Proteomics Bioinformatics* 3: 62–72, 2005.
66. **Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S.** Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res* 101: 59–68, 2007.
67. **Kulkarni RV, Kulkarni PR.** Computational approaches for the discovery of bacterial small RNAs. *Methods* 43: 131–139, 2007.
68. **Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T.** Identification of novel genes coding for small expressed RNAs. *Science* 294: 853–858, 2001.
69. **Lai EC.** Predicting and validating microRNA targets. *Genome Biol* 5: 115, 2004.
70. **Latronico MV, Catalucci D, Condorelli G.** Emerging role of microRNAs in cardiovascular biology. *Circ Res* 101: 1225–1236, 2007.
71. **Lee RC, Feinbaum RL, Ambros The CV.** elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75: 843–854, 1993.
72. **Lewis BP, Burge CB, Bartel DP.** Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15–20, 2005.
73. **Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P, Klein LO, Davis MM, Chen CZ.** miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell* 129: 147–161, 2007.
74. **Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM.** Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433: 769–773, 2005.
75. **Little PF.** Structure and function of the human genome. *Genome Res* 15: 1759–1766, 2005.
76. **Mann DL.** MicroRNAs and the failing heart. *N Engl J Med* 356: 2644–2645, 2007.
77. **Mattick JS, Makunin IV.** Non-coding RNA. *Hum Mol Genet* 15: R17–R29, 2006.
78. **Mattick JS, Makunin IV.** Small regulatory RNAs in mammals. *Hum Mol Genet* 15: R17–R29, 2006.
79. **Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C.** Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Mol Cancer* 5: 24–26, 2006.
80. **Megraw M, Sethupathy P, Corda B, Hatzigeorgiou AG.** miRGen: a database for the study of animal microRNA genomic organization and function. *Nucleic Acids Res* 35: D149–D155, 2007.
81. **Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T.** Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* 130: 2113–2129, 2006.
82. **Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ.** Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 1: 882–891, 2003.
83. **Miller W, Makova KD, Nekrutenko A, Hardison RC.** Comparative genomics. *Annu Rev Genomics Hum Genet* 5: 15–56, 2004.
84. **Moschos SA, Williams AE, Perry MM, Birrell MA, Belvisi MG, Lindsay MA.** Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. *BMC Genomics* 8: 240–246, 2007.
85. **Nuovo GJ.** In situ detection of precursor and mature microRNAs in paraffin embedded, formalin fixed tissues and cell preparations. *Methods* 44: 39–46, 2008.
86. **O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D.** MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA* 104: 1604–1609, 2007.

87. **O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT.** c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 435: 839–943, 2005.
88. **Ota A, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, Yoshida Y, Seto M.** Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer Res* 64: 3087–3095, 2004.
89. **Pasquinelli AE, Hunter S, Bracht J.** MicroRNAs: a developing story. *Curr Opin Genet Dev* 15: 200–205, 2005.
90. **Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, Calin GA, Hagan JP, Kipps T, Croce CM.** Tcl1 expression in chronic lymphocytic leukemia is regulated by mir-29 and mir-181. *Cancer Res* 66: 11590–11593, 2006.
91. **Peltonen L, McKusick VA.** Genomics and medicine. Dissecting human disease in the postgenomic era. *Science* 291: 1224–1229, 2001.
92. **Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, Mercatanti A, Hammond S, Rainaldi G.** MicroRNAs modulate the angiogenic properties of HUVECs. *Blood* 108: 3068–3071, 2006.
93. **Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M.** A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432: 226–230, 2004.
94. **Rajewsky N.** microRNA target predictions in animals. *Nat Genet* 38: S8–S13, 2006.
95. **Ramos KS, Steffen MC, Falahatpisheh MH, Nanez A.** From genomics to mechanistic insight: a global perspective on molecular deficits induced by environmental agents. *Environ Mol Mutagen* 48: 395–399, 2007.
96. **Rane S, Sayed D, Abdellatif M.** MicroRNA with a MacroFunction. *Cell Cycle* 6: 1850–1855, 2007.
97. **Rédei GP, Koncz C, Phillips JD.** Changing images of the gene. *Adv Genet* 56: 53–100, 2006.
98. **Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A.** Requirement of bic/microRNA-155 for normal immune function. *Science* 316: 608–611, 2007.
99. **Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, Calin GA, Volinia S, Liu CG, Scarpa A, Croce CM.** MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. *J Clin Oncol* 24: 4677–4684, 2006.
100. **Ruby JG, Jan CH, Bartel DP.** Intronic microRNA precursors that bypass Drosha processing. *Nature* 448: 83–86, 2007.
101. **Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M.** MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ Res* 100: 416–424, 2007.
102. **Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC.** Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem* 282: 1479–1486, 2007.
103. **Silahtaroglu AN, Nolting D, Dyrskjøt L, Berezikov E, Møller M, Tommerup N, Kauppinen S.** Detection of microRNAs in frozen tissue sections by fluorescence in situ hybridization using locked nucleic acid probes and tyramide signal amplification. *Nat Protoc* 2: 2520–2528, 2007.
104. **Stark A, Kheradpour P, Parts L, Brennecke J, Hodges E, Hannon GJ, Kellis M.** Systematic discovery and characterization of fly microRNAs using 12 Drosophila genomes. *Genome Res* 17: 1865–1879, 2007.
105. **Storz G.** An expanding universe of noncoding RNAs. *Science* 296: 1260–1263, 2002.
106. **Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC.** Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res* 100: 1164–1173, 2007.
107. **Sun Y, Koo S, White N, Peralta E, Esau C, Dean NM, Perera RJ.** Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. *Nucleic Acids Res* 32: e188–e120, 2004.
108. **Szymański M, Barciszewska MZ, Zywicki M, Barciszewski J.** Noncoding RNA transcripts. *J Appl Genet* 44: 1–19, 2003.
109. **Taganov KD, Boldin MP, Baltimore D.** MicroRNAs and immunity: tiny players in a big field. *Immunity* 26: 133–137, 2007.
110. **Taganov KD, Boldin MP, Chang KJ, Baltimore D.** NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 103: 12481–12486, 2006.
111. **Tatsuguchi Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, Rojas M, Hammond SM, Wang DZ.** Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J Mol Cell Cardiol* 42: 1137–1141, 2007.
112. **Thompson RC, Deo M, Turner DL.** Analysis of microRNA expression by in situ hybridization with RNA oligonucleotide probes. *Methods* 43: 153–1561, 2007.
113. **Tian Z, Greene AS, Pietrusz JL, Matus IR, Liang M.** MicroRNA-target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. *Genome Res* 18: 404–411, 2008.
114. **Van Rooij E, Olson EN.** MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest* 117: 2369–2376, 2007.
115. **Van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN.** A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci USA* 103: 18255–18260, 2006.
116. **Van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN.** Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316: 575–579, 2007.
117. **Vigorito E, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KG, Rada C, Enright AJ, Toellner KM, Maclennan IC, Turner M.** microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity* 27: 847–859, 2007.
118. **Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM.** A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 103: 2257–2261, 2006.
119. **Watanabe Y, Tomita M, Kanai A.** Computational methods for microRNA target prediction. *Methods Enzymol* 427: 65–86, 2007.
120. **Watson JD, Crick FH.** Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* 171: 737–738, 1953.
121. **Wightman B, Ha I, Ruvkun G.** Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans. Cell* 75: 855–862, 1993.
122. **Xiao J, Luo X, Lin H, Zhang Y, Lu Y, Wang N, Zhang Y, Yang B, Wang Z.** MicroRNA miR-133 represses HERG K^+ channel expression contributing to QT prolongation in diabetic hearts. *J Biol Chem* 28: 12363–12367, 2007.
123. **Xiao J, Yang B, Lin H, Lu Y, Luo X, Wang Z.** Novel approaches for gene-specific interference via manipulating actions of microRNAs: examination on the pacemaker channel genes HCN2 and HCN4. *J Cell Physiol* 212: 285–292, 2007.
124. **Xu C, Lu Y, Pan Z, Chu W, Luo X, Lin H, Xiao J, Shan H, Wang Z, Yang B.** The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. *J Cell Sci* 120: 3045–3052, 2007.
125. **Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC.** Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9: 189–198, 2006.
126. **Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z.** The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med* 13: 486–491, 2007.
126a. **Zaug AJ, Cech TR.** The intervening sequence RNA of Tetrahymena is an enzyme. *Science* 231: 470–475, 1986.
127. **Zeng Y, Cullen BR.** Sequence requirements for micro RNA processing and function in human cells. *RNA* 9: 112–123, 2003.
128. **Zeng Y, Yi R, Cullen BR.** MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. *Proc Natl Acad Sci USA* 100: 9779–9784, 2003.
129. **Zhang B, Pan X, Wang Q, Cobb GP, Anderson TA.** Computational identification of microRNAs and their targets. *Comput Biol Chem* 30: 395–407, 2006.
130. **Zhao Y, Samal E, Srivastava D.** Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 436: 214–220, 2005.
131. **Zhu S, Si ML, Wu H, Mo YY.** MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem* 282: 14328–14336, 2007.