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Long Noncoding RNA in Digestive Tract Cancers: Function, Mechanism, and Potential Biomarker

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Long noncoding RNAs • Digestive tract cancers • Biomarker • Epigenetic regulation

Abstract

Digestive tract cancers (DTCs) are a leading cause of cancer-related death worldwide. Current therapeutic tools for advanced stage DTCs have limitations, and patients with early stage DTCs frequently have a missed diagnosis due to shortage of efficient biomarkers. Consequently, it is necessary to develop novel biomarkers for early diagnosis and novel therapeutic targets for treatment of DTCs. In recent years, long noncoding RNAs (lncRNAs), a class of noncoding RNAs with >200 nucleotides, have been shown to be aberrantly expressed in DTCs and to have an important role in DTC development: the expression profiles of lncRNAs strongly correlated with poor survival of patients with DTCs, and lncRNAs acted as oncogenes or tumor suppressor genes in DTC progression. In this review, we summarized the functional lncRNAs and expounded on their regulatory mechanisms in DTCs. ***The Oncologist*** 2015;20:898–906

Implications for Practice: Digestive tract cancers (DTCs) are a leading cause of cancer-related death worldwide. It is necessary to exploit novel biomarkers for early diagnosis and novel therapeutic targets for treatment of DTCs. Long noncoding RNAs (lncRNAs), a class of noncoding RNAs with approximately 200 nucleotides to 100,000 bases, participate in the progression of a variety of diseases. This review summarizes functional lncRNAs, which were shown to serve as novel biomarkers for diagnosis and prognosis of DTCs and to act as oncogenes or tumor suppressor genes in DTC development. In addition, the potential mechanism of functional lncRNAs in DTCs is highlighted.

Introduction

Digestive tract cancers (DTCs), including multiple malignancies such as esophageal cancer (EsC), gastric cancer (GC), and colorectal cancer (CRC), are the most common tumors worldwide. In eastern Asian countries, DTCs are still the leading cause of cancer-related death [1, 2]. There were 182,410 new diagnosed cases and 77,030 deaths caused by esophageal, gastric, and colorectal cancers in the U.S. in 2013 [3]. Complete surgical resection remains the most effective treatment for early DTCs, but many patients are not diagnosed until tumors have developed to the advanced stage. The lack of effective prevention methods and treatments is a great threat to human health, and the 5-year survival rate for advanced DTCs is very low [4–7], despite multiple treatments involving surgery, radiation, and chemotherapy. Tumor metastasis and drug-resistance contribute to high mortality; therefore, identification of potential biomarkers for DTCs may help prevention and early diagnosis of such diseases, and understanding the molecular regulatory mechanism in tumor development may contribute to exploring effective treatments for advanced DTCs.

Long noncoding RNAs (lncRNAs) are a class of noncoding RNAs with approximately 200 nucleotides (nt) to 100,000 bases, without coding for proteins [8, 9]. In recent years, numerous studies reported that aberrant expressions of lncRNAs were associated with the recurrence [10], metastasis [11], and prognosis [12] of malignancies. LncRNAs were also found to play crucial roles in tumor development, including tumor cell proliferation [13], apoptosis [14], migration [15], invasion [16], and epithelial-to-mesenchymal transition [17]. The regulatory mechanism of lncRNAs in tumor development is complicated and involves chromatin modification [18, 19], transcriptional [20] and post-transcriptional regulation [21], and influence of protein stability [22]. Understanding the expression profiles of lncRNAs and their function and mechanism in DTC development will help develop novel biomarkers for early diagnosis and effective therapeutic targets. We summarized aberrantly expressed lncRNAs and expounded on their possible function and mechanism in DTCs.

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Properties of lncRNAs

Numerous studies have indicated that 70%–90% of the human genome transcribes RNA products; however, less than 2% of the total genome encodes protein-coding genes [23–25], suggesting that the human transcriptome predominantly consists of abundant noncoding RNAs. Noncoding transcripts were initially thought to be "noise" generated from gene transcription, but a large number of more recent studies showed that noncoding RNAs have important roles in human diseases [26–30]. Noncoding RNAs can be divided into two classes: housekeeping noncoding RNAs and regulatory noncoding RNAs. Housekeeping noncoding RNAs contain ribosomal, transfer, small nuclear, and small nucleolar RNAs. Short regulatory noncoding RNAs include microRNAs (miRNAs), small interfering RNAs, and Piwi-associated RNAs [31]. Long regulatory noncoding RNAs were defined as lacking a significant open reading frame and having a length of more than 200 nt [32]. In 1988, Pachnis et al. reported the first lncRNA, H19, which has a very unusual structure consisting of five exons and is activated early during muscle cell differentiation [33]. Subsequently, Air [34], NRON [35], and HOTAIR [19] were gradually discovered. Recently, more than 32,000 lncRNAs have been documented, and more than 1,000 lncRNA disease entries and 475 lncRNA interaction entries were integrated in the LncRNADisease database (http://www.cuilab.cn/lncrnadisease).

Several studies indicated that lncRNAs could be classified into five categories based on their genomic location and context. *Intergenic* and *intronic* lncRNAs are transcribed from intergenic regions and from introns of protein-coding genes, respectively. *Sense* lncRNAs are transcribed from the sense strand of protein-coding genes, and their sequences may overlap with parts of or entire sequences of protein-coding genes. *Antisense* lncRNAs are transcribed from the antisense strand of protein-coding genes, and their sequences may be complementary base pairing with exon, intron, or entire sequences of protein-coding genes. Last, *bidirectional* lncRNAs have transcriptional orientations opposite those of their neighboring protein-coding genes [31, 36] (Fig. 1).

LncRNAs have a diverse set of functions. Perturbation experiments, such as loss of function and gain of function, showed that lncRNAs participated in epigenetic regulation. XIST, for example, is required in the process of X chromosome inactivation [37]; HOTAIR is crucial for reprograming the chromatin state in cancer metastasis [38]; and NRON represses NFAT transcription factor activity [35]. Interestingly, Guttman et al. summarized the modular regulatory principles of lncRNAs [29]. First, lncRNAs have a *cis*-function and affect their neighboring regions [39]. Second, lncRNAs serve as *trans*-regulators and modulate their neighboring genes [40]. Third, lncRNAs bind regulatory proteins and change their activity, resulting in the change in modification state and expression of the target genes [41]. Fourth, lncRNAs act as "decoys" and bind protein complexes and prevent them from binding to their proper regulatory targets [42]. Briefly, lncRNAs can interact with proteins, RNA (eg, mRNA, miRNA), and DNA to affect chromatin modification, gene expression, and the stability of proteins and mRNA. These regulatory principles may greatly enhance our understanding of the underlying role of lncRNAs in DTC development.

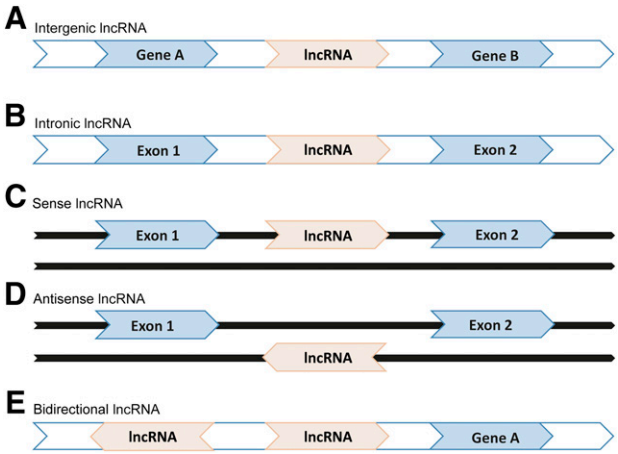

Figure 1. Genomic location of lncRNAs. Blue represents protein-coding genes and their exons, and light red represents lncRNAs. **(A):** Intergenic lncRNA, which is transcribed from intergenic regions. **(B):** Intronic lncRNA, which is transcribed from introns of protein-coding genes. **(C):** Sense lncRNA, which is transcribed from the sense strand of protein-coding genes. **(D):** Antisense lncRNA, which is transcribed from the antisense strand of protein-coding genes. **(E):** Bidirectional lncRNA, for which transcriptional orientation is opposite that of neighboring protein-coding genes.
Abbreviation: lncRNA, long noncoding RNA.

lncRNAs in DTC Development

Increasing numbers of lncRNAs have been identified in DTCs in recent years, especially in EsC, GC, and CRC, in which lncRNAs participated in regulating proliferation, apoptosis, migration, and/or invasion of tumor cells in vitro and in vivo. In addition, lncRNA interacted with proteins, mRNAs, or miRNAs to modify expression of the target genes, resulting in the change in activation of some signaling pathways, such as the Wnt/β-catenin signaling pathway. The functional lncRNAs and their mechanisms are summarized in Tables 1–3. Notably, several lncRNAs, including HOTAIR and H19, were frequently reported in the three cancers.

HOTAIR

The long intergenic noncoding RNA (lincRNA) HOTAIR, which is transcribed from the HOXC locus, can interact with polycomb repressive complex 2 (PRC2; consisting of H3K27 methylase EZH2, SUZ12, and EED) to silence HOXD and select genes on other chromosomes [19, 38, 79]. Tsai et al. subsequently reported that a 5′ domain of HOTAIR binds PRC2, whereas a 3′ domain of HOTAIR binds LSD1 (KDM1/BHC110) [40], which is a demethylase that mediates the enzymatic demethylation of H3K4me2 [80] and a component of CoREST/REST repressor complexes [81].

In EsC, HOTAIR expression was markedly upregulated in esophageal squamous cell carcinoma (ESCC) and strongly correlated with tumor progress and poor prognosis of patients with ESCC. HOTAIR inhibition can reduce cell proliferation and migration in vitro and in vivo. Further exploration via microarray demonstrated that HOTAIR reprogrammed the gene expression profile of ESCC cells, some of which are

Table 1. The identified lncRNAs in esophageal cancer

LncRNAs	Expression	Correlation	Function	Interaction	Regulated gene	Pathway	Biomarker	Reference
HOTAIR	Upregulated	TNM stage, histological differentiation, and survival time	Proliferation, apoptosis, migration, invasion, and tumorigenesis		WIF-1	Wnt/β-catenin signaling pathway	Prognosis	43, 44
Linc-POU3F3	Upregulated		Development of ESCC	EZH2 mRNA	POU3F3			45
AFAP1-AS1	Upregulated		Proliferation, colony-forming ability, apoptosis, migration, and invasion					46
HNF1A-AS1	Upregulated		Proliferation, anchorage-independent growth, migration, and invasion		H19			47
lincRNA-uc003opf.1	Upregulated	Risk of ESCC	Cell proliferation and tumor growth	miR-149*				48
ENST00000435885.1, XLOC_013014, ENST00000547963.1	Upregulated, downregulated, downregulated	TNM stage					Prognosis	49

Abbreviations: ESCC, esophageal squamous cell carcinoma; lncRNAs, long noncoding RNAs.

responsible for tumorigenesis [43]. HOTAIR was also shown to promote WIF-1's histone H3K27 methylation in the promoter region, resulting in decreased WIF expression and activation of the Wnt/β-catenin signaling pathway [44].

In GC, HOTAIR-expressing GC cells exhibited an enhanced ability of metastasis and peritoneal dissemination in a nude mouse model [57]. Liu et al. confirmed that HOTAIR was markedly increased in GC tissues compared with matched normal tissues, and its loss led to suppressed proliferation of GC cells in vitro and in vivo. The investigation of the molecular mechanism revealed that HOTAIR indirectly regulated human epithelial growth factor receptor 2 (HER2) by competitively binding to miR-331-3p [58]. In addition, the plasma levels of HOTAIR were increased in the patients with GC compared with the healthy controls [52], suggesting that HOTAIR may be a noninvasive biomarker for GC diagnosis.

In CRC, HOTAIR expression correlated with tumor invasion, lymph node metastasis, organ metastasis, histological differentiation, vascular invasion, and advanced tumor stage in colon cancer. Colon cancer patients with high HOTAIR expression had higher recurrence rates and reduced metastasis-free and overall survival compared with those with low HOTAIR expression. Further investigation revealed that HOTAIR increased the expression of E-cadherin and decreased the expression of vimentin and MMP9 [75]. Svoboda et al. demonstrated that HOTAIR expression was increased in primary tumors and blood of CRC patients, and its levels in tumors were associated with higher mortality of patients, suggesting that HOTAIR blood levels might serve as a potential surrogate prognostic marker in sporadic CRC [76].

Colon cancer patients with high HOTAIR expression had higher recurrence rates and reduced metastasis-free and overall survival compared with those with low HOTAIR expression.

In summary, HOTAIR can interact with proteins and miRNAs to regulate expression of the target genes and activate the Wnt/β-catenin signaling pathway; HOTAIR can serve as independent and noninvasive biomarker for diagnosis of DTCs (Fig. 2).

H19

H19, a maternally imprinted gene, is located in an imprinted region of chromosome 11p15.5 near the insulin-like growth factor 2 (IGF2) gene and contains five exons and four introns [82, 83]. In GC, Yang et al. reported that H19 was frequently increased in the GC tissues compared with the matched normal tissues and that its upregulation promoted GC cell proliferation, whereas H19 small interfering RNA led to cell apoptosis in AGS-line cells. Mechanism investigation revealed that H19 could interact with p53 and partially induce inactivation of p53 in AGS cells [53]. Thereafter, Arita et al. stated that the plasma levels of H19 were markedly increased in patients with GC compared with healthy controls, and levels were significantly decreased in postoperative samples, suggesting that lncRNAs may be novel, noninvasive biomarkers for GC diagnosis [52].

Zhang et al. showed the molecular mechanism by which c-Myc-induced upregulation of H19 can promote cell

Table 2. Dysregulation of lncRNAs in gastric cancer

LncRNAs	Expression	Correlation	Function	Interaction	Regulated gene	Pathway	Biomarker	Reference
ANRIL	Upregulated	TNM stage	Proliferation	miR-99a/ miR-449a				50
GAPLINC	Upregulated	Poor survival	Migration	miR-211-3p	CD44		Prognosis	15
GHET1	Upregulated	Tumor size, tumor invasion, and poor survival	Proliferation	IGF2BP1				51
H19	Upregulated	Poor survival and advanced TNM stage	Proliferation and apoptosis	P53, ISM1	miR-675 and RUNX1	p53 inactivation	Diagnosis	52–56
HOTAIR	Upregulated	TNM staging and lymph node metastasis	Proliferation, metastasis and peritoneal dissemination	miR-331-3p	HER2		Diagnosis	52, 57, 58
HULC, ABHD11-AS1	Upregulated	Lymph node metastasis, distant metastasis, and advanced tumor node metastasis stages	Proliferation, invasion and apoptosis					59, 60
LINC00152	Upregulated	Tumor invasion, gastric juice					Diagnosis	61
MRUL	Upregulated		Apoptosis, accumulation and doxorubicin release					62
NCR143/145	Upregulated			miR-143/145 cluster				63
AC096655.1-002	Downregulated	Lymph node metastasis, distant metastasis, and differentiation					Diagnosis	64
BM742401	Downregulated		Metastasis-related phenotype		MMP9			65
FER1L4	Downregulated	Tumor size, histologic grade, general classification, depth of invasion, lymphatic metastasis, distant metastasis, TNM stage, vessel or nerve invasion, and serum CA72-4					Prognosis	66
GAS5	Downregulated	Poor disease-free survival and overall survival	Proliferation and apoptosis		E2F1 and P21			67
HMlincRNA717	Downregulated	Cancer distal metastasis, venous invasion, and nervous invasion					Early diagnosis	68
ncRuPAR	Downregulated						Diagnosis	69
Uc001lsz	Downregulated	TNM stage						70

Abbreviation: lncRNAs, long noncoding RNAs.

proliferation, which strongly correlates with poor survival of patients with GC [54]. Interestingly, H19-derived miR-675 could promote GC cell proliferation through the miR-675-targeting tumor suppressor gene runt-related transcription factor 1 (RUNX1) [55]. Recently, Li et al. reported that H19/miR-675 upregulation can promote the proliferation, migration, and invasion of GC cells in vitro and in vivo. RNA immunoprecipitation (RIP) and dual-luciferase reporter experiments confirmed

Table 3. The function of lncRNAs in colorectal cancer

LncRNAs	Expression	Correlation	Function	Interaction	Regulated gene	Pathway	Biomarker	Reference
CCAT2	Upregulated		Tumor growth, metastasis, and chromosomal instability	TCF7L2	MYC, miR-17-5p, and miR-20a	WNT signaling activity	Diagnosis	71
H19	Upregulated		Precursor of miR-675, tumor cell growth, and soft agar colony formation		RB mRNA	p53 inactivation		72–74
HOTAIR	Upregulated	Tumor invasion, lymph node metastasis, organ metastasis, histological differentiation, vascular invasion, and advanced tumor stage			E-cadherin, vimentin, and MMP9		Early diagnosis	75, 76
loc285194	Downregulated		Tumor cell growth	miR-211				77
ncNRFRM	Upregulated		Malignant transformation, inhibited the function of the tumor suppressor let-7		let-7			78

Abbreviations: lncRNAs, long noncoding RNAs; RB, retinoblastoma.

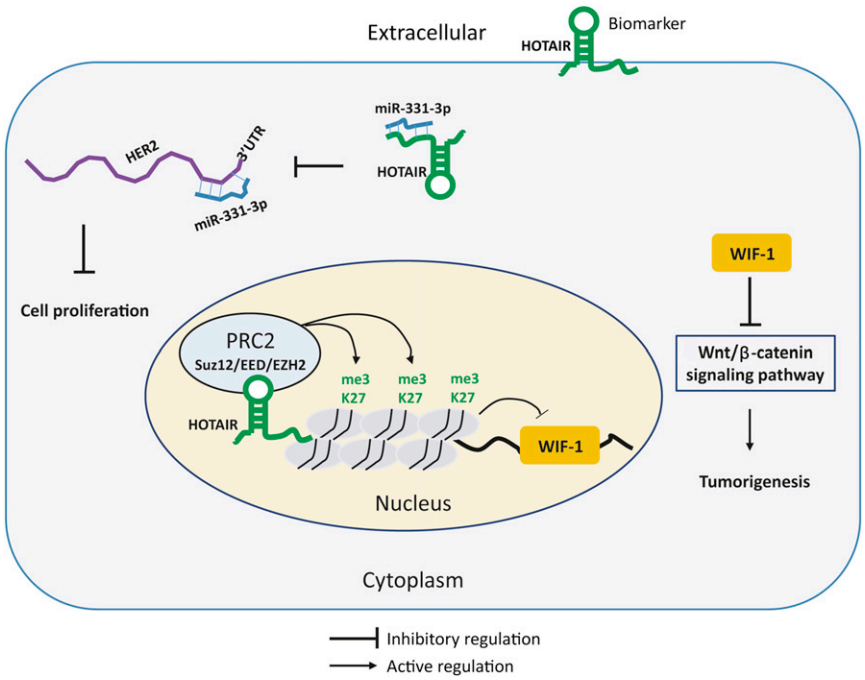

Figure 2. Diagrammatic sketch shows the hypothetical mechanism of HOTAIR in digestive tract cancers. HOTAIR interacts with PRC2 complex (Suz12/EED/EZH2) to methylate H3K27 and silence WIF-1 expression, further inducing activation of Wnt/β-catenin signaling pathway. HOTAIR acts as competing endogenous RNA to sponge miR-331-3p, which represses HER2 expression by binding to HER2 mRNA 3′UTR. HOTAIR can be released to blood from cells and serve as biomarker.

that H19 could bind isthmin 1 (ISM1), an angiogenesis inhibitor. Further study revealed that H19 expression was positively correlated with ISM1, and H19-derived miR-675 could target calneuron 1 (CALN1); however, the function of H19/ISM1 in GC development has not been explored in detail [56].

In CRC, H19 was the first reported lncRNA. In the insulator region of IGF2/H19, loss of imprinting of the IGF2 gene strongly

correlated with biallelic hypermethylation of a core of five CpG sites, and the hypermethylation created a field defect, predisposing to cancer [72]. Cui et al., however, reported that the IGF2-H19 enhancer competition model for IGF2 imprinting does not apply to the human colon [73], suggesting that lncRNAs have different functions in different types of cancer. Interestingly, Tsang et al. reported that H19, a precursor of miR-675, and miR-675 were both upregulated in human CRC tissues compared with adjacent noncancerous tissues, and the enhanced expression of miR-675 downregulated tumor suppressor retinoblastoma mRNA expression by targeting its 3′UTR, further increasing tumor cell growth and soft agar colony formation [74].

In summary, upregulation of H19 is frequently found in DTCs and promotes tumor progression. H19 can interact with p53 and induce p53 inactivation and can serve as noninvasive biomarker for GC detection.

lncRNA Interacts With Protein and miRNA

Under the regulatory principle of lncRNA, lncRNA interacting with proteins was frequently reported. In EsC, Li et al. reported that linc-POU3F3, a lincRNA encoded by a gene located next to POU3F3, could enhance proliferation and the ability to form colonies of ESCC cells and reduce the expression of POU3F3 mRNA. The ESCC cell lines with overexpressed linc-POU3F3 revealed dense hypermethylation of CpG islands in POU3F3, whereas EZH2 inhibition increased the expression of POU3F3 mRNA and reduced the binding of DNA methyltransferase (DNMT) 1, DNMT3A, and DNMT3B to POU3F3. Importantly, linc-POU3F3 can interact with EZH2 in Eca-109 and TE-1 cells, causing increased binding of DNA methyltransferase [45]. Yang et al. reported that lncRNA-GHET1 was markedly upregulated in gastric carcinoma, and its overexpression correlated with tumor size, tumor invasion, and poor survival in addition to enhanced GC cell proliferation in vitro and in vivo. Further experiments based on RNA pull-down and RIP confirmed that lncRNA-GHET1 was physically associated with insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) and promoted a physical interaction between c-Myc mRNA and IGF2BP1 [51]. Ling et al. reported that CCAT2, a novel lncRNA encompassing the rs6983267 SNP, was increased in microsatellite-stable CRC and promoted tumor growth, metastasis, and chromosomal instability. CCAT2 could upregulate MYC, miR-17-5p, and miR-20a through TCF7L2-induced transcriptional regulation. Interestingly, CCAT2 and TCF7L2 had a physical interaction, enhancing the WNT signaling activity, which could also affect CCAT2 expression, suggesting the existence of a feedback loop [71].

Several papers reported that lncRNAs function as competing endogenous RNAs (ceRNAs) to sponge miRNAs and reduce the biological function of the miRNAs, resulting in changes in the expression of cancer-related genes. In GC, for example, Iio et al. reported that NCR143/145, the stability of which was preferentially reduced by DDX6, encompassed the miR-143/145 cluster and downregulated the expression of mature miR-143/145 in GC cells [63]. ANRIL (CDKN2B-AS1), which can recruit and bind to PRC2, was increased in human GC tissues and correlated with a higher TNM stage and tumor size. Further experiments confirmed that E2F1 could induce ANRIL-mediated growth promotion by which ANRIL acted as a ceRNA to repress the biological function of miR-99a/miR-449a, which was shown to bind to PRC2 mRNA. These findings provided support for a positive feedback loop by which ANRIL can continue to promote GC cell proliferation [50]. Liu et al. reported that lncRNA loc285194, a known lncRNA in osteosarcoma, is a p53 transcription target with two binding sites of miR-211 in its exon 4. The ectopic expression of loc285194 suppressed tumor cell growth in vitro and in vivo by repressing miR-211-induced cell growth [77]. Recently, Hu et al. reported that lincRNA GAPLINC was overexpressed in GC tissues and correlated with poor survival of patients with GC. GAPLINC suppression led to alterations in cell migration pathways in which CD44 expression was the most strongly correlated. Mechanistic investigations showed that GAPLINC acted as ceRNA to sponge miR211-3p, which can directly bind to CD44 mRNA [15].

Function of Other lncRNAs

During the past decade, a large number of functional lncRNAs had been identified and the identified lncRNAs showed multiple functions in DTC development. Several studies confirmed that lncRNAs, including lncRNA HULC [59] and ABHD11-AS1 [60], correlate with GC metastasis and differentiation and modulate the ability to perform cell proliferation, apoptosis, and invasion. Interestingly, Pang et al. reported that LINC00152, an overexpressed lncRNA in GC tissues, was increased in the gastric juice from patients with GC compared with non-GC controls [61], suggesting that GC tissue can secrete lncRNA into gastric juice, in which its expression is also stable. Some lncRNAs were shown to correlate with drug resistance in the treatment of GC. lncRNA MRUL, for instance, was markedly overexpressed in two multidrug-resistant GC cells, SGC-7901/ADR and SGC-7901/VCR, and its loss in these cells with the presence of adriamycin (ADR) or vincristine (VCR) resulted in increased rates of apoptosis, increased accumulation, and reduced doxorubicin release [62]. Sun et al. stated that GAS5 was markedly downregulated in GC tissues, and its low expression correlated with the poor disease-free survival and overall survival of patients with GC. The ectopic expression of GAS5 repressed GC cell proliferation and induced apoptosis in vitro and in vivo through regulating E2F1 and P21 expression [67]. Franklin et al. reported that ncNRFRM exhibited a malignant transformation ability when it was stably overexpressed in nontransformed and conditionally immortalized mouse colonocytes, and it inhibited the function of tumor suppressor *let-7* [78].

In addition, the effects of single nucleotide polymorphisms (SNPs) of lncRNAs on ESCC susceptibility were also explored. Wu et al. scoured the exons of lincRNAs located in ESCC susceptibility loci for all probable functional SNPs and stated that in the lincRNA-uc003opf.1 exon, the functional polymorphism rs1175942A > G was considered a genetic modifier for the development of ESCC [48], suggesting that the function of lncRNAs in ESCC might be of individual significance. These findings highlight that the expression of lncRNAs was stably expressed in gastric juice; the function of some lncRNAs may be associated with multidrug resistance.

Identification of lncRNAs Using lncRNA Microarray or RNA Sequencing

Some studies used high-throughput RNA sequencing (RNA-seq) and/or lncRNA microarray to screen the dysregulated

lncRNAs. Wu et al. found the CpG methylation status of 1.8 million loci distributed throughout the genome in Barrett's esophagus (BE) tissues using a high-resolution assay with massively parallel sequencing. The authors reported that AFAP1-AS1 was hypomethylated and overexpressed in BE and esophageal adenocarcinoma (EAC) as well as in EAC cells. AFAP1-AS1 dysregulation, without changing the expression of its protein-coding counterpart AFAP1, could affect the proliferation, colony-forming ability, apoptosis, migration, and invasion of EAC cells [46].

Yang et al. used next-generation RNA-seq analysis and showed that 61 unique lncRNAs were significantly overexpressed in EACs compared with normal esophagus. Among the 61 lncRNAs, HNF1A-AS1 was strikingly overexpressed in human primary EACs and EAC cells, and its loss suppressed the proliferation, anchorage-independent growth, migration, and invasion of EAC cells in vitro. They further confirmed that HNF1A, the sense-cognate gene for HNF1A-AS1, was not an HNF1A-AS1 target. Mechanistic investigation via gene ontology enrichment analysis revealed that HNF1A-AS1 knockdown dysregulated the expression of genes involved in cell cycle regulation and affected the expression of a cancer-related lncRNA H19, suggesting that one lncRNA may influence the function of another lncRNA in EACs [47].

Park et al. used RNA-seq to screen lincRNAs in GC and normal tissues. By analyzing the RNA-seq and public microarray data, they found that 31 transcripts, including BM742401, were significantly decreased in GC tissues. The ectopic expression of BM742401 suppressed metastasis-related phenotypes and decreased the concentration of extracellular MMP9 [65].

Song et al. used lncRNA microarray and found that the expressions of 135 lncRNAs were markedly changed, including the most downregulated lncRNAs—FER1L4, uc001lsz, BG491697, AF131784, uc009ycs, BG981369, af147447, HMlincRNA1600, and AK054588—and the most upregulated ones—H19, HMlincRNA717, BM709340, BQ213083, AK054978, and DB077273. Further exploration revealed that the downregulation of uc001lsz was associated with TNM stages and suggested that uc001lsz might be a novel biomarker for the diagnosis of early GC [70].

Wang et al. used lncRNA microarray to analyze the expression levels of 21,558 lncRNAs in gastric cardia adenocarcinoma and found 2,289 upregulated lncRNAs and 1,546 downregulated lncRNAs. Among these, the most upregulated lncRNA was ASHG19A3A028863 (fold change: 169.673093), and the most downregulated lncRNA was ASHG19A3A007184 (fold change: 59.385806). Pathway analysis revealed that 18 pathways corresponded to the upregulated transcripts, and the most enriched network was "Ribosome-Homo sapiens (human)." In addition, eight pathways corresponded to the downregulated transcripts, and the most enriched network was "Mineral absorption-Homo sapiens (human)" [84].

Other data on dysregulated lncRNAs from microarray analysis revealed that 2,621 lncRNAs were differentially expressed in GC tissues compared with the matched normal tissues. KEGG pathway analysis indicated that the lncRNA-mRNA target pairs were the most significantly enriched in the p53 pathway [85]. These results suggested that many lncRNAs are dysregulated in DTCs and may have important roles in DTC development. Aberrant expression profiles of the identified lncRNAs via RNA-seq or/and lncRNA microarray need to be further confirmed, and their function and regulatory mechanism in DTC development need to be better understood.

lncRNAs as Clinical Biomarkers

Molecular markers have different types, including diagnostic markers, which aid in the diagnosis or subclassification of a particular disease state; prognostic markers, which have an association with some clinical outcomes, such as overall survival or recurrence-free survival; predictive markers, which predict the activity of a specific class or type of therapy and help determine more specific treatment; and companion diagnostic markers, which may be diagnostic, prognostic, or predictive but are used to identify a subgroup of patients for benefit therapy [86].

In recent years, several studies investigated whether lncRNAs can serve as effective diagnostic and prognostic biomarkers for DTCs. HOTAIR, for example, was shown to be an independent prognostic factor in patients with ESCC, and its high levels correlated with TNM stage, histological differentiation, and overall survival rates in ESCC patients [87, 88]. Based on the lncRNA microarray, Li et al. demonstrated the altered expressions of lncRNAs in the tumor tissues and matched normal tissues from 119 patients with ESCC, and they developed a prognostic signature from the training group (60 patients) using a random forest supervised classification algorithm and nearest shrunken centroid algorithm. In detail, the three-lncRNA signature (including the lncRNAs ENST00000435885.1, XLOC_013014, and ENST00000547963.1) had strong prognostic value and could serve as an independent prognostic factor for patients with ESCC [49]. Some lncRNAs were shown to serve as diagnostic biomarkers, such as ncRuPAR, which had sensitivity of 88.41%, specificity of 73.91%, and accuracy of 81.16% for the diagnosis of GC [69]; AC096655.1-002, which had sensitivity of 51.3%, specificity of 87.2%, and an area under the curve (AUC) of 0.731 for diagnosis of GC [64]; FER1L4, which had sensitivity of 67.2%, specificity of 80.3%, and AUC of 0.778 for the prognosis for GC [66]; and HMlincRNA717 [68]. Together, lncRNAs may serve as potential diagnostic and prognostic biomarkers for DTCs.

Conclusion

In this review, we summarized the lncRNAs that play important roles in DTCs. Some of them act as oncogenes or tumor suppressor genes, contributing to tumor invasion, metastasis, and histological differentiation as well as cell proliferation, apoptosis, and clone formation. These lncRNAs are correlated with TNM staging, tumor size, lymph node metastasis, and poor patient survival. Some lncRNAs have high sensitivity and high specificity in serving as potential biomarkers for the prognosis and diagnosis of DTCs. lncRNAs, serving as molecular biomarkers for prognosis and diagnosis of tumors may have more advantages because they have limited conservation among human organs. Consequently, lncRNA-derived biomarkers may have higher specificity. Through screening and comparison of transcripts in a variety of human organs, some lncRNAs that are only upregulated or downregulated in a single organ may be found, and specific expression profiles of these lncRNAs can be used to specifically diagnose disease without

the interference of other diseases. More important, several papers reported that lncRNAs were stably detected in the plasma of patients or healthy subjects, suggesting that lncRNAs can serve as noninvasive biomarkers for the prognosis and diagnosis of DTCs.

These lncRNAs are correlated with TNM staging, tumor size, lymph node metastasis, and poor patient survival. Some lncRNAs have high sensitivity and high specificity in serving as potential biomarkers for the prognosis and diagnosis of DTCs.

There are more than 32,000 lncRNAs in the human genome, many of which have important functions in the initiation and progression of cancer. For DTCs, the identification of functional lncRNAs is the tip of iceberg, and many more functional lncRNAs must be identified and their regulatory mechanisms explored. Strikingly, several papers have shown that lncRNAs interact with proteins, mRNAs, and miRNAs in DTCs, resulting in the gain or loss of function of the corresponding elements. The molecular mechanisms by which lncRNAs contribute to DTC development seem to be more complicated, provoking researchers' interests. As the next step, we should investigate the crucial function of lncRNAs in the initiation and progression of DTCs and find potentially effective biomarkers for prognosis and diagnosis of DTCs. More important, the molecular regulatory mechanisms of lncRNAs underlying DTC development should be explored in detail. Improving our understanding of these mechanisms will make it possible to identify an effective therapeutic target for DTCs.

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Manuscript writing: Shuo Zeng, Bo-Sheng Li
Final approval of manuscript: Shi-Ming Yang, Bo-Sheng Li

Disclosures

The authors indicated no financial relationships.

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