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REVIEW

MicroRNAs as potential biomarkers for diagnosis, therapy and prognosis of gastric cancer

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Abstract: Despite the widespread use of endoscopy and conventional tumor biomarkers, gastric cancer (GC) remains one of the most frequent causes of cancer-related deaths worldwide due to its late diagnosis and poor response to treatment. Valuable and practical biomarkers are urgently needed to screen patients with a high risk of GC that can complement endoscopic diagnosis. Such biomarkers will enable the efficient prediction of therapeutic response and prognosis of GC patients and favor the establishment of an effective treatment strategy for each and every patient. MicroRNAs (miRNAs) are a class of small non-coding RNA sequences that play important roles in modulating key biological processes by regulating the expression of target genes. Expectedly, miRNAs are abnormally expressed within the tumor tissue and in associated biological fluids of GC patients including their blood, gastric juice, and urine. Accumulating evidence indicates that miRNAs are potential biomarkers with multiple diagnostic functions for GC. Here, we review recent advances and challenges in using miRNAs, particularly biofluid miRNAs, as GC biomarkers with potential clinical applications including diagnosing, clinically staging, and predicting malignant behaviors, therapy response, recurrence after surgery and survival time.

Keywords: gastric cancer, microRNA, biomarker, diagnosis, prognosis

Introduction

Gastric cancer (GC) is one of the most frequent causes of death in cancer patients and a major public health problem worldwide.[1] Nearly 95% of GCs are adenocarcinomas which are classified into three histological types: diffuse, intestinal, and hybrid gastric cancers according to the Lauren system,[2] and cardia and non-cardia gastric cancers according to the World Health Organization.[3] Although surgery, chemotherapy, and other therapies continuously progress in the management of GC patients, their prognosis remains poor.[4] An early and precise diagnosis is essential for improving the survival rate of patients with GC.

Endoscopy followed by biopsy is a common tool for detecting GC and remains the most accurate method for GC diagnosis.[5] However, this procedure is invasive and expensive, and it may cause the patients discomfort and fear. In addition, asymptomatic patients generally do not receive an endoscopic examination.[6] Conventional serum tumor biomarkers, such as carcino-embryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 72-4 (CA72-4), and carbohydrate antigen 50 (CA50), are frequently used for gastrointestinal tumor detection but are not recommended for GC due to a lack of sensitivity and/or specificity.[7,8] Thus, simple, cost-effective, and noninvasive methods are urgently required for GC detection.

MicroRNAs (miRNAs; miRs) are non-coding RNA molecules that regulate eukaryotic gene expression at the post-transcriptional level in humans, animals,

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and plants, and these molecules are abnormally expressed frequently in cancer tissues. MiRNAs can be delivered from tumor cells into biofluids by exosome particles or microvesicles, which protect miRNAs from the degradation by RNases and have been found in serum, plasma, urine, tear, and gastric juice.[9] There are accumulating evidences that miRNAs can be potential biomarkers for various cancer types.[10] Several miRNAs are abnormally expressed in the tumor tissues and body fluids of GC patients and show promise in clinical application.[11]

In this review, we summarize and discuss the recent advances and challenges in the use of miRNAs, particularly miRNAs in the biological fluids, as potential GC biomarkers for screening and diagnosing, clinically staging, and predicting malignant behaviors, therapy response, recurrence after operation, and survival time.

MicroRNAs are emerging biomarkers for cancer
MicroRNAs and biofluid microRNAs

MiRNAs were discovered more than 20 years ago but did not receive attention until 2001.[12,13] To date, thousands of human miRNAs have been identified. Most human genes are directly targeted by miRNAs.[14] MiRNAs play an important role in the regulation of almost all aspects of biology, including the pathogenesis of cancer.[15] MiRNAs affect cancer progression by regulating cancer genes (tumor suppressor miRNA) or tumor suppressor genes.[16] Additionally, miRNAs can regulate different levels of gene expression (transcription and translation) and serve as tumor suppressors or oncogenes.[17]

Biofluid miRNAs, also known as circulating miRNAs, are a type of miRNA with stability in body fluids. These molecules are secreted into body fluids by tumor cells via specific pathways.[18] MiRNAs can be secreted through exosomes.[19] Another pathway is passive secretion after cell necrosis and apoptosis.[20] The most widely studied miRNAs are those encapsulated in exosomes. The amount of miRNAs secreted by exosomes was inconsistent with the miRNAs in the exosomes,[21] indicating that miRNAs are selectively packed into exosomes. However, the mechanism by which miRNAs are selectively secreted from these vesicles remains unknown. Interestingly, a recent study revealed that some of the highly expressed serum miRNAs in patients with GC might be derived not from GC tissues but other non-cancerous tissues.[22] Therefore, the origin of circulating miRNAs needs to be further elucidated.

Both tissue and biofluid miRNAs are abnormally expressed in many diseases, especially in tumors. The expression levels of miRNAs in cancer tissues are related to the tumor status, size, invasion, and metastasis and may be used for early cancer detection.[23] However, the miRNAs in tumor tissues are not ideal candidates for clinical diagnostic application, as obtaining tissue samples involves an invasive procedure. In 2008, a study reported that miRNAs in the blood of tumor patients can be used as biomarkers for cancer,[24] and they have recently been considered promising biomarker candidates for cancer.[25] Compared with intracellular miRNAs, biofluid miRNAs are non-invasive biomarkers for clinical application in cancer. For example, serum miR-30e and miR-223 can be used to distinguish hepatocellular carcinoma from healthy controls;[26] the expression of serum miR-365 was related to the overall survival of patients with lung cancer;[27] and the serum expression levels of miR-21, miR-17 and miR-92 were highly correlated with recurrence after adjuvant chemotherapy in patients with colon cancer.[28]

MicroRNAs in gastric cancer

MiRNAs play important roles in the molecular and cellular biology of GC, including proliferation, migration and invasion.[29] Studies have revealed that miR-17-5p could serve as an oncogene (oncomiR) to promote cell growth and invasion by regulating transforming growth factor-beta receptor 2 in GC.[30] In contrast, miR-203 is a tumor suppressor that is capable of inhibiting tumor cell proliferation, migration, and invasion by regulating the levels of Slug in GC.[31] Ye et al[32] recently demonstrated that miR-647 could serve as a tumor suppressor to inhibit GC metastasis by targeting the SRF/MYH9 axis.

Interestingly, the miRNAs that are expressed in GC may also serve as tumor suppressors and oncogenes. For example, miR-10b in GC could promote tumor cell invasion via the up-regulation of RhoC and AKT phosphorylation through targeting HOXD10,[33] and could also dramatically suppress cell proliferation, migration, and invasion and induce apoptosis.[34] Several studies have shown that miR-107 could act as an oncogene to promote the invasion and metastasis of GC through a variety of targets (including DICER1 and NF1),[35,36] but Feng et al[37] found that miR-107 was a tumor suppressor that inhibited the proliferation and invasion of gastric cancer cells. The dual effects of these miRNAs may be related to the fact that a single miRNA can target multiple genes and that a single gene can be regulated by multiple miRNAs.[38] The mechanism underlying the complex interaction between miRNAs and targets remains unclear in cancer.[39]

MiRNAs as diagnostic biomarkers for gastric cancer

MiRNAs are abnormally expressed in the tumor tissues and body fluids of GC patients. Because the detection of miRNAs in plasma, serum, urine, and gastric juice can be used in diagnosis, several studies have been conducted to evaluate the diagnostic performance of miRNAs in body fluids for GC, and the results have shown that many miRNAs have certain value for GC in diagnosis, recurrence monitoring and/or clinical staging.

MiRNAs for the detection of gastric cancer

MiRNAs in plasma/serum

Tsujiura et al[40] analyzed the levels of miRNAs in the plasma of 69 patients with GC and found that some miRNAs, such as miR-17-5p, miR-21, miR-106a and miR-106b, are up-regulated. Zhang et al[41] showed that miR-16-5p and miR-19b-3p are consistently elevated in the plasma of GC patients compared with controls and these molecules had potential diagnostic value for GC, with areas under the receiver operating characteristic curves (AUROCs) of 0.772 and 0.749, sensitivities of 49.0% and 81.3%, and specificities of 91.0% and 58.6%, respectively. Tsai et al[42] investigated the expression levels of miR-196a/b in plasma and showed a much higher specificity for the diagnosis of GC than CEA and CA19-9, with sensitivity and specificity of 69.5% and 97.6% for miR-196a and 62.2% and 96.1% for miR-196b, respectively. Hung et al[43] found that compared with healthy controls, miR-376c was up-regulated in the tissue, plasma, and urine of GC patients and the levels of miR-376c in plasma exhibited a diagnostic value for GC, with AUROC of 0.77, sensitivity of 71.0% and specificity of 78.0%. Another study reported that the miR-206 levels in serum were lower in GC patients (n = 150) than in healthy controls (n = 150), with AUROC of 0.861, sensitivity of 78% and specificity of 86% for GC detection.[44]

The performance of single miRNAs in blood is not ideal for GC diagnosis because of tumor heterogeneity. Thus, a combination of plasma miRNAs may help improve their diagnostic performance for GC. Zhu et al[45] conducted a four-phase study on the diagnostic value of circulating miRNAs in 124 patients with gastric non-cardia adenocarcinoma (GNCA), 36 patients with gastric cardia adenocarcinoma (GCA) and 160 cancer-free controls. These authors found that the levels of five miRNAs (miR-16, miR-25, miR-92a, miR-451 and miR-486-5p) were consistently elevated in the plasma of GC patients and could be used as potential markers

for diagnosing GNCA, with AUROCs of 0.850 to 0.925 in the training set and 0.694 to 0.790 in the validation phases, respectively. The panel of the five miRNAs presented a high diagnostic accuracy for early-stage GNCA, with AUCs of 0.989 and 0.812 in the training and validation phases, respectively. However, in another report, the six serum miRNAs (miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, and miR296-5p) used as a biomarker panel for discriminating GC from normal controls were not as good, exhibiting AUROCs of 0.764 and 0.702 in the training and validation phases, respectively.[46] The different results between the two groups may be due to different miRNAs used in the panels. Additionally, various analysis platforms for miRNA detection may also result in different results.[47] However, miRNAs in combination may be crucial for improving diagnostic performance.

MiRNAs in gastric juice

Chen et al[10] examined the stability of miRNAs under harsh conditions, including boiling, low/high pH and freeze–thaw cycles, and the results showed that miRNAs still remained stable after treatment for 3 h in low (pH = 1) or high (pH = 13) pH solution, indicating that miRNAs in gastric juice might be used as novel biomarkers for GC.

Several studies have shown that the presence of miR-21, miR-106a, miR-133a, miR-421, miR-129-1-3p, miR-129-2-3p and miR-129 in gastric juice could be good candidates for GC diagnosis, even in early GC. Cui et al[48] found that miRNAs in gastric juice were valuable for discriminating GC from benign gastric diseases, with AUROCs, sensitivities and specificities of 0.969, 85.7% and 97.8% for miR-21 and 0.871, 73.8% and 89.3% for miR-106a, respectively; and an AUROC up to 0.982 was observed for the combination of miR-21 and miR-106a. Shao et al[49] found that the miR-133a levels in gastric juice were dramatically lower in GC patients than in both healthy subjects and individuals with benign gastric diseases, with AUROC of 0.907, sensitivity of 85.9% and specificity of 84.8% for discriminating GC from gastric benign diseases. Zhang et al[50] revealed that the levels of miR-421 in gastric juice were lower in patients with GC than in patients with gastric benign diseases, which distinguished GC from gastric benign patients with AUROC of 0.767, sensitivity of 71.4%, and specificity of 71.7%; they found the same positive rates in early and late gastric cancer [71.4% (5/7) vs. 71.4% (25/35)]. In contrast, the positive rates in early and late gastric cancer patients were, respectively, 14.3% (1/7) and 40.0% (14/35) for serum CEA and 42.8% (3/7) and 74.3% (26/35) for gastric juice CEA.

The expression levels of miR-129-1-3p and miR-129-2-3p in gastric juice were also lower in GC patients than in healthy subjects, with AUROCs of 0.639 for miR-129-1-3p and 0.651 for miR-129-2-3p.[51]

Compared with blood, the collection of gastric juice from the patients through gastroscopy or the insertion of a nasogastric tube is invasive. Thus, efforts are needed to develop non-invasive methods for collecting gastric juices in the future.

MiRNAs in urine
The urinary miR-376c also exhibited diagnostic value in GC patients, with AUROC of 0.7, sensitivity of 60.0% and specificity of 64.0%.[43] A recent study showed that miR-21-5p was highly expressed in urine of gastric cancer patients compared with healthy controls and was significantly reduced after surgical resection of gastric cancer.[52] Because the collection of urine is convenient and completely non-invasive, additional studies on using urinary miRNAs as biomarkers for GC diagnosis are warranted.

MiRNAs in exosome
Exosomes are lipid bilayer membrane-enclosed small vesicles in the extracellular environment, which are secreted by cells and contain a variety of molecules, including miRNAs. Tumor cells secrete excessive amount of exosomes related to tumor biological behaviors. miRNAs in exosomes are protected from ribonuclease degradation.[53] Therefore, they are stable in body fluid and suitable as GC biomarkers. Ma et al[54] found that miR-221 in exosomes from the peripheral blood was highly expressed (2.5 fold) in GC patients compared with healthy controls and significantly reduced after surgical resection. Exosomal miR-106a-5p and miR-19b-3p in plasma were dramatically elevated in GC patients compared with controls, with AUROCs of 0.786 for miR-106a-5p, 0.769 for miR-19b-3p, and 0.814 for both miRNAs in combination in the diagnosis of GC.[55] However, more studies are required to evaluate the diagnostic value of exosomal miRNAs for GC.

MiRNAs for monitoring the recurrence of gastric cancer after resection
Biofluid miRNAs are valuable for monitoring the recurrence of gastric cancer after operation. Hou et al[44] detected the serum miRNA-206 levels in pre-operative (n = 150), post-operative (n = 150), and recurrent (n = 110) patients with GC and showed that miR-206 expression in serum was significantly up-regulated in paired post-operative samples and reduced

in recurrent samples, with AUROC of 0.85 for discriminating recurred and non-recurred patients. Liu et al[56] screened a large number of plasma miRNAs in 407 patients with GC at stages II and III and created a 7-miRNA classifier and an index with 7 miRs and pathological factors to predict the 3-year post-operative recurrence; the results showed that the AUROCs of the classifier and the index were, respectively, 0.725 and 0.841 in the training set and 0.627 and 0.771 in the validation set. The high-risk patients determined by the signatures had a significantly shorter disease-free survival and overall survival time than did the low-risk patients.

Tissue miRNAs also have potential value for diagnosing the recurrence of gastric cancer after resection. Zhang et al[57] detected hsa-miR-375 and hsa-miR-142-5p in 34 frozen fresh tissues and 38 formalin-fixed paraffin-embedded tissues and combined these two miRNAs as a classifier to predict GC recurrence, which showed AUROCs of 0.75 and 1.00 in the training and validation phases, respectively. Similarly, Park et al[58] conducted a study using 50 paraffin-embedded tissues and found that hsa-miR-21-5p was significantly higher in recurrence group than in the group without recurrence, with AUROC of 0.723, specificity of 65.5% and sensitivity of 86.7%.

MiRNAs can be used to predict recurrence in early gastric cancer after endoscopic submucosal dissection (ESD). Xue et al[59] detected the expression levels of miR-328 in the early gastric cancer tissues from ESD and found significantly lower levels in the recurrence group (N = 96) than in the non-recurrence group (N = 134). They also noted that the recurrence rate was significantly lower in the group with higher levels of miR-328 than in the group with lower levels of miR-328.

MiRNAs for gastric cancer staging
The TNM system is the most widely used cancer staging system, and it is based mainly on the primary tumor size (T), the extent of lymph node involvement (N) and the presence of distant metastasis (M). In gastric cancer tissues, the expression of miRNAs, including microRNA-630, microRNA-196a/-196b and microRNA-29, was significantly related to the TNM staging system.[60–62]

Biofluid miRNAs are also associated with tumor staging in gastric cancer. Zhang et al[41] found that the plasma miR-16-5p and plasma miR-19b-3p showed good performance in differential diagnosis of GC cases with different TNM stages (IV stage: AUC=0.832 for miR-16-5p; III stage: AUC=0.822 for miR-19b-3p); these authors also showed that the diagnostic performances of the two miRNAs improved

when gastric cancer progressed from earlier stages to advanced stages.

MiRNAs as prognostic biomarkers for gastric cancer

Accumulating studies have shown that some biofluid miRNAs are capable of predicting the survival of GC patients. An earlier study found that the expression levels of plasma miR-21 were related to the overall survival (OS) of GC patients: the 3-year survival rate was lower in patients with higher miR-21 levels than in patients with lower levels (64.9% vs 85.9%).[63] In a recent study, Zhuang et al[64] found that the plasma miR-23b levels were higher in GC patients (n = 138) than in normal subjects (n = 50) and were remarkably related to the survival of GC patients: the 5-year OS was much lower in GC patients with higher miR-23b levels than in patients with lower levels (29.4% vs 75.7%).

MiRNAs in GC tissues are also related to the OS of GC patients. Yang et al[65] reported that the miR-206 expression levels were negatively correlated with the survival rates of GC patients: 1, 3, and 5-year survival rates were 76.9% (40/52), 69.2% (36/52) and 57.7% (30/52), respectively, in patients with higher levels of miR-206, but 91.3% (42/46), 84.8% (39/46), and 80.6% (37/46), respectively, in patients with lower levels. These findings were further confirmed by Shi et al.[66] The expression of miR-92a in GC tissue was also significantly related to OS of GC patients: GC patients with higher levels of miR-92a had shorter survival times than those with lower levels of miR-92a in stage II (OS 30.6 vs 64.9 months) and stage III (OS 29.7 vs 58.9 months).[67]

A recent meta-analysis demonstrated that many miRNAs, such as miR-20b, miR-141, miR-145 and miR-486-5p, were associated with survival prediction in patients with gastric cancer.[68]

Recently, Ding et al[69] developed an eight-miRNA prognostic model (miR-145, miR-184, miR-20b, miR-9-1, miR-9-2, miR-1537, miR-549 and miR-802) for predicting the survival of stomach adenocarcinoma patients, and the results showed that patients with high scores had a poor OS (median OS: 1,811 days vs 562 days), similar to the finding for early-stage patients (median OS: 2,197 days vs 652 days).

MiRNAs as biomarkers for histological behaviors of gastric cancer
Invasion
Some miRNAs are involved in the activation of GC invasion. The overexpression of miR-148a inhibited GC cell migration and invasion in vitro and metastasis formation in vivo by targeting ROCK1.[70] Interestingly, miR-148a also targets matrix metalloproteinase (MMP) 7, which may lead to the invasion of GC.[71] In a recent study, Qiu et al[72] found that both miR-148a and miR-26a were down-regulated in tumor tissues and that the up-regulated expression of miR-26 inhibited the proliferation, migration, invasion, and colony formation of MGC-803 cells. Similarly, the up-regulation of miR-153 in MKN-45 cells reduced cell migration and invasion, whereas the down-regulation of miR-153 in SGC-7901 cells promoted cell migration and invasion.[73]

Metastasis
MiRNAs are related to GC metastasis. The overexpression of some miRNAs in GC tissues is significantly associated with lymph node metastases, such as miR-196a/b, miR-10a, miR-23a/b,[61,74,75] and some miRNAs with low expression in GC tissues are also related to lymph node metastasis. miR-125a-3p was down-regulated in GC tissues and associated with lymph node and liver metastasis.[76] Zhang et al[77] found that the expression levels of miR-145-5p were significantly lower in GC tissues than that in adjacent normal tissues, which was associated with lymph node metastasis, metastasis stage, and distant metastasis. Recently, Li et al[78] observed the expression levels of miR-30c-2-3p and miR-133a-3p in GC tissues from 338 patients with metastases and 23 patients without metastases and found that the levels of these miRNAs were related to lymph node metastasis.

The expression levels of biofluid miRNAs are also associated with GC metastasis. Yang et al[79] found that the serum miR-20a levels were correlated with lymph node metastasis in GC. The levels of serum miR-203 were dramatically lower in GC patients (n = 130) than in healthy controls, with odds ratios of 9.86 (95% CI 2.73–26.59) for lymph node metastasis and 12.04 (95% CI 2.07–230.0) for distant metastasis.[80]

Histological type
Stomach adenocarcinoma is divided into three histological types: intestinal, diffuse and undifferentiated,[2] and each type has different clinical features and biological behaviors, particularly intestinal type and diffuse type.[81] In the mouse model of diffuse-type gastric cancer (DGC), four miRNAs (miR-103, miR-107, miR-194, and miR-210) were highly expressed in the serum and cancer tissues compared with the corresponding controls, and when these four miRNAs in serum were used as biomarkers for the early detection of DGC, they showed high AUROCs, sensitivities and

specificities: 0.881, 81.8% and 95.7% for miR-103, 0.909, 90.9% and 95.7% for miR-107, 0.925, 90.9% and 95.7% for miR-194, and 0.846, 72.7% and 87.0% for miR-210, respectively.[82] Azarbarzin et al[83] showed that the miR-383 levels were down-regulated in intestinal-type gastric adenocarcinoma compared with non-tumor tissues; this feature was able to distinguish intestinal gastric cancer patients from healthy controls, with a sensitivity of 76% and specificity of 84%.

MiRNAs as therapeutic biomarkers for gastric cancer

Studies in cellular biology have shown that the levels of the miRNAs that are expressed in GC cells are associated with the sensitivity to anticancer drugs. The expression levels of miR-15b or miR-16 could attenuate multidrug resistance in GC cells by targeting the BCL2 gene: the apoptotic cells were higher than 30% in the overexpression group but approximately 10% in the control group.[84] MiR-185 shows low levels in gastric cancer cell lines and clinical tissues, and its overexpression increased the sensitivity of GC cells to anticancer drugs by targeting ARC (the apoptotic GC cells were approximately 40% in the miR-185 overexpression group and 15% in the control group).[85] MiR-31, as a tumor suppressor involved in GC tumorigenesis, increased the sensitivity to chemotherapy (5-fluorouracil) in MKN-45 cells compared to the control groups.[86]

In clinical studies, miRNAs were associated with chemosensitivity or chemoresistance. Kim et al[87] analyzed the association of the levels of miRNAs in the biopsy tissues with time to progression (TTP) of disease after chemotherapy in 90 patients with GC and found that 30 miRNAs were inversely correlated and 28 miRNAs were positively correlated with TTP in 82 cancer patients, including six up-regulated miRNAs (let-7g, miR-342, miR-16, miR-181, miR-1, and miR-34) related to chemosensitivity and another six up-regulated miRNAs (miR-518f, miR-520a, miR-520d, miR-519e, miR-363, and miR-517) related to chemoresistance. By using tissue samples from 54 patients who underwent palliative chemotherapy, Smid et al[88] found that the miRNAs with high levels of expression (miR-150, miR-342-3p, miR-181b, miR-221, and miR-224) and the miRNA with a low expression level (miR-520h) were related to TTP and OS in GC patients, indicating that these miRNAs could be used as biomarkers to predict the effectiveness of treatment. Shang et al[89] found that the overexpression of miR-27b was sufficient to sensitize GC cells to chemotherapy in vitro and in vivo and that the expression levels of miR-27b and

miR-508-5p were associated with chemosensitivity and overall survival in 114 patients with GC.

MiRNAs may predict the outcome of GC target therapy. Sui et al[90] analyzed the association of tissue miR-125b with the outcome of 28 patients with GC who were treated with trastuzumab (a targeted drug used for HER2-positive cancer); they found that the overall survival rate was dramatically lower in the patients with high miR-125b expression than those with low miR-125b expression, suggesting that miRNAs could be used as biomarkers to more precisely predict the efficiency of tumor target therapy.

Surgically treated GC patients who had high levels of serum miR-203 showed longer overall survival time than those with low levels of serum miR-203, and the serum miR-203 level was an independent prognostic biomarker.[80] Similarly, the serum miR-204 level was a biomarker for predicting prognosis in GC patients with systemic treatment.[91] A recent study showed that miR-21-5p was highly expressed in the urine of gastric cancer patients compared with healthy controls and noted that its expression was reduced following surgery, suggesting that urine miR-21-5p can be used as a valuable noninvasive biomarker to evaluate the prognosis of GC patients undergoing surgical treatment.[52]

MicroRNAs as multifunctional biomarkers for gastric cancer

Interestingly, many miRNAs, such as miR-16-5p, miR-19b-3p, miR-196a/b, miR-376c, miR-206, miR-21-5p, miR-18a and miR-144, have multiple diagnostic potential (Figure 1) for GC.[41–44,52,92–94] These miRNAs have predictive values not only in diagnosis but also in evaluating prognosis and/or treatment responses in patients with GC.

Su et al[92] reported that plasma miR-18a was significantly up-regulated in GC patients compared with controls and had potential diagnostic value for GC, with AUROC of 0.907, specificity of 84.6% and sensitivity of 80.5%. This miRNA was also associated with the prognosis of GC patients: the 5-year OS was much worse in GC patients with higher levels of plasma miR-18a than in patients with lower levels of plasma miR-18a. Moreover, the expression of miR-18a was also significantly associated with pathological grades and lymph metastasis. Tsujiura et al reported similar results.[93]

MiR-144 is another multifunctional miRNA in GC. Liu et al[94] found that miR-144 was significantly down-regulated in the plasma of GC patients, with AUROC of 0.821, specificity of 83.6% and sensitivity of 71.5% for diagnosing GC, and the miR-144 levels in both tissue and serum were associated with the prognosis of GC patients following
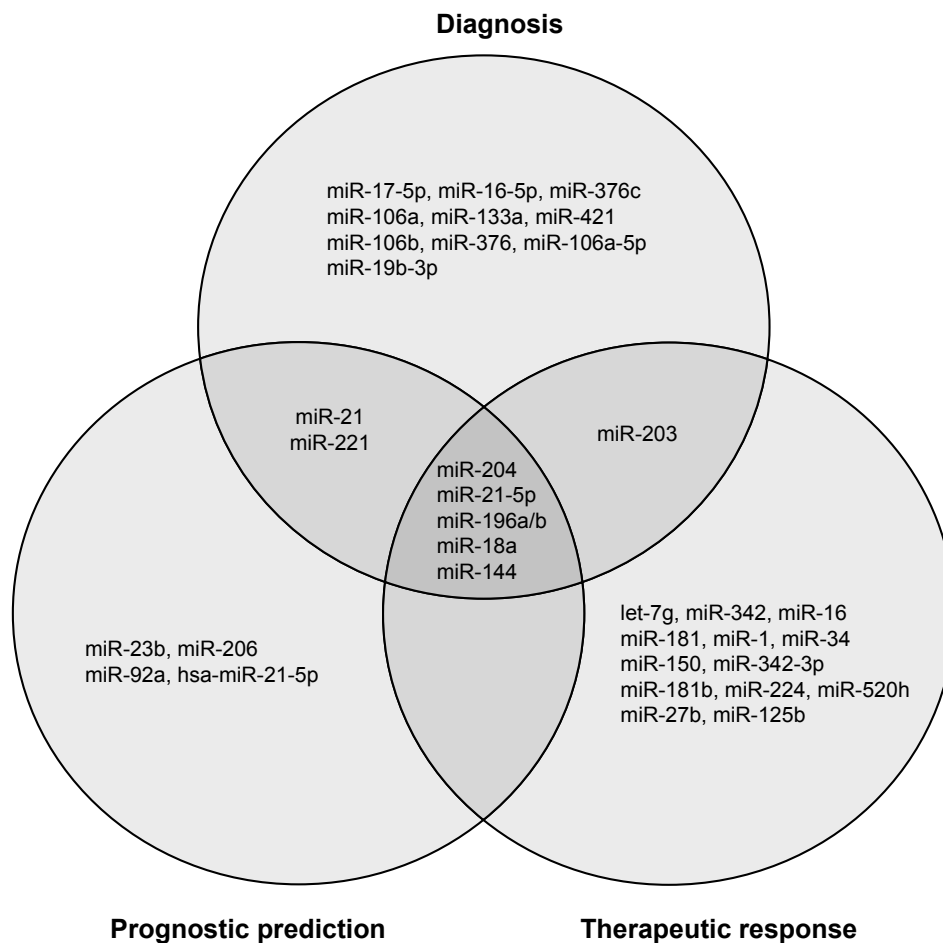
Diagnosis

Figure 1 The clinical function sorting of miRNAs as biomarkers of gastric cancer.

surgery: the 5-year OS was much lower in GC patients with lower tissue miR-144 levels than that in patients with higher levels (26.1% vs 76.0%); similarly, the 5-year OS was much worse in GC patients with lower serum miR-144 levels than that in patients with higher levels. These findings suggest that miR-144 can serve as a multifunctional biomarker for patients with gastric cancer, which has a predictive value for diagnosis, treatment, and prognosis.

Challenges of miRNAs as clinical biomarkers for gastric cancer

Although miRNAs hold promise as GC biomarkers, some challenges should be overcome before these molecules are used as clinical tests for GC. The first issue is to validate the diagnostic value of miRNAs. The reliability of using miRNAs as biomarkers for the diagnosis, prognosis, and treatment response of gastric cancer should be confirmed via further larger-scale studies. The current reports are generally single-center studies with small samples. The second issue is the sensitivity and specificity of miRNAs to GC.

The identification of sensitive miRNAs that can be used as biomarkers may be a challenge due to the heterogeneity of GC itself. In recent reports, few miRNAs in GC are highly sensitive and ideal for clinical application. Similarly, the specificity of miRNAs to GC is also an issue due to the "universality" of the miRNAs themselves. For example, the high expression of miR-21 was related to poor prognosis not only in GC patients[63] but also in patients with other cancers, including hepatocellular carcinoma and breast cancer.[95,96] The third issue is the methodology for miRNA detection. The biological specimens used for GC detection include tissue, plasma and serum, urine and gastric juice. For each type of specimen that is utilized in miRNA analysis, a standardized procedure is needed before miRNAs as biomarkers are translated into clinical application, including specimen collection, pre-analytical preparation, and analysis.[97,98] The fourth issue is the further realization of miRNA functions. There is an enormous number of miRNAs in the human body. The fact that a single miRNA can target multiple genes and that a single gene can be targeted by multiple

miRNAs indicates a regulation network of miRNAs in the body. Thus, substantial studies on functions and mechanism of miRNAs in GC are crucial to facilitate the application of miRNAs in the clinic.

Conclusions

Classic tumor biomarkers, such as CEA, CA72-4, and CA19-9, are poor in sensitivity and specificity for GC diagnosis.[7,8] Accumulating evidence indicates that miRNAs possess great promise as tumor biomarkers. The abnormal expression of miRNAs can be detected in patients with GC not only in tissue samples but also in body fluids, including plasma/serum, urine, and gastric juice. The miRNAs in biofluids are stable even under harsh conditions, and these molecules are ideal clinical biomarkers due to the non-invasive nature of their sample collection. The expression levels of many miRNAs in the tissues or biofluids of GC patients are valuable for diagnosis, prediction of prognosis and response to therapy, and/or monitoring recurrence after resection, and these molecules may be "multifunctional" biomarkers; ie, a single miRNA has predictive value in diagnosis, therapy response, and prognosis simultaneously. However, some obstacles should be overcome for miRNAs to translate into the clinic, including the validation of diagnostic value in larger-scale studies, the identification of more sensitive and specific miRNAs to gastric cancer, the development of standardized methods for miRNA detection and analysis, and more knowledge of miRNA biology. After overcoming these challenges, the use of miRNAs as multifunctional biomarkers for the precise management of GC may be realized.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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