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EDITORIAL

Micro RNAs: a Revolutionary Discovery in Biology

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ABSTRACT

MicroRNAs (miRNAs) are endogenous, single-stranded, short, noncoding ribonucleic acids (RNAs), which can bind to their target messenger RNAs (mRNAs), leading to the inhibition of translation or degradation of the mRNA. Only recently have scientists discovered the important role that miRNAs play in gene regulation. To date, more than 700 miRNAs have been identified from the human genome. Malfunctioning miRNAs have been implicated in a number of diseases, due to their regulatory functions in transcription, signal transduction, cell cycle regulation, proliferation, cell growth and metabolism, cell apoptosis, and neurogenesis. Absence of miRNAs or their mutations, detected by genetic analysis, has been associated with a broad spectrum of disease processes, such as various cancers and autoimmune, cardiovascular, infectious, metabolic, neurodegenerative, skin, and psychiatric diseases. The large progress made in understanding miRNAs also points to their great potential as new biomarkers in the diagnosis and early detection of various diseases, as well as their promising role in future therapeutics.

KEY WORDS: *micro RNA; gene regulation; biomarkers; cancer; cardiovascular disease; autoimmune diseases; neurodegenerative diseases; antagomirs*

ABBREVIATIONS
CVD = cardiovascular disease
DNA = deoxyribonucleic acid
IL = interleukin
mRNA = messenger RNA
miRNA = micro RNA
RNA = ribonucleic acid
TLRs = Toll-like receptors
TNF = tumor necrosis factor

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INTRODUCTION

MicroRNAs (miRNAs) are endogenous, single-stranded, short (19-25 nucleotides in length), noncoding ribonucleic acids (RNAs), which can bind to complementary sequences in the three prime untranslated regions of their target messenger RNAs (mRNAs), leading to the inhibition of translation or degradation of the mRNA.[1] Only recently have scientists discovered the important role that miRNAs play in gene regulation, deemed as negative regulators of gene expression. To date, more than 700 miRNAs have been identified from the human genome. Malfunctioning miRNAs have been implicated in a number of diseases, due to their regulatory functions in transcription, signal transduction, cell cycle regulation, proliferation, cell growth and metabolism, cell apoptosis, and neurogenesis. MicroRNAs, an abundant class of gene regulators, are highly expressed in regulatory T cells and a wide range of miRNAs are involved in the regulation of immunity and in the prevention of autoimmunity. Certain miRNAs serve in important negative feedback loops in the immune system, whereas others serve to amplify the response of the immune system by repressing inhibitors of the response. Absence of miRNAs or their mutations, detected by genetic analysis, has been associated with various cancers and diseases such as autoimmune, cardiovascular,

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infectious, metabolic, neurodegenerative, skin, and psychiatric diseases (Table 1).[1-12] This disease association may further lead to potential diagnostic (new biomarkers) and therapeutic applications of miRNA delivery. Different approaches have been proposed such as the modification of microRNAs and the use of viral delivery vectors. Commonly used methods for measuring miRNAs in the research setting, include Northern blot, polymerase chain reaction, and microarray.

TABLE 1. Micro-RNAs in Various Diseases

DISEASE	Micro-RNA	FUNCTION / STATUS
Autoimmune Diseases		
Asthma	miR-21 miR-126	modulates interleukin (IL)-12 suppresses the asthmatic phenotype
Diabetes (autoimmune)	miR-375 61 glucose regulated miRNAs	Negatively regulates glucose-stimulated insulin release in a calcium independent manner (the most abundant intra-islet miRNA) most of these miRNAs are up-regulated / only few (miR-296, miR-184 & miR-160) are down-regulated
Inflammatory Bowel Disease (IBD)	miR-192, miR-375, miR-422b miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126, miR-195, & let-7f	significantly decreased in the ulcerative colitis (UC) tissues significantly increased in active UC tissues / miR-192 & miR-21 are the most highly expressed of the active UC-associated miRNAs in human colonic tissues
Multiple sclerosis (MS)	hsa-miR-145 other 165 miRNAs	the best single miRNA marker that allows discriminating MS from controls 165 miRNAs significantly up- or down-regulated
Psoriasis	miR-203 miR-146a miR-125b	keratinocyte-derived miRNA / up-regulation of miR-203 in psoriatic plaques up-regulated, involved in the innate immune responses & the TNF-pathway Involved in TNF-pathway/also deregulated in psoriasis & atopic eczema
Rheumatoid arthritis	miR-146 / miR-155 miR-223	Inhibition of TLR signalling / regulation of Th1 cells & mRNA for TNF-α over-expressed in T cells ($CD4^+$)
Scleroderma	miR-29	down-regulates type I collagen mRNA, contributing to excessive collagen production
SLE	miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112, miR-184 miR-189, miR-61, miR-78, miR-21, miR-142-3p, miR-342, miR-299-3p, miR-198, miR-298	7 miRNAs down-regulated 9 miRNAs up-regulated
Cardiovascular Diseases		
Arrhythmia	miR-1 miR-133	QRS & QT prolongation Depression of Ikr and QT prolongation
Angiogenesis	miR-92a miR-130 miR-210	Inhibition of sprout formation and neovascularization Promotion of angiogenesis caused by endothelial cell tube formation in vitro Increase of endothelial cell migration and tubulogenesis
Cardiac fibrosis	miR-21 miR-24 miR-29 / miR-133	Enhancement of ERK-MAP kinase pathway and fibroblast proliferation Attenuates cardiac fibrosis after MI Inhibition of fibrosis

TABLE 1. (continued) Micro-RNAs in Various Diseases

DISEASE	Micro-RNA	FUNCTION / STATUS
Cardiac hypertrophy	miR-21	Promotion of cellular outgrowth
	miR-23a / miR-195	Induction of hypertrophy
	miR-133	Inhibition of cardiac hypertrophy
	miR-208	Encoded by an intron of the a-MHC Modulation of activity of the thyroid hormone receptor
Cholesterol regulation	miR-33	Inhibition of HDL formation
Myocardial infarction & cell death	miR-1	Promotion of apoptosis, induced by H2O2 in H9c2 cells
	miR-1/206	Increase in mitochondrial depolarization in H9c2 cells
	miR-21	Decrease of myocardial infarct size
	miR-199a	Stabilization of p53 and inhibit apoptosis
	miR-499, miR-208a, miR-133a, miR-126	Increase in circulation with myocardial injury (muscular miRNAs: miR-499, miR-208a & miR-133a; vascular miRNA: miR-126)
Neurodegenerative & Psychiatric Diseases		
Alzheimer's disease	miR-107 / & other 18 miRNAs	miR-1097 levels decreased significantly in Alzheimer's disease / other 9 upregulated & 9 downregulated miRNAs
Huntington's disease (HD)	miR-9/miR-9*	decreased in HD patient cortices
Schizophrenia	miR-26b, miR-30b, miR-29b, miR-106b	disruption (global increase) in miRNA bio-genesis & expression in the cerebral cortex
Psychological stress	miR-134 / miR-183	miRNA levels both increased in the amygdala following acute stress / chronic stress decreased miR-134 levels, whereas miR-183 remained unchanged

ERK-MAP = extracellular signal-regulated kinase - microtubule-associated protein (kinase); HDL = high-density lipoprotein; IBD = inflammatory bowel disease (Crohn's disease & ulcerative colitis); IL = interleukin; SLE = systemic lupus erythematosus; *MHC* = major histocompatibility complex; MI = myocardial infarction; TLRs = Toll-like receptors; TNF = tumor necrosis factor

CANCER

Increasing evidence is revealing that the expression of miRNAs is deregulated in ***cancer***.[4-6] The miRNA profiles of human solid and hematologic malignancies indicate their potential value as tumor markers in cancer patient therapy. Large-scale studies in human cancer have demonstrated that specific miRNA expressions are associated not only with specific tumor subtypes but also with clinical outcomes.[4] Thus, the potential applications of miRNAs for cancer diagnosis, prognosis, and treatment are evident; however, miRNA-based cancer therapy currently remains an option for the future. A few examples of miRNA utility as diagnostic and prognostic markers are herein depicted. The microRNA let-7 family is associated with poor prognosis in lung and ovarian cancers, while it discriminates high-risk uveal melanomas; high expression of miR-15a and miR-16 are linked with poor prognosis in de novo aggressive chronic lymphocytic leukemia; miR-29c low expression correlates with short interval from diagnosis to therapy in chronic lymphocytic leukemia; poor prognosis is conferred by miR-21 high expression in colon and breast cancers; poor prognosis characterizes miR-155 high expression in lung cancer, diffuse large B-cell lymphoma, and aggressive chronic lymphocytic leukemia; while good prognosis is indicated by miR-21 high expression in de novo diffuse large B-cell lymphoma.[5]

AUTOIMMUNE DISEASES

An example of the miRNA role and involvement in the pathogenesis of autoimmune diseases relates to the expression of miR-29, which targets type I collagen mRNA, reported to be down-regulated in cultured dermal fibroblasts derived from *scleroderma* skin, contributing to excessive collagen production in this disease. Supplementation of the miRNA results in the decrease of collagen expression in scleroderma fibroblasts. In addition, serum miR-29a levels are significantly decreased in the very early stage of scleroderma. Similarly, in *rheumatoid arthritis* there is an increased expression in synovial fibroblasts of miR-146 and miR-155, shown to be

induced by proinflammatory stimuli such as Toll-like receptors (TLRs), interleukin (IL)-1 and, tumor necrosis factor (TNF)-α.[1] Findings that over-expression of miR-146 and miR-155 suppresses T cell apoptosis indicate a possible role of these miRNAs in the pathogenesis of rheumatoid arthritis and provide potential novel therapeutic targets. In *psoriasis*, investigators have indicated that the affected skin has a specific miRNA expression profile; leukocyte-derived miRNAs and one keratinocyte-derived miRNA, miR-203, have been identified, and emerging data suggest that miRNA deregulation is involved in the pathogenesis of psoriasis. In patients with *bronchial asthma*, investigators have reported that selective blockade of miR-126 suppressed the asthmatic phenotype, resulting in diminished T helper-2 cell responses, inflammation, airways hyperresponsiveness, eosinophil recruitment, and mucus hypersecretion. These findings suggest that targeting miRNA in the airways may lead to anti-inflammatory treatment of allergic asthma. Several miRNAs have been studied in *diabetes mellitus*; miR-375 has been identified as the most abundant intra-islet miRNA, deemed to be an important regulator of insulin release and glucose.

INFLAMMATORY BOWEL DISEASE

In *inflammatory bowel disease*, miR-192 and miR-21 are highly expressed in colonic tissues of patients with ulcerative colitis, while expression of miR-23b, miR-106, and miR-191 is increased in tissues from patients with active Crohn's disease. Even peripheral blood miRNAs can be used to distinguish active Crohn's disease and ulcerative colitis from healthy controls.

NEURODEGENERATIVE AND PSYCHIATRIC DISORDERS

In neurodegenerative and psychiatric disorders, miRNAs also appear to play important roles.[1] A number of reports have alluded to altered miRNA expression in neurological diseases (e.g. Parkinson's disease, Alzheimer's disease, Tourette's syndrome, Huntington's disease, Down syndrome, etc.), which may be a direct consequence of disease or may be derived from the loss of a specific cell population. It has been found that the best single miRNA marker, hsa-miR-145, allows for discerning *multiple sclerosis* from controls with high sensitivity and specificity. Psychiatric and neurological disorders, comprising schizophrenia, depression and mental health disorders also appear to involve changes in miRNA expression. Studies have indicated that schizophrenia is associated with a global increase in miRNA biogenesis and expression in the cerebral cortex. Altered expression of miRNAs prior to the onset of or during the course of a disease raises the possibility that expressing or inhibiting specific miRNAs might favorably affect the disease process and provide an effective mode of therapy.

CARDIOVASCULAR DISEASES

Recent studies indicate that miRNAs modulate a diverse spectrum of cardiac functions.[2,3] Identifying circulating miRNAs can serve as novel biomarkers and diagnostic tools for diverse cardiovascular diseases (CVDs), including acute myocardial infarction, heart failure, coronary artery disease, arrhythmias, diabetes, stroke, hypertension, and pulmonary embolism.[2,3,7-12] It appears that cardiac hypertrophy, heart failure, and myocardial infarction are each associated with distinct miRNA expression patterns. A few examples of specific miRNA cardiac pathologies are illustrated in Table 1. Two miRNAs that display cardiac and skeletal-muscle-specific expression during development are miR-1 and miR-133; these miRNAs have also been linked with *arrhythmias*. On the other hand, miR-199a is a master regulator of a hypoxia-triggered pathway and can be utilized for preconditioning cells against hypoxic damage. [3]

Recent studies have shown that some miRNAs are present in the circulation and the levels of circulating miRNAs have been reported for certain forms of CVD, such as in patients with myocardial infarction, hypothesizing that miRNAs in systemic circulation may reflect tissue damage and, thus, they can be used as a biomarker of *myocardial infarction*.[8-10] In patients with acute coronary syndromes, myocardial injury appears to be associated with cell type-specific expression or release of miRNAs. Thus, muscle cell-enriched miR-499 and miR-133a are released from the heart into the coronary circulation in such patients, while endothelial cell-enriched miR-126 is reduced during transcoronary passage.[9]

Another miRNA type, miR-21, is among the most strongly upregulated miRNAs in response to a variety of forms of cardiac stress, upregulated in cardiac fibroblasts in the failing heart. In general, miRNAs are important regulators of cardiac fibrosis and are involved in structural heart disease. Important modulators of vascular disease and vessel remodeling may include miR-21, miR-155, miR126, miR-221 and miR-222. Studies have indicated that miR-33 controls *cholesterol* homeostasis. In a recent study, in patients with *heart failure*, 4 miRNAs were identified circulating in the plasma, miR-423-5p, miR-320a, miR-22, and miR-92b, which may be used as markers for heart failure diagnosis.[11]

In cases where reduction of a particular miRNA appears pathogenic, they can be remedied by increasing miRNA levels. In contrast, when miRNA overexpression plays a pathogenic role in a particular CVD, targeted treatment that leads to decreased levels of a specific miRNA would be beneficial (Table 2). Thus, miRNA-mimics and anti-miRs are being developed, produced and employed in clinical studies.[12] In order to reduce the effective concentration of an overexpressed endogenous miRNA and, subsequently, increase targeted gene expression, the delivery of either cholesterol- conjugated antisense miRNA inhibitors (antagomirs) or miRNA sponges (i.e., RNA containing 4–10 binding sites for the miRNA of interest) is required.

TABLE 2. Targets of MicroRNA Cardiovascular Therapeutics

Neointimal proliferation	**Vascularization**		
• miR 21 ↓	miR 92a ↓		
• miR 145 ↑	miR 503 ↓		
• miR 221/222 ↓	miR 24 ↓		
Lipid metabolism	**Cardiac hypertrophy and fibrosis**		
• miR 122 ↓	• miR 133 ↑	• miR 21 ↓	• miR 24 ↑
• miR 33 ↓	• miR 199b ↓	• mir 29 ↑↓	
	• miR 98 ↑	• miR 208 ↓	
Arrhythmogenesis	**Cell survival**		
• miR 328 ↓	• miR 15 ↓		
• miR-1 ↓	• miR 320 ↓		

↑ promote/increase; ↓ inhibit/block

Preliminary studies have provided validating evidence of the efficacy of utilizing antagomirs and sponges to manipulate and silence miRNA function in vivo, thus therapeutically exploiting miRNAs. A few examples of effective miRNA therapeutics include the following: in vivo inhibition of miR-199b normalized significantly attenuated cardiac functional impairment and fibrosis; miR-98 overexpression reduced angiotensin II-mediated fibrosis and amount of apoptotic myocytes; antagomir-21 decreased development of cardiac fibrosis and improved cardiac function; inhibition of miR-503 normalized post-ischemic flow; antagomir-320 reduced infarction size; antagomir-328 abrogated or stopped atrial fibrillation and improved cardiac function post-myocardial infarction; a miR-33 inhibitor increased plasma HDL levels. However, compared to classic drug delivery, severe challenges lie ahead since miRNAs have multiple molecular targets, which increases the probability that targeting of a miRNA may affect multiple cellular functions, some pathological and some beneficial. Such problems and caveats may pose significant hindrance to miRNA therapeutic approaches.

CONCLUSION

In conclusion, microRNAs, long considered as an unimportant element of 'junk DNA', have been recently demonstrated to represent a group of endogenous noncoding RNAs pivotal in post-transcriptional regulation of gene expression. A plethora of research articles over the past several years, have shed light on the biogenesis of microRNA genes, their function, and their involvement in a broad spectrum of disease processes. The large progress made in understanding microRNAs also points to their great potential in the diagnosis and early detection of various diseases, including different types of cancer and diseases such as autoimmune, cardiovascular, infectious, metabolic, neurodegenerative, skin, and psychiatric diseases, as well as their promising role in future therapeutics. Continuing research will lead to enlightened new insights regarding the roles of these molecules in health and disease.

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