The cross talk between long, non-coding RNAs and microRNAs in gastric cancer

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Abstract

Gastric cancer is one of the most common malignant diseases and remains the second leading cause of cancer-related mortality worldwide. Although great effort has been made during the past decades to facilitate the early detection and treatment of gastric cancer, the prognosis is not yet satisfactory and the underlying molecular mechanisms of gastric cancer pathogenesis are not fully understood. Meanwhile, non-coding RNAs have been established as key players in regulating various biological and pathological processes, such as cell-cycle progression, chromatin remodeling, gene transcription, and posttranscriptional processing. Furthermore, numerous studies have also revealed a complicated interplay among different species of non-coding RNAs; therefore, the cross-regulation between long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) has begun to emerge. This lncRNA–miRNA cross talk, which has attracted increasing attention in recent years, is involved in a great number of human diseases including gastric cancer. In this review, we summarize the latest research progress of the interactions between lncRNAs and miRNAs, highlighting their influences on the development and progression of gastric cancer to provide novel approaches for cancer diagnosis and treatment.

Key words: long non-coding RNA, microRNA, cross talk, gastric cancer

Introduction

Gastric cancer (GC) remains a major public health issue worldwide, with an estimated 990,000 new cases and 738,000 deaths registered in 2008 [1,2]. Although great advances in the diagnosis and treatment of GC have been achieved recently, the 5-year mortality of GC has been slightly reduced over the past few decades. One of the reasons may be that most patients are diagnosed at advanced stage accompanied with malignant proliferation, thereby effective therapeutic approaches are limited. Additionally, recurrence and metastasis are also great challenges during cancer treatment [3]. Although many oncogenes and tumor suppressors have been identified as key players in the tumorigenesis of GC, almost no commonly accepted biomarker has been established to facilitate the comprehensive management of patients, such as early diagnosis, targeted therapy, and prognostic prediction. Therefore, a deep understanding of the molecular mechanisms underlying gastric carcinogenesis and the identification of new diagnostic and therapeutic targets for GC will have remarkable importance.

Non-coding RNAs

Recently, studies with high-resolution microarray and genome-wide sequencing technology have discovered that ~21,000 genes with <2% of human genome has protein-coding ability, indicating that non-coding RNAs represent majority of the human transcriptome. Non-coding transcripts can generally be divided into two major categories by their transcript sizes, including ~9000 small, non-coding RNAs and ~10,000–32,000 long, non-coding RNAs (lncRNAs), along with around 11,000 pseudogenes [4,5]. There are several types of small, non-coding RNAs with different functions, such as microRNAs (miRNAs), transfer RNAs, small-interfering RNAs, small nuclear RNAs, small nucleolar RNAs, and PIWI-interacting RNAs [6].
LncRNAs

LncRNAs are generally shorter than mRNAs, with lengths ranging from 200 bp to 100 kb. Based on the genomic location relative to nearby transcripts, lncRNAs can be classified into five subtypes: intergenic lncRNAs, intronic lncRNAs, sense or antisense transcripts, pseudogenes, and retrotransposons [7]. Functionally, lncRNAs can regulate gene expression at various levels, including chromatin modification, transcription, and posttranscriptional processing [8,9]. Studies have shown that lncRNAs play critical roles in modulating diverse biological processes ranging from pluripotency to immune responses [10], and the dysregulations of lncRNAs have also been observed in different types of cancers including GC [11–14]. Some lncRNAs are upregulated in cancers and may exhibit tumor-promoting abilities, among them Hox transcript antisense intergenic RNA (HOTAIR) is one of the best known oncogenic regulators [12,15]. Some other lncRNAs are downregulated in cancers and play tumor-suppressive roles, such as maternally expressed gene 3 (MEG3) and growth arrest-specific transcript 5 (GAS5) [16–18]. The role of lncRNAs in GC has also been reviewed recently [19].

Mechanisms of the Interplay Among Non-Coding RNAs

To provide a detailed elucidation of the dynamic ncRNA regulatory network, great effort has been made to reveal the interplay and cross-regulation among non-coding RNAs, especially the cross talk between lncRNAs and miRNAs. Mounting evidence has uncovered that lncRNAs and miRNAs can interact with and regulate each other through multiple posttranscriptional mechanisms. Four archetypes of the mechanisms will be discussed in the sections below (Fig. 1).

MiRNAs

MiRNAs are ~19–23 nucleotides in length. Up to now, >2000 miRNAs have been registered in the ‘miRbase’ database [20], and many miRNAs have been relatively well investigated during the past few years. miRNAs can regulate 60% of coding genes by binding to mRNA molecules, altering the expression of target genes. Although the functional role of most miRNAs is still unclear, many studies have shown that they are involved in a wide variety of physiological processes including cell fate specification, proliferation, and cell death, thus participating in the pathogenesis of diseases such as cancers [21]. Several miRNAs have been identified as either oncogenes or tumor suppressors. For example, miR-21 is a well-characterized oncogenic miRNA which is overexpressed in many types of malignancies, such as breast cancer, colorectal cancer, lung cancer, glioblastoma, as well as GC [22–25]. While miR-34 family members are known as tumor-suppressive miRNAs, decreased expression of miR-34a is associated with metastasis and recurrence of prostate cancer, and restoration of miR-34a expression could repress the proliferation and invasion of cancer cells [26,27].

Regulation Mechanisms of Non-Coding RNAs

Like protein-coding RNAs, the regulation mechanisms of non-coding RNAs are also complicated. On one hand, non-coding RNAs may be regulated by a number of mechanisms, such as gene mutation, alteration of DNA copies, defective transcription, and epigenetic alteration [28]. On the other hand, non-coding RNAs can also regulate gene expression at different levels via interacting with DNA, RNA, and proteins. In most cases, miRNAs can interact with and regulate other transcripts through binding to the binding sites of target genes, while lncRNAs can exert their functions via four mechanisms: (i) lncRNAs can serve as molecular signals in the process of gene regulation by marking functionally significant biological events, including space, time, developmental stage, and expression; (ii) some lncRNAs are transcribed and subsequently bind to and titrate away protein targets or small regulatory RNAs from the chromatin without exerting any additional functions; (iii) lncRNAs can also serve as guides by binding to specific proteins and then localizing ribonucleoprotein complex chromatin targets; and (iv) some lncRNAs can bring protein factors altogether to form scaffolding complexes that may activate or repress transcription by affecting histone modification [29].

In addition to the regulation mechanisms mentioned above, lncRNAs and miRNAs may also participate in an intricate network that involves diverse RNA species. A recent review has described the multilayered complexity of RNA–RNA cross talk and competitive networks [6]. Among non-coding RNAs, miRNAs are able to bind and sequentially destabilize or arrest the translation of hundreds of their potential mRNA targets [30]. Nevertheless, non-coding RNAs that share miRNA response elements (MREs) with coding RNAs can be similarly targeted; thus, the miRNAs are sequestered and then prevented from acting on the protein-coding mRNAs, resulting in the formation of a complex network of competing endogenous RNAs (ceRNAs) [31]. Moreover, by searching and integrating the data from multiple databases, a ceRNA network in GC has been constructed recently, which revealed a novel strategy to predict ceRNA network in cancer [32]. Taken together, ceRNAs have been identified as important posttranscriptional regulators of gene expression, which have exciting implications for the development of new therapeutic methods for various diseases including cancers [33].

LncRNAs serve as miRNA sponges/decoys

LncRNAs that share MREs with coding RNAs could harbor similar miRNA target sequences and then sequester miRNAs away from acting on mRNAs. These lncRNAs are known as ceRNAs. They can serve as ‘sponges’ or ‘decoys’ for miRNAs, decreasing the amount of available miRNAs and contributing to enhanced translations of their target mRNAs (Fig. 1B). The first example of ceRNAs was the tumor suppressor PTEN (phosphatase and tensin homolog). It was reported that PTEN expression was regulated by the expression level of the transcript encoded by pseudogene PTENP1, while PTENP1 functioned as a decoy to exert this influence [37]. Additionally, linc-MD1, a muscle-specific lncRNA, could sponge miR-133 and miR-135 away from target mRNAs to lower the available levels of miRNAs, eventually leading to the increased expression of MAML1 and MEF2C mRNAs [38].
LncRNAs compete with miRNAs for binding to mRNAs
Some lncRNAs that are complementary to mRNAs in regions that include the binding sites of miRNAs can directly compete with miRNAs for binding to mRNAs, sequentially deleting the regulation effect of miRNA on mRNAs (Fig. 1C). For example, the lncRNA BACE1AS could compete with miR-485-5p for binding to BACE1 mRNA, rescuing the decreased expression of BACE1 through antagonizing miR-485-5p-induced mRNA degradation [39]. Besides, the tumor-promoting lncRNA ncNRFR (non-coding Nras functional RNA) may also exhibit similar property. Researchers discovered that ncNRFR possessed a 22-nt sequence that was identical to let-7a by 1–4 nucleotides, and upregulation of ncNRFR in the colonic epithelial cell line YAMC enhanced the activity of a heterologous reporter containing a let-7 binding site, implicating that ncNRFR could suppress the functions of let-7 by competing with let-7 for endogenous target mRNAs [40].

LncRNAs generate miRNAs
Some lncRNAs are able to generate miRNAs (Fig. 1D). It has been found that linc-MD1 could generate miR-206 and miR-133, respectively, from an intron and an exon [38]. In addition, lncRNA H19 has been indicated to generate miR-675 in mouse [41].

Cross Talk Between LncRNAs and MiRNAs in GC
By now, the cross talk between lncRNAs and miRNAs has been described in a wide range of human carcinomas. Here, we will outline the emerging role of lncRNA–miRNA interplay in GC development and progression based on the types of lncRNAs (Fig. 2).

HOTAIR
HOTAIR that is transcribed from the HOXC locus is an oncogenic lncRNA with 2158 nucleotides. It is well known for its involvement in various tumors, including breast cancer, colorectal cancer, hepatocellular carcinoma, pancreatic cancer, and GC. Generally, highly expressed HOTAIR induces cancer pathogenesis via promoting polycomb repressive complex 2 (PRC2) relocation and histone 3 lysine 27 (H3K27) trimethylation [11,12,15,42,43].

A recent study in GC indicated that HOTAIR was able to positively regulate human epithelial growth factor receptor 2 (HER2) through a competition for miR-331-3p binding, a miRNA with dual target specificities for both HOTAIR and HER2. The expression of HOTAIR was elevated in GC tissues compared with adjacent normal gastric tissues. Ectopic expression of HOTAIR in GC could enhance cell proliferation, migration, and invasion, while knockdown of HOTAIR repressed cell invasion and cell viability [44]. Like HOTAIR, HER2 was also overexpressed and exerted tumor-promoting capability in GC [45]. As confirmed by luciferase assays, HER2 was found to be directly targeted and suppressed by miR-331-3p, and the high abundance of HOTAIR could restore the expression of HER2. Furthermore, increased expression of miR-331-3p or depletion of HOTAIR in GC led to a significant reduction of HER2 protein expression, while the transfection with HOTAIR could increase HER2 protein expression in GC cells. Taken together, these findings suggested that HOTAIR could relieve the repression of HER2 mRNA by serving as a ceRNA to sponge miR-331-3p away from binding to HER2, thus increasing the translation of HER2 and accelerating the development and progression of GC [44].

H19
H19 ncRNA that is encoded by the paternally imprinted gene H19 is highly expressed in embryogenesis but dramatically downregulated after birth [46]. H19 was initially found to possess tumor-suppressing ability [47], but later was established as an oncogenic regulator with a notably overexpression in bladder cancer, breast cancer, and colorectal cancer, implying that the influence of H19 on cancers may be cell or tissue specific [48–50].

As mentioned before, lncRNA H19 is capable of generating miR-675, which is commonly observed in colorectal cancer [51]. More recently, the lncRNA H19-derived miR-675 has also been found in GC, suggesting that H19/miR-675 pathway may play an oncogenic role in gastric carcinogenesis by further modulating...
Consistent with the results in colorectal cancer, expressions of H19 and miR-675 were both significantly upregulated in GC and positively correlated with each other, which were closely related to poor prognosis by promoting cell proliferation, migration, invasion, and metastasis. Meanwhile, transfection with miR-675 could abolish the inhibitory effect on cell proliferation when H19 RNA was silenced in GC cells, thereby supporting the hypothesis that H19 could regulate the progression of GC via miR-675.

Further investigations have also shown that the H19/miR-675 pathway could affect the biological behaviors of GC cells through interactions with multiple targets of miR-675, such as Runt Domain Transcription Factor 1 (RUNX1) and Calneuron 1 (CALN1) [52,53].

MEG3 LncRNA Maternally expressed gene 3 (MEG3) that is imprinted at the DLK1-MEG3 imprinting locus is expressed in many normal human tissues [54]. The decreased expression of MEG3 has been observed in different human carcinomas, and mounting evidence has demonstrated that MEG3 serves as a tumor suppressor by inducing cell growth arrest and apoptosis in cancers [55–58]. In addition, it has also been found that MEG3 expression is closely associated with the methylation levels of differentially methylated regions (DMRs) [59,60].

Recently, a study in GC has revealed an interaction between MEG3 and miR-148a. MEG3 expression was downregulated in GC, which resulted from the hypermethylation of MEG3 DMR. Overexpression of miR-148a could rescue the abnormal methylation status of MEG3 DMR by inhibiting DNA methyltransferase 1 (DNMT1), thus indirectly increasing MEG3 expression and subsequently leading to the suppression of cell proliferation in GC [61].

GAPLINC By global microarray and in situ hybridization (ISH) analysis in GC specimens, a novel lncRNA named Gastric adenocarcinoma predictive long intergenic non-coding RNA (GAPLINC) has been discovered recently. GAPLINC is overexpressed in GC tissues and participates in enhancing proliferation and invasion of cancer cells, which can be used as an indicator for poor prognosis and shorter survival. Intriguingly, GAPLINC is positively correlated with CD44 by acting as a molecular decoy for miR-211-3p, a miRNA that targets both GAPLINC and CD44 to trigger the degradation of the bound RNA. As a well-established oncogene, CD44 plays a driving role in regulating cancer proliferation, migration, and angiogenesis [62,63]. Elevated expression of GAPLINC competes with CD44 for miR-211-3p binding, which decreases the availability of miR-211-3p for CD44. Thus, GAPLINC reduces CD44 mRNA degradation and eventually enhances the translation of the CD44 oncoprotein [64].

ANRIL Antisense, non-coding RNA in the INK4 locus (ANRIL), a 3.8-kb lncRNA, is transcribed in the opposite orientation of the INK4B-ARF-INK4A gene cluster. Similar to HOTAIR, ANRIL is required for relocating PRC2 complexes to specific loci, leading to the inhibition of gene expression [65–67]. With an independent role in predicting poor prognosis of GC patients, ANRIL expression is increased in GC tissues, while deletion of ANRIL remarkably inhibits cell growth and induces cell apoptosis. Moreover, researchers have also found that ANRIL could epigenetically silence miR-99a/miR-449a by binding to PRC2, thereby further modulating the target genes of miR-99a/miR-449a to promote cell proliferation in GC [68].

LncRNA-AC130710 AC130710, another newly discovered tumor-promoting lncRNA, is also upregulated in GC tissues. Its expression level is correlated with tumor size, TNM stages, distal metastasis, and tissue CEA level. In addition, it has been found that AC130710 is targeted and
downregulated by miR-129-5p, which contributes to the suppression of tumor growth [69].

FER1L4

During the study of ceRNA network in GC, lncRNA FER1L4 has been found to be associated with miR-106a-5p. Researchers discovered that both FER1L4 and RB1 were targets of miR-106a-5p. Moreover, knockdown of FER1L4 could reduce the levels of both FER1L4 and RB1 in GC cells, suggesting that downregulation of FER1L4 could free more miR-106a-5p to bind to other targets, such as RB1 mRNA [32].

Perspective

As a central player in regulating gene expression at multiple levels, non-coding RNAs can influence all aspects of cellular processes, including cell division, proliferation, differentiation, senescence, and apoptosis. Dysregulation of non-coding RNAs is involved in various cancers, forming a complex regulation network by interacting with other transcription factors. With an emerging role in cancer progression, the interplay among different species of non-coding RNAs, particularly between IncRNAs and miRNAs, has also come into sight. In this review, we discussed the interactions between IncRNAs and miRNAs in GC, highlighting the functional role of lncRNA–miRNA cross talk in gastric carcinogenesis. The examples described above indicate that IncRNAs can act as either regulators or effectors of miRNAs through several posttranscriptional mechanisms, thus jointly modulating gene expression and eventually affecting GC progression.

Although great progress has been made in the study of non-coding RNAs, our understandings of the detailed mechanisms underlying IncRNA–miRNA cross talk in GC are still insufficient. Further investigations, such as animal experiments and systematic analysis of IncRNA-related miRNAs, are needed to fully elucidate the IncRNA–miRNA regulation network, which will help to develop better strategies for the diagnosis and therapy of GC.

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