Review Article
Circulating microRNAs as diagnostic and prognostic biomarkers for gastric cancer-opportunities and challenges

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Abstract: Gastric cancer is the fourth most common malignant tumor in the world. Despite tremendous efforts invested in developing targeted therapies, advanced-stage gastric cancer remains one of the most deadly diseases being the second leading cause of cancer-related deaths worldwide. While the 5-year survival for early-stage gastric cancer is up to 90%, the median overall 5-year survival for advanced stages of gastric cancer is less than one year, underscoring the urgent need of developing novel and affordable biomarkers for early detection of gastric cancer. MicroRNAs (miRNAs) are a large class of small regulatory noncoding RNAs and are found to be critically involved in almost all aspects of cancer. Studies have shown that miRNAs exist in blood circulation with unusually high stability and that the levels of circulating miRNAs are altered significantly during cancer development and progression. With rapid advancement of technologies in miRNA profiling and direct sequencing, circulating miRNAs are being actively explored as an emerging novel class of biomarkers for cancer diagnosis and prognosis prediction. During past four years, an increasing number of studies showed the presence and alterations of circulating miRNAs in gastric cancer patients. However, despite exciting findings have been made recently in the field of circulating miRNA studies, these findings have not been translated into routine clinical practices to help cancer patients. Here, we briefly summarize and discuss the opportunities and challenges for using circulating miRNAs as potential diagnostic and prognostic biomarkers for gastric cancer.

Keywords: Gastric cancer, noncoding RNA, microRNA, circulating microRNA, biomarker

Introduction

About 1 million new cases of gastric cancer are diagnosed and 700,000 people die from gastric cancer each year worldwide, making gastric cancer the fourth most common malignant tumor and the second leading cause of cancer-related deaths in the world [1-3]. Early-stage gastric cancers are usually asymptomatic or exhibit non-specific symptoms, while the majority of gastric cancers are diagnosed at advanced disease stages with the 5-year survival being less than 30% and the median overall survival being less than one year [4, 5]. Despite the tremendous efforts in developing targeted therapies for gastric cancer, no significant improvements have been achieved in the outcomes of patients with advanced disease stages with the exception of Trastuzumab treatment for Her2 overexpression gastric cancer, which represents only about 20% of all gastric tumors [6-8]. The high prevalence and poor clinical outcomes of gastric cancer underscore the needs for developing new biomarkers for early diagnosis. During the past 4 years, a novel class of potential biomarkers for gastric cancer being actively explored is circulating microRNAs (miRNAs).

MiRNAs are a big class of small noncoding RNAs (ncRNAs), which refer to RNA molecules that do not possess a clearly defined open reading frame and thus are not translated into proteins. The majority of miRNA genes are transcribed by RNA polymerase II producing primary miRNAs, which undergo two processing steps performed by two type III ribonucleases to generate mature miRNAs. Mature miRNAs are ~22 nucleotide long and are able to negatively regu-
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late protein-coding gene expression post-transcriptionally through interacting with 3’-untranslated region (3’UTR) of messenger RNAs (mRNAs), causing mRNA degradation or translation inhibition [9-11]. Studies have shown that miRNAs are major cellular regulators of gene expression, and it has been estimated that about two thirds of human protein-coding genes can be regulated by miRNAs [12]. It is now well accepted that miRNAs are critically involved in almost all aspects of cancer [13-15]. A large number of studies have reported dysregulation of miRNAs in gastric cancer [16-20]. In this short review, we will briefly summarize and discuss the opportunities and challenges for using circulating miRNAs as potential diagnostic and prognostic biomarkers for gastric cancer.

Circulating miRNAs

Cellular miRNAs can be packaged into exosomes or microvesicles and then excluded from cells entering blood circulation. The presence of miRNAs in circulation was first reported by Chim et al. and Lawrie et al. in 2008 [21, 22], respectively. Shortly after these two initial publications, three other groups reported and characterized miRNAs in sera and other body fluids [23-25]. The findings from these pioneer studies showed that (i) miRNAs are present in serum, plasma and other body fluids; (ii) body fluid miRNAs are not associated with cells and are extremely stable even under various harsh conditions; (iii) miRNAs in plasma or serum are associated with cancer, diabetes or pregnancy stages. These distinctive features of circulating miRNAs make them ideal candidates serving as diagnostic and/or prognostic biomarkers for various diseases, particularly cancer. Since then, extensive studies have compared circulating miRNAs between various cancer patients and healthy populations [26-30].

Circulating miRNAs as potential diagnostic biomarkers for gastric cancer

Tsujiiura et al. first reported alterations of circulating miRNA levels in patients with gastric cancer in 2010 [31], which was followed by a good number of studies showing differential levels of circulating miRNAs in various types of and stages of gastric cancer [32-47]. Dysregulated circulating miRNAs reported from recent studies for gastric cancer are summarized and presented in Table 1. More than 30 miRNAs were reported to be significantly increased or decreased in gastric cancer patients’ blood, serum or plasma samples. However, the reported changes of circulating miRNAs were mostly sporadic with very low consensus among different studies. Very few circulating miRNAs were reported to be significantly changed in three or more than three studies. Among these miRNAs are miR-21 and miR-106b (Table 1). The reported alterations of the rest circulating miRNAs

### Table 1. Dysregulated circulating miRNAs reported in gastric cancer patients

<table>
<thead>
<tr>
<th>Up-regulated miRNAs (miR-)</th>
<th>Down-regulated miRNAs (miR-)</th>
<th>miRNA detecting methods</th>
<th>Samples &amp; sample sizes (gastric cancer patients/healthy controls)</th>
<th>References</th>
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<tr>
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<td>Plasma (69/30)</td>
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<tr>
<td>17, 106a</td>
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<td>Q-PCR</td>
<td>Whole blood (90/27)</td>
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<td>Solexa sequencing/Q-PCR</td>
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<td></td>
<td>Q-PCR</td>
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<td>187*, 371-5p, 378</td>
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<td>Array/Q-PCR</td>
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<tr>
<td>370</td>
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<td>Q-PCR</td>
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<td>27a, 27b, 191, 221, 222, 376c, 744, let-7e</td>
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<td>Array/Q-PCR</td>
<td>Serum (68/68)</td>
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<td>Q-PCR</td>
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<td>375</td>
<td>Array/Q-PCR</td>
<td>Serum (42)</td>
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<td>Q-PCR</td>
<td>Whole blood (40/17)</td>
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<td>20a, 106b, 221</td>
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<td>Q-PCR</td>
<td>Plasma (90/90)</td>
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<td>195-5p</td>
<td>Q-PCR</td>
<td>Plasma (20/190)</td>
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<tr>
<td>106b, 223</td>
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<td>Q-PCR</td>
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<td>199a-3p</td>
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<td>Q-PCR</td>
<td>Plasma (80/70)</td>
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</table>
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were only observed in one or two studies (Table 1). Zhu et al. recently performed a systemic review and meta-analysis on published studies comparing circulating miRNAs levels in gastric cancer patients before and after operation: the levels of nine miRNAs were found to be significantly reduced after operation [48]. However, only one miRNA (miR-21) was reported to be reduced similarly in two studies, the reduction of other eight miRNAs was observed only in one study [48]. Taken together, Zhu et al. concluded that the systematic review on published circulating miRNA studies indicated that up-regulated miR-21 in circulation may serve as a potential novel biomarker for detection of gastric cancer [48]. However, most of the studies did not show at what gastric cancer stages that increased circulating miRNAs were detected.

Circulating miRNAs as potential prognostic biomarkers for gastric cancer

The potential value of circulating miRNAs for predicting gastric cancer prognosis has also been explored [49-51]. The levels of an increasing number of circulating miRNAs were reported to be associated with a better or worse prognosis of gastric cancer. However, inconsistent or contradictory findings exist across different studies. For example, Wang et al. found that plasma levels of miR-17-5p/20a significantly correlated with poor overall gastric cancer survival [52]. However, Komatsu et al. reported that plasma levels of miR-21 but not those of miR-17-5p and miR-106b were significantly correlated with postoperative survival of gastric cancer patients [53]. The findings from Song et al. showed that while high serum levels of miR-21 were associated with an increased gastric tumor size, no significant differences of patient survival were found between the groups having high and low serum levels of miR-21 [54]. Similar inconsistent observations were also reported for the members of miR-200 family, a widely studied family of miRNAs playing important roles in cancer cell invasion and tumor metastasis [55, 56]. For example, Valladares-Ayerbes et al. found that while the blood levels of miR-200 family were significantly higher in gastric patients than healthy controls, the levels of miR-200 family were not significantly different between the paired non-tumor mucosa and cancer tissues [40]. Moreover, no significant differences of miR-200 family levels were observed between diffuse and intestinal gastric tumor tissues. Combining multivariate analyses with Kaplan-Meier and Breslow-Wilcoxon tests, Valladares-Ayerbes et al. found that high blood levels of miR-200c in gastric cancer patients were significantly associated with poor progression-free and overall survivals [40]. However, a very recent study by Song et al. showed that the miR-200c level in gastric tumors is not associated with gastric cancer prognosis [57]. In contrast, Song et al. found that the levels of miR-200a/b in gastric tumors were significantly associated with good prognosis in female patients [57].

Challenges and perspectives for circulating miRNAs as clinical useful diagnostic and prognostic biomarkers for gastric cancer

While many studies showed different levels of circulating miRNAs between gastric cancer patients and healthy control populations, these findings have not been translated into routine clinical practices to help patients with gastric cancer. This could be in part due to the fact that the reported alterations of the majority of circulating miRNAs are either sporadic or contradictory in their roles linked with gastric cancer across different studies. The potential value of circulating miRNAs for serving as diagnostic or prognostic biomarkers for gastric cancer thus remains to be questionable.

The observed inconsistence of circulating miRNA alterations in gastric cancer patients could be attributed by multiple factors. First, the patient sample size and the number of miRNAs investigated in many studies were small and had less statistical power; their findings may not be well representative. Second, different sample processing and analytic methods were used in different studies. Currently, plasma, serum and whole blood samples have been used because there is no consensus about which kind of samples is preferable for circulating miRNA analysis. No standardized procedures are available for sample processing and RNA extraction. Moreover, it was recently found that the majority of reported circulating miRNAs in cancer patients are also highly expressed in one or more types of blood cells; and disturbances in blood cell counts and hemolysis can drastically change the levels of plasma miRNAs [58]. Therefore, different kinds of samples and
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methods used for processing the samples may have a significant impact on reported circulating miRNA levels. Third, different methods have been used for normalizing the measurements of circulating miRNAs. The most widely normalization methods such as normalization against a house-keeping noncoding small nuclear RNA U6 for tissue miRNA measurement is not suitable for normalization in circulating miRNA analysis, as no house keeping ncRNAs are available in circulation for this approach of normalization. Different normalization methods may lead to identification of different circulating miRNAs whose expression levels are significantly altered in cancer patients. And fourth, circulating miRNA levels can also be changed under different physiological conditions and by other diseases in addition to cancer. Therefore, critical evaluation and recording of certain confounding factors during sample collection and processing is essential for reducing variations of detected circulating miRNA levels from different studies.

Although there are some improvements in the outcomes of patients with gastric cancer, advanced-stage gastric cancers remain to be one of the most deadly diseases. Therefore, early diagnosis is the key to remarkably increase gastric patient survival. Because of their unusually high stability, easy and minimally invasive access and relatively simple but accurate analytic technology available, circulating miRNAs represent one of the best biomarkers to be developed for early detection of and prediction of prognosis of gastric cancer. Future studies with well-designed larger sample sizes and more standardized and exhaustive analytic approaches, such as the newest version of miRNA microarrays and direct sequencing, may generate more reproducible findings for using circulating miRNAs as reliable biomarkers for early detection and prediction of prognosis of gastric cancer.

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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References

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[39] Song MY, Pan KF, Su HJ, Zhang L, Ma JL, Li JY, Yuasa Y, Kang D, Kim YS and You WC. Identification of serum microRNAs as novel non-inva-
miRNAs as biomarkers for gastric cancer


