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MicroRNAs in Disease and Health: Diagnostic and Therapeutic Potentials

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1. Introduction

In the past decade, relevance and application of genomics and proteomics technologies in early detection of diseases have verified that numerous categories of diseases could be diagnosed at early stage which would be helpful in initiation of treatment. As a result of human genome studies, a shift has occurred from mRNAs to noncoding RNAs as a main regulator of human genome. The sequence analyses of genomes in eukaryotes indicated that simple unicellular organisms, invertebrates and mammals have approximately 25%, 80% and 98%, respectively, of their genomes composed of noncoding DNA regions [1]. In higher eukaryotic organisms, approximately, the entire genome is transcribed as consisting of rRNA, tRNA, introns, 5' and 3' untranslated regions, and microRNAs. It also has been proposed that mammalian miRNAs are originated from transposons and repeats [2,3]. Moreover, it is showed that miRNAs could be developed from pseudogenes [4]. Existing analyses of the human genome reflects that the protein coding genes are low as 23500, indicating that a large segment of human genome consists of non-coding protein genes. Open reading Frames comprise less than 2%, repetitive sequences around 46% and non-coding parts of protein-coding genes (introns, 5' and 3'-UTRs) around 25-27% of 3.2 billion bases in the human genome [5,6]. As the human genome and its functions are being explored, the roles of non-coding RNAs are becoming more evident in specific cellular functions. Members of non-coding RNAs include microRNAs and small nucleolar RNAs which are believed to have well preserved functions in various species. MiRNAs are functional molecules that have recently emerged as important regulators of gene expression at the posttranscriptional and translational levels. Endogenous miRNAs are involved in a variety of physiological and pathological processes in human. Target mRNA specification is defined by sequence complementarity between the seed sequence and the target mRNA. The technological advances and applications of functional RNA molecules for medicine provide important insights into molecular mechanisms affecting human health and disease and could eventually lead to the discovery of diagnostic biomarkers and the development of novel gene therapies [7].

In this chapter, we first address the synthesis, mechanisms of action and functions of miRNAs. Then, we focus on recent advances and technologies in miRNA. Next, we discuss

the clinical applications of miRNA. At last, we review current knowledge of the roles of miRNAs in various diseases and determine the advantages and potential challenges of microRNA-based approaches compared to conventional drug-based therapies.

2. MiRNA biology and function

Micro ribonucleic acids (miRNAs) are a large class of endogenously expressed single stranded, evolutionarily conserved small non-coding RNAs, typically 17-25 ribonucleotides in length that are found in many plants, animals and DNA viruses – regulating gene expression in a sequence-dependent manner [8]. MiRNA has revealed the great potential regulating roles in various aspects of biological and pathological processes. They were first discovered in worms in 1993, *Caenorhabditis elegans*, *lin-4*, capable of modulating the protein expression of *lin-14* opened up a new role for small RNAs in regulating gene expression [9]. While more than 3,000 miRNAs have now been identified in animals, plants and viruses, the human genome has been estimated to code up to 1000 miRNAs that are expected to regulate a third of all genes. A plethora of predicted mRNA targets, it is believed that these small RNAs have an enormous regulatory potentials [10,11]. In mammals, miRNAs derive from a large primary transcript, referred to as a pri-miR, transcribed by RNAPolymerase II/III in nucleus. The pri-miR, which is likely hundreds to thousands of nucleotides long, experiences nuclear cleavage by a ribonuclease III called Drosha and the double-stranded DNA binding protein DGCR8/Pasha to generate a hairpin shaped ~70-nucleotide pre-miRNA. Then, pre-miRNA is transported to the cytoplasm by the nuclear export factor exportin 5, and undergoes another processing where they serve as substrates of RNase Dicer and its cofactors (PACT and TRBP) to generate ~22 nt miRNA duplexes. RNA duplex is unwound into a mature single-stranded miRNA, and loaded into RNA-induced silencing complex (RISC). Although most miRNAs in plants hybridize to target mRNAs with perfect complementarity, in animals the 5' proximal “seed” region (nucleotides 2 to 8) appears to be the primary determinant of the pairing specificity of the miRNA to the 3' untranslated region (3'-UTR) of a target mRNA. MiRNA then binds to complementary sites in the 3'-untranslated region (3'-UTR) of target mRNA and regulate its expression either by causing degradation of mRNA or repression of their translation, depending on the degree of complementarity between the miRNA and its target [12-17]. Alternatively, pre-miRNAs are derived directly from size-matched introns. These are referred to as ‘mitrons’ skip the Drosha-DGCR8 processing step and are spliced out of their host genes. These lariats are debranched, refolded into the stem-loop structure of typical pre-miRNAs, and then enter the canonical pathway [18]. Of note, Drosha, DGCR8, and Dicer are the well-established regulators of miRNA processing. Defects in the miRNA biogenesis machinery could be intimately related to various diseases including cancer.

3. MiRNA-mediated gene regulation

The expression of miRNAs is vibrant depending on tissue types, metabolic status and disease condition. A potent trait of miRNA-based regulation is the capability of individual miRNAs to regulate multiple functionally related mRNAs, which itself regulates multiple metabolic genes [19,20]. Approximately half of all known miRNA genes are grouped in the genome and transcribed as polycistronic primary transcripts with the remainder expressed as individual transcripts from intergenic or intronic site. Regardless of genomic

organization, miRNAs function in a distinct yet cooperative manner to regulate cellular processes by coordinately targeting related proteins [21,22]. Furthermore, miRNAs often go to families of narrowly related or the same sequences. As of their homology in the seed sequence, the associated miRNAs are able to target the same mRNAs, which increases the effectiveness of repression. The number of validated mature human miRNA approaches over 1 thousand, with individual miRNAs capable to repress multiple genes. Of note, miRNAs have emerged as important regulators of every biological process involved with differentiation, cellular proliferation, tissue development and cell-type specific function and homeostasis. As a consequence, dysregulation of miRNAs have been implicated with impairment of regulatory networks. In fact, a multiple number of pathologies are associated with altered miRNAs expression [23]. It is becoming progressively more evident that the aberrant expression of miRNAs is causally related to a diversity of disease states. Stress-induced upregulation of miRNAs be able to result in the downregulation of a set of targeted mRNAs, whereas downregulation of miRNAs can lead to upregulation of target mRNAs. At last, it is the pattern of miRNA-induced gene expression that contributes to the consequential disease phenotype [24]. It was shown that diverse forms of stress alleviate mRNAs from miRNA repression by releasing mRNAs from P-bodies (cytoplasmic bodies containing mRNA degradation enzymes) and promotes their entry into polysomes, thereby enhancing translation of the preexisting mRNA [25]. Both changes in expression and function in response to stress increase their influence under conditions of disease states. The apparent roles of miRNAs in disease have led to an increasing interest in miRNA regulation as a therapeutic and diagnostic approach [26]. The role of miRNAs-mediated regulation in viral infection is becoming more evident. The outcome of miRNA repression is not well understood. Some reports showed that miRNA regulation led to translational inhibition, without affecting mRNA levels. Although there are accumulating reports that mRNA degradation is a consequence of miRNA regulation, it is unclear whether it is a consequence of translational repression or a separate pathway of miRNA regulation [27-30].

4. MiRNAs expression profile analysis

Investigating and implementing efficient tools for detection, quantification, and functional analysis of miRNAs is essential to explore the role of miRNAs signatures in health and disease process. Various studies exploited high throughput methods to analyze global miRNA expression in clinical samples. As a common platform, Oligonucleotide miRNA microarray analysis has been extensively used as high-throughput method for the evaluation of global expression levels in a large number of samples [31,32]. An original miRNA microarray platform by means of locked nucleic acid-modified capture probes has also been emerged. This procedure allows miRNAs with single nucleotide differences to be discriminated. Furthermore, MiRAGE, a form of serial analysis of gene expression to evaluate miRNA levels, and RAKE an RNA primed, array-based, Klenow enzyme high-throughput assay, have also been built up to find out miRNA expression profiles [33,34]. Of note, the above-mentioned techniques were mainly developed to determine the cancer-specific expression levels, however, the use of high-throughput arrays for investigating miRNA expression in non-neoplastic diseases is beginning to emerge. A number of miRNAs are up regulated in the brains of demised Alzheimer's patients, implicating impairment of miRNA expression in neurodegenerative disorders [35]. A microarray-based approach has

also been used to profile miRNA expression in response to environmental stresses such as hypoxia. A method to weigh miRNA expression is the bead-based flow cytometric approach. Individual polystyrene beads coupled to miRNA complementary probes are marked with fluorescent tags. After hybridization with size-fractionated RNAs and streptavidin-phycoerythrin staining, the beads are analyzed using a flow-cytometer to measure bead color and phycoerythrin, demonstrating miRNA characteristics and loads respectively. The technique provides great specificity for intimately related miRNAs because hybridization happens in solution. [36]. Quantitative real-time polymerase chain reaction (qRT-PCR) have also been widely used to miRNA research due to its cost-effectiveness, high throughput and higher detection [37]. Up to now, the most successful approach in terms of specificity and sensitivity is a two-step approach via looped miRNA-specific reverse transcription primers and TaqMan probes. Multiplex PCR approaches for miRNAs have also been reported [38]. All of these technologies facilitating miRNAs expression profiling are essential for validation of microarray data. The high throughput capability of array-based platforms make them an attractive option for miRNA studies compared with lower throughput techniques such as Northern blotting and cloning. High-throughput analysis of miRNA expression in various diseases only demonstrates that some miRNAs are over expressed while others are markedly reduced in other tissues. These are exclusively correlative data. In other words, high throughput platforms to gauge miRNA expression provide only correlative evidence that atypical miRNA processing. These techniques only convey us modest about the underlying miRNA expression problem. Besides, the threshold of differential miRNA expression required so as to impact biological processes is unknown for most disease-associated miRNAs. Of importance, the development of methods to manipulate miRNA expression has made possible examinations into the cellular processes involved by differentially expressed miRNAs. 2-O-Methyl antisense single-strand oligonucleotides and locked nucleic acid-modified oligonucleotides have been developed as miRNA inhibitors, making the suppression of endogenous miRNA activity and its downstream effect on mRNA expression achievable [39-42]. The effect of target miRNA knockdown on cell morphology and function can be determined using standard assays for processes such as cell proliferation, migration, invasion, and angiogenesis. Cell-based assays that examine the role of miRNA in human disease are also recently emerged. Numerous studies in cancer cell lines, using either antisense inhibition or over expression of individual miRNAs, show a direct functional link between aberrant miRNA expression and an individual tumor [43-46]. It is well-known that cancer-based cell assays are suitable for biochemical experimentation. MiRNA mimicry, a complementary method to inhibition, newly used in vitro to identify cellular processes and phenotypic changes associated with specific miRNAs transfected into cell lines; which will be explained at the last section. Disruption in miRNA expression is not the only change that can modify the regulation of target mRNAs. Mutations in the 3' UTR of the candidate disease genes that disrupt miRNA binding sites can also affect diseases through reduced or total loss of miRNA-mediated regulation.

5. Biological functions of miRNAs

While the "seed" region is most important for miRNA-mRNA interactions, applying this principle only leads to prediction of many miRNA targets that cannot be validated in vivo, raising the possibility that further imperative rules manage miRNA-mRNA interaction. A

prevailing aspect of all targets is that miRNAs usually target UTR sites that do not have a complex secondary structure and are located in accessible regions of the RNA. Since this model was proposed, several additional targets have been characterized *in vivo*, and approximately all are consistent with the proposed accessibility criteria [47,48]. MiRNAs are regulators of gene expression that control many biological processes in growth, development, differentiation, and metabolism. Their expression levels, small size, and mode of action pose unique challenges in studies elucidating the function of miRNAs. New technologies for identification, expression profiling and target gene validation will make easy the study of their involvement to biological processes and disease. Such information will be crucial to utilize the upcoming knowledge of miRNAs for the development of new human therapeutics. It is remarkable to think about whether individual miRNAs function separately on specific targets or if they can also function in a combinatorial manner. For instance, the effects of single miRNAs can be found in the regulation of *lin-28* mRNA by *lin-4*, the first known miRNA, discovered in 1993 and was shown to have significant functions in developmental timing of stage-specific cell lineages [49]. By contrast, many miRNA clusters inhabit in related introns of paralogous genes. These polycistronic miRNAs could be classified into the similar miRNA family based on sequence similarity in the miRNA 50 region, implying that they might cooperatively control frequent sets of targets or molecular events. Besides to coordinately regulating a single mRNA, several members of a miRNA family can control sequential events [50]. Given the ability of miRNAs to regulate multiple genes, it will be interesting to examine whether they function through many of the same paradigms as transcription factors, such as combinatorial regulation and regulation of whole genetic programs. Genetic studies in *Caenorhabditis elegans*, *Drosophila melanogaster* have identified important functions of specific miRNAs in the coordination of cell proliferation and death during development, stress resistance, fat metabolism and brain morphogenesis [51,52]. For the past few years, one primary focus of the investigators studying mammalian miRNAs was to recognize and catalog the complete miRNA list and its expression pattern using cloning, bioinformatics and gene expression approaches. With these efforts nearing conclusion in the near future, the focus is shifting to the clarification of miRNA function. Several technological advances including bioinformatic prediction algorithms, reporter assays, *in situ* hybridizations, overexpression and silencing technologies, were developed to infer miRNA function. Here, we review the basic knowledge on miRNAs functions, especially highlighting recently discovered miRNA functions. The recent progress of miRNA involvements in organs development will be discussed.

5.1 Pivotal functions of miRNAs in development and or differentiation

Examining mice lacking the essential miRNA-processing enzyme Dicer is essential to investigate the role of miRNAs in development. Targeted deletion of Dicer in mice causes embryonic lethality before embryonic day (E) 7.5. Dicer-deficient embryonic stem (ES) cells are defective in differentiation both *in vitro* and *in vivo*, and do not form the three germ layers normally located in embryoid bodies derived from ES cells, suggesting an essential role for miRNAs in development [53]. In fact, without *lin-4*, *C. elegans* is unable to make the transition from the first to the second larval stage due to a differentiation defect, which is caused by a failure to posttranscriptionally repress the *lin-14* gene, which is the target gene of *lin-4* [54]. Similarly, without *let-7*, a failure of larval-to-adult transition was observed [55]. Therefore, miRNA function might be essential for 'fine tuning' developmental incidents

during the defined events of organogenesis. MiR-1-1 and miR-1-2 are rich in the developing heart. Enrichment of miR-1-1 is primarily observed in the atrial precursors before becoming omnipresent in the heart, whereas a miR-1-2 enhancer is particular for the ventricles in the developing heart; therefore, miR-1-1 and miR-1-2 might have chamber-specific effects in vivo [56]. MiR-1 in flies regulates the Notch signaling pathway by directly targeting mRNA of the Notch ligand, Delta, demonstrating that miR-1 functions to persuade differentiated cardiac cells from an equivalency group of progenitor cells [57,58]. MiRNAs regulate key events during neurogenesis in various species. In mammals, miR-134 is particularly expressed in the rat dendritic spine in hippocampal neurons. It binds to 3' UTR of Limk1 and represses local LimK1 translation, which results in inhibition of dendritic spine growth. Stimuli such as brain-derived neurotrophic factor can mitigate this suppression. This finding revealed that miRNAs can be key regulators of neuronal structure and plasticity [59]. MiRNAs control key events during neurogenesis. In zebrafish, maternal zygotic Dicer mutant embryos have severe defects in the development of the neurocoel and the neural tube. These developmental defects are saved by members of the miR-430 family, indicating that the latter are the major miRNAs involved in the neurogenic defects [60]. MiRNAs can also be important regulators of neuronal structure and plasticity in mammals [61]. A discrete set of miRNAs is exclusively expressed in pluripotent embryonic stem cells but not in differentiated embryoid bodies, suggesting a role in stem cell self-renewal [62]. Understanding how miRNAs are processed and how they are integrated into the complex regulatory networks that direct the developmental, homeostatic and physiological processes of organisms are extremely crucial.

5.2 Cell proliferation and apoptosis

MiRNAs have been shown to regulate main genes for cancer progression that coordinately manages cell proliferation and apoptosis. MiRNAs regulate pathways controlled by genes such as p53, MYC and RAS. Moreover, miR17-92 cluster was shown to be competent to operate as a functional switch between cell proliferation and apoptosis. The fly miRNA, Bantam, was originally characterized from a fly P-ELEMENT screen for genes that promote cell proliferation and suppress apoptosis during tissue growth [63]. Its sequence complementarity with the 3' UTR and its functional antagonism of the pro-apoptotic *hid* gene were the first clues for the translational repression of *hid* by *Bantam* [63, 64]. In addition, fly *miR-14* was recognized through a *P-element* screen for inhibitors of apoptotic cell death. Deficiency in miR-14 enhances cell death that is induced by the cell death activator, *Reaper*, and results in defective stress responses and fat metabolism. Loss of *miR-14* also leads to an elevated level of Drice, an apoptotic effector caspase, indicating a direct or indirect repression of Drice by *miR-14* [65].

5.3 Contribution to maintain tissue identity

Yu et al. conducted a genome-wide analysis of the expression profiles of miRNA targets in human, mouse and *Drosophila*. They found that the expression levels of miRNA targets are significantly lower in all mouse mature tissues and *Drosophila* later life stages than in the embryos. These results point out that miRNAs may play roles in determining the timing of tissue differentiation during the larva period of *Drosophila* development and maintaining the tissue identity during adulthood [66].

5.4 As regulators for noise filtering and buffering

Eukaryotic cells are noisy milieus in which transcription often happens in a bursting manner, causing the number of mRNAs per cell to swing significantly [67]. In positive regulatory loops, noise or stochastic fluctuations of gene transcripts and protein molecules leads to randomly switching cell phenotypes in yeast, while a negative regulator adding in the positive regulatory loops often helps in reducing such noise in biological systems and making a strong result for cell development. Because miRNAs can adjust target protein levels more rapidly at the posttranscriptional level, they may significantly shorten the response delay and, in turn, provide more effective noise buffering. The miRNA *miR-17* might play a role in preventing noise-driven transition from apoptosis to cell proliferation. It is possible that miRNAs serve to buffer stochastically fluctuating expression of genes in positive regulatory loop. MiRNAs provide a common mechanism in buffering gene expression noise by frequently regulating positive regulatory loops [68,69].

Up to date, miRNAs have been shown to be involved in a range of cellular processes primarily developmental and metabolic processes including: cell proliferation, cell differentiation, developmental timing, fat metabolism, apoptosis, insulin secretion, stem cell maintenance, neuronal patterning, and haematopoietic lineage differentiation. Table 1 lists a number of the main important miRNAs that are associated with defined biological processes and some of the target genes through which they exert their regulatory function.

6. MiRNAs- associated disease mechanisms

MiRNAs- Associated Disease Mechanisms are quietly abundant so as because of time and place limitations, it is impossible to discuss in detail the all respective involved mechanisms. We then describe the essentials and needful accordingly. The high sequence conservation of many miRNAs among faintly related organisms suggests strong evolutionary pressure and involvement in main physiological processes by miRNAs. In fact, miRNA deficiencies or excesses are correlated with a range of clinically imperative diseases including myocardial infarction, virus infection, Alzheimer's disease, metabolic diseases, cancers, and many others [70-73]. Calin and colleagues found that a region containing miR-15 and miR-16 at chromosome 13q14 was commonly deleted in the majority of chronic lymphocytic leukemia cases [74]. It is quite clear that many miRNAs are associated with primary human tumors, and more than 50% of human miRNAs genes are located at genomic regions implicated in cancers, such as common breakpoint regions and fragile sites [75,76]. Discovery of the role of miRNAs in various pathological processes has shed light to the possible applications of miRNA in molecular diagnostics and prognostics, particularly for cancer. It is obvious that miRNAs in deleted or amplified regions in cancer samples contain altered expression levels. The impaired expression of various miRNAs throughout oncogenesis may advance or impede the tumor formation by modulating the expression of critical genes involved in cancer cell proliferation or survival. Though the vast majority of associations between miRNAs and human disease are coupled with tumorigenesis, there is accumulating evidence that miRNAs most probably are involved in multiple diseases. An investigation has added indirect support that miRNA changes are causal, rather than consequential, of cellular transformation [77]. One of them is fragile X mental retardation (FXMR) caused by absence or mutation of the fragile X mental retardation protein. Experimental results from *Drosophila melanogaster* indicate that FXMR may be a part of RISC [78]. The syndrome most often results from trinucleotide expansion of the CGG repeat in the 5' UTR of the *FMR1*

microRNA	Target gene (s)	Biological Functions
Mammalian		
miR-1	HAND, HDAC4	Cardiomyocyte /skeletal muscle differentiation and proliferation
miR-10a	HOXA1	Megakaryocytopoiesis
miR-27b	CYP1B1	Regulation drug metabolising enzymes
miR-32	Retrovirus PFV-1	Antiviral defence
miR-103	Multiple targets of cell cycle	Cell cycle progression
miR-132	P250GAP	Neuronal morphogenesis
miR-143	ERK5	Adipocyte differentiation
miR-155	AT1R	Angiotensin II related processes
miR-221	c-KIT	Erythropoiesis
miR-196a	HOXB8	Developmental patterning
miR-132	TRAF6	Regulation innate immune response
miR-133	nPTB, SRF	Myoblast/skeletal muscle differentiation and proliferation
miR-134	LIMK1	Regulation of dendritic spine development
miR-375	MTPN	Regulation of insulin secretion
miR-130a	MAFB	Megakaryocytopoiesis
miR-15a	BCL2	Regulation of granulopoiesis
miR-146a-b	IRAK1	Regulation macrophage inflammatory response
Caenorhabditis elegans		
let-7	lin-41, hbl-1	Regulation of developmental transition between the last larval stage (L4) and the adult stage
lin-4	lin-14, lin-28	Regulation of developmental transition between the first two larval stages, L1 and L2
Drosophila melanogaster		
Bantam	hid	Promotion of cell proliferation and suppression of apoptosis
miR-14	Unknown	Suppression of apoptosis and regulation of fat metabolism
Mus musculus		
miR-181	Unknown	Promotion of haematopoietic differentiation towards the B-cell lineage
miR-196	Hoxb8	Unknown
Zea mays		
miR-166	rld1	Regulation of leaf morphogenesis

Abbreviations: HAND2, hand transcription factor; HDAC4, histone deacetylase 4; CYP1B1, cytochrome P450 1B1; PFV1, primate foamy virus type 1; P50GAP (RICS), Rho GTPase-activating protein; ERK5, extracellular signal regulated kinase 5; AT1 R, angiotensin II type 1 receptor; c-KIT, stem cell factor receptor; HOXB8, homeobox B8; TRAF6, TNF receptor-associated factor 6;nPTB, neuronal polypyrimidine tract-binding protein;SRF, serum response factor; LIMK1, Lim domain containing protein kinase 1; MTPN, myotrophin; Hoxb8, homeobox B8; rld1, rolled leaf1; MAFB, v-maf musculoaponeurotic fibrosarcoma oncogene homologue B; BCL2, B-cell lymphoma 2; IRAK1, IL-1 receptor associated kinase;

Table 1. MicroRNAs associated with experimentally defined functions and targets.

gene, causing hypermethylation and defeat of FMR1 expression [79]. Biochemical studies using recombinant FMRP and FXR1P suggest that FMR1P, FXR1P, and FXR2P proteins perform as acceptor molecules for Dicer-processed miRNAs and help direct gathering of miRNA [80]. The dAGO1 is indispensable for miRNA-directed gene silencing in *Drosophila*, showing a functional interaction with dFMR1, strengthens the dispute that failing in miRNA-mediated translation repression during neural development could result into fragile X syndrome [81,82]. It has been shown that expression of many miRNAs is altered in heart disease and that various types of heart disease are associated with discrete alterations in miRNA expression [83]. Such changes intimately look like the miRNA expression patterns observed in fetal cardiac tissue, indicating a novel mode of regulation for the transcriptional changes in cardiac failure. Numerous miRNAs were found to be dysregulated in two mouse models of cardiac hypertrophy. MiR-9 and miR-128a were overexpressed in the brains of Alzheimer's patients and 16 miRNAs were differentially expressed in the brains of schizophrenics. A mutation in the 3' UTR of the SLITRK1 gene that is associated with Tourette's syndrome led to enhanced repression by miR-189 [84-87]. Several hundreds of miRNAs were identified in human but currently, only a couple of specific targets have been experimentally validated. As predicted by bioinformatical analysis, a single miRNA can potentially target different mRNAs.

MiRNA regulation has also been implicated in virus-induced diseases. In fact, cellular miRNA expression may confer host immunity against viral infections; whereas viruses may have evolved to utilize miRNA machinery for their replication. It was observed that production of miRNAs by RNA viruses or cytoplasmic DNA viruses is unlikely, due to the compartmentalization of miRNA processing in the nucleus, as well as the fact that Drosha processing of miRNAs from RNA viruses would result in cleavage of the viral genome [88]. Increasing evidence suggests that viruses interact with the miRNA machinery [89]. Host miRNAs can also be used for viral replication as well as antiviral defense. The small size of miRNA precursors makes them also potentially ideal for use by oncogenic viruses as inhibitors of host cell defense pathways. For example, Human Papilloma Virus 16 (HPV16), is associated with cervical cancer, incorporates into miRNA genes at a rate 3 times higher than to the rest of the genome [90]. In fact, Cellular microRNAs have regulatory functions and direct antiviral consequences. A cellular microRNA effectively restricts the accumulation of the retrovirus primate foamy virus type 1 (PFV-1) in human cells. Viral Tas protein suppresses microRNA-directed functions in mammalian cells and displays cross-kingdom antisilencing activities [91]. Epstein-Barr virus (EBV) is a large DNA virus that preferentially infects human B cells and expresses a number of microRNA genes. The small RNA profile of cells infected by EBV was recorded and discovered that EBV miRNAs originated from five different double-stranded RNA precursors clustered in two regions of the EBV genome. Epstein-Barr virus use RNA silencing as a method for gene regulation of host and viral genes in a non-immunogenic manner [92]. Nearly and likely more, 11 viruses have been shown to encode their own miRNAs. These viral miRNAs stand for potential antiviral targets and might also serve as diagnostic markers for viral infection or stage of infection. Viruses use miRNAs in various ways, such as to inhibit viral or host transcripts or to recruit host miRNAs for viral replication.

7. MiRNAs and cancer

The aberrant expression of miRNA in cancers and the fact that approximately half of miRNA genes are localized in cancer-associated genomic regions or in fragile sites indicates

the potential role of miRNAs as oncogenes or tumor suppressor genes in human carcinogenesis. MiRNA acts as oncomiRNA or tumor suppressors to affect the tumorigenesis if their target mRNAs were encoded by tumor suppressor genes or ontogenesis. MiRNAs still mainly intact in conventionally collected, formalin-fixed and paraffin-embedded tissues and even a modest number of miRNAs are adequate to discriminate human tumors according to the developmental lineage and differentiation state of the tumors [93-95]. The first association between miRNAs and cancer was identified by Croce and colleagues when the miR-15a-16-1 cluster was implicated as a putative tumor suppressor gene mapping to chr 13q14, a small genomic region frequently deleted or translocated in chronic lymphocytic leukemia [96]. Since the predicted number of miRNAs in the human genome is as many as 1000. Regulation mediated by these genes has possibly a great impact on gene expression because, based on computational predictions, a single miRNA can target dozens of genes. Each cancer tissue has a particular miRNA signature and miRNA based cancer sorting is a very effective and potential tool. A subset of cancer-related miRNAs are persistently involved in a variety of cancers so that the miR-17-92, miR-106b-25 and miR-221-222 clusters, miR-155, let-7, miR-34a, miR-200 and others were revealed to play key roles in a variety of oncogenic processes [97,98]. Other miRNAs are expressed only in tumors of a limited tissue type and may be the most precise approach of defining not only the cell of origin, though the state of differentiation of a tumor. Moreover, miRNA signatures specify tumor subtypes and can help envisage prognosis and reaction to therapy. It is likely that numerous further essential oncomir-regulated targets/pathways with additional oncomirs will persist to be described in this fast-moving field [99,100]. To initiate and maintain a tumor, cancer cells must obtain the ability to proliferate autonomously, elude apoptosis and self-renew. To expand progress and metastasize, solid tumors also require stimulating angiogenesis, attacking normal tissue boundaries and becoming motile. Cancer-related miRNAs are implicated in each of these steps. For example, miR-15a-16 expression is downregulated in nearly all cases of CLL and prostate cancer; while miR-25/92 are upregulated in the majority human cancers. MiR-34a, which is transactivated by p53, one of the main tumor suppressor genes recurrently inactivated in human tumors, operates as a tumor suppressor miRNA by inducing cell cycle arrest and apoptosis in breast and lung cancer cells. This miRNA inhibits the expression of cyclin D1 and the cyclin-dependent kinase CDK6, which force cell cycle progression from G1 to S phase, as well as SIRT1, suppresses p53 transcriptional activity and the expression of its targets p21 and PUMA [101-103]. MiRNAs also play a main role in regulating cancer cell self-renewal. A self-renewing pool of immature cancer cells within a tumor, are thought to serve as a self-renewing reserve to generate new cancer cells. These cells are also relatively resistant to chemotherapy and radiotherapy and likely play a major role in tumor recurrence after therapy. The let-7 family of miRNAs, which is not expressed in CSC (or other types of normal stem cells), acts as a master regulator of self-renewal in breast CSC. Processing of the let-7 primary transcripts to the mature miRNA is inhibited at several steps by lin28, which is only expressed in stem cells [104-106]. Invasion of contiguous tissue and metastasis by epithelial cancer cells is another key step in tumor progression and is thought to involve a process termed epithelial mesenchymal transition. Epithelial cancer cells at the periphery of the tumor employ in a complex crosstalk with stromal cells leading to a global re-programming of gene expression with loss of epithelial traits and acquisition of mesenchymal properties, including invasion and motility [107]. Tumor-associated miRNAs along with validated target genes are presented in Table 2.

miRNAs	Cancer	Expression	Targets
miR-21	Breast, Colon, Lung, Pancreas, Brain, Liver	Up	CDK6, PDCD4, FAS, IL6R TPM1, CDKN1A, SOCS5
miR-25/92	Leukemia, Lung, Stomach, Colon, Prostate, Thyroid	Up	CDKN1C, BCL2L11
miR-142	Aggressive B cell leukemia	Up	Translocated c-MYC gene
miR-155	Chronic lymphocytic leukemia	Up	BIC RNA
miR-186	Chronic lymphocytic leukemia	Up	IgVh gene
miR-221	Thyroid, Stomach, Pancreas, Prostate, Melanoma	Up	CDK1B, CDKN1C, KIT
Let-7	Breast, Lung	Down	LIN28, HMGA2, kRAS, NF2
miR-335	Breast	Down	SOX4, MERTK
miR-372/3	Breast, Testicular germ cells	Down	LATS2, CD44, CD24
miR-15/16	Chronic lymphocytic leukemia	Down	BCL2, PDCD4, JUN, MCL1,RAB21, PRIM1
miR-17/20/93/106	Lung, Stomach, Colon, Pancreas, Prostate, Leukemia, Thyroid	Up	E2F1, CDKN1A, RUNX1, NCOA3

Table 2. Tumor-associated miRNAs along with validated target genes.

Overall, it is proposed that miRNAs may regulate tumorigenesis through a overabundance of possible oncogenic mechanisms. Genomic deletion or epigenetic silencing of a miRNA that normally represses expression of one or more oncogenes might lead to increased oncogenic expression. Alternatively, amplification, overexpression, or loss of epigenetic silencing of a gene encoding an miRNA that targets one or more tumor suppressor genes could inhibit the activity of an anti-oncogenic pathway [108]. Besides, mutations affecting the sequence of the mature miRNA or target mRNA could modify binding of the miRNA to its related targets leading to alterations in the balance of critical growth regulatory proteins [109]. The onco-microRNA expression profiling of human malignancies has also identified several diagnostic and prognostic cancer signatures. Furthermore, some miRNAs are downregulated in cancer, such as let-7 and miR-34a. The widespread differential expression of miRNA genes between malignant and normal cells is a complex phenomenon and may engage multiple mechanisms, including miRNA transcriptional control by tumour suppressor genes, oncogenes, epigenetic mechanisms and genomic abnormalities [109,110].

Moreover, germ-line and somatic mutations in miRNAs or polymorphisms in the mRNAs targeted by miRNAs may also participate to cancer predisposition and progression. It has been suggested that alterations in miRNA genes play a serious role in the pathophysiology of many human cancers [111].

7.1 MiRNAs and esophageal carcinoma

MiRNA plays role as oncomiRNA or tumor suppressors to influence the tumorigenesis if their target mRNAs were encoded by tumor suppressor genes or ontogenesis. Aberrant miRNA expression has been identified and confirmed in esophageal carcinoma. MiRNAs expression profiling differs markedly between esophageal squamous cell carcinoma and esophageal adenocarcinoma, indicates that these two main forms of esophageal carcinoma have distinct etiologic and pathologic characteristics. The miRNAs which are highly expressed in tumor can work as tumor promoter by targeting and inhibiting diverse tumor suppressor genes that itself were involved in carcinogenesis of esophagus [112-114]. Apart from identifying different cancer-related miRNAs, efforts were made to recognize their target genes, messenger RNAs and receptors, which will result into their contribution in cancer treatment. For example, one of the putatively identified targets of miR-21 is PTEN [115]. A decrease in functional PTEN causes constitutive activation of downstream components of the PI3K/AKT pathway, leading to tumor progression and metastasis. Thus, down-regulation of PTEN by miR-21 may contribute to transformation and increased tumor cell survival [116]. Epigenetic silencing of tumor suppressor genes is an additional mechanism of cancer development. In fact, the expression of tumor suppressor being able to get altered in epigenetic silencing mechanism via expression of miRNA. It was found that miR-127, located in a CpG island, identified in hematological cancers and deleted by loss of heterozygosity in solid tumors [117]. Further investigation is required for improved thoughtful their role in carcinogenesis of esophageal carcinoma and for better application in the future.

7.2 MiRNAs and breast cancer

MiR-10b, *miR-125b* and *miR-145*, are down-regulated and *miR-21*, *miR-155*, get up-regulated in breast cancer. *MiR-125b* gene is located at chromosome 11q23-24, one of the regions most frequently deleted in breast, ovarian, and lung tumors [118, 119]. *MiR-145* was progressively down-regulated from normal breast tissue to cancer with high proliferation index. The expression of various *let-7* miRNAs was down-regulated in breast cancer samples with either lymph node metastasis or higher proliferation index [120]. It was found that upregulation of *miR-10b* commences breast cancer invasion and metastasis [121]. The justification for using miRNAs as potential therapeutic targets is underlined by the fact that miRNA overexpression in cancer cells has a pathogenic effect.

7.3 MiRNAs and lung cancer

let-7 expression is recurrently reduced in lung cancers and associated with decreased post operative survival [122, 123]. It was shown that *miR-155* was over-expression, while *miR-17-92* cluster was over-expressed [124]. An *in vitro* experiment has revealed that overexpression of *let-7* results in the inhibition of lung cancer cell growth. Furthermore, Dicer protein expression is reduced in a fraction of lung cancers with a prognostic impact on the survival of surgically treated patients [125].

7.4 MiRNAs and hematological malignancies

It was shown that two miRNA genes located at 13q14.3 within a 30-kb region of minimal loss in. Both of these genes, miR-15a and miR-16-1, are down-regulated in more than 60% of CLL cases. Cluster miR-15b, miR-16-2, along with a different promoter, was found on chromosome 3q25–26.169. These miRNAs are less intensively expressed in normal cells; however, they may play a role in cases of 13q14 deletions [126,127]. It was also found that down-regulation of miR-16-1 and miR-15a expression correlates with allelic loss at 13q14, could be essential for clinical classification of CLL. Patients with a normal karyotype or deletion of 13q14 as the sole genetic abnormality have a better prognosis than those with a complex karyotype [128,129]. Indeed, the miRNA expression profile is associated with progression in CLL and be able to serve as a potential prognostic marker. As the investigation on lymphoma showed an elevation in the amount of miR-155/ BIC RNA happening in lymphomas derived from B cells, therefore *miR-155* possibly will play a role in the pathogenesis of B cell lymphomas [130]. *MiR-142* located at a spot of rearrangement linked to human leukemia that causes an aggressive B cell leukemia due to up-regulation of a translocated c-MYC gene. Moreover, it was revealed that miR-142 expression is higher in B-lymphoid and myeloid lineages compared to other hematopoietic tissues [131, 132].

7.5 MiRNAs and brain tumors

Microarray studies of glioma tissue have demonstrated a number of miRNAs implicated in glioma formation and propagation. MiR-7, -10b, -15b, -21, -26a, -124, -128, -137, -181a, -181b, -221, -451, play a role in human glioma. The majority of miRNAs are underexpressed in proliferating glioma cells with the important exception of miR-10b, -21, and -221 [133]. MicroRNAs regulate oncogenes implicated in brain tumor formation. Interestingly, microRNAs may also dictate the invasiveness and aggressiveness of tumors. MicroRNAs are significant regulators of cellular proliferation and differentiation so that it was found that miR-124 and miR-137 are downregulated [134]. On the other hand, microRNAs have been shown to work as tumor suppressors where downregulation of miR-181a and miR-181b involved in glioma formation was reported suggesting a tumor suppressor role for miRNAs [135]. Evidence for a role of miRNA in brain tumorigenesis recently emerged via the involvement of miRNA in medulloblastoma by demonstrating that miR-124 modulates cell-cycle regulation in medulloblastoma cells. They showed that miR-124 expression is significantly decreased in medulloblastoma [136]. Therefore, strong evidence raising that miRNAs are integrally involved in brain tumor development and progression was proven. The discovery of the role miRNAs in brain tumors has also revealed a new category of therapeutic targets.

7.6 MiRNAs and cardiovascular diseases

MiRNAs are fundamental for heart development and regulating the expression of genes which play a part in cardiac function including the conductance of electrical signals, heart muscle contraction, and heart growth and morphogenesis. The most abundant miRNAs in heart are miR-1, let-7, miR-133, miR-126-3p, miR-143, miR-30c, and miR-22 [136]. It was found that loss-of-function of miR-1 prevented heart arrhythmia, whereas miR-1 overexpression led to heart arrhythmia in normal and infarcted hearts. The same also showed that both gain- and loss-of function of miR-1 affect conductivity through affecting potassium channels [137]. MiR-1, miR-133 and miR-208 are considered muscle specific,

being primarily expressed in cardiac and skeletal muscles and also the intronic miRNAs including miR-208a, miR-208b, and miR-499 control muscle performance [138-140].

MiRNA expression profiles in a given tissue are dependent on the disease state. In order to adjust to the workload and impaired cardiac function, the heart may react by undergoing large cardiac remodeling known as cardiac hypertrophy. Investigators established an association between miRNAs and hypertrophy by demonstrating that stress regulated miRNAs could activate both positive and negative pathways on the hypertrophic growth response [141,142]. Investigation on three various types of failing hearts showed that around 50% of miRNA were differentially expressed in at least one disease group, while seven miRNAs revealed similar regulation in all three disease states [143]. Another study revealed that a large fraction of miRNAs were either up- or down regulated in the same direction in fetal and failing heart compared with normal heart [144]. Changes in miRNA expression profiles in experimental models may also provide further insights into our understanding of heart failure. Arrhythmias arise due to heart disease or mutation in ion channel genes, however, miRNAs, such as miR-1 and miR-133 have been implicated to function in cardiac conduction system. The involvement of miR-1 in cardiac conductance was further confirmed by over expression studies in normal and infarcted rat hearts so as upregulation of miR-1 in individuals with coronary heart disease [145,146].

Myocardial vascularization subsequent MI needs signaling by angiogenic growth factors, such as vascular endothelial growth factor and fibroblast growth factor. MiR-126 is an endothelial cell-specific miRNA that plays an critical role in neoangiogenesis following MI and in maintenance of vascular integrity. The actions of miR-126 reflect its potentiation of mitogen-activated protein kinase signaling downstream of VEGF and FGF. Spry-1, an intracellular inhibitor of the Ras/mitogen-activated protein kinase pathway, serves as a key target for repression by miR-126. These findings suggest that strategies to raise miR-126 expression in the ischemic myocardium could enhance cardiac repair [147,148]. For the first time, it was showed that aberrant expression of miRNAs in the vascular walls after carotid artery balloon angioplasty in rats. This team identified p27 (Kip1) and p57 (Kip2) as potential targets for these miRNAs in these cells. Further by knocking down miR-221 and miR-222 in rat carotid arteries, they revealed therapeutic potential for these miRNAs in suppressing cell proliferation in vivo and neointimal lesion formation after angioplasty [149, 150].

The role of miRNAs in regulating endothelial cells in response to hypoxia was also studied [151]. They observed miR-210 upregulation in human endothelial cells in response to hypoxia affecting cell survival, migration, and differentiation. MiR-210 overexpression in normoxic endothelial cells stimulated angiogenesis whereas the opposite was observed with miR-210 blockade. Furthermore, a Dicer knockdown approach was used to examine the implication of miRNA in regulating the redox state and angiogenic response of human microvascular endothelial cells. The reduced miRNA levels induced expression of the transcription factor HIF-1 that negatively regulates expression of p47phox of the NADPH oxidase complex. This study provides the first evidence that redox signaling in cells is subjected to regulation by miRNA [152].

Taken together, it seems clear that miRNAs have a central role in regulating gene expression in the heart. These studies indicate that miRNAs are important during heart development and adult cardiac physiology, and modulate a diverse spectrum of cardiovascular functions in vivo. Furthermore, these studies also have implications for understanding complex pathways, e.g., interactions between miRNAs, cell signaling and transcription factors,

involved in heart diseases and can lead to potential opportunities in manipulating miRNAs as therapeutic targets.

8. Diagnostic and prognostic value of miRNA: as novel promising biomarkers

Make use of blood biomarkers was proved to be a reasonable means for early detection of some diseases. Since abnormal miRNA expression seems to characterize many diseases, increasing evidence indicates that the specific miRNA expression can be applied not only for diagnosis but also for classification of different tumors. The distinctive expression profile of miRNAs in different types and at different stages of cancer, and in other diseases, suggest that miRNA can be exploited as novel biomarkers for disease diagnostics and might present a new strategy for miRNA gene therapy. At the same time, risk stratification and prognosis assessment have become a major concern in the era of personalised medicine. Although, gene expression profiling has reached a plateau in this regard, recent miRNA studies illustrate great promise whereas aberrant miRNA profiling and or abnormal miRNA levels in tissues or in plasma can perform as a robust predictor of overall survival and disease outcome in various disorders. Because numerous miRNAs are expressed in a tissue-specific manner and their levels in different organs vary in association with disease states, these small molecules represent an attractive new class of highly specific biomarkers [153-157]. Some reports have previously illustrated the potential of these small RNA molecules as biomarkers in cancerous patients [158]. Moreover, serum of patients with autoimmune disorders exhibits increased amounts of certain miRNAs. Another study showed that miR-155 and miR-146a are raised in samples from patients with rheumatoid arthritis [159]. One report revealed that the miRNA profile in the serum of type 2 diabetic patients is considerably different from those of healthy individuals and includes three miRNAs that were not associated with other disorders [160-162]. Recent evidence revealed that profile of miRNAs expression correlated with clinicopathological attributes and disease consequence. Because abundant studies demonstrated that miRNAs are implicated in cancer development and metastasis, miRNAs have great cancer diagnostic and prognostic potentials. Several miRNAs was showed to be associated with prognostic factors and disease progression in chronic lymphocytic leukemia [163]. Furthermore, high expression of miR-155 and low expression of let-7a-2 were associated with poor prognosis in human lung cancer [164]. For instance, a number of studies demonstrated that MicroRNA profiling acts as a tool to understand prognosis, and manage therapy response and resistance in breast cancer [165-167]. They revealed that expression level of miR-210 in early initiation of the disease was associated negatively with overall survival, suggesting that miR-210 could be free prognostic factor for breast cancer. MiRNA microarray profiling in breast cancer was also shown a positive correlation with epidemiological and pathological features of the disease so that miR-21 was associated with pathological features including advanced tumor stage, lymph node metastasis, and poor survival [168]. Taken together, in cancerous conditions, microRNAs expression profiling is certainly considered widely as a novel prognostic and diagnostic tool in clinical setting compared to mRNA; because, firstly that miRNAs stability in paraffin-embedded tissues is higher and remain primarily intact in plasma/serum compared with mRNA, due to lack of endogenous RNase activity. Secondly, a small number of miRNAs likely is adequate to distinguish cancers from normal [169,170]. These findings suggest that miRNA profiling is far more informative than usual mRNA profiling. Furthermore, predictive miRNA

expression signatures may be identified within tumor groups that predict the rate of progression risk, survival or existence of metastases. Lastly, miRNAs could be a target or tool for cancer prevention or therapeutic intervention. Hence, with mounting facts concerning miRNAs associated with molecular signatures and clinicopathological characteristics of various disorders, it is deemed that miRNAs may verify useful as diagnostic and prognostic means in the future.

9. MiRNA-based therapeutics

MiRNAs have great potential to be developed as a novel class of therapeutic targets. The exclusive biogenesis and mechanism of miRNA action allocate it to be free from the problems including off-target effects and drawbacks of siRNA which greatly hamper therapeutic use of siRNA. The benefit on specificity and toxicity, and the striking feature of multiple targeting potential, miRNA will also become an excellent tool for gene intervention. It is well known that miRNA levels are either upregulated or downregulated in various diseases. Therefore, miRNAs need to be finely modulated so as to either knockdown pathogenic or aberrantly expressed miRNAs or induce expression of optimistic miRNAs to threshold levels. There are several approaches to attain these *in vivo* and *in vitro*. In cancer therapy, reasonable approaches for therapy would comprise achieving “gain” or “loss” of miRNA roles in the cancer cells. Because many miRNAs have been identified to impart tumor suppressive effects, restoring their expression could produce therapeutic effects. It was found that ectopic overexpression of let-7 tumor suppressor miRNA inhibited the growth of lung, liver and pancreatic cancer cells. Moreover, systemic administration of miR-26a using adeno-associated virus in an animal model of hepatocellular carcinoma inhibited tumor progression. These findings demonstrated an important target miRNA with tumor suppressive activity and signified that adenoviral vector-based delivery might be a clinically viable approach [171,172]. Besides, synthetic miRNA mimics have also been introduced to re-establish miRNA function within the tumor. In case of oncogenic miRNAs, a number of approaches have been designed and tested to attain their downregulation [173]. For oncogenic miRNAs, multiple approaches have been designed and tested to achieve their downregulation. These approaches include the use of anti-miRNA oligonucleotides (AMOs), small molecule inhibitors, miRNA sponges and miRNA masking. Of note, downregulation of miRNAs could be approached via its biogenesis. In a one investigation, several small organic molecules were screened to block miR-21 function and azobenzene was identified as an efficient inhibitor of miR-21 expression [174]. An additional approach is the application of synthetic mRNA consisting multiple pairing sites for endogenous miRNA [175]. These synthetic mRNAs, known as miRNA sponges, act by sucking up the target miRNAs and blocking their regulatory function in the cell [176].

Taken together, to date, two strategies have been introduced to exploit miRNAs as a novel and effective therapeutic tool. These include inhibition strategy and replacement strategy. Antisense inhibition of mature miRNA is one the inhibition strategies. Antisense miRNA Oligonucleotides (ASOs) are currently the most easily approachable technology for inhibiting miRNAs therapeutically. Anti-miRNA ASOs (AMOs) have been used by many groups to inhibit miRNA activity in cell culture. A group of investigators demonstrated that oligonucleotides carrying 2'sugar modifications (including 2'- (2'-F) and locked nucleic acid (LNA) plus phosphorothioate backbone modification, most probably works as successful inhibitors of miRNAs in cell culture [177–180]. It seems that there are other factors vital for

effective miRNA targeting, which will be imperative to appreciate to develop the most compelling AMOs for therapeutics. The most likely AMO binding site is the RISC-loaded miRNA, and there is indirect proof to support that. How this results in degradation is not clear, it may engage accelerated turnover of the miRNA complex [181]. Further approaches for modulating or improving oligonucleotide distribution *in vivo* include liposomal formulation and conjugation chemistry. Some progress has been made in liposomal delivery of ASOs [182,183]. However, route of administration, toxicity, and the cost made limited employing of oligonucleotide/liposome complex in practice. These limitations are needed to be addressed effectively in order to develop from a laboratory implement to a commercially practical therapeutic and clinical setting [184,185]. An alternative approach to targeting miRNAs therapeutically is by inhibiting its processing. This could be achieved by inhibiting Drosha, Dicer, or other miRNA pathway components, which could access by small molecule drugs. The anticipated pleiotropic effects of this approach may be challenging. On the other hand, an oligonucleotide complementary to an individual pri-miRNA designed to interrupt the hairpin structure could prevent Drosha recognition or processing [186,187]. It could also be potential to target pri-miRNAs with ASOs that function by an RNase H mechanism. One team showed inhibition of a pre-miRNA hairpin in the cytoplasm with a siRNA targeted to the loop region, requiring much higher doses of siRNA than a typical mRNA target [188]. The next effective approach is replacement strategy. One approach to achieve this is through the introduction of a double-stranded miRNA mimetic, corresponding to the endogenous Dicer product and analogous in structure to a siRNA. This approach will likely face all the same impediments that siRNA therapeutics currently encounter, mainly the trouble of systemic delivery to tissues. Nucleotide modifications to improve stability to nuclease degradation and avoid quick renal clearance of the dsRNA from the blood were widely investigated, and a general understanding of what kinds of modifications are beneficial [189- 191]. The current progress in therapeutic delivery of siRNA indicates that augmentation of miRNA function with dsRNA mimetics will ultimately be promising.

A substitute strategy for therapeutic miRNA replacement is a gene therapy approach. This involves expression of a short hairpin RNA (shRNA) from a polymerase II or III promoter, in a DNA or viral vector that is subsequently processed by Dicer prior to loading into RISC. Benefits to this approach are the potential for more persistent silencing compared to double-stranded siRNA or miRNA mimetics, plus the simplicity of expressing several miRNAs from one transcript. Modified adenovirus or adeno-associated virus (AAV) vectors also were effective for gene delivery to the liver and the brain. They are restricted by the immune response to the adenovirus, and still limited in the tissues so as they can competently infect [192,193].

Recently, further therapeutic potentials for miRNAs clinical applications over stem cells signatures have been emerged. Particularly, in majority of malignant tumors, heterogeneous population of cells exist so as a proportion of which have been assigned the related stem cells. These cells are involved in tumorigenesis owing to their capability to self-renew and proliferation. At the same time, the ability of miRNAs to control many target genes gets them smart candidates for regulating stem cell self-renewal. Compelling evidence supports that specific miRNAs are differentially expressed in stem cells. It was showed that miRNAs are required to enable stem cells to overcome the G1-S checkpoint and achieve self-renewal. The exploitation of such miRNAs would smooth the progress of the regulation of the stem cells motivating tumorigenesis. An extra stem cell/miRNA therapeutic possibility is the use of mesenchymal stem cells as promising tumor-targeted vectors for miRNA therapeutics. Of

note, the advantages and challenges of miRNA-based therapeutics compared with conservative and routine treatments are needed taken into account comprehensively [194-198]. The advantages involve regulation of many components of the same pathway/cellular via miRNA, stable and durable, and effective in vivo regulation of the miRNA. In contrast, the potential challenges include delivery of the miRNA modulator, off-target effects of unintended targets of a miRNA, toxicity of the miRNA modulator. It is important to keep in mind that a single miRNA can comprise both favorable and pathogenic effects. Besides, 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 are invaluable means for various areas of basic and applied research and, more importantly, for therapeutic intervention [199, 200].

10. References

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