

Functions and mechanisms of long noncoding RNAs in lung cancer

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Abstract: Lung cancer is a heterogeneous disease, and there is a lack of adequate biomarkers for diagnosis. Long noncoding RNAs (lncRNAs) are emerging as an important set of molecules because of their roles in various key pathophysiological pathways, including cell growth, apoptosis, and metastasis. We review the current knowledge of the lncRNAs in lung cancer. In-depth analyses of lncRNAs in lung cancer have increased the number of potential effective biomarkers, thus providing options to increase the therapeutic benefit. In this review, we summarize the functions, mechanisms, and regulatory networks of lncRNAs in lung cancer, providing a basis for further research in this field.

Keywords: ncRNA, tumorigenesis, biomarker, network, proliferation, apoptosis

Introduction

Besides small-cell lung cancer, non-small-cell lung cancer (NSCLC) is any type of epithelial lung cancer and accounts for 85% of all lung cancers. The 5-year survival rate of this heterogeneous disease is 16.6%, and it has only improved slightly in the past few years.¹ NSCLC can be classified into discrete subclasses according to histological phenotypes, including squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large-cell carcinoma. The most common type of NSCLC is ADC, which is less associated with smoking and chronic inflammation than SCC.² The clinical integration of ADC in terms of predictive biomarker signatures is characterized by positive immunostaining for cytokeratin 7 and TTF1; however, SCC is characterized by positivity of cytokeratin 5, cytokeratin 6 and/or SOX2, and p63.^{3,4} In addition to tissue biopsy for clinical diagnosis, specific gene mutations in tumors have highlighted their usefulness as diagnostic markers and molecular therapy targets. For instance, *EGFR*, *ALK*, and *MET* mutations are always found in ADC patients.² However, *DDR2*, *FGFR1*, and *FGFR2* mutations, as well as mutations in genes in the PI3K signaling pathway, are generally found more frequently in SCC.⁵

Characteristics of lncRNAs

Noncoding RNAs (ncRNAs) are a superclass of endogenous, non-protein-coding RNA transcripts, many of which have essential functions in various cellular processes. Based on their function, ncRNAs can be classified into two subclasses, namely housekeeper ncRNAs (rRNA, tRNA, snRNA, snoRNA) and the regulated ncRNAs. The regulated ncRNAs can be categorized by length as follows: any ncRNA <200 nucleotides (nt) in length is a short ncRNA (siRNA, miRNA, piRNA) and those >200 nt in length are long ncRNAs (lncRNAs). Recently, Iyer et al⁶ demonstrated that there are ~60,000 ncRNAs in the human genome, and >68% of these are lncRNAs, of which ~80%

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are not annotated. Approximately 1% of lncRNAs harbor ultraconserved elements, and 7% of lncRNAs harbor disease-associated single-nucleotide polymorphisms.⁶

An increasing number of lncRNAs have been identified as key regulators of a wide range of cellular processes, including dosage compensation, imprinting, transcription, mRNA splicing, translation, nuclear and cytoplasmic trafficking, and cellular localization. Notably, ectopic expression of lncRNAs is associated with a great variety of diseases.

However, compared with mRNAs, lncRNAs have their own peculiar characteristics. First, the mean length of lncRNAs is shorter than that of mRNAs, with an average of 592 nt compared to 2,453 nt for mRNAs. Second, lncRNAs have fewer exons, although they harbor standard canonical splice sites.⁷ Third, the methylation level of the transcription start site of lncRNAs is higher than that of mRNAs; therefore, the expression level of lncRNAs is significantly lower. In addition, lncRNAs show strict tissue specificity.⁸ Furthermore, lncRNAs tend to correlate with transposable elements, especially with endogenous retroviruses, compared with protein-coding genes.⁹ Moreover, lncRNA conservation includes more than introns or random intergenic regions, but they are less conserved than mRNAs.¹⁰ Despite the fact that the conservation of some lncRNAs at the sequence level is not high, they may have the same functions.^{11,12} Diederichs¹¹ indicated that the conservation of lncRNAs should include four dimensions: sequence, structure, function, and expression from synthetic loci. Despite the differences between lncRNAs and mRNAs, they share certain common characteristics. Large-scale studies revealed that many identified lncRNAs are transcribed by RNA polymerase II, which is the same as that for mRNA. They also share the same posttranscriptional mRNA processing, including 5'-capping, splicing, and poly-adenylation at the 3' end. Certain transcription factors can bind to the promoter region of lncRNAs, such as c-myc, p53, and Sox2.

Classification and functions of lncRNAs

The most recent classification of lncRNAs is based on their location relative to that of target protein-coding genes. According to these criteria, lncRNAs can be classified as exonic, intronic, overlapping, or intergenic. Moreover, based on the transcriptional direction with respect to protein-coding genes, lncRNAs are divided into two groups, namely sense and antisense.⁷

According to transcriptional modes, lncRNAs can be categorized as *cis*-acting and *trans*-acting lncRNAs. *Cis*-acting lncRNAs mediate gene expression based on their

position in the vicinity of the target gene transcriptional site. However, *trans*-acting lncRNAs can control the expression of genes at any loci based on the recruitment of proteins to the target sites to participate in transcriptional regulation. lncRNAs hybridize with DNA or RNA molecules to form triple-stranded RNA–DNA structures that play essential roles in transcription.^{12,13}

lncRNAs can be classified into various discrete subclasses on the basis of their function as follows (Figure 1).¹⁴

Decoys

lncRNA decoys exert biological functions by binding to proteins indirectly and playing a role in multiple processes of life.

Scaffolds

The second set of lncRNAs function as scaffolds, as lncRNAs act as platforms to bind relevant molecular complexes. Typically, *HOTAIR* acts as a bridging scaffold for the PRC2 and LSD1/CoREST/REST complex to suppress gene expression. PRC2 binds to an 89 bp fragment in the 5' end of *HOTAIR*, and the LSD1/CoREST/REST complex binds to a 646 bp fragment in its 3' end.^{15,16} Genome-wide RNA immunoprecipitation analysis has shown that ~20% of the various types of lncRNAs are bound to PRC2.¹⁷ Another example of a molecular scaffold is *Kcnq1ot1*, which combines PRC2 and G9a to generate H3K27me3 and H3K9me3, leading to epigenetic silencing of genes.¹⁸ Indeed, several studies show that not only proteins but also lncRNAs play critical roles in bridging molecular components.

Signal

lncRNAs play critical roles in signal regulation and in the responses to various stimuli. lncRNAs are often expressed in a spatial- and temporal-specific pattern. Upon specific expression, they can modulate translation and integrate developmental cues.¹⁹ For instance, *lincRNA-p21*, which binds to hnRNP-K, promotes the proper localization of hnRNP-K and results in the silencing of p53-regulated genes.²⁰ Certain lncRNAs are emerging as signals of functionally significant biological events because of their roles in regulating transcriptional activity or pathways.

Sponges

PTEN1 (pseudogene of PTEN) upregulates the tumor suppressor PTEN by attracting miRNAs to its 3'-untranslated region.²¹ The lncRNA *GAPLINC* acts as a “sponge” and modulates CD44 expression by attracting *miR211-3p*.²² The lncRNA antisense ncRNA in the INK4 locus (*ANRIL*) can

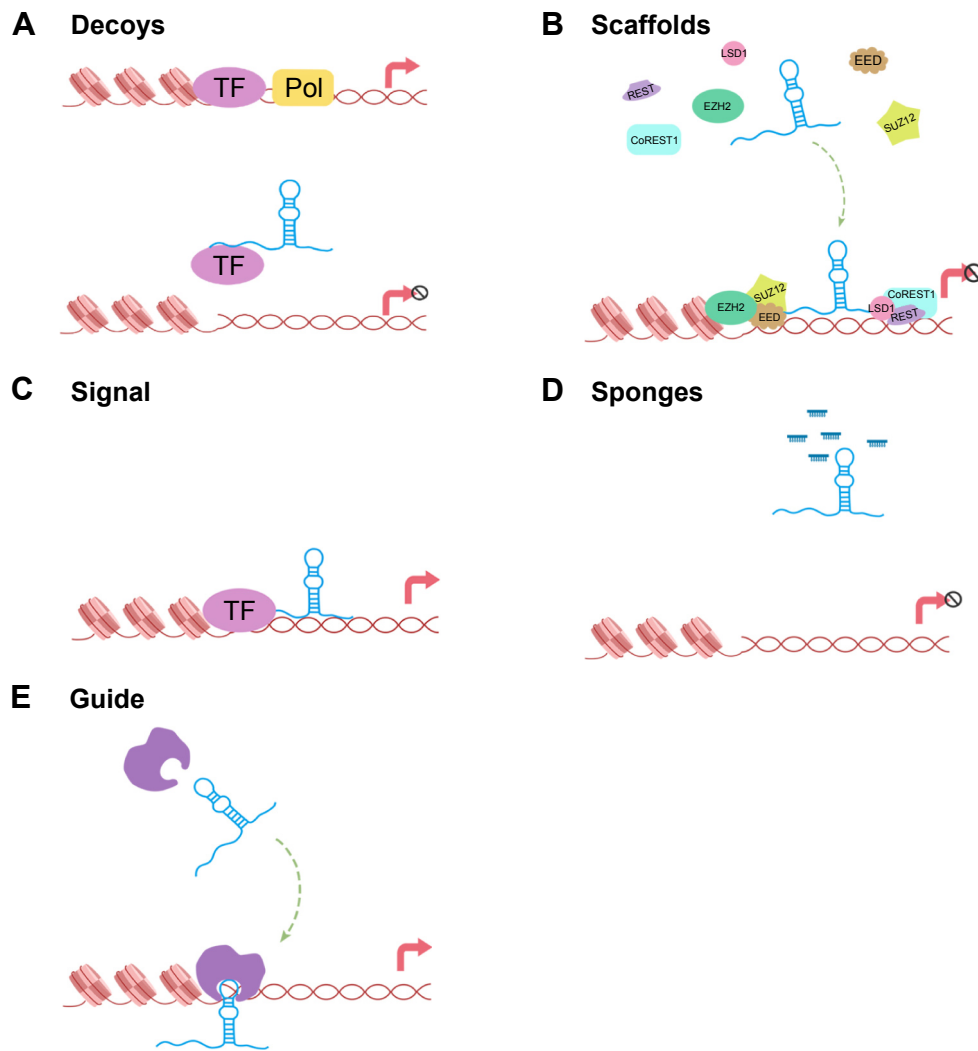


Figure 1 Overview of the five molecular functions of lncRNAs.

Notes: (A) lncRNAs act as decoys to attract transcription factors and influence protein activity. (B) In addition, the ability to aggregate different proteins has highlighted their usefulness as scaffolds. (C) lncRNAs also have a critical role in signal regulation. (D) lncRNAs can interact with miRNAs, acting as “sponges”. (E) lncRNAs play essential roles in guiding chromatin-remodeling enzymes to target sites.

Abbreviations: lncRNAs, long noncoding RNAs; TF, transcription factor; Pol, polymerase.

function as a “sponge” to titrate *miR-99a/miR-449a*, thus activating *CDK6* and inactivating *p15^{INK4B}/p16^{INK4A}*. Consequently, *E2F1* is released inappropriately and contributes to gastric cancer cell proliferation.²³ lncRNAs therefore function as sponges by interacting with miRNAs and suppressing their effects on target sites. Recent evidence highlights a classification of circRNAs as miRNA sponges that contribute to the downregulation of target genes. Compared to linear RNAs, the half-life of circRNAs is longer.²⁴ It is plausible that the duration and the effect of circRNAs have more advantages.

Guide

Multiple studies indicate that nuclear-retained lncRNAs function in guiding chromatin modifiers to specific genomic loci.

Typical is PRC2, which contains a histone methyltransferase (enhancer of zeste 2, *EZH2*) that inhibits gene expression via trimethylation of histone H3Lys27 (H3K27me3).²⁵ In addition, chromatin conformation changes induced by nuclear-enriched lncRNAs can promote gene expression.²⁶

Regulation modes of lncRNAs

lncRNAs involved in epigenetic regulation

An example of the chromatin-modifying capabilities of lncRNAs is dosage compensation in mammals, which requires the preferential silencing of one parental allele. *Xist* gives rise to stable epigenetic silencing of large-scale genes in the X-chromosome by tethering PRC2 to the transcriptional site, inducing the formation of H3K27me3 to inactivate

heterochromatin.²⁷ Another example is *lncRNA p21*, which can change DNA methylation levels by promoting histone methyltransferase and DNA methyltransferase binding to target sites, thus affecting the expression of reprogramming genes.²⁸

lncRNAs participate in transcriptional regulation

Evidence to date indicates that lncRNAs, which are transcribed from enhancers or promoters, can act in *cis* pattern to control transcriptional efficiency. lncRNAs transcribed from enhancers can affect their activity or help recruit protein factors. Among lncRNAs in prostate cancer, two overexpressed lncRNAs bind to the androgen receptor, promoting androgen receptor binding to an enhancer.²⁹

lncRNAs affect posttranscriptional processing

Most genes are transcribed via tissue-specific and cell-specific alternative-splicing patterns in humans.³⁰ Noteworthy is that the majority of lncRNAs are often similarly expressed in a spatial- and temporal-specific manner. Accumulating evidence indicates that lncRNAs may regulate alternative splicing by *cis*-acting mechanisms or by recruiting regulatory splicing factors.^{31,32} Recently, Gonzalez et al showed that a conserved antisense lncRNA transcribed from the *FGFR2* locus in humans regulates alternative splicing by recruiting the histone demethylase KDM2a and polycomb group proteins. These findings suggested that lncRNAs can regulate alternative splicing through the establishment of a splicing-specific chromatin signature.³³

Dysregulation and functional roles of lncRNAs in lung cancer

Lung cancer is often associated with aberrant lncRNA transcriptomes, including onco-lncRNAs and tumor suppressor lncRNAs. Here, we discuss recent discoveries that implicate aberrant lncRNAs in lung cancer (Table 1). In addition, we provide a framework of systematically functionalized lncRNAs and integrate them with the protein-coding RNA dimension in complex networks (Figure 2).

Onco-lncRNAs

MALAT1

Characteristics of MALAT1

MALAT1, also known as *NEAT2*, is located on chromosome 6p24.3 in humans with a length of 8.7 kb.^{11,12} *MALAT1* is a well-characterized lncRNA with markedly high expression in most NSCLC types. *MALAT1* generates a primary noncoding transcript that is enriched in the nucleus.³⁴ Furthermore, the *MALAT1* gene shows strong sequence

conservation from humans to zebra fish.³⁵ *MALAT1* transcription is initiated from multiple promoters and produces different *MALAT1* transcript variants. However, most *MALAT1* transcripts, which harbor two distinct nuclear speckle localizational elements, are enriched in nuclear speckles, indicating essential role of *MALAT1* in RNA metabolism. *MALAT1* is modified at the 3' end and forms a triple-helical structure, which may be the reason why *MALAT1* appears as a very stable lncRNA.^{36,37} The half-life of *MALAT1* ranges from 9 to 16.5 hours.³⁸ After modification, *MALAT1* generates a second small mRNA that is localized to the cytoplasm.³⁹ *MALAT1* binds to the unmethylated PRC2 protein, therefore contributing to PRC2 preferential binding to H2AK5ac and H2AK13ac, marking transcriptional activation.⁴⁰ At the transcriptional level, several studies have shown that *MALAT1* regulates gene expression, such as that of growth control genes. *MALAT1* affects the phosphorylation levels of serine/arginine (SR) splicing factors, such as B-MYB, leading to changes in gene expression.¹² *MALAT1* also regulates gene expression at the posttranscriptional level.⁴¹ For instance, *MALAT1* affects a minor subset of transcripts in the process of alternative splicing, such as *RNPS1*, *PRP6*, and *SON*.⁴² In summary, *MALAT1* can modulate gene expression both at the transcriptional and posttranscriptional level.

MALAT1 in lung cancer

MALAT1, which is found in abundance in various tissues and cell lines, is successfully knocked out by the zinc finger nuclease technique in A549 cells. *MALAT1* has been shown to be a critical regulator of the metastasis phenotype in lung cancer cells.⁴³ In A549 cells, inhibition of *MALAT1* downregulates the expression of motility-associated genes, including *HMMR*, *AIM1*, *SLC26A2*, *LAYN*, *CCT4*, *ROD1*, *CTHRC*, and *FHL1*.^{44,45} Xenograft models have confirmed this effect. The prevailing view is that *MALAT1* participates in the regulation of motility-related genes to enhance the motility of lung ADC cells.⁴⁵ Recently, Shen et al⁴⁶ demonstrated that *MALAT1* overexpression promotes lung cancer brain metastasis by inducing epithelial-to-mesenchymal transition (EMT). Another study suggested that *MALAT1* is involved in cell cycle regulation at the level of G2/M phase progression. *MALAT1* interacts with nuclear hnRNP C and promotes hnRNP C translocation to the cytoplasm in the G2/M phase.⁴⁷ In addition to its effect on cell cycle progression, *MALAT1* affects the expression of the proapoptotic factor Bcl-2, which is specifically associated with prognosis in NSCLC.⁴⁸ Moreover, a meta-analysis and various studies

Table 1 Summary of lncRNAs in lung cancer

Type of lncRNA	Name	Location	Length (kb)	Expression level	Property	Intersection molecules and pathway	Cell processes and clinical features	References
Intergenic	MALAT1	6p24.3	8.7	Up	Oncogenic	Affects expression of Bcl-2 and metastasis-related genes	Promotes cell growth, metastasis, and EMT; inhibits apoptosis; poor survival	11, 12, 34–49
	HOTAIR	12q13.13	2.15	Up	Oncogenic	Induced by Col-1, HIF-1 α . Promotes expression of gelatinases. Represses cell-adhesion related genes, p21, and HOXA5	Promotes cell growth, invasion, metastasis, EMT and relapse	25, 50–64
	MEG3	14q32.3	1.6	Down	Tumor suppressive	Affects expression of p53	Inhibits proliferation. Induces apoptosis	88–91
Intronic	SOX2-OT	3p26.33	4.2	Up in SCC	Oncogenic	Inhibits the expression of EZHP	Promotes cell growth. Poor survival	65–68
	SPRY4-IT	15q31	0.69	Down	Tumor suppressive	Silenced by EZHP. Inhibits the expression of EMT markers	Inhibits growth, invasion, and metastasis. Induces apoptosis	92–96
	HNFI A-AS	12q24	2.46	Up in ADC	Oncogenic	Affects EMT-related genes via DNMT1	Promotes cell growth and metastasis	1
Overlapping	ANRIL	9p21.3	126	Up	Oncogenic	Represses KLF2 and p21 via PRC2	Promotes cell growth and metastasis. Inhibits apoptosis	69–73
	H19	11q15.5	2.3	Up	Oncogenic	Affects expression of miR675 and HGF/SF	Poor survival	74–81
	PANDAR	6p21.2	1.5	Down	Tumor suppressive	Induced by cigarette smoke	Unknown	
Exonic	CARLO-5	8q24.21	1.6	Up	Oncogenic	Affects expression of Bcl-2 via NF-YA	Inhibits proliferation. Induces apoptosis	97, 98
	GAS5	1q25	0.65	Down	Oncogenic	Suppresses p16, p21 and p27 and EMT markers	Affects cell cycle, proliferation, and invasion, EMT. Poor survival	82, 83
	MV1H	1q25	0.65	Down	Tumor suppressive	Affects expression of E2F1, p21, and p53. Suppresses miR-21 expression	Inhibits proliferation. Induces apoptosis	99–108
Unknown	MV1H	10q22		Up	Oncogenic	Affects expression of MMP2/MMP9	Promotes proliferation and invasion. Poor survival	84
	TUG1	22q12.2	7.1	Down	Tumor suppressive	Induced by p53, represses HOXB7 via PRC2	Inhibits proliferation. Induces apoptosis	109–112
	BANCR	9q21.11	0.69	Down	Tumor suppressive	Inhibits the expression of EMT markers	Inhibits migration, invasion, metastasis, EMT. Induces apoptosis	113–117
	EVADR	6q13	0.39	Up in ADC	Oncogenic	Induced by MER48 ERV element	Unknown	87
	PVT1	8q24.21	210	Up	Oncogenic	Induced by p53	Induces proliferation, invasion, and metastasis. Poor survival	85–86

Notes: Up, upregulated; down, downregulated.

Abbreviations: lncRNAs, long noncoding RNAs; EMT, epithelial-to-mesenchymal transition; SCC, squamous cell carcinoma; ADC, adenocarcinoma; MMP, matrix metalloproteinase.

have shown that high *MALAT1* expression is related to poor overall survival in NSCLC.⁴⁹

HOTAIR

Characteristics of HOTAIR

HOTAIR is located on chromosome 12q13.13 in humans, and has a length of 2.1 kb. There are four gene clusters (*HOXA*, *HOXB*, *HOXC*, and *HOXD*) and 39 HOX genes in the genome.²⁵ These clusters generate numerous lncRNAs

that are often expressed in a spatial- and temporal-specific pattern.⁵⁰ Although *HOTAIR* is located in the *HOXC* locus, it has been shown to repress gene expression in the *HOXD* locus. In addition, the secondary structure of *HOTAIR* contains four independently folding modules, two of which are evolutionarily conserved protein-binding domains.⁵¹ *HOTAIR* is enriched in the nucleus but is also expressed in the cytoplasm.⁵² *HOTAIR* has little sequence conservation in humans and mice, but the molecular mechanism is similar

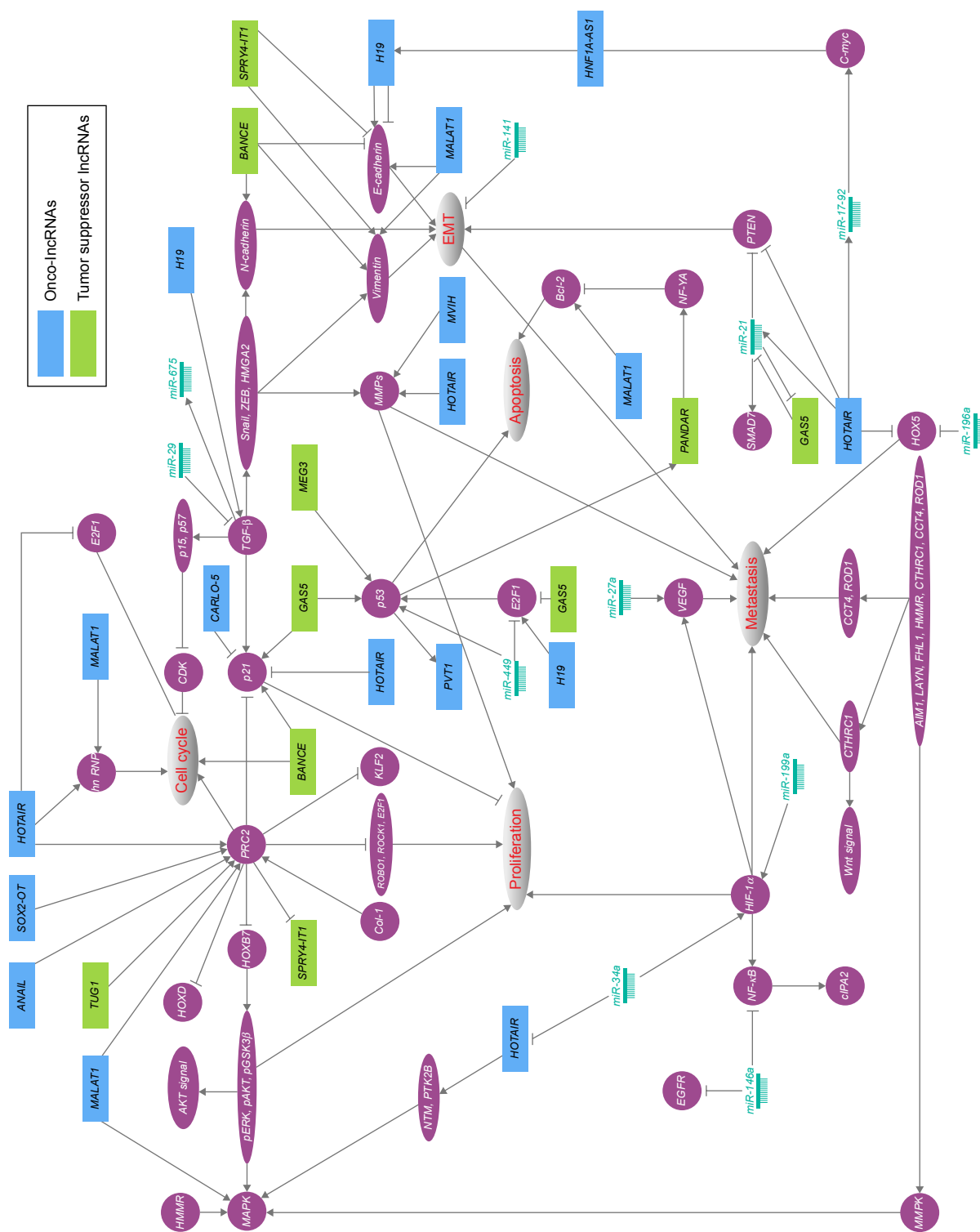


Figure 2 Overview of the regulatory network of lncRNAs in lung cancer.

Notes: Blue frames represent onco-lncRNAs. Green frames represent tumor suppressor lncRNAs. Purple frames represent lncRNAs.

Abbreviations: lncRNAs, long noncoding RNAs; miR, microRNA; MMPs, matrix metalloproteinases; EMT, epithelial-to-mesenchymal transition.

in the two species. Reciprocally, the conserved lncRNA sequence does not always possess the same function in other species as expected.

HOTAIR-mediated epigenetic gene silencing is dependent on its function as a bridge scaffold for PRC2 and LSD1/CoREST/REST. PRC2 binds to the 5' end of *HOTAIR* and regulates chromosome occupancy by EZH2 (a subunit of PRC2), which leads to histone H3 lysine 27 trimethylation of the *HOXD* locus. Meanwhile, the LSD1/CoREST/REST complex binds to the 3' end of *HOTAIR*.⁵³ *HOTAIR* represses gene expression by increasing the occupation of H3K27me3 and decreasing the occupation of H3K4me3 on the promoter in a *trans*-acting manner.⁵⁴ In addition to its role in the PRC2 and LSD1/CoREST/REST complexes, *HOTAIR* acts as a scaffold for E3 ubiquitin ligases and their corresponding substrates, namely E3 ubiquitin ligase Dzip3 and its substrate Ataxin-1, resulting in proteolysis.⁵⁵ Furthermore, *HOTAIR* harbors a target site for *miR-34a*⁵⁶ and *miR-141* in its exon 6, both of which can attenuate the expression of *HOTAIR*. Exon 6 of *HOTAIR* contains a target site for let-7i that mediates the formation of a hetero-tetramer containing let-7i, Ago2, *HOTAIR*, and HuR.⁵⁷

HOTAIR in lung cancer

Ectopic expression of *HOTAIR* has been reported in a plethora of cancerous tissues. High *HOTAIR* levels are associated with invasion and metastases and linked to an advanced stage of disease and poor survival in patients with lung cancer.^{58–60} EMT mediates the invasive phenotype of lung cancer cells. *HOTAIR* may promote the EMT process of lung epithelial cells by distinct mechanisms. One of these mechanisms is transcriptional repression of *HOXA5* gene. *HOXA5* is related to postnatal lung development. It is likely that *HOTAIR* and *miR-196a* act in common to repress the expression of *HOXA5*, therefore contributing to dedifferentiation during lung tumorigenesis.⁶¹ Moreover, *HOTAIR* facilitates the process of EMT by inhibiting the expression of cell adhesion-related genes in small-cell lung cancer epithelial cells.⁵⁹ Meanwhile, tumor-promoting type 1 collagen, which is a potent inducer of EMT, can modulate the translational control of *HOTAIR* in NSCLC cells.⁶² In addition to the repression of EMT inhibitors and the promotion of EMT, *HOTAIR* also affects the expression of gelatinase, a matrix metalloproteinase (MMP) that plays a role in triggering invasion in lung cancer cells.⁵⁸ *HOTAIR* can promote resistance to cisplatin via downregulation of p21 (WAF1/CIP1) protein levels in lung ADC cells.⁶³ Furthermore, *HOTAIR* as a direct target of HIF-1 α , promotes cell proliferation, migration,

and invasion in hypoxic NSCLC. Evidence indicates that HIF-1 α binds to *HOTAIR* via interaction with the upstream region of *HOTAIR* in NSCLC cells.⁶⁴ The upregulation of *HOTAIR* has been shown to have a negative impact on lung cancer by regulating genes involved in invasion, metastasis, and poor survival.⁵⁹

SOX2-OT

SOX2-OT is located on chromosome 3p26.33 in humans and has a length of 4.2 kb. *SOX2-OT* is an intronic lncRNA that overlaps with the *SOX2* gene, which is a major regulator of pluripotency.⁶⁵ Recent studies have shown that *SOX2* and *SOX2-OT* have similar expression patterns in lung SCC and ADC tissues.⁶⁶ There are multiple transcription initiation sites in the human and mouse *SOX2-OT* locus, leading to many spliced variants.⁶⁷ In addition, the human genomic region of *SOX2-OT* is characterized by multiple conserved transcription factor-binding sites. These sites have essential roles in the tumorigenesis process.⁶⁵ *SOX2-OT* is expressed at higher levels in human primary lung cancer tissues than in adjacent non-tumor tissues. Typically, *SOX2-OT* exhibits significantly higher expression in SCC of the lung than in ADC.⁶⁸ With respect to the mechanism of *SOX2-OT*, Hou et al showed that knockdown of *SOX2-OT* expression leads to cell cycle arrest at G2/M phase through the modulation of the expression of EZH2. Meanwhile, its expression level is significantly correlated with cell proliferation and colony formation ability in lung cancer cell lines.⁶⁶ This study further indicates that high *SOX2-OT* expression predicts poor survival in lung cancer patients.

HNFI A-AS1

HNFI A-AS1 is located on chromosome 12q24 and has a length of 2.46 kb. Overexpression of *HNFI A-AS1* has been reported in lung ADC tissues compared with the corresponding non-tumor tissues. In addition, elevated expression of *HNFI A-AS1* is linked to tumor-node-metastasis (TNM) stage, tumor size, and lymph node metastasis. *HNFI A-AS1* can regulate EMT-related protein expression via binding to DNMT1, therefore regulating cell growth and metastasis both in vitro and in vivo.¹

ANRIL

ANRIL is derived from the p15/CDKN2B-p16/CDKN2A-p14/ARF gene cluster, which maps to human chromosome 9p21.3 with a length of 126 kb. This gene family is associated with cutaneous malignant melanoma and neural system tumors. *ANRIL* includes several isoforms with

tissue-specific expression because it consists of 19 exons.⁶⁹ Recently, elevated levels of *ANRIL* have been reported in NSCLC tissues, and its expression level is significantly correlated with poor prognosis. siRNA-mediated knockdown of *ANRIL* results in the inhibition of cell proliferation and the promotion of apoptosis both in vitro and in vivo.⁷⁰ An ongoing study indicates that the subcellular localization of *ANRIL* is mostly in the cell nucleus. *ANRIL* is indicated as a “decoy”, and it represses KLF2 and p21 transcription by binding to PRC2 in NSCLC PC9 cells, which sheds light on the effect of *ANRIL* on NSCLC cell proliferation and apoptosis partly in *trans*.⁷⁰ Generally, KLF2 as a tumor suppressor is significantly downregulated in various cancers, leading to inhibition of cell proliferation via KRAS.⁷¹ *ANRIL* can regulate the transcription of *miR-99a* and *miR-449a* by recruiting the PRC2 complex in gastric cancer.⁷² Recently, Ren et al⁷³ demonstrated that high expression of *miR-449a* attenuates lung cancer cell proliferation, and the downregulation of *miR-449a* is correlated with a shorter disease-free survival of patients. It is plausible that *ANRIL* modulates the expression of *miR-449a*, thereby inhibiting the proliferation of lung cancer cells during lung tumorigenesis.

H19

Characteristics of *H19*

H19 is located on chromosome 11q15.5 in humans and has a length of 2.3 kb. *H19* is a paternally imprinted gene that is spliced into five exons. The *H19* gene locus is complex, harboring conserved *miR-675* and antisense protein-encoding transcript (*HOTS*), which is a tumor suppressor.⁷⁴ Another pro-tumorigenic antisense transcript, 91H, overlaps with the *H19* gene locus.⁷⁵ *H19* and its nearby gene *IGF2* show uniparental mono-allelic expression. There is an imprinting control region (ICR) between them. The ICR is unmethylated on maternal chromosomes, where it binds to the transcription factor CTCF and inhibits the enhancer from binding to the ICR. As a result, the enhancer binds to *H19* and induces its expression. Conversely, on paternal chromosomes, the ICR is methylated and binds to the enhancer, resulting in *H19* downregulation.⁷⁶ *H19* can act in both *cis* and *trans* patterns: for instance, *H19* regulates the imprinting of *IGF2* by silencing the expression of neighboring genes. An example of *H19* acting in *trans* pattern is its role as a molecular sponge for miRNA *let-7*, which is involved in inducing EMT.⁷⁷

H19 in lung cancer

Evidence to date indicates that *H19* is associated with various tumorigenesis signaling pathways, including the p53 and HIF-1 α , TGF- β , Bcr-Abl, Wnt/ β -catenin, and

HGF pathways.⁷⁸ In lung cancer cells, *H19* is induced by hypoxic stress via a p53-dependent manner. Knockdown of *H19* expression in hypoxia has a suppressing effect on cancer cell proliferation, anchorage-independent growth, and colony formation.^{79,80} Furthermore, knockdown of *H19* can reverse the tumorigenic and scattering effect of HGF/SF on A549 cells.⁷⁹ The overexpression of *H19* has a negative impact on lung cancer. Notably, the upregulated *H19* is loss of imprinting independent in the airway epithelia of smokers in comparison with nonsmokers.⁸¹

CARLO-5

CARLO-5 is located on chromosome 8q24.21 in humans and has a length of 1.6 kb. *CARLO-5* is significantly upregulated in NSCLC tissues. Overexpression of *CARLO-5* in NSCLC tissues is significantly correlated with advanced TNM stage. The expression of p16, p21, and p27, which are G0/G1 arrest markers, decreases with the downregulation of *CARLO-5*.⁸² High level of *CARLO-5* expression is a prognostic indicator of poor patient survival. Moreover, elevated expression of *CARLO-5* is associated with increased proliferation and invasion ability, partially through the modulation of EMT.⁸³

MVIH

MVIH is located on chromosome 10q22 in humans. Overexpression of *MVIH* has been reported in NSCLC tissues, and its expression level is significantly correlated with TNM stage and tumor size. High levels of *MVIH* expression are prognostic indicators of poor survival.⁸⁴ siRNA-mediated knockdown of *MVIH* inhibits cell proliferation and invasion, partly via modulating the expression of *MMP2* and *MMP9*. *MMPs* are involved in multiple biological processes, including remodeling of extracellular matrix, cell proliferation, differentiation, and metastasis.

PVT1

PVT1 is located on chromosome 8q24.21 in humans and has a length of 210 kb. The similar expression patterns of *PVT1* and *MYC* gene might be explained by the shared genomic locus between them. A study identified a p53 transcription factor-binding site in the *PVT1* promote region.⁸⁵ Overexpression of *PVT1* in NSCLC tissues is significantly correlated with TNM stage. In addition, patients with high levels of *PVT1* expression show poor survival.⁸⁶ Knockdown of *PVT1* expression inhibits lung cancer cell proliferation, migration, and invasion.

EVADR

EVADR is located on chromosome 6q13 in humans and has a length of 0.39 kb. Recent evidence shows that *EVADR* is

overexpressed in ADC tissues, including lung ADC, and is correlated with decreased patient survival. Among nine MER48-associated lncRNAs, *EVADR* is the only one that is consistently expressed in ADC tissues. *EVADR* expression is regulated via an active promoter provided by the MER48 endogenous retrovirus element.⁸⁷

Tumor suppressor lncRNAs

MEG3

MEG3 is located on chromosome 14q32.3 in humans and has a length of 1.6 kb. Previous evidence indicates that *MEG3* is a tumor suppressor because of its role in modulating angiogenesis.⁸⁸ *MEG3* can act in both p53-dependent and p53-independent manner during different processes. However, overexpression of *MEG3* decreases NSCLC cell proliferation and induces apoptosis via the activation of p53.⁸⁹ In addition, *MEG3* expression is deregulated in NSCLC tissues, and a low expression level is significantly related with higher TNM stage, increased tumor size, and poor patient survival.^{90,91}

SPRY4-IT1

SPRY4-IT1 is located on chromosome 5q31 in humans and has a length of 0.69 kb. *SPRY4-IT1* is upregulated in melanoma, esophageal SCC, and clear cell renal cell carcinoma, suggesting a common oncogenic role.^{92–94} Sun et al⁹⁵ showed that *SPRY4-IT1* is significantly downregulated in 94.2% of NSCLC cancerous tissues compared with normal tissues, which suggests an anti-oncogenic role. Ectopic expression of *SPRY4-IT1* is associated with tumor size, advanced pathological stage, lymph node metastasis, and overall survival time in NSCLC patients. Reduced *SPRY4-IT1* expression is an independent prognostic marker for NSCLC. EZH2, a methyltransferase and a catalytic subunit of PRC2, is overexpressed in NSCLC,⁹⁶ and its downregulation prevents it from binding to the *SPRY4-IT1* promoter region. This decreases the H3K27me3 modification, resulting in the inhibition of *SPRY4-IT1* expression. *SPRY4-IT1* has also been shown to promote NSCLC cell proliferation and metastasis by modulating the process of EMT.⁹⁵

PANDAR

PANDAR is located on chromosome 6p21.2 in humans and has a length of 1.5 kb.⁹⁷ Although *PANDAR* is transcribed in antisense to *CDKN1A*, it is not a linked transcript of *CDKN1A*.⁹⁸ *PANDAR* is a direct transcriptional target of p53 in NSCLC cells, and can modulate Bcl-2 expression by binding to NF-YA, thus affecting NSCLC cell apoptosis.⁹⁷ *PANDAR* interacts with NF-YA (NF-YA is related to tumorigenesis) to decrease proapoptotic gene expression in a

p53-dependent manner in normal human fetal lung fibroblasts. In NSCLC tissues, *PANDAR* interacts with NF-YA and is deregulated. *PANDAR* downregulation is associated with increased tumor size and advanced TNM stage. *PANDAR* expression is an independent prognostic predictor for NSCLC. Moreover, *PANDAR*, a transcriptional target of p53, affects NSCLC cell apoptosis partly by modulating Bcl-2 transcription through binding to NF-YA, thus affecting the proliferation of NSCLC cells in vitro and in vivo.⁹⁷

GAS5

Characteristics of GAS5

GAS5, comprising 12 exons, is located on chromosome 1q25 in humans and has a length of 0.65 kb. With respect to this locus, there are ten C/D box snoRNAs transcribed from its intronic regions. In addition, these snoRNAs have increasingly been linked to the functions of *GAS5*.⁹⁹ *GAS5* plays an essential role in cell apoptosis and growth. Evidence to date indicates that *GAS5* modulates the activity of glucocorticoid-responsive genes. In this process, *GAS5* represents a clear example of a decoy lncRNA, which competitively binds to the glucocorticoid receptor and prevents it from binding to glucocorticoid response elements.¹⁰⁰

GAS5 in lung cancer

Multiple studies have indicated that *GAS5* acts as a tumor suppressor in various cancers, such as gastric cancer,¹⁰¹ hepatocellular cancer,¹⁰² and colorectal cancer,¹⁰³ because of its role in the inhibition of proliferation and promotion of apoptosis. In NSCLC patient samples, decreased expression of *GAS5* is linked to advanced TNM stage and increased tumor size.¹⁰⁴ Increased expression of *GAS5* deregulates E2F1 and drives the expression of p21 p53 in NSCLC cells, indicating that *GAS5* has a regulatory effect on NSCLC cell proliferation.¹⁰⁵ The activity of *GAS5* can be regulated by miR-21, which has a putative binding site in *GAS5*. In turn, *GAS5* suppresses miR-21 expression in a feedback loop between them.¹⁰⁶ Dong et al¹⁰⁷ showed that overexpression of *GAS5* in the lung ADC A549 cell line reverts gefitinib resistance, suggesting its tumor-suppressive function. Moreover, overexpression of *GAS5* reverses the resistance to EGFR-tyrosine kinase inhibitors in ADC in vitro and in vivo. Generally, increased EGFR is related to poor prognosis in NSCLC patients.¹⁰⁸ This study further indicated that *GAS5* has an anti-oncogenic role.

TUG1

TUG1 is located on chromosome 22q12.2 in humans and has a length of 7.1 kb. The *TUG1* gene displays a high level

of conservation in the human, mouse, rat, dog, and cow genomes.¹⁰⁹ *TUG1* is involved in photoreceptor development and is deregulated in many kinds of human cancers. In NSCLC, *TUG1* possesses tumor suppressor features such as inhibition of cell proliferation and promotion of apoptosis. *TUG1*, which is induced by p53, is found binding to PRC2 and epigenetically regulates the expression of *HOXB7*.¹¹⁰ Thus, it is plausible that *TUG1* modulates NSCLC cell growth via the AKT and MAPK signaling pathways because *HOXB7* participates in these pathways. Moreover, patients with low level of *TUG1* expression display a higher TNM stage, increased tumor size, and relatively poor overall survival.¹¹⁰ *TUG1* has an oncogenic role in NSCLC, but it is a bona fide ncRNA in other cancer entities, including urothelial carcinoma of the bladder¹¹¹ and osteosarcoma.¹¹²

BANCR

BANCR is located on chromosome 9q21.11 and has a length of 0.69 kb. *BANCR* is upregulated in malignant melanoma, colorectal carcinoma, and papillary thyroid carcinoma tissues, suggesting a common oncogenic role.^{113–115} However, Sun et al showed that *BANCR* is significantly downregulated in NSCLC cancerous tissues compared with normal tissues.¹¹⁶ In addition, deregulated expression of *BANCR* is associated with increased tumor size, advanced pathological stage, lymph node metastasis, and poor survival in NSCLC patients. Reduced *BANCR* expression is an independent prognostic marker for NSCLC. Furthermore, knockdown of *BANCR* expression leads to the promotion of cell migration and invasion but inhibition of metastasis. It is plausible that downregulated *BANCR* promotes cell proliferation by downregulating p21 expression.¹¹⁷ Subsequent studies further indicate that *BANCR* has a critical role in EMT via modulation of E-cadherin, N-cadherin, and Vimentin expression. In sum, *BANCR* is proposed to modulate NSCLC cell-invasive and metastatic ability partially by modulating the EMT process.¹¹⁶

Discussion

Lung cancer is responsible for the largest number of cancer-related deaths around the world. One of the main barriers to the success of lung cancer therapy is the lack of tumor biomarkers for early diagnosis. In the previous studies, evidences have mainly focused on elucidation of lncRNAs in cellular and mouse models. As shown in Table 1, the expression of *MALAT1*, *HOTAIR*, *SOX2-OT*, *HNFI1A-AS1*, *ANRIL*, *MVIH*, and *PVT1* is associated positively with tumor size; reciprocally, the expression of *MEG3*, *SPRY4-IT1*, *GAS5*,

and *TUG1* is negatively correlated. Besides, the expression of *MALAT1*, *HOTAIR*, *HNFI1A-AS1*, *ANRIL*, and *PVT1* is associated positively with lymph node metastases, whereas the expression of *SPRY4-IT1* and *BANCR* is negatively correlated. Indeed, to accurately and comprehensively understand the role of lncRNAs in human, large clinical cases of pathological characteristics as well as the prognosis are needed. The previous studies have indicated that overexpression of *MALAT1*, *SOX2-OT*, *ANRIL*, *CARLO-5*, *MVIH*, and *PVT1* is a negative prognostic marker for patient survival. Indeed, the clinical integration of lncRNAs with respect to prognostic and predictive biomarker signatures will increase the therapeutic benefit. Here, we summarize the recent research and regulatory networks of lncRNAs (Figure 2) in lung cancer. Interestingly, the key role of *PRC2* in modulating proliferation and cell cycle of lung cancer cells has been emerging. *PRC2* indirectly functions as a double-edged sword through *ROB1*, *ROCK1*, and *E2F1*. First, *MALAT1*, *ANAIL*, *SOX2-OT*, and *HOTAIR* promote proliferation and cell cycle by regulating *PRC2*. *TUG1* inhibits proliferation by regulating *PRC2*. Meanwhile, *PRC2* is able to inhibit the expression of *SPRY4-IT1*. Several growth-related genes including p21 and p53 have been shown in the hinge of network. *BANCE*, *GAS5*, and *MEG3* enhance activity of these tumor suppressor genes. *CARLO-5* and *HOTAIR* have been proposed to highlight oncogenic feature by inhibiting the expression of p21 and p53. Generally, MMPs play a critical role in tumor cell growth and metastasis by altering the environments in which the cells grow.¹¹⁸ We have shown that *HOTAIR* and *MVIH* possibly regulate proliferation and metastatic ability of lung cancer cells. Noteworthy is that the altering expression of *E-cadherin*, *Vimentin*, and *N-cadherin* is a fundamental event in EMT.¹¹⁹ It is plausible that *MALAT1*, *BANCE*, *SPRY4-IT1*, and *H19* act in concert with them to regulate the progress of EMT during lung tumorigenesis. Additionally, miRNAs as high-potential biomarkers have critical position in regulatory network of lung cancer; for example, *miR-196a* represses *HOX5* expression, thereby promoting metastasis. It is very likely that lncRNAs harbor miRNA seed regions and enrich target RNA-binding motifs. Thus, miRNAs are proposed to modulate lung tumorigenesis by lncRNAs.

Although an impressive number of studies in the last decade focused on the characteristics and functions of ncRNAs, research is still in its infancy and presents great challenges. First, as the sequence and structure of lncRNAs are only poorly conserved, the canonical knockdown and knockout methods may have no effect. In addition, ectopic expression of lncRNAs may not show obvious phenotypes

as with protein-coding transcripts. Second, reference value is not always high between different researches, owing to different and multiple functions of lncRNAs in different tissues and cells. Therefore, elucidating the biological functions of lncRNAs is not easy. Third, the limited bioinformatical resources are another reason. Current lncRNA annotation is lacking compared with other RNA databases. Similarly, bioinformatic tools, such as lncRNA secondary structure prediction, remain to be developed. Unraveling the functions and regulatory mechanisms of lncRNAs in lung cancer might be a future breakthrough to improve our understanding of this network. The integration of miRNA and lncRNA signature profiling in lung cancer may be a useful tool for clinical applications.

Conclusion

lncRNAs are increasingly being recognized as critical molecules in various biological processes. In addition to the various types, there is a large number of lncRNAs, and they show numerous modes of interaction. Based on the location concerning the nearest protein-coding gene, lncRNAs can be classified into four subclasses, namely exonic, intronic, overlapping, and intergenic lncRNAs. According to their function, lncRNAs can be categorized as signal, decoy, sponge, guide, and scaffold molecules. It has become increasingly clear that lncRNAs are involved in tumorigenesis in many cancers. This network should serve as a guide for “navigating” through the lncRNAs research in the literature.

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Disclosure

The authors report no conflicts of interest in this work.

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