Noncoding RNA in Gastric Cancer with Potential Prognostic and Predictive Role

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Introduction

GC is one of the most frequent malignant tumors; every year in the world, there are 723,000 cancer-related deaths caused by GC according to the World Health Organization (WHO). It is the fifth most common cancer in the world and the third cause of death among cancer pathologies [1]. Due to the lack of specific diagnostic markers, most patients with GC do not receive an appropriate diagnosis and treatment; this leads to a progression of the pathological state with development of metastases [2]. Previous studies have hypothesized that GC is a genetic disease involving multi-step changes in the genome [3]. However, the human genome contains nearly 20,000 protein-coding genes, but they represent less than 2% of the whole genome [4]. In contrast, according to the Encyclopedia of DNA Elements (ENCODE) project, more than 80% of functional DNA elements in the human genome do not code for proteins [5]. A large part of these functional DNA elements is represented by ncRNAs [6].

In the last years, several studies have shown that ncRNAs play a significant role in different cellular and physiological processes including gene regulation, genomic imprinting, chromatin packaging, dosage compensation, cell differentiation, and embryonic development [6, 7]. Accordingly, the dysregulation of ncRNAs, as pivotal modulators of gene expression, has been documented in different human complex diseases including cancer [8]. In fact, they are able to influence different mechanisms in cancer cells,

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such as proliferation, apoptosis, invasion, and metastasis as well as neoangiogenesis [9]. Expression profiling studies on ncRNAs in a variety of cancer types have revealed a broad range of lncRNAs with aberrant expression [10]. Moreover, it has been shown that ncRNAs are promising candidate prognostic biomarkers for GC detection and potential therapeutic targets. Several ncRNAs could be secreted into body fluids, suggesting that tumor cells may change their extracellular environments through RNA-based, hormone-like mechanisms [11].

In this chapter, we discussed the different roles of ncRNAs in GC and the possible diagnostic, prognostic, and therapeutic applications.

ncRNAs

ncRNAs refer to a class of RNAs with no protein-coding function that are widely expressed in organisms [12]. ncRNAs can be divided into two groups: housekeeping ncRNAs and regulatory ncRNAs. The latter can further be divided into three types, according to their length: (1) short ncRNAs, including miRNAs, small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs), (2) mid-size ncRNAs, and (3) lncRNAs [13–15].

Short ncRNAs are shorter than 50 nucleotides (nt), mid-size ncRNAs have a length between 50 and 200 nt, and lncRNAs are longer than 200 nt [16].

Currently, numerous studies have found that miRNAs and lncRNAs play important roles in GC progression.

Table 11.1 summarizes the characteristics of different groups of ncRNAs.

miRNAs in GC

miRNAs are a class of small ncRNAs of approximately 18–24 nt. Genes encoding miRNAs could be single copy, multiple copies, or clusters; other forms exist in the region of protein-coding genes, including introns. They are highly conserved sequences and have temporal and tissue specificity [17].

Although miRNAs do not code for proteins, they have an important role in the regulation of gene expression at the posttranscriptional level. Through complete or incomplete complementary binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs, miRNAs promote the degradation of targeted-mRNA or their translational suppression. As a consequence of this process, which involves the recruitment of a number of other proteins, miRNAs are able to regulate negatively the expression of target genes [18, 19].

One miRNA interacts with several different mRNAs in different regions. A mRNA could also combine with several miRNAs on the basis of complete or incomplete sequence complementarity.

The synthesis of miRNA involves the production of a primary transcript (pri-miRNA) from genomic DNA by polymerase II within the nucleus. Then, the pri-miRNA is cut by the Drosha enzyme of RNase 3 endonuclease enzyme family into hairpin precursors of miRNA (pre-miRNA), which are approximately 70 nt [20]. Finally, the synergistic effect of Ran-GTP and transporter protein Exportin 5 transports pre-miRNA out of the nucleus, and the enzyme Dicer cuts it to produce the approximately 22 nt mature miRNA [21]. At this point, the synthesized miRNA is ready to exert its function.

Through the latest approaches of microarray technology, bioinformatics, and other genetics methods, the ectopic expression of miRNAs in GC has been found to be closely related to different steps of cancer initiation and progression including metastasis. By upregulation of the expression of oncogenes or downregulation of the expression of tumor suppressor genes, miRNAs play an important role in the regulation of cancer-related genes. A first example can be given by miRNA-106b-25. Petrocca et al. reported that an abnormal regulation of the transcription factor E2F1 and transforming growth factor-β (TGF-β) plays a critical role in gastric carcinogenesis. E2F1 activates its own promoter and miR-106b-25 cluster expression simultaneously with its host gene, Mcm7. Furthermore, the TGF-β tumor suppressor pathway was impaired by overexpression of the miR-106b-25 cluster, but also the expres-

Table 11.1 Classification of human genomic ncRNAs

RNA type	Symbol	Length (nt)	Function	References
Housekeeping ncRNAs				
Transfer RNAs	tRNA	70–80	Connect amino acids with mRNA [1]	(1) Lodish H, Berk A, Zipursky SL, et al. Molecular cell biology. 4th ed. New York: W. H. Freeman; 2000
Ribosomal RNAs	rRNA	121–5070	Component of ribosomes [1]	
Small nuclear RNAs	snRNA	≈150	Assemble with proteins into spliceosomes to remove introns during mRNA processing [2]	(2) Valadkhan S, Gunawardane LS. Role of small nuclear RNAs in eukaryotic gene expression. Essays in Biochemistry. May 03, 2013, 5479–90. https://doi.org/10.1042/bse0540079
Small nucleolar RNAs	snoRNA	70–200	Guide modifications of other ncRNAs, alternative splicing; or function as miRNA [3]	(3) Scott MS, Ono M. From snoRNA to miRNA: dual function regulatory non-coding RNAs. Biochimie. 2011;93(11):1987–92. https://doi.org/10.1016/j.biochi.2011.05.026
Telomerase RNAs	TERC	451	Provide template for de novo synthesis of telomeric DNA [4]	(4) Theimer CA, Feigon J. Structure and function of telomerase RNA. Curr Opin Struct Biol. 2006;16(3):307–18. https://doi.org/10.1016/j.sbi.2006.05.005
Ribonuclease P	RPPH1	341	RNA component of ribonuclease P [5]	(5) Altman S, Ribonuclease P. Philos Trans R Soc Lond B Biol Sci. 2011;366(1580):2936–41. https://doi.org/10.1098/rstb.2011.0142
Regulatory ncRNA				
Small interfering RNAs	siRNA	21–22	Silencing genes in a sequence-specific manner [6]	(6) Dana H, Chalbatani GM, Gharagouzlo E. Molecular mechanisms and biological functions of siRNA. Int J Biomed Sci. 2017;13(2):48–57. Available on: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5542916/#__ffn_sectitle
MicroRNAs	miRNA	20–23	Regulating gene expression [7]	(7) MacFarlane L-A, Murphy PR. MicroRNA: Biogenesis, function and role in cancer. Curr Genomics. 2010;11(7):537–61. https://doi.org/10.2174/138920210793175895
Piwi-interacting RNAs	piRNA	25–33	Repress transposons and maintain germline genome integrity [8]	(8) Iwasaki YW, Siomi MC, Siomi H. PIWI-Interacting RNA: its biogenesis and functions. Ann Rev Biochem. 2015;84:405–33. d https://doi.org/10.1146/annurev-biochem-060614-034258
Promoter-associated RNAs	paRNA	<200	Regulating gene expression [9]	(9) Yan BX, Ma JX. Promoter-associated RNAs and promoter-targeted RNAs. Cell Mol Life Sci. 2012;69(17):2833–42. https://doi.org/10.1007/s00018-012-0953-1
Long noncoding RNAs	lncRNA	>200	Various [10]	(10) Ahmad Bhat S, Mudasir Ahmad S, et al. Long non-coding RNAs: mechanism of action and functional utility, Non-coding RNA Res. 2016;1(1):43–50. https://doi.org/10.1016/j.ncrna.2016.11.002

sion of the factors CDKN1A (p21Waf1/Cip1) and BCL2L11 (Bim) is altered. Finally, CDKN1A and BCL2L11 disrupted the G1/S checkpoint and conferred resistance to TGF-β-dependent apoptosis, respectively (Fig. 11.1) [22].

A different example can be given by miRNA-9, which is downregulated in GC. A direct target of the miRNA-9 molecule is the nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NF-κB1). A study conducted by Wan et al. has

Fig. 11.1 Functions of microRNA-106b-25. miRNA-106b-25 interferes with the expression of CDKN1 and BCL2L11. The interaction of miRNA-106b-25 with E2F1 and transforming growth factor-β (TGF-β) affects the cell cycle and apoptosis
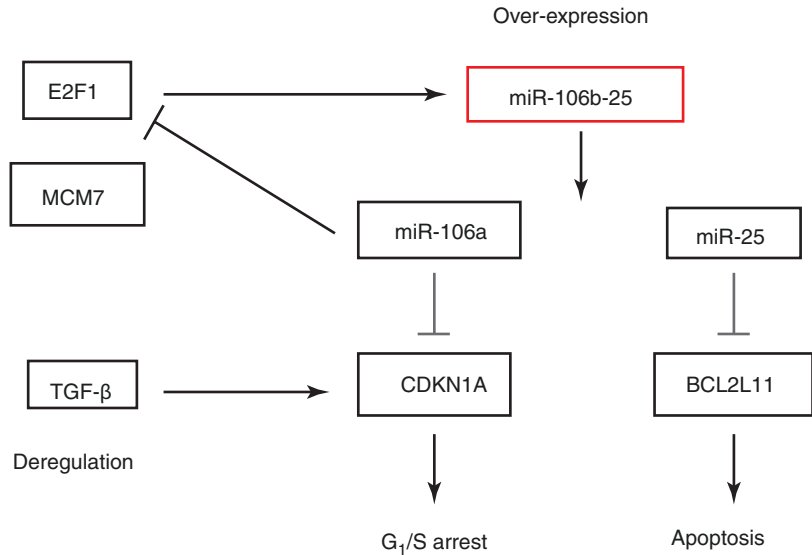
shown that cell growth and proliferation were significantly inhibited by overexpression of miR-9 that not only inversely regulates endogenous NF-κB1 protein expression but also reduces endogenous NF-κB1 mRNA levels [23].

In a more recent study by Tae-Su Han and his colleagues, several GC-specific miRNAs have been identified through comprehensive miRNA profiling using a next-generation sequencing (NGS) platform. It was discovered that miR-29c expression was downregulated in GC tissues. Moreover, a tumor suppressor role was identified for miR-29c, which regulates its downstream target gene, ITGB1, in GC. The suppression of miR-29c is an early event in gastric carcinogenesis [24].

Chemotherapeutic resistance is a big problem that has not yet been solved in GC treatment. Multiple reports have suggested that miRNAs are associated with the sensitivity of GC cell lines to chemotherapy. For example, miR-375 was conspicuously downregulated in cisplatin (DDP)-resistant cells compared with the DDP-sensitive human GC cell line. Western blot analyses showed that upregulation of miR-375 increased GC cell sensitivity to DDP treatment by targeting ERBB2 and phosphorylated Akt. The antiproliferative and apoptosis-inducing effects of DDP could be reversed by reducing the level of miR-375 [25].

Many other miRNAs, like miR-448, miR-15a, and miR-485-5p, were found to suppress proliferation, invasion, or migration in GC cell lines via their target genes such as IGF1R, Bmi1, and Flot1, respectively [26–28].

Other miRNAs, such as miR-1290 and miR-543, could promote gastric tumor cell proliferation or metastasis by targeting their downstream genes FOXA1 and SIRT1 [29, 30].

lncRNA in GC

lncRNAs are the largest class of ncRNAs ranging from 200 nt to several kilobases in length. It is possible to classify them into different groups based on their genomic localization, mode of action, and function. On the base of their genomic location, five main types can be distinguished: antisense, intronic, intergenic, bidirectional, and sense-overlapping lncRNAs. Based on their mode of action on DNA sequences, there are two classes of lncRNAs: cis-acting lncRNAs and trans-acting lncRNAs. Functionally, lncRNAs may be grouped into four types: signaling, decoy, guide, and scaffold (Fig. 11.2) [6, 31]. lncRNAs take part in various cellular and physiological processes such as gene regulation, genomic imprinting, chromatin packaging, dosage compensation, cell differentia-
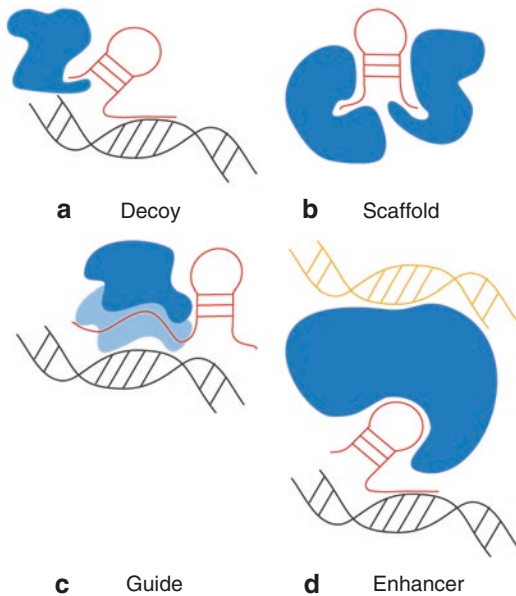
Fig. 11.2 Four types of lncRNA mechanisms: (**a**) The lncRNAs can act as decoys, titrating away DNA-binding proteins (e.g., transcription factors); (**b**) lncRNAs may act as scaffolds to bring two or more proteins to spatial proximity or into a complex; (**c**) lncRNAs may act as guides to recruit proteins to DNA (e.g., chromatin modification enzymes); and (**d**) lncRNA guidance can also be exerted through chromosome looping in an enhancer-like model in cis. lncRNA (red), DNA (black), section of DNA loop (yellow), DNA-binding proteins (blue). (Source: Luka Bolha et al. [31], Article ID 7243968, 14 pages, Fig. 1, https://doi.org/10.1155/2017/7243968, an open access article distributed under the Creative Commons Attribution License)

tion, and embryonic development [6]. Being pivotal regulators of gene expression, alterations of lncRNA can be found in different diseases including cancer. In fact, they influence the main mechanisms related to cancer including proliferation, apoptosis, invasion, and metastasis as well as neoangiogenesis [9].

lncRNAs expression profiling in a variety of cancer types has revealed a broad range of lncRNAs with aberrant expression.

lncRNA Upregulated in GC

Ak058003 is transcribed from its locus at chromosome 10q22, and it has a length of 1197 base pairs (bp). Wang et al. have discovered that the expression of Ak058003 increased during hypoxia. Moreover, this lncRNA is upregulated in GC, and its elevated level is accompanied by an increase in cell migration in vivo and in vitro. Furthermore, this lncRNA targets the γ-synuclein (SNCG), a prometastatic oncogene. Increased AK058003 expression decreases SNCG promoter methylation and consequently upregulates the expression of this oncogene, which promotes hypoxia-induced GC cell metastasis [32].

ANRIL is transcribed in an antisense direction by a locus located on 9p21.3 [33]. It has been shown that ANRIL can act as a scaffold or guide to chromatin [34]. According to recent studies, ANRIL binds to PRC2 and epigenetically represses the expression of miR-99a and miR-449a. In GC, the levels of ANRIL and miR-99a/miR-449a are inversely related so that the expression of these two miRNAs is decreased and the level of ANRIL expression is high in GC samples. This leads to a high tumor-node-metastasis (TNM) stage and tumor size [35].

BANCR The BRAF-activated noncoding RNA (BANCR) gene is located on 9q21.1 and contains four exons. It encodes a lncRNA with a length of 693 bp. BANCR expression is elevated in many GC tissues and cell lines. It has been assessed that this lncRNA influences GC cell growth and apoptosis through regulating NF-κB1 expression via miR-9. Upregulation of BANCR contributes to a decline in NF-κB1 expression that leads to an increase in cell numbers and a decrease in apoptosis in GC cells [36]. Several studies have shown that overexpression of BANCR in GC tissues is correlated with clinical stage, lymph node, and distant metastases [37].

CCAT1 Colon cancer-associated transcript 1 (CCAT1) is 2628 nt long, and its gene is located at 8q24 [38]. CCAT1 is overexpressed in some GC tissues with a significant correlation with primary tumor growth, lymph node, and distant metastases. c-Myc oncogene physically interacts with E-box element in the CCAT1 promoter and increases its expression. In vitro, CCAT1 regulates cell proliferation and migration [39]. Other studies have demonstrated that CCAT1 activates the ERK/MAPK pathway and suppresses cell cycle arrest and apoptosis.

GACAT3 Located at 2p24, GACAT3 encodes a lncRNA of 1096 nt in length. It was observed that it is upregulated in GC tissues and this upregulation is positively correlated with TNM stages, tumor size, and distant metastasis [40].

H19 As a maternally imprinted gene, H19 is located on 11p15.5. H19 plays an important role during embryogenesis, and its expression is low in most adult tissues except for cardiac and skeletal muscles [41, 42]. It is associated with p53 protein, and reciprocally, p53 protein has repressing effects on H19 levels [43, 44]. H19 gene contains a 23 nt RNA, miR-675 [45]. It has been shown that H19 works via its miR-675 product to silence the transcription factor RUNX1, a tumor suppressor in GC, in turn inducing cell proliferation [41]. The amount of H19 and miR-675 is increased in GC tissues with a significant correlation with lymph node metastases and clinical stage [46]. H19 and miR-675 have different targets, but they both function as oncogenes to increase proliferation, migration, invasion, and metastases in human GC [47].

HOTAIR HOX transcript antisense RNA (HOTAIR) is transcribed from 12q13.13 and plays an important role in GC progression [48]; for this reason it is one of the most studied lncRNAs. HOTAIR is expressed from the HOXC locus, and its length is of 2158 nt [49]. Functioning as a scaffold, HOTAIR is involved in epigenetic silencing. It directs polycomb repression complex 2 (PRC2) to trimethylate histone H3 lysine-27 of specific HOXD genes and thus repressing their expression.

It is believed that HOTAIR can promote metastasis through this pathway by inhibiting certain metastasis suppressor genes [50]. It has been demonstrated that HOTAIR expression is markedly raised in GC tissues, which is associated with poor prognosis, higher TNM stage, perineural invasion, larger tumor size, and lymph node and distant metastases [49, 51].

MALAT1 Encoded at chromosome 11q13 with 8000 nt in length, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a lncRNA [52, 53]. It was observed that MALAT1 is overexpressed in GC tissues, which correlates with peritoneal metastasis in patients [54]. Furthermore, MALAT1 increases cellular proliferation by regulating alternative splicing factor 1 (ASF1) and pre-mRNA-splicing factor (SF2) (SF2/ASF1) [55]. These proteins are pivotal players in inflammatory disorders and also in cancer [56].

PVT1 Plasmacytoma variant translocation 1 gene (PVT1) is located on human 8q24, 57 kb downstream of c-Myc [57]. The 8q24 region with both genes is involved in a variety of cancer types.

It has been reported that this lncRNA has a role in the suppression of apoptotic genes in different types of cancer. Upregulation of PVT1 is essential for the increased level of c-Myc in cancer cells [58]. PVT1 expression is elevated in GC tissues as well. Furthermore, PVT1 may be involved in the silencing process of *CDKN2B/p15* and *CDKN2A/p16* genes through its association with EZH2 during the progression of GC [59]. Its overexpression is linked to lymph node metastases [57].

UCA1 Urothelial carcinoma associated 1 (UCA1) is located on 19p13.12, and it contains three exons [60]. UCA1 presents higher expression in GC tissues and cell lines. The expression was associated with tumor size, worse differentiation, invasion depth, and TNM stages. Analyses conducted in GC have reported that excessive amount of UCA1 correlates with poor overall survival and disease-free survival in patients [61].

lncRNA Downregulated in GC

AA174084 is a lncRNA downregulated in GC tissues compared with adjacent normal tissues. Studies conducted on samples of gastric juice in patients with gastric ulcer, chronic atrophic gastritis, or GC have shown that levels of this lncRNA were highest in GC patients, suggesting its potential value as a GC biomarker. AA174084 expression levels in GC tissues were associated with age, Borrmann type, and perineural invasion. Expression in gastric juice was associated with tumor size, tumor stage, Lauren type, and CEA levels. Overall, the current data show that the AA174084 level in gastric juice may be used as a screening biomarker for detecting GC at early stages [62].

FENDRR FOXF1 adjacent noncoding developmental regulatory RNA (FENDRR) is located on 16q24.1 and contains seven exons. Through binding to PRC2 and/or TrxG/MLL complexes, FENDRR lncRNA regulates histone methylation and chromatin structure [63]. Furthermore, FENDRR is diminished in GC tissues and cell lines, which correlate with depth of invasion, advanced tumor stage, and lymphatic metastasis.

FER1L4 Fer-1-like protein 4 (FER1L4) is located at 20q11. Its expression is reduced in GC tissues, and it is correlated with histological grade, tumor size, severity of invasion, vessel or nerve invasion, and lymph node and distant metastases [64]. FER1L4 is one of the targets of miR-106a-5p. Low quantity of this lncRNA increases the amount of free miR-106a-5p, making it more available for its targets such as the retinoblastoma gene, *RB1* [65].

GACAT2 Gastric cancer-associated transcript 2 (GACAT2) is encoded at 18p11, and it has a length of 818 nt. GACAT2 is markedly decreased in GC tissues and cell lines, which is associated with distal metastasis and neural and blood vessel invasion in GC tissues [66].

MEG3 Maternally expressed gene 3 (MEG3) is a tumor suppressor lncRNA transcribed from an imprinted gene cluster at 14q32, with a length of 1700 nt [67]. It has been demonstrated a significant decrease of MEG3 levels in GC tissues, and this was linked with TNM stage, tumor size, depth of invasion, and shorter overall survival time in GC patients [68].

MT1JP Metallothionein 1 J gene is located on 16q13. It has considerably lower expression in GC tissue samples than in matched normal tissues. Zhongchuan et al. have demonstrated that MT1JP is necessary for maintaining the normal life activities of cells and played a critical function as a tumor suppressor. lncRNA MT1JP is involved in many steps of tumor progression, including cell proliferation, migration, and invasion. For this reason, it may be a potential diagnostic marker and could have a potential therapeutic value in the prevention of GC [2].

ncRuPAR Noncoding RNA upstream of the PAR-1 (ncRuPAR) [69] increases the expression of protease activator-1 (PAR1) during embry-onic growth. The study conducted by Liu et al. reports that it works as a tumor suppressor in cancer.

Its gene is located on human 5q13. Decreased expression of this lncRNA in GC samples was inversely correlated with the amount of PAR-1. Its level was negatively associated with tumor size, tumor invasion depth, lymph node, and distant metastases [70].

TUSC7 Tumor suppressor candidate 7 (TUSC7) is located on 3q13.31 and contains four exons. Some studies have reported that TUSC7 is downregulated in GC tissues contributing to an augmentation in cell growth. In addition, p53 is a regulator of TUSC7 in GC, and *TP53* mutations or deletions are the likely cause of TUSC7 down-regulation. Furthermore, TUSC7 negatively regulates the level of miR-23b, which promotes cell growth in GC samples [71].

miRNA as Biomarker in GC

Numerous miRNAs are aberrantly expressed in the plasma and serum of GC patients [72–74]. For example, miR223, miR-233, miR-378, miR-421, miR-451, miR-4865p, and miR-199-3p are overexpressed in sera of GC patients [75–78]. Wang et al. found that miR-233 was overexpressed in GC patient sera, and its level was positively associated with tumor differentiation grade, TNM stage, tumor size, and metastasis status [75].

Wu et al. found that miR-421 was overexpressed in 90 cases of GC patient sera compared to 90 controls. The high expression of miR-421 in cancer cells acts as a biomarker for GC circulating tumor cells, which may be used for early diagnosis for gastric metastasis [76]. Furthermore, in vivo and in vitro experiments demonstrated that the onco-miR-421 promotes tumor proliferation, invasion, and metastasis but had no significant association with the clinic-pathological features [79, 80].

In contrast, the expression of miRNAs such as let-7a, miR-375, miR-20a-5p, and miR-320 was relatively reduced in GC patient sera [81, 82]. A study demonstrated that let-7a exhibited rela-

tively low expression in plasma of GC patients compared with healthy controls, whereas the expression of miR-17-5p, miR-106a, miR-106b, and miR-21 was significantly elevated in GC plasma [83]. Other studies demonstrated that miR375 was suppressed in GC. Overexpression of miR-375 suppresses GC progression by targeting p53, JAK2, ERBB2, and STAT3 [84, 85]. These studies indicate that miRNA could be useful diagnostic biomarkers. However, large-scale clinical research is needed to demonstrate that miRNA can serve as a diagnostic biomarker for GC.

Several studies have demonstrated that miR-NAs could be used not only as biomarkers but also as potential therapeutic targets for cancer. miRNA-based drugs that act by suppressing miRNAs or inhibit the onco-miRNAs can inhibit tumor progression by suppressing the relative signal pathway [86, 87]. For example, miR-34 is one of the most characterized tumor suppressor miRNAs in a variety of tumors including GC. In literature, it is reported that it is lost or expressed at minimum levels in numerous tumor tissues, and the reintroduction of miR-34 mimics was found to inhibit cancer cell growth both in vitro and in vivo. Therefore, miR-34a has proved to be a tumor suppressor in cancer cells and an ideal therapeutic tool to reduce metastasis, chemoresistance, and tumor recurrence [88–90].

However, some problems should be considered; as one miRNA can target multiple genes and signaling, the off-target effect is not easily predictable. Thus, miRNA therapy needs more detailed studies [91].

lncRNA as Biomarkers in GC

In recent years, detection of cancer-associated lncRNAs in body fluids of cancer patients has proven itself as a valuable method to effectively diagnose cancer. Cancer diagnosis and prognosis through the use of circulating lncRNAs are preferred when compared to classical biopsies of tumor tissues, because of their noninvasiveness and great potential for routine applications in clinical practice.

Among main advantages of lncRNAs, which make them suitable as cancer diagnostic and prognostic biomarkers, is their high stability while circulating in body fluids, especially when included in exosomes or apoptotic bodies [92]. It has been shown that lncRNAs are able to resist the multiple ribonucleases in body fluids [93]. In addition, lncRNA deregulation in primary tumor tissues is clearly mirrored in various bodily fluids, including whole blood, plasma, urine, saliva, and gastric juice [94, 95]. These characteristics make the lncRNAs of potential prognostic and predictive biomarkers for GC, easy to take and evaluate, bringing great benefits to patients compared to a classic tissue biopsy [96].

The detection of circulating lncRNAs could represent an excellent method in the evaluation of cancer to distinguish tumor patients from healthy people at early stages with both high sensitivity and specificity. In addition, the prognosis of tumor patients and the risk of tumor metastasis and recurrence after surgery could be assessed [93]. Good results have been obtained from the diagnostic performances of lncRNAs BANCR, H19, CCAT, and AA174084 evaluated in body fluid samples (e.g., plasma and gastric juice) of GC patients. These lncRNAs had the ability to differentiate GC patients from healthy individuals and to effectively detect different stages of GC (from early to metastatic cancer forms). However, despite their overall positive diagnostic performances, similar to those obtained by several conventional cancer biomarkers, false-positive and false-negative detections were observed [95, 97, 98].

Stability of lncRNAs in body fluids of tumor patients has not been thoroughly explored. Studies revealed that some lncRNAs remained stable in plasma under extreme conditions, such as several freeze-thawed cycles and prolonged incubation at elevated temperatures [99]. So far, three mechanisms have been identified by which lncRNAs are released into body fluids. First, extracellular RNAs may package themselves into specific membrane vesicles, such as exosomes and microvesicles, in order to be secreted and resist to RNase activity. Different studies revealed that exosomes most frequently protect plasma

lncRNAs [100–103]. Second, extracellular RNAs can be actively released by tumor tissues and cells [104]. Third, extracellular RNAs may encapsulate themselves into high-density lipoprotein (HDL) or apoptotic bodies or be associated with protein complexes, for example, Argonaute (Ago)-miRNA complex and nucleophosmin 1 (NPM1)-miRNA complex [105, 106]. However, despite many performed studies, secretion and transport mechanisms of lncRNAs to the circulation system remain yet poorly understood.

In order to introduce circulating lncRNAs into clinical practice, further studies and improvements should be performed regarding the standardization of sample preparation protocols and the extraction methods [93].

Conclusion

In recent years, the role of ncRNAs in GC has been clarified. Multiple studies have already demonstrated the potential clinical applications of several ncRNAs in GC diagnosis and prognosis. Circulating ncRNAs are regarded as an emerging biomarker for GC, but the applications of circulating ncRNAs need to be further investigated because of the interactions between ncRNAs and GC that are very complex.

Among these, several ncRNAs are promising neoplastic biomarkers to be detected in the patient's body fluids, including miR-34, H19, HOTAIR, MALAT1, UCA1, and AA174084. For many of these ncRNAs, it has been proven that they could be used in clinical practice as diagnostic and prognostic GC biomarkers. ncRNA research will likely take a big step forward with the identification of more molecules in the next years.

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