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MICRORNAs: A REVIEW STUDY

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ABSTRACT: *MicroRNAs (miRNAs) are endogenous short (20–22 nucleotides) non-coding RNA molecules that mediate gene expression. This is an important regulatory mechanism to modulate fundamental cellular processes such as differentiation, proliferation, death, metabolism, and pathophysiology of many diseases. The miRNA expression profile of the kidney differs greatly from that of other organs, as well as between the different regions in the kidney. In kidneys, miRNAs are indispensable for development and homeostasis. In this review, we explore the involvement of miRNAs in the regulation of blood pressure, hormone, water, and ion balance pertaining to kidney homeostasis. We also highlight their importance in renal pathophysiology, such as in polycystic disease, diabetic nephropathy, nephrogenic diabetes insipidus, hypertension, renal cancer, and kidney fibrosis (epithelial–mesenchymal transition). In addition, we highlight the need for further investigations on miRNA-based studies in the development of diagnostic, prognostic, and therapeutic tools for renal diseases. [1]*

KEYWORDS: *miRNAs, siRNAs, Cancer, Immune system, therapeutic method.*

I. INTRODUCTION

Gene expression in cells and tissues of every complex organism is precisely controlled and largely dependent on different conditions (such as development, changes in the environment, diseases or drugs). Various cells and organ systems within such organism (including humans) contain different gene expression profiles, thus proper understanding of regulatory mechanisms involved in such expression represents one of the key issues in genomic medicine.

Non-coding RNA molecules have a role in plethora of regulatory events – from controlling the number of copies in bacterial division to X-chromosome inactivation in mammals. Recent analyses of the human and animal genomes have shown that most of RNA transcripts do not code for proteins (i.e. they are messenger RNAs or mRNAs), but are instead noncoding RNAs (ncRNAs).

MicroRNAs (or miRNAs) comprise a novel class of small, non-coding endogenous RNAs that regulate gene expression by directing their target mRNAs for degradation or translational repression. Their discovery added a new dimension to the understanding of complex gene regulatory networks in humans and animals alike.

1. History of microRNAs:

MicroRNA (miRNA) was initially discovered in Caenorhabditis elegans by Victor Ambros' laboratory in 1993 while studying the gene lin-14. At the same time, Gary Ravkun identified the first miRNA target gene. Those two groundbreaking discoveries identified a novel mechanism of posttranscriptional gene regulation.

However, the importance of miRNA was realized seven years later when Ravukon and Horvitz laboratories identified a second miRNA in the same model nematode species (named let-7), and when another class of short RNA (siRNA) involved in the process of RNA interference was discovered. Only then it became obvious that short non-coding RNA molecule identified in 1993 was part of a much bigger phenomenon.

Since then, an increasing number of miRNAs have been recognized in mammals. In humans alone over 700 miRNAs have been identified and fully sequenced, and the estimated number of miRNA genes in a human genome is more than one thousand. Based on computer models, miRNAs in humans have a direct influence on at least 30% of the genes in the whole genome. [1]

2. What are microRNAs?

miRNAs represent small RNA molecules encoded in the genomes of plants and animals. These highly conserved 22 nucleotides long RNA sequences regulate the expression of genes by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs. A growing body of evidence shows that miRNAs are one of the key players in cell differentiation and growth, mobility and apoptosis (programmed cell death).

Differentiating miRNAs from other classes of small RNAs that are present in the cell is often cumbersome – particularly the distinction from endogenous small interfering RNAs (siRNAs). The most significant distinction between miRNAs and siRNAs is whether they silence their own expression. Almost all siRNAs (regardless of their viral or other origin) silence the same locus from which they were derived. On the other hand, most miRNAs do not silence their own loci, but other genes instead.

miRNAs regulate diverse aspects of development and physiology, thus understanding its biological role is proving more and more important. Analysis of miRNA expression may provide valuable information, as dysregulation of its function can lead to human diseases such as cancer, cardiovascular and metabolic diseases, liver conditions and immune dysfunction. [2]

II. DIFFERENCE BETWEEN SIRNA AND MICRORNA

The process by which double-stranded RNAs initiate the degradation of homologous RNA is known as RNA interference. In 2006, the Nobel Prize in Medicine and Physiology was awarded to Drs Andrew Fire and Craig Mello for their discovery of siRNA, which guides the cleavage of mRNA. siRNAs regulate the degradation of mRNA molecules identical in sequence to that of the corresponding siRNA, resulting in the silencing of the corresponding gene and the shutting down of protein synthesis. The main mechanism of action of siRNA is the mRNA cleavage function. There are no genes that encode for siRNAs. siRNAs can also silence gene expression by triggering promoter gene methylation and chromatin condensation.

In contrast with siRNAs, miRNAs are encoded by specific miRNA genes as short hairpin pri-miRNAs in the nucleus. miRNAS are also small noncoding RNAs, but they seem to require only a 7- to 8-base-pair "seed" match between the 5' region of the miRNA and the 3'UTR of the target. It would seem that the majority of miRNA targets are translationally repressed; however, degradation of the target mRNA can also occur. The main mechanism of action of miRNA may be the inhibition of mRNA translation, although the cleavage of mRNA is also an important role siRNAs are synthesized from double-stranded segments of matched mRNA via RNA-dependent RNA polymerase. miRNAs are synthesized from an unmatched segment of RNA precursor featuring a hairpin turn. siRNAs are often derived from repetitive DNA sequences and associated transposons and centromeres, forming heterochromatin structures. Both siRNAs and miRNAs are produced by Dicer-mediated cleavage of longer double-stranded RNA precursors. However, miRNAs are entirely endogenous, whereas siRNAs may be endogenous or exogenously derived from viruses. Also, miRNAs cluster in "families" closely related as far as the sequence is concerned or as individual units. siRNAs can exist as repeated elements. [3]

Attribute	siRNA	miRNA
Primary mode of action	Inhibition of translation	mRNA cleavage
Secondary mode of action	mRNA cleavage	Chromatin silencing
Synthesis	Endogenous	Endogenous or exogenous of dsRNA
Hairpin Structure	No	Yes
Length	19-23 bases	19-24 base pairs
Impact on protein synthesis	Indirect	Indirect
Potential use in cancer diagnosis	Unknown	Yes

Table 1.[4]

Hundreds of miRNAs have now been identified in various organisms, and the RNA structure and regulatory mechanisms that have been characterized in lin-4 and let-7 still provide unique molecular signatures as to what defines miRNAs. miRNAs are generally 21–25nucleotide, non-coding RNAs that are derived from larger precursors that form imperfect stem-loop structures. The mature miRNA is most often derived from one arm of the precursor hairpin, and is released from the primary transcript through stepwise processing by two ribonuclease-III (RNase III) enzymes.At least in animals, most miRNAs bind to the target-3′ UTR with imperfect complementarity and function as translational repressors.

Almost coincident with the discovery of the second miRNA, let-7, small RNAs were also characterized as components of a seemingly separate biological process, RNA interference (RNAi). RNAi is an evolutionarily conserved, sequence-specific gene-silencing mechanism that is induced by exposure to dsRNA. In many systems, including worms, plants and flies, the stimulus that was used to initiate RNAi was the introduction of a dsRNA (the trigger) of ~500 bp.The trigger is ultimately processed in vivo into small dsRNAs of ~21–25 bp in length, designated as small interfering RNAs (siRNAs). It is now clear that one strand of the siRNA duplex is selectively incorporated into an effector complex (the RNA-induced silencing complex; RISC). The RISC directs the cleavage of complementary mRNA targets, a process that is also known as post-transcriptional gene silencing (PTGS).The evolutionarily conserved RNAi response to exogenous dsRNA might reflect an endogenous defense mechanism against virus infection or parasitic nucleic acids. Indeed, mutations of the RNAi components greatly compromise virus resistance in plants, indicating that PTGS might normally mediate the destruction of the viral RNAs. In addition, siRNAs can also regulate the expression of target transcripts at the transcriptional level, at least in some organisms.Not only can siRNAs induce sequence-specific promoter methylation in plants, but they are also crucial for heterochromatin formation in fission yeast, and transposon silencing in worms. [5]

III. FUNCTIONS OF MICRORNAS

1. Involvement of microRNAs in the control of gene expression

The basic mechanism leading to alteration of gene expression is based on the recruitment of mature miRNA at the level of the RISC silencing complex. This process occurs in the cytoplasm, where the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer, which interacts with the 30 end of the hairpin and cuts away the loop joining the 30 and 50 arms, yielding an imperfect miRNA/ miRNA duplex. One of the strands is incorporated into the RISC, where it binds to target mRNA sequences. Animal miRNAs are usually complementary to a site in the 30UTR. Perfect or near perfect base pairing with the target RNA promotes cleavage of the RNA. It is proposed that in the case of partially complementary microRNAs, in order to recognize their targets, nucleotides 2–7 of the miRNA (the 'seed region') are important. This is the key process permitting mature miRNAs to exert their effects in gene regulation. The final effect of miRNAs activity is the inhibition of the synthesis of the protein(s) encoded by the target mRNA(s). This has of course important biological implications depending on the role of the protein in the cellular network. Since a single 30UTR of a given mRNA contains signal sequences for several microRNAs, applied biological studies are needed to determine which microRNA should be targeted to achieve alteration of gene expression. Possible effects on the expression of other mRNA targets should be considered. An alteration of a single microRNA may exhibit multiple effects, possibly in combination with the targeting activity of other miRNAs, enabling the achievement of strong biological effect. [4]

2. Biogenesis of microRNAs and drug design

Some miRNAs are encoded by unique genes (intergenic miRNAs) and others are embedded into the intronic regions of protein-coding genes (intragenic miRNAs). Examples of intergenic miRNA are miR-210, miR-10a, miR-21, and miR-222/miR-221, which are encoded by unique genes located in the chromosome 11, 17, 17, 6 and X, respectively. The transcription is controlled, as protein-coding genes, by a promoter which is regulated by specific interactions with transcription factors. The transcription by RNA polymerase II of these miR genes gives rise to long primary miRNAs (pri-miRNAs) with typical stem-loop structures. These are rapidly processed by the nuclear RNase endonuclease-III Drosha, which, removing the branches, gives rise to precursor miRNAs (pre-miRNA) of around 60–100 nts in length. An example of intragenic miRNA is miR-301. Its genomic sequences are embedded into the intronic regions of ska2. In this specific case, the transcription of miRNA sequences depends on the cellular promoter of the host gene. The miR sequences follow the splicing pathways giving rise to a ''Mirtron'' (microRNA/ intron) sequence further processed by debranch enzymes to generate a pre-miRNA. The microRNA transcription can be controlled by targeting regulatory transcription factors, the microRNA promoter itself, or the promoter of the host gene. An example is that reported by Xi et al., showing that knocking-down of C-EBP-b induces a decrease of the recruitment of this transcription factor on the promoter of the LOC554202 gene (hosting miR-31) and down-regulation of miR-31. [4]

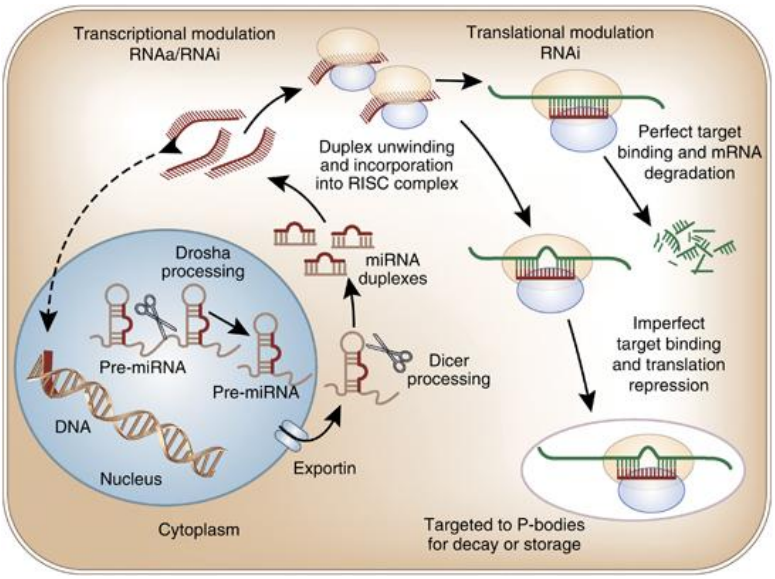

Fig. 1 **MicroRNA (miRNA)—biogenesis and function**. The biosynthesis of miRNAs, as well as their activity in translational repression (RNA interference (RNAi)) and transcriptional modulation (RNAa or RNAi), is represented diagrammatically. Pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; RNAa, RNA activation. [6]

3. Post-transcriptional repression by miRNAs

The precise molecular mechanisms that underlie posttranscriptional repression by miRNAs still remain largely unknown. One of the best-studied examples is lin-4, which negatively regulates its target, lin-14, by repressing its translation16. Base pairing between lin-4 and lin-14 has proved to be crucial for their interaction in vivo, as mutations that affect their complementarity compromise or abolish this negative regulation. Interestingly, lin-4 only inhibits the synthesis of the LIN-14 protein but fails to affect the synthesis, polyadenylation state or abundance of lin-14mRNA. Furthermore, the initiation of lin-14 translation seems to occur normally in the presence of lin-4, because lin-14 mRNAs are efficiently incorporated into polyribosomes regardless of lin-4 expression16.Therefore, it is reasonable to speculate that the translational repression by lin-4 occurs after translational initiation, probably during translational elongation and/or the subsequent release of the LIN-14 protein. Translational repression of target genes is not specific to lin-4; in fact, it turns out to be the predominant mechanism by which miRNAs negatively regulate their targets throughout the animal kingdom. let-7 and Bantam (which encodes an anti-apoptotic miRNA) bind to the 3′ UTRs of their targets and negatively regulate their translation in worms and flies, respectively. In addition,miR-30, a mammalian miRNA, inhibits protein synthesis of a reporter gene that bears an artificial 3′ UTR with miR-30 complementary sites. Although most animal miRNAs repress target translation, one miRNA, mir-196, was found recently to direct mRNA cleavage of its target, Hoxb8. In plants, however, most miRNAs that have been studied so far mediate the destruction of their target mRNAs.

Despite progress in identifying protein and RNA components of the RISC, the biochemical mechanism by which this complex functions still remains unknown. Genetic screens combined with biochemical purification have pinpointed several important components. Argonaute (AGO) proteins belong to an evolutionarily conserved family that is defined by the presence of a PAZ domain and a Piwi domain. AGO-family proteins have been consistently co-purified with RISC activity in many organisms, and mutations in AGO homologues have been associated with distinct developmental and/or RNAi phenotypes. As a core component of the RISC, the AGO family has multiple homologues in each metazoan species (24 for C. elegans, 5 for D. melanogaster and 8 for mammals). Given the diverse mutant phenotypes and expression patterns of AGO homologues, RISCs might come in different flavours and act in a tissue-specific or a developmentally regulated manner. [4]

4. Functional characterization of miRNAs

Although the studies of lin-4 and let-7 shaped our understanding of miRNA molecular structures and functional mechanisms, their roles in temporal regulation of development only revealed one of many possible aspects of miRNA function.Mutations in Dicer homologues disrupt the biogenesis of miRNAs, and cause diverse developmental defects, including germline defects in C. elegans, abnormal embryogenesis in A. thaliana, developmental arrest in zebrafish and depletion of stem cells in mice. Mutations in AGO family proteins are also associated with pleiotropic developmental phenotypes, such as premature MERISTEM differentiation in A. thaliana zwille mutants, defective embryogenesis and larval development in C. elegans alg-1 and alg-2mutants and decreased stem-cell self-renewal during oogenesis in Drosophila piwi mutants. Because Dicer and AGO are essential components in miRNA and siRNA biogenesis and function, these defects might reflect the collective functions of multiple miRNAs and/or siRNAs that are expressed during early development. [4]

IV. MICRORNAS AND CANCER

MicroRNAs play a pivotal role in cancer. The literature on this specific issue is impressive (see the Human MicroRNA Disease Database. MicroRNAs play a double role in cancer, behaving both as oncogenes or tumor suppressor genes. In general, miRNAs promoting cancer target mRNA coding for tumor-suppression proteins, while microRNAs exhibiting tumor-suppression properties usually target mRNAs coding oncoproteins. MicroRNAs which have been demonstrated to play a crucial role in the initiation and progression of human cancer are defined as oncogenic miRNAs (oncomiRs). Moreover, microRNAs have been definitely demonstrated to be involved in cancer metastasis (metastamiRs. For instance, miR-372 and miR-373 were identified as oncogenes, after the screening of hundreds of miRNAs. The mechanism of action of these microRNAs is to negatively regulate the expression of the LAST2 tumor suppressor gene, thus blocking the pathway of one of the key tumor suppressors, p53. Using breast cancer MCF7 as a model system, Huang et al. were able to demonstrate that miR-373 promotes tumor invasion and metastasis. A similar tumor-promoting activity is exhibited by miR-221 and miR-222, able to stimulate proliferation following inhibition of the expression of the tumor suppressor p27Kip1. An opposite effect on tumor development is displayed by other miRNAs; for instance miR-31 expression levels correlate inversely with the metastatic ability of breast tumor cell lines, and the inhibition of miR-31 promotes metastasis. Further studies have revealed that miR-31 blocks several steps of metastasis, including local invasion, extravasation or initial survival at a distant site, and metastatic colonization. Another interesting feature of the miRNA life was found by studying cancer associated miRNAs in different experimental model systems, i.e. that cancer-specific miRNAs are present in extracellular body fluids, and may play a very important role in the cross-talk between cancer cells and surrounding normal cells [5]. The extracellular miRNA are protected by exosome-like structures, small intraluminal vesicles shed from a variety of cells (including cancer cells), with a biogenesis connected with the endosomal sorting complex required for transport (ESCRT) machinery in multivesicular bodies (MVB). These extracellular structures, originally considered as a ‘‘garbage bag’’ devoted to discard degraded proteins, are now considered of interest as an intercellular communication tool. It is still unclear whether these exosome-associated miRNAs are the result of tumor cell death and lyses, or are actively excreted from tumor cells into the microenvironment. However, this novel secretory machinery of miRNAs may be involved in tumorassociated features, such as enhancement of angiogenesis, increase of cytokine secretion and migration to a pre-metastatic niche [7]. In conclusion, miRNAs are deeply involved in tumor onset and progression, so that therapeutic strategies involving miRNA silencing have been proposed. Since miRNAs can behave as tumor suppressor genes, miRNA replacement therapy has been also proposed as a possible therapy of cancer. [5]

V. MIRNA IN INFLAMMATORY DISEASES AND IMMUNE RESPONSE SYSTEM

Recently miRNAs have emerged as fine-tune regulators of innate and acquired immune response systems. The recognition on miRNAs in inflammation has in turn furthered our understanding of the molecular pathogenesis of autoimmune diseases, cancers, asthma to name a few. However, due to inherent complexities of miRNA regulation of targets, we are still in an early stage of exploring the functional roles of miRNAs in inflammatory diseases. Certain miRNAs serve as important components of negative feedback loops in the immune system (miR-146a), whereas others serve to amplify the response of the immune system (miR-155). In addition, role of miRNA can be cell- or tissue- specific. It is likely that multiple miRNAs rather than single miRNAs, synergistically act together to regulate gene expression and thus biological networks in response to stress. Thus, a better understanding about how miRNAs regulate gene networks in general may provide insights into novel regulatory mechanisms. [8]

VI. MICRORNAS IN VIRAL DISEASES

Viral genes encode miRNAs and these miRNAs have a regulatory effect on the viral protein-coding genes .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. Expression of a total of 30 cellular miRNAs in hepatocytes has been influenced by IFN-α/β or IFN-γ. In this , 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.

VII. MICRORNAS IN NEURODEVELOPMENTAL DISEASES

MicroRNAs are highly expressed in human and other mammalian brains relative to other organs. The results of high-throughput sequencing experiments suggest that the number of miRNAs expressed in human brain should be over 1000, although currently this number stands at about 550 in all humans. The expression of miRNAs in brain changes during brain MicroRNAs in Cardiovascular Disease development. Therefore, some miRNAs are expressed more abundantly during early development in the mammalian brain, and some are expressed less during later development. The changes in miRNA expression levels in brain during development may represent biochemical signals for cell fate determination, apoptosis and/or cell division programming. Some miRNAs are differentially expressed in neuronal nuclei, and/or different cell populations in brain. Because miRNAs are known to be dynamically regulated in neurogenesis and brain development, it is believed that miRNAs are also involved in neural development and play an important role in mediating neuronal plasticity. One of the major common traits linking many of the neurodevelopmental disorders [e.g. intellectual disability, autism, Attention Deficit Hyperactivity Disorder (ADHD) and epilepsy] is that disease onset occurs during periods of maturation and development. Therefore miRNAs contribute significantly to the pathogenesis of neurodevelopmental disorders at the molecular level.

VIII. MICRORNAS IN 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. A systemic miRNA profiling in peripheral blood mononuclear cells from PD patients revealed miR-30b, miR-30c, and miR-26a to be associated with the susceptibility of the disease. Deregulation of miR-133b expression may contribute to the pathogenesis as the miR-133b-Pitx3 feedback loop is essential for maintaining dopaminergic neurons in the brain. An analysis of miRNA and mRNA expression in brain cortex from AD and age-matched control subjects demonstrated strong correlations between the expression levels of miRNAs and predicted mRNA targets, implying functional relevance of microRNA-mediated regulations in AD pathogenesis. The expression of miR-29a, miR-29b-1 and miR-9 was significantly decreased in AD patients, resulting in abnormally high expression of their target BACE1, a protein playing an important role in AD pathogenesis. These findings highlight the importance of miRNA research in understanding ND pathogenesis.

IX. MICRORNAS IN CARDIOVASCULAR DISEASE

MicroRNAs play an important role in regulation of heart function and Cardiovascular Diseases. miRNAs are important regulators of cardiovascular growth, proliferation, cell differentiation, and apoptosis. 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. Deregulated expression of miR-1 and miR-133 were reported in human heart failure .miR-23a, miR-23b, miR-24, miR-195, miR-199a, and miR-214 were upregulated during cardiac hypertrophy, and their over-expression in cardiomyocytes *in vitro* caused an induction of hypertrophic growth. Interestingly, miR-24, miR-125b, miR-195, miR-199a, and miR-214 were similarly upregulated in the tissue of patients with end-stage failing human hearts. The investigation into the role of miRNAs as a novel class of gene regulators in cardiovascular disease is a new frontier for research.

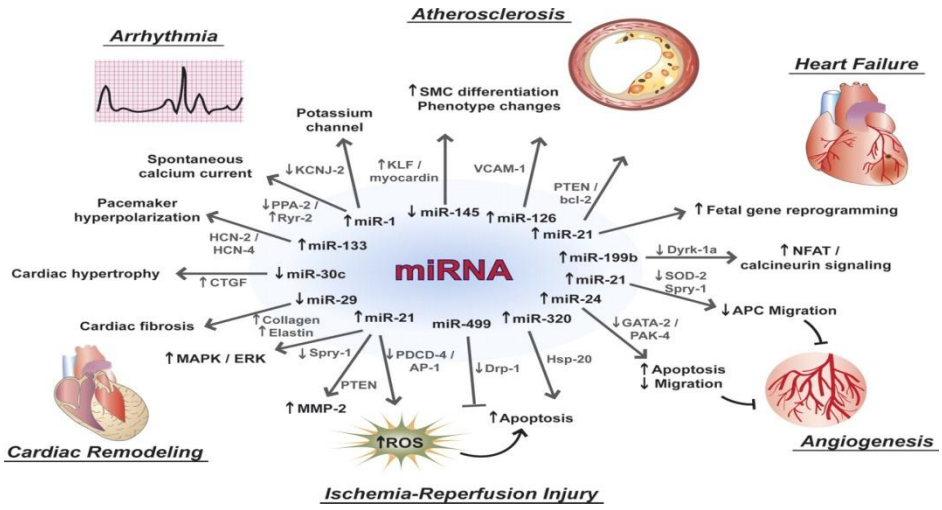

Fig 2

DISEASE		MI-RNA
Cancer	B-CLL	miR-15, miR-16
	Breast cancer	miR-125b, miR-145, miR-21, miR-155, miR-210
	Lung cancer	miR-155, let-7a
	Gastric cancer	miR-145
	Liver cancer	miR-29b
Viral diseases	HCV	miR-122, miR-155
	HIV-1	miR-28, miR-125b, miR-150, miR-223, miR-382
	Influenza virus	miR-21, miR-223
Immune-related diseases	Multiple sclerosis	miR-145, miR-34a, miR-155, miR-326
	Systemic lupus erythematosus	miR-146a
	Type II diabetes	miR-144, miR-146a, miR-150, miR-182, miR-103, miR-107
	Nonalcoholic fatty liver disease	miR-200a, miR-200b, miR-429, miR-122, miR-451, miR-27
	Non-alcoholic steatohepatitis	miR-29c, miR-34a, miR-155, miR-200b
Neurodegenerative diseases	Parkinson's disease	miR-30b, miR-30c, miR-26a, miR-133b, miR-184*, let-7
	Alzheimer's disease	miR-29b-1, miR-29a, miR-9

Table 2.

X. MICRORNAS FOR CLASSIFICATION OF DISEASE

MicroRNA expression signatures often reflect embryonic or developmental origin of the tumor type. This greatly facilitates tumor classification based on microRNAs: a blind study of 22 different tumor types showed microRNA expression classified tumors according to tissue of origin with accuracy higher than 90%. WHO classification of leukemia is done according to progenitor cell lineage, and microRNA expression across leukemia subtypes also reflects the cell of origin. Association of expression signatures in acute myeloid leukemia (AML) revealed specific microRNA expression patterns in each disease subtype. Similarly, in prostate cancer, microRNA patterns were distinct between different cellular subsets when stem/progenitor cells were isolated from prostate tumors, showing that microRNA expression patterns are indicative of the cellular populations in a tumor. Distinct micro-RNA expression patterns have also recently been identified in luminal

(epithelial origin), basal-like (myoepithelial origin), and human epidermal growth factor receptor 2 (HER2) breast cancers. The classification of breast cancer is better defined that most malignancies; however, meta-analyses of recent clinical trials have shown incorrect classification of a substantial number of tumors in laboratories with high volume testing (fluorescence in situ hybridization and immunohistochemistry-based tests), and therefore microRNA analysis may add robustness to current testing. Various cancer subtypes have been identified using the huge body of data at TCGA, and microRNAs are either associated with these changes or have been used to create subtypes. The identification of microRNAs that target current biomarkers may pave the way for microRNA-based tests as an alternative to mRNA/protein expression for prognosis assessment. MicroRNAs have been shown to have a role in cancer progression and might be useful for the prediction of metastatic outcomes for patient management. Specific microRNAs have been shown to support endothelial recruitment to metastases in breast cancer and might serve as efficient biomarkers for predicting this event. MicroRNA signatures associated with known inducers of EMT have also been developed and shown to be relevant in both in vitro and in vivo models of EMT in endometrial cancer.[9]

XI. MICRORNAS AS DRUGS

Efforts are ongoing to develop miRNA-based drugs, either in the form of miRNA mimics, amplifying the impact of a miRNA, or miRNA inhibitors, essentially quenching the effect of a miRNA. miRNA drugs have the advantage that one miRNA may target and modify the expression of several genes with different roles in the same pathway. The most advanced miRNA drug to date is a miRNA inhibitor which targets miR-122 in liver to treat hepatitis C virus (HCV). One challenge as such drugs approach clinical use is testing for interactions between the novel miRNA drugs and traditional drugs already in the market. The link from miRNAs to drugs allows Pharmaco-miR to predict putative interactions between novel miRNA drugs and more traditional drugs, which can then be tested experimentally. miR-122 is for instance predicted to target estrogen receptor 1 (ESR1), whose gene product is essential for the important drug families of estrogens (e.g. estradiol) and anti-estrogens (e.g. tamoxifen). If this predicted target is functional, treating patients for HCV with miR-122 may cause adverse drug effects if the patient is also undergoing treatment with estrogens or anti-estrogens.[10]

1. Discovering the first micro-RNA trageted drug:

MicroRNAs (miRNAs) are important post-transcriptional regulators of nearly every biological process in the cell and play key roles in the pathogenesis of human disease. As a result, there are many drug discovery programs that focus on developing miRNA-based therapeutics. The most advanced of these programs targets the liver-expressed miRNA-122 using the locked nucleic acid (LNA)–modified antisense oligonucleotide miravirsen. Here, we describe the discovery of miravirsen, which is currently in phase 2 clinical trials for treatment of hepatitis C virus (HCV) infection.

2. MicroRNAs as predictors of drug efficacy:

Although not yet used in clinical decision making, several studies have associated microRNAs with well-known biomarkers for treatment therapy decisions. For example, in chronic myeloid leukemia (CML), levels of cells with the BCR-ABL rearrangement, which characterizes this dis-ease, decrease over time with imatinib treatment. It has been discovered that miR-451 levels inversely correlate with BCR-ABL levels at both the time of diagnosis and on treatment. SNPs in microRNA target sites may also be predictors of response; the LCS6 polymorphism in the let-7 binding site in the 30UTR of KRAS predicted response to anti-EGFR-based therapy in 100 metastatic CRC cancer patients. Base excision repair genes have been associ-ated with treatment resistance, and variations in the microRNA binding sites of the 30UTRs of these genes have been shown to reflect CRC cancer prognosis and treatment response. A notable and interesting example of altered target sites in cancer is the creation of an illegitimate target site for miR-191 in the 30UTR of MDM4 by the presence of SNP34091, which affects chemosensitivity in ovarian cancer. MicroRNA polymorphisms predisposing cancer Aside from treatment resistance, it is worth noting that a number of SNPs in microRNA binding sites are involved in cancer risk and may be markers for genetic susceptibility studies in some cancers. They can be used as markers to predict subsets of patients at risk of poor outcome or lack of treatment response. These SNPs may be present in microRNA target sites, in the processing machinery, or in the microRNA sequence, altering the target of the microRNA and its ability to be processed.[9]

3. MicroRNAs as non-invasive biomarkers

The use of circulating microRNAs as markers in different cancer types is a rapidly developing area. Tumor cells can release microRNAs, stabilized by their incorporation into microvesicles, which have shown stability in the cir-culation following multiple freeze–thaw cycles and pro-longed exposure to room temperature. MicroRNAs have also shown stability in other bodily fluids, such as urine and saliva; however, most studies have centered around serum microRNAs as biomarkers. A study of 391 patients with non-small cell lung cancer (NSCLC) identi-fied 35 highly expressed microRNAs with predicted bind-ing sites for at least one of 11 genes of the TGF-b pathway, which were significantly differentially expressed at the extremes of survival. Of these, 17 were associated with patient survival and were combined into a risk score that significantly predicted survival in advanced NSCLC. Furthermore, isolation of exosomes from serum showed that a signature involving two microRNAs and one small non-coding RNA can be used for non-invasive diagnosis of glioblastoma. The detection of microRNAs in the blood presents some challenges, and there is an overwhelming discordance between reports in well-studied cancers. Appropriate endogenous controls for microRNA quantification in serum are under debate because many mRNA and rRNA species are absent in blood due to circulating RNases. Clini-cally, fluctuations of circulating microRNAs can occur as a result of treatment, diet, and other factors, increasing noise in these assays. The presence of myeloid and lymphoid cells can alter the levels of certain microRNAs, and viral infections of the patient might also affect endogenous microRNA expression. Expression changes of microRNAs are rapid in blood, and even a traumatic venepuncture may have the potential to influence expression. Despite these hurdles, it is clear that further study is warranted for detection of the presence of microRNAs in the blood for future non-invasive biomarker development, and the field is moving rapidly towards that goal.

4. MicroRNA-based therapeutics and clinical trials

Most current clinical trials are for the use of microRNAs as biomarkers for patient stratification, prognosis, and drug efficacy, and breast cancer seems to be the cancer under the most study. In few cases are specific microRNAs stated, and a global expression-profiling platform has been employed for the mining of appropriate biomarkers. In addition to biomarker studies, microRNAs and anti-micro- RNA constructs are now under investigation as potential therapeutic agents for cancer. Despite the challenges pre-sented by delivery of these types of molecule, there are currently two clinical trials for microRNA-based therapeutics. Targeting microRNAs may be used directly to target tumor cells, and also to enhance other therapies, for example, they may have a potential use in reducing the drug resistance of tumors as has been shown by the chemo-resistant properties of miR-100 in small cell lung cancer and the epigenetic silencing of miR-199b- 5p in chemoresistant ovarian cancer. The most advanced microRNA trial involves use of anti-miR-122 (Miravirsen) for hepatitis C therapy, which shows reduction in viral RNA with no evidence of resis-tance. Miravirsen is complementary in sequence to miR- 122 but also has a modified locked-nucleic acid structure, which provides resistance to degradation and increased affinity for its target. More recent studies have shown that although the intended target of Miravirsen is mature miR- 122, it also has affinity for pri- and pre-miR-122 leading to reduced processing and enhancement of its therapeu-tic effect. The first microRNA-based therapy specifically for can-cer is MRX34: a synthetic miR-34a mimic loaded in lipo-somal nanoparticles. miR-34a is a tumor suppressor microRNA downstream of p53. Its replacement in cancer cells antagonizes key hallmarks including self-renewal, migratory potential, and chemo-resistance. MRX34 is in a phase I clinical trial for primary liver cancer and liver metastases and should complete by the end of 2014. MRX34 nanoparticles readily accumulate in the liver, and quantification of MRX34 in non-human primates has established a satisfactory 7.7 h half-life in whole blood. Lipid-based local and systemic delivery of miR-34a in animal studies has also shown positive results for lung cancer. For microRNA-based therapeutics, resistance may be-come a factor, which may be overcome by using combina-torial microRNA-based therapies. With similar effect, certain anti-microRNA therapies have the potential to target whole families of microRNAs, reducing the likelihood of resistance. The study of microRNA-based therapies is still in its infancy, and side effects of these therapies need to be evaluated. MicroRNAs have been shown to be exported from cells in exosomes and therefore have the potential for systemic effects which might only become apparent in clinical trials. Also, the processing of other microRNAs is likely to be dampened by overloading the microRNA processing machinery with replacement microRNAs, and the effects of this are uncertain [9].

XII. CONCLUSION

MicroRNAs are a new subject of interest since their discovery in 2002 to be related to certain diseases. There are several clinical trials and prognostic treatment underway that will prove microRNA based drugs to be effective against several deadly diseases. Due to this increasing interest in microRNAs there have been several long-coding and small-coding RNAs that are being discovered for their successful therapeutic application in the long term and short term treatment of variety of diseases. Mainly, these drugs have been tested for breast cancer and lung cancer. These drugs are found to act upon the target mRNA as against the traditional drugs that act upon the target protein. This helps in the response to chemotherapy. This is an evolutionary method for treating cancer though it still has a long way to go. MicroRNA is a nascent topic with many things yet to be discovered whose potential should not be underestimated.

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