Emerging role of long non-coding RNAs in pulmonary hypertension and their molecular mechanisms (Review)

YUHAN QIN[1], GAOLIANG YAN[2], YONG QIAO[2], DONG WANG[2], ERFEI LUO[1], JIANTONG HOU[1] and CHENGCHUN TANG[2]

[1]Department of Cardiology, Medical School of Southeast University; [2]Department of Cardiology, Zhongda Hospital Affiliated to Southeast University, Nanjing, Jiangsu 210009, P.R. China

Received January 7, 2020; Accepted August 19, 2020

DOI: 10.3892/etm.2020.9293

Abstract. Pulmonary hypertension (PH) is a life-threatening cardiopulmonary condition caused by several pathogenic factors. All types of PH are characterized by the excessive proliferation of pulmonary artery endothelial cells and pulmonary artery smooth muscle cells, apoptosis resistance, pulmonary vascular remodeling, sustained elevated pulmonary arterial pressure, right heart failure and even death. Over the past decade, next generation sequencing, particularly RNA-sequencing, has identified some long non-coding RNAs (lncRNAs) that may act as regulators of cell differentiation, proliferation and apoptosis. Studies have shown that lncRNAs are closely associated with the development of several diseases, including cardiovascular diseases. In addition, a number of studies have reported that lncRNAs, including maternally expressed gene 3, metastasis-associated lung adenocarcinoma transcript 1, taurine upregulated 1 and cancer susceptibility candidate 2, serve important roles in the pathogenesis of PH. Despite the development of novel drug treatments, the mortality rate of PH remains high with no evident downward trend. Therefore, certain lncRNAs may be considered as therapeutic targets for the treatment of incurable PH. The present review summarizes the latest research on lncRNAs and PH, aiming to briefly describe PH-associated lncRNAs and their mechanisms of action.

Contents

Correspondence to: Professor Chengchun Tang, Department of Cardiology, Zhongda Hospital Affiliated to Southeast University, 87 Dingjiaqiao, Nanjing, Jiangsu 210009, P.R. China
E-mail: tangchengchun@hotmail.com

Key words: long non-coding RNAs, pulmonary hypertension, mechanism, review

1. Introduction

Pulmonary hypertension (PH). PH is a condition with relatively high morbidity and an annual global incidence of 3-10 cases per 1 million adults. In individuals >65 years of age the prevalence of PH is estimated to be as high as ~10%. The prognosis of PH is poor and the 5-year survival rate in newly diagnosed patients is only 57% (1,2). The main symptoms of PH include progressive dyspnea during exercise, fatigue, including chest pain and syncope (3). However, patients with PH do not usually exhibit specific symptoms, which may result in delays in diagnosis, ranging from several months to years (4).

Regarding the pathogenesis of the disease, PH is classified into the following five categories (4): i) pulmonary arterial hypertension (PAH); ii) left heart disease-induced PH; iii) hypoxia- or lung disease-induced PH; iv) chronic thromboembolic PH (CTEPH); and v) PH caused by unclear or multifactorial mechanisms. The pathogenesis of PH is very complex, and includes impaired angiogenesis, metabolic disorders, chronic inflammation, abnormal proliferation, and the resistance of pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs) to apoptosis (5). The abnormal proliferation of VSMCs is considered to be the main cause of vascular remodeling. Under hypoxic conditions, abnormal PASMC proliferation promotes thickening of the media layer, pulmonary vasoconstriction and pulmonary vascular remodeling, which together result in sustained increased pulmonary artery pressure and right ventricular hypertrophy (6). Although several molecules and signaling pathways, including bone morphogenetic protein receptor type 2, platelet-derived growth factor (PDGF), Rho/Rho-associated coiled-coil containing protein kinase, serotonin, endothelin, nitric oxide and NADPH oxidase, have been found to be involved in the pathogenesis of PH, others may as yet be unidentified (7-9).

Despite significant progress in understanding the basic mechanisms of PH and the reduction in the number of PH-associated hospitalizations that has occurred over the past few decades (10), PH remains an incurable disease with high treatment costs. In addition, an upward trend in hospitalization time with no significant decrease in mortality has been reported (11). Therefore, further clarification of

the pathophysiological mechanisms of PH is important for understanding the disease and developing novel treatments.

Long non-coding RNAs (lncRNAs). Non-coding RNAs (ncRNAs) were originally considered as 'transcriptional noise'. However, in the past few decades, the roles of ncRNAs, including microRNAs (miRNAs) and lncRNAs, in normal physiology and disease pathology have been widely investigated, and ncRNAs have been reported to participate in cell homeostasis and disease processes (12). Several studies have confirmed the importance of ncRNAs as transcriptional regulators and further explored their potential molecular mechanisms, thereby demonstrating the vital role of ncRNAs in several biological processes (13). The development of next-generation sequencing, particularly RNA-sequencing (RNA-seq), has led to the discovery of numerous lncRNAs for which biological functions have been identified (14). There is considerable evidence of associations between lncRNAs and the onset of diverse human diseases, including cancer, cardiovascular and neurodegenerative diseases. Abnormal and uncontrolled cell proliferation and migration, as well as an imbalance between cell growth and apoptosis are characteristic pathological changes of PH. Several studies on the roles of lncRNAs and their mechanisms of action in the regulation of PH pathogenesis have indicated that lncRNAs act as key regulators in the aforementioned biological changes.

lncRNAs are a class of ncRNAs >200 nucleotides in length that are synthesized by RNA polymerase II and subjected to post-transcriptional processing, including capping, splicing and polyadenylation. The majority of lncRNAs are located in the cell nucleus, expressed at low levels and show poor sequence conservation (15). lncRNAs serve an important epigenetic role by acting as activators or repressors of gene transcription and mRNA translation, RNA stabilizers, miRNA precursors and sponges (16). In addition, the interaction of lncRNAs with RNA, DNA or proteins may promote or inhibit protein-coding gene expression (17).

It is now recognized that lncRNAs regulate cell differentiation and apoptosis, chromatin remodeling and carcinogenesis at transcriptional and post-transcriptional levels (18,19). In addition, lncRNAs are closely associated with hypoxic diseases, and their abnormal expression has been associated with different types of cancer and cardiovascular diseases (20-22). For example, it has been reported that the lncRNAs Nkx2.1-associated non-coding intergenic RNA (NANCI) and growth arrest-specific transcript 5 (GAS5) are associated with lung diseases (23,24). PH and cancer share some common features, such as excessive cell proliferation and apoptosis resistance (25), and it has been suggested that lncRNAs participate in the proliferation, apoptosis and cell cycle of PASMCs and PAECs in PH (26). PAECs, PASMCs and inflammatory cells contain lncRNAs, some of which serve important roles in the pathological process of PH and are of great significance in its occurrence and development. Abnormal levels of lncRNAs in the blood have been suggested to be a novel diagnostic marker for PH.

lncRNAs and miRNAs. miRNAs are single stranded RNA molecules that are ~22 nucleotides in length, and so are shorter than lncRNAs. miRNAs are derived from primary miRNAs, and when mature are mainly located in the cytoplasm. By contrast, lncRNAs are derived from different cells and are mainly localized in the cell nucleus (15).

miRNAs inhibit the translation of mRNA or promote its degradation to silence downstream gene expression through binding to the 3'untranslated region of the mRNA (27). Regarding the pathological mechanisms of PH, lncRNAs mainly act as miRNA sponges (28). Each miRNA has been reported to regulate the expression of >100 mRNAs (29). Studies using animal models of PH have shown that miRNA mimics or inhibitors may delay pulmonary vascular remodeling to some extent and exhibit therapeutic effects by reducing pulmonary artery pressure and ameliorating right heart failure (30,31). In addition, clinical studies have identified a variety of differentially expressed miRNAs in the peripheral blood of patients with PH, suggesting the diagnostic and prognostic value of miRNAs in PH (32). lncRNAs interact with miRNAs to regulate the expression and biological activities of the miRNA, and also act as competitive endogenous RNAs by binding to miRNA, thereby inhibiting the ability of the miRNA to bind to its target genes (33). Thus, miRNAs are regulated by lncRNAs. These two types of RNA molecule complement and interact with each other to form a complex network of interactions in which lncRNAs act as miRNA sponges, some miRNAs are derived from lncRNAs while others degrade them, and lncRNAs and miRNAs both bind to mRNAs, indicating a competitive relationship between them (19,34).

2. lncRNAs and PH

Numerous studies have indicated that lncRNAs have essential roles in the occurrence and development of PH. Therefore, the present review summarizes the characteristics of lncRNAs that have been identified to be involved in the pathogenesis of PH.

Maternally expressed gene 3 (MEG3). The lncRNA MEG3 is located on human chromosome 14q32 and is 1.6 kb in length. It acts as a tumor suppressor and has a vital role in several types of cancer, including breast, liver, gastrointestinal and lung cancer (35-37). It has been reported that the downregulation of MEG3 increases the sensitivity of lung cancer to cisplatin treatment (38,39). MEG3 also serves a key role in cardiovascular diseases and is involved in hypoxia, abnormally expressed in cardiac fibroblasts and downregulated during late cardiac remodeling (40). Furthermore, elevated MEG3 levels have been detected in vascular endothelial cells (41).

A study conducted by Piccoli *et al* (40) demonstrated that MEG3 was mainly localized in the cytoplasm of hypoxic PASMCs, and expressed at significantly increased levels in a hypoxia-induced animal model of PH and PASMCs from idiopathic PH patients. The increased expression of MEG3 was further confirmed in hypoxia-induced human and mouse PASMC cell lines. Furthermore, fluorescence *in situ* hybridization analysis revealed that MEG3 was primarily localized in PASMCs and the media layer of the vascular wall, and translocated to the cytoplasm when exposed to hypoxia. Additionally, the authors suggested that MEG3 associated with miR-328-3p to regulate multiple targets, particularly

insulin-like growth factor 1 receptor (IGF1R), the upregulation of which further induced PASMC proliferation and pulmonary vascular remodeling (42). However, Zhu *et al* (43) demonstrated that MEG3 was downregulated in hypoxia-induced human PASMCs (HPASMCs), and that the downregulation of MEG3 significantly promoted the proliferation and migration of PASMCs under both normal and hypoxic conditions. The underlying mechanism of MEG3 was suggested to involve a MEG3/miR-21/PTEN axis. Similarly, a study by Sun *et al* (44) detected the downregulation of MEG3 in pulmonary arteries derived from PH patients and demonstrated that MEG3 promoted PASMC proliferation and migration via the p53 pathway.

In an animal model, experiments using a lung-specific small interfering (siRNA)-loaded liposomal delivery system demonstrated that hypoxic PH was significantly ameliorated when MEG3 was knocked down, as the right ventricle systolic pressure, right ventricular hypertrophy index and pulmonary artery pressure were relieved. These findings indicate the potential of MEG3 as a novel therapeutic target for the pharmaceutical treatment of PH (42).

Notably, the findings regarding MEG3 up- or downregulation in PH are not consistent in the aforementioned studies. This may be associated with differences in the distribution of MEG3 in cells, the time of measurement and study objects. Further research is needed to clarify the role of MEG3 in PH.

Hoxa cluster antisense RNA 3 (HOXA-AS3). The HOX gene cluster is a group of highly homologous transcription factors (45). It has been reported that members of the HOXA cluster, namely HOTAIR and HOTTIP, regulate the proliferation of lung cancer cells (46). A study by Zhang *et al* (47) demonstrated that Hoxaas3, a member of the HOX gene cluster, was upregulated in hypoxia- and monocrotaline (MCT)-induced PH models as well as in PASMCs isolated from patients with idiopathic PH. Furthermore, the knockdown of Hoxaas3 reduced the expression of proliferating cell nuclear antigen (PCNA), Ki-67 and cyclins A, D and E in PASMCs, suggesting that Hoxaas3 promotes PASMC proliferation and accelerates the cell cycle. In addition, histone acetyltransferase p300 inhibitors downregulated Hoxaas3 expression under hypoxic conditions, suggesting histone acetyltransferase was involved in Hoxaas3 upregulation under hypoxia. Another study demonstrated that HOX genes are overexpressed in the lung tissues of patients with PH, suggesting that they may have an important role in endothelial cell proliferation and vascular remodeling (48). These studies provide new data that improve our understanding of the role of Hoxaas3 lncRNA in PASMC proliferation. However, further research is required to confirm the role of Hoxaas3 in patients with different clinical subtypes of PH.

MANTIS. In a study by Leisegang *et al* (49), exon array analyses were performed to investigate the effect of histone demethylase JARID1B knockdown on endothelial RNA expression. The expression levels of several lncRNAs were found to be significantly altered, including MANTIS which was significantly downregulated. The study also revealed that MANTIS lncRNA was downregulated in the lung tissues of patients with end-stage idiopathic PH and in MCT-induced rat models of PH, in which endothelial dysfunction and apoptosis serve important roles (49). MANTIS lncRNA has also been reported to be overexpressed during the regression of atherosclerosis in high-fat feeding monkeys restored to a normal diet (50). These findings indicate a novel and potent epigenetic regulatory mechanism for MANTIS in endothelial cells.

Taurine upregulated gene 1 (TUG1). TUG1 is a 7.1-kb lncRNA that is expressed in the cytoplasm and nucleus of PASMCs (51). Studies have shown that TUG1 is involved in the regulation of protein and miRNA expression, serves an important role in the regulation of cell proliferation, apoptosis and chromatin remodeling, and is associated with hypoxia (52-54).

TUG1 is a tumor suppressor gene that is highly conserved in mammals (55). Two studies have demonstrated that TUG1 is upregulated in the pulmonary arteries of hypoxic PH mice and in hypoxic PASMCs, where it leads to pulmonary vascular remodeling via the regulation of PASMC proliferation and apoptosis. In one study, Yang *et al* (56) reported that TUG1 knockdown downregulated Foxc1 expression through a TUG1/miR-374c/Foxc1/Notch pathway. Furthermore, TUG1 knockdown inhibited the proliferation and migration of HPASMCs, promoted apoptosis and regulated pulmonary vascular remodeling via the regulation hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor expression (57). In the other study, Wang *et al* (58) showed that TUG1 regulated the proliferation and cell cycle of HPASMCs by directly binding to miR-328. However, whether TUG1 also regulates PH via other mechanisms, such as phenotypic switch, endothelial-to-mesenchymal transition (EndMT), immunological dysregulation and inflammatory responses remains to be further clarified.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1). MALAT1 is a highly conserved lncRNA involved in the pathogenesis of several types of cancer, including prostate, gastric and non-small cell lung cancer (59,60). The expression of MALAT1 is significantly increased in hypoxia-induced human endothelial cells and is involved in the phenotype switch of endothelial cells (41).

Brock *et al* (61) first reported that MALAT1 expression was significantly increased in hypoxic HPASMCs and the lung tissues of mice with hypoxia-induced PH. They also demonstrated that MALAT1 promoted the proliferation and migration of PASMCs, and regulated their phenotype. Furthermore, MALAT1 knockdown was indicated to inhibit PASMC proliferation *in vitro* and cardiac hypertrophy in the mouse model via upregulation of the expression of cyclin-dependent kinase inhibitors.

In addition to its role in PASMCs, MALAT1 also serves a vital role in PAECs. In one study, the downregulation of MALAT1 increased the migration of human umbilical vein endothelial cells (HUVECs) and slightly upregulated their apoptosis under hypoxic conditions. A microarray analysis performed to investigate the mechanism of MALAT1 indicated that cell cycle inhibitory genes were significantly increased in the HUVECs transfected with MALAT1 siRNA, suggesting that MALAT1 is involved in regulation of the cell cycle (41). MALAT1 has also been proposed to regulate the phenotypic transition of endothelial cells (62).

A previous study has confirmed that MALAT1 binds to hsa-miR-124-3p and the latter directly targets kruppel-like factor 5 (63). Zhuo *et al* (64) conducted a case-control study involving 587 patients with PH and 736 healthy individuals. The results showed that the MALAT1 gene rs619586 A>G polymorphism was significantly associated with increased PH risk. The study further demonstrated that MALAT1 acts as a competitive endogenous RNA for miR-124 and thereby regulates X-box binding protein 1 expression and participates in the pathogenesis of PH. Furthermore, MALAT1 was significantly increased in the pulmonary artery tissues and PASMCs of patients with PH (63). The knockdown of MALAT1 significantly decreased the proliferation and migration of PASMCs, and the expression levels of PCNA and cyclin in HPASMCs, suggesting that the mechanism of MALAT1 in PH involves modulation of the proliferation, migration and cell cycle of PASMCs. Furthermore, bioinformatics analysis and luciferase reporter assays confirmed that hsa-miR-124-3p.1 was a downstream target of MALAT1.

In summary, there is considerable evidence that MALAT1 contributes to PH. MALAT1 is significantly differently expressed in hypoxia-induced PAECs, PASMCs, hypoxia-induced animal models and pulmonary arterial tissues isolated from patients with PH. MALAT1 gene polymorphism is also significantly associated with the risk of PH.

CPS1-intronic transcript 1 (CPS1-IT1). It has been documented that CPS1-IT1 lncRNA is involved in the pathogenesis of lung cancer and other malignancies. In one study, the overexpression of CPS1-IT1 in lung cancer cell lines restrained cell proliferation and migration, and induced cell apoptosis, indicating that CPS1-IT1 has protective effects on lung cancer. Therefore, it was suggested that CPS1-IT1 may be used for the early diagnosis of lung cancer and could be applied as a targeted therapy (65). With regard to PH, a study found that CPS1-IT expression levels were decreased in the pulmonary tissues of rats with obstructive sleep apnea-induced PH. In addition, the overexpression of CPS1-IT1 significantly alleviated pulmonary arterial remodeling in the rat model. Analysis of the mechanism of action *in vitro* indicated that CPS1-IT overexpression attenuated PH by downregulating interleukin (IL)-1β via the inhibition of the nuclear factor κB (NF-κB) signaling pathway and HIF-1 transcriptional activity (66). However, further *in vitro* studies are required to clarify the cells in which CPS1-IT1 exerts biological functions, and research of CPS1-IT1 in patients with PH is also necessary.

Cancer susceptibility candidate 2 (CASC2). The lncRNA CASC2 is a tumor suppressor gene, located on human chromosome 10 (67). CASC2 is closely associated with PH. CASC2 upregulation has been shown to inhibit cell proliferation and migration, promote apoptosis and ultimately suppress pulmonary artery remodeling. The contractile-to-synthetic phenotypic switching of PASMCs is vitally important for the biological processes associated with hypertension, atherosclerosis, PH and other cardiovascular diseases (68). The upregulation of CASC2 has been demonstrated to reverse the phenotypic transition of PASMCs, thereby delaying the progression of PH (25). The specific mechanism of CASC2 in the regulation of PH, however, requires further exploration.

TCONS_00034812. Liu *et al* (26) performed a microarray analysis on the pulmonary arteries of rats with PH and found that the expression of TCONS_00034812 was 8.7881-fold higher in the control rats compared with the rats with PH. Consistent with this, reverse transcription-quantitative PCR (RT-qPCR) demonstrated that TCONS_00034812 expression levels were 6.1-fold higher in the controls compared with the rats with PH. In addition, TCONS_00034812 knockdown was demonstrated to promote the proliferation of PASMCs and inhibit their apoptosis. Gene Ontology (GO) analysis was also performed to identify potential target genes of TCONS_00034812. The results indicated that storkhead box 1 (Stox1), Met and neurotrophin 3 were upregulated in PH and have an association with cell proliferation. The direct targeting of Stox1, a transcription factor of the expanded forkhead box gene family, by TCONS_00034812 was confirmed by RT-qPCR analysis.

The mitogen-activated protein kinase (MAPK) signaling pathway is important for cell survival and apoptosis (69) and is involved in pulmonary vascular remodeling (70). In PASMCs, TCONS_00034812 silencing activates the MAPK signaling pathway and thereby increases Stox1 expression, which in turn regulates PASMC proliferation and apoptosis, resulting in PH. Therefore, TCONS_00034812 may be a potential novel therapeutic target for PH (26).

H19. It is well documented that H19 serves a key role in tumorigenesis and metastasis (71,72). In addition, H19 is involved in pathophysiological processes associated with hypoxia (73). A study demonstrated that the expression levels of H19 were 5-fold higher in the lung tissues of rats with MCT-induced PH compared with those in healthy rats. H19 levels were also significantly increased in the serum of the MCT-induced PH rats. The stimulation of PASMCs with different concentrations of IL-1β and PDGF-BB significantly increased H19 expression in a dose-dependent manner. Furthermore, H19 upregulated angiotensin II (Ang II) type 1 receptor expression by sponging the miRNA let-7b to regulate PASMC proliferation, resulting in PH. The knockout of H19 was demonstrated to protect mice from MCT-induced pulmonary vascular remodeling and alleviate PH (74).

Melatonin has been indicated to have a therapeutic effect on PH, and may act via antiproliferative effects on PASMCs and anti-inflammatory activity (75). Wang *et al* (76) suggested that H19 plays a significant role in the pathological process of PH through the miR-675-3p/IGF1R and miR-200a/programmed cell death 4 (PDCD4) signaling pathways. In a rat model of MCT-induced PH, melatonin treatment upregulated the expression of H19 and miR-675-3p and downregulated that of miR-200a, resulting in increased PDCD4 and decreased IGFR1 expression. The differential expression levels of PDCD4 and IGFR1 ultimately led to the apoptosis of PASMCs and inhibition of their proliferation, resulting in reduced vascular remodeling and PH.

Urothelial carcinoma associated 1 (UCA1). There is evidence to suggest that the lncRNA UCA1 is involved in the pathogenesis

of lung cancer (77,78). In addition, it has been reported that UCA1 is highly expressed in hypoxia-induced HPASMCs and promotes cell proliferation under hypoxic conditions (79). By contrast, inhibitor of growth family 5 (ING5) promotes tumor cell apoptosis and inhibits tumor growth, thus playing a protective role in tumor development (80). ING5 also inhibits the proliferation and promotes the apoptosis of PASMCs. However, UCA1 competes with ING5 for binding to heterogeneous ribonucleoprotein I (HnRNP I), which contains an RNA-binding domain with mRNA splicing activity (61), thus decreasing ING5-HnRNP I binding and promoting PASMC proliferation, pulmonary vascular remodeling and ultimately PH (79). Elevated UCA1 might aid in the diagnosis of PH, and targeting UCA1 may provide novel therapeutic approaches.

Lnc-Ang362. Lnc-Ang362 was first identified as a lncRNA that is differentially upregulated in Ang II-induced VSMCs. It regulates cell proliferation and the expression of miR-221 and miR-222 (81). In another study, lnc-Ang362 was found to be abnormally elevated in the lung tissues of patients with PH. Furthermore, the overexpression of lnc-Ang362 promoted HPASMC proliferation and migration, reduced apoptosis and was involved in the pathological process of PH. In addition, lnc-Ang362 upregulated miR-221 and miR-222, thereby activating the NF-κB signaling pathway (82). However, the expression of lnc-Ang362 in PASMCs and PAECs induced by hypoxia requires further clarification.

lncRNA regulated by PDGF and transforming growth factor β (LnRPT). PDGF plays an important role in PASMC hyperproliferation and pulmonary vascular remodeling (83). Using RNA sequencing, 95 differentially expressed lncRNAs were identified in PDGF-BB-induced rat PASMCs. These included LnRPT, the expression of which was significantly lower in rat PASMCs following PDGF-BB treatment compared with that in the control PASMCs. Furthermore, LnRPT knockdown promoted PASMC proliferation (84). These results suggest that LnRPT participates in PH pathogenesis by inhibiting the proliferation of PASMCs.

The Notch receptor is a cell surface receptor responsible for cell signaling between adjacent cells (85). The Notch signaling pathway is also involved in the development of PH (86). In one study, RNA sequencing and RT-qPCR assays demonstrated that the downregulation of LnRPT increased the expression of two important Notch signaling pathway genes, namely notch3 and jag1, and that inhibition of the Notch signaling pathway attenuated, to some extent, the proliferation of PASMCs. These findings indicate that LnRPT regulates the Notch signaling pathway and that PDGF-BB participates in PH by affecting the expression of LnRPT. Furthermore, inhibition of phosphoinositide 3-kinase (PI3K) diminished the PDGF-BB-induced downregulation of LnRPT, indicating that a PDGF-BB/PI3K/LnRPT pathway participates in the pathological process of PH (84). However, the involvement of LnRPT in apoptosis and PAECs, and in animals and patients with PH require further investigation.

NONRATT015587.2. In addition to hypoglycemic effects, it has been reported that metformin has a vascular protective activity and may delay cell senescence (87). Studies have shown that metformin reverses hypoxia and has therapeutic effects in MCT-induced PH (87,88). To elucidate the specific mechanisms of metformin, microarray analyses were performed to analyze the differential expression of lncRNAs and mRNAs. The results showed that NORNATT015587.2 was significantly increased in hypoxia-induced PASMCs. It was also reported that NORNATT015587.2 promotes PASMC proliferation, inhibits apoptosis and affects pulmonary vascular remodeling. In addition, GO enrichment analysis, Kyoto Encyclopedia of Genes and Genomes pathway analysis and western blot assays verified that p53 and HIF-1 participated in the NORNATT015587-induced vascular remodeling (89). *In vivo* experiments and clinical research are required to verify whether NONRATT015587.2 is involved in PH.

Although RNA sequencing technology has identified large numbers of lncRNAs, and lncRNA has been indicated to perform key epigenetic modifications in PH, the role of lncRNA in PH requires further elucidation. The lncRNAs involved in the pathological process of PH reviewed in this article mainly exert an influence through regulating the proliferation, migration and apoptosis of PAECs and PASMCs. A series of studies have shown that lncRNA can be involved in regulation of the phenotype switch of smooth muscle cells, which contributes to cell migration and proliferation (90). EndMT is involved in the occurrence and development of a variety of cardiovascular diseases, including PH. Three specific lncRNAs have been found to be associated with EndMT: MALAT1, GATA6 antisense RNA and H19 (91). Immunological and inflammatory disorders also play an important role. The activation and release of a variety of cellular inflammatory factors are involved in PH (92), but the roles of lncRNA in the regulation of the vascular inflammation associated with PH remain unclear. Therefore, in the future, researchers may investigate whether lncRNAs participate in the regulation of PH through phenotypic switch, EndMT and immunological effects and the specific mechanisms.

Translational potential of the reviewed lncRNAs. Numerous studies have shown that some ncRNAs, including lncRNA, have the potential to translate into proteins (93,94). Methodologies combining ribosome profiling and scoring schemes have been developed for the evaluation of lncRNA translation. When we used the Coding Potential Assessment Tool 2.0.0 (http://lilab.research.bcm.edu/cpat/index.php) to determine whether or not specific lncRNAs have translational potential, the results indicated that only MANTIS and H19 have translational potential.

lncRNA and PH with different etiologies. PH can be divided into five categories according to its etiologies, as briefly summarized in the Introduction. Table I shows the underlying causes for each category (95). Basic research on lncRNA and PH is mostly based on hypoxia-induced cell or animal models. However, only data obtained on lncRNAs identified from research using tissues from patients with PH are summarized in Table I. The study populations were all patients with PAH and CTEPH. No studies on lncRNAs in patients with the other three classes of PH were identified, which may be due to the small number of patients with these PH types. There have been few clinical studies on lncRNAs

Table I. Underlying causes of PH and their associated lncRNAs.

Classification	Underlying cause	Associated lncRNA	(Refs.)
1.	Pulmonary arterial hypertension	PAXIP1-AS1, MEG3, HypERlnc, MALAT1, lncRNA-Ang362	(42,44,63,64,82,100,101)
2.	PH due to left heart disease		
3.	PH due to lung diseases and/or hypoxia		
4.	Chronic thromboembolic PH	CTEPH1, NR_036693, NR_027783, NR_033766, NR_001284, PAFAH1B1, lncRNA NONHSAT073641	(102-104)
5.	PH with unclear multifactorial mechanisms		

PH, pulmonary hypertension; lncRNA, long non-coding RNA; PAXIP1-AS1, PAX-interacting protein 1- antisense RNA1; MEG 3, maternally expressed 3; HypERlnc, hypoxia-induced endoplasmic reticulum stress regulating long non-coding RNAs; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; CTEPH1, chronic thromboembolic pulmonary hypertension 1; PAFAH1B1, platelet-activating factor acetyl hydrolase 1B1.

Table II. Involvement of lncRNAs in each pathological stage of pulmonary hypertension.

Pathological stage	lncRNA	(Refs.)
PAEC dysregulation and EndMT	NR_001284, NR_036693, NR_033766, NR_027783, MIR22HG, MIR210HG, H19, MEG9, MALAT1, GATA6-AS	(103,105-108)
PASMC proliferation and migration	H19, MALAT1, lnc-Ang362, PAXIP1-AS1, TUG1, HOXA-AS3, MEG3, LnRPT, CASC2	(25,47,56,74,82,84,100,109)
PASMC apoptosis	TCONS_00034812, UCA1	(26,79)
Adventitial thickening	-	-
Plexiform lesions	-	-
Perivascular inflammation	H19	(74)

lncRNA, long non-coding RNA; PAEC, pulmonary artery endothelial cell; EndMT, endothelial-to-mesenchymal transition; PASMC, pulmonary artery smooth muscle cell; MEG9,3, maternally expressed 9,3; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; GATA6-AS, GATA6 antisense RNA 1; PAXIP1-AS1, PAXIP1 antisense RNA 1; TUG1, taurine upregulated 1; HOXA-AS3, HOXA cluster antisense RNA 3; LnRPT, lncRNA regulated by PDGF and transforming growth factor β; CASC2, cancer susceptibility candidate 2; UCA1, urothelial carcinoma associated 1.

in patients with PH, and the included populations are all patients with PAH (64,96,97).

lncRNAs and the pathological stages of PH. The pathological process of PH includes the following stages: PAEC dysregulation, EndMT, PASMC proliferation, migration and apoptosis, adventitial thickening of lexiform lesions and perivascular inflammation. At present, research on the pathological stages of PH in association with lncRNA expression has mainly focused on PASMC proliferation, migration and apoptosis, and PAEC dysfunction. However, no studies have been conducted on the association of lncRNAs with the adventitial thickness of plexiform lesions. The lncRNAs associated with each pathological stage are listed in Table II.

Therapeutic targets for lncRNAs in PH. lncRNAs have the potential to be used in combination with other PH-treating drugs such as endothelin receptor antagonists, PDE-5 inhibitors and prostacyclin analogs as novel treatments for PH; however, no studies have yet been performed to investigate this. The use of a combination of lncRNA and PH-treating drugs has the following theoretical basis: lncRNAs are stably expressed in the circulation and have the potential to be used as biomarkers, which is of great significance for the treatment of PH and judgement of the curative effect. Taking into account the tissue specificity of lncRNA, lncRNA-based therapies should be tissue- and dose-specific interventions. However, as of yet no clinical research has been conducted on targeted lncRNAs in the treatment of PH as a number of obstacles remain. First, lncRNAs are poorly conserved among different species, thus increasing the difficulty of drug development and clinical application. Second, the functions and underlying mechanisms of lncRNAs are significantly more complex and diverse than those of miRNAs (34). The use of siRNAs to silence the expression of target lncRNA is not always possible,

Table III. lncRNA expression, targets and effects on PH.

lncRNA	Expression	Target	Effect	(Refs.)
MEG3	Upregulated	miR-328-3p/IGF1R	Promotes proliferation of PASMCs	(42)
MEG3	Downregulated	miR-21/PTEN; p53 pathway	Inhibits proliferation and migration of PASMCs	(43,44)
HOXA-AS3	Upregulated	Hoxa3	Promotes proliferation and regulates the cell cycle	(47)
MANTIS	Downregulated	BRG1	Facilitates endothelial angiogenic function, promotes apoptosis	(49)
TUG1	Upregulated	miR-374c/Foxc1/Notch; miR-328	Promotes proliferation and migration, and inhibits apoptosis of HASMCs	(56)
MALAT1	Upregulated	Cyclin-dependent kinase inhibitors; cell cycle regulator; hsa-miR-124-3p.1/KLF5	Promotes proliferation and migration, slightly inhibits cell apoptosis, regulates cell cycle and phenotype switch of PASMCs	(41,61,63)
CPS1-IT	Downregulated	IL-1β; inhibits HIF-1 transcriptional activity and the NF-κB signaling pathway	Alleviates PH in a rat model	(66)
CASC2	Downregulated	Unknown	Inhibits proliferation and migration, promotes apoptosis and inhibits the phenotypic switch of hypoxia-induced PASMCs.	(25)
TCONS_00034812	Downregulated	Stox1/MAPK signaling	Promotes PASMC proliferation and inhibits apoptosis	(26)
H19	Upregulated	miRNA let-7b AT1R; H19/miR-675-3p/IGF1R and H19/miR-200a/PDCD4	Promotes PASMC proliferation and pulmonary vascular remodeling	(74,76)
Lnc-Ang362	Upregulated	miR-221, miR-222	Promotes PASMC proliferation and migration, inhibits apoptosis	(82)
UCA1	Upregulated	HnRNP I	Promotes PASMC proliferation	(79)
LnRPT	Downregulated	Notch signaling pathway	PASMC proliferation	(84)
NONRATT01558 7.2	Upregulated	p53 and HIF-1 signaling pathway	Promotes PASMC proliferation, inhibits cell apoptosis	(89)

lncRNA, long non-coding RNA; PH, pulmonary hypertension; miR, microRNA; MEG3, maternally expressed 3; PASMC, pulmonary artery smooth muscle cell; PTEN, phosphatase and tensin homolog; Hoxaas3, Hoxa cluster antisense RNA 3; Hoxa3, homeobox a3; BRG1, Brm/SWI2-related gene 1; TUG1, taurine upregulated 1; Foxc1, forkhead box C1; HASMCs, human aortic muscle cells; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; KLF5, kruppel-like factor 5; CPS1-IT, carbamoyl phosphate synthetase 1-intronic transcript; IL-1β, interleukin 1β; HIF-1, hypoxia-inducible factor 1; NF-κB, nuclear factor κB; CASC2, cancer susceptibility candidate 2; Stox1, storkhead box 1; MAPK, mitogen-activated protein kinase; AT1R, angiotensin II type 1 receptor; IGF1R, insulin-like growth factor 1 receptor; PDCD4, programmed cell death protein 4; UCA1, urothelial carcinoma associated 1; HnRNP I, heterogeneous ribonucleoprotein I; LnRPT, lncRNA regulated by PDGF and transforming growth factor β.

as most lncRNAs are localized in the nucleus. Furthermore, the toxicity of chemically modified siRNAs has not yet been fully clarified (98). In addition, the secondary structure of lncRNAs, their delivery into the human body, the speed of onset and duration of their action, as well as the prevention of off-target effects require further investigation.

Outlook. Using second generation sequencing, especially RNA-seq and other methods, a number of studies have identified lncRNA networks involved in the regulation of PH. However, the sequences, structures and functions of lncRNAs are poorly conserved among different species; therefore, the *in vivo* study of lncRNAs is a challenging task. The number of large-scale clinical studies regarding the diagnosis, assessment of disease severity and prognosis of lncRNA-induced PH is limited. This may be explained by the following: First, it is difficult to achieve a perfect match between patients with and without PH as clinical patients may also exhibit different

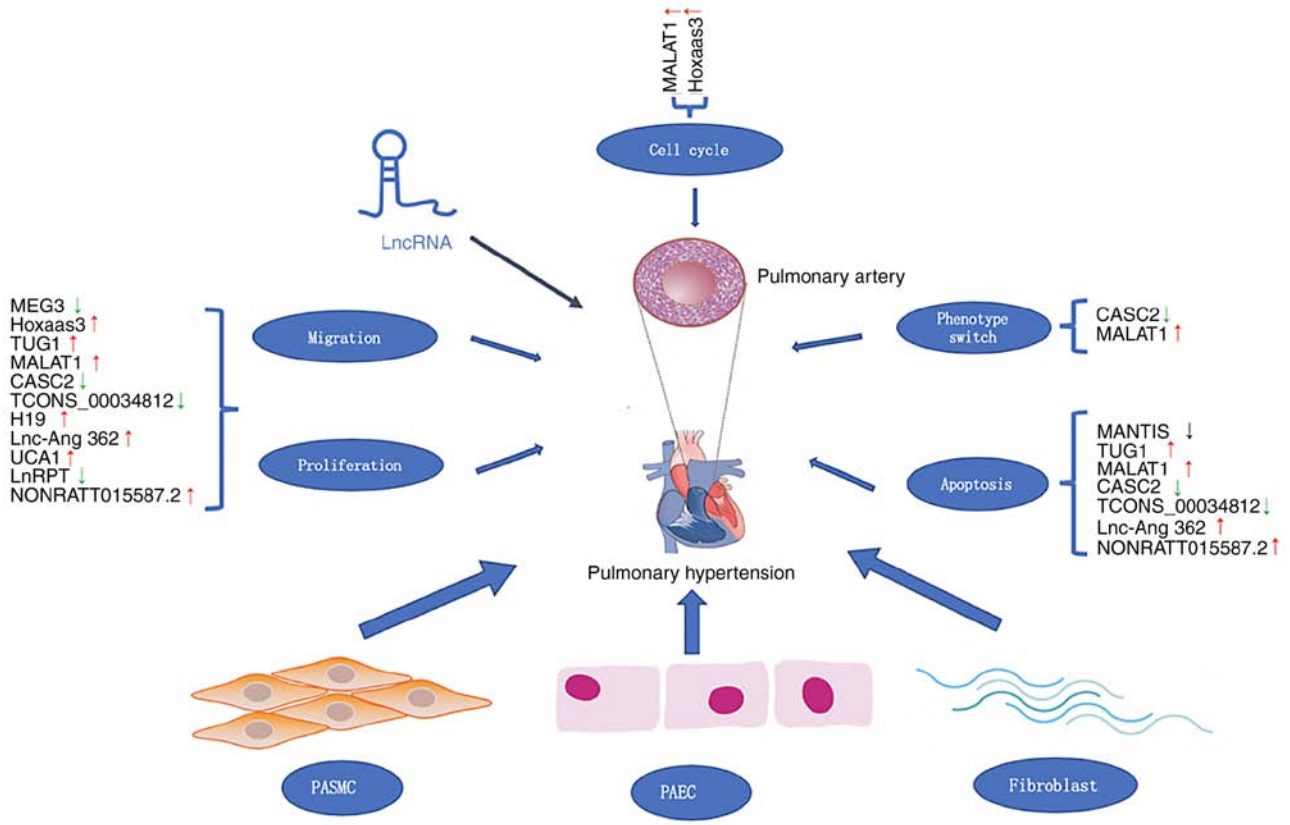

Figure 1. Roles of lncRNAs in PH. PH is caused by thickening of the pulmonary blood vessel wall, narrowing of the lumen and increasing pulmonary artery pressure. The dysregulation of lncRNA expression is involved in cell proliferation, migration and apoptosis, phenotype switch and regulation of cell cycle. Red and green arrows indicate upregulated and downregulated lncRNAs in PH, respectively. PH, pulmonary hypertension; lncRNA, long non-coding RNA; PASMC, pulmonary artery smooth muscle cell; PAEC, pulmonary artery endothelial cell; MEG3, maternally expressed 3; Hoxaas3, Hoxa cluster antisense RNA 3; TUG1, taurine upregulated 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; CASC2, cancer susceptibility candidate 2; UCA1, urothelial carcinoma associated 1; LnRPT, lncRNA regulated by PDGF and transforming growth factor β.

comorbidities and complications. Second, lncRNAs are poorly conserved among different species; therefore, the results of *in vitro* animal experiments cannot be directly applied to clinical practice. Third, lncRNA tests are more complicated and expensive compared with commonly used clinical detection methods. However, the screening of lncRNAs with excellent sensitivity, specificity or predictive value and the development of drugs targeting specific lncRNAs for PH treatment would be of great significance in the future.

3. Conclusions

PH is a multifactorial disease characterized by pulmonary vascular remodeling, resulting in sustained increased pulmonary arterial pressure, right heart failure and even death. lncRNAs are key molecules that control cellular biological activities by regulating gene expression at the transcriptional and post-transcriptional levels (99). A considerable body of evidence has demonstrated that lncRNAs are essential regulators of the pathogenesis and progression of PH. Numerous studies have greatly improved our understanding of the roles of lncRNAs in PH. Novel PH-associated lncRNAs, their expression and targets are listed in Table III. The dysregulation mechanisms of differentially expressed lncRNAs involved in cell proliferation, migration and apoptosis, regulation of the cell cycle, and phenotypic switching are shown in Fig. 1. lncRNAs have the potential to become novel diagnostic markers in clinical practice and lncRNAs may become potential pharmacological targets for PH treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 81970237 and 81600227).

Availability of data and materials

Not applicable.

Authors' contributions

All the authors (YHQ, GLY, YQ, DW, EFL, JTH and CCT) contributed to the conception and design of the study. YHQ, EFL and JTH searched the relevant literature, and YHQ wrote the manuscript. GLY, YQ and DW provided advice and are responsible for revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE and McGoon MD: An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. Chest 142: 448-456, 2012.
2. Hoeper MM, Humbert M, Souza R, Idrees M, Kawut SM, Sliwa-Hahnle K, Jing ZC and Gibbs JS: A global view of pulmonary hypertension. Lancet Respir Med 4: 306-322, 2016.
3. Hoeper MM and Simon R Gibbs J: The changing landscape of pulmonary arterial hypertension and implications for patient care. Eur Respir Rev 23: 450-457, 2014.
4. Hoeper MM, Ghofrani HA, Grunig E, Klose H, Olschewski H and Rosenkranz S: Pulmonary hypertension. Dtsch Arztebl Int 114: 73-84, 2017.
5. Luna RCP, de Oliveira Y, Lisboa JVC, Chaves TR, de Araújo TAM, de Sousa EE, Miranda Neto M, Pirola L, Braga VA and de Brito Alves JL: Insights on the epigenetic mechanisms underlying pulmonary arterial hypertension. Braz J Med Biol Res 51: e7437, 2018.
6. Zhao L, Oliver E, Maratou K, Atanur SS, Dubois OD, Cotroneo E, Chen CN, Wang L, Arce C, Chabosseau PL, *et al*: The zinc transporter ZIP12 regulates the pulmonary vascular response to chronic hypoxia. Nature 524: 356-360, 2015.
7. Savai R, Al-Tamari HM, Sedding D, Kojonazarov B, Muecke C, Teske R, Capecchi MR, Weissmann N, Grimminger F, Seeger W, *et al*: Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. Nat Med 20: 1289-1300, 2014.
8. Shintani M, Yagi H, Nakayama T, Saji T and Matsuoka R: A new nonsense mutation of SMAD8 associated with pulmonary arterial hypertension. J Med Genet 46: 331-337, 2009.
9. Pullamsetti SS, Berghausen EM, Dabral S, Tretyn A, Butrous E, Savai R, Butrous G, Dahal BK, Brandes RP, Ghofrani HA, *et al*: Role of Src tyrosine kinases in experimental pulmonary hypertension. Arterioscler Thromb Vasc Biol 32: 1354-1365, 2012.
10. de-Miguel-Díez J, López-de-Andrés A, Hernandez-Barrera V, Jimenez-Trujillo I, de-Miguel-Yanes JM, Mendez-Bailón M and Jimenez-Garcia R: National trends and outcomes of hospitalizations for pulmonary hypertension in Spain (2001-2014). Int J Cardiol 263: 125-131, 2018.
11. Anand V, Roy SS, Archer SL, Weir EK, Garg SK, Duval S and Thenappan T: Trends and outcomes of pulmonary arterial hypertension-related hospitalizations in the United States: Analysis of the nationwide inpatient sample database from 2001 through 2012. JAMA Cardiol 1: 1021-1029, 2016.
12. Hon CC, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJ, Gough J, Denisenko E, Schmeier S, Poulsen TM, Severin J, *et al*: An atlas of human long non-coding RNAs with accurate 5'ends. Nature 543: 199-204, 2017.
13. Zhang K, Shi ZM, Chang YN, Hu ZM, Qi HX and Hong W: The ways of action of long non-coding RNAs in cytoplasm and nucleus. Gene 547: 1-9, 2014.
14. Ezkurdia I, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, Vazquez J, Valencia A and Tress ML: Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. Hum Mol Genet 23: 5866-5878, 2014.
15. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, *et al*: Landscape of transcription in human cells. Nature 489: 101-108, 2012.
16. Bunch H: Gene regulation of mammalian long non-coding RNA. Mol Genet Genomics 293: 1-15, 2018.
17. Atianand MK, Caffrey DR and Fitzgerald KA: Immunobiology of long noncoding RNAs. Annu Rev Immunol 35: 177-198, 2017.
18. Sun W, Yang Y, Xu C and Guo J: Regulatory mechanisms of long noncoding RNAs on gene expression in cancers. Cancer Genet 216-217: 105-110, 2017.
19. Yoon JH, Abdelmohsen K and Gorospe M: Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol 34: 9-14, 2014.
20. Ferdin J, Nishida N, Wu X, Nicoloso MS, Shah MY, Devlin C, Ling H, Shimizu M, Kumar K, Cortez MA, *et al*: HINCUTs in cancer: Hypoxia-induced noncoding ultraconserved transcripts. Cell Death Differ 20: 1675-1687, 2013.
21. Bell RD, Long X, Lin M, Bergmann JH, Nanda V, Cowan SL, Zhou Q, Han Y, Spector DL, Zheng D and Miano JM: Identification and initial functional characterization of a human vascular cell-enriched long noncoding RNA. Arterioscler Thromb Vasc Biol 34: 1249-1259, 2014.
22. Huarte M: The emerging role of lncRNAs in cancer. Nat Med 21: 1253-1261, 2015.
23. Shi X, Sun M, Liu H, Yao Y, Kong R, Chen F and Song Y: A critical role for the long non-coding RNA GAS5 in proliferation and apoptosis in non-small-cell lung cancer. Mol Carcinog 54 (Suppl 1): E1-E12, 2015.
24. Zhang Y, Cheng HP, Bao TP, Wang XG and Tian ZF: Expression of long non-coding RNA NANCI in lung tissues of neonatal mice with hyperoxia-induced lung injury and its regulatory effect on NKX2.1. Zhongguo Dang Dai Er Ke Za Zhi 19: 215-221, 2017 (In Chinese).
25. Gong J, Chen Z, Chen Y, Lv H, Lu H, Yan F, Li L, Zhang W and Shi J: Long non-coding RNA CASC2 suppresses pulmonary artery smooth muscle cell proliferation and phenotypic switch in hypoxia-induced pulmonary hypertension. Respir Res 20: 53, 2019.
26. Liu Y, Sun Z, Zhu J, Xiao B, Dong J and Li X: LncRNA-TCONS_00034812 in cell proliferation and apoptosis of pulmonary artery smooth muscle cells and its mechanism. J Cell Physiol 233: 4801-4814, 2018.
27. Lund E, Guttinger S, Calado A, Dahlberg JE and Kutay U: Nuclear export of microRNA precursors. Science 303: 95-98, 2004.
28. Su Z, Zhi X, Zhang Q, Yang L, Xu H and Xu Z: LncRNA H19 functions as a competing endogenous RNA to regulate AQP3 expression by sponging miR-874 in the intestinal barrier. FEBS Lett 590: 1354-1364, 2016.
29. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS and Johnson JM: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433: 769-773, 2005.
30. Rothman AM, Arnold ND, Pickworth JA, Iremonger J, Ciuclan L, Allen RM, Guth-Gundel S, Southwood M, Morrell NW, Thomas M, *et al*: MicroRNA-140-5p and SMURF1 regulate pulmonary arterial hypertension. J Clin Invest 126: 2495-2508, 2016.
31. Caruso P, Dempsie Y, Stevens HC, McDonald RA, Long L, Lu R, White K, Mair KM, McClure JD, Southwood M, *et al*: A role for miR-145 in pulmonary arterial hypertension: Evidence from mouse models and patient samples. Circ Res 111: 290-300, 2012.
32. Schlosser K, White RJ and Stewart DJ: miR-26a linked to pulmonary hypertension by global assessment of circulating extracellular microRNAs. Am J Respir Crit Care Med 188: 1472-1475, 2013.
33. Lennox KA and Behlke MA: Cellular localization of long non-coding RNAs affects silencing by RNAi more than by antisense oligonucleotides. Nucleic Acids Res 44: 863-877, 2016.
34. Ballantyne MD, McDonald RA and Baker AH: lncRNA/MicroRNA interactions in the vasculature. Clin Pharmacol Ther 99: 494-501, 2016.
35. Al-Rugeebah A, Alanazi M and Parine NR: MEG3: An oncogenic long non-coding RNA in different cancers. Pathol Oncol Res 25: 859-874, 2019.
36. Zhao Y, Zhu Z, Shi S, Wang J and Li N: Long non-coding RNA MEG3 regulates migration and invasion of lung cancer stem cells via miR-650/SLC34A2 axis. Biomed Pharmacother 120: 109457, 2019.
37. Guo W, Dong Z, Liu S, Qiao Y, Kuang G, Guo Y, Shen S and Liang J: Promoter hypermethylation-mediated downregulation of miR-770 and its host gene MEG3, a long non-coding RNA, in the development of gastric cardia adenocarcinoma. Mol Carcinog 56: 1924-1934, 2017.

38. Xia Y, He Z, Liu B, Wang P and Chen Y: Downregulation of Meg3 enhances cisplatin resistance of lung cancer cells through activation of the WNT/β-catenin signaling pathway. Mol Med Rep 12: 4530-4537, 2015.
39. Zhou Y, Zhang X and Klibanski A: MEG3 noncoding RNA: A tumor suppressor. J Mol Endocrinol 48: R45-R53, 2012.
40. Piccoli MT, Gupta SK, Viereck J, Foinquinos A, Samolovac S, Kramer FL, Garg A, Remke J, Zimmer K, Batkai S and Thum T: Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. Circ Res 121: 575-583, 2017.
41. Michalik KM, You X, Manavski Y, Doddaballapur A, Zörnig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, *et al*: Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. Circ Res 114: 1389-1397, 2014.
42. Xing Y, Zheng X, Fu Y, Qi J, Li M, Ma M, Wang S, Li S and Zhu D: Long noncoding RNA-maternally expressed gene 3 contributes to hypoxic pulmonary hypertension. Mol Ther 27: 2166-2181, 2019.
43. Zhu B, Gong Y, Yan G, Wang D, Qiao Y, Wang Q, Liu B, Hou J, Li R and Tang C: Down-regulation of lncRNA MEG3 promotes hypoxia-induced human pulmonary artery smooth muscle cell proliferation and migration via repressing PTEN by sponging miR-21. Biochem Biophys Res Commun 495: 2125-2132, 2018.
44. Sun Z, Nie X, Sun S, Dong S, Yuan C, Li Y, Xiao B, Jie D and Liu Y: Long non-coding RNA MEG3 downregulation triggers human pulmonary artery smooth muscle cell proliferation and migration via the p53 signaling pathway. Cell Physiol Biochem 42: 2569-2581, 2017.
45. Duboule D: The rise and fall of Hox gene clusters. Development 134: 2549-2560, 2007.
46. Gong WJ, Yin JY, Li XP, Fang C, Xiao D, Zhang W, Zhou HH, Li X and Liu ZQ: Association of well-characterized lung cancer lncRNA polymorphisms with lung cancer susceptibility and platinum-based chemotherapy response. Tumour Biol 37: 8349-8358, 2016.
47. Zhang H, Liu Y, Yan L, Wang S, Zhang M, Ma C, Zheng X, Chen H and Zhu D: Long noncoding RNA Hoxaas3 contributes to hypoxia-induced pulmonary artery smooth muscle cell proliferation. Cardiovasc Res 115: 647-657, 2019.
48. Golpon HA, Geraci MW, Moore MD, Miller HL, Miller GJ, Tuder RM and Voelkel NF: HOX genes in human lung: Altered expression in primary pulmonary hypertension and emphysema. Am J Pathol 158: 955-966, 2001.
49. Leisegang MS, Fork C, Josipovic I, Richter FM, Preussner J, Hu J, Miller MJ, Epah J, Hofmann P, Günther S, *et al*: Long noncoding RNA MANTIS facilitates endothelial angiogenic function. Circulation 136: 65-79, 2017.
50. Hathaway CA, Heistad DD, Piegors DJ and Miller FJ Jr: Regression of atherosclerosis in monkeys reduces vascular superoxide levels. Circ Res 90: 277-283, 2002.
51. Katsushima K, Natsume A, Ohka F, Shinjo K, Hatanaka A, Ichimura N, Sato S, Takahashi S, Kimura H, Totoki Y, *et al*: Targeting the Notch-regulated non-coding RNA TUG1 for glioma treatment. Nat Commun 7: 13616, 2016.
52. Li FP, Lin DQ and Gao LY: LncRNA TUG1 promotes proliferation of vascular smooth muscle cell and atherosclerosis through regulating miRNA-21/PTEN axis. Eur Rev Med Pharmacol Sci 22: 7439-7447, 2018.
53. Xie C, Chen B, Wu B, Guo J and Cao Y: LncRNA TUG1 promotes cell proliferation and suppresses apoptosis in osteosarcoma by regulating miR-212-3p/FOXA1 axis. Biomed Pharmacother 97: 1645-1653, 2018.
54. Jiang L, Wang W, Li G, Sun C, Ren Z, Sheng H, Gao H, Wang C and Yu H: High TUG1 expression is associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. Cancer Chemother Pharmacol 78: 333-339, 2016.
55. Cai H, Liu X, Zheng J, Xue Y, Ma J, Li Z, Xi Z, Li Z, Bao M and Liu Y: Long non-coding RNA taurine upregulated 1 enhances tumor-induced angiogenesis through inhibiting microRNA-299 in human glioblastoma. Oncogene 36: 318-331, 2017.
56. Yang L, Liang H, Shen L, Guan Z and Meng X: LncRNA Tug1 involves in the pulmonary vascular remodeling in mice with hypoxic pulmonary hypertension via the microRNA-374c-mediated Foxc1. Life Sci 237: 116769, 2019.
57. Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR and Sadeghi MM: VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res 107: 408-417, 2010.
58. Wang S, Cao W, Gao S, Nie X, Zheng X, Xing Y, Chen Y, Bao H and Zhu D: TUG1 regulates pulmonary arterial smooth muscle cell proliferation in pulmonary arterial hypertension. Can J Cardiol 35: 1534-1545, 2019.
59. Ren S, Liu Y, Xu W, Sun Y, Lu J, Wang F, Wei M, Shen J, Hou J, Gao X, *et al*: Long noncoding RNA MALAT-1 is a new potential therapeutic target for castration resistant prostate cancer. J Urol 190: 2278-2287, 2013.
60. Qi Y, Ooi HS, Wu J, Chen J, Zhang X, Tan S, Yu Q, Li YY, Kang Y, Li H, *et al*: MALAT1 long ncRNA promotes gastric cancer metastasis by suppressing PCDH10. Oncotarget 7: 12693-12703, 2016.
61. Brock M, Schuoler C, Leuenberger C, Bühlmann C, Haider TJ, Vogel J, Ulrich S, Gassmann M, Kohler M and Huber LC: Analysis of hypoxia-induced noncoding RNAs reveals metastasis-associated lung adenocarcinoma transcript 1 as an important regulator of vascular smooth muscle cell proliferation. Exp Biol Med (Maywood) 242: 487-496, 2017.
62. Potente M, Gerhardt H and Carmeliet P: Basic and therapeutic aspects of angiogenesis. Cell 146: 873-887, 2011.
63. Wang D, Xu H, Wu B, Jiang S, Pan H, Wang R and Chen J: Long noncoding RNA MALAT1 sponges miR1243p.1/KLF5 to promote pulmonary vascular remodeling and cell cycle progression of pulmonary artery hypertension. Int J Mol Med 44: 871-884, 2019.
64. Zhuo Y, Zeng Q, Zhang P, Li G, Xie Q and Cheng Y: Functional polymorphism of lncRNA MALAT1 contributes to pulmonary arterial hypertension susceptibility in Chinese people. Clin Chem Lab Med 55: 38-46, 2017.
65. Xiaoguang Z, Meirong L, Jingjing Z, Ruishen Z, Qing Z and Xiaofeng T: Long noncoding RNA CPS1-IT1 suppresses cell proliferation and metastasis in human lung cancer. Oncol Res 25: 373-380, 2017.
66. Zhang Z, Li Z, Wang Y, Wei L and Chen H: Overexpressed long noncoding RNA CPS1-IT alleviates pulmonary arterial hypertension in obstructive sleep apnea by reducing interleukin-1beta expression via HIF1 transcriptional activity. J Cell Physiol 234: 19715-19727, 2019.
67. Yu X, Zheng H, Tse G, Zhang L and Wu WKK: CASC2: An emerging tumour-suppressing long noncoding RNA in human cancers and melanoma. Cell Prolif 51: e12506, 2018.
68. Jie W, Guo J, Shen Z, Wang X, Zheng S, Wang G and Ao Q: Contribution of myocardin in the hypoxia-induced phenotypic switching of rat pulmonary arterial smooth muscle cells. Exp Mol Pathol 89: 301-306, 2010.
69. Zhang W and Liu HT: MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res 12: 9-18, 2002.
70. Ma C, Liu Y, Wang Y, Zhang C, Yao H, Ma J, Zhang L, Zhang D, Shen T and Zhu D: Hypoxia activates 15-PGDH and its metabolite 15-KETE to promote pulmonary artery endothelial cells proliferation via ERK1/2 signalling. Br J Pharmacol 171: 3352-3363, 2014.
71. Gibb EA, Brown CJ and Lam WL: The functional role of long non-coding RNA in human carcinomas. Mol Cancer 10: 38, 2011.
72. Matouk IJ, Halle D, Gilon M and Hochberg A: The non-coding RNAs of the H19-IGF2 imprinted loci: A focus on biological roles and therapeutic potential in lung cancer. J Transl Med 13: 113, 2015.
73. Matouk IJ, Mezan S, Mizrahi A, Ohana P, Abu-Lail R, Fellig Y, Degroot N, Galun E and Hochberg A: The oncofetal H19 RNA connection: Hypoxia, p53 and cancer. Biochim Biophys Acta 1803: 443-451, 2010.
74. Su H, Xu X, Yan C, Shi Y, Hu Y, Dong L, Ying S, Ying K and Zhang R: LncRNA H19 promotes the proliferation of pulmonary artery smooth muscle cells through AT1R via sponging let-7b in monocrotaline-induced pulmonary arterial hypertension. Respir Res 19: 254, 2018.
75. Jin H, Wang Y, Zhou L, Liu L, Zhang P, Deng W and Yuan Y: Melatonin attenuates hypoxic pulmonary hypertension by inhibiting the inflammation and the proliferation of pulmonary arterial smooth muscle cells. J Pineal Res 57: 442-450, 2014.
76. Wang R, Zhou S, Wu P, Li M, Ding X, Sun L, Xu X, Zhou X, Zhou L, Cao C and Fei G: Identifying involvement of H19-miR-675-3p-IGF1R and H19-miR-200a-PDCD4 in treating pulmonary hypertension with melatonin. Mol Ther Nucleic Acids 13: 44-54, 2018.
77. Wang ZQ, He CY, Hu L, Shi HP, Li JF, Gu QL, Su LP, Liu BY, Li C and Zhu Z: Long noncoding RNA UCA1 promotes tumour metastasis by inducing GRK2 degradation in gastric cancer. Cancer Lett 408: 10-21, 2017.

78. Liu X, Huang Z, Qian W, Zhang Q and Sun J: Silence of lncRNA UCA1 rescues drug resistance of cisplatin to non-small-cell lung cancer cells. J Cell Biochem 120: 9243-9249, 2019.
79. Zhu TT, Sun RL, Yin YL, Quan JP, Song P, Xu J, Zhang MX and Li P: Long noncoding RNA UCA1 promotes the proliferation of hypoxic human pulmonary artery smooth muscle cells. Pflugers Arch 471: 347-355, 2019.
80. Xing YN, Yang X, Xu XY, Zheng Y, Xu HM, Takano Y and Zheng H: The altered expression of ING5 protein is involved in gastric carcinogenesis and subsequent progression. Hum Pathol 42: 25-35, 2011.
81. Leung A, Trac C, Jin W, Lanting L, Akbany A, Sætrom P, Schones DE and Natarajan R: Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells. Circ Res 113: 266-278, 2013.
82. Wang H, Qin R and Cheng Y: LncRNA-Ang362 promotes pulmonary arterial hypertension by regulating miR-221 and miR-222. Shock 53: 723-729, 2019.
83. Heldin CH and Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79: 1283-1316, 1999.
84. Chen J, Guo J, Cui X, Dai Y, Tang Z, Qu J, Raj JU, Hu Q and Gou D: The long noncoding RNA LnRPT is regulated by PDGF-BB and modulates the proliferation of pulmonary artery smooth muscle cells. Am J Respir Cell Mol Biol 58: 181-193, 2018.
85. Baeten JT and Lilly B: Differential regulation of NOTCH2 and NOTCH3 contribute to their unique functions in vascular smooth muscle cells. J Biol Chem 290: 16226-16237, 2015.
86. Li X, Zhang X, Leathers R, Makino A, Huang C, Parsa P, Macias J, Yuan JX, Jamieson SW and Thistlethwaite PA: Notch3 signaling promotes the development of pulmonary arterial hypertension. Nat Med 15: 1289-1297, 2009.
87. Arunachalam G, Lakshmanan AP, Samuel SM, Triggle CR and Ding H: Molecular interplay between microRNA-34a and Sirtuin1 in hyperglycemia-mediated impaired angiogenesis in endothelial cells: Effects of metformin. J Pharmacol Exp Ther 356: 314-323, 2016.
88. Dean A, Nilsen M, Loughlin L, Salt IP and MacLean MR: Metformin reverses development of pulmonary hypertension via aromatase inhibition. Hypertension 68: 446-454, 2016.
89. Sun Z, Liu Y, Yu F, Xu Y, Yanli L and Liu N: Long non-coding RNA and mRNA profile analysis of metformin to reverse the pulmonary hypertension vascular remodeling induced by monocrotaline. Biomed Pharmacother 115: 108933, 2019.
90. Lino Cardenas CL, Kessinger CW, Cheng Y, MacDonald C, MacGillivray T, Ghoshhajra B, Huleihel L, Nuri S, Yeri AS, Jaffer FA, *et al*: An HDAC9-MALAT1-BRG1 complex mediates smooth muscle dysfunction in thoracic aortic aneurysm. Nat Commun 9: 1009, 2018.
91. Ranchoux B, Antigny F, Rucker-Martin C, Hautefort A, Péchoux C, Bogaard HJ, Dorfmüller P, Remy S, Lecerf F, Planté S, *et al*: Endothelial-to-mesenchymal transition in pulmonary hypertension. Circulation 131: 1006-1018, 2015.
92. Rabinovitch M, Guignabert C, Humbert M and Nicolls MR: Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. Circ Res 115: 165-175, 2014.
93. Jackson R, Kroehling L, Khitun A, Bailis W, Jarret A, York AG, Khan OM, Brewer JR, Skadow MH, Duizer C, *et al*: The translation of non-canonical open reading frames controls mucosal immunity. Nature 564: 434-438, 2018.
94. Li LJ, Leng RX, Fan YG, Pan HF and Ye DQ: Translation of noncoding RNAs: Focus on lncRNAs, pri-miRNAs, and circRNAs. Exp Cell Res 361: 1-8, 2017.
95. Thenappan T, Ormiston ML, Ryan JJ and Archer SL: Pulmonary arterial hypertension: Pathogenesis and clinical management. BMJ 360: j5492, 2018.
96. Schlosser K, Hanson J, Villeneuve PJ, Dimitroulakos J, McIntyre L, Pilote L and Stewart D: Assessment of circulating LncRNAs under physiologic and pathologic conditions in humans reveals potential limitations as biomarkers. Sci Rep 6: 36596, 2016.
97. Han B, Bu P, Meng X and Hou X: Microarray profiling of long non-coding RNAs associated with idiopathic pulmonary arterial hypertension. Exp Ther Med 13: 2657-2666, 2017.
98. Viereck J, Kumarswamy R, Foinquinos A, Xiao K, Avramopoulos P, Kunz M, Dittrich M, Maetzig T, Zimmer K, Remke J, *et al*: Long noncoding RNA Chast promotes cardiac remodeling. Sci Transl Med 8: 326ra322, 2016.
99. Mercer TR and Mattick JS: Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 20: 300-307, 2013.
100. Jandl K, Thekkekara Puthenparampil H, Marsh LM, Hoffmann J, Wilhelm J, Veith C, Sinn K, Klepetko W, Olschewski H, Olschewski A, *et al*: Long non-coding RNAs influence the transcriptome in pulmonary arterial hypertension: The role of PAXIP1-AS1. J Pathol 247: 357-370, 2019.
101. Bischoff FC, Werner A, John D, Boeckel JN, Melissari MT, Grote P, Glaser SF, Demolli S, Uchida S, Michalik KM, *et al*: Identification and functional characterization of hypoxia-induced endoplasmic reticulum stress regulating lncRNA (HypERlnc) in pericytes. Circ Res 121: 368-375, 2017.
102. Wang M, Gu S, Liu Y, Yang Y, Yan J, Zhang X, An X, Gao J, Hu X and Su P: miRNA-PDGFRB/HIF1A-lncRNA CTEPHA1 network plays important roles in the mechanism of chronic thromboembolic pulmonary hypertension. Int Heart J 60: 924-937, 2019.
103. Gu S, Li G, Zhang X, Yan J, Gao J, An X, Liu Y and Su P: Aberrant expression of long noncoding RNAs in chronic thromboembolic pulmonary hypertension. Mol Med Rep 11: 2631-2643, 2015.
104. Josipovic I, Fork C, Preussner J, Prior KK, Iloska D, Vasconez AE, Labocha S, Angioni C, Thomas D, Ferreirós N, *et al*: PAFAH1B1 and the lncRNA NONHSAT073641 maintain an angiogenic phenotype in human endothelial cells. Acta Physiol (Oxf) 218: 13-27, 2016.
105. Voellenkle C, Garcia-Manteiga JM, Pedrotti S, Perfetti A, De Toma I, Da Silva D, Maimone B, Greco S, Fasanaro P, Creo P, *et al*: Implication of Long noncoding RNAs in the endothelial cell response to hypoxia revealed by RNA-sequencing. Sci Rep 6: 24141, 2016.
106. Su Q, Sun Y, Ye Z, Yang H and Li L: Oxidized low density lipoprotein induces endothelial-to-mesenchymal transition by stabilizing Snail in human aortic endothelial cells. Biomed Pharmacother 106: 1720-1726, 2018.
107. Neumann P, Jaé N, Knau A, Glaser SF, Fouani Y, Rossbach O, Krüger M, John D, Bindereif A, Grote P, *et al*: The lncRNA GATA6-AS epigenetically regulates endothelial gene expression via interaction with LOXL2. Nat Commun 9: 237, 2018.
108. Lin R, Roychowdhury-Saha M, Black C, Watt AT, Marcusson EG, Freier SM and Edgington TS: Control of RNA processing by a large non-coding RNA over-expressed in carcinomas. FEBS Lett 585: 671-676, 2011.
109. Rivero Puente A, Asín Marcotegui J, Reparaz B and Achutegui G: Malignant nephroangiosclerosis. Rev Clin Esp 140: 251-255, 1976 (In Spanish).