**Name of journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 6935**

**Columns:** **TOPIC HIGHLIGHT**

WJG 20th Anniversary Special Issues (8): Gastric cancer

**MicroRNAs as potential biomarkers for gastric cancer**

Liu HS *et al.* MiRNAs in gastric cancer

Han-Shao Liu, Hua-Sheng Xiao

**Han-Shao Liu, Hua-Sheng Xiao,** National Engineering Research Center for Biochip Design and Engineering at Shanghai, Shanghai 201203, China

**Han-Shao Liu, Hua-Sheng Xiao,** Shanghai-MOST Key Laboratory for Disease and Health Genomics, Chinese National Human Genome Center at Shanghai, Shanghai 201203, China

**Han-Shao Liu,** Advanced Institute of Translational Medicine, Tongji University, Shanghai 200092, China

**Author contributions:** Liu HS wrote the manuscript; Xiao HS revised the manuscript for critical intellectual content.

**Supported by** Grants from the National Natural Science Foundation of China No. 30900745; and the National High-Tech Research and Development Program (863 Program) No. 2012AA020103

**Correspondence to:** **Hua-Sheng Xiao, PhD,** National Engineering Center for Biochip Design and Engineering at Shanghai, No. 151 Libing Rd., Shanghai 201203, China. huasheng\_xiao@shbiochip.com

**Telephone:** +86-21-51320288 **Fax:** +86-21-51320266

**Received:** October 29, 2013  **Revised:** May 6, 2014

**Accepted:** June 12, 2014

**Published online:**

**Abstract**

Gastric cancer is the fourth most common cancer in the world and the second leading cause of cancer-related death. More than 80% of diagnoses occur at the middle to late stage of the disease, highlighting an urgent need for novel biomarkers detectable at earlier stages. Recently, aberrantly expressed micro (mi)RNAs have received a great deal of attention as potential sensitive and accurate biomarkers for cancer diagnosis and prognosis. This review summarizes the current knowledge about potential miRNA biomarkers that have been reported in the publicly available literature between 2008 and 2013. Available evidence indicates that aberrantly expressed miRNAs in gastric cancer correlate with tumorigenesis, tumor proliferation, distant metastasis and invasion. Furthermore, tissue and cancer types can be classified using miRNA expression profiles and next-generation sequencing. As miRNAs in plasma/serum are well protected from RNases, they remain stable under harsh conditions. Thus, potential functions of these circulating miRNAs can be deduced and may implicate their diagnostic value in cancer detection. Circulating miRNAs, as well as tissue miRNAs, may provide for the detection of gastric cancer at an early stage, prediction of prognosis, and allow physicians to monitor recurrence and/or lymph node metastasis. Taken together, the data suggest that the participation of miRNAs in biomarker development will enhance the sensitivity and specificity of diagnostic and prognostic tests for gastric cancer.

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**Key words:** microRNAs; Gastric cancer; Biomarker; Clinical application

**Core tip:** Gastric cancer is the second leading cause of cancer-related deaths, and > 80% of cases is diagnosed at the middle to late disease stage. Novel biomarkers for the detection of early stage gastric cancer are therefore urgently needed. Recent recognition of a correlation between aberrantly expressed micro (mi)RNAs and cancer-related processes has highlighted the potential of miRNAs as diagnostic and prognostic markers. Detection of miRNAs, in tissue as well as in serum/plasma, may enhance the sensitivity and specificity of diagnostic and prognostic tests for early stage gastric cancer, and provide a means to monitor recurrence and/or lymph node metastasis.

Liu HS, Xiao HS. MicroRNAs as potential biomarkers for gastric cancer. *World J Gastroenterol* 2014; In press

**INTRODUCTION**

Although the rate of gastric cancer (GC) has declined over the last 50 years, GC remains the fourth most common cancer worldwide, with a total of 989600 new cases and 738000 deaths estimated in 2008[1]. Over 70% of these new cases and deaths occurred in developing countries, with half of the world total occurring in Eastern Asia (mainly in China)[2]. Due to the absence of specific symptoms and early detection diagnosis，gastric cancer is often diagnosed at an advanced stage when a cure is not possible, and in these cases, the prognosis remains unfavorable. Median survival is 7–9 mo and survival at 2 years is exceptionally > 10%. A combination of early diagnosis and the improvement in surgical techniques has extended survival in GC patients[3-5]. As the prognosis for these patients differs depending on the disease stage at the time of diagnosis, early detection of GC is crucial. While the combined use of tumor markers, such as -feto-protein, carcinoembryonic antigen and carbohydrate antigens 125 and 19-9, improves the sensitivity for the diagnosis of advanced gastric cancer, these markers yield inconsistent results when used for early detection of GC[6-10]. Therefore, identification of novel biomarkers for early GC diagnosis is a major focus of current investigation.

A class of small (about 22 nucleotides) noncoding RNA species, known as micro (mi)RNAs, have been shown to regulate gene expression and play important roles in a wide range of physiological and pathological processes[11-17,36]. For example, the miR-15a-miR-16-1 cluster has been demonstrated to promote prostate cancer by targeting genes related to a multitude of oncogenic activities[18], and p53-mediated transactivation of miR-34a has been shown to influence the expression of genes related to apoptosis[19]; furthermore, miR-373, miR-520c and miR-10b have been shown to promote tumor invasion and metastasis[20-22]. Although the biological function of miRNA has yet to be fully understood, miRNA analyses indicate that a wide range of tumor types display significantly different expression profiles compared to normal tissues. Thus, miRNAs are now being utilized in development of various diagnostic tests as highly tissue specific biomarkers, with the potential for clinical determination of metastasis origin[23-25]. MiRNAs remain stable after incubation at room temperature for up to 24 h or subjecting it to up to eight cycles of freeze-thawing. MiRNAs protected by various lipids and proteins in exosomes and other microparticles, have been detected in plasma, serum, urine, saliva, and milk[26-29]. their stability and about 22bp easily testable length make miRNAs well suited for being utilized as biomarkers. Furthermore, circulating miRNAs meet the basic conditions for utility as a biomarker as tumor-derived miRNAs can be detected in plasma, with no significant difference in circulating levels in healthy male and female subjects[30-35]. Current knowledge concerning the diagnostic and prognostic applications of miRNAs in GC is reviewed and summarized below.

**MiRNAs AND GC**

An increasing number of studies have shown dysregulation of miRNAs in *H. pylori*-infected gastric mucosa and GC, which correlate with cancer development and tumor progression[37-41] (Figure 1). For example, upregulation of miR-17-5p/20a promotes GC cell cycle progression and inhibits cell apoptosis *via* post-transcriptional modulation of tumor suppressor protein TP53INP1 and the cyclin-dependent kinase inhibitor p21[42]. MiR-106b and miR-93 impair TGF-induced apoptosis in GC cells through inhibition of BCL2L11[43]. In addition, miR-150 has been shown to promote GC growth by targeting EGR2[44], a tumor suppressive transcription factor that induces apoptosis by direct transactivation of BNIP3L and Bak[45]. Other miRNAs, such as miR-204, are downregulated in gastric tumors, and ectopic expression inhibits the colony formation, migration and tumor engraftment of GC cells by targeting Bcl-2 messenger RNA[46]. Additionally, miR-375 is one of the most downregulated miRNAs in GC and its overexpression substantially reduces cell viability by targeting PDK1 and 14-3-3zeta[47]. Furthermore, ectopic expression of miR-146a, whose levels are significantly lower in cancerous tissues, inhibits migration and invasion of GC cells and downregulates their expression of the epidermal growth factor receptor and the interleukin receptor-associated kinase IRAK1[48].

miRNAs have also been shown to correlate with tumor proliferation and pathology. Low expression levels of miR-125a-5p are associated with an enhanced malignancy potential, such as tumor size and depth, and a poor clinical prognosis. *In vitro* assays have shown that the proto-oncogene ERBB2 is a direct target of miR-125a-5p, which potently suppresses the proliferation of GC cells[49]. Song *et al*[50] showed that miR-148b is significantly downregulated in GC tissues and cell lines compared with their non-tumor counterparts, and is associated with tumor size. Reduced expression of miR-148a, as well as miR-152, was also shown in human GC by Chen *et al*[51], whichcorrelated with an increased tumor size and advanced tumor stage.

Invasiveness and metastasis are essential aspects of cancer cells that have also been associated with miRNA gene targeting. Recent studies have demonstrated that the expression of the novel tumor suppressor protein programmed cell death 4 (PDCD4) is suppressed by miR-21 and downregulated in GC. Tumor size and depth, lymph node metastasis, venous invasion, advanced stage, and poor clinical prognosis are all significantly correlated with reduced PDCD4 and elevated miR-21 expression[52], as well as a higher expression of miR-106a[53]. In contrast, the levels of let-7a miRNA are significantly lower in the serum and tumor tissues of gastric adenocarcinoma patients compared to the peritumoral tissues and serum from healthy controls. *In vitro* transfection of MNK-45 cells with let-7a significantly inhibits the protein expression of the chemokine receptor CCR7, and impedes their migratory and invasive capabilities[54-56].

Furthermore, *in vivo* studies have also shown the significant impactions of miRNAs in gastric carcinogenesis[57-102}. For example, tumor suppressor gene miR-338 can decrease migratory, invasive, proliferative behaviors as well as EMT by targeting NRP1 in GC[103]. By using gain or loss-of-function in in vitro and in vivo experiments, overexpressed miR-19a/b in GC tissues have been observed to have a pro-metastatic function by attenuating the expression of MXD1[104]. The in vivo roles of miR-133b and miR-202-3p have been shown to inhibit GC metastasis by direct targeting Gli1 and suppressing Gli1 target genes, OPN and Zeb2[105,106]. In addition, miR-1207-5p and miR-1266 are significantly decreased in GC tissues, and ectopic expressions inhibit gastric tumor growth in vitro and in vivo by suppressing hTERT[107]. Other miRNAs, such as miR-26a and miR-212, can inhibit proliferation of GC by directly repressing the expressions of their targets FGF9 and RBP2[108,109].

**CLINICAL APPLICATION OF MiRNAs AS BIOMARKERS IN GC**

Due to the absence of specific symptoms and early detection[55], GC is often diagnosed at an advanced stage with a median survival of about 7 - 9 mo[56]. Thus, a longstanding goal of GC research has been to identify methods for the early diagnosis and management of cancer. Over the past five years, scientists have begun to explore the feasibility of utilizing miRNAs as biomarkers, as many are involved in GC tumorigenesis, proliferation, invasion and metastasis (Tables 1-4).

***MiRNAs as diagnostic markers***

MiRNA expression profiles and next-generation sequencing have revealed that aberrant expression could be used for tissue specificity and to classify cancer types, highlighting the potential of miRNAs for cancer diagnosis. For example, miR-375 is significantly downregulated in distal gastric adenocarcinoma tissues as well as in the circulating serum. At a normalized cutoff of 0.218, miR-375 yields a receiver operating characteristic (ROC) area under the curve (AUC) of 0.835 with a specificity and sensitivity of 80% and 85%, respectively, in the discrimination of distal gastric adenocarcinoma from control tissues[57]. The levels of miR-106a and miR-21 are significantly higher in GC tissues[53,58], while the level of miR-31 is significantly lower[59]. MiR-421 is overexpressed in GC tissues, and while it is not associated with clinicopathological features, it may be involved in the early stage of stomach carcinogenesis[60]. As the positive detection rate of miR-421 is higher than that of serum carcinoembryonic antigen, it thus may serve as an efficient early diagnostic biomarker. These new data lend further support to the notion that miRNAs may represent efficient diagnostic biomarkers.

***MiRNAs as prognostic markers***

The connection between miRNA expression and GC progression and metastasis suggests that miRNAs can be used as prognosis monitoring tools. The expression levels of miR-10a, miR-221, miR-212 and miR-195 are associated with lymph node metastasis[61-63], and in addition, miR-21 expression is also significantly correlated with histologic type, tumor stage, and pathologic tumor-node-metastasis (pTNM) stage[64]. Meanwhile, a seven-miRNA signature (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p, miR-126) has been identified for overall survival and relapse-free survival in GC patients[65]. A high frequency of recurrence and poor survival are observed in GC cases with high levels of miR-335, miR-196a or miR-375, while low expression levels of miR-146a, miR-142-5p or miR-204 are correlated with increased tumor size, pTNM stage and worse overall survival[66-68,46,48]. Furthermore, low expression of miR-125a-5p is associated with a poor prognosis and enhanced malignancy potential, measured by tumor size, tumor invasion and liver metastasis.

***MiRNA-based therapeutic approaches***

Several studies to date have investigated the molecular mechanisms underlying miRNA-targeting as an anticancer therapy. Overexpression of miR-34a has been shown to induce apoptosis and accumulation of cells in the G1 phase, ultimately inhibiting tumorsphere formation[69]. Both miR-15b and miR-16 were shown to promote chemotherapy (mitotic inhibitor: vincristine)-induced apoptosis in a human GC cell line (SGC7901/VCR), suggesting the potential of these miRNAs to modulate the sensitivity of GC cells to certain anticancer drugs[70]. The potential anticancer property of miR-508-5p has been shown to involve its targeting of the 3'-untranslated regions of ABCB1 and zinc ribbon domain-containing 1 (ZNRD1), which sensitizes cancer cells to an array of known chemotherapeutic agents[71]. Furthermore, increased expression of miR-150 and miR-146a, and reduced expression of miR-142-3p and miR-199b-5p have been observed in blood samples from chronic myeloid leukemia patients after two weeks of imatinib therapy, suggesting that miRNAs may serve as a novel clinically useful biomarker in this disease[110]. In this regard, miR-451 has also been considered as a potential predictor marker of imatinib therapy[111]. In addition, miR-1274a is shown to be involved in sorafenib target-therapy of hepatocellular carcinomas (HCC) by targeting ADAM9[112]. Other miRNAs, such as miR-122, can directly inhibit angiogenesis in vitro with concomitant suppression of its target genes, namely ADAM10, SRF, and Igf1R[113]. And ectopic expression of miR-122 potentiates growth inhibitory function of sorafenib in HCC cells. Additionally, Kaplan-Meier survival analysis reveals that low expression levels of miR-21 and miR-181b are closely associated with better GC patient’s overall survival for both S-1 and Doxifluridine based therapies[114]. Collectively, these data suggest the potential application of miRNAs as targets of anticancer therapeutic approaches but also as predictive markers of drug resistance and a patient’s response to treatment.

***Circulating miRNAs as biomarkers***

The search for non-invasive tools for the diagnosis and management of cancer has led to the investigation of circulating nucleic acids, including miRNAs, in plasma and serum. Finding miRNA in plasma/serum has suggested the potential of miRNA signatures in cancer diagnosis. Endogenous circulating miRNAs, which are protected from RNases and remain stable in harsh conditions, exhibit specific tissue and cancer type expression patterns[30,31], demonstrating their diagnostic potential. Circulating levels of miR-103, miR-107, miR-194 and miR-210 were upregulated in sera from double conditional knockout mice with early or advanced-stage diffuse-type GC[72]. Tsujiura *et al*[73] have shown that miR-17-5p, miR-21, miR-106a, and miR-106b are significantly higher, whereas let-7a is lower, in GC patients, with AUCs of 0.721 and 0.879 for the miR-106b and miR-106a/let-7a ratio assays, respectively. Furthermore, the expression of miRNA-199a-3p, miR-106b, miR-20a, and miR-221 are significantly elevated in plasma of GC patients with AUCs of 0.818, 0.7733, 0.8593, and 0.7960, respectively[74-76]. However, there were no significant differences in the plasma levels of these miRNAs among the four TNM stages. Our group has found that serum miR-378 is significantly elevated in early GC patients, in an assay yielding an ROC curve area of 0.861 with 87.5% sensitivity and 70.73% specificity[77]. These findings indicate that elevated circulating miRNAs can be detected in early stages of tumor growth, suggesting their potential as noninvasive biomarkers for early GC detection.

There is also evidence to suggest that circulating miRNA levels are associated with the progression and prognosis of GC. miR-21, miR-17-5p/20a, miR-146a, and miR-148a have been reported as non-invasive biomarker candidates to predict prognosis, monitor chemotherapeutic effects and predict the presence of lymph node metastasis[78-80,48,50]. In addition, Valladares-Ayerbes *et al*[81] have found that expression levels of miR-200c correlate with the number of lymph node metastases, and are significantly associated with poor overall and disease-free survival rates, suggesting that miR-200c has the potential to be a predictor of cancer progression and survival. Moreover, Liu *et al*[82] used Solexa sequencing and qRT-PCR to identify a profile of five serum miRNAs (miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p) correlating with tumor stage that can serve as biomarkers for detecting GC. In their study, the AUCs for this five-serum miRNA signature were 0.879 and 0.831 in two sets of serum samples, which are markedly higher than those of the currently used biomarkers carcinoembryonic antigen (0.503) and carbohydrate antigen 19-9 (0.600). Furthermore, their data demonstrated that higher sensitivity and specificity of monitoring prognosis can be achieved by circulating miRNAs as compared to the other commonly used non-miRNA testing methods.

**FUTURE PERSPECTIVES**

Recently, the detection of circulating tumor cells in peripheral blood has received a great deal of attention for the prediction of postoperative cancer recurrence and the evaluation of novel adjuvant therapies. A significant correlation has been identified between the number of circulating cancer cells and the levels of miR-106a, miR-17, miR-421 and miR-21[83-85]. In this regard, miRNAs are being evaluated as a new molecular diagnostic marker for the detection of these cells. These data highlight a novel potential use for miRNAs in monitoring circulating tumor cells. In addition to serum, the levels of miRNAs in gastric juice are under investigation. Levels of miR-21 are higher in specimens of intestinal type GC compared to diffuse or mixed GC types, whereas the levels of miR-129-1-3p and miR-129-2-3p in gastric juice are significantly lower, with AUCs of up to 0.969 for miR-21 and 0.656 for a combination test of miR-129-1-3p and miR-129-2-3p[86,87]. Furthermore, the addition of gastric juice miR-421 for the detection of early GC shows a remarkable improvement over serum carcinoembryonic antigen alone[88]. These results indicate that gastric juice miRNAs provide additional novel non-invasive biomarkers for screening GC.

**CONCLUSION**

Although miRNAs show promise as detection and prognosis biomarkers, there are methodological and technical limitations regarding the analyses. The variety of methodologies, types of carcinomas included, analysis software and normalization strategies used in the various studies in the published literature have led to a considerable amount of variability and inconsistency among the findings reported. Therefore, detection methods should be standardized and include normalization controls, such as the housekeeping miRNAs, miR-16[89] and RUN6B[77]. miR-93 is also recommended as a suitable reference gene for serum miRNA analysis between GC patients and healthy controls[89]. Other protocols call for samples to be processed from identical input volumes, then corrected for technical variability using spiked-in synthetic non-human (*Caenorhabditis elegans*) miRNA as a normalizing control[30,90]. The use of “invariant” miRNAs as endogenous controls has been proposed by some investigators, however biological variability may preclude this approach. As no consensus concerning the ideal normalization control has been reached, additional studies are needed for sufficient sensitivity and precision in the quantification of miRNAs.

Though thousands of miRNA have been demonstrated to be related to GC, the variability among different patients, even with the same type of cancer, makes it impossible to use just one marker an reliable method of determining cancer status. For this reason only the combination of several miRNA could be effective for diagnostic purpose. For example, low expression of miR-106b and high expression of miR-181b are observed in patients with liver cirrhosis. The AUC for miR-106b and -181b are 0.715 and 0.833, respectively. The ROC curve of the combined miRNAs has an AUC of 0.882. These data demonstrate that the combined detection of miR-106b and miR-181b has more considerable clinical value to diagnose patients with liver cirrhosis[115]. The combination of four serum miRNAs (miR-22, miR-572, miR-638 and miR-1234) signature and TNM stage had a better prognostic value on personalized therapy in nasopharyngeal carcinoma than the TNM stage or miRNA signature alone[116]. In addition, high expressed miR-223, miR-21 and low expressed miR-218 in GC patients yield the AUC values of 0.9089, 0.7944, and 0.7432, respectively. While the combined ROC analysis reveals the highest AUC value of 0.9531 in discriminating GC patients from healthy controls[92]. therefore, a cluster of biomarkers for one disease would be a better diagnostic tool with much higher sensitivity, specificity, and accuracy.

In conclusion, GC-specific miRNAs have been associated with tumorigenesis, tumor proliferation and metastasis. While further investigation with large-scale validation is needed before miRNAs can serve as a non-invasive screening tool in routine clinical trials, their inclusion in biomarker development will enhance the sensitivity and specificity of diagnostic and prognostic tests for GC.

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**P-Reviewer:** Ierardi E, Link A, Nagahara A,Sugimura H,Wang YH **S-Editor: L-Editor: E-Editor:**

**Figure 1 microRNAs and gastric cancer.**



**Table 1 Tissue microRNAs as diagnostic biomarkers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Sample** | **Method (Normalization)** | **MiRNA** | **Clinical application** |
| Jiang Z *et al*[60] (CN) | 60 GC (46A,6M,8S)/18 C | qRT-PCR (U6) | miR-421  | diagnostic biomarker |
| Zhang Y *et al*[59] (CN) | 63 GC/10 C  | real-time qRT-PCR (RNU6B) | miR-31  | diagnostic biomarker |
| Xiao B *et al*[53] (CN) | 55 GC/17 C | real-time qRT-PCR (U6) | miR-106a | diagnostic biomarker |
| Chan SH *et al*[58] (CN) | 37 GC/37 C | qPCR (U6) | miR-21 | diagnostic marker |
| Tsai KW *et al*[67] (CN) | 72 GC/72 C | qRT-PCR | miR-196a | detecting GC and  |
| monitoring recurrence |
| Xiao B *et al*[99] (CN) | 20 GC/20 C | qRT-PCR (U6) | miR-146a | diagnostic biomarker  |
| and therapeutic target |
| Song YX *et al*[50] (CN) | 4 GC cell lines | real-time qRT-PCR (U6) | miR-148b | diagnostic biomarker |
| 106 GC/106 C tissues |
| Chen Y *et al*[51] (CN) | 101 GC(34I, 67B)/101CC/101 C | real-time qRT-PCR (U6) | miR-148a and miR-152 | detection of gastrointestinal |
| cancer |
| Su Y *et al*[101] (CN) | 20 GC/20 C | miRNA microarray  | miR-574-3p | early detection of GC |
| and qRT-PCR (U6) |

C: Control; GC: Gastric cancer; A: Adenocarcinoma; M: Mucinous carcinoma; S: Signet-ring cell carcinoma; I: Intestinal; D: Diffuse.

**Table 2 Tissue microRNAs as prognostic biomarkers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Sample** | **Method (Normalization)** | **MiRNA** | **Clinical application** |
| Wang M *et al*[42] (CN) | 110 GC/110 C  | qRT-PCR (U6) | miR-17-5p/20a | therapeutic marker |
| Yan Z *et al*[66] (CN) | 31 with/43 without  | miRNA microarray  | miR-335 | recognition of recurrence  |
| recurrent GC | and qPCR (U6) | risk |
| Liu K *et al*[62] (CN) | 92 GC/92 C | real-time qRT-PCR (U6) | miR-221 | prognostic factor  |
| for overall survival |
| Inoue T *et al*[96] (JP) | 161 GC/161 C | miRNA qPCR array, | miR-107 | prediction of prognosis |
| *Taq*man miRNA assay  |
| and real-time qRT-PCR (U6) |
| Brenner B *et al*[97] (IL) | 45 GC(39A, 6S) | miRNA microarray | miR-451, miR-199a-3p | prediction of recurrence |
| and qRT-PCR (six miRs) | and miR-195 |
| Zhang X *et al*[68] (CN) | 29 with/36 without | miRNA microarray  | miR-375 and miR-142-5p | prediction of recurrence  |
| recurrent GC(A) | and qPCR (U6) | risk for GC |
| Kim K *et al*[99] (KR) | 15 GC cell lines | miRNA methylome profiling, | miR-10b  | assessing the risk of GC |
| 100 GC(44I, 56D)/100 C tissues | bisulfite pyrosequencing  |
|  | and qRT-PCR (U6) |
| Nishida N *et al*[49] (JP) | 87 GC | qRT-PCR (RNU6B) | miR-125a-5p | prognostic marker |
| Li X *et al*[65] (CN) | 100 GC |  real-time RT-PCR | miR-10b, miR-21, miR-223, miR-338,  | survival prediction |
| let-7a, miR-30a-5p, miR-126 |
| Xu Y *et al*[64] (CN) | 86 GC  | RT-PCR (Let-7a) | miR-21 | prediction of LN metastasis |
| Wu WY *et al*[63] (CN) | 52 GC/52 C | real-time RT-PCR (U6, Let7a,miR-191, 103) | miR-212 and miR-195 | prediction of LN metastasis |
| Liu K *et al*[102] (CN) | 84 GC/84 C | qRT-PCR (U6) | let-7i | prediction of chemotherapeutic  |
| sensitivity and prognosis |
| Chen W *et al*[61] (CN) | 3 GC/ 1 gastric mucosal lines  | miRNA array and qPCR (U6) | miR-10a | prediction of LN metastasis |
| 38 GC(28A,8T,2S)/10 C tissues |
| Valladares-Ayerbes M *et al*[100] (ES) | 28 CC/7 GC/3 pancreas | qRT-PCR (5S and U6) | miR-17-92 | prognostic markers  |
| Kogo R *et al*[48] (JP) | 90 GC/90 C | qRT-PCR (RNU6B) | miR-146a | prognostic factor |
| Sacconi A *et al*[46] (IT)  | 123 GC (75I,24D,24U)/123 C | miRNA microarray | miR-204 | prognostic value |
| and qPCR (RNU6B) |

C: Control; CC: Colorectal cancer; GC: Gastric cancer; LN: Lymph node. A: Adenocarcinoma; T: Tubular adenocarcinoma; S: Signet-ring cell carcinoma; I: Intestinal; D: Diffuse; U: Undefined.

**Table 3 Circulating microRNAs as diagnostic biomarkers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Sample** | **Method (Normalization)** | **MiRNA** | **Clinical application** |
| Li C *et al*[74] (CN) | 80 EGC/70 C plasma | real-time qRT-PCR(U6, U44) | miR-199-3p | early detection of GC |
| Rotkrua P *et al*[72] (JP) | 6 DCKO mice with DGC/ 6 C tissue | miRNA microarray and | miR-103, miR-107, | early detection of DGC |
| 21 DCKO mice with DGC/ 23 C serum | *Taq*Man qRT-PCR (cel-miR-39) | miR-194 and miR-210 |
| Li C *et al*[75] (CN) | 230 GC/130 C and  | miRNA microarray and  | miR-199-3p | GC detection |
| 20 gastric precancerous plasma | real-time qRT-PCR (U6, U44) |  |  |
| Cai H *et al*[76] (CN) | 90 GC(70A,16M,3S,1SC)/90 C plasma | qRT-PCR (cel-miR-39) | miR-106b, miR-20a, | early detection of GC |
| and miR-221 |
| Li BS *et al*[91] (CN) | 8 GC/8 C tissue | qRT-PCR (U6,cel-miR-39) | miR-223, miR-21 | GC detection |
| 70 GC(56A,13M,1S)/ 70 C plasma | and miR-218 |
| Wang B *et al*[92] (CN) | 174 solid cancers/ 39 C serum | qRT-PCR (miR-16) | miR-21 | detection of some solid cancers |
| Song MY *et al*[93] (CN) | 82 GC(71A,3S,1SC)/46 DYS/ 128 C serum | *Taq*Man low-density array  | miR-221, miR-376c | early detection of GC |
| and *Taq*Man qRT-PCR (cel-miR-39) | and miR-744 |
| Konishi H *et al*[94] (JP) | 56 GC/30 C plasma | miRNA microarray | miR-451 and miR-486 | screening GC |
| and qRT-PCR (U6) |
| Liu H *et al*[77] (CN) | 61 GC(A,M)/61 C serum | miRNA microarray  | miR-378 | early detection of GC |
| and real-time qRT-PCR (U6) |
| Liu R *et al*[82] (CN) | 164 GC/127 C serum | Solexa sequencing  | miR-1, miR-20a, miR-27a,  | GC detection |
| and qRT-PCR (the serum volume) | miR-34 and miR-423-5p |
| Tsujiura M *et al*[73] (JP) | 8 GC/8 C tissue | qRT-PCR (RNU6B) | miR-17-5p, miR-21, miR-106a, | GC detection |
| 79 GC/ 30 C plasma | miR-106b and let-7a |
| Gorur A *et al*[95] (TR) | 20 GC/190 C plasma | real-time qPCR (global means) | miR-195-5p | early detection of GC |
| Wang WN *et al*[54] (CN) | 80 gastric adenocarcinomas/40 peritumoral tissues  | real-time qRT-PCR (U6) | let-7a | GC detection |
| 80 gastric adenocarcinomas/45 C serum |

C: Control; DCKP: Double-conditional knockout; DGC: Diffuse-type gastric cancer; DYS: Dysplasia; GC: Gastric cancer; LN, lymph node; A: Adenocarcinoma; M: Mucinous carcinoma; S: Signet-ring cell carcinoma; SC: Squamous cell carcinoma.

**Table 4 Circulating microRNAs as prognostic biomarkers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Sample** | **Method (Normalization)** | **MiRNA** | **Clinical application** |
| Valladares-Ayerbes | 52 GC/31 C peripheral blood | qRT-PCR (U6,5S) | miR-200c | predictor of progression  |
|  M *et al*[81] (ES) |  and 2 GC cell lines | and survival |
| Komatsu S *et al*[78] (JP) | 69 GC plasma | qRT-PCR (U6) | miR-21 | prognostic marker |
| Wang M *et al*[79] (CN) | 79 pre-operative GC/30 post-/6 relapse plasma | qRT-PCR (RNU6B) | miR-17-5p/20a | prediction of prognosis and  |
| monitoring of chemotherapeutic effects |
| Kim SY *et al*[80] (KR) | 16 LN-positive GC/ | qRT-PCR | miR-21, miR-146a, | predicting LN metastasis |
| 15 LN-negative GC/10 C serum | and miR-148a |

C: Control; GC: Gastric cancer; LN: Lymph node.