Long non-coding RNAs: Novel links in respiratory diseases (Review)

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Abstract. Long non-coding RNAs (lncRNAs) represent a surprisingly novel field in mammalian transcriptome research. With the development of RNA sequencing technology and computational methods, lncRNAs have been demonstrated to have important roles in biological processes at the epigenetic, transcription and post-transcriptional levels. In addition, the dysregulation of lncRNAs contributes to numerous diseases, including cancer and cardiovascular diseases. The present review discusses the important functions of lncRNAs in respiratory diseases, highlights the mechanistic roles which underlie lncRNAs in lung cancer as well as considers the current and future potential use of lncRNAs as novel biomarkers and therapeutic targets for the treatment of lung cancer.

Contents

1. Introduction

The central principle of molecular biology considers RNA as the intermediaries between DNA sequences and their encoded proteins (1). However, due to the vast amounts and variety of non-coding RNA transcripts uncovered by advances in RNA sequencing technology and computational methods, it has been elucidated that numerous non-coding (nc)RNA transcripts have important roles in a variety of biological processes. ncRNAs are conventionally divided into two major classes based on transcript size; small ncRNAs and long (l)ncRNAs (2). Small ncRNAs are represented by the well-documented miRNAs, which are ~22 nucleotides(nt) in length (2). By contrast, lncRNAs are messenger (m)RNA-like transcripts, which range in length from 200 nt to ~100 kilobases (kb) and lack significant open reading frames (2).

In the last decade, numerous studies have shown that lncRNAs were able to regulate gene expression at the levels of epigenetics, transcription and post-transcriptional processes (3) (Fig. 1). LncRNAs have been reported to mediate epigenetic changes by recruiting chromatin-modifying complexes to specific genomic loci or tumor cell-specific promoter regions (4). For example, Hox transcript antisense RNA (HOTAIR), which is derived from the HOXC locus, interacts with the polycomb repressive complex 2 (PRC2) in order to regulate their target genes in cancer (5); in addition, lncRNAs were shown to interact with transcription factors or act as transcriptional co-regulators in order to mediate the process of transcription. Furthermore, lncRNAs directly interact with RNA polymerase II in order to regulate transcription (3). LncRNAs have also been recognized as effective regulators of pre-mRNA splicing, mRNA decay and translation (6).

To date, several techniques have been used for discovery, identification and detection of lncRNAs. The predominantly used techniques include microarrays, RNA sequencing (RNA-seq), Northern blotting, reverse transcription quantitative polymerase chain reaction (RT-qPCR), *in situ* hybridization, bioinformatics prediction and target sequencing (Table I). Scientists have created databases providing comprehensive annotations of lncRNAs in order to fully elucidate the functions of lncRNAs in diseases and to identify potential lncRNAs which may be used as diagnostics, therapeutics and prognostic markers (Table II).

Of note, several human diseases have been demonstrated to be associated with mutated and dysregulated lncRNA expression, including numerous types of cancer (18-23), cardiovascular diseases (24-26) and neurological diseases (27-29). Candidate lncRNAs, including prostate

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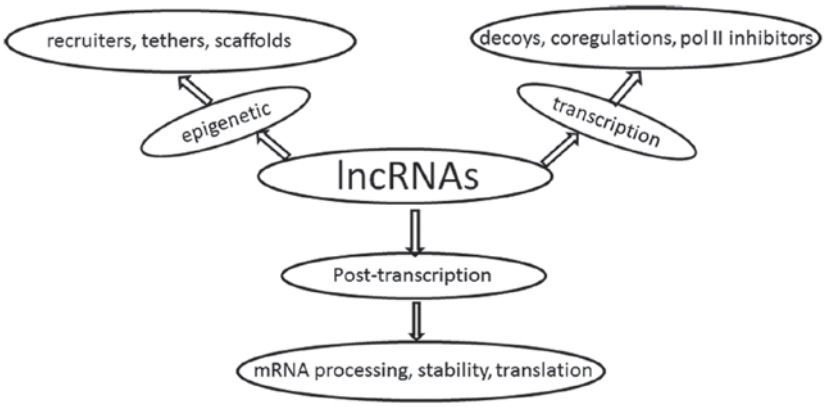

Figure 1. Roles of lncRNAs in the regulation of gene expression. Epigenetic function: Recruit or tether to the epigenome, or act as scaffolds for chromatin modification. Transcription function: Act as decoys for 'sponge' protein factors in order to suppress gene expression as well as act as transcription co-regulators or bind with pol II inhibitors in order to regulate transcription. Post-transcription function: Regulate the levels of mRNA processing, stability and translation. LncRNA, long non-coding RNA; Pol II, RNA polymerase II; mRNA, messenger RNA.

Table I. Methods for discovery, identification and detection of lncRNAs.

Method	Characteristics
Northern blotting	Verifies the existence of novel lncRNAs Low sensitivity, time consuming Requires relatively large amounts of total RNA
RT-qPCR	Validates the existence of novel lncRNAs with high sensitivity and specificity Cannot discover novel lncRNAs
In situ hybridization	Can locate lncRNA in tissue and cell compartments Low sensitivity and quantification
RNA sequencing	Can be employed for high throughput discovery of novel lncRNAs and transcriptome analysis Expensive; requirement of large number of data for analysis
Microarrays	Can be effective for high-throughput analysis of lncRNA expression Usually the results rely on the PCR
Bioinformatic prediction	Can give helpful information for further exploration and reduce experimental cost
Target sequencing	Combined multiplexed and target-specific amplification process with a high-throughput sequencing technology

lncRNA, long non-coding RNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

cancer-associated 3 and urothelial cancer-associated 1, have been regarded as potential biomarkers for the diagnosis of prostate and bladder cancer, respectively (21,30). The present review focuses on the emerging roles of lncRNAs in lung diseases.

2. LncRNA in lung development

Human lung development may be subdivided into five distinct stages: Embryonic, pseudoglandular, canalicular, terminal saccular and alveolar (31). During the first stage, the lung primordium develops from the foregut. Thereafter, the original lung buds further branch into a larger number of smaller areas. The canalicular stage is characterized by enlargement of the bronchi and vascularization of the lung tissue. Alveolar ducts and air sacs are established during the saccular phase. At the final stage, the terminal saccules, alveolar ducts and alveoli increase in number (31). It has been demonstrated that microRNAs have important roles during early and late lung development (32,33). However, the functions of lncRNAs in lung development remain to be elucidated. Alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV) is a rare, congenital lung development malformation, which results in pulmonary veins adjacent to small pulmonary arteries, medial thickening of small pulmonary arteries, deficient lobular development, insufficient alveolar wall capillaries and occasionally lymphangiectasis (34). ACD/MPV has been associated with Forkhead box protein F1 (FOXF1) on 16q24.1, which is predominantly expressed in mesenchymal tissues of the developing lungs (35,36). Szafranski *et al* (37) demonstrated that the loss of a small non-coding gene region at 16q24.1, including lncRNAs, led to the development of ACD/MPV.

Table II. Long non-coding RNA databases.

Name	Website	Reference
Non-coding RNA database	http://biobases.ibch.poznan.pl/ncRNA/	(7)
fRNAdb	http://www.ncrna.org/frnadb/	(8)
NRED	http://jsm-research.ibm.uq.edu.au/nred/cgi-bin/ncrnadb.pl	(9)
LncRNAdb	http://www.lncrnadb.org/	(10)
ncFANs	http://www.ebiomed.org/ncFANs/	(11)
NONCODE	http://www.noncode.org/NONCODERv3/	(12)
CHIPBase	http://deepbase.sysu.edu.cn/chipbase/	(13)
LNCipedia	http://www.lncipedia.org	(14)
DIANA-LncBase	http://www.microrna.gr/LncBase	(15)
LncRNADisease	http://cmbi.bjmu.edu.cn/lncrnadisease	(16)
lncRNome	http://genome.igib.res.in/lncRNome	(17)

Therefore it was proposed that the FOXF1 promoter was regulated by the interplay between chromatin looping, which may be mediated by lncRNAs and methylation-controlled glioma-associated oncogene family zinc finger 2 (GLI2) (37). Overall, previous studies have indicated that lncRNAs may be responsible for numerous disorders of human development.

3. LncRNA in lung inflammation

The immune system protects the body against organisms and foreign substances which may cause infections and diseases. The immune system is divided into the innate and adaptive immune systems (38). Inflammation is one of the first responses of the immune system to infection. LncRNAs have been demonstrated to have important roles in innate and adaptive immunity, including regulation of the differentiation of immune cell subsets and their immunological functions (39-46).

Activation of the innate immune system and pathological inflammation are the first steps in the protection of the human body against a vast number of microorganisms (47). The respiratory epithelial surface is exposed to an enormous number of foreign substances, including allergens and pathogens. Toll-like receptors (TLRs) are a type of pattern recognition receptor (PRR) (48). TLR signaling is known to be involved in pathogen recognition and the activation of innate immune cell responses following the invasion of microbes across physical barriers, including the skin and surfaces of other organs (49-50). Large intergenic non-coding RNA (lincRNA)-Cox2, which is induced by TLRs, interacts with various regulatory complexes, including heterogeneous nuclear ribonucleoproteins (hnRNP)-A/B and A2/B1, in order to regulate immune genes (39). Rapicavoli *et al* (40) reported that the expression of a pseudogene lncRNA called Lethe increased when tumor necrosis factor (TNF)-α activated the pro-inflammatory factory transcription factor nuclear factor (NF)-κB. In addition, Lethe interacts with the NF-κB subunit RelA in order to prevent DNA binding and reduce the expression of various inflammatory proteins (40). Innate immune system anti-viral host defense is mediated by type I interferon (IFN) induction and signaling machinery (51). IFN, produced primarily from dendritic cells, establishes an effective anti-viral state in cells. It was reported that signal transducer and activator of transcription factor 1 (STAT1) may be a key modulator of IFN signaling and have a key role in clearance of severe acute respiratory syndrome coronavirus (SARS-CoV) in the innate response (52). Peng *et al* (41) demonstrated the widespread differential expression of lncRNA in response to viral infections, which were found to be involved in innate immunity. These results were obtained through performing qPCR on lung samples from mice lacking the IFN (IFNAR-/-) or STAT1 (STAT1-/-), which were infected with SARS-CoV (41).

LncRNA expression has been identified to be involved during the development and differentiation of T cells. TMEVPG1, a novel lincRNA, was first identified using a positional cloning approach in Theiler's viral infection (42). Collier *et al* (43) reported that TMEVPG1 is a type 1 T-heper (Th1)-specific lincRNA which is regulated by STAT4 and T-box expressed in T cells (T-bet) and was found to be involved in the transcription of the gene encoding IFN-γ. A further study demonstrated that TMGVPG1 contributed to histone methylation at the Ifng locus in CD8+ T cells via interactions with WDR5 (44). In a study by Pang *et al* (45), hundreds of lncRNAs were found to be expressed in mammalian CD8+ T cells, several of which surrounded or overlapped with the expression of important protein-coding genes, which indicated their possible function as regulatory decoy genes. In addition, Hu *et al* (46) revealed highly dynamic and cell-specific expression patterns for lincRNAs during T cell differentiation. LincR-Ccr2-5'AS, a lincRNA regulated by GATA-3, was reported to be an important component in gene expression specific to the Th2 subset of T helper (Th) cells as well as the migration of Th2 cells (46).

4. LncRNA and cigarette smoke

Cigarette smoke is a significant risk factor for the development of lung diseases, including lung cancer, chronic obstructive pulmonary disease (COPD) and emphysema (53). Several lncRNAs have been demonstrated to be differentially expressed between smokers and non-smokers. One of these lncRNAs was

significantly increased in epithelia of smokers and was also associated with lung cancer (54). This lncRNA was therefore named smoke and cancer-associated lncRNA-1 (SCAL1). In addition, SCAL1 was found to be a key downstream mediator of NF erythroid 2-related factor 2 (Nrf-2) in the regulation of genes responsible for oxidative stress protection (54). Nrf-2 was demonstrated to be a transcription factor which protected against the cytotoxic effects of oxidative stress (55). Imprinted genes inherit a single allele, while the other allele is not or only weakly expressed. The H19 gene was reported to be highly expressed during embryonic development and strongly down-regulated in the majority of tissues following birth (56). H19 is one of the most highly conserved imprint genes, which has been shown to have important roles in normal development as well as oncogenesis (57,58). Kaplan *et al* (59) demonstrated that lncRNA H19 expression was significantly increased in the bronchial epithelial cells of smokers due to activation of a H19 single allele, rather than due to loss of imprinting (LOI). Furthermore, previous studies have shown that LOI of H19 was associated with lung cancer (60,61). Therefore, lncRNA H19 and SCAL1 may be potential biomarkers for the early diagnosis of lung cancer in smokers.

5. LncRNA and lung cancer

Lung cancer is the leading cause of cancer-associated mortality worldwide. To date, no early detection mechanisms have been elucidated and the current therapeutic strategies for lung cancer treatment are ineffective; as a result, the mortality rate of this disease is high. Recent studies have indicated that lncRNAs may have an important role in the development and progression of lung cancer (62,63). The primary lncRNAs which have been associated with lung cancer to date include metastasis-associated lung adenocarcinoma transcript (MALAT)-1, H19, growth arrest-specific gene 6 antisense RNA 1 (GAS-AS1), HOTAIR and MEG3.

Development of lung cancer. LOI refers to the loss of parental-origin-specific differential allele expression (64). LOI has been considered to be abundant and precocious in the development of human tumors (64). Overexpression of H19 has been observed in lung cancer with LOI of H19 (60,61). Barsyte-Lovejoy *et al* (65) demonstrated that the oncogene c-Myc bound to conserved E-boxes at the H19 promoter close to the imprinting control region and upregulated the expression of this lncRNA, which contributed to the tumorigenic phenotype of lung cancer cells. However, this study showed that the oncogene c-Myc did not affect the imprinting of H19, which remained to be monoallelic. In addition, the imprinted H19 lncRNA is a precursor of micRNA-675 (66), which has been shown to regulate the tumor suppressor retinoblastoma protein in order to induce tumorigenesis (67).

Epigenetics refers to the heritable changes in gene expression without permanent changes to the DNA sequence. These changes may include DNA methylation, histone modification and nucleosome positioning. Epigenetic alterations have been recognized to contribute to several pathological processes, including cancer (68). MEG3 is a tumor suppressor lncRNA gene; hypermethylation of the MEG3 promoter has been shown to contribute to the low expression of MEG3 in lung cancer (69). In addition, overexpression of MEG3 may induce reactivated p53 (69), which may indicate another potential mechanism of MEG3 in tumor suppression.

HOTAIR was proposed to be an oncogene due to its increased expression in several types of cancers, which was reported to promote invasion and metastasis (70-72). Type I collagen (Col-1), a type of interstitial extracellular matrix (ECM), was found to be abnormally enriched in the tumor microenvironment and promoted tumor activity (73). Zhuang *et al* (74) demonstrated that Col-1 induced the expression of HOTAIR in non-small-cell lung carcinoma (NSCLC) cells, which indicated that HOTAIR may contribute to the tumorigenesis of lung cancer.

LncRNA and lung cancer metastasis. MALAT-1 was first identified as a predictive marker for metastasis development in lung cancer (75); however, its role in metastasis remains to be elucidated. Tano *et al* (76) suggested that MALAT-1 promoted cell motility through transcriptional and post-transcriptional regulation of motility-associated gene expression. HOTAIR has been shown to have important roles in the metastasis of several types of human tumors, including lung cancer (77). HOTAIR was reported to interact with PRC2 and act as a co-repressor of silencing transcription factors in order to inhibit tumor metastasis-suppressor gene transcription, therefore increasing the risk of tumor metastasis (78).

LncRNA in the prognosis and treatment of lung cancer. A close association has been reported between high expression of MALAT1 and prognosis of lung cancer patients. Schmidt *et al* (79) demonstrated that the expression of MALAT-1 was associated with the prognosis of squamous cell carcinoma; however, it was independent of the prognosis of non-squamous cell carcinoma patients. In addition, downregulation of MALAT-1 may inhibit the metastasis and invasion of lung cancer cells (80). NSCLC patients with low expression of MEG3 were reported to have a poor prognosis (69). Therefore, MALAT-1 and MEG3 may be novel diagnostic prognostic markers for lung cancer. Growth arrest-specific 6 (GAS6) was shown to interact with the TAM (Axl, Tyro3/Sky and Mer) subfamily of receptor tyrosine kinases (81). GAS6 was found to be involved in biological processes, including proliferation, apoptosis and adhesion. In addition, lncRNA GAS6-AS1 expression was reported to be an independent risk factor for the overall survival and metastasis in NSCLC patients (82). Furthermore, lncRNA GAS6-AS1 was shown to be negatively correlated with GAS6 mRNA (82). These studies provided evidence to suggest that lncRNA GAS6-AS1 may be involved in NSCLC through regulating or interacting with its host gene GAS6.

Despite novel chemotherapeutic treatments and targeted drugs which have achieved great improvements in the treatment of lung cancer, the overall five-year survival rate of NSCLC has not improved (83). Chemoresistance is one of the most significant challenges for the successful treatment of lung cancer. In addition, the correlation of lncRNAs with chemoresistance has been demonstrated (84-86). HOTAIR was reported to contribute to cisplatin resistance in human lung adenocarcinoma cells through affecting apoptosis and cell cycle distribution via regulation of p21 expression (86). Studies have shown that lung adenocarcinoma cell resistance

to cisplatin was associated with Nrf-2 as well as its downstream genes (87,88). Thai *et al* (54) demonstrated that Nrf-2 activated the expression of SCAL1 through binding to the promoter, which suggested the possible role of Nrf-2 in lung cancer chemoresistance. Overall, these studies have indicated that lncRNAs may be potential drug targets for increasing the effectiveness of lung cancer treatment.

6. LncRNA and pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a disease with numerous pathological and physiological factors, which has a poor prognosis and ineffective treatment options. PAH is characterized by increasing pulmonary artery pressure and elevated pulmonary vascular resistance, leading to right heart failure (89). Studies have revealed the complex nature of the disorder, including inflammation, hypoxia, dysregulated pulmonary endothelial cell proliferation and gene mutations (89-91).

The renin-angiotensin system (RAS) has been shown to cause endothelial dysfunction and vascular remodeling during the development of PAH (92). RAS is primarily composed of angiotensin-converting enzyme (ACE), angiotensin II (Ang II) and angiotensin II type 1 receptor (AT1R) (93). A recent study has identified a novel lncRNA, Lnc-Ang 362, which is differentially expressed in the response of vascular smooth muscle cells (VSMC) to Ang II; in addition, this novel lncRNA, as a host transcript for miR-221 and miR-222, was shown to have a crucial role in cell proliferation (94). Furthermore, these two microRNAs were previously reported to be associated with VSMC proliferation and the regulation of Ang II in endothelial cells (95,96).

Inflammation may be another important factor which contributes to PAH due to the release of cytokines, chemokines and various growth factors that may result in cell proliferation. LncRNAs have been demonstrated to be involved in the regulation of inflammation and therefore may have an impact on the pathogenesis of PAH.

7. LncRNA and lung fibrosis

Idiopathic pulmonary fibrosis (IPF) is defined as chronic, progressive fibrotic interstitial pneumonia without a known cause. Until recently there were no effective drug therapies for the treatment of IPF. The disease is characterized by the expansion of activated mesenchymal cells and alveolar epithelial cell injury leading to excessive ECM protein deposition in the basement membrane and impaired gas exchange (97). Cao *et al* (98) established a model of bleomycin-induced lung fibrosis, in which they detected 568 differentially expressed lncRNAs in the bleomycin-treated lung samples compared with those in the normal control group through microarray analysis. In addition, levels of lncRNA AJ005396 and lncRNA S69206 were found to be significantly increased compared with those in the control animals (98).

Studies on lncRNAs associated with the pathogenesis of IPF are limited; however, the detection of mutated and dysregulated lncRNAs may elucidate potential molecular targets for the treatment of lung fibrosis, as lncRNAs have been demonstrated to have important roles in disease pathogenesis.

8. Conclusion

In conclusion, ncRNAs were previously thought of as 'noise'; however, following decades of research, evidence has been provided for the biological functions of ncRNA transcripts. In recent years, the functions of miRNAs in disease have been well documented; however, compared with that of studies into the dysregulation of miRNAs, current knowledge of the role of lncRNAs in disease is still the tip of the iceberg, as only a small portion of lncRNA functions have been elucidated. The present review described the involvement of lncRNAs in respiratory diseases, with a specific focus on lung cancer, as previous studies have demonstrated that lncRNAs have key regulatory roles in the pathogenesis of cancer. An in-depth understanding of the biological functions of lncRNAs and how they interact with other ncRNAs as well as target genes in lung cancer may elucidate novel biomarkers and therapeutic targets for the early diagnosis and treatment of lung cancer.

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