Review Article:

MicroRNAs in health and disease

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ABSTRACT

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNAs about 21-25 nucleotides in length and account for 1%-5% of the genome. Ever since its discovery in 1993, these molecules have attracted researchers due to their crucial role in regulating gene expression. Dysregulation in miRNA expression profile could serve as molecular signatures for identifying diseases. These have found applications in a number of diseases including cancer, wherein they may be useful for as biomarkers in cancer diagnosis as well as serve as therapeutic targets. This review provides an update on the evolution of miRNAs in the field of medicine and its future therapeutic applications.

Key words: *MicroRNAs, Biomarkers, Transcriptome*

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INTRODUCTION

MicroRNAs were discovered during RNA extraction procedures. Ribonucleic acid (RNA) when extracted from tissues or cells showed a pool of small RNA molecules which were assumed to be products of RNA degradation, arising as a result of RNA extraction procedure. These small RNA molecules were later shown to be non-coding RNA molecules involved in gene expression.[1] The small non-coding RNA molecules include small nuclear RNAs (snRNAs) which are involved in mRNA splicing and the small nucleolar RNAs (snoRNAs) which are involved in ribosomal RNA processing. Other classes of non-coding RNA are involved in the silencing of gene expression. These are subdivided into three types: short interfering RNAs (siRNAs) which target mRNA structure, others which target chromatin for epigenetic modification and the micro RNAs (miRNAs) which regulate mRNA translation.[1]

MicroRNAs are small non-coding RNAs about 21-25 nucleotides in length accounting for 1% -5% of the genome in plants and animals.[2] They regulate the expression of genes involved in cell proliferation and apoptosis, development, differentiation, metabolism, immunity, stress response, aging and cell cycle control. Approximately 50% of protein coding genes are regulated by miRNAs in a mammalian genome[3] and are located in either the introns of protein coding gene or non-coding region of genes or the intragenic regions of the genome.[4]

Discovery of microRNAs

The first miRNA to be discovered was lin-4 identified during screening for defects in the temporal control of post-embryonic development in *Caenorhabditis elegans* in 1993.[5] *C.elegans* cell lineages have four different larval stages (L_1-L_4). Lin-4 activity is required for the transition from $L_1$to L_2 .[5] Null mutation in Lin-4 causes a failure in temporal

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development.[5,6] Animals with this mutation have certain adult structures missing and developmental delays. In 1987, Ferguson et al,[7] identified a suppressor mutation in the gene Lin-14 which could revert the null mutations in Lin-4. These null mutations led to a phenotype which was opposite to that caused by Lin-4 null mutations, indicating a negative regulation of Lin-14 by Lin-4.[8]

The second miRNA let-7 was also discovered in *C.elegans* in the year 2000. Let-7 encoded a 21 nucleotide RNA which controls the L_4 to adult transition of larval development.[9] Loss of let-7 activity causes reappearance of larval cell fates during adult stage of development. Let-7 activity has been detected in vertebrates, ascidian, hemichordate, mollusk, annelid and arthropods. RNAs from plant and unicellular organisms did not show Let-7.[10] Let-7 expression has also been shown in human tissues including brain, kidney, heart, liver, lung, small intestine, stomach and thymus.[11]

Nomenclature of microRNAs

MicroRNAs are named using the prefix "miR" and a unique identification number, e.g., miR-1, miR- 12 etc. Mature miRNAs differing only in one or two positions are given suffixes; example, miR 10a and miR 10b. The mature sequences are designed as "miR" in the database, where as the precursor hairpins are labeled as "mir".[12]

Biogenesis of microRNAs

The biogenesis of miRNA (Figure 1) involves three sequential steps:[1,13] (i) transcription : RNA polymerase II is involved in transcription of majority of miRNAs; (ii) processing of primary miRNA (pri-miRNA) into a precursor miRNA in the nucleus (pre-miRNA); and (iii) Maturation of pre-miRNA into a functional miRNA in the cytoplasm.

The primary miRNA transcripts (pri-miRNAs) are several hundred nucleotides long and characterized by the formation of stem loop structures. Modification of pri-miRNAs occurs similar to that of protein-coding transcripts i.e., addition of a 5' cap and a 3' poly-A-tail. The enzyme "drosha" then processes the pri-miRNAs into pre-miRNAs in the nucleus. Pre-miRNA is usually 60-100 nucleotides long and is exported from the nucleus in a RAS-related nuclear protein (Ran) Guanosine triphosphate (GTP)-dependent process after binding with exportin-5.[14] The pre-miRNA is further processed in the cytoplasm, by the enzyme dicer into a 19-24 nucleotide long double stranded mature miRNA. The two strands of this miRNA are named the 'guide strand' and the 'passenger strand'. The guide strand incorporates into the RNA induced silencing complex (RISC) while the passenger strand is unwound from the guide strand and undergoes degradation[13] or both strands may remain functional.[15] The complex formed after incroporating miRNA guide strand with RISC is known as a 'miRISC complex'.[1] This complex binds to the 3' untranslated region of target mRNA and induces mRNA degradation or represses

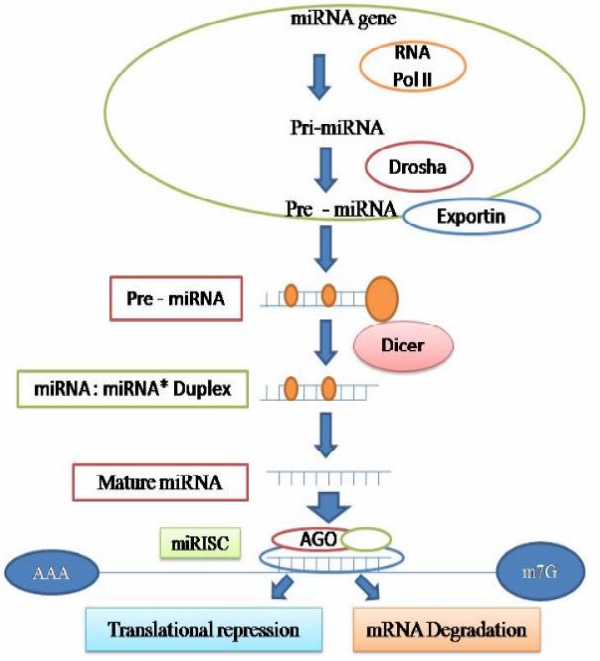

Figure 1: Biogenesis of miRNAs miRNA=microRNA, RNA=Ribonucleu acid; miRISC=micro RNA-induced silencing complex; AGO=arogonuate protein; AAA=polyA tail; m7G=7-methyl guanosine

translation (Figure 1) depending on the degree of sequence complementarity between miRNA and mRNA target.[16]

Circulating miRNAs

Most of the microRNAs are found intracellularly. However, a number of miRNAs have been observed in extracellular locations including their presence in various body fluids such as serum, plasma, tears, breast milk, bronchial lavage, colostrum, seminal, amniotic, pleural, peritonial and cerebrospinal fluids.[3,17-19] These miRNAs are more stable than RNAs, since they are shielded from ribonuclease degradation by packaging in lipid vesicles such as microvesicles, exosomes, in combination with RNA-binding proteins or both.[20,21] MiRNAs have been identified in cell derived lipid vesicles, microvesicles which are relatively large (100 nm to 1 μ) and exosomes which are smaller (30-100 nm).[13] These extracellular miRNAs are involved in cell-cell communications.[22,23]

Circulating miRNAs are highly stable even when subjected to extremes of pH, temperature, extended storage and multiple freeze-thaw cycles.[3] They have been isolated successfully from clinical specimens such as sputum,[24] plasma[25,26] and serum.[27] This makes them suitable to be used as non-invasive biomarkers.[26,28]

The expression profiles of circulating miRNAs have been studied in non-malignant and malignant diseases to identify disease-specific expression patterns or miRNA signatures.[13,28] These miRNA signatures can be used to discriminate healthy controls from patient samples with a high level of accuracy. Thus, circulating miRNAs in blood specimens could serve as diagnostic markers.[29]

Circulating microRNAs in health and disease

Dysregulation of specific miRNAs in the form of either up-regulation or down-regulation have been reported in a number of diseases including malignancies, cardiovascular diseases, skin diseases and autoimmune diseases (Table 1).

MicroRNAs in cardiovascular diseases

MicroRNAs have been shown to play a diagnostic and therapeutic role in

Table 1: Expression of miRNAs in human diseases

Disease	Up-regulated	Down-regulated
Cardiovascular Disease		
Coronary artery disease	miR-1[34]	
Cardiac hypertrophy		miR-29[34], miR-133[34]
Atherogenesis		miR – 126[34]
Alzheimer	miR-9,[50] miR-128a,[50] miR-125b[50]	
Psoriasis	miR-203,[55] miR – 146[55]	let-7d,[55] miR-142-3p and miR-181a
Dermatomyositis		miR – 223[59]
Rheumatoid arthritis	miR-155,[67] miR-146[67]	
Systemic lupus erythematosus	miR-189,[68] miR-61,[68] miR-78,[68] miR-21,[68] miR-142-3p,[68] miR 342,[68] miR-299-3p,[68] miR-198[68] and miR-298[68]	miR-196a,[68] miR-17-5p,[68] miR-409-3p,[68] miR-141,[68] miR-3 miR - 112,[68] and miR-184[68]
Breast cancer	miR-125b,[77] miR-145,[77] miR-21,[77] and miR-155[77]	
Colorectal cancer		miR-143[78] and miR-145[78]
Papillary thyroid cancer	miR – 21[85]	

miR=micro RNA; Let-7d=Lethal-7d

cardiovascular diseases. A number of miRNAs including miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143, miR-208 and the let-7 family are expressed in healthy cardiac tissue and play a role in normal cardiac maintenance as well as in diseases of the heart.[30] The miR-143/-145 (miR-143/-145) which are abundantly expressed in vascular smooth muscle cells (VSMCs) have been shown to be involved in the differentiation of VSMCs and determine the VSMC phenotypic switching.[31-33] They could thus serve as potential drug targets for vascular diseases such as atherosclerosis, hypertension, and CAD.[33]

Other miRNAs implicated in causing cardiovascular diseases include miR-1 in arrhythmia, miR-29 in cardiac fibrosis, miR-126 in angiogenesis and miR-133 in cardiac hypertrophy.[34] The miR-1 has been shown to have a role in cardiac morphogenesis, controlling cell fate of different lineages and thus helping in normal development of cardiac chambers.[35] miR-1 has been reported to be over expressed in individuals with coronary artery disease,[36] It thus represents a potential drug target in patients with arrhythmias.

The role of miR-126 in regulating endothelial cell biology and maintaining vascular structure has been demonstrated.[37] It was shown to target sprouty-related Extractable Nuclear Antigen/ Vasodilator-stimulated phosphoprotein (VASP) Homology protein (EVH1) domain-containing protein 1 (*SPRED1)* a protein that negatively downregulates mitogen activated protein (MAP) kinase pathway, vascular cell adhesion molecule 1 *(VCAM1)* and phosphoinositol-3 kinase regulatory subunit 2 *(PIK3R2)* for repression and functions to promote vascular endothelial growth factor (VEGF) signaling by inhibiting *SPRED1* and *PIK3R2*.[37] A study demonstrated the beneficial effects of aerobic exercise training on cardiac remodeling through its effect on certain miRNAs.[38] The effects included decreasing cardiac fibrosis by inhibiting collagen through upregulation of miR-29, inhibition of negative regulators of VEGF pathway. These effects of miR-126 in turn increase angiogenesis and modulation of the renin-angiotensin system by miRNAs-27a/ b and -143.[38]

MicroRNAs in neurodegenerative diseases

The miRNAs have been shown to be expressed in the central nervous system and play a role in brain development with specific temporal and spatial patterns of expression.[39] A number of microRNAs including let-7B, miR-9, 134,127,137 and 184 have been shown to have a role in neural stem cell proliferation and differentiation.[40-42] The miR-79, an epidermal microRNA regulates neuronal migration through control of the cellular glycosylation state,[43] while miR-132[44] mediates the integration of newborn neurons into the adult dentate gyrus. MicroRNAs have been shown to play a fundamental role in migration and integration; processes that are critical for the architecture of the brain.[45] miRNAs especially miR-9,[46] miR-132[47] and miR-134[48] have been shown to play a regulatory role in dendritic development and maturation in experimental models.

Neurodegenerative diseases related to aging are the result of different genetic and environmental influences.[49] Dysregulation in miRNA expresssion has been reported in Alzheimer's, Parkinson's and Huntington's disease. Dysregulation of miR-9, miR-125b , miR-128a, and miR-124a has been reported in Alzheimer brain samples when compared with age-matched controls among a subset of 12 miRNAs studied.[50] The miR-9 was found to be up-regulated in both fetal hippocampus and AD hippocampus, miR-128a in AD while miR-125b showed an increasing non-significant trend in AD.[50] The authors[51] studied the same set of miRNAs in cultured human fetal brain-derived primary neural (HN) cells, including astrocytes and neurons, treated with metal salts,

such as aluminum and iron sulfates to stimulate reactive oxygen species (ROS). ROS increased the expression of miR-9, miR-128 and, to a lesser extent, miR-125b. HN treated cell compared to controls supporting the hypothesis that ROS influence AD brain through pathways specifically mediated by miRNAs.[51]

Using Purkinji cells, the role of miRNAs in the survival of differentiated neurons through conditional Purkinje cell-specific ablation of Dicer, the key enzyme involved in miRNA generation causing Purkinje cell death has been demonstrated.[52] Apart from their role in neurodevelopment, miRNAs have been shown to be useful biomarkers.[53,54] The role of miRNAs in development of neurodegenerative diseases as well as use as biomarkers make them potential targets for treating neurodegenerative diseases.

MicroRNAs in skin disease

Dysregulation in miRNA expression has also been shown in skin diseases. An up-regulation of miR-203 which is expressed exclusively in keratinocytes in patients with psoriasis-affected skin compared to healthy skin has been reported.[55] The miR-146 was also found to be upregulated in psoriasis patients.[55] The miR-146 has been shown to be a NF-kappaB-dependent gene involved in cytokine signalling. It has been shown to down-regulate IL-1 receptor-associated kinase and tumour necrosis factor (TNF) receptor-associated factor 6 protein levels through a negative feedback regulation loop.[56] Circulating miRNAs dysregulated in psoriasis include miR-128a(up-regulated), let-7d, miR-142-3p and miR-181a (down-regulated). Treatments using anti-TNF agent etanercept showed decrease in miR-106b, miR-26b, miR-142-3p, miR-223 and miR-126 in responders.[57]

Circulating miRNA-221 has been proposed as a tumor marker in patients with malignant melanoma.[58] It has been shown to differentiate in-situ malignancy from stage I-IV and correlated with tumor thickness.[58] Serum miRNA-223 levels have been reported to be lower in patients with dermatomyositis.[59]

MicroRNAs in autoimmune diseases

MicroRNAs have a role in regulating immune response and prevent autoimmunity. As discussed above miRNA-146 is involved in down-regulating inflammatory response.[55] Other miRNA involved in regulating the innate immunity to bacterial and viral pathogens is miRNA-155.[60] This action is through its binding to Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1) and suppressor of cytokine signaling 1 (SOCS1) targets which are negative regulators of the immune response.[60] MiRNAs are also involved in regulating adaptive immune response[61,62] through the development and differentiation of T and B lymphocytes.[63,64]

Studies have reported an association of miRNAs with autoimmune inflammatory conditions like rheumatoid arthritis (RA). Increased expression of miR-155 and miR-146 have been reported in synovial fibroblasts and synovial tissue[65,66] and peripheral blood mononuclear cells in RA.[67] Dysregulation in miRNA expression has also been reported in SLE, another autoimmune disease characterized by overproduction of auto antibodies towards several self antigens. Using microarray assay for miRNA expression in polymorphonuclear leukocytes, down regulation of seven miRNAs including miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112,and miR-184 and up-regulation of nine miRNAs namely miR-189,miR-61, miR-78, miR-21, miR-142-3p, miR-342, miR-299-3p, miR- 198, and miR-298 has been repoted.[68] Similarly, dysregulation of 66 miRNAs was described in patients with lupus nepritis.[69]

MicroRNAs in cancer

MiRNAs have been implicated in the pathogenesis of cancer development being

involved in apoptosis, cell cycle control, cell proliferation and DNA repair. Studies have shown that the pattern of miRNA expression is altered in cancer and is different from that of the normal subjects[70-72] thus pointing towards their possible use as diagnostic markers. Studies indicate that a majority of miRNAs genes are located in cancer-associated genomic regions or in fragile sites.[73] The miRNAs have been shown to act as oncogenes (onco miRs) or tumour suppressors.[74] They have also been have a role in metastasis. These have been described as metastasis activators,[75] and their metastasis suppressor role has also been documented.[76]

Dysregulation in miRNA expression have been reported in cancer of breast,[77] colon,[78] lung,[79] thyroid[80] etc. Circulating miRNAs are being recognized as potential biomarkers for the diagnosis of cancer.[81-83] Dysregulated expression of miR-125b, miR-145, miR-21, and miR-155 in breast cancer clearly differentiated normal from breast cancer tissues. [77] Down-regulation in miR-143 and miR-145 expression has been reported in colorectal cancer. [78] Up-regulation of 60 and down-regulation of 31 miRNAs in the serum from non-small cell lung cancer (NSCLC) patients compared to non-cancer (NC) patients has been repoted.[84] Of these, a molecular signature of 4 miRNAs namely miR-193b, miR-301, miR-141 and miR-200b could discriminate lung cancer patients with high accuracy from NC patients. Recently the expression profile of seven miRNAs in serum of patients with papillary thyroid cancer (PTC), multinodular goiter (MG) and healthy controls has been reported.[85] The authors reported significantly higher levels of miR-21 in the pre-operative samples of PTC and MG patients compared to the control group. The MiR-151-5p, miR-221 and miR-222 were found to be significantly lower in the postoperative samples obtained from PTC patients. Their findings imply the role of specific miRNAs in the development of PTC.[85]

Future of circulating miRNAs

Thus, with increasing knowledge of the role of miRNAs in disease process and the discovery of circulating miRNAs in various diseases, a new branch of diagnostics based on molecular signatures of different miRNAs in diseases is coming up. The challenges' in this field would be to pinpoint disease-specific miRNAs and focus on methods to standardize collection techniques, analysis and interpretation of molecular signatures. Research in identifying potential miRNAs as therapeutic targets will be another new area in the over expanding field of microRNAs in coming years.

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