MicroRNA roles in gene expression and tumor progression

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Abstract

MicroRNAs (miRNAs) are a family of approximately 22 nucleotides (nt.) in length, endogenous, short noncoding RNA molecules that are essential regulators of gene expression in eukaryotes. RNA polymerases II and III transcribe microRNAs, producing precursors that undergo a series of cleavage events to form mature microRNAs. By binding to complementary sequences in the coding or 3' untranslated region of target messenger RNAs, miRNAs post-transcriptionally modulate gene expression (mRNAs). Over the past decade, the biological and biomedical research societies have drawn great attention to miRNAs. It is now clear that the biogenesis and function of miRNAs are connected to the molecular mechanisms of different clinical diseases and that all aspects of cellular activities, like differentiation, development, metabolism, proliferation, apoptotic cell death, viral infection, and tumor genesis, can potentially be regulated. Approximately 3707 miRNA genes have recently been confirmed to exist in the mammalian genome, out of which over 1000 belong to the human genome. In the eukaryotic system, MiRNAs are well preserved and are assumed to be an important and evolutionary ancient part of gene regulatory networks. This research explores the participation of miRNAs in most biological processes and their association with many human diseases, such as cancer, diabetes, and cardiovascular diseases. In all human cancers studied, profiling of miRNAs has demonstrated a distinct miRNA signature.

Keywords: MicroRNA, Biogenesis, Expression, Human diseases.

Introduction

MicroRNAs (miRNAs) are short, single-stranded RNAs that are about 22 nucleotides (nt.) in length that play an important role in gene expression regulation at post transcriptional levels these fascinating molecules may fine-tune the expression of as much as 30% of all mammalian protein-encoding gene. MicroRNA Like other types of RNAs are transcribed from DNA, but they do not participate in protein translation (Catalanotto et al., 2016).

The first microRNA was identified in 1993 in the roundworm *Caenorhabditis elegans*. Ever since then it has become evident that miRNAs are naturally abundant and evolutionarily conserved non-coding RNA molecules found in both plants and animals (Paranjape et al., 2009).

MiRNAs function as a novel class of global gene regulators by binding to partially complementary sequences in 3′ untranslated regions (UTRs) of downstream target mRNAs. MicroRNAs were first reported in mammalian systems in 2001, various cloning and bioinformatics studies predict that the human genome may contain up to 1000 miRNAs, and to date 706 human miRNAs are listed in miRbase highlighting the rapid growth of this field of research. However, the functions of most of these microRNAs still remain to be discovered (MacFarlane and Murphy, 2010; Ranganathan and Sivasankar, 2014).

Now, researchers from Thomas Jefferson University in Philadelphia have released data that adds another 3,707 novel miRNA sequences to the human genome, in addition to the 1,900 sequences previously described (5).

MicroRNAs play a pivotal role in most critical biological events, including development, proliferation, differentiation, cell fate determination, apoptosis, signal transduction, organ development, hematopoietic lineage differentiation, host-viral interactions and tumorgenesis. Improvements in the characterization of miRNAs and in techniques for their functional analysis have not only uncovered their roles in various cellular processes, but also revealed abnormal patterns of miRNA expression in various diseases (Dong et al., 2013; Oliveto et al., 2017).

Also, miRNAs have been determined to play a crucial role in regulation of DNA damage response. Scientists believe that the transmission of generic information in eukaryotic cells requires accuracy in DNA replication and chromosome as well as the ability to sense and repair spontaneous and induce DNA damage. Cells undergo a DNA damage response, a complex network of signaling pathways in order to maintain genomic integrity. This network is composed of coordinates sensors, transducers and effectors in cell cycle arrest, apoptosis and DNA repair (Wan et al., 2011; Catalanotto et al., 2016).

Recently, miRNAs have been linked to several human diseases. According to researchers, there is connection between dysregulation of miRNAs with certain diseases such as cardiovascular diseases, viral infection, neuromuscular diseases and cancer (MacFarlane and Murphy, 2010; Oliveto et al., 2017). The aim and objectives from this review will summarize the following points; the pathways of biogenesis of miRNA and the diverse biological roles played by miRNAs in human, and overview the progress of miRNA research related to human diseases.

History of miRNAs

In 1993, Lee, Feinbaum and Ambros discovered that lin-4 in *Caenorhabditis elegans* did not code for a protein but instead produced a pair of short RNA transcripts that each regulate the timing of larval development by translational repression of lin-14, which encodes a nuclear protein (Bartel, 2004; Rani, 2016). They postulated that this regulation was due in part to sequence complementarity between lin-4 and unique repeats within the 3′ untranslated regions (UTR) of the lin-14 mRNA. At the end of the first larval stage, down-regulation of lin-14 initiates developmental progression into the second larval stage (Barh, 2014).

Let-7 RNA, which was the second known miRNA, is expressed later in development and considered as complementary to the third end untranslated region of the heterochronic genes lin-14, lin-28, lin-41, lin-42, and daf-12, indicating that the expression of these genes may be controlled directly by let-7. Lin-4 and let-7 are nonhomologous and act in a similar manner to trigger the transition to late-larval and adult stages. More than ten thousand miRNAs have been identified in organisms as diverse as viruses, worms, and primates through random cloning and sequencing or computational prediction since the discovery of let-7 (Schulman et al., 2005; Barh, 2014).

In human, it is predicted that miRNAs account for 1-5% of the human genome and regulate at least 30% of protein- coding genes. Although little is currently known about the specific targets and biological functions of miRNA molecules, but it is evident that miRNA plays a crucial role in the regulation of gene expression controlling diverse cellular and metabolic pathways (Catalanotto et al., 2016).

Biogenesis of miRNAs

The biogenesis of miRNA in animals is a complex multi-step process starting in the nucleus, passing through many post-transcriptional modifications, and ending in the cytoplasm. MicroRNAs are transcribed by RNA polymerases II and III, generating precursors that undergo a series of cleavage events to form mature microRNA (Finnegan and Pasquinelli, 2013). The formation of microRNAs consists of three important steps:

1. Formation of primary microRNA

The primary transcript is synthesized from DNA template. The miRNAs are first transcribed as primary transcript or pri-miRNA with a cap and poly-A tail and then processed to pre-miRNAs. Either the sense strand or antisense strand

of DNA can function as templates to give rise to miRNAs (Finnegan and Pasquinelli, 2013).

2. Formation of precursor microRNA from pre-microRNA

A short, 70-nucleotide stem loop structure known as pre-miRNA is formed from primary miRNA in the nucleus. By a protein complex known as the Microprocessor complex, such process is performed in animals consisting of the nuclease Drosha and the double- strand RNA binding protein pasha (Dasgupta, 2015).

3. Formation of mature microRNA from pre-microRNA

Through interaction process with the endonuclease Dicer Later on, in the cytoplasm pre-miRNAs processed to mature miRNAs, which also initiates the formation of the RNA-induced silencing complex (RISC). This complex is responsible for the gene silencing observed due to miRNA expression and RNA interference. The genes encoding miRNAs are much longer than the processed mature miRNA molecule (Finnegan and Pasquinelli, 2013).

Thus microRNA (miRNA) is produced from precursor microRNA (pre-miRNA), which is formed from a microRNA primary transcript (pri-miRNA). Assembly of the mature, single stranded miRNA from the duplex into the RNA-induced silencing complex (RISC) completes miRNA biogenesis. The process of formation of miRNA can be represented as in Figure (1) (Finnegan and Pasquinelli, 2013).

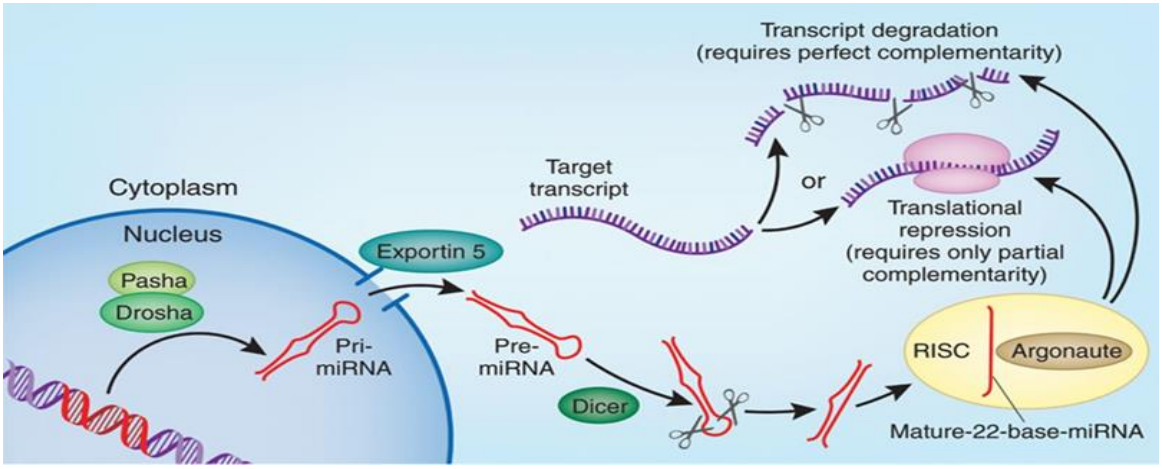

Figure 1. The microRNA (miRNA) biogenesis pathway (Sen, 2014).

Functions of microRNAs

It is estimated that miRNAs regulate 10–30% of all protein-coding genes. They do this in two ways; first mechanism, miRNAs that bind to protein-coding

mRNA sequences that are exactly complementary to the miRNA induce the RNA-mediated interference (RNAi) pathway, leading to cleavage of mRNA by Argonaute in the RISC (MacFarlane and Murphy, 2010). This mechanism is commonly observed in plants, although some studies do report it in animals. Second mechanism, which is more common one, miRNAs exert their effect by binding to imperfect complementary sites within the 3′UTRs of their target protein-coding mRNAs, leading to repression of expression of these genes at the level of translation (Figure 2). Consistent with translational control, miRNAs can reduce protein levels of their target genes with low impact on the genes' mRNA levels. In humans, miRNAs mainly inhibit protein translation of their target genes and infrequently cause degradation or cleavage of the mRNA (Paranjape et al., 2009; MacFarlane and Murphy, 2010).

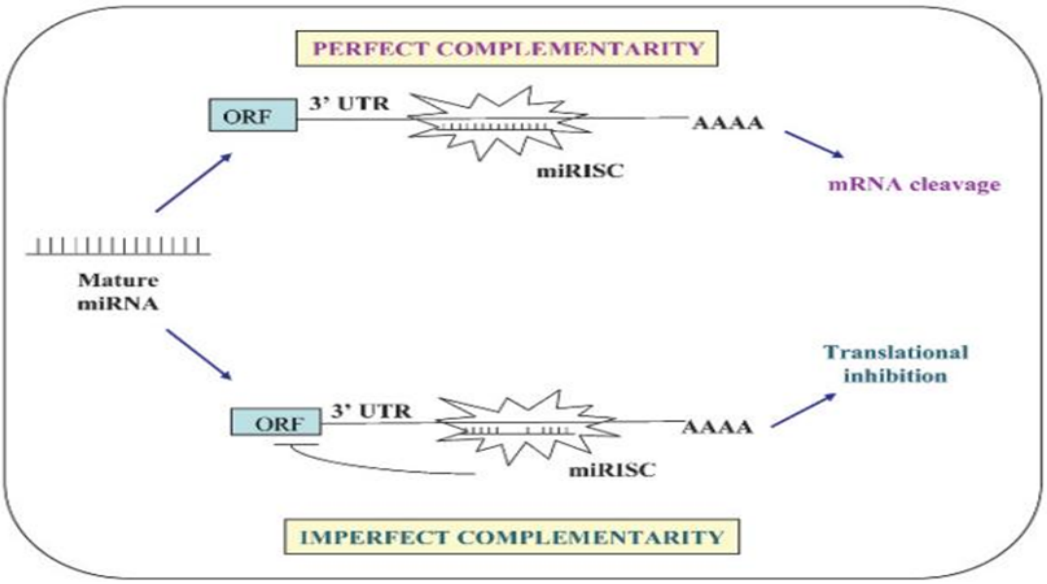

Figure 2. Mechanism of action of microRNAs (miRNAs) (Appasani, 2008).

Biological roles of microRNAs

MicroRNAs have been shown to be involved in a wide range of fundamental biological processes such as development, cell cycle control, neurogenesis, insulin secretion, cholesterol metabolism, aging, immune responses and viral replication cell proliferation, physiological processes including stem cell differentiation, hematopoiesis, hypoxia, cardiac and skeletal muscle development, fat metabolism, neural development, apoptosis, death and tumorgenesis (Ranganathan and Sivasankar, 2014).

Accumulating evidence demonstrates the importance of miRNAs in cancer. In contrast to the tight regulation during development and in normal tissues it is now well established that miRNAs are misregulated in cancer. MiRNAs that are overexpressed in cancer may function as oncogene (Figure 3). In addition, highly tissue-specific expression and distinct temporal expression patterns

during embryogenesis suggest that microRNAs play a key role in the differentiation and maintenance of tissue identity (Oliveto et al., 2017).

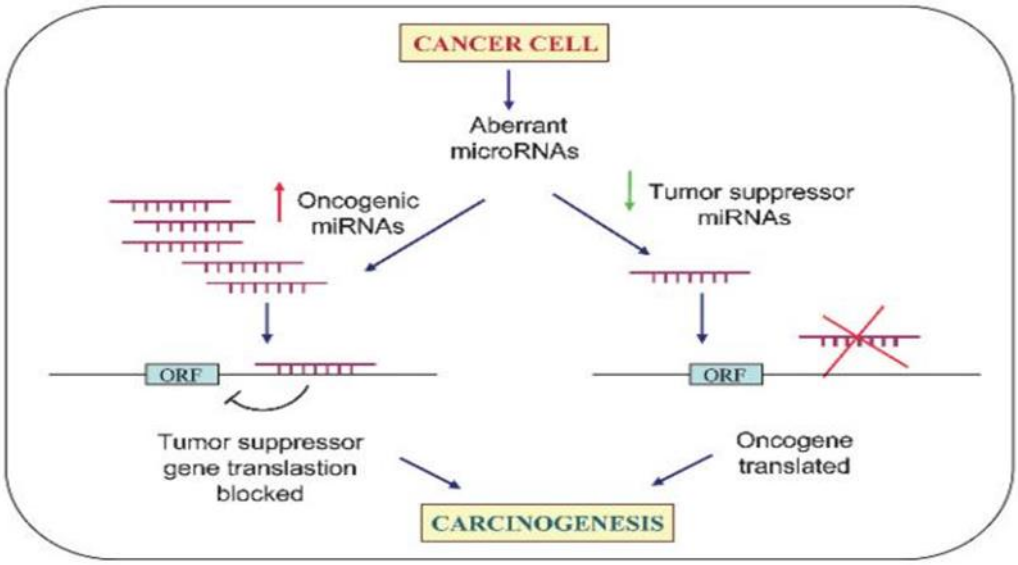

Figure 3. MicroRNAs (miRNAs) as tumor suppressors and oncogenes (Sen, 2014).

MicroRNAs and gene regulation

The important cellular function of miRNAs is related to gene regulation. The miRNA is attached to the mRNA at a specific point and inhibits protein translation. In other words, the miRNA complex blocks the protein translation machinery (MacFarlane and Murphy, 2010).

The formation of the double-stranded RNA through the binding of the miRNA leads to the degradation of the mRNA transcript In such cases it is also believed that miRNA can prevent translation without causing degradation of the mRNA (MacFarlane and Murphy, 2010).

MicroRNA as biomarkers

MicroRNAs also associated with a number of diseases in addition to their important roles in healthy individuals including many types of cancers, heart disease and neurological diseases. Consequently, microRNAs are intensely studied as candidates for diagnostic and prognostic biomarkers and predictors of drug response. Concurrent developments of miRNA biomarkers and miRNA drugs have made great strides towards improving public health. The ultimate goal of biomarker identification is to develop better clinical tests that improve diagnosis or prognosis of diseases (Oliveto et al., 2017).

In fact, miRNAs have been considered a top candidate for the next generation of biomarker as they possess a few advantages over other candidates such as

proteins and metabolites. First, miRNA biomarkers would more likely lead to early diagnosis due to their upstream positions in regulation cascades. Second, novel miRNA biomarkers would be more readily discovered by genomic tools such as oligonucleotide microarrays and deep sequencing which deliver higher throughput than mass spectrometry, the primary tool for protein and metabolite biomarker identification. Third, low abundant miRNA biomarkers can be amplified and then detected in a clinical setting by real-time quantitative PCR (qPCR), an approach used in FDA-approved clinical test whereas, no equivalent approach is available in detecting low abundant proteins or metabolites. The adoption of the locked-nucleic acid (LNA) technology in miRNA probe design could improve the sensitivity and specificity of miRNA qPCR assays even further. Non-invasive miRNA biomarkers are more sought after due to fewer complications associated with the specimen (Huang et al., 2016; Siravegna et al., 2017). The road from laboratory to clinic for using of miRNA as a biomarker are as shown in (Figure 4).

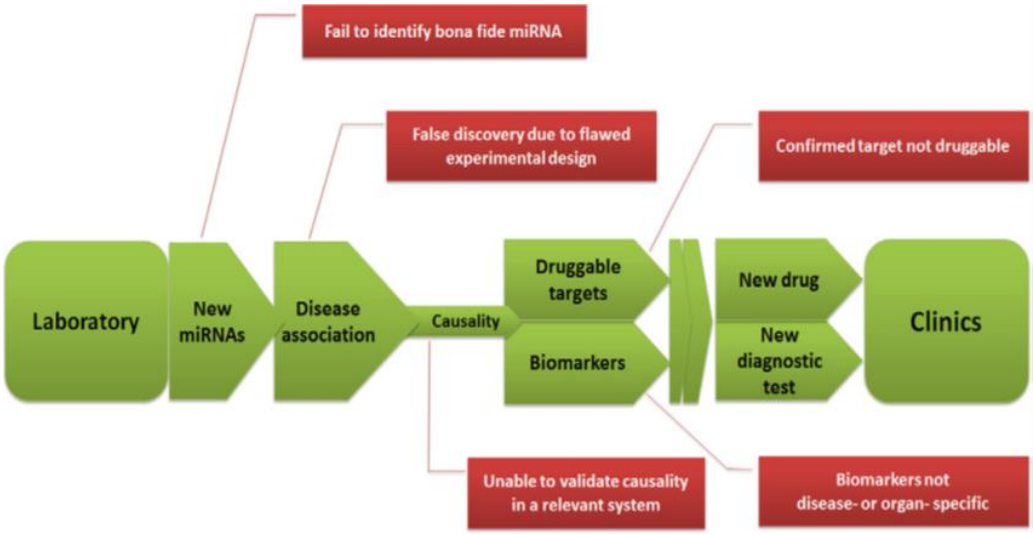

Figure 4. The road from laboratory to clinic for using of miRNA as a biomarker (Siravegna et al., 2017).

MicroRNAs in human diseases

The connection between miRNAs and disease was obvious. Deficiencies or excesses MicroRNAs have been linked to a number of other clinically important diseases ranging from myocardial infarction to autoimmune disease. Single point mutations in miRNA or its target or epigenetic silencing of miRNA transcription units is a mechanism by which the functions of miRNA in cell are affected. Great discoveries and rapid progress in the past few years on miRNAs provide the hope that miRNAs will in the near future have a great potential in the diagnosis and treatment of many diseases (Ardekani and Naeini, 2010; Li and Kowdley, 2012).

MicroRNAs and cancer

Since the early stages of miRNA research, cancer has been the most prominent of human diseases with a clear role for miRNA regulation. The first evidence came from a study by Calin and his coworkers, in which they demonstrated a frequent deletion of miRNA genes miR15 and miR16 among 65% of B-cell chronic lymphocytic leukemia (B-CLL) patients (Calin et al., 2002). Interestingly, down-regulation of miR-15 and miR-16 expression was observed among B-CLL patients without the deletion, suggesting that the pathogenesis of B-CLL may be attributed to the intracellular abundance of two miRNAs. Encouraged by this finding, this group applied a systemic search on the complete human genome and established correlations of miRNAs with various cancers Subsequent expression profiling studies further demonstrated the correlation between aberrant miRNA expression patterns and increased occurrence of different types of cancers (Li and Kowdley, 2012; Barh, 2014).

Table 1. Aberrant expression of microRNA in cancer (Acunzo et al., 2015)

Tumor suppressor miRs	**OncomiRs**				
Let-7	Lung	Down		Lung	Up
	Breast	Down		Esophagus	Up
				colon	Up
miR-181c	Stomach	Down	miR-21	Liver	Up
				Pancreas	Up
miR-31	Breast	Down		Breast	Up
	Stomach	Down		Glioblastoma	Up
	Ovary	Down		Myeloma	Up
	Colon	Down		lung	Up
miR-34	Ovary	Down	miR 221/222	Breast	Up
	glioblastoma	Down		liver	Up
miR-200c	breast	Down	miR-181b	Liver	Up
				Myeloma	Up
miR-107	Colon	Down	miR-200 a/b	Ovary	Up
	Pancreas	Down		breast	Up
	stomach	Down			Up
miR-126	breast	Down	miR-10b	Esophagus	Up
				Glioblastoma	Up
miR-96	pancreas	Down			
				Esophagus	Up
miR-146	Breast	Down	miR-196	Glioblastoma	Up
	pancreas	Down		Pancreas	Up
				colon	Up

Notably, the deregulation of miR-125b, miR-145, miR-21, and miR-155 expression was associated with the increased risk of breast cancer. In addition, up-regulation of miR-155 and down-regulation of let-7a were correlated with poor survival of lung cancer patients indicating an imbalance of cell death and

proliferation during cancer development attractively, miRNA expression patterns were also able to stage cancer progression, indicating that miRNA levels were not only useful in diagnosis but also potentially in prognosis of diseases. These cancer-related miRNAs were categorized into tumor suppressors and oncogenes due to their associations with opposite clinical outcomes with altered expressions. For example, miR-15, miR-16 and let-7 are known tumor suppressors while miR-21 and miR-155 serve as oncogenes (Yan et al., 2008; Paranjape et al., 2009; Santulli, 2015).

MicroRNAs and cardiovascular diseases

Cardiovascular diseases are a major cause of human mortality. Many studies suggest that miRNAs have specific roles in cardiac development and disorders. MiR-1 is the first miRNA that has been shown to have numerous functions in the heart, including regulation of cardiac morphogenesis, electrical conduction, and cell-cycle control. Several subsequent studies showed altered expressions of several miRNAs in cardiac tissue of mice and in diseased human myocardium. MicroRNAs that are highly expressed in muscle tissue are called myomiRs. The expression levels of myomiRs such as miRs-208a, miR-208b, and miR-499 are usually altered in heart diseases. Alternation of miRNAs expression could constitute a new treatment approach in cardiovascular disorders. For example, miR-15 inhibition protects mice from cardiac ischemic injury by reducing infarct size and enhancing cardiac function.

The homoeostasis of the vascular system depends on the functionality of endothelial cells and coordinated regulation of angiogenesis, vasculogenesis, and vessel regression. Little is known about the regulatory machinery at the gene expression level during neovascularization and vascular remodeling. In recent years, the discovery of microRNAs has made it evident that these molecules have a great important function in regulation of heart function and mammalian cardiovascular system in general (Ardekani and Naeini, 2010; Wu et al., 2013).

The miRNA expression levels have been linked to deregulation of developmental processes and disease states, such as cardiac hypertrophy and failure. Many miRNAs are expressed in a tissue-cell-specific manner and in adult cardiac tissue, miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143, and the let-7 family are abundantly expressed (Ardekani and Naeini, 2010).

Studies have shown that three mirRNAs (miR-1, miR-133, and miR-208) are highly expressed in the heart, and are important regulators of heart development and myocyte differentiation. Recently, deregulated expression of miR-1 and miR-133 were reported in human heart failure (Wu et al., 2013).

MicroRNAs and autoimmune diseases

MicroRNAs have an important role in the development of immune cells and the maintenance of immune system functions. Altered expression of microRNAs has been associated with autoimmune disorders such as rheumatoid arthritis (RA), SLE, and multiple sclerosis (MS). RA is a systemic autoimmune disorder that is characterized by chronic inflammation within the joint tissue. Initial studies showed that miR-146 and miR-155 were upregulated in synovial fluids, fibroblast, and peripheral blood mononuclear cells (PBMC) of RA patients. Later, elevation of miR-203 and miR-346 and decrease of miR-124a levels were found in RA.

Many common immune-related diseases, including multiple sclerosis (MS), systemic lupus erythematosus (SLE), type I diabetes, type II diabetes, and nonalcoholic fatty liver disease (NAFLD), have shown established correlations with cellular miRNAs. Dozens of miRNA signatures were identified by comparing the miRNA expression profiles of relapsing remitting MS and healthy controls. Specifically, the expression of miR-145 alone was found to distinguish affected patients from healthy controls with high specificity and sensitivity. Increased expression of miR-34a, miR-155 and miR-326 was observed in MS lesions (Li and Kowdley, 2012).

MicroRNAs and liver diseases

Recently, an increasing understanding of miRNA functions in liver the identification and validation of miRNA targets attracted a lot of attention. In hepatitis, viral genes encode miRNAs and these miRNAs have a regulatory effect on the viral protein-coding genes. Using computational approaches, *Hepatitis B Virus (HBV)* has been found to encode a candidate pre-miRNA, suggesting that *HBV* has the capacity to use viral miRNAs to regulate its own gene expression. MiRNAs from the host cells may also play a role in regulating viral genes. It has recently been reported that miRNA-122 facilitates the replication of *Hepatitis C Virus (HCV)* by targeting the viral 5' non-coding region (Bala et al., 2009; Hayes and Chayama, 2016).

Interestingly, eight of the miRNAs (miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431 and miR-448) were shown to be upregulated which also have an almost perfect complementarity with HCV RNA genomes. This suggests that these miRNAs are capable of inhibiting HCV replication and infection (Hayes and Chayama, 2016).

MicroRNAs and neurodegenerative diseases

Neurodegenerative diseases (ND) such as Parkinson's disease (PD) and Alzheimer's disease (AD) have placed substantial social-economic burdens on countries with aging populations. As the pathogeneses of NDs on molecular levels remain poorly understood, successful treatments are still unavailable. Researches on neurodegenerative diseases have become proprietary with increasing investments from pharmaceutical companies (Gottschalk et al., 2014).

Recent progresses from studies elucidating miRNA functions in NDs have shed new light on disease pathogenesis and may lead to novel treatment strategies. For instance, miRNA profiling in peripheral blood from PD patients revealed miR-30b, miR-30c, and miR-26a to be related with the susceptibility of the disease. Deregulation of miR-133b expression may contribute to the pathogenesis of PD, as the miR-133b-Pitx3 feedback loop is essential for maintaining dopaminergic neurons in the brain (Gottschalk et al., 2014).

MicroRNAs and gastrointestinal diseases

MiRNAs have the ability to inhibit the expression of oncogenes or anti-oncogenes, and can play a role in the tumorgenesis and progression of gastrointestinal cancers. Studies have shown that some miRNAs are down-regulated in gastrointestinal cancers, which suggests that they may function as tumor suppressors. miR-15b and miR-16, which are down-regulated in human gastric cancer cells, play a role in the development of multidrug resistance (MDR) by modulation of apoptosis via targeting BCL2 (Runtsch et al., 2014).

On the other hand, some miRNA genes are overexpressed in gastrointestinal cancers, indicating that they may have roles as oncogenes and accelerate the development of gastrointestinal cancer. Researches performed a systematic profile and analysis using 353 gastric samples and identified 22 miRNAs that were up-regulated and 13 that were down-regulated in gastric cancer (GC). Also, the two histological subtypes of GC tissues showed different miRNA signatures: eight miRNAs were up-regulated in diffuse-type and four in intestinal-type cancer (Ahmad et al., 2013).

MicroRNAs and skeletal muscle disease

The muscular dystrophies are a heterogeneous group of disorders involving degeneration of skeletal muscle. Recent studies provide evidence to support a role for miRNAs in the regulation of muscle development. The roles of miRNAs in myogenesis have been mainly from studies on muscle-specific

miR-1, miR-133 and miR-206. A unique miRNA signatures have been found in Duchenne muscular dystrophy (DMD) (Eisenberg et al., 2009).

It has been demonstrated that diagnosis of Facioscapulohumeral muscular dystrophy (FSHD) could be distinguished from DMD based on the level of miRNAs-381 and miRNAs-382 expressions in FSHD patients. Other studies have also shown a significant up-regulation of miRNAs-100, 103 and 107 in certain myopathies (Eisenberg et al., 2009).

Discussion

MiRNAs, these short sequences molecules attracted huge attentions because of its importance and role in variety of developmental processes (biological functions) also its contributions with many diseases.

Studies of the biogenesis of miRNA have shed new light on how gene expression is regulated, because the main function f miRNA is to regulate the translation of mRNA. MiRNAs have been shown to be involved in a wide range of biological process such as controlling of cell cycles, apoptosis and many physiological processes. MicroRNAs are now recognized to play a pivotal role in the regulation of certain processes related to development in all eukaryotes (Finnegan and Pasquinelli, 2013; Catalanotto et al., 2016).

Studies now provide evidence that miRNAs play a pivotal role in many diseases. Since the early stage of miRNA researches, cancer has been the most prominent of human diseases with a clear role for miRNA regulation, despite this, the role of miRNAs as a major source in the development of cancer is still very much unappreciated. But altered patterns of miRNAs in cells have been shown to be responsible for changes that cause cells to make a decision to turn malignant. For example up-regulation expression of miRNAs or down-regulation of it leads to different types of cancers like breast, gastrointestinal, lung and B-CLL (Dong et al., 2013; Barh, 2014).

Beside cancer, other important diseases ranging from myocardial infarction to autoimmune disease have been linked to deficiencies or excesses of miRNAs. Great discoveries and rapid progress in the past few years on miRNAs provide the hope that miRNAs will in the near future have a great potential in the diagnosis and treatment of many diseases (Bala et al., 2009; Ardekani and Naeini, 2010; Runtsch et al., 2014).

Beside these critical roles in human, miRNAs also have been used as prognostic biomarkers and for estimating of drug responses. In the future, with continuing technological advances facilitating easy and cost-effective methods for the detection of miRNAs, the potential of miRNAs as novel diagnostic biomarkers looks very promising (Hayes and Chayama, 2016; Huang et al., 2016).

Also, because of their potential role as agents controlling cell growth and differentiation, miRNA have been proposed to be good candidates for cancer therapy. use of miRNAs for the development of new therapeutic strategies is based on 2 approaches, either by use of miRNAs as drug molecules, based on the synthesis and delivery of specific oligonucleotides, able to increase or decrease miRNA levels in BC or modulation of miRNAs in combination with non-miRNA-based therapies to increase the efficacy of the conventional treatments (Bertoli et al., 2015).

Conclusions

MiRNA are non-coding molecules (approximately 22 nt.). These molecules have great biological functions in regulation of gene expression. They also contribute with several human diseases. Beside these roles, they are used as biomarkers for diagnostic approaches. In the near future, Researches about miRNAs appear to have a major role in medicine.

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