Int J Clin Exp Med 2019;12(5):4685-4696
www.ijcem.com /ISSN:1940-5901/IJCEM0087054

Original Article

Integrated analysis of long noncoding RNA and mRNA expression profiles in human pulmonary artery smooth muscle cells reveals the potential roles of lncRNAs in the pathogenesis of pulmonary artery hypertension

Yue Yu[1*], Fei Zhao[1*], Junjie Du[2], Chenjun Huang[1], Yue Zhou[1], Jun Li[1], Chengxiang Zhu[1], Qifan Li[1], Yu Zhuang[1], Wei Wang[1]

Departments of [1]Thoracic Surgery, [2]Cardiac Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China. [*]Equal contributors.

Received October 20, 2018; Accepted February 8, 2019; Epub May 15, 2019; Published May 30, 2019

Abstract: Purpose: Molecular biological mechanisms underlying the development and progression of pulmonary arterial hypertension (PAH) have remained largely elusive. This study aimed to investigate long noncoding RNAs (lncRNA) and mRNA expression profiles, examining potential functional roles of these RNA molecules in PAH. Method: Microarray and bioinformatics analysis was used to find potential lncRNAs useful in PAH in this study. Human pulmonary arterial smooth muscle cells (HPASMCs) were cultured and stimulated by endothelin (ET)-1 to establish a PAH cellular model. This study examined proliferation and apoptosis, identifying expression patterns of lncRNAs and mRNAs via microarray analysis. Bioinformatics analysis was performed for further examination. Results: After ET-1 treatment, proliferation and apoptosis resistance of HPASMCs was enhanced. Microarray data showed that 13 lncRNAs were significantly induced and 41 lncRNAs were suppressed in HPASMCs after ET-1 treatment (Fold change ≥ 2 and $P \leq 0.05$). Additionally, 26 mRNAs were upregulated and 6 mRNAs were downregulated. Expression levels of the four chosen lncRNAs and mRNAs were validated by quantitative reverse transcription-polymerase chain reaction, confirming microarray analysis. Functional analysis suggests that several groups of lncRNAs participate in biological pathways related to PAH by *cis*- and/or *trans*-regulation of protein-coding genes. Conclusion: Aberrantly expressed genes and key lncRNAs identified in this preliminary study might play important roles in PAH.

Keywords: Long noncoding RNAs, pulmonary arterial hypertension, microarray, bioinformatics analysis

Introduction

Pulmonary arterial hypertension (PAH) is a progressive and poorly characterized disease, with a poor prognosis in terms of quality of life. Despite the availability of several PAH-specific vasodilator therapies, 1-year mortality remains high at 10% to 15% [1]. Elevated pulmonary artery pressure and pulmonary vascular resistance with decreased pulmonary vascular compliance can cause obstructive vascular remodeling of the small resistance pulmonary arteries, eventually leading to right ventricular failure and death [2].

Apoptosis resistance and proliferation of human pulmonary arterial smooth muscle cells (HPASMCs) are important features of PAH [3]. Endothelin (ET)-1 contributes to the vascular remodeling of artery walls, thereby playing an important role in the pathological process of PAH [4]. ET-1 is a vasoconstrictor and pro-proliferative agent in PAH by activating two G-protein-coupled receptors, namely, ET_A and ET_B, which are targets of highly selective antagonists [5]. *In vitro* experiments demonstrated that ET-1 can induce excessive proliferation of HPASMCs, mainly by activating the ET_A receptor, although such effects can be eliminated remarkably by the ET_A receptor blocker BQ123 [6, 7]. Therefore, researchers always establish a PAH cellular model using HPASMCs stimulated with ET-1 [6].

Development of PAH involves a complex and heterogeneous constellation of multiple genetic, molecular, and humoral abnormalities. Non-

acquired array images. Agilent GeneSpring GX software package (v. 13.1, Agilent Technology, USA) was employed to finish the basic analysis with raw data. First, raw data was normalized with the quantile algorithm. Probes in which at least 1 out of 2 conditions having flags in "P" were chosen for further data analysis. Differentially-expressed genes or lncRNAs were then identified through fold changes. The threshold set for up- and downregulated genes was a fold change ≥ 2 and P ≤ 0.05. Next, the functional roles of these differentially-expressed mRNAs were determined by GO and KEGG analyses.

Quantitative real-time PCR (qRT-PCR)

Following manufacturer instructions, 2 μg of RNA samples was reverse-transcribed into cDNA using the 1st Strand cDNA Synthesis Kit (Thermo Fisher, USA) after RNA extraction. Differentially-expressed candidate lncRNAs were verified by qRT-PCR using 2 × SYBR Green mix (TOYOBO, Japan). The following reaction conditions were used with the ABI 7300 instrument: Cycling conditions of 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 20 seconds, and 72°C for 40 seconds. GAPDH, a housekeeping gene, was applied in each sample as an internal control. Sequences of specific primers were as follows:

NONHSAT158348: 5'-CTTGGCATTATCTTGACACCA-3' (forward), 5'-CTTCTCTGGCATTAATGTTCT-3' (reverse); NONHSAT155407: 5'-AATATAAGCCTCTGGACCTCACTAAGT-3' (forward), 5'-ATATGCTCTTCGTTTGACACACAGTCT-3' (reverse); DLC1: 5'-CGAACGGTACCTGCTTGATGTGCAGAAAG-3' (forward), 5'-AAGGATCCTCACCTAGATTTGGTGTCTTTG-3' (reverse); EFHC2: 5'-TGAAATGGTGTCATCACCGAGATA-3' (forward), 5'-TTTCACTTTGGACAGTTTATGCCT-3' (reverse).

After PCR amplification, melt curve analysis was generated to identify possible contributions of unspecific products to the fluorescence signal. Expression fold changes of each gene were calculated via the $2^{-\Delta\Delta Ct}$ method. All assays were performed three times.

Bioinformatics analysis

Feature Extraction software (v. 10.7.1.1, Agilent Technologies) was used to analyze array images to obtain raw data. Genespring (v. 14.8, Agilent Technologies) was employed to finish the basic analysis of raw data. To begin, raw data was normalized with the quantile algorithm. Probes in which at least 1 out of 2 conditions had flags in "P" were chosen for further data analysis. Differentially-expressed genes or lncRNAs were then identified through fold changes, as well as *P*-values calculated with *t*-test. The threshold set for up- and downregulated genes was a fold change ≥ 2.0 and P < 0.05. Hierarchical clustering and volcano plots were performed to display distinguishable gene expression patterns among samples. GO and KEGG analyses were applied to determine the roles of these differentially-expressed mRNAs. Finally, this study further explored how these dysregulated lncRNAs may exert their function through cis- and/or trans-regulating protein-coding genes. Cis-regulation regions were identified by the following procedures. For each lncRNA, this study identified mRNAs as "cis-regulated mRNAs" when: (1) The mRNAs loci were within 300 kb windows up- and downstream of the given lncRNA; (2) Pearson's correlation of lncRNA-mRNA expression was significant (*P*-value of correlation ≤ 0.05). To categorize lncRNAs, which potentially have trans-regulating functions, this study superimposed lncRNA target predictions onto the lncRNA-TF or lncRNA-TF-mRNA correlation network using Cytoscape (http://www.cytoscape.org).

Statistical analysis

All experiments are representative of at least three trials. Data are expressed as the mean ± standard deviation (SD). Significant differences between the groups were analyzed using Student's *t*-test. Statistical analysis was performed using SPSS (v. 18.0; SPSS, Inc.) and P < 0.05 indicates statistical significance.

Results

ET-1 induced proliferation and apoptosis resistance of HPASMCs

CCK-8 assays were performed to examine the effects of ET-1 on proliferation of HPASMCs. Cell proliferation increased more than 1.3 times when treated with 1 μM of ET-1 for 72 hours, compared with that of cells in the control cultures (**Figure 1A**). This result was further confirmed using an EdU incorporation assay, in which ET-1 stimulation significantly promoted the proliferation of HPASMCs (**Figure 1B** and **1C**).

Annexin V-APC/7-AAD double staining was used to detect the apoptosis of HPASMCs after treatment with 1 μM ET-1. Compared with control cells, HPASMCs treated with ET-1 significantly decreased the population of Annexin V-positive cells, consequently leading to apoptosis resistance (**Figure 1D** and **1E**).

Analysis of aberrantly expressed lncRNAs and mRNAs in untreated or ET-1-treated HPASMCs

To explore potential lncRNAs involved in PAH, this study examined lncRNA and mRNA expression profiles in untreated and 1 μM ET-1-treated HPASMCs using microarray analysis. All assays were performed three times. According to present data, 54 lncRNAs were significantly induced or suppressed in HPASMCs after ET-1 treatment, of which 75.9% (41 lncRNAs) were suppressed and 24.1% (13 lncRNAs) were induced (fold change ≥ 2, $P < 0.05$). NONHSAT158348 (4.8-fold change) was the most significantly downregulated lncRNA and NONHSAT155407 (2.9-fold change) was the most significantly upregulated.

Regarding mRNAs, expression profiling data showed that 32 mRNAs were aberrantly expressed in ET-1-treated cells, relative to control cells (fold change ≥ 2, $P < 0.05$), of which 81.3% (26 mRNAs) were upregulated and 18.7% (6 mRNAs) were downregulated. Of these mRNAs, DLC1 (4.1-fold change) was the most upregulated protein-coding gene, while EFHC2 (2.3-fold change) was the most downregulated.

Heat maps and volcano plots of differentially-expressed noncoding and coding genes are shown in **Figures 2** and **3**. Complete microarray data will be publicly available at Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).

Validation of RNA expression levels via qRT-PCR

To confirm the accuracy and repeatability of microarray data, two lncRNAs (NONHSAT158348 and NONHSAT155407) and two mRNAs (DLC1 and EFHC2) were selected for validation by qRT-PCR, based on different expression patterns between untreated and ET-1-treated HPASMCs. Expression patterns of these RNAs, detected by qRT-PCR analysis, were identical to those determined by microarray analysis. Thus, NONHSAT155407 and DLC1 were induced, while NONHSAT158348 and EFHC2 were suppressed after ET-1 stimulation (**Figure 4**).

GO and KEGG pathway analyses of differentially-expressed mRNAs

GO analysis was used to find the potential function of aberrantly expressed mRNAs on the regulation of pathological responses against ET-1 stimulation. The significance of enrichment of each GO term was assessed by $P < 0.05$ and FDR < 0.05. GO terms were filtered by enrichment scores (-Lg [P]) in aberrantly expressed mRNAs. Enrichment analyses of GO terms are listed in **Figure 5**. GO analysis showed that chorio-allantoic fusion (GO:0060710), proteinaceous extracellular matrix (GO:0005578), and inhibin binding (GO:0034711) were the most enriched GO terms targeted by differentially upregulated mRNAs in biological process, cellular component, and molecular function, respectively. Additionally, negative regulation of cell growth (GO:0030308), apical plasma membrane (GO:0016324), and metalloendopeptidase inhibitor activity (GO:0008191) indicated the most enriched GO terms targeted by differentially downregulated mRNAs in biological process, cellular component, and molecular function, respectively.

KEGG pathway analysis is a knowledge base for systematic analysis of gene function, linking genomic information with a higher-order functional information. This study identified the most significantly enriched pathways of upregulated mRNAs and downregulated mRNAs analyzed by KEGG analysis, using either Chi-squared test or Fisher's exact test. The top three upregulated pathways were axon guidance (pathway ID: hsa04360), TGF-beta signaling pathway (pathway ID: hsa04350), and HTLV-I infection (pathway ID: hsa05166). The top three downregulated pathways were vitamin digestion and absorption (pathway ID: hsa04060), alpha-linolenic acid metabolism (pathway ID: hsa00592), and linoleic acid metabolism (pathway ID: hsa00591). Enrichment analyses of pathways are shown in **Figure 6**.

Cis-regulation of lncRNAs

According to chromosomal coordinates, the closest differentially-expressed neighboring mRNAs, within 300 kb from transcription start and stop sites, were determined for each differentially-expressed lncRNA using a custom R

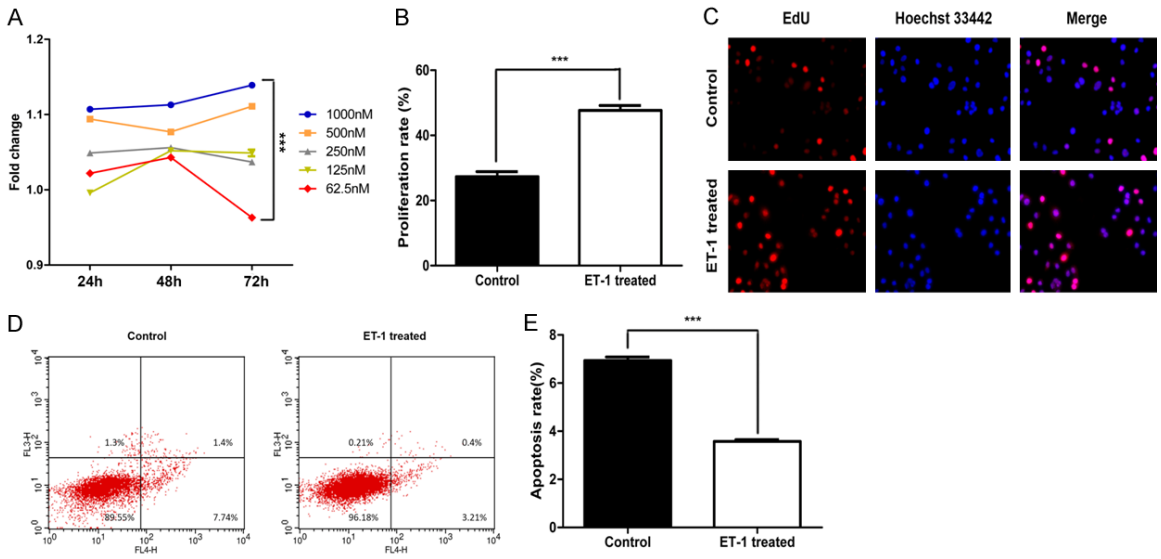


Figure 1. ET-1 induced proliferation and apoptosis resistance of HPASMCs. (A) CCK-8, (B & C) EdU, (D & E) Annexin V-APC/7-AAD double staining. All data are represented as the mean ± SD from the three independent experiments, *P < 0.05, **P < 0.01, ***P < 0.001, Student's *t*-test.

script. It was found that two differentially-expressed lncRNAs may play cis roles as gene regulators. Moreover, lncRNA NONHSAT151314 was predicted as a positive moderate role in the cis-regulation of AHDC1, while NONHSAT201343 played as a negative moderate role in CXCL8 regulation.

Trans-regulation of lncRNA

Using a threshold of P < 0.01 and FDR < 0.01, this study found 218 lncRNA-TF pairs corresponding to 36 TFs. Next, this study generated a core network using the top 100 lncRNA-TF pairs with the most credentiality (lowest *P* values and FDRs), as shown by **Figure 7**. Most of these potential trans-regulatory lncRNAs participated in pathways regulated by three TFs, namely, SUZ12, FOSL2, and STAT3. In the core network of lncRNA-TF pairs, SUZ12 participates in 32 of the 100 pairs, FOSL2 in 15 pairs, and STAT3 in 14 pairs.

The lncRNA-TF-mRNA correlation network was built using Cytoscape. This co-expression network consisted of 253 mRNA-lncRNA/TF pairs. As shown in **Figure 8**, several TFs, including SUZ12, STAT3, and XRCC4, were potential targets in the transcriptional regulation of gene expression in the lncRNA-TF-mRNA network. SUZ12 was a transcription regulator playing an important role in the core network.

Discussion

Recent discoveries have shown that lncRNAs could play different roles in pathogenesis and tumorigenesis. They could be novel clinical biomarkers and potential therapeutic targets in diseases [9]. However, only a few lncRNAs were confirmed to be related to diseases. Most remain unrevealed, including those in PAH. Thus, the present study aimed to uncover the roles of lncRNAs in the pathological development of PAH. To the best of our knowledge, this study is first to report altered lncRNA levels in ET-1-stimulated HPASMCs, examining their possible roles in PAH. Such roles have contributed to a better understanding of the pathogenesis of this devastating disease. Molecular mechanisms of ET-1-induced proliferation of PASMCs have been studied in depth [6, 10], but most of the involved gene transcriptional regulations are still indefinite. In the present study, lncRNA and mRNA expression profiles of ET-1-stimulated HPASMCs were built using gene expression microarrays, such as lncRNA profiling, which has the potential to identify PASMC-associated factors related to PAH.

ET-1 is observably upregulated in patients with PAH and exerts diverse actions on HPASMCs by interacting with G protein-coupled receptors [11]. ET-1 is also the most potent vasoconstrictor and directly modulates HPASMC growth by

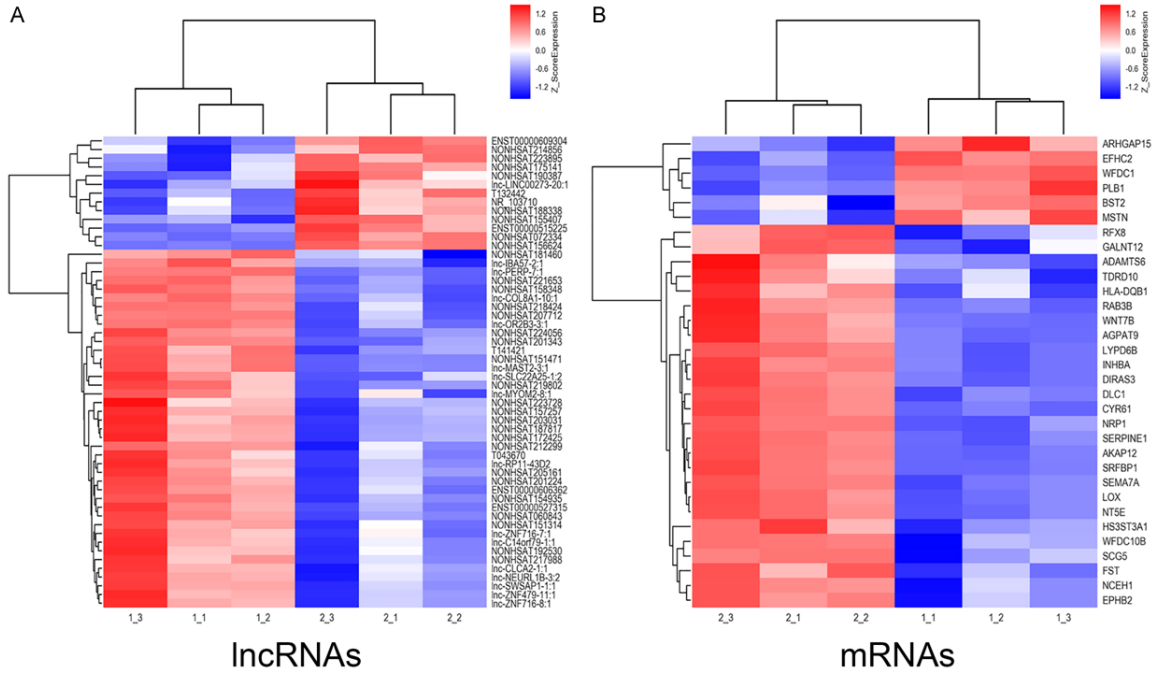


Figure 2. Transcriptomic landscape of HPASMCs with/without ET-1 treatment. (A) Hierarchical clustering analysis of 54 aberrant lncRNAs and (B) 32 aberrant mRNAs (Fold change ≥ 2 and P < 0.05). Red and green colors indicate high and low expression levels, respectively. In the heatmap, columns represent the samples and rows represent individual genes. The scale of the expression level is shown on the horizontal bar.

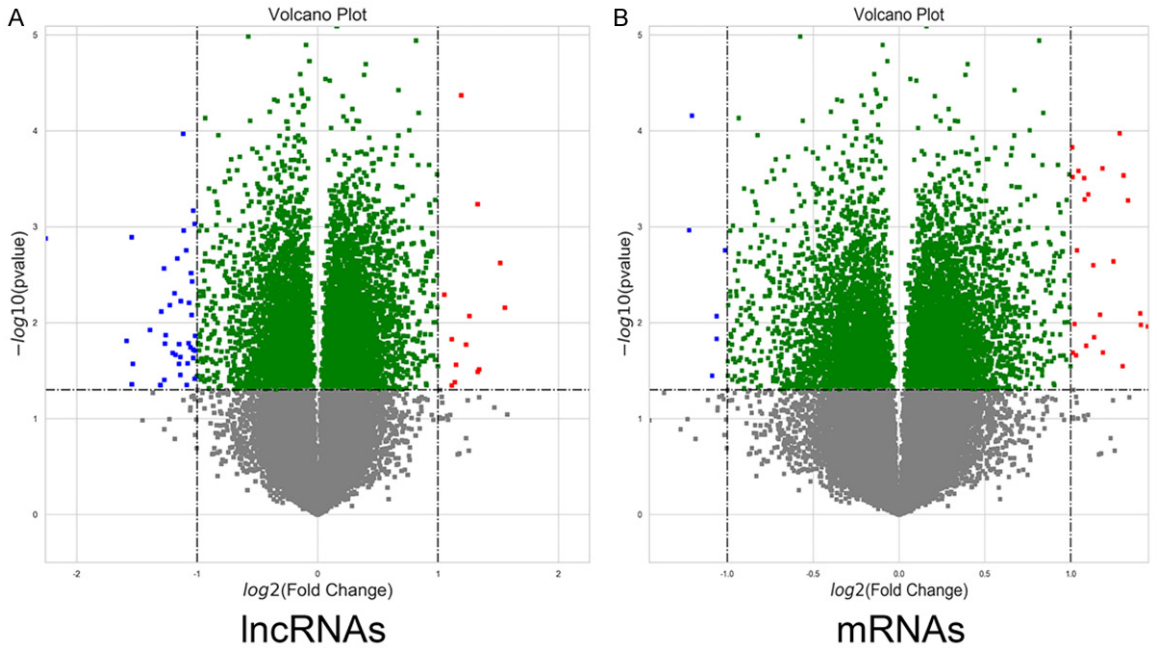


Figure 3. (A) Volcano plots show the distribution of lncRNAs and (B) mRNAs in HPASMCs with/without ET-1 treatment. Significantly up- and downregulated RNAs are presented as red or blue dots, respectively, and RNA expression levels that were not significantly differentially expressed are presented as green or grey dots (Fold change ≥ 2 and P < 0.05).

acting as an autocrine and paracrine secretion mitogen [12]. Along with its strong vasoconstrictive action, ET-1 can promote HPASMC proliferation. It also has anti-apoptotic effects on

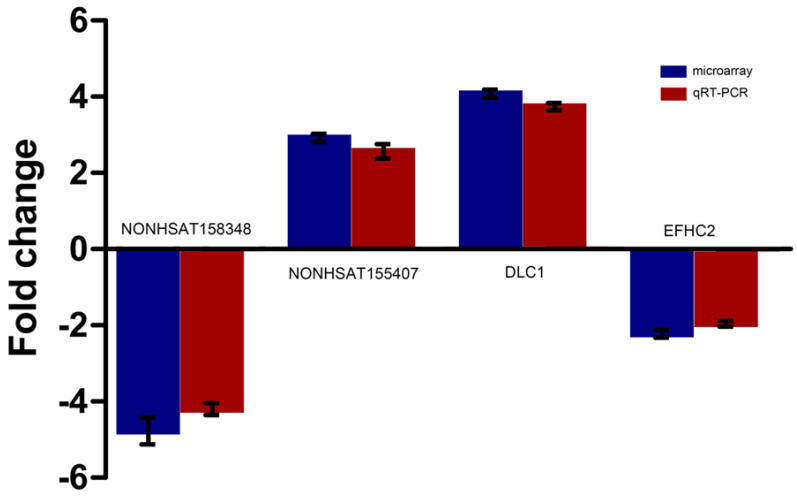


Figure 4. qRT-PCR results of the four chosen lncRNAs and mRNA all validate those of the microarray ($P < 0.05$, Student's *t*-test). The heights of the columns in the chart represent the mean fold changes in the expression for each of these genes. Bars represent SD. Fold change is positive when expression is upregulated and negative when downregulated.

HPASMCs [13]. Present results prove that ET-1 could induce proliferation and apoptosis resistance of HPASMCs. The cellular model of PAH was built using ET-1-treated HPASMCs.

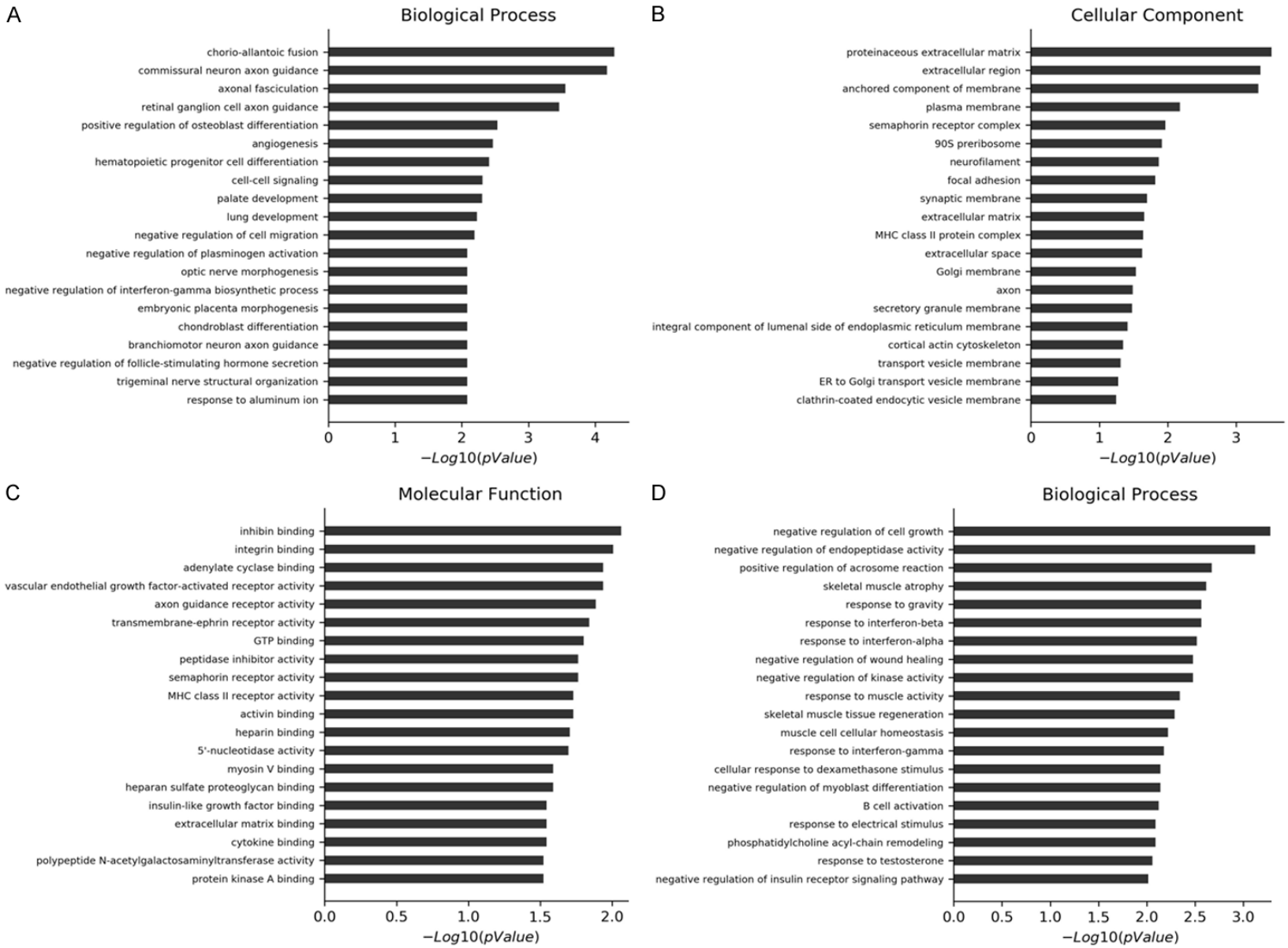
The present study identified aberrantly expressed lncRNAs (41 downregulated and 13 upregulated) and mRNAs (6 downregulated and 26 upregulated) between ET-1-treated HPASMCs and control cells, using bioinformatics analysis. Further function annotation showed that upregulated mRNAs were mainly involved in chorioallantoic fusion, proteinaceous extracellular matrix, and inhibin binding, while downregulated mRNAs were involved in the negative regulation of cell growth, apical plasma membrane, and metalloendopeptidase inhibitor activity. This finding is consistent with the knowledge that HPASMC proliferation is the main cause for PAH development and progression [14].

Furthermore, enriched KEGG pathways of upregulated mRNAs included axon guidance, TGF-β signaling pathways, and HTLV-I infection. TGF-β signaling pathways play a major role in the initiation and progression of PAH. TGF-β is not only an important regulator of vascular remodeling and inflammation in the lungs but also of hypertrophy and fibrosis in the heart [15]. Downregulated mRNAs have been related to vitamin digestion and absorption, alpha-linolenic acid metabolism, and linoleic acid metabolism. Patients with PAH are characterized by having changes in fatty acid compositions, with elevated linoleic, oleic, and docosahexanoic acids in phospholipids [16]. Therefore, monitoring these signaling pathways may aid in the prediction and treatment of PAH progression.

Cis-regulatory elements are regions of noncoding DNA that regulate the transcription of nearby genes [17]. It was found that two differentially-expressed lncRNAs may play cis-regulator roles for the closest differentially-expressed neighboring mRNAs, within 300 kb from the transcription start and stop sites. Moreover, lncRNA NONHSAT151314 was predicted as a positive moderate role in the cis-regulation of AHDC1, while NONHSAT201343 played a negative moderate role in the regulation of CXCL8.

The former mRNA AHDC1 was never reported to join the biological mechanisms of occurrence and development of PAH. However, the latter mRNA CXCL8 is a pro-inflammatory factor that is frequently over-expressed in PAH [18]. Inflammation plays an important role in the pathogenesis of human PAH and anti-inflammation treatment exerts reverse-remodeling effects by augmenting apoptosis and reversing inflammation in PASMC [19]. Present microarray results showed that cis-regulation of the over-expressive status of CXCL8 genes may be possible by downregulating the nearby lncRNA NONHSAT201343. Hence, lncRNA NONHSAT201343 may be a key factor to prevent and reverse pulmonary vascular remodeling.

Although some lncRNAs are cis-regulators, most functionally characterized lncRNAs are actually trans-regulators [20]. This study predicted the function of trans-regulatory lncRNAs through TFs that possibly regulate their expression. In the core network of both lncRNA-TF and lncRNA-TF-mRNA pairs, SUZ12 was the transcription regulator acting a central role in sig-


A
Biological Process
chorio-allantoic fusion
commissural neuron axon guidance
axonal fasciculation
retinal ganglion cell axon guidance
positive regulation of osteoblast differentiation
angiogenesis
hematopoietic progenitor cell differentiation
cell-cell signaling
palate development
lung development
negative regulation of cell migration
negative regulation of plasminogen activation
optic nerve morphogenesis
negative regulation of interferon-gamma biosynthetic process
embryonic placenta morphogenesis
chondroblast differentiation
branchiomotor neuron axon guidance
negative regulation of follicle-stimulating hormone secretion
trigeminal nerve structural organization
response to aluminum ion
0 1 2 3 4
−Log10(pValue)
B
Cellular Component
proteinaceous extracellular matrix
extracellular region
anchored component of membrane
plasma membrane
semaphorin receptor complex
90S preribosome
neurofilament
focal adhesion
synaptic membrane
extracellular matrix
MHC class II protein complex
extracellular space
Golgi membrane
axon
secretory granule membrane
integral component of lumenal side of endoplasmic reticulum membrane
cortical actin cytoskeleton
transport vesicle membrane
ER to Golgi transport vesicle membrane
clathrin-coated endocytic vesicle membrane
0 1 2 3
−Log10(pValue)
C
Molecular Function
inhibin binding
integrin binding
adenylate cyclase binding
vascular endothelial growth factor-activated receptor activity
axon guidance receptor activity
transmembrane-ephrin receptor activity
GTP binding
peptidase inhibitor activity
semaphorin receptor activity
MHC class II receptor activity
activin binding
heparin binding
5'-nucleotidase activity
myosin V binding
heparan sulfate proteoglycan binding
insulin-like growth factor binding
extracellular matrix binding
cytokine binding
polypeptide N-acetylgalactosaminyltransferase activity
protein kinase A binding
0.0 0.5 1.0 1.5 2.0
−Log10(pValue)
D
Biological Process
negative regulation of cell growth
negative regulation of endopeptidase activity
positive regulation of acrosome reaction
skeletal muscle atrophy
response to gravity
response to interferon-beta
response to interferon-alpha
negative regulation of wound healing
negative regulation of kinase activity
response to muscle activity
skeletal muscle tissue regeneration
muscle cell cellular homeostasis
response to interferon-gamma
cellular response to dexamethasone stimulus
negative regulation of myoblast differentiation
B cell activation
response to electrical stimulus
phosphatidylcholine acyl-chain remodeling
response to testosterone
negative regulation of insulin receptor signaling pathway
0.0 0.5 1.0 1.5 2.0 2.5 3.0
−Log10(pValue)

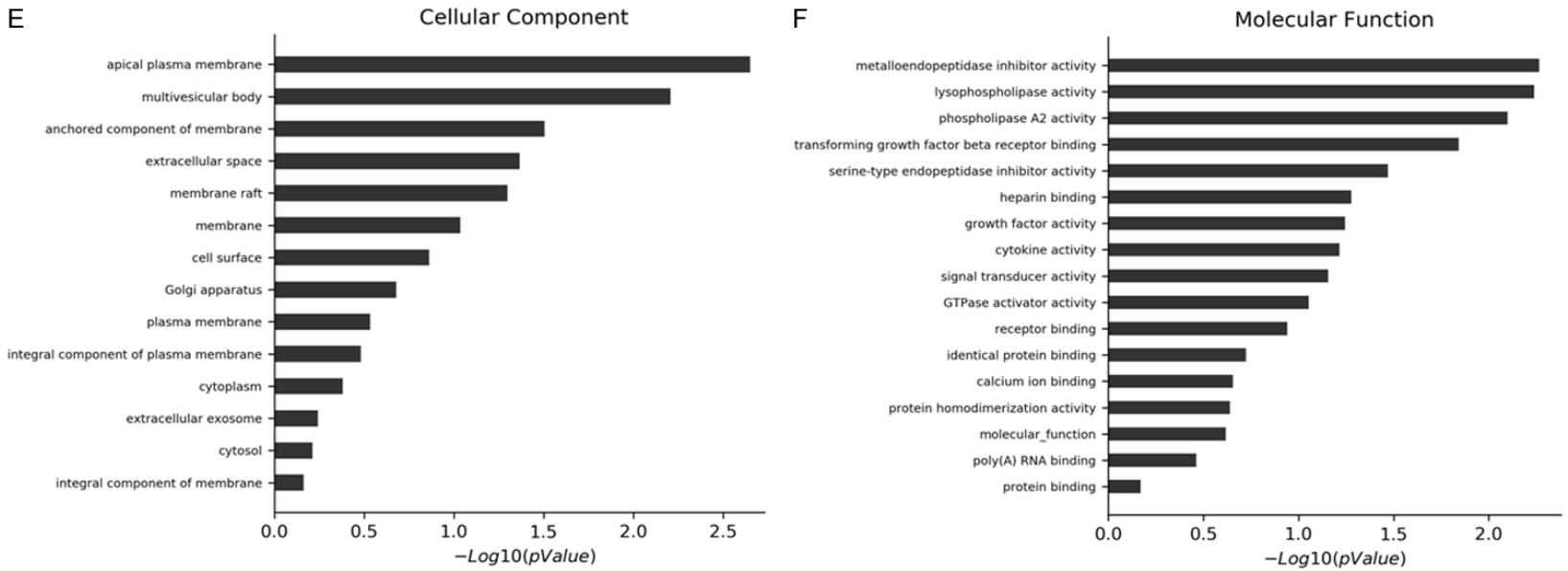


Figure 5. Gene ontology analysis of differentially-expressed genes associated in HPASMCs with/without ET-1 treatment.

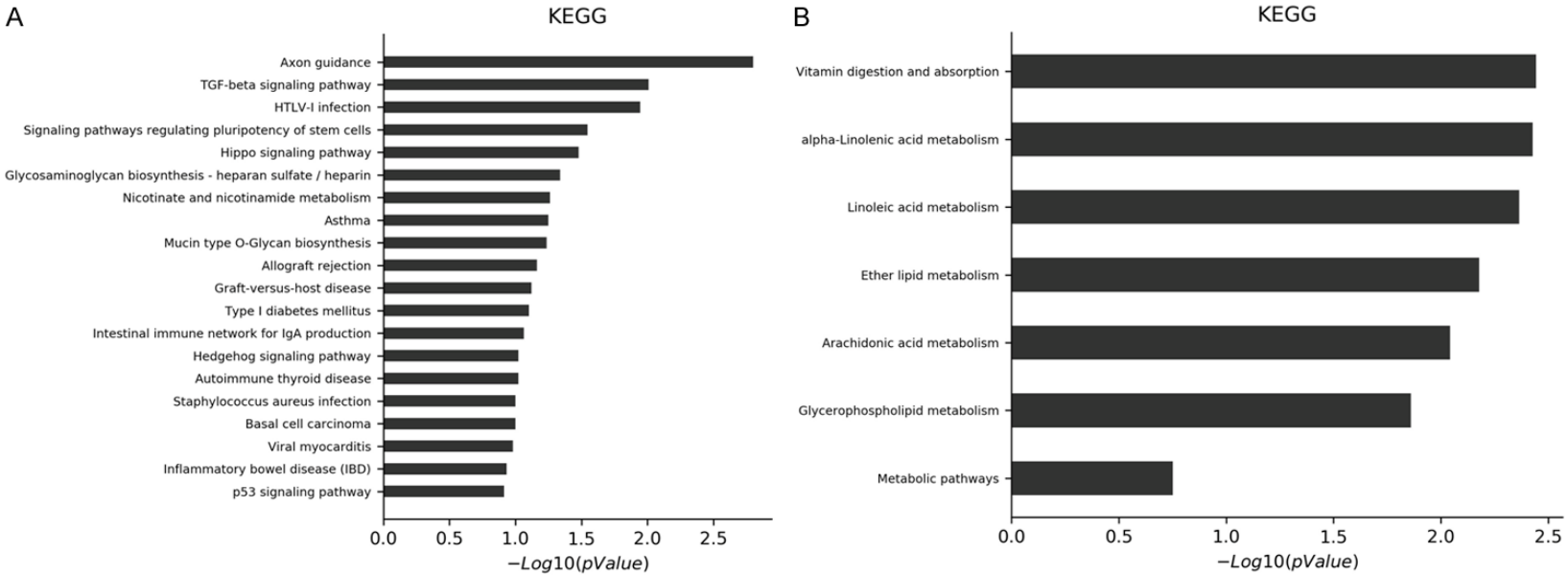


Figure 6. Pathway analysis of differentially-expressed genes in HPASMCs with/without ET-1 treatment. (A) Significant pathways of upregulated genes and (B) down-regulated genes. The pathways are considered statistically significant at $P < 0.05$.

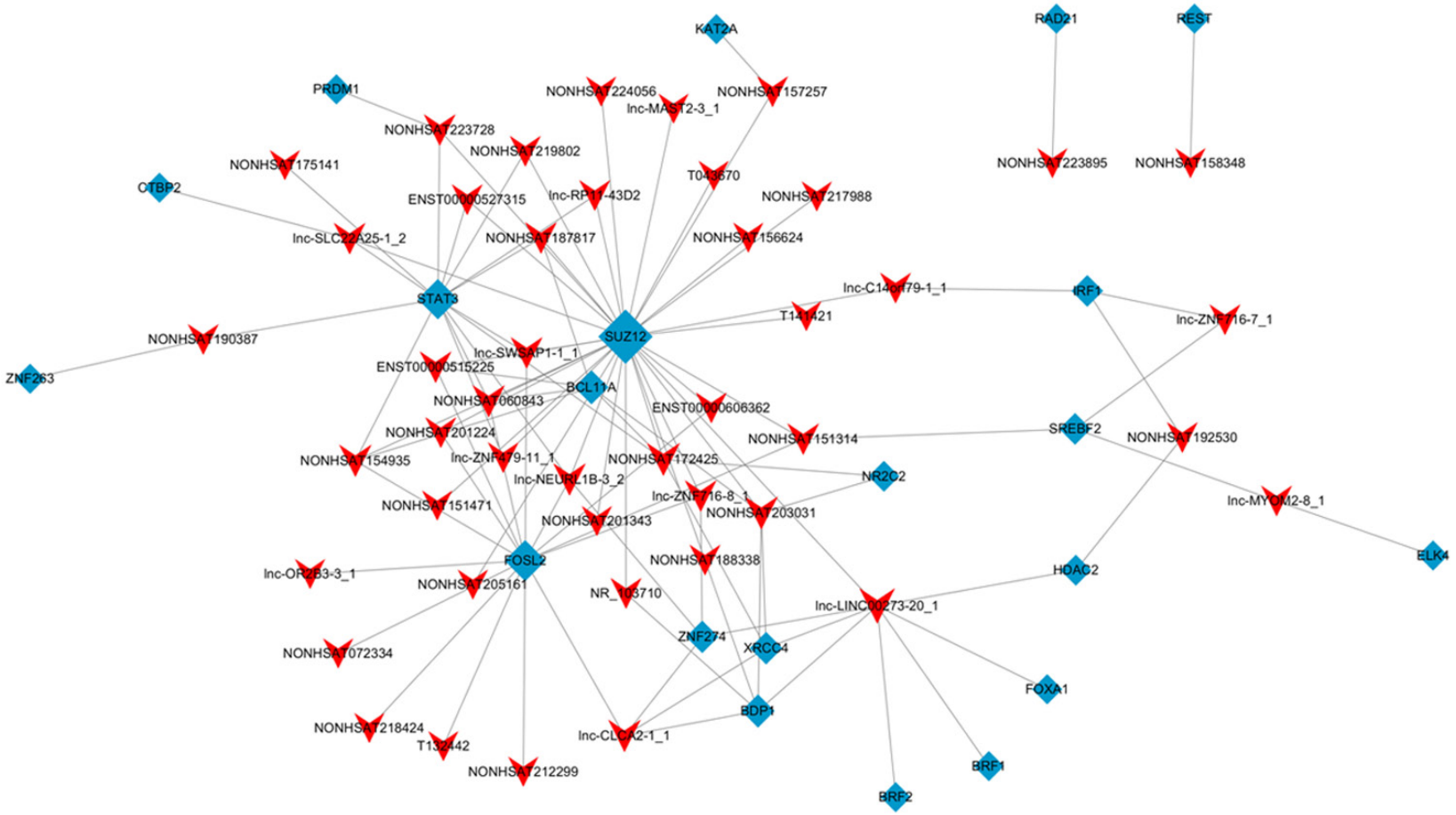


Figure 7. lncRNA-TF core network consisting of the top 100 pairs of lncRNA and TF with the most relevance. The red arrows represent lncRNAs and blue squares represent TFs. Edges between them indicate that the lncRNAs were potentially regulated by the TFs. lncRNAs = long noncoding RNAs, TF = transcript factor.

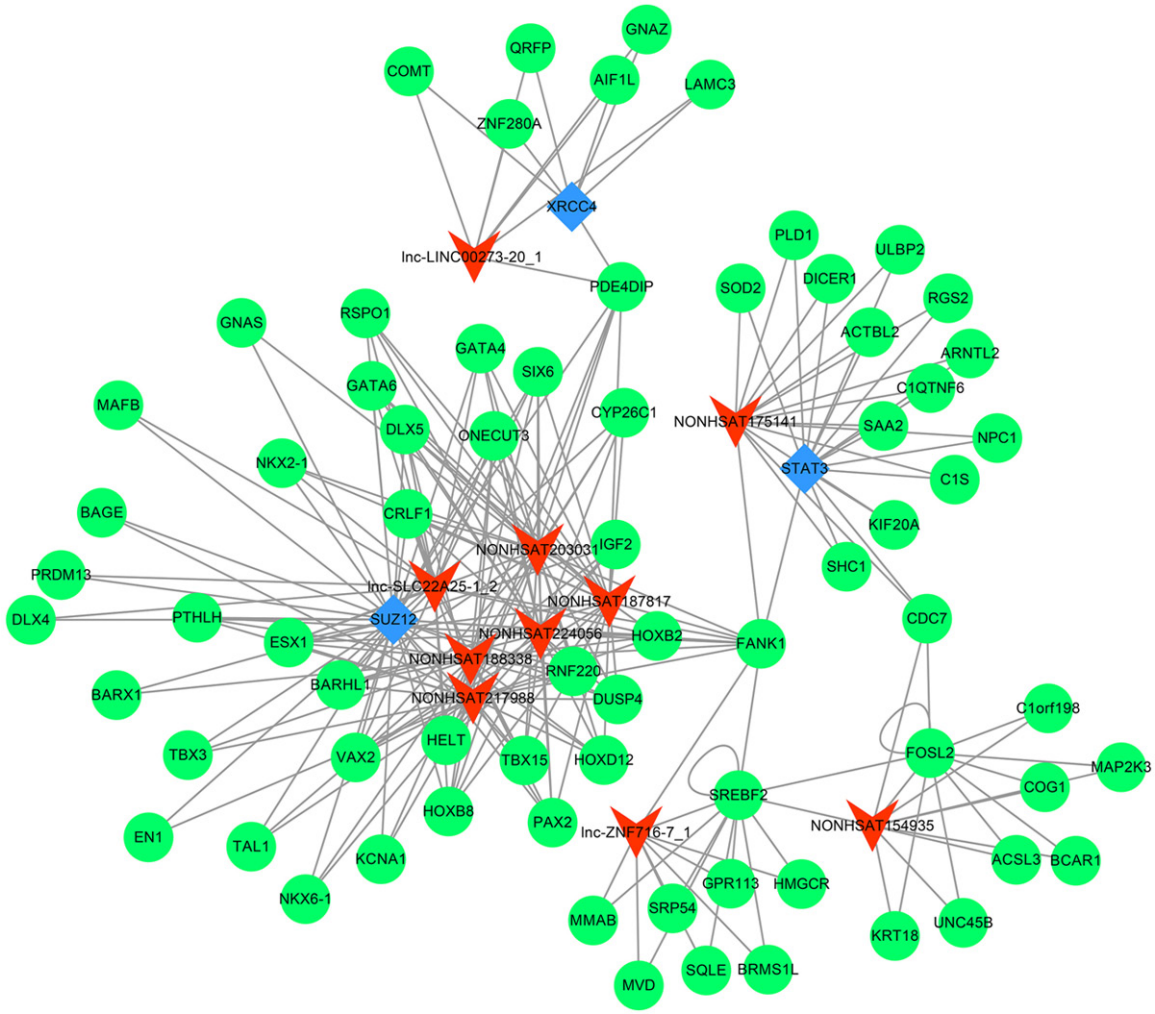


Figure 8. lncRNA-TF-mRNA core network consisting of all pairs of lncRNA, TF, and mRNA. The red arrows represent lncRNAs, blue squares represent TF, and green circles represent mRNAs. lncRNAs = long noncoding RNAs, TF = transcript factor.

naling pathways. SUZ12, one of the core polycomb repressive complex 2 (PRC2) components, has been increasingly appreciated as a key mediator during human tumorigenesis. PRC2, a member of the polycomb group, harbors multiple core members executing their functions by histone modifications [21]. SUZ12 has been found to be frequently over-expressed in several solid cancers. Its aberrant overexpression has been significantly associated with aggressive clinicopathological features and inferior survival [22-25]. However, expression patterns and biological mechanisms of SUZ12 in PAH remain largely unexplored. Present data shows that SUZ12 was recruited by 32 lncRNAs, according to trans-regulation analysis of the top 100 lncRNA-TF pairs, suggesting that coordinated patterns of lncRNAs and transcription factors involved in PAH development may be present. Further functional studies are necessary to determine expression patterns, as well as clinicopathological significance and biological roles in PAH.

This preliminary study identified a subset of aberrantly expressed lncRNAs in ET-1-stimulated PASMCs. These lncRNAs might contribute to the proliferation and anti-apoptosis of HPASMCs and PAH development by cis- and/or trans-regulation of protein-coding genes. Further mechanism studies of these lncRNAs are needed. These studies may expand the understanding of PAH pathogenesis, providing new approaches for treatment of this disease.

Acknowledgements

The work was supported by the Natural Science Foundation of Jiangsu Province (BK20151589, BK20181083).

Disclosure of conflict of interest

None.

Address correspondence to: Wei Wang, Department of Thoracic Surgery, The First Affiliated Hospital of Nanjing Medical University, No. 300, Guangzhou Road, Nanjing 210029, China. Tel: +86-1526188-3958; E-mail: wangwei15261883958@163.com

References

[1] Thenappan T, Shah SJ, Rich S, Tian L, Archer SL and Gomberg-Maitland M. Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. Eur Respir J 2010; 35: 1079-1087.

[2] Maron BA and Galie N. Diagnosis, treatment, and clinical management of pulmonary arterial hypertension in the contemporary era: a review. JAMA Cardiol 2016; 1: 1056-1065.

[3] He RL, Wu ZJ, Liu XR, Gui LX, Wang RX and Lin MJ. Calcineurin/NFAT signaling modulates pulmonary artery smooth muscle cell proliferation, migration and apoptosis in monocrotaline-induced pulmonary arterial hypertension rats. Cell Physiol Biochem 2018; 49: 172-189.

[4] Shihoya W, Nishizawa T, Okuta A, Tani K, Dohmae N, Fujiyoshi Y, Nureki O and Doi T. Activation mechanism of endothelin ETB receptor by endothelin-1. Nature 2016; 537: 363-368.

[5] Clozel M. Endothelin research and the discovery of macitentan for the treatment of pulmonary arterial hypertension. Am J Physiol Regul Integr Comp Physiol 2016; 311: R721-R726.

[6] Sun K, Xue H, Wang H, Wang Q, Zuo XR, Xie WP and Wang H. The effects of siRNA against RPL22 on ET-1-induced proliferation of human pulmonary arterial smooth muscle cells. Int J Mol Med 2012; 30: 351-357.

[7] Lan NSH, Massam BD, Kulkarni SS and Lang CC. Pulmonary arterial hypertension: pathophysiology and treatment. Diseases 2018; 6.

[8] Deng L, Bradshaw AC and Baker AH. Role of noncoding RNA in vascular remodelling. Curr Opin Lipidol 2016; 27: 439-448.

[9] Sun PR, Jia SZ, Lin H, Leng JH and Lang JH. Genome-wide profiling of long noncoding ribonucleic acid expression patterns in ovarian endometriosis by microarray. Fertil Steril 2014; 101: 1038-1046, e1037.

[10] Schmeck J, Konrad C, Schoffel S, Wendel-Wellner M, Gluth H, Koch T and Krafft P. Interaction of acetylcholine and endothelin-1 in the modulation of pulmonary arterial pressure. Crit Care Med 2000; 28: 3869-3875.

[11] Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, Pollock DM, Webb DJ and Maguire JJ. Endothelin. Pharmacol Rev 2016; 68: 357-418.

[12] Huetsch JC, Walker J, Undem C, Lade J, Yun X, Baksh S, Jiang H, Lai N and Shimoda LA. Rho kinase and Na(+)/H(+) exchanger mediate endothelin-1-induced pulmonary arterial smooth muscle cell proliferation and migration. Physiol Rep 2018; 6: e13698.

[13] Jankov RP, Kantores C, Belcastro R, Yi M and Tanswell AK. Endothelin-1 inhibits apoptosis of pulmonary arterial smooth muscle in the neonatal rat. Pediatr Res 2006; 60: 245-251.

[14] Qian Z, Li Y, Yang H, Chen J, Li X and Gou D. PDGFBB promotes proliferation and migration via regulating miR-1181/STAT3 axis in human pulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2018; 315: L965-L976.

[15] Rol N, Kurakula KB, Happe C, Bogaard HJ and Goumans MJ. TGF-beta and BMPR2 signaling in PAH: two black sheep in one family. Int J Mol Sci 2018; 19.

[16] Semen K, Yelisyeyeva O, Jarocka-Karpowicz I, Kaminskyy D, Solovey L, Skrzydlewska E and Yavorskyi O. Sildenafil reduces signs of oxidative stress in pulmonary arterial hypertension: evaluation by fatty acid composition, level of hydroxynonenal and heart rate variability. Redox Biol 2016; 7: 48-57.

[17] Hajdu M, Calle J, Puno A, Haruna A and Arenas-Mena C. Transcriptional and post-transcriptional regulation of histone variant H2A.Z during sea urchin development. Dev Growth Differ 2016; 58: 727-740.

[18] Li WJ, Hu K, Yang JP, Xu XY, Li N, Wen ZP and Wang H. HMGB1 affects the development of pulmonary arterial hypertension via RAGE. Eur Rev Med Pharmacol Sci 2017; 21: 3950-3958.

[19] Mumby S, Gambaryan N, Meng C, Perros F, Humbert M, Wort SJ and Adcock IM. Bromodomain and extra-terminal protein mimic JQ1 decreases inflammation in human vascular endothelial cells: Implications for pulmonary arterial hypertension. Respirology 2017; 22: 157-164.

[20] Guttman M and Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-346.

[21] Simon JA and Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 2009; 10: 697-708.

[22] Li H, Cai Q, Wu H, Vathipadiekal V, Dobbin ZC, Li T, Hua X, Landen CN, Birrer MJ, Sanchez-Beato M and Zhang R. SUZ12 promotes human epithelial ovarian cancer by suppressing apoptosis via silencing HRK. Mol Cancer Res 2012; 10: 1462-1472.

[23] Liu C, Shi X, Wang L, Wu Y, Jin F, Bai C and Song Y. SUZ12 is involved in progression of non-small cell lung cancer by promoting cell proliferation and metastasis. Tumour Biol 2014; 35: 6073-6082.

[24] Liu YL, Gao X, Jiang Y, Zhang G, Sun ZC, Cui BB and Yang YM. Expression and clinicopathological significance of EED, SUZ12 and EZH2 mRNA in colorectal cancer. J Cancer Res Clin Oncol 2015; 141: 661-669.

[25] Wu Y, Hu H, Zhang W, Li Z, Diao P, Wang D, Zhang W, Wang Y, Yang J and Cheng J. SUZ12 is a novel putative oncogene promoting tumorigenesis in head and neck squamous cell carcinoma. J Cell Mol Med 2018; 22: 3582-3594.