Novel perspectives of long non-coding RNAs in esophageal carcinoma

Chen-Yu Lin\textsuperscript{1,2} and Han-Mei Xu\textsuperscript{1,2,*}

\textsuperscript{1}Department of The Engineering Research Center of Peptide Drug Discovery and Development and \textsuperscript{2}Department of State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, P. R. China

*To whom correspondence should be addressed. Tel: +86-25-83271007; Fax: +86-25-83271007; Email: 13913925346@126.com

Esophageal carcinoma (EC) is one of the most aggressive cancer types worldwide. However, the underlying genomic events of EC are not fully understood. It is becoming evident that long non-coding RNAs (lncRNAs) play vital roles in tumorigenesis, metastasis, prognosis and diagnosis. Accumulating EC-related lncRNAs have been verified to involve in various biological processes through diverse functions including signal, decoy, scaffold and guide. However, the molecular mechanism of lncRNAs in EC has not been fully explored. In this review, we outline the functions and underlying mechanism of EC-related lncRNAs to pave the way for identification of novel potential biomarkers for EC.

Introduction

Esophageal carcinoma (EC) has two histological forms: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Currently, ESCC is a prevalent histological type of EC in China (\textsuperscript{1}), whereas EAC widely occurs in Western nations. Due to growing incidences and poor prognosis of EC, it is necessary to find novel earlier diagnosis parameters and methods for EC. Therefore, a better understanding of molecular mechanism of EC carcinogenesis is extremely critical to improve diagnosis, personal treatment and prognosis of EC. Although numerous EC-related oncogenes and tumor suppressor genes have been reported (\textsuperscript{2}), the biological functions and underlying mechanisms of EC tumorigenesis have not been entirely elucidated.

The human transcriptome events involved in the tumorgenesis are heterogeneous and complex (\textsuperscript{3,4}). Previous studies paid more attention to the role of the human protein-coding genes in various carcinogenesis, and numerous messenger RNA (mRNA) signatures have been revealed as potential predictors of survival in various cancers (\textsuperscript{5,6}). However, only \textasciitilde1.2\% of the human genome encodes for protein-coding genes (\textsuperscript{7}) and vast majority of the human genome encodes non-coding transcripts. With advancement in human transcriptome analysis, increasing non-coding RNAs (ncRNAs) have been identified (\textsuperscript{8,9}). The ncRNAs can be classified into three categories according to the length of nucleotides (nts): (i) small ncRNAs with size of 15\textendash30 nts including microRNAs (miRNAs), piwi-interacting RNAs and transcription initiation RNAs; (ii) medium-size ncRNAs with size of 30\textendash200 nts; and (iii) long ncRNAs (lncRNAs) with the length of 200 nts. Compared with small ncRNAs, little was known on medium-size ncRNAs. Recent study reported that nc886 (also known as vtRNA2-1, pre-miR-886 or CBL3) with size of 101 nts plays an antitumor role in ESCC. Lee et al. found that nc886 expression was attenuated in ESCC tissues, and decreased expression of nc886 was related to poor survival of patients. Downregulation of nc886 could promote PKR activation, induce FOS and MYC oncogenes as well as inflammatory genes, while overexpression of nc886 could suppress cell proliferation. Consequently, nc886 can act as a novel kind of antitumor ncRNA. (\textsuperscript{10}). LncRNAs were initially regarded as spurious transcriptional ‘noise’. However, increasing IncRNAs have been found to have evolutionary conservation features (\textsuperscript{11,12}), which are associated with functional interactions with proteins and other RNAs. With the development of high-resolution microarray and massively parallel sequencing technology, the sheer number of IncRNAs are emerging as potential tumor suppressor genes or oncogenes with tissue-specific and cell-type-specific expression patterns in tumorigenesis (\textsuperscript{13,14}). Meanwhile, lots of IncRNAs are correlated with prediction and prognosis of various cancers (\textsuperscript{15,16}), and a part of IncRNAs can be detected in serum, plasma and urine (\textsuperscript{17}). Although these evidences suggested that IncRNAs may be novel potential biomarkers for cancer diagnosis and treatment, the molecular regulatory mechanisms of IncRNAs in cancers remain to be illustrated. Intriguingly, growing number of EC-related IncRNAs have also been identified to act as potential biomarkers for EC diagnosis, prognosis and prediction (\textsuperscript{18\textendash20}).
In this review, we will summarize the emerging functions and molecular mechanisms of EC-related IncRNAs for better understandings and applications of these IncRNAs in EC.

**LncRNAs characterization**

**Classification of lncRNAs**

Although satisfactory annotation and classification for lncRNAs are currently unavailable, lncRNAs’ genomic locations relative to nearby protein-coding genes are one of the useful standards for lncRNAs classifications (21). Accordingly, five broad categories of lncRNAs can be found: (i) sense lncRNAs that overlap one or more exons of protein-coding genes on the same strand; (ii) antisense lncRNAs that are transcribed in the opposite strand of protein-coding genes; (iii) bidirectional lncRNAs that initiate in a reverse fashion from the promoter of a neighboring protein-coding gene; (iv) intronic lncRNAs that initiate inside of an intron of a protein-coding gene in either direction and terminate without overlapping exons; and (v) intergenic lncRNAs also termed large intervening non-coding RNAs or lincRNAs, which behave as genomic interval units between two protein-coding genes (22,23) (Figure 1).

**Locus module of gene expression**

LncRNAs regulate gene expression via different mechanisms in the nucleus and cytoplasm. LncRNAs are primarily localized in cell nucleus and chromatin (8,24). In the nucleus, lncRNAs can regulate gene expression through diverse mechanisms: transcriptional interference (25); function in either cis-regulation or trans-regulation (26); function as a decoy via binding and sequester transcription factors (27). In the cytoplasm, lncRNAs can influence both half-life and translation of mRNAs. In detail, the lncRNA TINCR (terminal differentiation-induced ncRNA) binds with Staufen 1 protein (STAU1) and targets mRNAs containing the TINCR box motif to affect the stability of mRNAs (28); lncRNAs of the half-STAU1-binding site RNAs (1/2-sbsRNAs) class bind to 3’-UTR-containing Alu elements and promote the degradation of these target mRNAs (29); under stress conditions, antisense Uch11 lncRNA translocates from the nucleus to the cytoplasm and hybridizes with Uch11 mRNA to induce its translation (30); lincRNA-p21 can interact with translational repressor Rck to inhibit the translation of the targeted mRNAs (31).

**The functions and mechanisms of lncRNAs in ESCC and EAC**

Accumulating lncRNAs with dysregulated expression in EC were identified. However, only a fraction of EC-related lncRNAs have been relatively well illuminated. The emerging functions of EC-related lncRNAs are diverse, including histone modification, epigenetic silencing, splicing regulations, genomic imprinting and genetic alteration. EC-related lncRNAs appear to be participated in numerous biological processes, including proliferation, cell cycle, apoptosis, migration, invasion and so on (Table 1). To better interpret and apply lncRNAs as diagnosis and therapy parameters for future researches, we outline the known lncRNAs-mediated transcriptional or posttranscriptional gene regulations in EC from four molecular functions: signal, guide, scaffold and decoy (32,33) (Figure 2).

**Signal**

Under diversified stimuli, lncRNAs have specific expression patterns, including cell-type, spatially and temporally specific pattern, and their expressions are under extensively transcriptional control (27,34).

**FOXCUT**

LncRNA FOXCUT upstream transcript (FOXCUT) and FOXC1 gene were reported to be extremely upregulated in ESCC, which was strongly relevant with less differentiation, metastasis, advanced lymph node classification and worse prognosis. Knockdown of FOXCUT or FOXC1 could significantly diminish cell proliferation, migration, invasion and colony formation. In addition, as a molecular signal, knockdown of FOXCUT decreased the expression of its cognate neighboring genes FOXC1, indicating that FOXCUT plays an essential role in modulating FOXC1 mRNA synthesis (35). However, the mechanism of how FOXCUT interacts with FOXC1 to regulate the ESCC progression is still unclear.

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**Table 1**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>EAC</td>
<td>esophageal adenocarcinoma</td>
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<tr>
<td>EC</td>
<td>esophageal carcinoma</td>
</tr>
<tr>
<td>ESCC</td>
<td>esophageal squamous cell carcinoma</td>
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<tr>
<td>EZH</td>
<td>Enhancer of Zeste Homolog 2</td>
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<tr>
<td>HOTAIR</td>
<td>HOX transcript antisense RNA</td>
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<tr>
<td>lncRNA</td>
<td>long non-coding RNA</td>
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<tr>
<td>MALATA1</td>
<td>metastasis-associated lung adenocarcinoma transcript 1</td>
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<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>miRNA</td>
<td>microRNA</td>
</tr>
<tr>
<td>ncRNA</td>
<td>non-coding RNA</td>
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<tr>
<td>nt</td>
<td>nucleotide</td>
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<tr>
<td>PRC2</td>
<td>polycomb repressive complex 2</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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Figure 1. LncRNAs were classified based on their genomic location relative to nearby protein-coding genes. (a) Sense lncRNAs overlap one or more exons of protein-coding genes on the same strand. (b) Antisense lncRNAs are transcribed in the opposite strand of protein-coding genes. (c) Bidirectional lncRNAs are initiated in a reverse fashion from the promoter of a neighboring protein-coding gene. (d) Intronic lncRNAs are initiated inside of an intron of a protein-coding gene in either direction and terminate without overlapping exons. (e) Intergenic lncRNAs (also termed large intervening non-coding RNAs or lincRNAs) behave as genomic interval units between two protein-coding genes.
PlncRNA-1
PlncRNA-1 (also termed as CBR3-AS1) was found to be overexpressed in prostate cancer (CaP). Silence of PlncRNA-1 attenuated the expression of androgen receptor mRNA and protein as well as androgen receptor downstream targets. Suppression of androgen receptor signaling also resulted in inhibition of PlncRNA-1 expression in the CaP cell lines (36). High expression of PlncRNA-1 was also found in ESCC, and knockdown of PlncRNA-1 could prevent cell proliferation, induce cell apoptosis and promote cycle transit from G1 to S phase. Moreover, the high expression of PlncRNA-1 was also related to advanced clinical stage (P < 0.01) and lymph node metastasis (P < 0.05) of ESCC (37). However, the underlying mechanism of PlncRNA-1 function in ESCC remains unknown.

LOC285194
The lncRNA LOC285194, also termed as LSAMP antisense RNA, is a p53-regulated tumor suppressor in osteosarcoma and acts in part via inhibition of miR-211 (38). Recent evidence showed the downregulation of LOC285194 in ESCC, which was relevant to larger tumor size, advanced TNM stage, more lymph node metastasis and distant metastasis. Moreover, the low expression of LOC285194 as an independent prognosis marker was closely associated with preoperative chemoradiotherapy response and attenuation in disease-free survival and overall survival. Thus, LOC285194 was suggested to be a potential therapeutic marker to screen out ESCC patients who would be suitable for chemoradiotherapy and estimate the outcome of these ESCC patients (39). Nevertheless, the basic mechanism of LOC285194 in ESCC needs further exploration.

HNF1A-AS1
HNF1A-AS1 (HNF1A antisense RNA 1) is a bidirectional lncRNA corresponding to the hepatic nuclear factor 1 alpha gene (also known as transcription factor 1 or TCF1). The significant upregulation of HNF1A-AS1 was detected in human primary EACs, HNF1A-AS1 knockdown considerably reduced cell proliferation, anchorage-independent growth, inhibited S-phase entry and prevented cell migration and invasion. As a molecular signal, HNF1A-AS1 knockdown could significantly decrease expression of H19, which is a well-characterized cancer-related lncRNA (40, 41) and regulate chromatin and nucleosome assembly to induce cell cycle arrest (42). However, how HNF1A-AS1 regulates other lncRNAs is required to be further investigated.
LncRNAs interact with specific protein and guide the resultant complex into the target positions. This class of lncRNAs can regulate gene expression changes either in cis (on neighboring genes) or in trans (on distantly located genes) via either RNA-protein or RNA–DNA binding.

**HOTAIR**
A best-characterized lncRNA as a molecular guide is HOX transcript antisense RNA (HOTAIR) that emanates from the homebox C gene (HOXC) locus. HOTAIR binds the polycomb repressive complex 2 (PRC2) and lysine-specific demethylase 1 (LSD1) to promote epigenetic gene silence through modulating chromatin states via couplings H3K27 methylation and H3K4 demethylation, finally induces metastasis, invasion and progression of various cancer (43). In ESCC, upregulation of HOTAIR was closely linked to the advanced progression and poorer prognosis of ESCC. In vivo, HOTAIR-silenced ESCC cells reduced both size and weight of tumors and inhibited metastases (18,19). The metastasis mechanism of HOTAIR in ESCC was associated with suppression of WIF-1 expression by inducing its histone H3K27 methylation in the promoter region, which led to activation of the Wnt/β-catenin signaling pathway (20).

**91H**
Another lncRNA as a guide is 91H, a long non-coding antisense transcript, which is transcribed from the H19/IGF2 imprinted locus. The 91H was initially reported to be upregulated in human breast tumors and modulated IGF2 expression in trans without impacting H19 expression (44). Intriguingly, downregulation of 91H was detected in ESCC patients with higher depth of invasion, neoplastic grading and TNM. In ESCC cell lines, the expression of 91H was attenuated by treatment with demethylating agent, and IGF2 expression in 91H knockdown cell lines was markedly increased. The functional mechanism of lncRNA 91H indicated to be correlated with suppression of IGF2 expression via regulating H19 imprinting control regions methylation in ESCC progression (45). These evidences indicated that 91H can act as either an oncogene or a tumor suppression gene depending on cancer types.

**Scaffold**
As molecular scaffolds, lncRNAs recruit various effector molecules to form scaffolding complexes and then initiate the relevant specific and biological functions via utilizing specific signaling components (32).

**Linc-POU3F3**
Linc-POU3F3 is transcribed from a gene located next to POU3F3. Linc-POU3F3 overexpression was found in ESCC, and its expression was positively correlated with promoting cell proliferation and colony formation. In addition, the ESCC cells with knockdown of linc-POU3F3 formed xenograft tumors more slowly. As a molecular scaffold, linc-POU3F3 can interact enhancer of zeste homolog 2 (EZH2) with DNA methyltransferase (DNMT1), DNMT3A and DNMT3B to methylate CpG islands of POU3F3, resulting in downregulation of POU3F3 mRNA (46).

**PCAT-1**
Prostate cancer-associated ncRNA transcript 1 (PCAT-1), a newly identified lncRNA, is located within the chromosome 8q30 gene desert and is ~725 kb upstream of the c-MYC oncogene (47). PCAT-1 interacts with PRC2 and governs histone H3 methylation.
at lysine 27 to inhibit transcription (48). Recently, markedly high expression of PCAT-1 was found in human ESCC and was closely associated with invasion of cancer tissues, metastasis of lymph node, advanced pathologic stage of ESCC progression and a shorter overall survival time. Therefore, high expression of PCAT-1 may be used as a potential diagnostic signature to evaluate poor prognosis of ESCC patients (49). However, the corresponding mechanism of how PCAT-1 influences ESCC progression is yet to be investigated.

TUG1
LncRNA taurine-upregulated gene 1 (TUG1) was downregulated in non-small cell lung carcinoma. The molecular mechanism of TUG1 in non-small cell lung carcinoma is via recruiting and binding PRC2 to regulate homeobox B7 (HOX-B7) expression by interacting with EZH2. In non-small cell lung carcinoma, low expression of TUG1 was correlated with a higher TNM stage and tumor size, as well as poorer overall survival (P < 0.001) (50). Interestingly, TUG1 was significantly upregulated in ESCC tissues compared with adjacent normal tissues. High expression of TUG1 was strong related to family history and upper segment of EC (P < 0.05). Depletion of TUG1 could inhibit the proliferation and migration of ESCC cells as well as the cell cycle progression at the S phase (51). However, it is still remained to be elucidated whether TUG1 can regulate target genes or translational factors through recruiting and binding PRC2 in ESCC. The previous studies implicated that TUG1 can serve as either an oncogene or a tumor suppression gene in different cancers. The discrepancy may be partly due to the bifunctional nature of the lncRNA or the differences of cellular context. The accurate mechanism of TUG1 remains to be explored.

CDKN2B-AS1
As molecular scaffold, lncRNA can regulate protein activity or change protein localization by binding specific protein. CDKN2B-AS1, a member of antisense lncRNA class, is related to diverse carcinogenesis such as prostate cancer, intracranial aneurysm and leukemias (52,53). Hu et al. found that silence of CDKN2B-AS1 in ESCC cells could enhance cell proliferation and increase the expression level of telomerase reverse transcriptase (hTERT). In addition, β-elemene (a kind of chemotherapeutic drug) was found to inhibit the proliferation of EC cells by suppressing hTERT expression depending on lncRNA CDKN2B-AS1 (54).

SPRY4-IT1
LncRNA SPRY4-IT1 is a 687nt unspliced polyadenylated transcript, which is transcribed from the second intron of SPRY4 gene. Depletion of SPEY-IT1 could promote accumulation of lipid 2 protein and upregulate the expression of diacylglycerol O-acyltransferase 2 (DGAT2) enzyme, resulting in apoptosis and cellular lipotoxicity (55). Xie et al. found that the expression of SPRY-IT1 was upregulated in ESCC. Knockdown of SPRY-IT1 attenuated cell proliferation, invasiveness and migration in vitro and suppressed tumor cell growth in vivo. Furthermore, high expression of SPRY4-IT1 was closely associated with tumor differentiation, "T classification, lymph node metastasis, and clinical stage rather than patient" stage, gender, smoking status, alcohol consumption and tumor location (56). Nevertheless, whether and how SPRY4-IT1 regulate protein activity in ESCC are still unknown.

SOX2OT
SOX2 overlapping transcript (SOX2OT) (also known as NC00049) is embedded within the single-exon SOX2 gene, in its intronic region overlapping in the same transcriptional orientation (57). Hou et al. demonstrated that SOX2OT regulated cell cycle progression through moderating EZH2 level in lung cancer cells. Although no evidence of physical interaction between SOX2OT and EZH2 has been reported (58), EZH2 acts as a major component of PRC2, which may participate in transcriptional suppression of its target genes (59,60). In ESCC, two novel SOX2OT spliced variants (SOX2OT-S1 and SOX2OT-S2) were found to be unregulated and participate in tumor initiation and/or progression via modulating the cell cycle to arrest in sub-G phase of cell cycle (61). But the underlying mechanism of the overexpression of SOX2OT in ESCC remains to be further discussed.

Decoy
As molecular decoys, lncRNAs can interact with and titrate away a protein or RNA target without exerting any additional functions. These lncRNAs may negatively modulate the expression of their effectors.

MALAT1
MALAT1, a well-known lncRNAs with decoy function, was found to be dysregulated across many cancer types. The underlying mechanism of MALAT1 is via recruiting SR family proteins (62), regulating alternative splicing of oncopgenic mRNAs (63) and binding the active regions of chromosome (64). MALAT1 were extremely overexpressed in ESCC tissues, which were positively associated with clinical stages, primary tumor size, and lymph node metastasis. Knockdown of MALAT1 prevented proliferation, metastasis, migration and invasion of ESCC cells as well as leading to G0/M arrest and increment apoptosis ratio. MALAT depletion could activate the ATM-CHK2 signaling pathway, which is responsible for cell cycle arrest (65). Additionally, MALAT1 can mediate ESCC cells proliferation by ‘sponging’ miR-101 and miR-217 to increase expression of P21 and P27 and decrease expression in B-MYB. Overexpression of miR-101, miR-217 or depletion of MALAT1 in ESCC cells could reduce the expression of CTT4 and CTHRC1, down-stream genes of MALAT1, positive regulators of metastasis, resulting in suppressing ESCC cells migration and invasion (66).

UCA1
Urothelial carcinoma associated 1 (UCA1) was overexpressed in bladder cancer (67), breast tumor (68) and colorectal cancer (69). Wang et al. found that hsa-miR-1 prevented the UCA1 expression in bladder cancer cells in an Ago2-slicer-dependent manner (67). Analogously, Li et al. reported that upregulation of UCA1 in ESCC. Silence of UCA1 could diminish cell proliferation, migration and invasion ability. The high expression of UCA1 could be a powerful independent prognostic factor, which was associated with tumor differentiation, clinical stage and lymph node metastasis rather than patients’ age, gender and tumor location (P > 0.05) (70). Nevertheless, whether UCA1 modulates ESCC progression via interacting with specific miRNA is still unclear, and the molecular mechanism of UCA1 in ESCC development is yet to be elucidated.

Other lncRNAs in EC
Mutations in several lncRNAs were demonstrated to be associated with EC. Genetic polymorphism of these lncRNAs was found to be relevant to the risk of ESCC. Since the function and mechanism of some lncRNAs remains unclear, these lncRNAs cannot be classified into function group in above.
LncRNA-uco030pf1

Emerging evidence verified that the presence of single nucleotide polymorphisms (SNPs) in several lncRNAs primary sequences was closely related to ESCC progression. Wu et al. discovered markedly differences between patients and controls in the genotype frequencies for the rs11752942A>G site in the lincRNA-uco030pf1 exon. The rs11752942GG and AG genotypes had a markedly decreased risk of ESCC, compared with the rs11752842AA genotype. The rs11752942G allele could also reduce the level of lincRNA-U0030pf1 through targeting miRNA-149, resulting in modulating cell proliferation and tumor growth. Therefore, genetic polymorphism rs11752942A>G in lincRNA-uco0030pf1 exon could be a functional modifer for the development of ESCC (71).

HOTAIR

HOTAIR was found to be associated with massive carcinogenesis, including ESCC. Zhang et al. investigated the effect of functional SNP in HOTAIR on the development of ESCC. They found that HOTAIR rs920778TT carriers had much higher ESCC risk than rs920778CC carriers. Furthermore, the allele-specific regulation of rs920778 on HOTAIR expression were testified through a novel intronic HOTAIR enhancer in both ESCC cell lines and normal esophageal tissue with higher level of HOTAIR among T allele carriers. These results support new evidence for ESCC genetic basis (72).

CCAT2

Colon cancer-associated transcript 2 (CCAT2) is also a novel oncogenic lncRNA involved in tumor growth, metastasis and chromosomal instability. CCAT2 containing the rs6983276 SNP (73) was extremely overexpressed in microsatellite-stable colorectal cancer (74,75), CCAT2 expression was impacted by SNP status and the risk allele G promoted more CCAT2 transcript. Moreover, the binding between CCAT2 and TCF7L2 induced activation of WNT signaling pathway (76). In terms of ESCC, CCAT2 overexpression was found in ESCC and significantly correlated with ESCC patients with smoking history (73). However, whether CCAT2 can regulate the ESCC carcinogenesis via genetic variation is still not clear. The mechanism of CCAT2 in ESCC progression remains to be demonstrated.

AFAP1-AS1

Wu et al. found the significantly epigenetic differences among normal esophageus tissue and Barrett’s esophagus and EAC. Barrett’s esophagus and EAC exert genome-wide hypomethylation, including intragenic and repetitive genomic elements as well as non-coding regions, which could affect the target binding of small and long non-coding regions. LncRNA AFAP1-AS1 recruits from the antisense strand of DNA at the AFAP1 coding gene locus and was significantly hypomethylated and overexpressed in Barrett’s esophagus and EAC. Silencing AFAP1-AS1 expression could reduce cell growth, proliferation, colony-forming ability, migration and invasion, as well as promote apoptosis and G/M phase arrest without changing the expression of its protein-coding counterpart, AFAP1 (77). How AFAP1-AS1 promotes EAC progression remains to be elucidated.

PEG10

LncRNA PEG10 locates in human chromosome 7 between the 94285681 and 94298949 base sites and encompasses 763bp. Overexpression of lncRNA PEG10 was found in EC tissues and related to the occurrence of lymph node metastasis. The downregulation of lncRNA PEG10 could significantly inhibit proliferation and invasion and enhance apoptosis in EC cell lines (78). However, whether lncRNA PEG10 can modulate the expression of ESCC-related oncogenes or tumor suppressor genes remains to be illustrated.

Insight and prospect of lncRNAs in diagnostics and therapies of EC

Accumulating cancer-related miRNAs have been found to be present in the blood, sputum and urine of various cancers and are extremely stable in the presence of high levels of endogenous ribonuclease (79,80). Therefore, miRNAs might be considered as biomarkers of diagnostics and treatments in cancers. Similarly, a growing number of lncRNAs have also been detected in body fluid, such as urine and plasma (81). In detail, three ESCC-related lncRNAs, POUSF3, HNF1A-AS1 and SPRY4-IT1, could be detected in plasma of ESCC patients with high levels. Notably, the level expression of plasma POUSF3 was demonstrated to have the best diagnostic performance for detection of ESCC among the three candidate lncRNAs [the area under the ROC curve (AUC), 0.842; P < 0.001; sensitivity, 72.8%; specificity, 89.4%]. In addition, the combination between POUSF3 and serum squamous cell carcinoma antigen could significantly enhance the early stage diagnosis performance in ESCC (AUC, 0.926; P < 0.01; sensitivity, 85.7%; specificity, 81.4%) (81).

Although extensive evidence has indicated that lncRNAs can act as novel biomarkers, a number of problems should be settled. First, circulating lncRNAs were deemed to be unstable in presence of high amount of RNase in peripheral blood (82). However, increasing circulating lncRNAs were extremely stable in plasma of cancer patients (83). Previous investigation indicated that these ncRNAs may be packaged into various types of microparticles and then be secreted into human body fluids (84). Another evidence suggested that circulating ncRNAs could be changed for prevention of RNase digestion by a series of ways, including methylation, adenylation and uridylation (85). Therefore, the precise mechanism about how lncRNAs are released into body fluids need to be uncovered in further study. The second challenge is how to apply amplification to resolve the measurement of low levels of lncRNAs in plasma, serum or urine. Quantitative PCR has been widely used to quantify gene expression. Selecting the appropriate reference genes with the lowest variation and high stability features is extremely critical to minimize inter-procedure bias and implement quality control in clinical laboratory. Third, although increasing individual lncRNA is used as a biomarker, growing studies indicate that the combination of different lncRNAs or the combining lncRNAs and miRNAs may show more precise results of therapy and diagnose. For instance, Li et al. analyzed lncRNA expression profile in 119 pairs of ESCC tissues and adjacent normal tissues by microarray analysis and then validated by quantitative reverse transcription–PCR. Three lncRNA signatures (including the lncRNAs ENST00000435885.1, XLOC-013014 and ENST00000547963.1) were identified, which can be used to divide the patients into two groups with markedly different overall survival and also be used in the test group and independent cohort. Additionally, each signature was an independent prognostic factor for patients with ESCC (86). Therefore, the combination of several lncRNAs can be more accurate to evaluate the prognostic and overall survival in ESCC patients. Finally, in the future, lncRNAs may not only serve as biomarkers but also act as novel targets in ESCC therapy. Nevertheless, due to the large size of lncRNAs, the most common gene therapy delivery systems are difficult to delivery lncRNAs into special tumor cells. Therefore, it is still a hardship to use lncRNAs directly as therapeutic agents. All in all, further efforts need to be made to accomplish lncRNAs-related target therapy.
Conclusion
Although a growing number of IncRNAs are identified as biomarkers in various diseases including ESCC, numerous crucial questions are still unanswered. In this review, we summarize well-characterized ESCC-related and EAC-related IncRNAs, which play functional roles in various biological processes, such as metastasis, proliferation, apoptosis and so on. However, the majority of EC-related IncRNAs remain to be explored and should be further evaluated. Intensive efforts should be put into understanding potential mechanisms of IncRNAs in EC, which would contribute to find novel diagnostic and therapeutic methods for EC.

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