Roles of long non-coding RNAs in gastric cancer metastasis

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Abstract
Gastric cancer is the second leading cause of cancer-related deaths. Metastasis, which is an important element of gastric cancer, leads to a high mortality rate and to a poor prognosis. Gastric cancer metastasis has a complex progression that involves multiple biological processes. The comprehensive mechanisms of metastasis remain unclear, though traditional regulation modulates the molecular functions associated with metastasis. Long non-coding RNAs (IncRNAs) have a role in different gene regulatory pathways by epigenetic modification and by transcriptional and post-transcriptional regulation. IncRNAs participate in various diseases, including Alzheimer’s disease, cardiovascular disease, and cancer. The altered expressions of certain IncRNAs are linked to gastric cancer metastasis and invasion, as with tumor suppressor genes or oncogenes. Studies have partly elucidated the roles of IncRNAs as biomarkers and in therapies, as well as their gene regulatory mechanisms. However, comprehensive knowledge regarding the functional mechanisms of gene regulation in metastatic gastric cancer remains scarce. To provide a theoretical basis for therapeutic intervention in metastatic gastric cancer, we reviewed the functions of IncRNAs and their regulatory roles in gastric cancer metastasis.

Key words: Long non-coding RNAs; Gastric cancer; Metastasis; Function; Development

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Core tip: Long non-coding RNAs (IncRNAs), are emerging as players in multiple biological processes, and are involved in many diseases via the regulation of gene expression at the chromatin, transcriptional, or posttranscriptional level. Their roles in gastric carcinoma metastasis are quite complex; however, the comprehensive study of metastasis will provide us with new perspectives to develop future therapeutic treatments. IncRNAs, which play pivotal roles in gastric cancer metastasis, may help provide future treatments to improve the quality of life of patients with metastatic gastric cancer.
INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies worldwide. With its high lethality, GC ranks second in cancer-associated deaths, although the morbidity associated with this malignancy has decreased in most countries\cite{1,2}. Because specific biomarkers of GC are currently limited, patients are typically diagnosed after metastasis, which is associated with recurrence, GC-related death, and poor prognoses. The following important changes occur during GC metastasis: (1) GC cells invade the primary protective barriers and migrate to adjacent tissue; (2) GC cells migrate to the circulatory system to a distant location; (3) GC cells invade the lymphatic system and disseminate to a secondary site; and (4) GC cells leave the primary tumor and adhere to a sensitive location. Recent studies have revealed that the processes of angiogenesis and adhesion, the cell-to-cell tight junctions, and the extracellular matrix play essential roles in GC metastasis\cite{3-6}. However, the mechanisms of GC metastasis have yet to be comprehensively elucidated. GC tumorigenesis and metastasis are multistep processes that involve a myriad of signaling pathways and gene regulation in which oncogenic and tumor-suppressive factors, such as long non-coding RNAs (lncRNAs) play pivotal roles\cite{7}.

lncRNAs are transcriptional products longer than 200 nucleotides that primarily have a regulatory role rather than encode proteins\cite{8-10}. In some studies, lncRNAs encode short, evolutionarily divergent proteins\cite{11,12}. Additionally, lncRNAs play a pivotal role in many biological mechanisms, including gene imprinting, activation and repression\cite{13-15}. At the transcriptional level, lncRNAs can bind to DNA or proteins, regulate the subcellular localization of transcription factors, and inhibit transcript elongation\cite{16,17}. lncRNAs affect many posttranscriptional processes, including alternative splicing, RNA editing, transport, degradation, translation, and miR-mediated regulation\cite{18-20}, and are involved in diseases such as Alzheimer’s disease, cardiovascular diseases, and malignancies, including lung cancer\cite{21}, renal cell carcinoma\cite{22}, bladder cancer\cite{23}, esophageal carcinoma\cite{24}, hepatocellular carcinoma\cite{25}, and acute leukemia\cite{26}. lncRNAs also play important roles in the proliferation, migration, and invasion of human cancer cells, and are associated with cancer metastasis and therapeutic sensitivity, implicating lncRNAs as potential novel biomarkers for diagnosis and as targets for therapeutic approaches\cite{27,28}.

MECHANISM OF LNCRNAS IN GC METASTASIS

lncRNAs involved in angiogenesis

Angiogenesis in cancer, including GC, is important for proliferation, metastasis, and drug sensitivity. Angiogenesis not only provides a wealth of nutrients and oxygen for GC cells and transports tumor metabolites, but also provides favorable conditions for GC vessel metastasis, increasing the opportunity for cancer cells to enter the blood circulation and to seed in secondary locations. Thus, antiangiogenic treatment can improve therapeutic efficiency\cite{29,30}. As a newly discovered group of regulatory genes, lncRNAs play essential roles in angiogenesis; however, their mechanisms of action in GC angiogenesis and metastasis have not been fully elucidated.

lncRNAs promote carcinoma metastasis by activating the angiogenic system in which phosphoglycerate kinase (PGK) participates. PGK, which is a disulfide reductase conventionally thought to be involved in glycolysis, also functions as a carcinoma metastasis inhibitor by modulating angiogenesis. The hepatocellular carcinoma overexpressed IncRNA, MVIH, is associated with microvascular invasion, TNM stage, recurrence-free survival, and overall survival, and promotes distant metastasis by inhibiting PGK1 secretion and by activating angiogenesis\cite{31,32}. Certain GC-associated lncRNAs modulate angiogenesis. For example, H19 promotes vasculogenesis by activating tumor necrosis factor-α, which indirectly induces angiogenic factors\cite{33}. Other lncRNAs regulate basal sprouting and migration, endothelial cell proliferation, capillary density, vascular endothelial growth factor α and its receptor, which participate in angiogenesis\cite{34-36}.

lncRNAs involved in cell-to-cell junction and adhesion

 Tight junctions (TJs) between cells play pivotal roles in regulating the diffusion of ions and specific molecules and in maintaining the integrity of the cell-to-cell protective barrier. Recent studies have revealed that cell-to-cell junctions function as protectors of GC metastasis. The aberrant expression or distribution of TJ proteins leads to the loss of cell-to-cell adhesion and tissue integrity, assisting cancer cell invasion and promoting metastasis\cite{4,5}.

TUC339 is a 1198-bp ultraconserved IncRNA that regulates cancer cell growth and adhesion\cite{37}. Ephrins are cell surface protein ligands that can mediate cellular adhesion and function via interactions with
the Eph receptor tyrosine kinases. Hypoxia induces the expression of hypoxia-inducible factor, leading to EFNA3 IncRNA expression, Ephrin-A3 protein accumulation, and metastatic dissemination in breast cancer\[37\]. As the interactions between IncRNAs and mRNAs are accepted, the important function of IncRNAs in cell-to-cell junctions and adhesion can be recognized by studying the association between IncRNAs and adhesion-associated genes. Zhao et al\[38\] demonstrated that Down Syndrome Cell Adhesion Molecule (DSCAM) is a member of the cell adhesion immunoglobulin superfamily. Its antisense IncRNA (DSCAM-AS1) is overexpressed in lung adenocarcinoma and may play a pivotal role in regulating cell-to-cell adhesion because this type of antisense IncRNA can interact with its host genes.

Epithelial-to-mesenchymal transition (EMT) and its inverse process, mesenchymal-to-epithelial transition, play critical roles in embryonic growth, stem cell biology, and tumorigenesis progression, which involves the invasion and migration of carcinoma cells with multiple participating signaling pathways\[39\]-[40]. The upregulated breast cancer-associated IncRNA, linc-ROR, can induce EMT, enhancing migration and invasion. However, silencing its expression represses lung metastasis. Linc-ROR functions as a miR sponge, binding with miR-205 and inhibiting the degradation of its target genes, including ZEB2, which induces EMT\[41\]. The Hox transcript antisense intergenic RNA (HOTAIR) is overexpressed in GC carcinoma metastatic lymph nodes, and its silencing downregulates the expression of the transcription factor Snail, with tighter cell-to-cell adhesion and rounder morphology, as well as decreased expression of the mesenchymal markers vimentin and N-cadherin, and increased expression of the epithelial markers E-cadherin and ZO-1, indicating that HOTAIR silencing can reverse EMT in GC cells and inhibit distal metastasis\[42\]. The IncRNA, MALAT1, modulates the expression of the transcription factors ZEB1, ZEB2, Slug, and E-cadherin, and promotes EMT by activating the Wnt signaling pathway, which regulates gene expression at the transcriptional level\[43\]. Other IncRNAs, such as the IncRNA, LEIGC, which is slightly expressed in GC, also regulate EMT\[44\]. Therefore, EMT regulation provides a target for the therapeutic intervention of GC metastasis.

**IncRNAs involved in extracellular matrix degradation**

The extracellular matrix prevents GC invasion and metastasis. Its damage degrades the protective barrier against GC metastasis, and provides a favorable environment. Pericellular proteases can degrade matrix proteins and modulate cancer metastasis via regulating the cleavage of proteins such as cell adhesion molecules\[6\]. Matrix metalloproteinases (MMPs) are a subgroup of proteases that degrade the extracellular matrix and modulate GC invasion and metastasis\[45\].

IncRNAs have great importance in stabilizing or degrading the extracellular matrix. H19 is downregulated in prostate cancer, and H19-derived miR-675 inhibits the expression of the extracellular matrix protein transforming growth factor β-induced protein mRNA by binding to its 3'-untranslated region (3'-UTR), thereby inhibiting prostate cancer metastasis\[46\]. Park et al\[47\] found that the IncRNA, BM742401, was downregulated in cancer, and its overexpression inhibited GC cell migration, invasion and metastasis by regulating MMP9 secretion. Other IncRNAs aberrantly expressed in GC, including HOTAIR and FENDRR, correlate with GC metastasis; these IncRNAs regulate extracellular matrix degradation by modulating the expression of cancer metastasis-associated genes such as ICAM-1, MMP1, MMP2, MMP3 and MMP9\[48,49\].

**GC METASTASIS ASSOCIATED LNCRNAS**

One difference between cancer and normal tissue is that cancer cells can proliferate infinitely, damage normal tissue under suitable conditions, migrate to adjacent normal tissue, vessels, and lymphatic tubes, and promote cancer metastasis and carcinoma-related death. IncRNAs, which are emerging regulators of gene expression in various biological processes, play important modulatory roles in cancer cell invasion and metastasis, and in patient prognosis.

**Promoting GC metastasis**

**GC metastasis-associated IncRNA H19:** H19, which is a 2.3-kb IncRNA transcribed from the paternally imprinted gene H19 on chromosome 11p15.5, is highly expressed during embryogenesis; however, its expression almost disappears from all tissues after birth\[50\] (Figure 1). H19 plays pivotal roles in tumorigenesis and metastasis in the same manner as oncogenes or tumor suppressor genes. In bladder cancer, H19 activates Wnt/β-catenin by associating with enhancer of zeste homolog 2 (EZH2), increasing its metastasis\[51\]. H19 partly promotes HMGA2-mediated EMT by antagonizing let-7 in pancreatic cancer, and increasing the migration and invasion potential of pancreatic ductal adenocarcinoma cells\[52\]. However, H19 represses prostate cancer metastasis through the H19-miR-675 axis\[46\]. H19 is also overexpressed in GC, and can promote cell proliferation by partly inactivating p53, decreasing its activity, and suppressing the expression of the p53 target protein Bax. In contrast, H19 silencing results in GC cell apoptosis, suggesting that H19 promotes GC tumorigenesis by activating p53\[53\]. H19 is the precursor of miR-675\[50\], and the H19/miR-675 signaling axis plays a critical role in the carcinogenesis
of colorectal cancer. miR-675 serves as a tumor suppressor by targeting the 3’-UTR of runt domain transcription factor 1 (RUNX1), downregulating its expression at the mRNA and protein levels in GC cells and restoring the proliferation inhibitory effects induced by siRNA-H19, indicating that H19 acts as an oncogene by indirectly regulating RUNX1 expression. However, entirely different conclusions suggest that H19 regulates the binding protein ISM1, but that miR-675 promotes cell proliferation, invasion and migration by targeting CALN1 in GC. In other studies, c-Myc associates with H19-induced GC tumorigenesis, development, and metastasis. Taken together, the inhibition of H19 expression and the suppression of its effects on carcinoma progression and metastasis may improve GC therapeutic treatment and prognosis.

**GC metastasis-associated IncRNA HOTAIR:**
HOTAIR, which is a 2.2-kb ncRNA transcribed from the HOXC locus of chromosome 12q13.13 in the opposite direction of HOXC, acts as a scaffold. Its 5’ domain binds to polycomb repressive complex 2 (PRC2), and its 3’ domain binds the LSD1/CoREST/REST complex, coordinating PRC2 and LSD1 to target chromatin, which leads to the methylation of histone H3 lysine 27 and to the demethylation of lysine 4, respectively (Figure 2). Thus, HOTAIR transcription affects distant gene silencing. HOTAIR is overexpressed in primary breast cancer and can promote cancer invasiveness and metastasis depending on PRC2, making HOTAIR a possible predictor of cancer metastasis. Increased expression of HOTAIR is also observed in hepatocellular carcinoma, and its expression is associated with MMP9 and vascular endothelial growth factor protein, which affect cell activity and cancer metastasis. HOTAIR expression is increased in colon cancer and is related to the depth of tumor invasion, lymph node metastasis, organ metastasis, histological differentiation, vascular invasion, advanced tumor stage, higher recurrence rate and poorer overall survival. HORAIR is a potential target for therapeutic intervention by modulating EMT.

**Figure 1** H19 regulating network in gastric cancer. (1): Inhibitory effects; (2): H19 is treated as the precursor of miR-675; (3): H19 positively regulates its binding protein ISM1; (4): Promoting effects; (5): H19 regulates CALN1 indirectly by miR-675.

**Figure 2** Hox transcript antisense intergenic RNA regulates gastric cancer metastasis and cell apoptosis by human epithelial growth factor receptor 2 and SUZ12 (A), and hox transcript antisense intergenic RNA acts as a ceRNA (B). (1): miR-331-3p negatively regulates HER2 by binding to its 3’-UTR; (2): HOTAIR indirectly regulates HER2 by competitive binding to miR-331-3p.
inhibiting GC metastasis.

**GC metastasis associated-lncRNA highly upregulated in liver cancer:** Highly upregulated in liver cancer (HULC) is an lncRNA that was first identified by its aberrant expression in hepatocellular carcinoma (Figure 3). In subsequent studies, its ectopic expression was found in other cancers, including GC and pancreatic cancer[65-67].

Increased HULC expression has been observed in SGC7901, BGC823 and AGS GC lines; however, no significant difference in HULC expression has been observed between MKN28, MKN45 and the gastric epithelial mucosa cell line GES-1. The expression level of HULC correlates with GC lymph node metastasis, distant metastasis and TNM stage. HULC expression promotes GC cell proliferation, migration and invasion via modulating EMT, which is important for metastasis[66]. Therefore, we can repress GC cancer progression and metastasis by downregulating HULC and, correspondingly, modulating EMT and autophagy.

**GC metastasis-associated IncRNA ANRIL (CD KN2B-AS1):** ANRIL is a 3.8-kb IncRNA transcribed from the INK4A-ARF-INK4B gene cluster in the opposite direction[70] (Figure 4). ANRIL binds to PRC2 with its component SUZ12 and recruits PRC2, thereby decreasing the expression of p15/INK4B, which is a tumor suppressor gene[71]. ANRIL and PRC2 play repressive roles at the epigenetic transcriptional level by binding to chromobox 7 within PRC1 and recruiting PRC1 to the p16 (INK4A)/p14 (ARF) locus, subsequently leading to its silencing[70]. The lncRNA ANRIL is also related to atherosclerosis, periodontitis and certain cancers[72].

ANRIL is aberrantly expressed in some cancers and used to predict lung cancer metastasis[73]. ANRIL expression is increased in GC, and its upregulation is related to higher TNM stage, larger tumor size and poorer prognosis. ANRIL promotes GC cell proliferation 3 (LC3)-II, a marker of autophagosomes, to LC3-I, suggesting that HULC promotes GC cell proliferation by activating autophagy[66]. Therefore, we can repress GC cancer progression and metastasis by downregulating HULC and, correspondingly, modulating EMT and autophagy.

**Figure 3** Highly upregulated in liver cancer regulatory roles in gastric cancer. (1): Promoting effects. HULC: Highly upregulated in liver cancer.

**Figure 4** ANRIL forms a positive feedback loop with miR-449a and E2F1, and functions in gastric cancer. (1): Inhibiting effects; (2): Promoting effects.
and growth partly by the epigenetic silencing of p15/INK4B and p16/INK4A in \textit{Cis} and by the regulation of miR-99a/miR-449a expression by binding to PRC2 with EZH2 and SUZ12 in \textit{Trans}. This regulation forms a positive feedback loop and establishes prognostic roles for ANRIL in GC diagnosis and therapeutic treatment\cite{74}.

Inhibiting GC metastasis

GC metastasis-associated IncRNA maternally expressed gene 3: Maternally expressed gene 3 (MEG3) is an imprinted gene on human chromosome 14q32.3 that can encode a IncRNA\cite{75} (Figure 5). LncRNA MEG3 expression is downregulated in multiple cancers, and its downregulation is associated with advanced pathological stage, larger tumor size, and poor prognosis. MEG3 induces G2/M cell cycle arrest and promotes apoptosis by partially activating p53, thereby inhibiting cell proliferation and growth\cite{75,76}.

In GC, MEG3 expression is significantly lower than in adjacent normal tissue, and its downregulation correlates with higher TNM stage, deeper invasion, and larger tumor size. MEG3 acts as a tumor suppressor partly through activation of p53. Methylation of the MEG3 regulatory regions (MEG3-DMRs) is involved in MEG3 modulation. miR-148a increases the expression of MEG3 by downregulating DNA methyltransferase-1, which is traditionally thought to be essential for DNA methylation and genome stability\cite{78,79}, miR-148a is a therapeutic agent that can repress GC metastasis by indirectly upregulating MEG3, which functions as a tumor suppressor gene.

GC metastasis associated IncRNA FENDRR: The IncRNA, FENDRR, which is transcribed 1250 bp upstream of the 5' end of the gene encoding the transcription factor FOXF1, plays important roles in the differentiation of the lateral mesoderm and in the development of the heart and the body wall (Figure 6). FENDRR modulates chromatin signatures through binding to both the PRC2 and the Trithorax group/Mixed lineage leukemia complexes\cite{80}.

FENDRR is downregulated in GC, and its low expression is related to deeper tumor invasion, higher tumor stage, lymphatic metastasis, and poor prognosis. FENDRR negatively regulates fibronectin 1 and MMP2/MMP9, indicating that FENDRR is a mediator of cell differentiation, growth, and migration and of GC progression and metastasis. FENDRR expression is downregulated by histone deacetylation in GC\cite{49}. FENDRR will help us to comprehensively understand the biological mechanisms of GC metastasis and to discover novel therapeutic treatments for GC.

Other GC metastasis associated IncRNA

Other aberrantly expressed IncRNAs in GC are closely related to TNM stage, invasion depth, lymph node metastasis, and patient prognosis, and some of their mechanisms have been partially elucidated\cite{81-83}.

Colon cancer-associated transcript (CCAT1) is an IncRNA that is ectopically expressed in colon adenocarcinoma and is highly expressed in GC, being associated with early GC growth, lymphatic node metastasis, and distal metastasis. c-Myc binds to the E-box element in the promoter region of CCAT1, promoting GC activity, proliferation and migration by upregulating proliferating cell nuclear antigen\cite{84,85}.

Under hypoxic conditions, the IncRNA, AK058003, decreases the CpG island methylation of SNCG, which is a metastasis-related gene, correspondingly modulating the hypoxia-induced metastasis of GC.

Figure 5 Maternally expressed gene 3 regulatory roles in gastric cancer. (1) Promoting effects; (2) Inhibiting effects.

Figure 6 FENDRR regulatory roles in gastric cancer. (1): Inhibiting effects.
cells[86]. The IncRNA, MALAT1, is ectopically expressed in GC, promoting cancer cell proliferation by recruiting and partly modulating SF2/ASF[87]. Other IncRNAs, such as GHET1 and GASS, are aberrantly expressed in GC and are related to tumor size, TNM stage, invasion, and prognosis. These IncRNAs function as GC metastasis regulators by modulating the interaction between insulin-like growth factor 2 binding protein-1 and c-Myc mRNA, consequently upregulating the expression of c-Myc mRNA and increasing its stability, thereby promoting proliferation or enhancing caspase-3-dependent apoptosis[88,89]. These regulatory mechanisms of IncRNAs in gastric carcinogenesis and metastasis pave the way for future molecular target therapy for GC.

CONCLUSION

In the human transcriptome, aberrant sequence expression involves both protein-coding RNAs and non-protein-coding transcripts. However, non-coding elements were neglected previously, and even considered as noise with no biological functions. Recent research has paid increasing attention to a traditionally ignored area, namely IncRNAs, which are a new cluster of non-coding RNAs transcribed from non-protein-coding sequences. IncRNAs can not only regulate the subcellular localization and activity of other RNAs and proteins, but also regulate local or global gene expression in trans or cis by influencing chromatin modification, and transcriptional or posttranscriptional gene regulation[90]. Recently, the functions and expression levels of IncRNAs were shown to correlate with diseases such as neurological disorders, Mendelian disorders, cardiovascular disease, and cancer[94]. IncRNAs play different biological and physical roles in normal individuals. IncRNAs are involved in gene expression programs by binding to various chromatin regulatory proteins and modulating the pluripotency and differentiation of embryonic stem cells[93]. IncRNAs play critical roles in the development of life and organs, such as the brain[92], heart[93], and lungs[94]. The aberrant expression and regulatory roles of IncRNAs in clinical diseases were demonstrated simultaneously with their functions to some extent. In recent years, their functions and expression levels have been shown to be associated with neurological disorders, Mendelian disorders, cardiovascular disease, and cancer[14,95]. In cancer, IncRNAs participate in a multitude of processes, including cancer cell proliferation, migration, invasiveness, and metastasis[96-98]. The IncRNA, H19, is highly expressed in hepatic metastatic individuals, giving it prognostic value for cancer metastasis[96]. The IncRNA, MALAT1, modulates gene expression, thus changing the metastasis phenotype of lung cancer cells[99]. The upregulated expression of MALAT1 in gallbladder carcinoma suggests that this IncRNA might function as an oncogene, activating the ERK/MAPK pathway and promoting gallbladder cancer cell proliferation and metastasis[97].

IncRNAs also modulate GC tumorigenesis, growth, and metastasis by affecting GC cell proliferation, cell migration and invasion, tumor suppressor genes and oncogenes such as p53[53,78] and c-Myc[90], mediating EMT[66], acting as ceRNAs[10], and being processed into other small molecular RNAs[50,55]. Increasing evidence has shed light on the role of IncRNAs in diverse cancer, particularly GC; however, their functional links to gene regulation, disease occurrence and, specifically, GC metastasis have yet to be thoroughly studied. IncRNAs serve as a group of multiple genes involved in the regulation of GC metastasis[66,100]. Studies have shown that angiogenesis and the stroma play pivotal roles in cancer metastasis[101]. Researchers have demonstrated that IncRNAs participate in neovascularature forma-

tion[31,32], TJ and adhesion regulation[37,38,42], and extracellular matrix degradation[47-49]. Some IncRNAs are aberrantly expressed or play essential roles in cell activity and cancer metastasis, indicating that IncRNAs may promote or inhibit GC metastasis.

Unfortunately, few studies have systematically described a functional overview of the mechanisms underlying the regulatory roles of IncRNAs and their association with GC metastasis. Provision of reliable approaches for predicting and identifying the functions of the versatile IncRNAs, and exploring this mysterious field in a comprehensive manner remains necessary[8]. For GC metastasis-related IncRNAs, a stepwise approach to the comprehensive understanding of GC metastasis-related IncRNAs is also required. Uncompleted tasks include the following: (1) screening for more GC metastasis-associated IncRNAs; (2) exploring the mechanisms of IncRNAs involved in GC carcinogenesis and metastasis; (3) searching for IncRNAs with metastasis value and constructing a IncRNA regulatory network; (4) seeking IncRNA pathways participating in GC invasion and metastasis and selecting the key pathway; and (5) intervening in GC at the gene level to inhibit metastasis. Taken together, the study of newly discovered IncRNAs is a promising field for future molecular targeted therapy of GC via metastasis intervention.

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