MicroRNAs: potential biomarkers for disease diagnosis

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Abstract. MicroRNAs (miRNAs) are a group of endogenous noncoding small RNAs characterized by high conservation; furthermore, various studies have shown the capability of miRNAs to impact diseases. For example, a study shows that cell-free miRNAs are stable in bodily fluids, which gives circulating miRNAs the ability to be potential biomarkers for noninvasive diagnosis. Additionally, accumulating studies have supported that miRNAs can function as suppressor genes, again demonstrating their effect on disease. This review introduces this particular role of miRNAs as well as analyzes the prospect of miRNAs as biomarkers and the capacity for using miRNA-based resources to benefit mankind.

Keywords: MicroRNA, biomarker, disease diagnosis

1. Introduction

Many early diagnostic measures have been developed and are being increasingly used, such as endoscopic examinations and stool- and blood- based tests. Blood-based tests are becoming increasingly desirable because they are minimally invasive as well as have high levels of adherence in population-based screening. An amount of blood markers has been proposed and evaluated, including cytological, protein, mRNA, and DNA markers [1,2], but the diagnostic performance has mostly been insufficient as a primary tool. The miRNAs are a group of evolutionarily conserved, small, noncoding RNAs of 21 to 25nt and often negatively regulate gene expression at the post-transcriptional level by incomplete or complete complementary binding to target sequences with the 3' untranslated region of mRNAs [3]. Furthermore, there is increasing evidence that implicates miRNAs’ involvement in a variety of biological processes, such as cell proliferation, differentiation, apoptosis, and tumorigenesis [4]. Accordingly, aberrant and/or absent expression of miRNAs is usually linked with pathophysiological disorders [5–7].
2. Biological functions of miRNAs

It has been proven that miRNAs are in plants, green algae, viruses, animals, and humans [8]. As lin-4 and let-7 have been found to play important roles in the timing of *C. elegans*’ larval development, much research has supported miRNAs as being associated with the other biological processes. With more than half of mammalian messages under selective pressure to maintain the pairing to miRNAs [9], it may prove, at least to some degree that miRNAs have influenced all the biological functions or processes in some cell types.

miRNAs are produced from the genomic DNAs that are transcribed by Pol II in the same way as mRNAs. Any hairpin secondary structures present in the RNAs are recognized and cleaved sequentially by the Drosha and Dicer enzymes. The mature miRNAs are the duplex of two RNA strands, on which there are approximately 22 nucleotides in length and two nucleotide 3’ overhangs on each strand [10,11]. The target genes can be recognized by miRNAs with paring to the mRNAs of protein coding genes. Then, miRNAs direct their post-transcriptional repression via the degradation of mRNAs, the inhibition of protein translation, or a combination of the two [12,13]. One miRNA may modulate the expression of various proteins [10,14].

Currently, there are 24,521 entries representing hairpin precursor miRNAs expressing 30,424 mature miRNA sequences in 206 species listed in the miRNA registry (Sanger miRBase release 20; http://www.mirbase.org/, June 2014). The registry shows that 2,652 mature human miRNAs are associated with lots of cellular pathophysiological pathways and are capable of significantly influencing the pathogenesis of some diseases. Table 1 shows some circulating miRNAs that are involved in human diseases.

**Table 1**

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Location on Chromosome</th>
<th>Target genes proved</th>
<th>Number of researches</th>
<th>Tendency of expression level</th>
<th>Samples sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-18a</td>
<td>13q31.3</td>
<td>BASP1</td>
<td>1 [15]</td>
<td>Up in pancreatic cancer</td>
<td>76</td>
</tr>
<tr>
<td>miR-20a</td>
<td>13q31.3</td>
<td>SARA1</td>
<td>2 [16]</td>
<td>Up in multiple myeloma</td>
<td>40</td>
</tr>
<tr>
<td>miR-21</td>
<td>17q23.2</td>
<td>PDCD4, PTEN, SPRY1, SPRY2, TPM1</td>
<td>7 [15,17-22]</td>
<td>Up in HCC</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in ESCC</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in NSCLC</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in prostate cancer</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in pancreatic cancer</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in GC</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in pancreatic cancer</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in CRC</td>
<td>159</td>
</tr>
<tr>
<td>miR-29a</td>
<td>7q32.3</td>
<td>MAP4K4</td>
<td>2 [23–25]</td>
<td>Up in CRC</td>
<td>159</td>
</tr>
</tbody>
</table>
### miRNAs, Location on Chromosome, Target genes proved, Number of researches, Tendency of expression level, Samples sizes

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Location on Chromosome</th>
<th>Target genes proved</th>
<th>Number of researches</th>
<th>Tendency of expression level</th>
<th>Samples sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-92a</td>
<td>13q31.3</td>
<td>ERJ1, MUC16</td>
<td>141 [24,26–31]</td>
<td>Up in CRC</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in CRC</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Down in HCC</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Down in acute leukemia</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Down in CRC</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Down in CAD</td>
<td>53</td>
</tr>
<tr>
<td>miR-106b</td>
<td>7q22.1</td>
<td>F3, IL-8, CTGF, PAI-1</td>
<td>2 [15]</td>
<td>Up in GC</td>
<td>96</td>
</tr>
<tr>
<td>miR-133a</td>
<td>18q11.2</td>
<td>SEC61B, FBN1, TAGLN2, GSTP1, ARPC5, RFT1, C4orf34</td>
<td>3 [28,32]</td>
<td>Up in CAD</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up in AMI</td>
<td>63</td>
</tr>
<tr>
<td>miR-143</td>
<td>5q32</td>
<td>ERK5, HNRPA2B</td>
<td>2 [33]</td>
<td>Up in enterovirus infection</td>
<td>40</td>
</tr>
<tr>
<td>miR-145</td>
<td>5q32.33</td>
<td>TIRAP</td>
<td>1 [28]</td>
<td>Down in CAD</td>
<td>53</td>
</tr>
<tr>
<td>miR-146a</td>
<td>5q33.3</td>
<td>IRAK1, IRAK2, TRAF6, RIG-1, IRF-5, STAT-1, PTC1, Numb</td>
<td>1 [28]</td>
<td>Up in CAD</td>
<td>70</td>
</tr>
</tbody>
</table>

Abbreviations: CRC, colorectal cancer; HCC, hepatocellular carcinoma; ESCC, oesophageal squamous cell carcinoma; NSCLC, non-small cell lung cancer; GC, gastric cancer; CAD, coronary artery disease; CHC, chronic hepatitis C virus infection; AMI, acute myocardial infarction; PTEN, phosphatase and tensin homolog; PDCD4, programmed cell death 4; TPM1, tropomyosin.

### 3. miRNAs and disease diagnosis

miRNAs modulate gene expression and are linked in the different physiological and pathological processes, including apoptosis, proliferation, development, and metabolism of glucose and lipids. Over time, there have been numerous studies conducted exploring the link between miRNAs’ expression and disease; these studies have continued to be further enhanced and broadened [34–38].

#### 3.1. Cancer

It has been proven that miRNAs are strongly dysregulated in cancer and show potential for cancer diagnosis, prognosis, and treatment [39]. Moreover, with the rapid development of miRNA microarrays, massive profiling studies in cancer patients have recently been made possible.
Circulating miRNAs have been regarded as biomarkers for many cancers, including colorectal cancer, gastric cancer, and acute myeloblastic leukemia.

In addition, it has been demonstrated by abundant experimental evidence that miRNAs play roles as oncogenes or tumor suppressor genes, which suggests they have important effects on the development and progression of cancer. According to the expression profiles of human miRNAs, many miRNAs are expressed differentially in normal tissues and cancers and are deregulated in cancers. Over the past decades, many oncogenes and tumor suppressor genes have been recognized to regulate apoptosis, such as Bcl-2, p-53, and MYC [40–44]. This has improved the understanding of apoptotic signaling pathways and their dysregulation in cancer progression and treatment. Moreover, this class of miRNAs has revealed the apoptotic signaling pathways to be more complex and enigmatic than was previously appreciated [45]. Chim et al. reported that there are circulating miRNAs in serum/plasma, [46,47] and they have found some tumor-derived miRNAs, such as miR-15b, miR-16, miR-17-92, miR-21, miR-24, miR-155, and let-7 miRNA in the plasma and sera of tumor-bearing patients [48,49]. Subsequently, more studies have shown that miRNAs may be used as potential biomarkers for different diseases, especially cancers [26,46,50]. Currently, the discovery and development of non-invasive tools for the diagnosis and management of cancer is a goal of cancer research. The application of miRNAs as the potential biomarkers will greatly decrease the worldwide health burden imposed by cancers [51].

3.2. Cardiovascular disease

Cardiovascular disease is one of the primary reasons for the rising rates of human morbidity and mortality worldwide. It increases the necessity for progressing novel diagnostics and therapies that are designed to detect cardiovascular diseases early. Current studies have shown not only the linkage between dysregulated miRNA expressions and diseased hearts but also the relevance of miRNA in the heart’s growth, development, function, and stress responsiveness. Thus, it is very attractive to exploit miRNAs as diagnostic markers or to manipulate them to obtain therapeutic effects since they have very specific targets in a particular cellular pathway [52].

Willeit’s research showed an important platelet contribution to the circulating miRNA pool and proposed miRNAs were responsive to antiplatelet therapy [53]. They found that the platelet-rich plasma exhibited significantly higher levels of miRNAs than both the serum and platelet-poor plasma. Conversely, the antiplatelet therapy significantly decreased miRNA levels. Willeit also evaluated 92 miRNAs from 377 miRNAs performed in the research by the dose-escalation method among healthy volunteers at 4 different time points. Furthermore, the results were validated by the individual TaqMan quantitative real-time PCR assay. It demonstrated that plasma levels of platelet miRNAs, including miR-223, miR-191, miR-126, and miR-150, were reduced by supplementary platelet inhibition. The work emphasized that antiplatelet therapy and the preparation of blood samples could be confusing factors in the case-control research that link miRNAs to cardiovascular diseases.

3.3. Diabetes

Until now, sensitive methods for the identification of β-cell death and the early detection of diabetes have been absent. Erener et al. [54] studied miR-375 levels, an islet enriched miRNA, by quantitative RT-PCR in order to verify whether miR-375 is a possible biomarker in blood for identifying β-cell death and predicting diabetes in mice. Moreover, they found the levels of circulating miR-375 of the cytokine- and streptozotocin-induced islets were significantly enhanced, whereas the miR-375 levels
were decreased by cell death inhibitors. The results suggested that circulating miR-375 is possibly regarded as the biomarker of β-cell death and a predictor of diabetes.

3.4. Occupational disease

Exposure to asbestos is the predominant cause of pleural mesothelioma (PM). PM is a tumor that is difficult to diagnose. Thirteen novel asbestos-related miRNAs have been revealed (over-expressed: miR-148b, miR-374a, miR-24-1*, Let-7d, Let-7e, miR-199b-5p, miR-331-3p, and miR-96 and under-expressed: miR-939, miR-671-5p, miR-605, miR-1224-5p and miR-202) [55].

Inorganic Arsenic (iAs) is a metalloid widely diffused in all environmental matrices. The International Agency for Research on Cancer classifies iAs as a Group 1 carcinogen. Present studies have evaluated the influence of iAs exposure on the expression of total miRNA in Jurkat cells. Treated cells showed a reproducible increase in the expression levels of three miRNAs: miR-663, miR-222, and miR-638. The study supports the importance of continuing the investigation of the possible application of some miRNAs as biomarkers in environmental and occupational iAs exposure [56].

3.5. Acute rejection of organ transplant

Recently, a few markers have been applicable for the clinical diagnosis of the acute rejection (AR) of an organ transplant. Attempting to confirm whether the plasma miRNAs can be potential biomarkers for AR, Hu et al. used the rat orthotopic liver transplantation model and microarrays [57] to distinguish the variation from the miRNAs fingerprint and levels in plasma and grafts derived from the AR rats and the control, respectively. They found that three plasma miRNAs, miR-122, miR-192, and miR-146a, were notably up-regulated when AR was conducted, and the increase could be inhibited by immunosuppression. They also demonstrated the presence of miR-122 and miR-192 rather than miR-146a in the liver-injury rat model. The succeeding study showed that miR-146a was up-regulated sixfold in microvesicle in AR plasma. However, miR-122 and miR-192 indicated little specific change. The studies provided the total spectrum of plasma miRNAs in AR rats and also suggested that miR-122 and miR-192 were linked with liver injury, as well as miR-146a possibly being related to cellular rejection. Moreover, many current studies have proposed the possibility of miRNA serving as biomarkers for evaluating the renal allograft status for kidney transplantation [58,59].

HDmiRs can be a promising biomarker for evaluating the allograft status after a liver transplant. Recently, HDmiR miR-122 has been found in serum and the miR-122 level increased significantly if the patients suffered hepatocyte injuries by viral, alcoholic, or chemical-related hepatotoxicity [60,61]. Further study showed the levels of both serum and plasma miR-122 correlated closely with the aminotransferases and liver histology of the patients. Furthermore, Waqar et al. demonstrated that the expression of miR-122 and miR-148a in liver tissue changed significantly with prolonged graft warm ischemia times during ischemia/reperfusion injury and acute rejection [62].

3.6. Osteoarthritis

Osteoarthritis (OA) is currently the most prevalent degenerative joint disease, characterized by injury of the articular cartilage due to environmental, mechanical, or genetic factors. The genetics of OA are complicated and are not wholly understood. Recent studies, however, have shown the significance of miRNAs in cartilage functions [63]. Silvia et al. has conducted research on identifying
and characterizing the expression profile of miRNAs in normal and OA chondrocytes. They aimed to analyze and determine the role of the 723 miRNAs in OA, of which seven miRNAs showed a statistically significant differential expression. Moreover, they found that among these seven human miRNAs, hsa-miR-483-5p was upregulated in OA chondrocytes, and the other six miRNAs, including hsa-miR-149*, hsa-miR-582-3p, hsa-miR-1227, hsa-miR-634, hsa-miR-576-5p, and hsa-miR-641 were upregulated in normal chondrocytes. The profiling results were affirmed by the detection of the specific miRNAs by qPCR. The possible role of the detected miRNAs in OA pathology was revealed and supported by the potential miRNA target predictions and the signaling cascades altered by the differentially expressed miRNAs.

Recently, Jones et al. found that miR-149* was down-regulated in OA chondrocyte micropellets [64]. The expression profiles of 157 human miRNAs have been studied and identified. Of those studied, 17 miRNAs were shown to be differentially expressed in human OA in comparison to normal cartilage. In the study, hsa-miR-140 showed a tendency to be downregulated in OA, and hsa-miR-146 was upregulated in this pathology. These findings stood in opposition to previous reports’ results [65,66].

4. Conclusion

Over time, as shown in Table 1 and Table 2, there have been numerous studies conducted exploring the link between miRNAs’ expression and disease; these studies have continued to be further enhanced and broadened.

We should pay more attention to identifying the classifications and levels of miRNAs characterized in both low and high risk populations, and we should develop more capable and regulatory methods and databases for screening the useful miRNAs markers for early disease diagnosis, prognosis, prevention, and prediction of therapeutic responses to diseases from the wealth of information available from miRNAs (see in Figure 1).

Table 2

<table>
<thead>
<tr>
<th>Diseases</th>
<th>the correlated miRNAs proven (†', upregulate; ‡', downregulate)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cancers</td>
<td>miR-15b(†), miR-16(†), miR-17-92(†), miR-21(†), miR-24(†), miR-155(†), let-7 miRNA(‡)</td>
<td>[47-51]</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>miR-223(†), miR-191(†), miR-126(†), miR-150(†)</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Diabetes</td>
<td>miR-375(‡)</td>
<td>[54]</td>
</tr>
<tr>
<td>Occupational</td>
<td>miR-148b(†), miR-374a(†), miR-24-1(†), Let-7d(†), miR-199b-5p(†), miR-331-3p(†), miR-96(†), miR-939(‡), miR-671-5p(‡), miR-605(‡), miR-1224-5p(‡), miR-202(‡)</td>
<td>[55]</td>
</tr>
<tr>
<td>Disease: Asbestos-related PM</td>
<td>miR-663(†), miR-222(†), miR-638(‡)</td>
<td>[56]</td>
</tr>
<tr>
<td>Exposed to Inorganic Arsenic</td>
<td>miR-122(†), miR-192(†), miR-146a(†), miR148a(†)</td>
<td>[57-62]</td>
</tr>
<tr>
<td>Acute Rejection of Organ Transplant</td>
<td>hsa-miR-483-5p(†), miR-149(‡)</td>
<td>[63,64]</td>
</tr>
</tbody>
</table>
Fig. 1. The general strategy of research conducted on miRNAs biomarkers.

References


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