World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i34.12007

World J Gastroenterol 2014 September 14; 20(34): 12007-12017
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

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

MicroRNAs as potential biomarkers for gastric cancer

Han-Shao Liu, Hua-Sheng Xiao

Han-Shao Liu, Hua-Sheng Xiao, National Engineering Research Center for Biochip 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 Research Center for Biochip 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: September 14, 2014

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 microRNAs (miRNAs) 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 for gastric cancer 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 allow for the detection of gastric cancer at an early stage, prediction of prognosis, and monitoring of 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.

Key words: MicroRNAs; Gastric cancer; Biomarker; Clinical application

Core tip: Gastric cancer is the second leading cause of cancer-related death, and > 80% of cases are 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 microRNAs (miRNAs) 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; 20(34): 12007-12017 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i34/12007.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i34.12007

INTRODUCTION

Although the rate of gastric cancer (GC) has declined

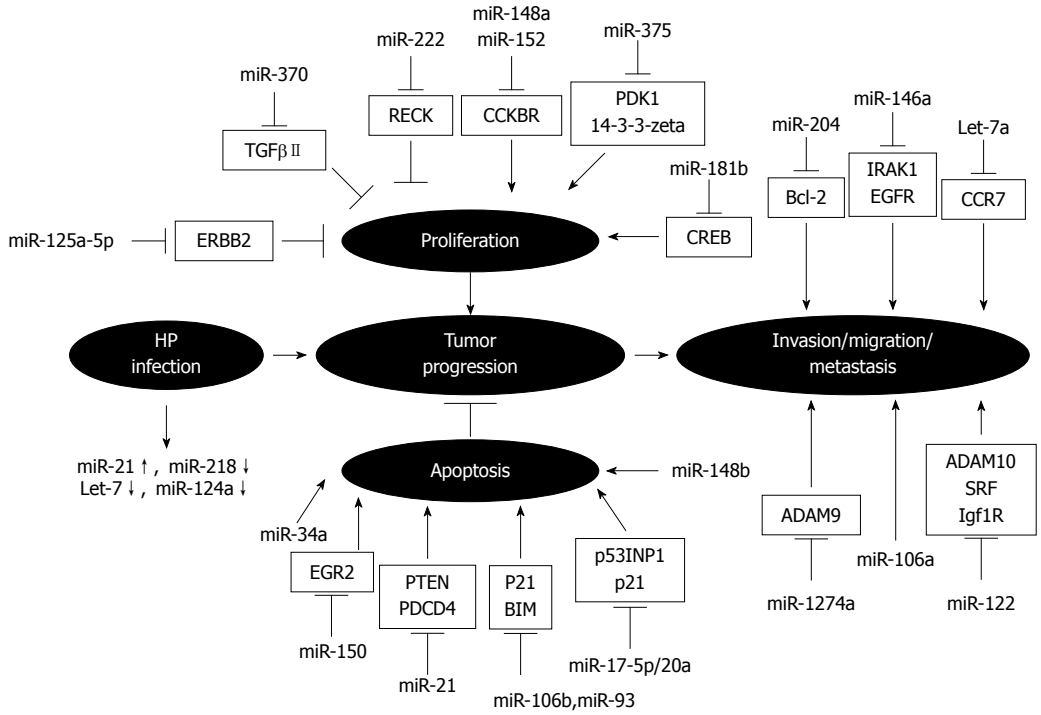

Figure 1 MicroRNAs and gastric cancer.

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, 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 α-fetoprotein, 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 microRNAs (miRNAs), have been shown to regulate gene expression and play important roles in a wide range of physiological and pathological processes[11-18]. 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[19], and p53-mediated transactivation of miR-34a has been shown to influence the expression of genes related to apoptosis[20]; furthermore, miR-373, miR-520c and miR-10b have been shown to promote tumor invasion and metastasis[21-23]. 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[24-26]. MiRNAs remain stable after incubation at room temperature for up to 24 h or after 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[27-30]. Their stability and easily testable length (about 22 bp) 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[31-36]. 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 *Helicobacter pylori*-infected gastric mucosa and GC, which correlates 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 TGFβ-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 their ectopic expression inhibits the colony formation, migration and tumor engraftment of GC cells by targeting Bcl-2 mRNA[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 the expression of the epidermal growth factor receptor and 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 malignant 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], which correlated 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 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 directly 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 their ectopic expression inhibits 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 expression 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 long-standing 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 miRNA 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 of 80% and a sensitivity of 85%, 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 gastric 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,

Table 1 Tissue microRNAs as diagnostic biomarkers

Ref.	Sample	Method (normalization)	MiRNA	Clinical application
Jiang *et al*[60] (CN)	60 GC (46A, 6M, 8S)/18 C	qRT-PCR (U6)	miR-421	Diagnostic biomarker
Zhang *et al*[59] (CN)	63 GC/10 C	real-time qRT-PCR (RNU6B)	miR-31	Diagnostic biomarker
Xiao *et al*[53] (CN)	55 GC/17 C	real-time qRT-PCR (U6)	miR-106a	Diagnostic biomarker
Chan *et al*[58] (CN)	37 GC/37 C	qPCR (U6)	miR-21	Diagnostic marker
Tsai *et al*[67] (CN)	72 GC/72 C	qRT-PCR	miR-196a	Detecting GC and monitoring recurrence
Xiao *et al*[98] (CN)	20 GC/20 C	qRT-PCR (U6)	miR-146a	Diagnostic biomarker and therapeutic target
Song *et al*[50] (CN)	4 GC cell lines 106 GC/106 C tissues	real-time qRT-PCR (U6)	miR-148b	Diagnostic biomarker
Chen *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 *et al*[101] (CN)	20 GC/20 C	miRNA microarray and qRT-PCR (U6)	miR-574-3p	Early detection of GC

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 *et al*[42] (CN)	110 GC/110 C	qRT-PCR (U6)	miR-17-5p/20a	Therapeutic marker
Yan *et al*[66] (CN)	31 with/43 without recurrent GC	miRNA microarray and qPCR (U6)	miR-335	Recognition of recurrence risk
Liu *et al*[62] (CN)	92 GC/92 C	real-time qRT-PCR (U6)	miR-221	Prognostic factor for overall survival
Inoue *et al*[96] (JP)	161 GC/161 C	miRNA qPCR array, Taqman miRNA assay and real-time qRT-PCR (U6)	miR-107	Prediction of prognosis
Brenner *et al*[97] (IL)	45 GC (39A, 6S)	miRNA microarray and qRT-PCR (six miRs)	miR-451, miR-199a-3p and miR-195	Prediction of recurrence
Zhang *et al*[68] (CN)	29 with/36 without recurrent GC (A)	miRNA microarray and qPCR (U6)	miR-375 and miR-142-5p	Prediction of recurrence risk for GC
Kim *et al*[99] (KR)	15 GC cell lines 100 GC(44I, 56D)/100 C tissues	miRNA methylome profiling, bisulfite pyrosequencing and qRT-PCR (U6)	miR-10b	Assessing the risk of GC
Nishida *et al*[49] (JP)	87 GC	qRT-PCR (RNU6B)	miR-125a-5p	Prognostic marker
Li *et al*[65] (CN)	100 GC	real-time RT-PCR	miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p, miR-126	Survival prediction
Xu *et al*[64] (CN)	86 GC	RT-PCR (Let-7a)	miR-21	Prediction of LN metastasis
Wu *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 *et al*[102] (CN)	84 GC/84 C	qRT-PCR (U6)	let-7i	Prediction of chemotherapeutic sensitivity and prognosis
Chen *et al*[61] (CN)	3 GC/1 gastric mucosal lines 38 GC (28A, 8T, 2S)/10 C tissues	miRNA array and qPCR (U6)	miR-10a	Prediction of LN metastasis
Valladares-Ayerbes *et al*[100] (ES)	28 CC/7 GC/3 pancreas	qRT-PCR (5S and U6)	miR-17-92	Prognostic markers
Kogo *et al*[48] (JP)	90 GC/90 C	qRT-PCR (RNU6B)	miR-146a	Prognostic factor
Sacconi *et al*[46] (IT)	123 GC (75I, 24D, 24U)/123 C	miRNA microarray and qPCR (RNU6B)	miR-204	Prognostic value

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.

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 for-

Table 3 Circulating microRNAs as diagnostic biomarkers

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

C: Control; DCKO: 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 *et al*[81] (ES)	52 GC/31 C peripheral blood and 2 GC cell lines	qRT-PCR (U6, 5S)	miR-200c	Predictor of progression and survival
Komatsu *et al*[78] (JP)	69 GC plasma	qRT-PCR (U6)	miR-21	Prognostic marker
Wang *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 *et al*[80] (KR)	16 LN-positive GC/15 LN-negative GC/10 C serum	qRT-PCR	miR-21, miR-146a, and miR-148a	Predicting LN metastasis

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

mation[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 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]. 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 not only as targets of anticancer therapeutic approaches but also as predictive markers of drug resis-

tance and treatment response.

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 miRNAs 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[31,32], 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 plasma 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 a 87.5% sensitivity and a 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[48,50,78-80]. 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 type, 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[31,90]. 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 miRNAs 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 as a reliable method for determining cancer status. For this reason only the combination of several miRNAs 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 better prognostic value in personalized therapy for 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.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

3 **Okines A**, Verheij M, Allum W, Cunningham D, Cervantes A. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; **21** Suppl 5: v50-v54 [PMID: 20555102 DOI: 10.1093/annonc/mdq164]

4 **Songun I**, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. *Lancet Oncol* 2010; **11**: 439-449 [PMID: 20409751 DOI: 10.1016/S1470-2045(10)70070-X]

5 **Takahashi T**, Saikawa Y, Kitagawa Y. Gastric cancer: current status of diagnosis and treatment. *Cancers* (Basel) 2013; **5**: 48-63 [PMID: 24216698 DOI: 10.3390/cancers5010048]

6 **He CZ**, Zhang KH, Li Q, Liu XH, Hong Y, Lv NH. Combined use of AFP, CEA, CA125 and CA19-9 improves the sensitivity for the diagnosis of gastric cancer. *BMC Gastroenterol* 2013; **13**: 87 [PMID: 23672279 DOI: 10.1186/1471-230X-13-87]

7 **Sisik A**, Kaya M, Bas G, Basak F, Alimoglu O. CEA and CA 19-9 are still valuable markers for the prognosis of colorectal and gastric cancer patients. *Asian Pac J Cancer Prev* 2013; **14**: 4289-4294 [PMID: 23991991 DOI: 10.7314/APJCP.2013.14.7.4289]

8 **Cervantes A**, Roselló S, Roda D, Rodríguez-Braun E. The treatment of advanced gastric cancer: current strategies and future perspectives. *Ann Oncol* 2008; **19** Suppl 5: v103-v107 [PMID: 18611865 DOI: 10.1093/annonc/mdn321]

9 **Nakane Y**, Okamura S, Akehira K, Boku T, Okusa T, Tanaka K, Hioki K. Correlation of preoperative carcinoembryonic antigen levels and prognosis of gastric cancer patients. *Cancer* 1994; **73**: 2703-2708 [PMID: 8194009]

10 **Marrelli D**, Pinto E, De Stefano A, Farnetani M, Garosi L, Roviello F. Clinical utility of CEA, CA 19-9, and CA 72-4 in the follow-up of patients with resectable gastric cancer. *Am J Surg* 2001; **181**: 16-19 [PMID: 11248169 DOI: 10.1016/S0002-9610(00)00549-3]

11 **Reinhart BJ**, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000; **403**: 901-906 [PMID: 10706289 DOI: 10.1038/35002607]

12 **Brennecke J**, Hipfner DR, Stark A, Russell RB, Cohen SM. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* 2003; **113**: 25-36 [PMID: 12679032 DOI: 10.1016/S0092-8674(03)00231-9]

13 **Carrington JC**, Ambros V. Role of microRNAs in plant and animal development. *Science* 2003; **301**: 336-338 [PMID: 12869753 DOI: 10.1126/science.1085242]

14 **He L**, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 2005; **435**: 828-833 [PMID: 15944707 DOI: 10.1038/nature03552]

15 **Pasquinelli AE**, Ruvkun G. Control of developmental timing by micrornas and their targets. *Annu Rev Cell Dev Biol* 2002; **18**: 495-513 [PMID: 12142272 DOI: 10.1146/annurev.cellbio.18.012502.105832]

16 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

17 **Cummins JM**, He Y, Leary RJ, Pagliarini R, Diaz LA, Sjoblom T, Barad O, Bentwich Z, Szafranska AE, Labourier E, Raymond CK, Roberts BS, Juhl H, Kinzler KW, Vogelstein B, Velculescu VE. The colorectal microRNAome. *Proc Natl Acad Sci USA* 2006; **103**: 3687-3692 [PMID: 16505370 DOI: 10.1073/pnas.0511155103]

18 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]

19 **Bonci D**, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, Bartucci M, Muto G, Peschle C, De Maria R. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med* 2008; **14**: 1271-1277 [PMID: 18931683 DOI: 10.1038/nm.1880]

20 **Chang TC**, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell* 2007; **26**: 745-752 [PMID: 17540599 DOI: 10.1016/j.molcel.2007.05.010]

21 **Huang Q**, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Puré E, Agami R. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol* 2008; **10**: 202-210 [PMID: 18193036 DOI: 10.1038/ncb1681]

22 **Ma L**, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007; **449**: 682-688 [PMID: 17898713 DOI: 10.1038/nature06174]

23 **Tavazoie SF**, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008; **451**: 147-152 [PMID: 18185580 DOI: 10.1038/nature06487]

24 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA

expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]
25 **Volinia S**, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006; **103**: 2257-2261 [PMID: 16461460 DOI: 10.1073/pnas.0510565103]
26 **Rosenfeld N**, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, Benjamin H, Shabes N, Tabak S, Levy A, Lebanony D, Goren Y, Silberschein E, Targan N, Ben-Ari A, Gilad S, Sion-Vardy N, Tobar A, Feinmesser M, Kharenko O, Nativ O, Nass D, Perelman M, Yosepovich A, Shalmon B, Polak-Charcon S, Fridman E, Avniel A, Bentwich I, Bentwich Z, Cohen D, Chajut A, Barshack I. MicroRNAs accurately identify cancer tissue origin. *Nat Biotechnol* 2008; **26**: 462-469 [PMID: 18362881 DOI: 10.1038/nbt1392]
27 **Sun Y**, Zhang K, Fan G, Li J. Identification of circulating microRNAs as biomarkers in cancers: what have we got? *Clin Chem Lab Med* 2012; **50**: 2121-2126 [PMID: 23087086 DOI: 10.1515/cclm-2012-0360]
28 **Zandberga E**, Kozirovskis V, Ābols A, Andrējeva D, Purkalne G, Linē A. Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. *Genes Chromosomes Cancer* 2013; **52**: 356-369 [PMID: 23404859 DOI: 10.1002/gcc.22032]
29 **Taylor DD**, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 2008; **110**: 13-21 [PMID: 18589210 DOI: 10.1016/j.ygyno.2008.04.033]
30 **Arroyo JD**, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA* 2011; **108**: 5003-5008 [PMID: 21383194 DOI: 10.1073/pnas.1019055108]
31 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]
32 **Gilad S**, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum microRNAs are promising novel biomarkers. *PLoS One* 2008; **3**: e3148 [PMID: 18773077 DOI: 10.1371/journal.pone.0003148]
33 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]
34 **Ng EK**, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS, Sung JJ. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* 2009; **58**: 1375-1381 [PMID: 19201770 DOI: 10.1136/gut.2008.167817]
35 **Resnick KE**, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol Oncol* 2009; **112**: 55-59 [PMID: 18954897 DOI: 10.1016/j.ygyno.2008.08.036]
36 **Lawrie CH**, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* 2008; **141**: 672-675 [PMID: 18318758 DOI: 10.1111/j.1365-2141.2008.07077.x]
37 **Carrasco G**, Corvalan AH. Helicobacter pylori-Induced Chronic Gastritis and Assessing Risks for Gastric Cancer. *Gastroenterol Res Pract* 2013; **2013**: 393015 [PMID: 23983680 DOI: 10.1155/2013/393015]
38 **Conteduca V**, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. H. pylori infection and gastric cancer: state of the art (review). *Int J Oncol* 2013; **42**: 5-18 [PMID: 23165522 DOI: 10.3892/ijo.2012.1701]
39 **Song J**, Bai Z, Zhang Z. MicroRNAs are implicated in the initiation and progression of gastric cancer. *Chin Med J* (Engl) 2014; **127**: 554-559 [PMID: 24451966 DOI: 10.3760/cma.j.issn.0366-6999.20122918]
40 **Song S**, Ajani JA. The role of microRNAs in cancers of the upper gastrointestinal tract. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 109-118 [PMID: 23165235 DOI: 10.1038/nrgastro.2012.210]
41 **Yasui W**, Sentani K, Sakamoto N, Anami K, Naito Y, Oue N. Molecular pathology of gastric cancer: research and practice. *Pathol Res Pract* 2011; **207**: 608-612 [PMID: 22005013 DOI: 10.1016/j.prp.2011.09.006]
42 **Wang M**, Gu H, Qian H, Zhu W, Zhao C, Zhang X, Tao Y, Zhang L, Xu W. miR-17-5p/20a are important markers for gastric cancer and murine double minute 2 participates in their functional regulation. *Eur J Cancer* 2013; **49**: 2010-2021 [PMID: 23333058 DOI: 10.1016/j.ejca.2012.12.017]
43 **Petrocca F**, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, Iliopoulos D, Pilozzi E, Liu CG, Negrini M, Cavazzini L, Volinia S, Alder H, Ruco LP, Baldassarre G, Croce CM, Vecchione A. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell* 2008; **13**: 272-286 [PMID: 18328430 DOI: 10.1016/j.ccr.2008.02.013]
44 **Wu Q**, Jin H, Yang Z, Luo G, Lu Y, Li K, Ren G, Su T, Pan Y, Feng B, Xue Z, Wang X, Fan D. MiR-150 promotes gastric cancer proliferation by negatively regulating the pro-apoptotic gene EGR2. *Biochem Biophys Res Commun* 2010; **392**: 340-345 [PMID: 20067763 DOI: 10.1016/j.bbrc.2009.12.182]
45 **Unoki M**, Nakamura Y. EGR2 induces apoptosis in various cancer cell lines by direct transactivation of BNIP3L and BAK. *Oncogene* 2003; **22**: 2172-2185 [PMID: 12687019 DOI: 10.1038/sj.onc.1206222]
46 **Sacconi A**, Biagioni F, Canu V, Mori F, Di Benedetto A, Lorenzon L, Ercolani C, Di Agostino S, Cambria AM, Germoni S, Grasso G, Blandino R, Panebianco V, Ziparo V, Federici O, Muti P, Strano S, Carboni F, Mottolese M, Diodoro M, Pescarmona E, Garofalo A, Blandino G. miR-204 targets Bcl-2 expression and enhances responsiveness of gastric cancer. *Cell Death Dis* 2012; **3**: e423 [PMID: 23152059 DOI: 10.1038/cddis.2012.160]
47 **Tsukamoto Y**, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, Hijiya N, Matsuura K, Fujioka T, Seto M, Moriyama M. MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. *Cancer Res* 2010; **70**: 2339-2349 [PMID: 20215506 DOI: 10.1158/0008-5472.CAN-09-2777]
48 **Kogo R**, Mimori K, Tanaka F, Komune S, Mori M. Clinical significance of miR-146a in gastric cancer cases. *Clin Cancer Res* 2011; **17**: 4277-4284 [PMID: 21632853 DOI: 10.1158/1078-0432.CCR-10-2866]
49 **Nishida N**, Mimori K, Fabbri M, Yokobori T, Sudo T, Tanaka F, Shibata K, Ishii H, Doki Y, Mori M. MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric cancer cells in combination with trastuzumab. *Clin Cancer Res* 2011; **17**: 2725-2733 [PMID: 21220473 DOI: 10.1158/1078-0432.CCR-10-2132]

50 **Song YX**, Yue ZY, Wang ZN, Xu YY, Luo Y, Xu HM, Zhang X, Jiang L, Xing CZ, Zhang Y. MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. *Mol Cancer* 2011; **10**: 1 [PMID: 21205300 DOI: 10.1186/1476-4598-10-1]

51 **Chen Y**, Song Y, Wang Z, Yue Z, Xu H, Xing C, Liu Z. Altered expression of MiR-148a and MiR-152 in gastrointestinal cancers and its clinical significance. *J Gastrointest Surg* 2010; **14**: 1170-1179 [PMID: 20422307 DOI: 10.1007/s11605-010-1202-2]

52 **Motoyama K**, Inoue H, Mimori K, Tanaka F, Kojima K, Uetake H, Sugihara K, Mori M. Clinicopathological and prognostic significance of PDCD4 and microRNA-21 in human gastric cancer. *Int J Oncol* 2010; **36**: 1089-1095 [PMID: 20372781 DOI: 10.3892/ijo_00000590]

53 **Xiao B**, Guo J, Miao Y, Jiang Z, Huan R, Zhang Y, Li D, Zhong J. Detection of miR-106a in gastric carcinoma and its clinical significance. *Clin Chim Acta* 2009; **400**: 97-102 [PMID: 18996365 DOI: 10.1016/j.cca.2008.10.021]

54 **Wang WN**, Chen Y, Zhang YD, Hu TH. The regulatory mechanism of CCR7 gene expression and its involvement in the metastasis and progression of gastric cancer. *Tumour Biol* 2013; **34**: 1865-1871 [PMID: 23519840 DOI: 10.1007/s13277-013-0728-9]

55 **di Mario F**, Cavallaro LG. Non-invasive tests in gastric diseases. *Dig Liver Dis* 2008; **40**: 523-530 [PMID: 18439884 DOI: 10.1016/j.dld.2008.02.028]

56 **Wagner AD**, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. *J Clin Oncol* 2006; **24**: 2903-2909 [PMID: 16782930 DOI: 10.1200/JCO.2005.05.0245]

57 **Zhang WH**, Gui JH, Wang CZ, Chang Q, Xu SP, Cai CH, Li YN, Tian YP, Yan L, Wu B. The identification of miR-375 as a potential biomarker in distal gastric adenocarcinoma. *Oncol Res* 2012; **20**: 139-147 [PMID: 23461060 DOI: 10.3727/096504012X13522227232156]

58 **Chan SH**, Wu CW, Li AF, Chi CW, Lin WC. miR-21 microRNA expression in human gastric carcinomas and its clinical association. *Anticancer Res* 2008; **28**: 907-911 [PMID: 18507035]

59 **Zhang Y**, Guo J, Li D, Xiao B, Miao Y, Jiang Z, Zhuo H. Down-regulation of miR-31 expression in gastric cancer tissues and its clinical significance. *Med Oncol* 2010; **27**: 685-689 [PMID: 19598010 DOI: 10.1007/s12032-009-9269-x]

60 **Jiang Z**, Guo J, Xiao B, Miao Y, Huang R, Li D, Zhang Y. Increased expression of miR-421 in human gastric carcinoma and its clinical association. *J Gastroenterol* 2010; **45**: 17-23 [PMID: 19802518 DOI: 10.1007/s00535-009-0135-6]

61 **Chen W**, Tang Z, Sun Y, Zhang Y, Wang X, Shen Z, Liu F, Qin X. miRNA expression profile in primary gastric cancers and paired lymph node metastases indicates that miR-10a plays a role in metastasis from primary gastric cancer to lymph nodes. *Exp Ther Med* 2012; **3**: 351-356 [PMID: 22969895 DOI: 10.3892/etm.2011.411]

62 **Liu K**, Li G, Fan C, Diao Y, Wu B, Li J. Increased Expression of MicroRNA-221 in gastric cancer and its clinical significance. *J Int Med Res* 2012; **40**: 467-474 [PMID: 22613407 DOI: 10.1177/147323001204000208]

63 **Wu WY**, Xue XY, Chen ZJ, Han SL, Huang YP, Zhang LF, Zhu GB, Shen X. Potentially predictive microRNAs of gastric cancer with metastasis to lymph node. *World J Gastroenterol* 2011; **17**: 3645-3651 [PMID: 21987613 DOI: 10.3748/wjg.v17.i31.3645]

64 **Xu Y**, Sun J, Xu J, Li Q, Guo Y, Zhang Q. miR-21 Is a Promising Novel Biomarker for Lymph Node Metastasis in Patients with Gastric Cancer. *Gastroenterol Res Pract* 2012; **2012**: 640168 [PMID: 22792096 DOI: 10.1155/2012/640168]

65 **Li X**, Zhang Y, Zhang Y, Ding J, Wu K, Fan D. Survival prediction of gastric cancer by a seven-microRNA signature. *Gut* 2010; **59**: 579-585 [PMID: 19951901 DOI: 10.1136/gut.2008.175497]

66 **Yan Z**, Xiong Y, Xu W, Gao J, Cheng Y, Wang Z, Chen F, Zheng G. Identification of hsa-miR-335 as a prognostic signature in gastric cancer. *PLoS One* 2012; **7**: e40037 [PMID: 22802949 DOI: 10.1371/journal.pone.0040037]

67 **Tsai KW**, Liao YL, Wu CW, Hu LY, Li SC, Chan WC, Ho MR, Lai CH, Kao HW, Fang WL, Huang KH, Lin WC. Aberrant expression of miR-196a in gastric cancers and correlation with recurrence. *Genes Chromosomes Cancer* 2012; **51**: 394-401 [PMID: 22420029 DOI: 10.1002/gcc.21924]

68 **Zhang X**, Yan Z, Zhang J, Gong L, Li W, Cui J, Liu Y, Gao Z, Li J, Shen L, Lu Y. Combination of hsa-miR-375 and hsa-miR-142-5p as a predictor for recurrence risk in gastric cancer patients following surgical resection. *Ann Oncol* 2011; **22**: 2257-2266 [PMID: 21343377 DOI: 10.1093/annonc/mdq758]

69 **Tarasov V**, Jung P, Verdoodt B, Lodygin D, Epanchintsev A, Menssen A, Meister G, Hermeking H. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* 2007; **6**: 1586-1593 [PMID: 17554199 DOI: 10.4161/cc.6.13.4436]

70 **Xia L**, Zhang D, Du R, Pan Y, Zhao L, Sun S, Hong L, Liu J, Fan D. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *Int J Cancer* 2008; **123**: 372-379 [PMID: 18449891 DOI: 10.1002/ijc.23501]

71 **Shang Y**, Zhang Z, Liu Z, Feng B, Ren G, Li K, Zhou L, Sun Y, Li M, Zhou J, An Y, Wu K, Nie Y, Fan D. miR-508-5p regulates multidrug resistance of gastric cancer by targeting ABCB1 and ZNRD1. *Oncogene* 2014; **33**: 3267-3276 [PMID: 23893241 DOI: 10.1038/onc.2013.297]

72 **Rotkrua P**, Shimada S, Mogushi K, Akiyama Y, Tanaka H, Yuasa Y. Circulating microRNAs as biomarkers for early detection of diffuse-type gastric cancer using a mouse model. *Br J Cancer* 2013; **108**: 932-940 [PMID: 23385731 DOI: 10.1038/bjc.2013.30]

73 **Tsujiura M**, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, Konishi H, Morimura R, Deguchi K, Fujiwara H, Okamoto K, Otsuji E. Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010; **102**: 1174-1179 [PMID: 20234369 DOI: 10.1038/sj.bjc.6605608]

74 **Li C**, Li JF, Cai Q, Qiu QQ, Yan M, Liu BY, Zhu ZG. MiRNA-199a-3p: A potential circulating diagnostic biomarker for early gastric cancer. *J Surg Oncol* 2013; **108**: 89-92 [PMID: 23733518 DOI: 10.1002/jso.23358]

75 **Li C**, Li JF, Cai Q, Qiu QQ, Yan M, Liu BY, Zhu ZG. miRNA-199a-3p in plasma as a potential diagnostic biomarker for gastric cancer. *Ann Surg Oncol* 2013; **20** Suppl 3: S397-S405 [PMID: 22956063 DOI: 10.1245/s10434-012-2600-3]

76 **Cai H**, Yuan Y, Hao YF, Guo TK, Wei X, Zhang YM. Plasma microRNAs serve as novel potential biomarkers for early detection of gastric cancer. *Med Oncol* 2013; **30**: 452 [PMID: 23307259 DOI: 10.1007/s12032-012-0452-0]

77 **Liu H**, Zhu L, Liu B, Yang L, Meng X, Zhang W, Ma Y, Xiao H. Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. *Cancer Lett* 2012; **316**: 196-203 [PMID: 22169097 DOI: 10.1016/j.canlet.2011.10.034]

78 **Komatsu S**, Ichikawa D, Tsujiura M, Konishi H, Takeshita H, Nagata H, Kawaguchi T, Hirajima S, Arita T, Shiozaki A, Kubota T, Fujiwara H, Okamoto K, Otsuji E. Prognostic impact of circulating miR-21 in the plasma of patients with gastric carcinoma. *Anticancer Res* 2013; **33**: 271-276 [PMID: 23267156]

79 **Wang M**, Gu H, Wang S, Qian H, Zhu W, Zhang L, Zhao C, Tao Y, Xu W. Circulating miR-17-5p and miR-20a: molecular markers for gastric cancer. *Mol Med Rep* 2012; **5**: 1514-1520 [PMID: 22406928 DOI: 10.3892/mmr.2012.828]

80 **Kim SY**, Jeon TY, Choi CI, Kim DH, Kim DH, Kim GH, Ryu DY, Lee BE, Kim HH. Validation of circulating miRNA biomarkers for predicting lymph node metastasis in gastric cancer. *J Mol Diagn* 2013; **15**: 661-669 [PMID: 23806809 DOI:

10.1016/j.jmoldx.2013.04.004]
81 **Valladares-Ayerbes M**, Reboredo M, Medina-Villaamil V, Iglesias-Díaz P, Lorenzo-Patiño MJ, Haz M, Santamarina I, Blanco M, Fernández-Tajes J, Quindós M, Carral A, Figueroa A, Antón-Aparicio LM, Calvo L. Circulating miR-200c as a diagnostic and prognostic biomarker for gastric cancer. *J Transl Med* 2012; **10**: 186 [PMID: 22954417 DOI: 10.1186/1479-5876-10-186]
82 **Liu R**, Zhang C, Hu Z, Li G, Wang C, Yang C, Huang D, Chen X, Zhang H, Zhuang R, Deng T, Liu H, Yin J, Wang S, Zen K, Ba Y, Zhang CY. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur J Cancer* 2011; **47**: 784-791 [PMID: 21112772 DOI: 10.1016/j.ejca.2010.10.025]
83 **Zhou H**, Xiao B, Zhou F, Deng H, Zhang X, Lou Y, Gong Z, Du C, Guo J. MiR-421 is a functional marker of circulating tumor cells in gastric cancer patients. *Biomarkers* 2012; **17**: 104-110 [PMID: 22263628 DOI: 10.3109/1354750X.2011.614961]
84 **Zheng Y**, Cui L, Sun W, Zhou H, Yuan X, Huo M, Chen J, Lou Y, Guo J. MicroRNA-21 is a new marker of circulating tumor cells in gastric cancer patients. *Cancer Biomark* 2011-2012; **10**: 71-77 [PMID: 22430134 DOI: 10.3233/CBM-2011-0231]
85 **Zhou H**, Guo JM, Lou YR, Zhang XJ, Zhong FD, Jiang Z, Cheng J, Xiao BX. Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. *J Mol Med* (Berl) 2010; **88**: 709-717 [PMID: 20349219 DOI: 10.1007/s00109-010-0617-2]
86 **Cui L**, Zhang X, Ye G, Zheng T, Song H, Deng H, Xiao B, Xia T, Yu X, Le Y, Guo J. Gastric juice MicroRNAs as potential biomarkers for the screening of gastric cancer. *Cancer* 2013; **119**: 1618-1626 [PMID: 23335180 DOI: 10.1002/cncr.27903]
87 **Yu X**, Luo L, Wu Y, Yu X, Liu Y, Yu X, Zhao X, Zhang X, Cui L, Ye G, Le Y, Guo J. Gastric juice miR-129 as a potential biomarker for screening gastric cancer. *Med Oncol* 2013; **30**: 365 [PMID: 23307240 DOI: 10.1007/s12032-012-0365-y]
88 **Zhang X**, Cui L, Ye G, Zheng T, Song H, Xia T, Yu X, Xiao B, Le Y, Guo J. Gastric juice microRNA-421 is a new biomarker for screening gastric cancer. *Tumour Biol* 2012; **33**: 2349-2355 [PMID: 22926798 DOI: 10.1007/s13277-012-0497-x]
89 **Song J**, Bai Z, Han W, Zhang J, Meng H, Bi J, Ma X, Han S, Zhang Z. Identification of suitable reference genes for qPCR analysis of serum microRNA in gastric cancer patients. *Dig Dis Sci* 2012; **57**: 897-904 [PMID: 22198701 DOI: 10.1007/s10620-011-1981-7]
90 **Kroh EM**, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods* 2010; **50**: 298-301 [PMID: 20146939 DOI: 10.1016/j.ymeth.2010.01.032]
91 **Li BS**, Zhao YL, Guo G, Li W, Zhu ED, Luo X, Mao XH, Zou QM, Yu PW, Zuo QF, Li N, Tang B, Liu KY, Xiao B. Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. *PLoS One* 2012; **7**: e41629 [PMID: 22860003 DOI: 10.1371/journal.pone.0041629]
92 **Wang B**, Zhang Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol* 2012; **138**: 1659-1666 [PMID: 22638884 DOI: 10.1007/s00432-012-1244-9]
93 **Song MY**, Pan KF, Su HJ, Zhang L, Ma JL, Li JY, Yuasa Y, Kang D, Kim YS, You WC. Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. *PLoS One* 2012; **7**: e33608 [PMID: 22432036 DOI: 10.1371/journal.pone.0033608]
94 **Konishi H**, Ichikawa D, Komatsu S, Shiozaki A, Tsujiura M, Takeshita H, Morimura R, Nagata H, Arita T, Kawaguchi T, Hirashima S, Fujiwara H, Okamoto K, Otsuji E. Detection of gastric cancer-associated microRNAs on microRNA microarray comparing pre- and post-operative plasma. *Br J Cancer* 2012; **106**: 740-747 [PMID: 22262318 DOI: 10.1038/bjc.2011.588]
95 **Gorur A**, Balci Fidanci S, Dogruer Unal N, Ayaz L, Akbayir S, Yildirim Yaroglu H, Dirlik M, Serin MS, Tamer L. Determination of plasma microRNA for early detection of gastric cancer. *Mol Biol Rep* 2013; **40**: 2091-2096 [PMID: 23212612 DOI: 10.1007/s11033-012-2267-7]
96 **Inoue T**, Iinuma H, Ogawa E, Inaba T, Fukushima R. Clinicopathological and prognostic significance of microRNA-107 and its relationship to DICER1 mRNA expression in gastric cancer. *Oncol Rep* 2012; **27**: 1759-1764 [PMID: 22407237 DOI: 10.3892/or.2012]
97 **Brenner B**, Hoshen MB, Purim O, David MB, Ashkenazi K, Marshak G, Kundel Y, Brenner R, Morgenstern S, Halpern M, Rosenfeld N, Chajut A, Niv Y, Kushnir M. MicroRNAs as a potential prognostic factor in gastric cancer. *World J Gastroenterol* 2011; **17**: 3976-3985 [PMID: 22046085 DOI: 10.3748/wjg.v17.i35.3976]
98 **Xiao B**, Zhu ED, Li N, Lu DS, Li W, Li BS, Zhao YL, Mao XH, Guo G, Yu PW, Zou QM. Increased miR-146a in gastric cancer directly targets SMAD4 and is involved in modulating cell proliferation and apoptosis. *Oncol Rep* 2012; **27**: 559-566 [PMID: 22020746 DOI: 10.3892/or.2011.1514]
99 **Kim K**, Lee HC, Park JL, Kim M, Kim SY, Noh SM, Song KS, Kim JC, Kim YS. Epigenetic regulation of microRNA-10b and targeting of oncogenic MAPRE1 in gastric cancer. *Epigenetics* 2011; **6**: 740-751 [PMID: 21562367 DOI: 10.4161/epi.6.6.15874]
100 **Valladares-Ayerbes M**, Blanco M, Haz M, Medina V, Iglesias-Díaz P, Lorenzo-Patiño MJ, Reboredo M, Santamarina I, Figueroa A, Antón-Aparicio LM, Calvo L. Prognostic impact of disseminated tumor cells and microRNA-17-92 cluster deregulation in gastrointestinal cancer. *Int J Oncol* 2011; **39**: 1253-1264 [PMID: 21743960 DOI: 10.3892/ijo.2011.1112]
101 **Su Y**, Ni Z, Wang G, Cui J, Wei C, Wang J, Yang Q, Xu Y, Li F. Aberrant expression of microRNAs in gastric cancer and biological significance of miR-574-3p. *Int Immunopharmacol* 2012; **13**: 468-475 [PMID: 22683180 DOI: 10.1016/j.intimp.2012.05.016]
102 **Liu K**, Qian T, Tang L, Wang J, Yang H, Ren J. Decreased expression of microRNA let-7i and its association with chemotherapeutic response in human gastric cancer. *World J Surg Oncol* 2012; **10**: 225 [PMID: 23107361 DOI: 10.1186/1477-7819-10-225]
103 **Peng Y**, Liu YM, Li LC, Wang LL, Wu XL. MicroRNA-338 inhibits growth, invasion and metastasis of gastric cancer by targeting NRP1 expression. *PLoS One* 2014; **9**: e94422 [PMID: 24736504 DOI: 10.1371/journal.pone.0094422]
104 **Wu Q**, Yang Z, An Y, Hu H, Yin J, Zhang P, Nie Y, Wu K, Shi Y, Fan D. MiR-19a/b modulate the metastasis of gastric cancer cells by targeting the tumour suppressor MXD1. *Cell Death Dis* 2014; **5**: e1144 [PMID: 24675462 DOI: 10.1038/cddis.2014.110]
105 **Zhao Y**, Huang J, Zhang L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B, Zhu Z. MiR-133b is frequently decreased in gastric cancer and its overexpression reduces the metastatic potential of gastric cancer cells. *BMC Cancer* 2014; **14**: 34 [PMID: 24443799 DOI: 10.1186/1471-2407-14-34]
106 **Zhao Y**, Li C, Wang M, Su L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B, Zhu Z. Decrease of miR-202-3p expression, a novel tumor suppressor, in gastric cancer. *PLoS One* 2013; **8**: e69756 [PMID: 23936094 DOI: 10.1371/journal.pone.0069756]
107 **Chen L**, Lü MH, Zhang D, Hao NB, Fan YH, Wu YY, Wang SM, Xie R, Fang DC, Zhang H, Hu CJ, Yang SM. miR-1207-5p and miR-1266 suppress gastric cancer growth and invasion by targeting telomerase reverse transcriptase. *Cell Death Dis* 2014; **5**: e1034 [PMID: 24481448 DOI: 10.1038/cddis.2013.553]
108 **Deng M**, Tang HL, Lu XH, Liu MY, Lu XM, Gu YX, Liu JF,

He ZM. miR-26a suppresses tumor growth and metastasis by targeting FGF9 in gastric cancer. *PLoS One* 2013; **8**: e72662 [PMID: 24015269 DOI: 10.1371/journal.pone.0072662]

109 **Jiping Z**, Ming F, Lixiang W, Xiuming L, Yuqun S, Han Y, Zhifang L, Yundong S, Shili L, Chunyan C, Jihui J. MicroRNA-212 inhibits proliferation of gastric cancer by directly repressing retinoblastoma binding protein 2. *J Cell Biochem* 2013; **114**: 2666-2672 [PMID: 23794145 DOI: 10.1002/jcb.24613]

110 **Flamant S**, Ritchie W, Guilhot J, Holst J, Bonnet ML, Chomel JC, Guilhot F, Turhan AG, Rasko JE. Micro-RNA response to imatinib mesylate in patients with chronic myeloid leukemia. *Haematologica* 2010; **95**: 1325-1333 [PMID: 20460641 DOI: 10.3324/haematol.2009.020636]

111 **Scholl V**, Hassan R, Zalcberg IR. miRNA-451: A putative predictor marker of Imatinib therapy response in chronic myeloid leukemia. *Leuk Res* 2012; **36**: 119-121 [PMID: 21944890 DOI: 10.1016/j.leukres.2011.08.023]

112 **Zhou C**, Liu J, Li Y, Liu L, Zhang X, Ma CY, Hua SC, Yang M, Yuan Q. microRNA-1274a, a modulator of sorafenib induced a disintegrin and metalloproteinase 9 (ADAM9) down-regulation in hepatocellular carcinoma. *FEBS Lett* 2011; **585**: 1828-1834 [PMID: 21530512 DOI: 10.1016/j.febslet.2011.04.040]

113 **Bai S**, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, Yadav A, Nuovo G, Kumar P, Ghoshal K. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem* 2009; **284**: 32015-32027 [PMID: 19726678 DOI: 10.1074/jbc.M109.016774]

114 **Jiang J**, Zheng X, Xu X, Zhou Q, Yan H, Zhang X, Lu B, Wu C, Ju J. Prognostic significance of miR-181b and miR-21 in gastric cancer patients treated with S-1/Oxaliplatin or Doxifluridine/Oxaliplatin. *PLoS One* 2011; **6**: e23271 [PMID: 21876743 DOI: 10.1371/journal.pone.0023271]

115 **Chen YJ**, Zhu JM, Wu H, Fan J, Zhou J, Hu J, Yu Q, Liu TT, Yang L, Wu CL, Guo XL, Huang XW, Shen XZ. Circulating microRNAs as a Fingerprint for Liver Cirrhosis. *PLoS One* 2013; **8**: e66577 [PMID: 23805240 DOI: 10.1371/journal.pone.0066577]

116 **Liu N**, Cui RX, Sun Y, Guo R, Mao YP, Tang LL, Jiang W, Liu X, Cheng YK, He QM, Cho WC, Liu LZ, Li L, Ma J. A four-miRNA signature identified from genome-wide serum miRNA profiling predicts survival in patients with nasopharyngeal carcinoma. *Int J Cancer* 2014; **134**: 1359-1368 [PMID: 23999999 DOI: 10.1002/ijc.28468]

P- Reviewer: Ierardi E, Link A, Nagahara A, Sugimura H, Wang YH
S- Editor: Qi Y **L- Editor**: Wang TQ **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327