MicroRNAs As Potential Biomarkers And Therapeutic Targets of Cancer

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Abstract- *microRNAs (miRNAs) are a group of small noncoding endogenous, 19–24 nucleotides RNA elements of eukaryotes and some viruses that regulate gene expression post transcriptionally by binding to the complementary 3′ untranslated region (UTR) of target messenger RNA leading to RNA silencing or post transcriptional regulation of targeted genes. miRNAs regulate around 30% of human protein coding genome. Unique miRNA signatures of various inherited, metabolic, infectious, and neoplastic diseases have added a new dimension to the studies that look at their pathogenesis and highlight their potential to be reliable biomarkers. MicroRNAs have been uncovered as important posttranscriptional regulators of nearly every biological process in the cell. Furthermore, mounting evidence implies that miRNAs play key roles in the pathogenesis of a variety of disease conditions. Altering miRNA functionality and the development of novel in vivo delivery systems to achieve targeted modulation of specific miRNA function are being actively pursued as novel approaches for therapeutic intervention in many diseases.*

Keywords- microRNAs, biomarker, RNA silencing, miRNA biogenesis, therapeutic targets

I. INTRODUCTION

microRNAs (miRNAs) are a class of small (~19–24 nucleotides in length), endogenous, evolutionarily conserved non coding RNAs that function as posttranscriptional regulators of gene expression. They control the expression of genes involved in several biological processes including immune modulation, metabolic control, neuronal development, cell cycle, muscle differentiation, and stem cell differentiation. It was reported that miRNAs regulated more than 30% of the human genome and are involved in almost all fundamental cell processes (Baek *et al*., 2008). They primarily function by binding to complementary target sequences in mRNA and interfering with the translational machinery, thereby preventing or altering the production of the protein product. Follow-up studies also revealed that in addition to repressing translation, miRNA binding to its target mRNA also triggered the recruitment and association of mRNA decay factors, leading to mRNA destabilization,degradation, and resultant decrease in expression levels.

miRNAs were discovered in 1993 by Lee and colleagues in the nematode *Caenorhabditiselegans* (Lee *et al.* 1993).In these organisms, the down regulation of LIN-14 protein was found to be essential for the progression from the first larval stage (L1) to L2. Furthermore, the down regulation of LIN-14 was found to be dependent on the transcription of a second gene called lin-4. Interestingly, the transcribed lin-4 was not translated into a biologically active protein. Instead, it gave rise to 2 small RNAs approximately 21 and 61 nucleotides in length. The longer sequence formed a stem loop structure and served as a precursor for the shorter RNA. It was also found that the smaller RNA had antisense complementarity to multiple sites in the 3′ UTR of lin-14 mRNA. The binding between these complementary regions decreased LIN-14 protein expression without causing any significant change in its mRNA levels. These two studies together brought forth a model wherein base pairing occurred between multiple lin-4 small RNAs to the complementary sites in the 3′ UTR of lin-14 mRNA, thereby causing translational repression of lin-14 and subsequent progression from L1 to L2 during *C. elegans* development.

This novel mode of regulating gene expression was first thought to be a phenomenon exclusive to *C. elegans*. In 2000, two separate groups discovered that a small RNA, let-7, was essential for the development of a later larval stage to adult in *C. elegans*. More importantly, homologues of this gene were subsequently discovered in many other organisms, including humans. The period that followed was marked by a deluge of information wherein multiple laboratories cloned numerous small RNAs from humans, flies, and worms. These RNAs were noncoding, around 19 to 24 nucleotides in length, and derived from a longer precursor with a stem-loop or fold-back structure.Many were found to be evolutionarily conserved across species and exhibited cell-type specificity. The recognition and confirmation of the existence of these small RNAs, now termed microRNAs (mi-RNAs), led to intense research aimed at identifying new members of this family. This resulted in the discovery of multiple miRNAs across different species of plants and animals. AnmiRNA

registry, named miRBase, set up in 2002 serves as the primary online repository for all potential miRNA sequences, annotation, nomenclature, and target prediction information. The current release (miRBase 20) contains 38589 entries. The biological significance of a vast majority of annotated miRNAs, however, remains unknown and requires functional validation.

II. BIOGENESIS OF miRNA

miRNA genes can vary widely in their location in the genome. Earlier studies had revealed 2 distinct classes of miRNAs: those that originated from overlapping introns of protein coding transcripts and others that are encoded in exons, underscoring the complexities associated with miRNA maturation.143 Clusters of miRNA genes that coex-press polycistronically with the potential to be transcribed as a single unit were also discovered. It is estimated that approximately 50% of miRNAs are expressed from non–protein coding transcripts (Saini *et al.* 2007).The rest are mostly located in the introns of coding genes and are generally co transcribed with their host genes and processed separately. Since this is a rapidly evolving field, there is potential for future developments to significantly overhaul the current understanding of miRNA genesis.

miRNA coding transcripts are initially transcribed by RNA polymerase II as long primary miRNAs (several hundred nucleotides long) with a 5′ guanosine cap and a 3′ polyadenylated tail. The primary miRNA is then processed into ~70- to 120-nucleotide-long precursor RNA (pre-miRNA) by a multiprotein complex called microprocessor protein. This complex contains a ~160-kDa nuclear RNase III enzyme called Drosha. This enzyme is highly conserved in animals but not in plants. Droshadimerizes with another double stranded RNA (dsRNA) binding protein, called DiGeorge syndrome critical region gene 8 (DGCR8) or Pasha, to form the functional Microprocessor complex. The newly transcribed pre-miRNA with a typical 5′ phosphate and ~2-nucleotide 3′ overhang is then exported into the cytoplasm by exportin 5 (Exp-5), a Ran- GTP dependent nuclear transport receptor protein. In the cytoplasm, the pre-miRNAs are finally processed into mature ~18- to 23-nucleotide-long duplexes by another RNase III enzyme, Dicer-1, with help from dsRNA-binding proteins, protein kinase RNA activator and transactivation response RNA binding protein. The 2 miRNA strands are then separated, depending on various factors such as thermodynamic asymmetry of the duplex and stability of base pairing at the 5′ end. One strand, termed the guide strand, along with the aforementioned and other RNA binding proteins that include tri nucleotide repeat containing gene 6A (TNRC6A), associates with catalytic Argonaute (AGO) proteins, forming a microribonuclear protein complex (miRNP) called RNA-induced silencing complex (RISC). The miRNA strand with the most unstable base pairing at the 5′ end usually acts as the guide strand, while the strand with stable base pairing at the 5′ end (known as the passenger or miR* strand) is usually degraded. The guide strand directs the complex to the target mRNA through sequence complementarity and causes its translational repression. Ago2 proteins have been localized to cytoplasmic bodies called GW/P-bodies (processing bodies), where miRNAs bound to their mRNA targets are believed to be stored for degradation or translational repression.

III. FUNCTIONING OF miRNA

Various mechanisms have been proposed on the mode of operation of miRNAs that results in posttranscriptional repression of target mRNA. The repression can be the result of either reduced translational efficiency or due to an actual decrease in the mRNA levels after miRNA binding. The specific region important for mRNA target recognition is located at the 5’ end of mature miRNA strand from bases 2 to 8 often referred as the seed region. Degree of complementarity between the miRNA and the target regions in the mRNA is thought to have a role wherein perfect complementarity is believed to result in mRNA degradation while less complementarity leads to translational inhibition (Hutvagner and Zamore;2002). An alternative mechanism of action has also been proposed where binding of miRNAs leads to faster deadenylation of mRNAs, thereby decreasing mRNA stability and accelerating their degradation.

IV. NOMENCLATURE OF miRNAs

A uniform system of annotation and nomenclature has been adopted to ensure uniformity and ease of cataloguing miRNAs. miRNAs are numbered sequentially in the order they are discovered. Those that have been experimentally confirmed are assigned a number that is attached to the prefix “miR” followed by a dash (eg, miR-21). In the identifier hsamiR-21, the first 3 letters indicate the organism (eg, hsa for Homo sapiens, mml for Macacamulatta). The mature miRNA is denoted as miR-21 (with a capitalized R), while the uncapitalized mir21 refers to both the miRNA gene and the predicted stem-loop component of the primary transcript, also known as the precursor miRNA. Identical mature miRNA sequences that originate from discrete precursor sequences and genomic loci are given identifiers that contain a numeric suffix such as hsa-miR-219-1 and hsa-miR-219-2. On the other hand, closely related mature sequences that differ by 1 or 2 nucleotides are named with a lettered suffix. This would mean that hsa-miR-130a and has-miR-130b are derived from

precursor's hsa-mir-130a and hsa-mir-130b, respectively. miRNAs can also be found as clusters and present in close proximity within the genome. For example, the miR-17 cluster contains 6 precursor miRNAs located within a 1-kilobase region of chromosome 13 that can give rise to 7 mature miRNAs—namely, miR-17, miR-91, miR-18, miR-19, miR-19b, miR-20, and miR92.96 These clusters have been denoted in the literature as either an miR-17 cluster (designated with only the smallest numbered miRNA) or an miR-17-92 cluster (contains both the lowest and highest numbered miRNA).

V. CIRCULATING miRNAs AS BIOMARKERS

The stability of miRNAs in plasma and the ease by which miRNAs can be detected in a quantitative manner by methods such as real-time PCR(qRT-PCR) and microarrays have sparked great interest in the use of circulating miRNAs as clinical biomarkers. An ideal biomarker fulfills a number of criteria, such as accessibility through noninvasive methods; a high degree of specificity and sensitivity; the ability to differentiate pathologies, allowing early detection; sensitivity to relevant changes in the disease; a long half-life within the sample; and the capability for rapid and accurate detection. Because circulating miRNAs are able to fulfill a number of those criteria, miRNAs can perform as circulating biomarkers for diagnosis or prognosis of many of the disease conditions including cancer, cardiovascular diseases, autoimmune disorders, neurodegenerative disorders, metabolic diseases and some viral infections also. miRNAs in different cell types can be secreted into the extracellular space and then transported to the circulating body fluid like peripheral blood. These miRNAs are detectable in plasma or serum in a remarkably stable form, encapsulated into the extracellular vesicles or bound with special lipid proteins, thus being resistant to RNase digestions. Therefore, these small molecules are capable to be ideal candidates to serve as biomarkers for cancer detection by liquid biopsies. Besides peripheral blood, various body fluids, including saliva, cerebrospinal fluid, ascites, urine, breast milk, and semen, allow for miRNA detection.Some of the advantages of using miRNA as biomarkers over other protein /genetic biomarkers are

- miRNAs are more stable due to their small size
- Present in exosomes or micro vesicles or bound to protein called Argonaute or sometimes bound to HDL which imparts additional stability to miRNA
- miRNA can be extracted and detected from blood (total blood/ plasma/serum), urine, sputum, saliva, semen, cerebrospinal fluid and even from frozen and paraffin embedded tissues
- As miRNAs are present in almost all body fluids noninvasive methods can be applied for its isolation.
- Circulating microRNAs (miRNAs) are attractive biomarker candidates as they can be easily collected, are stable under different storage conditions and can be measured using assays that are specific, sensitive and reproducible
- miRNAs are highly tissue specific thus its identification provides a clear picture regarding the tissue origin, demarcation of normal and diseased tissue, monitoring of treatment progress

VI. IMPLICATIONS OF miRNAs IN CANCER

The expression patterns of miRNAs are usually altered in different development stages and in various pathology conditions like senescence, cardiovascular diseases, metabolic diseases, autoimmune diseases, neurodegenerative diseases and cancers (Bracken *et al*. 2016). Cancer is one of the leading causes of death worldwide. In recent years, some significant improvements have been made in tumour diagnosis and treatment. However, early detection is still critical for improving outcomes and reducing recurrence and mortality of cancer patients. The absence of obvious symptoms and insufficiently sensitive biomarkers in early stages of carcinoma limits early diagnosis. Biopsy and imaging examination as golden standards greatly improve the detection rate, but their applications are limited by their own invasive or radiation-related characteristics, respectively. In addition, traditional tumour diagnostic markers like carcino embryonic antigen (CEA) and CA199 usually exhibit low sensitivity. Therefore, it is urgent to identify novel, more sensitive, and easy-to-detect biomarkers which can be used in diagnosis and prognosis of cancers. Many miRNAs that play a role in modulating apoptosis have also been linked to the initiation and progression of various neoplastic processes. Approximately 50% of miRNAs are located at genomic sites that are disrupted or amplified in various cancers. The differential expression of circulating miRNAs exhibited promising potential for cancer screening without additional injury for patients. The abnormal levels of distinct miRNAs could be observed at an early stage, during progression, and after metastasis of cancers. Thus, these small RNA molecules may function as favorable clinical biomarkers for distinguishing tumours, treatment strategy selection, and outcomes.

Tumour suppressive and oncogenic functions of miRNA

Development of cancer entails combined interaction of both tumour suppressors and cancer inducers. miRNAs may function as a novel class of oncogenes and tumour suppressor genes. The miRNAs with increased expression in tumours are thought to function as oncogenes and are termed as oncomirs.

These oncomirs negatively inhibit tumour suppressor genes and/or those controlling cell differentiation or apoptosis and thereby promote tumour development. In contrast, some miRNAs exhibit decreased expression in cancerous cells and are considered as tumour suppressor genes. Tumour suppressor miRNAs usually prevent tumour development by negatively inhibiting oncogenes and/or genes that control cell differentiation or apoptosis. Dysregulations of miRNAs were often observed in different kinds of cancers due to dysfunction of the miRNA biogenesis process or dysfunction of transcription of miRNA-encoding genes (Bracken *et al.* 2016). miRNA let-7 is one of the founding members of the miRNA family and is highly conserved. Let-7 is localized to a region of the chromosome, which is usually deleted in human cancers. The highest levels of let-7 expression occur in differentiated adult tissues and its inappropriate expression results in oncogenic loss of differentiation. The let-7 family of miRNAs is downregulated in many tumours, including lung and breast cancer. Instead members of the miR17-92cluster, which comprises six miRNAs (miR17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1), are highly expressed in a variety of solid tumours and haematological malignancies, including cancers of the breast, colon, lung, pancreas, prostate, and stomach as well as in B cell lymphomas.

The differential expression of miRNAs can be detected by real time polymerase chain reaction (qRT-PCR), Northern blotting, microarray, and high through put sequencing and have potential for clinical applications (Calin*et al.*, 2006).miRNAs in different cell types can be secreted into the extracellular space and then transported to the circulating body fluid like peripheral blood. These miRNAs are detectable in plasma or serum in a remarkably stable form, encapsulated into the extracellular vesicles or bound with special lipid proteins(argonaute proteins/ high density lipoproteins), thus being resistant to RNase digestions (Lindner *et al.* 2015). Therefore, these small molecules are capable to be ideal candidates to serve as biomarkers for cancer detection by liquid biopsies. Besides peripheral blood, various body fluids, including saliva, cerebrospinal fluid, ascites, urine, breast milk, and semen, allow for miRNA detection (Vanni*et al.*2017)

The differential expression of circulating miRNAs exhibited promising potential for cancer screening without additional injury for patients. The abnormal levels of distinct miRNAs could be observed at an early stage, during progression, and after metastasis of cancers. Thus, these small RNA molecules may function as favorable clinical biomarkers for distinguishing tumours, treatment strategy selection, and outcomes. In non-small cell lung cancers (NSCLC) patients, for example, a large group of miRNAs have been identified to be differentially expressed in different stages of disease and to contribute to the diagnosis, treatment determination, and prognosis.

Circulating miRNAs as biomarkers for early cancer diagnosis

A growing number of circulating miRNAs was reported to be dysregulated in the early stage of cancers. The altered expression may be observed before the obvious clinical symptoms or clear biopsy and image examination evidence. Plasma miR-21-5p, miR-20a-5p, miR-141-3p, miR-145-5p, miR-155-5p, and miR-223-3p significantly increased for NSCLC patients at stages I and II (Zhang *et al*. 2017). Serum miR-126-3p, miR-182-5p, miR-183-5p, and miR-210-3p were also found to possess early detective value for non-small cell lung cancers (NSCLC) patients, exhibiting similar sensitivity and specificity with traditional tumour marker CEA.

Significantly decreased levels of miR-125a-3p were observed in plasma exosomes of colon cancer patients (Wang *et al.* 2017) as well as increased levels of miR-23a-3p, miR-27a3p, miR-142-5p, and miR-376c-3p in serum (Vychytilova *et al.*, 2016).

A group of miRNA, including miR-642b-3p, miR-1202-5p, miR-1207-5p, miR-12255p, miR-4270-5p, and miR-4281-3p, was upregulated in plasma of breast cancer patients with stage I. Serum miR-1825-3p was specifically down regulated in glioma at early stage, and its level was correlated with tumour progression and poor prognosis. These evidences suggest that the circulating miRNA detection might be introduced for early-stage cancer screening.

Circulating miRNAs as biomarkers to distinguish different subtypes of cancer

Cancers can be divided into different subtypes by tissue origination or pathological mechanisms. For example, NSCLCs include two major pathologic subtypes, adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Breast cancers are well known as heterogeneous diseases, which can be sub-classified by the presence of estrogen receptor (ER), progesterone receptor (PR), and HER2/neu receptor (Hon *et al.*2016). The sub-classification of specific tumours is valuable for determining tumour mechanisms and making therapeutic decisions. Of note, certain differentially expressed miRNAs in parallel with the different subtypes draw more and more attention recently. They could be optimal to determine tumor subtype and pathology, contributing to the selection for a more efficient therapeutic approach. Taking NSCLC as an example, the accurate sub classification into

ADC and SCC is important for deciding treatment methods. Expression patterns of several miRNAs were different not only between the plasma of NSCLC patients and healthy individuals, but also between ADC and SCC patients. Levels of miR-16- 5p and miR-486-5p were elevated in ADC and SCC cases compared to those of healthy ones. miR-9-5p expression was stable between overall NSCLC patients and healthy controls, but exhibited significant declination in ADC patients instead of SCC ones. Another plasma miRNA, miR-205-5p, was up regulated only in SCC patients. Additionally, Jin *et al.* (2017) found several ADC- and SCC-specific differentially expressed miRNAs by RNA sequencing. miR-181-5p, miR-361- 5p, and miR-320b were significantly elevated in plasma exosomes of NSCLC patients. The levels of miR-181-5p and miR-361-5p were increased by more than 10 times in ADC patients than SCC patients, and miR-320b in SCC samples increased by over 10 times than in ADC ones. miR-30a-3p and miR-30e-3p were specifically downregulated in ADC patients, while miR-10b-5p and miR-15b-5p were decreased in SCC patients. Therefore, investigators suggest that these miRNA panels may be not only applicable in NSCLC diagnosis, but also helpful to subtype discrimination.
The subtyping of heterogeneous breast cancer is also of great importance for clinical therapy. The positively expressed ER, PR, or HER2/neu in tumors could be directed as therapeutic targets. On the contrary, patients bearing triple-negative breast cancer (TNBC), which expressed none of these receptors, were usually treated with traditional chemotherapy or radiotherapy. TNBC was associated with higher stage at diagnosis and poorer prognosis. Therefore, biomarkers of specific breast cancer subtypes have also been focused on, especially for TNBC from the miRNA point of view. Shin *et al.* (2015) observed the declined levels of miR-16-5p, miR21-5p, and miR-199a-5p in plasma and tumour tissues of TNBC patients compared with both non-TNBC and healthy individuals, as well as the elevated levels of miR92a-3p and miR-342-3p.

In other cancers, different tumor subtypes may also be distinguished by circulating miRNAs. Exosomal levels of miR-101-3p and miR-483-5p in plasma of adrenocortical carcinoma were significantly higher than those of adrenocortical adenomas, which could be adopted to preoperative diagnosis of adrenocortical malignancy (Perge*et al*.2017).

Circulating miRNAs as markers for tumour metastasis

The occurrence of tumour metastasis leads to a significant impairment of curative effect, resulting to the poor survival rate and high risk of recurrence. There are currently no reliable biomarkers for predicting metastatic spread to different sites. According to the characteristics of relative tissue specificity of miRNAs, the candidates for this purpose become favourable since more and more circulating miRNAs were found to be associated with clinical tumour stage and/or metastasis. In osteosarcoma patients, miR-497-5p was significantly down regulated in primary tumor tissues, metastatic tissues, and serum compared to healthy controls (Liu *et al.* 2016) This small molecule targeted multiple genes like IGF-1R, VEGFA, AMOT and P21 to inhibit osteosarcoma cell proliferation, migration, and invasion and enhance apoptosis. Furthermore, the declined miR-497-5p expression was associated with clinical stage, distant metastasis, and promoted cisplatin resistance (Shao *et al.*2015). Aberrant reduction of miR497-5p in plasma of osteosarcoma patients implied the poor response to chemotherapy .Furthermore, lower miR-146-5p levels in serum exosomes were associated with the cisplatin resistance and shorter progression-free survival (PFS) for NSCLC patients (Yuwen*et al*.2017). These putative resistant miRNAs may be favorable for monitoring the resistant and tolerance of treatment and for selection of clinical therapeutic approach. These findings above suggest the promise of applying circulating miRNAs as biomarkers for early prediction upon certain treatment and improvement of the outcomes in most types of cancer. In the future, further clarifying the miRNAs from the original tumor sites or the targeted metastatic tissue/organ might be helpful to predict the upcoming metastasis event.

The advantage of circulating miRNAs as diagnosis and prognosis biomarkers

The most validated traditional cancer markers include alpha-fetoprotein (AFP), carcino embryonic antigen (CEA), and carbohydrate antigen (CA) (Ugrinska2002). Carcino embryonic antigen, CA199, and CA125 were generally accepted to be validated to have positive predictive value as circulating biomarkers for different cancers. Other screening strategies include mammography for breast cancer , colonoscopy for CRC, and prostatespecific antigen (PSA) for prostate cancer .However, still missing are more effective, accurate,specific, and sensitive screening biomarkers to fulfill the detective and predictive functions in the care of cancer patients. Circulating miRNAs have the particular advantage as a potential clinical application. Circulating miRNAs are non-invasive biomarkers Circulating miRNAs are easy to obtain without severe damage. Besides, a great number of potential effective miRNA biomarkers are stable in healthy people. Their expression levels may not be obviously affected by age, gender, body mass index (BMI) or other basic characteristics when evaluating pathogenic potential. Hence, the altered expression pattern might be introduced to routine examination

for monitoring and early diagnosis of cancers. Although cell-free miRNAs from plasma and serum are the most common circulating miRNA biomarkers, other body fluid samples like urine and saliva are also applicable as the resource of circulating miRNAs. Several miRNAs including miR-210-3p were up regulated in urine from transitional cell carcinoma patients and capable to facilitate cancer diagnosis. A panel of four breast cancer-related miRNAs (miR-21-5p, miR-125b5p, miR-155-5p, and miR-451-5p) was also differentially expressed in urine samples of breast cancer patients and exhibited their diagnostic value (Erbes*et al*. 2015). Circulating miRNAs may be used for screening tumours with higher sensitivity. Monitoring of the aberrant expression can be easier and earlier compared with biopsy and/or image examination which reflects the actual size without amplification, although the latter are regarded as the golden standard so far. The dynamic expression pattern of circulating miRNAs may be associated with the progression of tumours. The generation of miRNAs is dynamic and prompt upon the internal or external stimuli. This feature endows miRNAs the ability to observe the whole time course changes in real time and dynamic manner from tumour genesis throughout the following progression. These observations revealed the possibility of circulating miRNAs for evaluating the stage and progression of tumours. The whole dynamic expression pattern of miRNAs could depict the development landscape of cancer during the entire progression.

miRNAs as Potential Therapeutic Targets

As miRNAs are generally inhibitors of gene expression, the use of therapies to increase or block gene expression will result in a decreased or derepression of their mRNA targets, respectively. Based on these opposite approaches we can classify the therapeutic application of miRNAs into two strategies.

The first strategy involves antimiRNA "gain of function" phenotype, also called "inhibitors," and aims to inhibit the function of miRNAs. Several approaches can be utilized for this purpose, including (1) small-molecule inhibitors directed to regulate miRNA expression, (2) miRNA masking due to molecules complementary to the 3'UTR of the target miRNA, resulting in competitive inhibition of the downstream target effects, (3) miRNA sponges that utilize oligonucleotide constructs with multiple complementary miRNA binding sites to the target miRNA, and (4) antisenseoligonucleotides, also known as miRNA antagonists or inhibitors, such as antimiRs, locked nucleic acids (LNA), or antagomiRs that by complementarity bind to miRNAs inducing either duplex formation ormiRNA degradation.

The second strategy involves anmiRNA "loss-of-function" phenotype, also called "mimic," and aims to enhance the function of miRNAs. The approaches that can be utilized for this strategy include (1) small-molecule activators or inductors of miRNA expression and
(2) miRNA mimics, which as exogenous miRNAs aim to repress the function of their mRNA targets. They are also called "miRNA replacement therapy."

Therapeutic miRNA Mimics or miRNA Replacement Therapy

In principle, delivery of miRNA mimics as pharmacological therapy could be used in situations in which a reduction in miRNA levels is responsible for the development of a pathological state, such as those produced in the human rare. Mendelian disorders or certain types of cancer, where regions containing miRNAs are deleted (Calin*et al.* 2006). Genetic mutation in either miRNA seed region or other miRNA regions that results in a reduced functional miRNA with a significant reduction of mRNA targeting required for normal function could also benefit from these therapies. The activity of a lost or down-regulated tumor suppressor miRNA can be restored by using miRNA mimics (Garzon*et al.*2010). These are chemically modified synthetic RNA duplexes designed to replace the lacking endogenous mature miRNA. The guide strand is identical to the miRNA of interest, whereas the passenger strand is chemically modified, to prevent its loading to the Argonaute protein in the miRISC. Pre-miRNAs are processed in the cytoplasm by Dicer to an around 22 nt doublestranded miRNA duplexes with 2 nucleotide 3' overhangs and 5'phosphate groups. These features are also important for efficient recognition of the miRNA mimic and adequate loading of the synthetic guide strand in the miRISC. Several studies have reported on potent double-stranded miRNA mimic designs, in which the passenger strand is substituted with chemical modifications, such as 2'-O-methyl (2'-O-Me) sugars, to prevent miRISC loading and ensure its degradation, whereas additional end modifications are used to protect the duplex from nucleolytic degradation (Sioud*et al.*2007). Protection of the3'overhangs against nuclease degradation has been achieved by using inverted dT modifications or other nonnucleotide groups or by incorporation of benzene-pyridine to the 3' ends of the miRNA duplex (Kitade*etal.*2010). Different experimental strategies to deliver miRNA mimics have been tested. Synthetic miRNA or pre-miRNA duplexes, normally modified for better stability and cellular uptake, have been incorporated into different delivery systems, including lipid nanoparticles with surface receptor ligands or other components to increase tissue/cell specificity. Another approach to replace a lost or down-regulated tumor suppressor miRNA is to use viral

vector based systems that express the miRNA gene in question, thereby restoring its function. Viral constructs are efficiently transduced to target cells, and both adeno associated virus (AAV) and lenti virus vector based systems have been used to replace tumor suppressor miRNAs by expressing them from systemically or intra nasally delivered viral constructs (Garzon*et al.* 2010). Another advantage of AAV-based constructs is that they do not integrate into the host genome and are therefore eventually eliminated, which minimizes the vector related toxicities.

Therapeutic miRNA Inhibition or Anti-miR Therapy/ Anti mimic Therapy

Mature miRNAs can be effectively inhibited using complementary antisense oligonucleotides, termed antimiRs, which act by sequestering the miRNAs, thereby blocking their ability to repress endogenous target mRNAs. Pharmacological inhibition of miRNA function requires chemical modification of the antimiR oligonucleotides to improve their binding affinity, specificity, nuclease resistance, and in vivo delivery. Antisense oligonucleotides (ASOs) complementary to the mature miRNA sequence, "antagomiRs," were the first miRNA inhibitors in mammals. Several studies have reported on the utility of different sugar modifications in designing antimiRs with increased duplex melting temperature (Tm) toward their cognate miRNAs and with enhanced nuclease resistance (Stenvang*et al.* 2012). The most commonly used modifications to improve antimiR potency include various 2' sugar modifications, such as2'-O-Me,2'-O-methoxyethyl(2'-MOE),or 2'fluoro (2'-F) chemistries, and the bicyclic locked nucleic acid (LNA) modification, in which the ribose is locked in a C3'-endo conformation by introduction of a 2'-O,4'-C methylene bridge. Among these, LNA possesses the highest affinity toward complementary RNA with an increase in Tm of + 20C to 8 0C per introduced LNA modification against complementary RNA.

Nuclease resistance is markedly improved by incorporation of phosphorothioate (PS) backbone linkages, in which sulfur replaces one of the non-bridging oxygen atoms in the phosphate group. Besides nuclease resistance, PS backbone modifications also promote plasma protein binding, thereby reducing clearance of antimiR oligonucleotides from plasma. This, in turn, enhances the pharmacokinetic properties of PS backbone -modified oligonucleotides.

To date, the most common approach to inhibit miRNA function is to use fully complementary antimiRs. However, the high duplex melting temperature of LNA-modified oligonucleotides allows efficient miRNA antagonism also by use of truncated antimiRs.

Indeed, several studies have reported on efficient miRNA inhibition using high-affinity 15- to 16 nucleotide LNA-modified DNA oligonucleotides targeting the 5' region of the mature miRNA Furthermore, recent studies have described a method for inhibition of miRNA function using short LNA oligonucleotides complementary to the seed region, termed tiny LNAs. This approach exploits the high duplex melting temperature of fully LNA-modified 8mer PS oligonucleotides complementary to the miRNA seed region and thereby enables simultaneous inhibition of entire miRNA seed families with concomitant derepression of direct target mRNAs.

Delivery of Mirna Therapeutics

Efficient and safe in vivo delivery of antimiR oligonucleotides and miRNA mimics is a critical factor for the development of miRNA-based therapeutic modalities. miRNA replacement therapy relies on restoring the function of lost or down-regulated tumor suppressor miRNAs and is currently being pursued using 2 delivery strategies: (i) delivery of formulated, synthetic, double-stranded RNA molecules that mimic pre-miRNAs and (ii) viral vector- based systems that express the miRNA gene in question, thereby restoring its function.

Several studies have reported on delivery and efficacy of synthetic miRNA mimics in restoring the functions of tumour-suppressive miRNAs in mouse cancer models in vivo. This approach requires optimized formulation for efficient and safe in vivo delivery, and proof of concept has been demonstrated for intravenously and intra tumourally injected miRNA mimics complexed with liposome, nanoparticles, polyethyleneimine, and atelocollagen. Viral delivery for restoring miRNA function has been reported using intra nasally or intravenously administered AAV or lentivirus constructs expressing different tumour suppressive miRNAs.

To date, 2 strategies have been used to deliver antimiR oligonucleotides for therapeutic targeting of miRNAs in vivo. Antagomirs are 3' cholesterol-conjugated 2'-O-Me oligonucleotides complementary to the mature miRNA sequence and modified with terminal PS linkages. .In this study, systemically delivered antagomir-16 showed uptake and inhibition of the ubiquitously expressed miR-16 in a number of peripheral tissues, including liver, kidney, lung, heart, skeletal muscle, colon, ovaries, adrenal glands, and bone marrow, whereas no efficacy was observed in the brain. Another delivery approach is to use unconjugated, PS backbone - modified antimiR oligonucleotides, which exhibit high nuclease resistance, good pharmacokinetic properties,

and tissue uptake along with high stability in tissues in vivo. More recently, a systemically delivered 8-mer tiny LNA targeting the seed region of miR-122 was shown to inhibit miR-122 function in the mouse liver, leading to concomitant derepression of predicted miR-122 target mRNAs with canonical 3'UTR seed match sites and dose-dependent lowering of serum cholesterol(Obad *et al.*2011). These findings highlight the utility of seed-targeting tiny LNAs in functional studies of miRNA seed families in vivo, because unlike other chemically modified antimiRs, 8-mer LNAs enable inhibition of co-expressed miRNA family members that may have redundant biological functions.

VII. FUTURE PROSPECTS

The discoveries and current developments in miRNA biology have essentially revolutionized the biological landscape and have created renewed interest within the scientific community to re-evaluate and significantly modify the conventional perceptions about gene expression, gene regulation, and RNA functionality. miRNA discovery effectively challenges the "central dogma of molecular biology," in which RNA was assigned the role of a mere intermediary to the flow of information between the genetic material (DNA) and the end product (protein). The discovery and validation of miRNA function also refute the faulty "junk DNA hypothesis," which stated that more than 80% of our DNA serves no biological purpose and is "junk" that has accumulated over time as evolutionary fossils. The implications of the insights coming out of studies in miRNA biology are far reaching. Many knockout or overexpression studies performed in the past to determine the function of individual genes invariably included deletion of introns. Since a significant number of miRNAs are now known to be present in introns, the inadvertent deletion of any miRNA coding sequences contained in these regions might also be a contributing factor to the phenotypic consequences observed in these individual knockout/ overexpression studies that were attributed solely to the protein coding gene. Data in this direction indicate that many knockout studies need to be re-examined to determine if loss of a miRNA contributed to the phenotype, warranting careful planning of knockout studies in future.

Since a single miRNA can regulate a number of genes and a single gene can be regulated by multiple miRNAs, loss of function of 1 or more miRNAs may not be evident at a functional level due to the inherent ability of the biological system to substitute 1 molecule with another capable of performing similar functions. Also, there is growing consensus that miRNAs, in many cases, are "fine-tuners" of gene expression, and hence, unlike genes encoding proteins, changes in spatial and temporal expression of miRNAs can be fleeting, can fluctuate rapidly, and might involve minimal changes in their expression levels. Hence, the ability to observe these changes is limited by the sensitivity and sophistication of the detection system. Although a lot of progress has been made in addressing these bottlenecks, more work is needed to better understand their biological function. Therapeutic implications of miRNAs are enormous. Individual miRNAs important in the development of diseases can be specifically targeted using anti-miRNAs or miRNA mimics. Many studies in this direction have yielded promising results. Although achieving stability and efficiency of the in vivo delivery of miRNAs is a major challenge, progress made in this direction indicates the rapid emergence of an entirely new field of small RNA-based therapeutics. These developments, together with their role as potential biomarkers, make them invaluable tools in probing multiple aspects of a disease process that includes diagnosis, classification, and treatment.

VIII. CONCLUSION

miRNA as a novel arm of gene expressional regulation tool has been widely used in gene functional studies of a variety of fields. More importantly, as an emerging technology, it has the great potential to be employed in drug development. Recent advances in the miRNA research have provided us more insights and improved understanding towards miRNA biogenesis, function and particularly their association with molecular pathogenesis of a variety of complex diseases including cancer, heart diseases, chronic viral infections, immune disorders, neurodegenerative disease and metabolic diseases. Currently, the identified oncogenic miRNAs, and viral encoded miRNAs, which are key factors for viral replication and latency, are the ideal targets for developing therapeutics. Moreover, understanding the miRNA signature in susceptible individuals including their expression profiles, dynamics, and even miRNA target variants (single-nucleotide polymorphisms) may eventually enable the miRNA-based individual-specific therapy, as well as disease diagnosis and prognosis. In addition, siRNA/miRNA specific delivery to target cell populations using approaches of nanobiotechnology is just beginning and looks promising. In a word, with the development in miRNA field, these small molecules could be an invaluable tool for various areas of basic and applied research and, more importantly, for therapeutic intervention.

REFERENCES

[1] Baek, D., Villen,J., Shin ,C., Camargo, F.D., Gygi, S.P. and Bartel, D.P. 2008. The impact of microRNAs on protein output. Nature;455:64–71.

[2] Blandin, S,,Grosbie, P.A., Balata, H., Chudziak , J., Hussell, T. and Dive, C. 2017. Progress and prospects of early detection in lung cancer. Open Biol.7: 9-27.

[3] Bracken, C.P., Scott, H.S. and Goodall, G. J. 2016. A network-biology perspective of microRNA function and dysfunction in cancer. Nat Rev Genet.17:719–32.

[4] Calin, G.A. and Croce,C.M.. 2006.MicroRNA signatures in human cancers. Nat Rev Cancer.6:857–66.

[5] Erbes, T., Hirschfeld, M., Rucker, G., Jaeger, M., Boas, J. and Iborra S.2015. Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker. BMC Cancer.15:193pp.

[6] Garzon, R., Marcucci, G. and Croce, C.M. 2010.Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov.9: 775-789.

[7] Hon, J.D., Singh, B., Sahin, A., Du, G., Wang, J. and Wang,V.Y. 2016. Breast cancer molecular subtypes: from TNBC to QNBC. Am J Cancer Res.6:1864–72.

[8] Hutvagner, G. and Zamore, P.D. 2002.A microRNA in a multiple-turnover RNAi enzyme complex. Science. 297:2056–2060.

[9] Jin ,X., Chen, Y., Chen, H., Fei, S., Chen, D. and Cai, X. 2017. Evaluation of tumorderivedexosomal.miRNA as potential diagnostic biomarkers for early-stage non-small cell lung cancer using next-generation sequencing. Clin Cancer Res. 23:5311–5319.

[10] Kitade, Y. and Akao, Y. 2010. MicroRNAs and their therapeutic potential for human diseases: microRNAs, miR-143 and -145, function as antioncomirs and the application of chemically modified miR-143 as an anti-cancer drug. J Pharmacol Sci. 114:276-280.

[11] Lee, R.C., Feinbaum, R.L. and Ambros, V. 1993.The C. elegansheterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell.75:843–854.

[12] Lindner, K., Haier, J., Wang, Z., Watson, D.I., Hussey, D.J. and Hummel,R.. 2015. Circulating microRNAs: emerging biomarkers for diagnosis and prognosis in patients with gastrointestinal cancers. ClinSci; 128:1–15.

[13] Liu, Q., Wang, H., Singh, A. and Shou, F. 2016. Expression and function of microRNA-497 in human osteosarcoma. Mol Med Rep. 14:439–45.

[14] Obad, S., Santos, C.O. and Petri, A.2011. Silencing of microRNA families by seed-targeting tiny LNAs. Nat Genet. 43:371-378.

[15] Perge, P., Butz, H., Pezzani, R., Bancos, I., Nagy, Z. and Paloczi,K. 2017. Evaluation and diagnostic potential of circulating extracellular vesicle-associated microRNAs in adrenocortical tumors. Sci Rep.7:1.

[16] Saini, H.K., Griffiths-Jones, S. and Enright, A.J. 2007. Genomic analysis of human microRNA transcripts. ProcNatlAcad Sci. 104:17719–17724.

[17] Shao, X.J., Miao, M.H., Xue, J. and Zhu, H. 2015.The down-regulation of microRNA-497 contributes to cell growth and cisplatin resistance through PI3K/Akt pathway in osteosarcoma. Cell Physiol Biochem.36:2051–2062.

[18] Shin, V.Y., Siu, J.M., Cheuk, I., andKwong, A. 2015.Circulating cell-free miRNAs as biomarker for triple-negative breast cancer. Br J Cancer.112:1751–1759.

[19] Sioud, M., Furset, G. and Cekaite, L. 2007. Suppression of immunostimulatorysiRNA-driven innate immune activation by 2'-modified RNAs. BiochemBiophys Res Commun. 361:122-126.

[20] Stenvang, J., Petri, A. and Lindow, M.2012. Inhibition of microRNA function by antimiR oligonucleotides. Silence.3:1.

[21] Ugrinska, A., Bombardieri, E., Stokkel, M.P., Crippa, F. and Pauwels, E.K. 2002.Circulating tumor markers and nuclear medicine imaging modalities: breast, prostate and ovarian cancer. Q J Nucl Med.46:88–104.

[22] Vanni, I., Alama, A., Grossi,F.and Coco S. 2017.Exosomes: a new horizon in lung cancer. Drug Discov Today. 22:927–936.

[23] Vychytilova-Faltejskova, P., Radova,L., Sachlova, M., Kosarova, Z., Slaba. K., Fabian, P. 2016. Serum-based microRNA signatures in early diagnosis and prognosis prediction of colon cancer. Carcinogenesis.37:941–950.

[24] Wang, J., Yan, F., Zhao, Q., Zhan, F., Wang, R. and Wang, L. 2017. Circulating exosomal miR-125a-3p as a novel biomarker for early-stage colon cancer. Sci Rep.7:1

[25] Yuwen, D.L., Sheng, B.B., Liu, J., Wenyu, W. and Shu, Y.Q. 2017.MiR-146a-5p level in serum exosomes predicts therapeutic effect of cisplatin in non-small cell lung cancer. Eur Rev Med Pharmacol Sci.21:2650–2658.

[26] Zhang, H., Mao, F., Shen, T., Luo, Q., Ding, Z., Qian, L. and Huang, J. 2017. Plasma miR145, miR-20a, miR-21 and miR-223 as novel biomarkers for screening early-stage non-small cell lung cancer. Oncol.Lett. 13:669–676.