Potential clinical application of lncRNAs in non-small cell lung cancer

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Abstract: Lung cancer has been identified as one of the most prevalent and deadly tumors worldwide. In recent years, lncRNAs have been demonstrated to play a significant role in the development of lung cancer. Specifically, lncRNAs act as a regulator of cancer-critical genes, and they regulate the biological behavior of tumors at the transcriptional and posttranscriptional levels. Recent studies have shown that lncRNAs possess great potential in the treatment of non-small cell lung cancer patients because of their roles in diverse cellular processes, such as proliferation, metastasis, stem cell maintenance, and epithelial to mesenchymal transition, and they serve as signaling biomarkers. Compared to other invasive diagnostic methods, detection of lncRNAs may become a very useful noninvasive methodology. Moreover, lncRNAs can serve as potential therapeutic targets in non-small cell lung cancer due to their roles in regulating many signaling pathways associated with lung carcinoma. In this review, we discuss the roles and expression profile of lncRNAs. We also discuss the promising application of lncRNAs as predictors of clinical diagnosis, prognosis, and as potential therapeutic targets, aiming to demonstrate their practical value for clinical treatment.

Keywords: biomarker, lncRNA, diagnosis, prognosis, therapy, NSCLC

Introduction

Pulmonary malignancies are one of the most lethal cancers of humans. The prognosis of most lung cancer patients, whether they are suffering from non-small cell lung cancer (NSCLC) or small cell lung cancer, is quite poor with limited survival. In China, it is thought that there were 733,000 new cases of lung cancer and >610,000 related deaths in 2015, which ranked as the first for male patients and the second for female patients.[1,2] Generally, the symptoms of early stage lung cancer are not obvious. Imaging modalities, such as computed tomography, and some serum tumor markers, such as carcinoembryonic antigen (CEA) and squamous cell carcinoma antigen (SCCA), are available for early diagnosis of NSCLC. However, traditional detection methods are inevitably limited by cumulative radiation damage and low sensitivity and specificity.[3,4] New biomarkers with high sensitivity and specificity are needed for molecular diagnosis and prognosis; this can be done through deeper research of the molecular mechanisms of NSCLC.

The human genome has been identified by a number of computational and evolutionary analyses in the last few decades. Protein-coding genes, which account for only 1.5% of the genome, are well known for their vital functions in humans.[5,6] The remaining 98% of the human genome that does not encode proteins creates the so-called ncRNAs, which have received more attention recently for their potential functional roles. These ncRNAs are grouped into short ncRNAs and lncRNAs according

to their size.[7] The lncRNAs are a type of ncRNAs longer than 200 nucleotides, which are not translated into a protein, and regulate gene expression by multiple mechanisms.[8] Many studies have shown lncRNAs to be implicated in several cellular biological functions, such as chromatin modification, gene expression regulation, cellular differentiation, and cell cycle progression. They can regulate mRNA splicing patterns and produce different splice variants, regulate downstream genetic transcription, modulate protein activity, serve as scaffolds for the assembly of multiple component complexes, be applied in transcriptional procession and regulate subcellular localization of protein.[9,10] For instance, MEG3 has been identified to simulate the expression of GDF-15 by binding to factor p53.[11] Similarly, lincRNA-p21 has been reported to be related to apoptosis, as it stimulates the inhibition of p53-dependent genes by activating p21 gene (CDKN1A).[12] In addition, the lncRNA CCND1 was demonstrated to serve as an allosteric modulator of the RNA-binding protein TLS and inhibit transcription by bringing about repression of the cyclin D1 promoter.[13] Consequently, considering the various functions that lncRNAs regulate, it is unsurprising that they are one of the primary features of some human cancers, including breast cancer, prostate cancer, lung cancer, colon cancer, and so on. These lncRNAs regulate tumor-critical genes in the development of cancers. In lung cancer, the reported lung cancer–associated lncRNAs include HOTAIR, H19, MALAT1, ANRIL, and GAS5, among others. Accordingly, lncRNAs' expression profiling is a reasonable approach to study tumor growth, invasion and metastasis, which might be feasible for diagnosis.[14]

A study of different targetable mutations, such as KARS, EGFR, HER2, MET, PI3KA, as well as ROS1 and ALK rearrangements, represents a critical junction for the therapy of lung carcinoma, paving the way to the era of personalized medicine.[15,16] However, the 5-year overall survival (OS) of lung cancer of all stages together remains low at 15.9%.[17] Further studies of lncRNAs have demonstrated them to be biomarkers or potential factors affecting cancer growth, invasion, metastasis, and recurrence. In this review, we aim to discuss the promising applications of lncRNAs as lung cancer biomarkers, thus bringing out novel findings about the noninvasive detection potentials for NSCLC diagnosis and prognosis as well as novel therapeutic targets for the attention of clinicians and researchers.

LncRNAs related to NSCLC diagnosis

Although many of the current markers for the diagnosis of NSCLC are proteins, ncRNAs are widespread in various

body fluids and are relatively stable.[18] Exploration of new ncRNAs for clinical diagnosis is a growing area of research. The stability of lncRNAs is similar to mRNA, while their tissue specificity is higher than mRNA, and they can also be detected in various body fluids such as blood, urine, and saliva; therefore, lncRNAs are suitable clinical indicators.[19] Recently, numerous lncRNAs have been reported to act as tumor diagnosis biomarkers, including plasma HULC, which is a novel potential biomarker used for the confirmation of hepatocellular carcinoma.[20] Additionally, PAC3 was used as a urinary biomarker for prostatic carcinoma diagnosis and prognosis.

Also, many abnormal lncRNAs have been identified in NSCLC. For example, MALAT1, on which nearly 100 studies have been published on its association with lung cancer, has been evaluated as a potential biomarker in body fluids. Research by Weber et al validated the expression of plasma lncRNA MALAT1 by distinguishing 45 NSCLC patients from healthy controls. These results indicated MALAT1 as a promising diagnostic biomarker due to its high specificity, stability, and minimal invasiveness (sensitivity, 56%; specificity, 96%; area under the receiver operating characteristic curve=0.79).[21] Also, serum exosomal MALAT1 may have clinical significance in the diagnosis of NSCLC. To study the expression of MALAT1 contained in exosome, Zhang et al[22] selected 77 NSCLC patients and found the levels of MALAT1 from patients to be higher than healthy controls, with a test sensitivity of 60% and specificity of 81% (AUC=0.703). In their study, exosomal MALAT1 expression was positively associated with lymphatic node metastasis and TNM stage. However, other clinicopathologic features, including gender, age, and tumor diameter, showed no significant difference compared to exosomal MALAT1 levels.

As an early discovered lncRNA, accumulating evidence has indicated that GAS5 is downregulated in many cancers and functions as a tumor suppressor.[23,24] For example, Liang et al[25] designed a study to investigate the diagnostic value of GAS5 in blood samples, and they found that GAS5 was highly expressed in blood from NSCLC patients, compared with healthy controls, and the AUC reached 0.832 with a sensitivity of 82.2% and specificity of 72.7%. In order to test this further, GAS5 and CEA were combined to identify the diagnostic efficiency, and the results showed that the combination yielded an AUC of 0.909 (95% CI: 0.857–0.962; $P<0.001$), which was significantly improved compared to GAS5 or CEA alone.

Similarly, the lncRNAs HIF1A-AS1 and XIST have been shown to play significant regulatory roles in tumor biology, with an elevated expression level in lung cancer. Tantai et al[26]

Table 1 lncRNAs related to NSCLC diagnosis

lncRNA	Diagnostic efficacy	Reference
MALAT1	Circulating MALAT1, AUC=0.79	21
	Exosomal MALAT1, AUC=0.703	22
GAS5	Circulating GAS5, AUC=0.832	25
	Combination of GAS5 and CEA, AUC=0.909	
XIST and HIF1A-AS1	Circulating XIST, AUC=0.834	26
	Circulating HIF1A-AS1, AUC=0.876	
	Combination of XIST and HIF1A-AS1, AUC=0.931	
SPRY4-IT1, ANRIL, and NEAT1	Circulating SPRY4-IT1, AUC=0.603	27
	Circulating ANRIL, AUC=0.798	
	Circulating NEAT1, AUC=0.693	
	Combination of SPRY4-IT1, ANRIL, and NEAT1; AUC=0.876	
SOX2OT and ANRIL	Circulating SOX2OT, AUC=0.745	28
	Circulating ANRIL, AUC=0.723	
	Combination of SOX2OT, ANRIL, CEA, CYFRA21-1, and SCCA; AUC=0.853	

Abbreviations: AUC, area under the receiver operating characteristic curve; NSCLC, non-small cell lung cancer; CEA, carcinoembryonic antigen.

explored the diagnostic values of these proteins in NSCLC. They found that compared to preoperative patients, patients with postsurgical intervention showed significantly lower expression levels of serum HIF1A-AS1 and XIST. The AUC reached 0.876 (95% CI: 0.793–0.965; $P<0.001$) for HIF1A-AS1 and 0.834 (95% CI: 0.726–0.935; $P<0.001$) for XIST. Also, the combination of HIF1A-AS1 and XIST had higher diagnostic value, yielding an AUC of 0.931 (95% CI: 0.869–0.990; $P<0.001$).

Furthermore, in order to improve diagnostic efficiency, Hu et al[27] selected 21 previously identified lncRNAs as potential targets for subsequent lncRNA detection. They eventually found that circulating NEAT1, ANRIL, and SPRY4-IT1 were significantly increased in plasma samples of NSCLC patients compared to control sets, and the combination of the three factors illustrated a higher power, with an AUC of 87.6% (sensitivity, 82.8%; specificity, 92.3%). These three factors might serve as promising biomarkers in the early identification of NSCLC due to their stability.

As tumor biomarkers, CEA, SCCA, and CYFRA21-1, among others, are not always able to work alone. Xie et al[28] demonstrated that the combination of novel lncRNA biomarkers and traditional detection could have great potential in making NSCLC diagnosis. In their study, 14 lncRNAs were combined with three tumor biomarkers (SCCA, CEA, and CYFRA21-1) to predict NSCLC. Their results showed that SOX2OT and ANRIL might be potential biomarkers for such a diagnosis. In addition to establishing the role of lncRNAs in diagnosis, some studies have examined their use for the pathological classification of NSCLC. Zhao et al[29] identified 72 lncRNAs with significant differences between lung squamous cell carcinoma and lung adenocarcinoma using gene microarray. White et al[30] found 27 lung cancer–associated lncRNAs

to be markers for the differential diagnosis of lung squamous cell carcinoma and lung adenocarcinoma.

Along with the aforementioned factors, many other novel lncRNAs, such as PVT1,[31] CAR10,[32] ZXF1,[33] MetaLnc9,[34] and PANDAR,[35] among others, have been identified as playing multiple roles in NSCLC diagnosis and prognosis in recent years (Table 1). Although studies on these lncRNAs are not mature enough, accumulating evidence suggests that lncRNAs do have significant functions in epigenetic regulation, promote proliferation and cellular growth, and result in uncontrolled and progressive tumor growth and metastasis. Based on these findings, we can conclude that a single lncRNA by itself is not enough to be used as a clinical indicator in NSCLC due to its low sensitivity and specificity. However, combination of multiple lncRNAs for the diagnosis of NSCLC shows more reliable specificity and sensitivity. However, there is still no commonly recognized group of lncRNAs for NSCLC diagnosis.

LncRNAs related to NSCLC therapy

The most commonly used treatment for NSCLC currently includes surgical excision, chemotherapy, and chest radiotherapy. Surgical excision is especially available for early NSCLC patients. However, these therapies are limited to defeat advanced cancer due to their poor therapeutic efficacy. Novel approaches need to be explored and applied in the clinic before improving patient survival and quality of life.[36] The lncRNAs have multiple roles in cancer progression and tumorigenesis and are widely expressed in lung carcinoma. They can be used as potential therapeutic targets in NSCLC due to their effects in the development and regulation of diverse molecular pathways correlated with

gene expression. Significantly, dysregulated lncRNAs are reported to be associated with many treatments, including molecular-targeted therapy and chemotherapy, among others. For these reasons, lncRNAs can serve as new therapeutic targets for NSCLC by restoring the specificity and sensitivity of cancer cells to chemotherapeutic drugs and they may even cure the condition.[37]

RNAi-mediated gene silencing therapy

Generally, RNA interference technologies (shRNA, siRNA, and antisense oligonucleotides) represent the most promising approach for the selective inhibition of target lncRNAs, although in some cases, such a modality may not be available due to the introcellular localization or secondary structure. lncRNA-targeted RNAi has been proven to be effective using cell lines; however, stable conditions are needed to transport siRNA to the targets in vivo. Recently, numerous lncRNAs have been identified as potential therapeutic targets. For instance, HOTAIR silencing through RNAi has been shown to reduce invasiveness and viability in breast, pancreatic, as well as lung cancer;[38] it also contributed to cisplatin resistance in lung adenocarcinoma cells via the downregulation of p21 expression.[39] In addition, MALAT1 shRNA-mediated knockdown has been shown to significantly reduce cell invasiveness and migration in NSCLC.[19]

Remarkably, Cheng et al recently compared the pretreatment levels of UCA1 protein in EGFR-mutant NSCLC patients who had developed acquired resistance to EGFR-tyrosine kinase inhibitors. UCA1 may play a significant role in acquiring resistance to EGFR-tyrosine kinase inhibitors.[40] The delivery problems associated with siRNA and its off-target effects may limit its further application, and inhibition of lncRNAs remains a challenge in vivo.[41] However, several strategies have been developed to overcome this shortcoming, such as lipid-based nanoparticle delivery,[42] conjugate-based delivery,[43] and polymer-based delivery,[44] and these approaches are available for potential molecular treatment.

Antisense oligonucleotide (ASO)-based treatment

ASOs are short single-stranded DNA that can induce lncRNA degradation via RNaseH; they can also be used for lncRNA silencing and regulation. Compared with siRNA, ASOs show fewer off-target effects and higher specificity.[41] In some cases, ASOs were shown to target lncRNA MALAT1 and the inhibition of MALAT1 weakened malignant phenotypes

via cycle arrest in cervical and lung cancer cells.[45] As for uncovering the function of lncRNA MALAT1, Tony et al developed a MALAT1 knockout model in human lung tumor cells as a unique loss-of-function model. In their study, animals treated with MALAT1 ASO had significantly lower tumor volume and nodules in the lung compared to control ASO-treated animals. Thus, inhibition via MALAT1 ASOs prevented NSCLC metastasis, revealing a novel therapeutic approach for NSCLC patients' treatment.[46]

Small molecule modulators of lncRNA–protein interactions

Small molecule modulators mediate the regulation of lncRNAs by blocking the binding of lncRNAs to their interacting proteins.[47] Small molecule inhibitors can be applied to bind lncRNA by mimicking or changing their secondary structure. Many chromatin-modifying enzymes are flexible, which can allow proteins to form stronger, more stable interactions and interact with specific partners. They can also form distinct structures when bound to different DNAs, RNAs, or proteins.[48–51]

Based on the above theory, small molecular modulators may develop novel therapeutic technology. For instance, targeting lncRNA–protein interactions would reduce off-target effects and increase the specificity of compounds. Chromatin-modifying enzymes targeting lncRNA–protein interactions are more reliable compared to those targeting either lncRNAs or proteins alone.[52] For this matter, HOTAIR interaction is inhibited along with LSD1 and PRC2 via small molecular modulators identified to reduce metastasis in breast carcinoma.[53] However, there are still no reports about the lncRNA-related modulator in NSCLC. Therefore, in order to create novel small molecular drugs, lncRNA–protein interactions and pharmacological trends are needed to be explored and studied.

Plasmid-mediated targeted therapy

Another interesting approach for the treatment of cancer is plasmid-mediated targeted therapy. H19 has been identified to show high expression in breast cancer and lung cancer, among others. The plasmid BC-819, which carries the gene for the A subunit of diphtheria toxin, has been studied to utilize the tumor-specific expression of H19 lncRNA.[54] BC-819 was also illustrated to play a role in the treatment of pancreatic, ovarian, and colon cancers and NSCLC.[52]

New biological technologies have emerged, such as RNAi, ASOs, and small molecular modulators. The potential use of lncRNAs for therapies is great, since they can provide

a novel therapeutic strategy for patients with NSCLC and can be effective in restoring the normal expression levels of lncRNAs. However, several limitations still need to be addressed, such as selective tumor suppressor targets, the stability of lncRNAs in body fluids, and distribution of therapeutic lncRNAs. Due to the fact that many mechanisms of lncRNAs are still undiscovered, further studies are required to confirm these findings; however, it is unquestionable that lncRNAs can play an increasingly significant role in NSCLC treatment. The more we learn about lncRNAs, the higher the chance that an improved therapy can be developed.

LncRNAs related to NSCLC prognosis

The lncRNAs are closely related to NSCLC invasion and metastasis, as well as affect the prognosis of NSCLC patients. For example, MALAT1 has been investigated for its prognostic value in many studies. However, these studies were inevitably limited by their small sample sizes. Li et al[55] performed an updated meta-analysis to summarize the potential value of MALAT1 as a biomarker for predicting survival in multiple human cancers, and they found raised MALAT1 could be regarded as an independent predictor for OS. In a study by Schmidt et al, it was found that high expression of MALAT1 were often associated with poor prognosis in squamous cell carcinoma of the lung.[56] Furthermore, they showed that patients with low expression of MALAT1 had a better prognosis than those with high expression of MALAT1, and suggested that MALAT1 could serve as a biomarker for the prognosis of NSCLC patients.

Besides MALAT1, Zhang et al[57] investigated the lncRNA H19, including its biological roles and clinical significance. They stated that elevated H19 predicted a poor prognosis and was an independent prognostic factor for OS. Also, Nakagawa et al found that the expression of HOTAIR in early brain metastases of NSCLC patients was much higher than in primary lesions, and suggested that HOTAIR could be used as a predictor of early tumor cell metastasis in NSCLC patients. HOTAIR could also promote tumor cell migration in vitro.[58] Liu et al[39] found that overexpression of HOTAIR presented with lymph node metastasis, advanced stage of disease, and predicted poor prognosis. Compared with the high expression levels of HOTAIR patients, the lower expression group experienced a longer OS.

Accordingly, Qiu et al studied the lncRNA CCAT2 expression levels in lung cancer and found that overexpression of CCAT2 was significantly associated with lung adenocarcinoma (P=0.033), but not with squamous cell cancer.

They also demonstrated that elevated CCAT2 expression promoted the invasion and proliferation of lung adenocarcinoma cells, indicating a poor prognosis.[59] Luo et al found that patients with high CARLo-5 expression levels presented poorer prognosis, whereas patients with lower expression of CARLo-5 experienced a longer OS.[60] Besides the above, the lncRNAs ZXF1,[33] BANCR,[61] and PVT1[62] were also investigated in various studies and proved to be potential prognostic biomarkers for NSCLC.

The combination of multiple lncRNA expressions was investigated for use in the prognosis of NSCLC patients. In order to determine the prognostic value of multiple lncRNAs in NSCLC patients, Zhang et al studied the correlation between clinical outcomes and lncRNA expression. According to the median gene expression, patients were divided into different groups. The researchers were able to demonstrate that high expression of H19, MALAT1, and HOTAIR and low expression of TUG1 and PANDA were predictors of poor prognosis. Different risk groups are managed according to the expression of lncRNAs. In the results, the disease-free survival time curves differed significantly among the low-risk, moderate-risk, and high-risk groups.[63] There are also many studies analyzing the correlation between lncRNAs and their clinical significance in silico. The association between the expression of MEG3 and prognosis in NSCLC found using the Gene Expression Omnibus database showed that low expression of MEG3 experienced an unfavorable prognosis in NSCLC (Table 2).[64]

Although many lncRNAs, such as MALAT1, HOTAIR, H19, CCAT2, and so on, are associated with NSCLC prognosis, there is no clinical study showing that lncRNAs can be used in a practical judgment of the prognosis of NSCLC. The prognostic value of lncRNAs thus remains limited to preclinical research and has no clinical application value currently.

Discussion and perspectives

In this review, we highlighted the clinical application of lncRNAs, especially their potential application for diagnosis, therapy, and prognosis in NSCLC. In sum, lncRNAs are actively involved in multiple signaling pathways and have been regarded as important regulators of diverse cellular processes. Growing evidence suggests that they participate in various cellular processes, including cell growth, migration, stem cell maintenance, and apoptosis.[65] In the clinic, imaging diagnosis and hematological examinations are the most commonly used methods to detect NSCLC. However, the efficiency of traditional methods is unsatisfactory. Since the identification

Table 2 lncRNAs related to NSCLC prognosis

lncRNA	Function in NSCLC	Function	Reference
MALAT1	Promotes cell proliferation, migration, and invasion	Poor prognosis	55, 56
H19	Promotes cell growth	Poor prognosis	57
HOTAIR	Promotes cell proliferation, invasion, and metastasis	Poor prognosis	38, 58
CCAT2	Promotes cell proliferation and invasion	Poor prognosis	59
CARLo-5	Promotes cell proliferation, migration, and invasion	Poor prognosis	60
BANCR	Suppresses cell proliferation, induces apoptosis	Poor prognosis	61
PVT1	Promotes cell proliferation, migration, and invasion	Poor prognosis	62
ZXF1	Promotes cell invasion and metastasis	Poor prognosis	33
TUG1	Suppresses cell proliferation	Poor prognosis	63
PANDAR	Represses cell proliferation	Poor prognosis	
MEG3	Promotes cell invasion and metastasis	Poor prognosis	64

Abbreviation: NSCLC, non-small cell lung cancer.

of CEA in 1965, various studies have demonstrated its role in the follow-up of NSCLC patients.[66] Approximately 70% of NSCLC patients have CEA that is overexpressed in advanced stages.[67] CEA is useful for monitoring disease and detecting disease recurrence, metastasis, and prognosis.[68,69] However, the use of CEA is clinically controversial as a predictive and prognostic marker for lung cancer patients as its specificity is higher but its sensitivity is lower than other biomarkers in the diagnosis of NSCLC patients.[69]

For a long time, chemotherapy has been one of the major therapeutic modalities in oncology. Classical chemotherapy can block cellular proliferation, mainly by interrupting mitotic division and DNA synthesis. However, positive clinical outcomes using this approach have remained limited. Accordingly, with the significant advances in the genetic study of lncRNAs, novel methods should be explored for diagnosis, therapy, and prognosis, but potential clinical applications of lncRNAs are significant.

For example, lncRNAs are aberrantly expressed in cancers and exhibit stability in tissues and body fluids. Another advantage is that targeting lncRNAs have no side effect on normal tissues. Therefore, selectively targeting dysregulated lncRNAs could provide a new therapeutic strategy for NSCLC treatment. Over the last decade, many lncRNAs have served as diagnostic and prognostic biomarkers in their regulation of lung cancer. Lung tissue-specific genes should be explored to guide clinical practice. As reviewed in this paper, lncRNAs represent potentially noninvasive biomarkers in NSCLC diagnosis and prognosis.

Moreover, lncRNAs might serve as predictive biomarkers by representing the sensitivity of targeted oncology therapies. Based on the study of lncRNA biology, lncRNA-based therapies, such as ASOs, hold promising potential for developing effective personalized anticancer treatment strategies in lung cancer patients as well as for overcoming chemoresistance. However, we have to acknowledge that studies on lncRNAs still do not tell the whole mechanistic story. Even though some lncRNAs have been identified, there are still difficulties in applying them to the clinic. We must consider the standardization of sample preparation protocols, stability of lncRNA vectors in body fluids, developing a delivery system for adequate distribution and dosing lncRNA, and extraction methods, among other issues.

Researchers are paying more attention to the novel molecular biomarkers outlined above and are translating these achievements for use in the clinic. As biomedical research progresses, lncRNAs are believed to have the potential to have considerable clinical value in oncology.

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Disclosure

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