

## MicroRNAs Reconceived: A Novel Promising Biomarker for Diagnostic and Therapeutic Prospects

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### ABSTRACT

MicroRNAs, are regulatory small non-coding RNAs, which have recently been identified for the post-transcriptional regulation of gene expression in a variety of cellular processes especially important roles in disease and tissue remodeling. Apart from involvement in a variety of biological processes, microRNAs were early recognized for their potential use as biomarkers in disease diagnostics and therapeutics. Currently, there are number of microRNAs helping clinicians to determine the origins of cancer and other diseases. Though more than a decade of research on miRNA, yet the potency of their use is still in progress. The development of microRNA therapeutics has proved more challenging mainly due to delivery issues. This review article mainly focuses on the emerging insights involving miRNA biogenesis and their role as clinical diagnostic and therapeutic non-invasive biomarkers in various pathological conditions such as cancer, neurodegenerative disorders, polycystic ovarian syndrome and diabetes mellitus.

### Keywords:

miRNA, Biomarker, Diagnosis, Therapeutic, Cancer, Diabetes Mellitus

### INTRODUCTION

MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNA molecules that regulate gene expression. Their discovery is revolutionizing both basic biomedical research and drug discovery. The human genome is believed to encode ~1,000 miRNAs. A repository of miRNAs from many organisms has been listed in the miRBase Sequence Database,<sup>1</sup> that contains sequences and annotation.<sup>2</sup> More than 25,000 miRNAs have been described in man, worms, *Drosophila*, and also in the small plant *Arabidopsis thaliana*. This review aims to describe the basics of miRNA and their role as biomarkers in clinical diagnostics and prognostic values.

wherein a hairpin fold-back structure from the precursor transcript separates the miRNAs from other small RNAs with expression confirmation of about 22 nucleotide-long mature sequence. Presently, based on deep-sequencing data, 28645 entries representing miRNA precursors, expressing 35828 mature miRNA products, in 223 species have been deposited into miRBase v21.<sup>1</sup> The nomenclature of these miRNAs is based on a “mir” or “miR” prefix with identifying numbers assigned sequentially at the time of discovery. “mir” represents a precursor miRNA whereas “miR” denotes a mature miRNA sequence. Similar or identical sequences can be given the same number.

### miRNA BASICS

Gary Ruvkun and Victor Ambros in 1990, first discovered miRNAs in *Caenorhabditis elegans* and their target gene.<sup>3,4</sup> Together, these two seminal discoveries identified a novel mechanism for post-transcriptional gene regulation,

### miRNA BIOGENESIS

miRNA genes exist in the intergenic regions, introns of coding and exons of non-coding genes.<sup>5</sup> Approximately one third of miRNAs are intergenic and most of all miRNA loci contain clustered miRNAs (miRBase v21). The majority of miRNAs are transcribed by

RNA polymerase II.<sup>6</sup> Analysis of miRNAs residing in intergenic primary transcripts indicates that the protein-coding transcripts are larger than the primary transcripts, of which, the transcription start sites about 2 kb upstream of the pre-miRNA and polyadenylation signals 2 kb downstream.<sup>7</sup> A subset of miRNAs is transcribed by RNA polymerase III. This cluster of miRNAs is located among Alu rich regions on chromosome 19.<sup>8</sup> Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems and are processed into 60- to 100-nt hairpins known as pre-miRNAs. The pre-miRNAs are exported from the nucleus to the cytoplasm by exportin 5, where they, in general, are cleaved by the endonuclease Dicer to yield imperfect miRNA-miRNA\* duplexes.<sup>9</sup> The miRNA strand is selected to become a mature miRNA, whereas, most often, the miRNA\* strand is degraded. The mature miRNA that is added to the RNA-induced silencing complex (RISC) identifies the potential precise targets and activates post-transcriptional gene silencing (Figure 1).<sup>10</sup> On the other hand, an alternative biogenesis pathway was revealed in which miR-451 enters RISC by direct loading of the pre-miR into RISC after Drosha processing, by skipping further processing by Dicer.<sup>11</sup> Transcription of miRNAs occurs through RNA polymerase II and subsequent processing is mediated by the nuclear ribonuclease III (RNase III) enzyme Drosha to form precursor miRNAs (70–100 nucleotides). Following transportation to the cytoplasm by exportin 5, a further cleavage occurs via another RNase III enzyme, Dicer, to form the mature miRNA. miRNAs modulate both physiological and pathological pathways by post-transcriptionally inhibiting the expression of a multitude of target genes (Figure 1). Known as master regulators in

the cell, miRNAs are involved in almost all the cellular processes in both normal and pathological conditions including differentiation, proliferation, and migration. Statistical studies using genome-wide alignments suggest that roughly 60% of all human 3'-UTRs are predicted to be regulated by miRNAs *via* Watson–Crick complementarity.<sup>12</sup> Changes in the miRNA expression level to an abnormal state can cause rapid and adaptive changes in gene expression, which can be the cause of various diseases.<sup>13–16</sup>

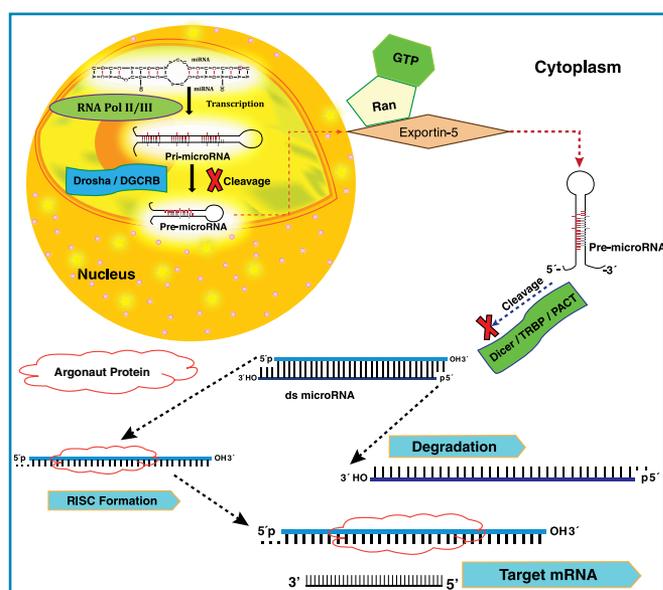
## USE OF miRNA AS BIOMARKERS IN CLINICAL DIAGNOSIS

miRNAs exhibit strict developmental and tissue-specific expression patterns in organ and immune system development. For example, miR-129-5p is involved in mammalian heart development,<sup>17</sup> miR-34c regulates pancreatic insulin secretion,<sup>18</sup> miR-23a/23b are necessary for the proper hematopoietic progenitor cell production and differentiation,<sup>19</sup> and miR-122 can be used as a biomarker for liver toxicity in Zebra fish.<sup>20</sup> These studies highlight the participation of miRNAs in diverse cellular processes. Hence, it is not surprising that dysregulation of miRNA function is associated with many human diseases such as diabetes, neurological disorders, cancer, pulmonary disease and polycystic ovarian syndrome.

## miRNA AS BIOMARKER FOR CANCER

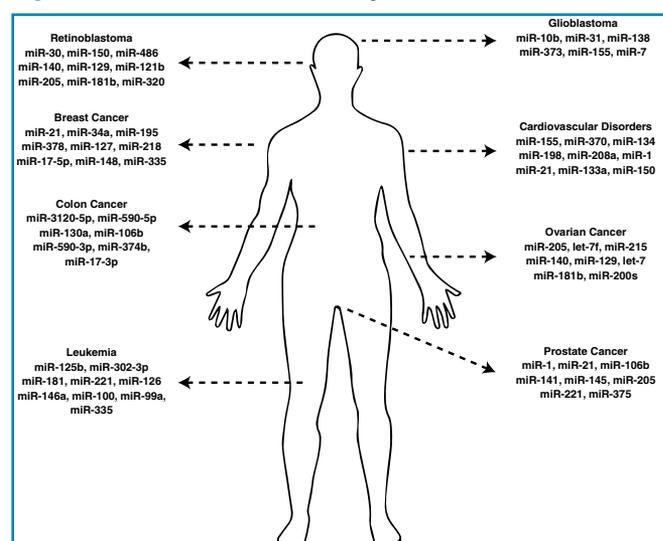
Cancer has been the leading cause of death and a major health problem worldwide for many years; basically, it results from out-of-control cell proliferation. miRNAs play a critical role in the development of cancer and can influence cancer-promoting and cancer-suppressing genes. The first documentation of a miRNA abnormality in cancer stemmed from studies of human chromosome 13q14.<sup>21</sup> Aberrant levels of miRNAs contribute to cancer formation and progression by regulating expression levels of key genes involved in tumorigenesis pathways which are responsible for cell proliferation, tumor migration, invasion, integrin-mediated adhesion and resistance to cancer therapy. In tumorigenesis, over-expression of certain miRNA down-regulates tumor suppressor genes. These miRNAs can be exploited as potential biomarkers due to their tissue specificity and to tumor type and its origin.<sup>22</sup> Similar to protein-coding genes, miRNAs are also subject to epigenetic regulatory modifications in cancer. The majority of miRNA loci are associated with CpG islands that are epigenetically regulated, suggesting a marked dependence on DNA methylation.<sup>23</sup> Notably, the identified cancer-associated miRNAs

**Figure 1:** miRNA Biogenesis



are diverse and specific for different tissues and cancer types, suggesting that they are potential biomarkers for diagnosis and therapeutic targets. Upregulated oncogenic miRNAs (oncomiRs) or downregulated tumor-suppressive miRNAs are the main cause for the failure of balanced expression of miRNA in carcinogenesis.<sup>24,25</sup> These key miRNAs have accelerated the development of several approaches to probing miRNAs and analyzing functions in cell culture and in animal models. miRNAs may act as oncogenes or tumor suppressors in cancer, depending on their target mRNA.<sup>26</sup> miRNAs that are downregulated in cancer normally act as tumor suppressors, whereas upregulated miRNAs usually act as oncogenes.<sup>27</sup> Hence, identifying the specific mRNAs for their miRNAs in cancer initiation and progression is necessary. Due to the regulatory functions of miRNAs in cancer initiation and progression, targeting of miRNAs should be investigated as a novel therapeutic strategy (Figure 2).

**Figure 2:** miRNA, a non-invasive diagnostic marker.



## Glioblastoma

Glioblastoma multiforme (GBM) is the most aggressive type of malignant brain tumor with complex profile and even with the current multimodal therapy, is an invariably lethal cancer with the life expectancy that depends on the tumor subtype but, even in the most favorable cases, rarely exceeds 2 years. The intricacy in determining an explicit biomarker for glioma lies in part with the complex heterogeneous nature of the cancer itself. In comparison to normal brain, deregulation of more than 290 miRNAs has been reported in GBM.<sup>28</sup> miRNA signatures have been recognized in both glioblastoma tissue and in blood circulation of glioblastoma patients. Recently, deep

sequencing method has produced one of the largest sets of miRNA profiles for glioblastoma and control brain tissue. This study identified 33 up-regulated and 40 down-regulated miRNA in the glioblastoma tissue. In addition, 18 novel miRNAs and 16 novel miRNA-3ps were identified (miRNA-3p, miR-3676, miR-204, miR-539, miR-758, miR-382, miR-1271, miR-98, miR-1307, miR-181b1-miR-873, miR-212, miR-135a-2, miR-511-1, miR-301a, miR-381, miR-487a).<sup>29</sup> Recently, Zubeita *et al.*<sup>30</sup> have reported that miRNA-135a regulates overexpression of Na<sup>+</sup>/H<sup>+</sup> Exchanger isoform 9 (NHE9) inhibiting proliferation and migration of GBM cells. miRNAs are potential therapeutic targets for GBM, but there are difficulties associated with their delivery to the targeted cells. Sharif *et al.*<sup>31</sup> have suggested that use of exogenous miRNA (miR-124) delivery with the exosomes derived from Wharton's jelly-mesenchymal stem cells decreases cell proliferation and provides a novel approach for miRNA replacement therapy in GBM. miRNAs regulates multiple genes signifying more prognostically powerful regulator than a single gene. A 4-signature miRNA (hsa-miR-107\_st, hsa-miR-548x\_st, hsa-miR-3125\_st and hsa-miR-331-3p\_st) was identified as promising biomarkers for glioblastoma stratifying patients into short and long-term survivors.<sup>32</sup>

## Ovarian Cancer

Ovarian cancer (OC) is the most common gynecological malignancy with poor overall survival.<sup>33</sup> Revealing novel mechanisms for the initiation and progression of OC are critical to developing new and improved diagnostics and therapeutics. The role of miRNAs in the initiation, growth and progress has already been identified.<sup>34,35</sup> miR-363, a cancer-related miRNA, plays an oncogenic as well as tumor suppressor role in many cancers. miR-363 has been found to play a tumor-suppressor role in OC by inhibiting NIN1/RPN12 binding protein 1 homolog (NOB1).<sup>36</sup> Chen *et al.*<sup>37</sup> have reported that epigenetic silencing of miR-193a-3p by DNA hypermethylation in ovarian cancer progression, may explore as a promising miRNA replacement therapy. miR-18b, an oncogene in many tumors was reported to be upregulated in OC metastasis by targeting PTEN and inhibiting miR-18b. It may be a novel diagnostic and therapeutic measure for OC.<sup>38</sup>

## Retinoblastoma

Retinoblastoma (RB), the most common primary malignancy in the retina, usually occurring in the childhood, and accounts for 2-4% of all childhood malignancies.<sup>39</sup> Emerging evidence supports a major

role of miRNAs in regulating the initiation and progression of RB.<sup>40</sup> Aberrant expression of miR-29a has been reported in many cancers, whereas, Liu *et al.*<sup>41</sup> has reported that miR-29a possess tumor suppressor effect by modulating STAT3, thereby suggesting a potent biomarker for RB. miR-320, which is believed to be associated with pathogenesis was found to regulate the development of autophagy by targeting HIF-1 $\alpha$  and autophagy-related proteins in RB under hypoxic conditions.<sup>42</sup> miRNAs have also played a major role in identifying the target gene specific for RB such as Runx3, a tumor suppressor gene that is upregulated after miR-106b suppression, suggesting that Runx3 is the target for miR-106b.<sup>43</sup> Further, miR-382 targets brain-derived neurotrophic factor (BDNF)-mediated PI3k/AKT signaling pathway.<sup>44</sup> Interestingly, Magdonel *et al.*<sup>45</sup> has elucidated miRNA landscape analysis in RB tumor samples, suggesting that all RB tumor samples possess a collective profile of miRNA expression, inspite of their tumor heterogeneity, among which miR-3613 was found to exhibit a novel therapeutic target for RB.

### miRNA AS BIOMARKER FOR DIABETES MELLITUS

Diabetes mellitus (DM), a common metabolic disorder, globally affects more than 400 million people. Currently available treatments permit to manage the disease but, in the long term, many patients develop severe micro- and macrovascular complications that decrease life quality and expectancy. Better therapeutic tools to prevent and treat diabetes are therefore urgently needed. Growing evidence indicates that miRNAs are involved in Type 2 DM.<sup>46</sup> However, the specific role of miRNAs in T2DM has yet to be elucidated. Previous studies have demonstrated that miR-34a and miR-125b were upregulated in peripheral blood mononuclear cells from patients with T2DM, suggesting as a biomarker for T2DM.<sup>47</sup> Further, loss of pancreatic beta cells is found to be involved in the pathogenesis of gestational DM, where miR-503 was found to regulate the functions of pancreatic  $\beta$ -cells by targeting the mTOR pathway, suggesting that targeting miR-503/mTOR axis could serve as a novel therapeutic target for gestational DM.<sup>48</sup> Diabetes is generally associated with accelerated arterial intimal thickening that contributes to the increased cardiovascular disease. Recently, Lightell *et al.*<sup>49</sup> suggested that diabetes is accompanied by increased arterial miR-221 and -222 expression that promotes intimal thickening. In Type 1 DM, the role of miRs in IL-1 $\beta$  and TNF inflammatory cytokines were reported to induce miR-21-5p, miR-30b-3p, miR-34, miR-101a and miR-146a-5p expressions in MIN6

cells and human pancreatic islets.<sup>50</sup> Latrielle *et al.*<sup>51</sup> have reported that mir-375 gene dosage in pancreatic beta cells leads to the regulation of beta-cell mass and biomarker development for T1DM. Few miRNAs are characterized based on their differential expression in insulin targeted DM patients. Some of the insulin sensitivity related miRNA in adipocytes (miR-377, miR-27a, miR-96),<sup>52-54</sup> muscle (miR-21, miR-29),<sup>55,56</sup> and liver (miR-203a-3p, miR-150, miR-21, miR-338-3p)<sup>57-60</sup> were essential in maintaining physiological homeostasis and energy balance.

### miRNA AS BIOMARKER FOR NEURODEGENERATIVE DISEASE

Neurodegenerative diseases include several central nervous system disorders characterized by the progressive loss of neural tissues and CNS damage. Hence, early diagnosis is essential to maximize the effectiveness of disease-modifying therapies. In recent years, much effort has been taken to recognize the neuropathological, biochemical, and genetic biomarkers of the diseases so that the diagnosis could be established in the earlier stages. The biomarkers for Alzheimer's, Parkinson disease and other neurodegenerative diseases must be reliable and specific, and they should be useful in guiding us to make more accurate diagnosis and better treatment of the diseases.

#### Alzheimer's disease

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by progressive memory loss and increasing dysfunction in mental behavior. AD presents an increasing clinical challenge in terms of diagnosis and treatment.<sup>61</sup> Neuropathological features of AD includes extracellular amyloid plaques consisting of deposits of beta-amyloid (A $\beta$ ), intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein, and neuronal cell loss.<sup>62</sup> The  $\beta$ -site APP-cleaving enzyme 1 (BACE1) is validated as Alzheimer's  $\beta$ -secretase and a therapeutic target for AD. Recently, several miRNAs have been reported to the pathogenesis of AD by modulating the function of AD-relevant molecules.<sup>63,64</sup> Gong *et al.*<sup>65</sup> have recently reported that microRNA-15 (miR-15 family) which includes miR-15a, 15b and 192 plays a major role in the pathogenesis of AD. Among them mir-15b was demonstrated as one of the major biomarker for the cellular AD phenotype that might be involved in AD. Rianchio *et al.*<sup>66</sup> have reported that exosomes act as nanovesicles that transport miRNAs and is found to be increased in AD.

MiR-9-5p and miR-598 were found to be significantly elevated in exosome enriched cerebrospinal fluid of AD patients, suggesting that these two miRNAs can be used as a biomarker for the prediction of AD. Further, Tang *et al.*<sup>67</sup> have demonstrated that miR-139 inversely modulated the responses to pro-inflammatory stimuli, thereby exerting a pathogenic effect in AD. Profile of 6 miRNA in blood plasma has been reported by Nagaraj *et al.*<sup>68</sup> to identify the molecular signature characteristics for the early AD stage patients with mild cognitive impairment. The 6 miRNA signatures for AD are hsa-miR-200a-3p, hsa-let-7f-5p, hsa-miR-146a-5p, hsa-miR-339-5p, hsa-miR-339-3p, hsa-miR-27b-3p. These miRNAs are found to be most promising biomarker candidates for differentiating early stages of AD with that of control. In addition, has-miR-501-3p was found to be overexpressed in cultured cells, which mimicked upregulation in AD brains, thereby downregulating 128 genes that overrepresented in gene ontology. This suggest that has-miR-501-3p can be used as a novel serum biomarker that corresponds to pathological events occurring in AD brains.<sup>69</sup>

### Schizophrenia

Schizophrenia (SZ), a debilitating mental disorder with an overall prevalence estimate of around 4.0 per 1,000 people, is characterized by early adulthood onset and complex clinical symptoms. A substantial number of studies have revealed altered gene/miRNA expression in different biological samples from patients with schizophrenia, leading to an extensive search miRNA fingerprints as biomarkers for SZ diagnosis and treatment monitoring.<sup>70-73</sup> Blood derived miRNA from peripheral blood mononuclear cells (miR-181b-5p, miR-21-5p, miR-195-5p, miR-137, miR-346 and miR-34a-5p) have been demonstrated to have high diagnostic sensitivity and specificity as a promising biomarker for SZ.<sup>74</sup> Few studies have reported that transcription factors miRNA such as TF-miR-30 targets early growth response protein -1 (EGR-1), thereby considering as a biomarker for diagnosis and treatment monitoring in SZ patients.<sup>75</sup> A hemizygous deletion of a 1.5–3-Mb region of chromosome 22 can lead to the 22q11 deletion syndrome (22q11DS), which is characterized by multiple physical and psychiatric abnormalities. A previous study determined that ~30% of 22q11DS patients may develop schizophrenia.<sup>76</sup> miR-25 and miR-185 are regulators of the sarco/endoplasmic reticulum Ca<sup>2+</sup> ATPase (SERCA2), which are responsible for loading Ca<sup>2+</sup> into the endoplasmic reticulum. Earls *et al.*, reported that miR-25 and

miR-185 were found to be depleted in mouse models of 22q11DS, while their restoration to presynaptic neurons saved the long-term potentiation of this mouse model, suggesting that miRNA-dependent SERCA2 dysregulation possess a pathogenic event in 22q11DS and schizophrenia.<sup>77</sup>

### miRNA AS BIOMARKER FOR CARDIOVASCULAR DISEASE

Cardiac fibroblast activation and transdifferentiation occurs due to pressure overload, thereby leading to an increase in the formation of interstitial fibrosis formation. This further leads to myocardial stiffness, diastolic and systolic dysfunction, and eventually heart failure. Recently, miR-221/222 family was found to be downregulated in heart failure patients, thereby enabling a profibrotic signaling in the pressure overloaded heart counteracts myocardial fibrosis.<sup>78</sup> Further, Yang *et al.*<sup>79</sup> have reported that miR-200a-5p was found to regulate myocardial necroptosis by selenium deficiency targeting ring finger protein 11 (RNF-11). Myocardial miR-30e-3p was found to be down regulated after coronary microembolization, an important cause for the loss of myocardial reperfusion therapy, and is accompanied by inhibiting autophagy and decreasing cardiac function. This study revealed that miR-30e-3p might be involved in CME-induced cardiac dysfunction by regulating myocardial autophagy.<sup>80</sup> In an earlier study, Sullivan *et al.*<sup>81</sup> suggested that plasma miR-499a-5p levels serve as a complementary biomarker for the occurrence of coronary syndromes. Recently, Hoekstra *et al.*<sup>82</sup> reported that miR-499a-5p exhibited a protective role in cardiomyocytes suggesting a new way to treat patients who are at risk of developing acute cardiovascular syndromes. Zampetaki *et al.*<sup>83</sup> reported three signature miRNAs (miR-126, miR-197 and miR-223) for prediction of myocardial infarction (MI), where miR-126 levels are found to be positive, while the other miRs are inversely associated with future MI.

### miRNA AS BIOMARKER FOR POLYCYSTIC OVARIAN SYNDROME

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease, which is characterized by hyperandrogenism (HA), chronic anovulation, polycystic ovaries, insulin resistance and obesity. PCOS is a genetic disorder with multifactorial essence and has a strong association with environmental impacts, thereby rendering a noteworthy importance to miRNA. In this regard, Naji *et al.*<sup>84</sup> has found

that intermediary position of miRNA (miR-93 and miR-21) in granulosa cells and follicular fluid plays an important role as androgenic responsive factors for the pathogenesis of PCOS in HA condition. miRNAs are found to be involved in the regulation of different pathways, biological functions and cellular components underlying the factors of PCOS. In a recent report by Xue *et al.*<sup>85</sup> 31770 genes were found to be targets for 263 miRNAs that are differentially expressed in PCOS. miR-27a-3p was found to significantly affect estradiol and androgen imbalance by targeting the specific gene of cyclic AMP response element (CRE)-binding protein 1 (Creb1) in granulosa cells of mouse PCOS model.<sup>86</sup> Deregulated expression of miRNA are found to play a significant role in PCOS, where dysregulation of miR-145 and miR-182 in granulosa-lutein cells are reported to be involved in pathogenesis, whereas differential up-regulation of miR-182 in follicular fluid exhibits a promising predictive role in discriminating PCOS from healthy.<sup>87</sup> Recently, Zhang *et al.*<sup>88</sup> has demonstrated that deficiency of miR-20a impairs steroidogenesis in cumulus granulosa cells by the deregulation of osteogenic transcription factor RUNX2, suggesting their influence for the treatment of PCOS.

## miRNA AS BIOMARKER FOR PULMONARY DISEASE

In the past few years, increasing number of reports have suggested that miRNAs plays a critical role in the pathogenesis of pulmonary disease such as pulmonary fibrosis, pulmonary arterial hypertension etc. Recently, Pires *et al.*<sup>89</sup> has reported that modulation of miR-106b-5p may serve as potent target for host-directed therapy for *M.tuberculosis* infection. Interestingly, a group of researchers have identified a miRNA signature of cigarette smoking and its associated clinical phenotypes, gene expression and inflammatory signaling, thereby exploring their diagnostic and therapeutic use.<sup>90</sup> Baptista *et al.*<sup>91</sup> have investigated that miR-424 322 has a diagnostic and prognostic value in pulmonary hypertension patients. In addition, they have also reported that this particular miRNA targets proteins that rely on heart function, thereby linking this miRNA as a messenger for pulmonary vascular disease and right ventricle hypertrophy. Extensive research has been performed on *in vitro* and *in vivo* models to reveal the role of miRNAs in modulation of hypoxia-induced pulmonary hypertension (HPH). With this regard, very recently, Blissenbach *et al.*<sup>92</sup> has identified that plasma miR-17 and miR-190 are found to epigenetically modulate hypoxia-induced increase in pulmonary artery pressures, thereby suggesting their diagnostic role.

## CONCLUSION

Taking into account of the use of miRNA as biomarkers for clinical diagnostics and therapeutic approaches outlined in this review will allow for more precise understanding for their potential use in clinical applications. Further elucidation of miRNA biogenesis and functionality will enable the development of more specific and sensitive assays. Enhancing the art of performing research and implying its application in clinical set-up will lead to exciting novel gene regulators. Also, their specific functions will augment the opportunities to safely pursue them as therapeutic modalities.

## CONFLICTS OF INTEREST

None.

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