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MicroRNAs in gastric cancer: from bench to bedside

Minireview

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Gastric carcinogenesis results from complex interactions between host and environmental and bacterial factors, and this leads to genetic and epigenetic deregulation of oncogenic and tumor-suppressive genes. MicroRNAs (miRNAs) are a class of small noncoding RNAs which regulate almost 30% of human genes post transcriptionally and they are crucial in the initiation and progression of various diseases; especially malignancies. Accumulated evidence documents changes in gene sequences and epigenetic modifications. These then lead to abnormal miRNA expression in gastric cancer (GC) and also to deregulated miRNAs which act as oncogenes or tumor suppressors by regulating related target genes and contributing to malignant phenotypes. This altered miRNA expression in body fluids could well provide a novel biomarker for GC patient diagnosis and prognosis. MiRNAs present a promising target for GC treatment, and more tempting, for eradication of gastric cancer stem cells. This latter sub-group of tumor cells is thought to initiate and maintain GC development. Herein, we review the aberrant expression of miRNA expression and the underlying mechanisms and consequential effects of miRNA de-regulation. This identifies the responsible gastric cancer target genes, and highlights potential clinical applications.

Key words: gastric cancer, microRNAs, expression dysregulation, therapeutic target

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer-related death worldwide. It is especially common in Asia, Latin America, and Central and Eastern Europe and it constitutes a major burden of disability-adjusted life-years [1, 2]. The pathogenesis of GC is a complex multi-step process with interaction between host and environmental and bacterial factors, wherein *Helicobacter pylori* (*H pylori*) infection is a well-documented high-risk factor [3, 4].

While gastrectomy is the principal treatment for patients with early-stage GC, the absence of specific symptoms in the early-stage renders most GC patients diagnosed at non-surgical stages. Systemic chemotherapy is then the major treatment option for these patients, but resistance makes treatment ineffective [5]. The current prognosis for advanced stage patients therefore remains very poor, with advanced GC patient median survival less than 12 months [6]. Investigating novel biomarkers for early diagnosis and other effective therapies based on better understanding of the mechanisms involved in gastric carcinogenesis and drug-resistance is urgently required to improve patient outcome.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs (ncRNAs) with approximately 18–25 nucleotides. The biogenesis of miRNAs has been identified in excellent reviews [7, 8], with miRNAs functioning by combining the 3'-untranslated region (UTR) of the messenger RNAs (mRNAs) of their cognate target genes. This leads to silenced expression by cleaving the mRNA molecules or inhibiting their translation [9]. Thus, approximately 30% of human genes are modulated by miRNAs which mediate their regulation in physiological processes such as differentiation, proliferation, migration and apoptosis [10]. Because more than 50% miRNAs are located in cancer-associated genomic regions or fragile sites, miRNA genetic alteration is considered to participate in tumorigenesis, and this has been confirmed in multiple cancers; including GC [11, 12]. De-regulated

miRNAs contribute to malignant GC phenotypes, including tumor growth, angiogenesis, metastasis and drug-resistance by regulating critical cellular processes as tumor suppressors or oncogenes [5, 13, 14].

Herein, we discuss aberrant miRNA expression and resultant effects, and investigate the targets genes in gastric oncogenesis and the mechanisms underlying miRNA de-regulation. We then present the clinical application of miRNAs as novel biomarkers for diagnosis and prognosis based on their role in GC and also susceptibility prediction for these patients. The potential roles of miRNAs as therapeutic targets for GC, especially in modifying chemo-resistance and cancer stem cell (CSC) treatment, are then addressed and we introduce the general role of long non-coding RNAs (lncRNAs) in gastric cancer.

Expression and function of miRNAs in GC

Accumulated evidence documents the differential expression of specific miRNAs in GC cell lines and tumor tissues. Due to the extensive regulatory function of miRNAs in gene expression, the de-regulated miRNAs cause oncogenic activity in almost all tumorigenesis and progression; especially cell proliferation, migration, invasion and apoptosis. Oncogenic miRNAs facilitate GC tumor development with their aberrantly high expression, and silenced or lost expression of tumor-suppressive miRNAs contribute to the oncogenetic effect. Tables 1 and 2 herein summarize miRNA de-regulation and the implicated carcinogenic processes.

Tsukamoto et al. investigated the miRNA expression profiles of 22 surgically resected GC tissues and identified 39 miRNAs with different expression in tumor and non-neoplastic tissues. Of these, 6 miRNAs had comparatively lower expression in GC and the remaining 33 were up-regulated [15]. MiR-28 is a particular contributor to GC cell proliferation and invasion and it is up-regulated in 31 GC tissues compared to matched adjacent normal gastric tissues ($p<0.05$). This higher expression is also observed in a series of GC cell lines compared to controls [16]. Zhang et al. then established that relatively higher miR-21 oncogene expression was detected in 80% of the 30 tested surgical specimens of gastric cancer compared to normal matched tissues.

Table 1. Summary of miRNAs profiling in GC.

Sample	Detection methods	Upregulated miRNAs	Deregulated miRNAs	Refs
3 pairs of GC tissue and normal tissue	microarray	miR-223, miR-106b, miR-147, miR-34a, miR-130b*, miR-106a, miR-18a, miR-17, miR-98, miR-616*, miR-181a-2*, miR-185, miR-1259, miR-601, miR-196a*, miR-221*, miR-302f, miR-340*, miR-337-3p, miR-520c-3p, miR-575 and miR-138	miR-638 and miR-378	[77]
42 undifferentiated GC tissues and paired normal tissue	qRT-PCR	miR-34b, miR-34c and miR-128a	miR-128b, miR-129 and miR-148	[78]
6 primary GC tissue samples and the corresponding non-neoplastic mucosa	microarray	miR-222, miR-25, miR-106b, miR-21, miR-107, miR-103, miR-145, miR-7, miR-214, miR-30b, miR-93, miR-24, miR-143, miR-320, miR-92, miR-125b, miR-27a, miR-23b, let-7a, let-7b	miR-146a, miR-148a	[79]
20 pairs of primary GC and corresponding noncancerous gastric tissues	RT-PCR	miR-223, miR-21, miR-23b, miR-222, miR-25, miR-23a, miR-221, miR-107, miR-103, miR-99a, miR-100, miR-125b, miR-92, miR-146a, miR-214 and miR-191	let-7a, miR-126, miR-210, miR-181b, miR-197, and miR-30aa-5p	[80]
90 pretreatment GC samples and 34 normal gastric mucosal biopsy samples	microarray	miR-25, miR-106b, miR-93, miR-503, miR-18a, miR-224, miR-451, miR-18b, miR-17-5p, miR-486-5p, miR-144, miR-552, miR-425-5p, miR-92, miR-106a, miR-223, miR-205, miR-196b, miR-19a, miR-191, let-7i, miR-185, miR-769-5p, miR-301, miR-21, miR-130b, miR-19b, miR-424, miR-484, miR-767-5p, miR-183, miR-210, miR-302c*, miR-520g, miR-324-5p, miR-103, miR-376b, miR-151, miR-596, miR-545, miR-221, miR-20a, miR-181b, miR-181d, miR-623, miR-519d, miR-563, miR-505, miR-107, miR-320, miR-96, miR-339, miR-181a, miR-345, miR-20b, miR-33b, miR-135b, miR-431, miR-193a, miR-550, miR-565	miR-146a, miR-133a, miR-625, miR-375, miR-133b, miR-195, miR-148a, miR-1, miR-26a, miR-204, let-7c, let-7a, let-7g, miR-497, miR-26b, miR-145, miR-34a, miR-143, miR-650, miR-150, miR-768-5p, let-7d, miR-203, miR-29c, let-7f, miR-30d, miR-642, miR-30c, miR-155, miR-34b, miR-551b, miR-28, let-7e, let-7b, miR-212, miR-564, miR-770-5p, miR-30b, miR-30a-5p, miR-199b, miR-125a, miR-621, miR-31, miR-365, miR-381, miR-626, miR-127, miR-660, miR-342, miR-146b, miR-361, miR-489, miR-29a, miR-95, miR-567, miR-152, miR-429, miR-200b, miR-504, miR-668, miR-186, miR-135a, miR-485-5p	[81]
184 GC sample and 169 non-tumor mucosae	microarray	miR-181d, miR-181a-1, miR-a-2, miR-181c, miR-b-1, miR-181b-2, miR-21, miR-25, miR-92-1, miR-92-2, miR-93, miR-17-5p, miR-106a, miR-20b, miR-135a-1, miR-135a-2, miR-425-5p, miR-106b, miR-20a, miR-19b-1, miR-19b-2, miR-224, miR-18a, miR-135b, miR-19a, miR-345, miR-191	miR-148a, miR-148b, miR-375, miR-29b-1, miR-29b-2, miR-29c, miR-152, miR-218-2, miR-451, miR-30d, miR-30a-5p, miR-30b, miR-30c-1, miR-30c-2, miR-422b	[82]

Table 2. Deregulated miRNAexpression and target genes in GC.

miRNAs	Studied samples	Detection methods	Expression level	Target genes	Implicated processes	Refs
miR-1	Tissues and cell lines	qPCR	Down-regulated	VEGF-A, EDN1, MET	proliferation and migration of tumor cells and endothelial cells,	[41]
miR-141	Tissues and cell lines	RT-PCR	Down-regulated	ZEB1, STAT4	Cell proliferation, apoptosis and invasion	[45, 83]
miR-106b miR-93	Tissues and cell lines	miRNA micro-array, qRT-PCR	Up-regulated	E2F1, CDKN1A (p21)	Cell cycle	[10]
miR-126	Tissues and cell lines	qRT-PCR	Up-regulated/ Down-regulated	PI3KR2, VEGF-A, Crk, SOX2	cell growth and colony formation, apoptosis migration and invasion, cell cycle, drug-resistance, angiogenesis	[22-25, 31]
miR-15a miR-16-1	Tissues and cell lines	qPCR	Down-regulated	Twist1, YAP1	Cell proliferation, EMT, migration, invasion, colony formation *in vitro*, tumorigenicity *in vivo*	[20, 21]
miR-185	Tissues and cell lines	qRT-PCR	Down-regulated	ARC	chemosensitivity	[44]
miR-200c	Cell lines	qRT-PCR	Down-regulated	ZEB1, ZEB2	EMT, Drug-resistance, invasion, and migration	[43]
miR-21	Tissues and cell lines	miRNA micro-array, qRT-PCR	Up-regulated	PDCD4, Bcl-2, Bax, PTEN	Cell proliferation, apoptosis, invasion, migration, drug-resistance	[5, 17]
miR-223	Tissues and cell lines	RT-PCR	Up-regulated	EPB41L3	Cell migration and invasion *in vitro* and *in vivo*	[80]
miR-25	Tissues and cell lines	miRNA micro-array, qRT-PCR	Up-regulated	Bim	Cell apoptosis	[10]
miR-146a	Tissues	qRT–PCR	Down-regulated	EGFR, IRAK1	migration and invasion	[11]
miR-29a	Tissues	qRT-PCR	Down-regulated	ITGB1	Cell invasion, metastasis	[39]
miR-29c	Tissues	NGS analysis, qRT-PCR	Down-regulated	ITGB1	Cell proliferation, adhesion, invasion and tumor growth	[40]
miR-296-5p	Tissues and cell lines	qRT-PCR	Up-regulated	CDX1	Tumor cell growth	[14]
miR-331-3p	Cell lines	-	-	HER2	-	[42]
miR-335	Cell lines	qRT–PCR	Down-regulated	SP1, Bcl-w	invasion and metastasis	[47]
miR-34a	Tissues	qRT–PCR	Down-regulated	PDGFR, MET	migration, invasion and proliferation	[19]
miR-375	Tissues and cell lines	miRNA micro-array, qRT-PCR	Down-regulated	PDK1, 14-3-3ζ (zeta)	apoptosis	[15]
miR-423-5p	Tissues and cell lines	qPCR	Up-regulated	TFF1	proliferation and colony formation but suppress invasion in gastric cancer cells	[84]
miR-424	Tissues	TCGA analysis	Up-regulated	LATS1	cell proliferation, invasion, colony formation	[26]
miR-425	–	–	Up-regulated	PTEN	cell proliferation	[28]
miR-532-5p	Tissues and cell lines	TCGA analysis	Down-regulated	NCF2	metastasis, angiogenesis	[13]
Let-7b	Tissues and cell lines	qRT–PCR	Down-regulated	ING1	invasion and metastasis	[85]

Abbreviation: ARC: apoptosis repressor with caspase recruitment domain; CDX1: caudal-related homeobox 1; EDN1: endothelin 1; EGFR: epidermal growth factor receptor; ING1: inhibitor of growth family, member 1; EPB41L3: erythrocyte membrane protein band 4.1-like 3; IRAK: interleukin-1 receptor-associated kinase 1; ITGB1: integrin β1; NCF2: neutrophil cytosol factor 2; NGS: next generation sequencing; SOX2: SRY (sex-determining region Y)-box 2; STAT4: Signal transducer and activator of transcription 4; SP1: specificity protein 1; TFF1: trefoil factor 1; YAP1: Yes-associated protein 1; ZBTB10: Zinc finger and BTB domain containing 10.

This miR-21 over-expression promoted cell proliferation, colony formation and growth and it increased migration and invasion [17, 18].

Expression of the miR-34a, miR-15a and miR-16-1 anti-tumor miRNAs differs to the elevated GC level of oncogenic miRNAs because these exert negative regulation on cell proliferation, epithelial-mesenchymal transition (EMT), migration, invasion, colony formation *in vitro* and tumorigenicity *in vivo*. These are all decreased in tumor tissues compared to adjacent normal tissues. [19–21]. In addition, MiR-126

is confirmed to be down-regulated in GC where it acts as a tumor-suppressor by repressing tumor cell growth, migration and invasion and inducing cell cycle arrest in the G0/G1 phase. It also promotes apoptosis *in vitro* and inhibits tumorigenicity and metastasis *in vivo* [22, 23]. Moreover, Otsubo et al. recorded that miR-126 was aberrantly up-regulated in several GC cell lines and tumor tissues, and its ectopic expression contributed to GC cell growth and colony formation [25] However, MiR-126 has a well-recognized role in vascular integrity and angiogenesis, and our review established its suppressive effect on GC angiogenesis.

Intriguingly, some miRNAs' expression and function is tissues specific. For example, MiR-424 promotes GC cell growth and invasion with aberrantly high levels in gastric adenocarcinoma (GAC) tissues [26], but it is significantly down-regulated in cervical cancer tissues where it has a tumor suppressive role in inducing G1/S cell-cycle arrest, inhibiting cell migration and inducing apoptosis [27].

Mechanisms underlying miRNAs de-regulation in GC

Although miRNAs are abnormally expressed in multiple cancers, their deregulation mechanisms remain mostly elusive. The frequent causes identified include transcriptional deregulation, epigenetic modification, mutation, DNA copy number abnormality and defects in miRNA biogenetic machinery [9]. Solely or in combination, these mechanisms contribute to miRNA deregulation.

Pro-inflammatory cytokine IL-1β induces activation of transcription factor NF-κB and subsequently up-regulates miR-425 expression in GC cells [28]. The miR-106b-25 cluster, including miR-106b, miR-93, and miR-25, have been demonstrated to be up-regulated by E2F1, in parallel with their host gene Mcm7. In contrast, however, E2F1 is targeted and negatively controlled by miR-106b and miR-93 which form a negative feedback loop in GC [10]. Epigenetic alterations also affect the miRNA expression in GC, and these include DNA methylation, histone modification and post-transcriptional gene regulation by other ncRNAs.

Intriguingly, *H. pylori* is involved in epigenetic silencing of tumor suppressor genes, where enhanced activity of epigenetic modification enzymes induced by the increased nitric oxide pro-inflammatory factor nitric oxide have a central role [29]. The cytotoxin-associated A gene is a well-characterized virulent factor in *H. pylori*-associated inflammation and gastric carcinogenesis, and this contributes to epigenetically silencing of let-7a and let-7c expression in rat gastric mucosal cells. This is ascribed to cooperative increase in H3K27 trimethylation and DNA methylation of CpG islands at the let-7 promoter region accomplished by increased expression of Enhancer of Zeste homologue 2 (EZH2) and DNA methyltransferase 3B (DNMT3B) [3].

Other ncRNAs also regulate miRNAs expression due to their complementary sequences to miRNAs and thus have a regulatory effect on miRNAs [30]. A significant decrease in miR-126 level is observed in SGC-7901 cells transfected with lncRNA HOX antisense intergenic RNA (HOTAIR) cDNA [31]. The level of phosphatase and tensin homolog pseudogene 1 (PTENP1) was then observed inversely correlated with miR-106b/miR-93 in GC tissues [32], and silencing the circular RNA (circRNA) hsa_circ_0000096 up-regulated miR-200a expression but deregulated miR-224 in GC [33].

The following observations were also clear in this review. Over 50% of miRNA genes are located in fragile chromosomal regions and miRNA expression is also susceptible to gene amplification, mutation or translocation during carcinogenesis and development [12]. A germline G mutation identified in the coding region of miR-125a led to 40% reduction in miR-125a expression and enhanced stability of pri-miR-125a in GC [34]. In 76 GC samples, it was observed that G/C single nucleotide polymorphism (SNP) (rs2910164) in the pre-miR-146a seed sequence could lead to the down-regulation of miR-146a in the GG genotype compared to the CC genotype [11]. The miR-106b-25 cluster located in intron 13 of Mcm7 on chromosome 7q22, and the amplification of this site has also been reported in gastric carcinomas [35].

Moreover, miRNA biogenesis is a complex process regulated by multiple related proteins such as Drosha, DGCR8, Dicer and Ago, and their deregulation can directly affect miRNA transcription, maturation and RISC assembly processes during miRNA biogenesis and promote tumorigenesis [9]. The down-regulation of Dicer was observed in GC, and this correlated with poor differentiation and lymph node invasion at $p<0.05$ [36].

miRNA target genes in GC

It is acknowledged that miRNAs perform functions by post-transcriptionally inhibiting the expression of oncogenic and tumor suppressor genes by base-pairing with their mRNAs. Therefore, identification of miRNA target genes is of great help in revealing the significant role of miRNAs in malignancy pathogenesis. Table 2 herein highlights miRNA GC target genes and their associated processes.

Disturbed balance between cell proliferation and growth and cell apoptosis leads to tumorigenesis, and this is delicately tuned by complex proteins and signaling cascades [37]. The Bcl-2 family members, which predominantly regulate apoptosis, are regulated by miRNAs in gastric carcinogenesis. MiR-21 inhibitors elevate the expression of the Bax pro-apoptotic molecule and coincidentally decrease expression of the anti-apoptotic molecule Bcl-2 which unlocks the stimulating effect of miR-21 on GC tumor growth [18].

Further, Bim is a BH3-only protein which can activate Bax to promote apoptosis, but this is down-regulated by miR-25, resulting in resistance to TGFβ-induced GC cell apoptosis [10]. However, over-expressing the miR-93 and miR-106b members of the miR-106b-25 cluster can abolish the G1/S cycle arrest induced by TGFβ by targeting p21 which blocks passage through the cell cycle through its repressive effects on

both cyclin-dependent kinases and proliferating-cell nuclear antigen [10, 38]. The suppressive effects of blocking the cell cycle and inducing apoptosis by caudal-related homeobox 1 (CDX1), which is an intestinal-specific transcription factor involved in gastric intestinal metaplasia (IM), are attenuated by miR-296-5p; thus resulting in GC growth [14].

In addition to genes implicated in carcinogenesis, miRNAs can also regulate targets affecting tumor progression, and especially metastasis which is the most lethal aspect of the oncogenic process. Several cell biological activities are controlled in multi-step metastasis. These include adhesion, angiogenesis, migration and invasion which enable tumor cells to disseminate from their primary locations, enter the circulation and form secondary colonies at other sites [1]. ITGB1 (Integrin β1) is a cell adhesion molecule which mediates mutual adhesion between cells and extra-cellular matrix (ECM). This is targeted by miR-29, and enhanced ITGB1 expression due to decreased miRNA-29 in GC also promotes tumor metastasis [39, 40].

In addition, Twist1 promotes EMT by regulating the N-cadherin, α-SMA and Fibronectin EMT-related genes and thus contributes to GC cells migration and invasion. This oncogenic effect can then be repressed by the interaction of miR-15a-3p and miR-16-1-3p with the miRNA recognition elements (MREs) on the Twist1 3'-UTR [20]. Xie et al. further reported that ectopic expression of miR-1 in GC cells can inhibit endothelial cell migration and tube formation due to down-regulated endothelial vascular growth factor-A (VEGF-A) and endothelin 1 (EDN1) [41].

Some target genes have now become significant therapeutic sites in malignancies. These include human epidermal growth factor receptor 2 (HER2), c-Met and VEGF-A. In addition, Trastuzumab, a monoclonal antibody against the HER2 transmembrane tyrosine kinase receptor, can be combined with chemotherapy in standard treatment for the 11–20% of patients affected by HER2 amplification [42, 43]. Although there is frequently acquired resistance to Trastuzumab in GC patients, enhanced TGF-β-induced-EMT from impaired miR-200c on ZEB1 and ZEB2 regulation may counteract this [43]. Additional target genes implicated in drug-resistance include the positive role of the apoptotic repressor in the caspase recruitment domain (ARC) in the impaired response to DDP/ doxorubicin, and PTEN's negative role in DDP tolerance [5, 44].

Notably, the crosstalk between several ncRNAs and mRNAs has attracted increasing attention in regulating ncRNA transcription (especially circRNAs and lncRNAs). Here, mRNAs can competitively bind to the same miRNA as competing endogenous RNAs (ceRNAs) or miRNA sponges because of shared MREs; thus affecting the post-transcriptional regulation of target mRNAs by miRNAs [30]. These interactions are important in the development of GC because they offer additional modification of gene post-transcriptional regulation. Here, Zhou et al. reported that lncRNA H19 attenuates the negative regulation of miR-141 on its target gene ZEB1, thus promoting GC proliferation and migration [45].

Research has also recorded that over-expressed lncRNA LINC01410 inhibited miR-532-5p, leading to impaired de-regulation of neutrophil cytosol factor 2 (NCF2) and subsequent activation of the NF-κB pathway. NF-κB then activated the LINC01410 transcription level through the NF-κB/p65-binding site in the LINC01410 promoter region and this formed a positive feedback circuit contributing to GC angiogenesis and metastasis [13]. Increased circLARP4 can also act as a ceRNA for miR-424 to up-regulate expression of the large tumor suppressor kinase 1 (LATS1) target gene, and consequently attenuate the GC cell proliferation induced by miR-424 [26].

Clinical application of miRNAs in GC

MiRNAs as useful biomarkers. As previously discussed, significantly different miRNA expression occurs in tumor tissues and controls, and the miRNA expression signatures can therefore distinguish GC and normal tissues. Moreover, miRNAs also correlate with GC progression and clinical-pathological characteristics, so miRNA profiling can classify GC patients in prognostic groups and choose appropriate treatment strategies.

Higher miR-214 levels positively associate with invasion depth, metastasis and TNM stages. In addition, the GC patient Kaplan-Meier survival curves determined strong correlation between miR-214 level and survival time ($p=0.031$) [46]. In a similar study of 70 GC samples, low miR-335 tumor suppressor level was associated with lymph-node metastasis ($p<0.001$). lymphatic vessel invasion, advanced pT stage and higher pN stage (all $p<0.05$) [47]. Li et al. analyzed follow-up data of 38 GC stage I patients after surgery, and concluded that combined high miR-23a levels and low PTEN indicated less disease-free survival time (DFS) [48]. Similarly, lower miR-146a level correlated with both increased lymph node metastasis and venous invasion, and multivariate analysis then indicated that miR-146a expression could well be an independent prognostic predictor of overall GC survival [11].

In addition to circulating miRNA levels in tumor tissues, these can also be detected in serum, plasma, urine and gastric juice. Numerous studies have proven the great promise of circulating miRNAs as novel non-invasive biomarkers because of their high stability, close relationship with disease status and easy measurement. For example, Zhu et al. identified accurate early-stage gastric cancer detection from signatures of over-expressed miR-16, miR-25, miR-92a, miR-451 and miR-486-5p in GC patient plasma [49]. Similarly, miR-185, miR-20a, miR-210, miR-25 and miR-92b in peripheral plasma were confirmed as diagnostically significant for GC [50].

Low serum miR-203 levels are a proven independent risk factor for lymph node, peritoneal, and distant metastases and consequent poor prognosis for GC patients [51], and the

miRNAs in gastric juice peculiar to stomach tissues may also provide more specific and effective GC biomarkers [52]. Cui et al. demonstrated that detecting miR-106a level in gastric juice had more reliable diagnostic value than in peripheral blood (AUC 0.871 to 0.684), and the combined detection of gastric juice miR-21/miR-106a and carcino-embrionic antigen (CEA) was more valuable than using serum CEA alone [53].

miRNA gene mutations and SNPs influence their transcription, maturation and miRNA-mediated regulation of target genes, and this makes miRNAs SNPs susceptible to numerous cancers [54]. Sun et al. demonstrated that the common rs895819 A/G polymorphism in the miR-27a genome correlates with susceptibility to GC, and subjects with AG+GG genotypes had significantly increased risk in gastric cancer compared to the AA genotype (adjusted OR =1.48) [54]. SNPs in miR-146a rs2910164, miR-196a-2 rs11614913 and miR-149 rs2292832 are also associated with GC susceptibility [55]. Therefore, detecting these SNPs will help screening high GC risk. It is noteworthy that the methylation level of miRNAs can also be predictive of GC. For example, miR-34b/c methylation in non-cancerous gastric mucosa can be a useful biomarker in predicting metachronous GC [56].

MiRNAs as potential therapeutic targets. The close association between miRNA deregulation and GC pathogenesis enables the restoration of tumor-suppressive miRNAs and/or inhibiting oncogenic miRNAs to be potential strategies against this tumor. Significantly impaired ability of proliferation, migration and colony formation is observed when transfecting BGC-823 and MKN-74 cells (GC cell lines) with miR-21 inhibitor compared to the control group ($p<0.05$) [18]. Ectopic expression of miR-141 by transfecting GC cells with miR-141 mimics can also provide almost 40% inhibition to proliferation and a prominent reduction in invasion [45].

miRNAs have also been tested *in vivo*. Han et al's xenograft nude mouse model recorded tumor growth in mice implanted with miR-29c over-expressing GC cells was significantly slower than in those with untreated parental GC cells ($p<0.0001$). Injecting the tumor with miR-29c mimics by liposome delivery also results in remarkable inhibition of tumor growth [40], and bioluminescence imaging and analysis showed miR-137 had anti-metastatic effect in *in vivo* lung tissue [57].

Consistent with their regulation of cell apoptosis, miRNAs also exert significant effects in chemotherapies mediated by apoptotic signaling pathways. It has been demonstrated that the level of miR-21 in the DDP resistant GC cell line SGC7901/DDP was significantly higher than its SGC7901 parental cell line ($p<0.001$); and further, knocking miR-21 down can promote apoptosis and decrease viability in the SGC7901/DDP cell line treated with DDP [5]. Similarly, miR-19a/b are up-regulated in SGC7901/VCR and SGC7901/ADR cell lines, and the enhanced response to CDDP, 5-FU and ADR can be acquired via suppressing miR-19a/b level in SGC7901/VCR cells [58].

In contrast, Li et al. reported that ectopic miR-185 expression in GC cells enhanced DDP or doxorubicin-induced apoptosis, and knockdown of endogenous miR-185 prevented cisplatin or doxorubicin-induced GC cell apoptosis. Further, tumor growth in the established GC xenograft model is inhibited by the combined therapy of enforced miR-185 expression and a low dose of doxorubicin. This is as effective as high-dose doxorubicin therapy [44], and miRNAs are therefore promising targets for improving chemotherapy treatment by modifying drug-sensitivity and helping to formulate appropriate chemotherapy by predicting therapeutic response.

The CSC hypothesis postulates that initiation and maintenance of cancers are attributed to a small subset of cancer cells, called CSCs [59]. Primarily, CSCs are defined as rare, quiescent and capable of self-renewal and multi-lineage differentiation [60]. These cells have been a critical cause of tumor metastasis, recurrence and chemo and radio-resistance. While conventional anti-carcinogenic treatment limits CSC effects, novel therapeutic strategies for eradicating CSCs are essential for successful cancer cure; and the first challenge lies in recognizing CSCs in the tumor mass. Here, stem markers, spheroid formation assay and selection of side-population (SP) cells with high efflux ability of Hoechst dyes are feasible [61, 62].

CD44 can identify tumor cells with CSCs properties in GC [60, 63], and several studies have observed significantly different expression profiling of specific miRNAs between gastric CSCs and other tumor cells, thus implying the potential application of miRNAs in recognizing CSCs [61, 63] (Table 3). Moreover, restoring deregulated miRNAs can modulate CSC characteristics, so miRNAs could be therapeutic targets in CSC treatment. For example, miR-106b knockdown could suppress EMT, self-renewal and the invasive capacity and CSC marker expression in CD44 (+) GC cells [63].

Blockages to miRNA clinical application. Despite the great potential miRNAs manifest in GC management, there are many blockages to the transition from laboratory studies to clinical practice. While miRNAs have good sensitivity and specificity as biomarkers for GC diagnosis and prognosis with greater promise than in current use, the following factors restrict increased application; (1) current research on miRNAs' predictive ability frequently involves small-size coherents. This is a major cause of study discrepancy; (2) significant effort is required to integrate accumulated research; (3) population-based differences in miRNA deregulation must be considered when these are used as biomarkers for GC diagnosis and prognosis and (4) an appropriate standard testing system is lacking for clinical practice, and any instituted system must have a feasible threshold and quality control.

Table 3. MiRNAs and cancer stem cells in GC.

miRNAs alteration	CSCs Recognition	Effects	Implicated signaling	Refs
miR-501-5p	CD44, CD133, Bmi1, Nanog, MYC and SOX2 expression; percentage of the side-population of cells; tumor spheres formation	Promotion in self-renewal	Wnt/β-catenin pathway	[86]
miR-34a	tumor spheres formation	Inhibition in self-renewal	Bcl-2, Notch, and HMGA2 (targets of miR-34a)	[87]
miR-106b, miR-93, miR-25 (miR-106b family)↑	CD44(+)	Promotion in EMT, self-renewal and invasion	TGF-β/Smad pathway	[63]
miR-21 and miR-302↑, let-7a↓	CD44(+)	Not described	Not described	[61]
miR-19b, miR-20a and miR-92a (miR-17-92 cluster)↑	EpCAM(+)/CD44(+)	Promotion in proliferation, metastasis, self-renewal	Wnt/β-catenin pathway	[62]
miR-4270, miR-483-5p, miR-642b-3p, miR-4669, miR-4290, miR-483-3p, miR-4778-5p, miR-451b, miR-4763-3p↑, other 173 miRNAs↓	spheroid body formation assay	Not described	Notch, Wnt/β-catenin, MAPK, mTOR, JAK-STAT pathway	[88]

The therapeutic effect of miRNAs also contains issues which must be settled before miRNAs are accepted in clinical application; (1) current studies testing miRNA potential curative effects are carried out in isolated GC cell lines or animal models, and therefore dose optimization should be investigated when transforming study results to clinical application; (2) safe and effective vectors are required for delivering miRNA to a specific tissue or disease site; (3) miRNA therapeutic effects vary in different contexts because of their wide interactions with different target genes and signaling pathways. Research results should be interpreted with caution and possible off-target effects must also be considered and (4) immune activation should be minimized when miRNA mimics or antagomirs are used in GC treatment.

The role of lncRNAs in GC

Genome-wide studies in the last decade showed that only 2% of the mammalian genome encodes mRNAs; with most transcribed as ncRNAs [64]. There is division into two categories: large RNAs over 50nt and smaller ones. The former category includes lncRNA, small nucleolar RNA, circRNA, transfer RNA and ribosomal RNA; and the latter category has miRNA, small interfering RNA and Piwi-interacting RNA [65]. Despite their inability to encode protein, RNAs act as transcriptional and post-transcriptional regulators and chromatin-modifying complex guides, and thus have prominent roles in physiological and pathological conditions [64]. Evidence has provided new information on miRNA, lncRNA and ncRNA involvement in CG pathogenesis and development.

LncRNAs have over 200 nucleotides but lack an open reading frame over 100 amino acids [66]. They are subdivided into exonic, intronic, overlapping and inter-genic lncRNAs, dependent on location with the nearest protein-coding transcripts [67]. These heterogeneous lncRNAs fulfill a broad spectrum of cellular functions, including chromatin modification and transcriptional and post-transcriptional regulation. Deregulated lncRNAs also regulate GC progression [65, 68] and recent studies demonstrate that aberrant lncRNA expression contributes to malignant GC phenotypes, such as carcinogenicity, tumor growth and metastasis. These functions are performed through regulation of proliferation, apoptosis, invasion and migration.

Up-regulation of oncogenic lncRNAs and tumor-suppressive lost expression co-operate in gastric cancer initiation and development. HOTAIR is a typical oncogenic lncRNA which binds Polycomb Repressive Complex 2 (PRC2) and LSD1 (lysine specific demethylase 1)/CoREST (Co-repressor of RE1-silencing transcription factor)/REST complexes. This directs the complexes to specific gene sites providing H3K27 methylation, H3K4 de-methylation and ultimate gene silencing [69]. HOTAIR is significantly up-regulated in GC, and its siRNA knockdown inhibits GC cell proliferation, migration and invasion and concurrently enhances anoikis rate. Moreover, injecting HOTAIR siRNA-transfected GC cells into nude mice represses both xenograft tumor growth and peritoneal metastases [70].

Up-regulated lncRNA highly expressed gastric carcinoma transcript 1 (GHET1) is another lncRNA in GC, and this facilitates tumor cell proliferation and xenograft tumor growth in nude mice. Mechanistic analysis indicates that the promotion effects of GHET1 are due to the increased c-Myc mRNA stability and expression mediated by GHET1's association with insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1). This enhanced the physical interaction between c-Myc mRNA and IGF2BP1 protein [71]. In contrast, the lncRNAs maternally expressed gene 3 (MEG3) and FENDRR were down-regulated in GC and inhibited

cell proliferation, migration and invasion and promoted cell apoptosis by modulating p53, fibronectin 1 and matrix metalloproteinase 2 (MMP2)/MMP9 [72, 73].

Potential lncRNA clinical implications have also been investigated in GC. For example, Arita et al. observed that lncRNA H19 plasma level was significantly higher in patients than in healthy controls (p=0.029) [74], and Dong et al. measured 39 lncRNAs in the serum from 110 GC patients, 106 healthy subjects and 15 gastric peptic ulcer patients. These authors identified a three-lncRNA diagnostic signature consisting of lncRNAs cancer up-regulated drug resistant (CUDR), long stress-induced non-coding transcript 5 (LSINCT-5) and PTENP1. They then demonstrated that the signature had better diagnostic accuracy than the commonly used CEA and CA19-9 biomarkers. (with areas under the curve (AUC): 0.92, 95%CI: 0.807–0.974 compared to 0.574, 95%CI: 0.432–0.708 and: 0.580, 95%CI: 0.438–0.713; respectively) [75].

lncRNA expression levels in GC tumor tissues also possess prognostic predictive value. Here, the highly-expressed HOTAIR could be an independent prognostic and risk factor for GC peritoneal metastases [70]. Decreased MEG3 level also correlated with larger tumor size, advanced pathological stage and deeper invasion, and GC patients therefore had significantly worse prognosis than those with higher levels (P <0.001) [73]. There are also lncRNAs, such as FENDRR and growth arrest-specific transcript 5 (GAS5), associated with the 5-year survival rate of GC patients [76]. Unfortunately, although the anti-tumor effects achieved by lncRNA modulation set them as novel GC therapeutic targets, some caveats impede their clinical application.

Conclusion

This review summarizes deregulated miRNAs' contribution to gastric cancer pathogenesis. It then investigated malignant tumor growth, metastasis and drug-resistant GC phenotypes by miRNA regulation of downstream target genes involved in cell differentiation, proliferation, migration, invasion and apoptosis. The genetic alteration and epigenetic modification mechanisms underlying miRNAs' aberrant expression are also identified

The cellular and molecular deregulation of miRNAs in GC participation promotes their trial in clinical application. The aberrant levels of miRNAs in tumor tissues, and also in plasma, serum and gastric juice, could well serve as additional diagnostic and prognostic biomarkers for GC patients, and SNPs and specific miRNAs' DNA methylation may determine GC risk factors. Thus, deregulated miRNAs present a promising anti-cancer therapeutic target in GC; especially considering their effects in modifying chemo-resistance and modulating gastric CSC action.

Unfortunately, blocking factors persist in transforming laboratory results into clinical application. They include ensuring safe and effective vectors, avoiding undesired off-target effects and instituting standardized testing systems. These factors are therefore appropriate research themes for future gastric cancer elucidation.

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