Roles of Long Noncoding RNAs in Regulating Epithelial-Mesenchymal Transition Process in Gastric Cancer

Wei Feng, Hui Cong, Xianjuan Shen, Wei Zong and Shaoqing Ju*

Center of Laboratory Medicine, Affiliated Hospital of Nantong University, Nantong, China

*Corresponding author: Shaoqing Ju

✉ jsq814@hotmail.com

Center of Laboratory Medicine, Affiliated Hospital of Nantong University, Nantong, China.

Tel: +86-0513-85052610

Abstract

Gastric Cancer (GC) is one of the most three reasons related to death caused by cancer, especially in East Asia area, where many people have been suffering in this disease. Although there are significant improvements in surgical techniques and medical standards, the five-year survival rate of GC patients is still low. Epithelial-Mesenchymal Transition (EMT) plays a key role in the process of metastasis, which is a biological process that epithelial cells lose their polarity and turn into mesenchymal phenotype. It also enjoys a potential effect on invasion and migration of multiple malignancies, including that of gastric cancer. Accumulating evidences in literature suggest that long noncoding RNAs (lncRNAs) play an important role in the process of EMT. In this review, roles of lncRNAs in GC EMT are highlighted and pathways of lncRNAs in regulating EMT in GC is clarified.

Keywords: Long noncoding RNAs; Epithelial-mesenchymal transition; Gastric cancer; Metastasis; lncRNAs pathways

Highlights

LncRNAs involved in gastric cancer EMT

LncRNAs related to mechanisms of EMT in GC

Regulation mechanisms of transcription factor

Epigenetic regulatory mechanisms

Signal pathways associated with LncRNAs in regulating EMT in GC

Introduction

Gastric cancer is the third leading cause for cancer deaths. People have been suffering a high mortality rate worldwide, especially in East Asia [1,2]. Gastric cancer is difficult to cure, and the majority of patients with gastric cancer was diagnosed at an advanced stage [3]. Although surgical techniques and medical standards are much higher now, the five-year survival rate of GC patients only 20%-30% [4]. Obviously, it is necessary to improve the efficiency and effectiveness of early diagnosis and master the mechanism of tumor metastasis. The formation of metastasis is a complex process [5]. Firstly, tumor cells escape from primary tumor tissue and enter into blood or lymphatic system. Secondly, some of them weather apoptosis and survive in a secondary site. Then they remove from blood vessels and

settle in a new microenvironment. In the end, they proliferate in the new environment [6]. All such processes are critical for us to understand the mechanism during metastasis. Moreover, even the steps of metastasis process being recognized and very important to understand the pathology, the knowledge about biomarkers is also crucial to deeply understand the process. In recent years, a series of convincing studies have shown that lncRNAs can be used as biomarkers for cancer diagnosis including GC.

The human transcriptional group not only contains a large number of protein-coding messenger RNAs(mRNAs), but also many non-protein coding RNAs (ncRNAs) occupying up more than 98% of the whole genome sequence and function as important regulatory factors in the process of tumor suppression and carcinogenic [7-9]. In terms of size, ncRNAs are divided into short non-coding RNAs (<200nt) and long non-coding RNAs (>200nt). There is increasing evidence that lncRNAs play an important role in many aspects, such as cell differentiation, chromatin remodeling, immune responses and carcinogenesis [10-12]. In addition, many studies have shown that the metastatic pathways are highly correlated with abnormal expression of lncRNAs [13,14].

Epithelial-Mesenchymal Transition (EMT), playing a key role in the process of metastasis, is a biological process that epithelial

cells lose their polarity and turn into a mesenchymal phenotype [15]. Recent studies found that EMT has a potential effect on invasion and migration of multiple malignancies [16,17]. During the formation of tumor, the adhesion between cells decreased, epithelial cells lose polarity, and the contact with the surrounding matrix cells decreased. Tumor cell migration and motor ability are enhanced. At the same time, cell phenotype changes gradually, and epithelial cells lose epithelial phenotype characteristics. During this process, there are many molecules that can be used as a marker for EMT, for example, the decreased expression of epithelial markers E-cadherin, Zonula Occludens protein 1 (ZO-1), Epithelial Cell Adhesion Molecule (EpCAM), the increased expression of mesenchymal markers Vimentin, N-cadherin [18].

In this review, roles of lncRNAs in GC metastasis and the mechanism of regulating EMT progress in GC are stressed.

LncRNAs Involved in Gastric Cancer EMT

Recently, accumulating evidences have discovered that lncRNAs can directly regulate the process of EMT in GC [19,20]. EMT is an important process for cancer cells to obtain the ability to migrate and metastatic. Moreover, EMT property is also linked with embryonic development, wound healing and fibrotic disease [21,22]. Recently, many studies have found that lncRNAs can regulate the process of EMT in GC. Therefore, the regulation of EMT by lncRNAs is generalized in **Table 1.**

The activation of the process of EMT is accompanied by a decrease in the expression of cell adhesion molecules (E-cadherin). Subsequently, cytokeratin would transform into Vimentin. Recently, it was found by Liu et al. [23] that lncRNA HOTAIR (HOX transcript antisense RNA) is highly expressed in diffuse-type GC and is negatively related to E-cadherin. In addition, HOTAIR can exogenously down-regulate miR34a by interacting with Polycomb Repressive Complex2 (PRC2) to activate its target gene C-Met (HGF/C-Met/Snail pathway) and Snail (a family of transcription factors), thus promoting EMT in gastric cancer. Furthermore, high-HOTAIR group predicted a poor over-all survival in GC patients. *In vitro*, it was found by Xu [24] and his colleagues that inhibition of HOTAIR in GC cells could reduce invasiveness, and the expression of Matrix metalloproteinase-1 (MMP1) and Matrix metalloproteinase-3 (MMP3). Suppressing the expression of HOTAIR could reverse the process of EMT. By bioinformatic analysis and luciferase assay, it was found by Zhou

Table 1 Regulation of EMT by lncRNAs.

LncRNA	Chromosomal location	Gene type	Putative functions related to metastatic prognosis	Related gene	Ref.
LEIGC	Chr2q14.1	Tumor suppressor	Tumor growth, Cell proliferation, Migration, EMT	E-adherin, Snail, Slug, Zeb, Twist	[30]
SPRY4-IT1	Chr5	Tumor suppressor	Tumor size, Lymphatic metastasis, Advanced pathological stage, Deeper depth of invasion	DNMT1, E-cadherin, Vimentin	[31]
XLOC-010235	Chr12	Oncogene	Cell viability, Migration, EMT, invasion	E-cadherin, N-cadherin, Vimentin, Snail1	[36]
MALAT-2	Chr11	Oncogene	Lymph node metastasis, Tumor stage, proliferation, Cell migration, EMT	E-cadherin, Vimentin	[54]
ZFAS1	Chr17	Oncogene	Distant metastasis, TNM stage, Lymph-node metastasis, Invasion	CDH1, CDH2, Snail,ZEB1, Vimentin, MMP14	[39]
HOTAIR	Chr12q13.13	Oncogene	EMT, Invasion, TNM stage, Lymph node metastasis	miR-331-3p, HER2, C-Met(HGF/C-Met/ Snail pathway), E-cadherin, Vimentin	[23,24]
TRERNA1	Chr20q13.13	Oncogene	Invasion, Migration, EMT	EZH2,Snail1,CDH1	[35]
H19	Chr11p15.5	Oncogene	Cell proliferation, migration, Invasion, Distant metastasis	ZEB1,miR-141	[7,25]
CCAT2	Chr8q24	Oncogene	Proliferation, Invasion, Tumor size, EMT, lymph node metastasis, TNM stage	CDH1, LATS2, E-cadherin, Vimentin	[26]
LncRNA00261	Chr20p11.21	Tumor suppressor	Poor prognosis, Cell proliferation, Cell cycle progression, Invasion,EMT	GSK3β,Slug, ZEB1, E-cadherin, Vimentin	[32]
SNHG20	Chr17q25.2	Oncogene	Cell development, Proliferation, Differentiation, Apoptosis, Invasion, Metastasis	p21,EZH2, H3K27me3, GSK-3β/β-catenin, E-cadherin, Vimentin	[53]
HOXA-AS2	Chr6	Oncogene	Proliferation, tumor size, poor prognosis, apoptosis, TNM stage	EZH2,P21/PLK3/DDIT3	[50]
SNHG6	Chr11	Oncogene	Cell proliferation, apoptosis, migration, EMT	EZH2,P27, miR-101-3p	[52]
ATB	Chr1	Oncogene	Vascular invasion, EMT	TGF-β, ZEB1, CDH1, miR-200,	[29]
LncRNA-p21		Tumor suppressor	Cell proliferation,Migration, Invasion,EMT	YAP,p53, Hippo- pathyway	[55]
LncRNA00152	Chr2	Oncogene	Cell cycle arrest, Apoptpsis, EMT, Migration, Invasion	N-cadherin, E-cadherin, Vimentin	[3]
NEAT1	Chr11	Oncogene	Lymph node metastasis, Distant metastasis, EMT	Vimentin,N-cadherin,Zo-1,E-cadherin	[56]
UCA1	Chr19	Oncogene	Cell proliferation, Invasion, Migration, EMT	TGFβ1, Vimentin,Snail,E-cadherin,Zo-1	[28]
PVT1	Chr8	Oncogene	Deep invasion depth, TNM stage, EMT	P15.p16,EZH2	[27]

[25] and collaborators that the expression of H19 is inversely correlated to the expression of miR-141 in GC cells and tissues. H19 promotes proliferation and invasion whereas miR-141 suppresses malignancy in GC cells. The long non-coding RNA CCAT2 (Colon Cancer Associated Transcript 2) was identified to be up-regulated in GC. In the study by Wang et al. [26], it was demonstrated that CCAT2 could promote the GC cells EMT by down-regulating E-cadherin expression and up-regulating Zinc finger E-box Binding homeobox2 (ZEB2), Vimentin and N-cadherin expression. Further experiments demonstrated that PVT1 knockdown could inhibit proliferation both *in vitro* and *in vivo*. It was confirmed by Kong et al. [27] that PVT1 is associated with Enhancer of Zeste Homolog2 (EZH2), in addition, also found that this association is required for the repression of p15 (MTS2, Multiple Tumor Suppressor 2) and p16 (MST1, Multiple Tumor Supressor 1). In Zuo's [28] research, Urothelial Cancer Associated 1 (UCA1) was specifically up-regulated in GC tissues and cell lines, and high UCA1 was related with EMT-related factors. Moreover, it was found that the effect of UCA1 could be partly restored by Transforming Growth Factor-β1 (TGF-β1) treatment. Futhermore, in Saito's [29] research, when treatment with TGF-βin GC cell lines resulted in morphological EMT changes, upregulation of lncRNA-ATB (lncRNA Activated by Transforming Growth Factor-β) would occur, therefore, it was concluded that lncRNA-ATB plays an important role in EMT to promote invasion and metastasis through the TGF-β/miR-200S/ZEB axis. Recently, it was found by Han et al. [30] that GC tissues suffer significantly lower levels of Lower Expression in Gastric Cancer (LEIGC) expression than adjacent non-cancerous tissues. It was further demonstrated LEIGC functions by inhibiting EMT, overexpression of LEIGC suppressed tumor growth and cell proliferation. QRT-PCR was performed to detect the expression of SPRY4-IT1 in 61 pairs of gastric cancer samples. Subsequently, GC cells transfected with pCDNA-SPRY4-IT1 were injected into nude mice via tail vein. Ectopic overexpression SPRY4-IT1 resulted in a reduction of metastatic nodules [31]. In addition, linc00261 was significantly down-regulated in GC tissues compared with that of adjacent normal tissues. *In vitro* functional assays, it was demonstrated by Yu [32] and his colleagues that linc00261 supress invasion while inhibit the EMT partly through reducing Slug (snail2) protein abundance, the progression of EMT by lncRNAs is generalized in **Figure 1**.

LncRNAs Related to Mechanisms of EMT in GC

Multivariate analysis indicates that abnormal expression of lncRNAs play important roles in epithelial-mesenchymal transition in GC. Therefore, the association of lncRNAs and GC EMT progression is discussed.

EMT enjoys main characteristics as the down-regulate in the expression of cell adhesion molecule (E-cadherin) and the transformation of cytokeratin cell skeleton into Vimentin. Many efforts have been devoted to study the process of E-cadherin and Vimentin regulated EMT.

Regulation Mechanisms of Transcription Factor

Transcription factors (including Snail, Twist, and ZEB) are the initiating factors that induce EMT. They can regulate the expression of EMT related molecular markers, inhibit epithelial phenotype markers (E-cadherin, β-catenin) and activates interstitial phenotypic markers (N-cadherin, Vimentin and Fibronectin) [33]. Large numbers of lncRNAs have been reported as regulator to the expression of transcription factors and the process of EMT.

Snail, as a member of the zinc finger protein family, is a DNA-binding factor that recognizes E-box motifs in target promoters (CDH-1, Cadherin-1), which can regulates the following E-cadherin repression [34]. The Snail family comprises three members:
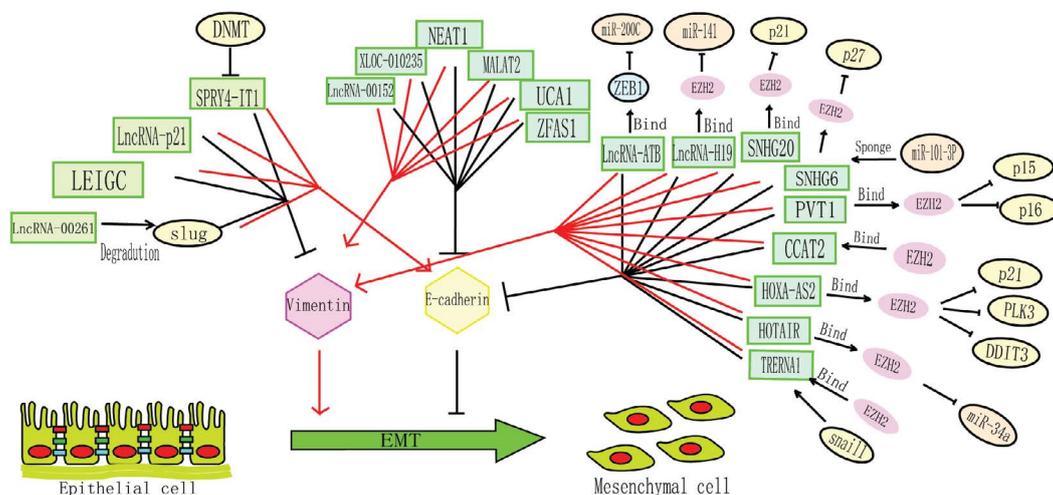

Figure 1 Progression of EMT by lncRNAs.

Snail1 (originally identified as Snail), Snail2 (as Slug) and Snail3 (as Smuc). In order to investigate whether Snail is a repressor of CDH-1 in GC cells, the Snail expression level was detected by Wu et al. [35] either in RNA interference or in enforced expression. Corresponding result showed that lncRNA TRERNA1 (Translation Regulatory Long Non-coding RNA 1) acts like an enhancer of Snail1 to moudulate CDH-1 expression, thereby promoting cell invasion and migration and contribute to metastasis of GC. It was also found by Liu et al. [36] that when lncRNA XLOC-010235 is over-expressed or under-expressed, mRNA of Snail1 is increased 2.5-fold or decreased about 60% compared to that of control groups. At the same time, western blot analysis was performed to validate the expression of the Snail1 on protein level. When knocking out or overexpressing Snail occured, Snail protein levels were reduced by about 54% or increased by a factor or two. Relevant researches show that Snail1 is positively regulated by lncRNA XLOC-010235 at the mRNA and protein levels.

Twist is a member of the basic helix-loop-helix transcription factor family, including Twist1 and Twist2. In accordance with current studies, it was found that Twist was up-regulated in many tumors. A series of researches illustrated that Twist plays an important role in tumorigenesis and progression by affecting tumor cell apoptosis, inhibiting differentiation, inducing EMT, participating in tumor drug resistance, and tumor angiogenesis [37,38]. According to such studies, it was confirmed that Twist participates in the EMT of some epithelial-derived tumor cells and promotes its invasion and metastasis, inducing a series of mesenchymal markers. Twist, like other regulators, can act on the upstream synthesis of E-cadherin, and inhibit its expression [28]. In order to study the relationship between lncRNA ZFAS1 and the EMT associated factors, 40 pairs of gastric cancer tissue were randomly selected by Zhou et al. [39] to detect Twist expression levels. Subsequently, it was found that the expression level of lncRNA ZFAS1 is positively correlated with Twist.

ZEB (Zinc finger E-box-binding protein) including ZEB1 and ZEB2 enjoys similar structure in different patterns. The middle of variable sequence will be C-terminal and N-terminal zinc finger clusters connected [40]. Overexpression of ZEB protein is closely related to tumorigenesis and metastasis [40]. ZEB promotes the expression of Matrix Metalloproteninases (MMPS) by decreasing E-cadherin while increasing the mesenchymal phenotype markers Vimentin, Fibronectin and N-cadherin, inducing EMT in tumor cells and promoting tumor transfer [41]. Effects of lncRNA ZFAS1 knockdown on cell migration was investigated by Pan et al. [42]. Relevant data showed that the expression of epithelial marker E-cadherin was up-regulated while that of the mesenchymal marker N-cadherin was downregulated, and transcription factor ZEB1 was remarkably decreased.

Epigenetic Regulatory Mechanisms

It has been found that the expression of E-cadherin is not only regulated by transcription factors, but also by epigenetic modifications, such as microRNAs, DNA methylation, and histone modification [43-47].

DNA methylation is one of the earliest methods modification. A large number of studies have shown that DNA methylation would cause changes in the chromatin structure, DNA conformation, DNA stability and DNA-protein interactions to control the gene expression. DNA methylation can cause gene inactivation, lead to DNA conformation changes in some regions, thus affecting the protein-DNA interaction. In addition, the sequence-specific methylated binding protein (MBD/MeCP) binds to the methylated CpG island in promoter region to prevent the transcription factor from acting on the promoter, thereby suppressing gene transcription [48]. Through Bioinformatic analysis, a canonical CpG island was identified by Xie et al. [31] in the promoter region of the lncRNA SPRY4-IT1 loci. In addition, it was found that lncRNA SPRY4-IT1 expression is significantly increased in gastric cancer cells compared with controls. Moreover, inhibiting the expression of DNA methyltransferase1 (DNMT1) could up-regulate SPRY4-IT1 expression. Moreover, Chromatin Immunoprecipation (CHIP) assays were also performed to invalidate that DNMT1 could directly bind to lncRNA SPRY4-IT1 promoter region.

Histone modification refers to processes, such as methylation, acetylation, phosphorylation, adenylation, ubiquitination and ADP ribosylation under the action of related enzymes. Among them, histone methylation plays an important role in the expression and progression of EMT related genes [49]. Through RNA Immunoprecipitation (RIP) and Chromatin Immunoprecipitation (CHIP) assays, it was revealed by Wu et al. [35] that lncRNA TRERNA1 functions are confirmed as a scaffold to recruit Enhancer of zeste homolog2 (EZH2) to epigenetically silence epithelial-mesenchymal transition marker CDH1 by H3K27me3 of its promoter region. It was reported by Xie et al. [50] that the expression of lncRNA HOXA-AS2, a 1048bp RNA was related to the tumor size and higher clinical stage in GC patients. In addition, lncRNA HOXA-AS2 could epigenetically repress the expression of P21 (Calcium binding protein), PLK3 (Polo like kinase 3), and DDIT3 (DNA damage inducible transcript 3) through binding with EZH2, inducing H3K27 trimethylated.

MicroRNAs(miRNAs) are a class of non-coding single-stranded RNA molecules of about 22 nucleotides in length encoded by endogenous genes that are involved in post-transcriptional gene expression regulation in animals and plants [51]. In recent years, many studies have shown that miRNAs play an important role in tumor development. It was revealed by Yan et al. [52] that lncRNA SNHG6 (Small nucleolar RNA host gene 6) was over-expressed in GC tissues and cell lines, and the expression levels of lncRNA SNHG6 were associated with invasion depth, lymph node metastasis, and TNM stage. In addition, through CHIP, RIP, RNA-pulldown and luciferase reporter assays, it showed that lncRNA SNHG6 could competitively sponge miR-101-3p and regulate Zinc finger E-box binding homeobox 1 (ZEB1), thus participating in tumor EMT process.

Signal Pathways Associated with lncRNAs in Regulating EMT in GC

In recent years, many studies have shown that lncRNA–related signaling pathway can participate in the regulation of EMT process.

It was found by Liu et al. [53] that lncRNA SNHG20 (Small nucleolar RNA host gene 20) could promote GC progression by inhibiting p21 expression and regulating the GSK-3β/β-catenin signaling pathway. In relevant study, they proved that lncRNA SNHG20 plays an important role in regulating GC cells invasion by stimulating EMT process. In addition, it was also investigated that lncRNA SNHG20 could epigenetic silence the expression of p21 and E-cadherin by binding with EZH2 and regulating the GSK-3β/β-catenin signaling pathway. Chen et al. [54] revealed that lncRNA MALAT2 could induce EMT through an MEK/ extracelluar signal-regulated kinase-dependent mechanism as treatment with the MEK inhibitor, U0126, decreased migration and reversed the EMT in the lncRNA MALAT2 (Metastasis associated lung adenocarcinoma transcript 2) over-expressed cells. It was demonstrated by Saito and colleagues that miR-200c or ZEB1 levels in GC tissues are correlated inversely or positively with lncRNA-ATB levels. Being treated with TGF-βcan upregulate lncRNA-ATB and ZEB1, while downregulate miR-200c and CDH1.

These results might validate that lncRNA-ATB can through the TGF-β/miR-200/ZEB axis to regulate the process of EMT [29].

Conclusion

Although lncRNAs are classified as non-coding RNA, many studies have shown that lncRNAs plays an important role in tumorigenesis and its development. In this review, lncRNAs are summarized, involving in the regulation of the EMT process, and relevant signaling pathways and regulatory mechanisms are collated. The role of lncRNA and EMT in the formation and development of gastric cancer is further clarified. It provides a strategy for finding molecules capable of regulating EMT. Current studies suggest the important role of lncRNA in the process of gastric cancer EMT. Therefore more studies should be performed in the future [55-56].

Acknowledgment

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