LncRNA and mRNA interaction study based on transcriptome profiles reveals potential core genes in the pathogenesis of human thoracic aortic dissection

YANG LI[1*], NAN YANG[2*], XIANBAO ZHOU[1], XUEZHI BIAN[1], GENQIANG QIU[1], MO ZHANG[3], HUAGANG LIN[3] and DINGFENG LI[3]

[1]Department of General Surgery, Beijing Yuho Rehabilitation Hospital of Integrated Chinese and Western Medicine, Beijing 100039; [2]Department of Stomatology, PLA 309th Hospital, Beijing 100091; [3]Department of Orthopedics, Beijing Yuho Rehabilitation Hospital of Integrated Chinese and Western Medicine, Beijing 100039, P.R. China

Abstract. The aim of the present study was to determine the potential core genes in the pathogenesis of human thoracic aortic dissection (TAD) by analyzing microarray profiles of long non-coding (lnc)-RNAs between TAD and normal thoracic aorta (NTA). The differentially expressed lncRNA profiles of the aorta tissues between TAD patients (TAD group, n=6) and age-matched donors with aortic diseases (NTA group, n=6) were analyzed by lncRNAs microarray. Gene ontology (GO), pathway and network analyses were used to further investigate candidate lncRNAs and mRNAs. Differentially expressed lncRNAs and mRNAs were validated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). In total, the present study identified 765 lncRNAs and 619 mRNAs with differential expression between TAD and NTA (fold change >2.0, P<0.01). GO analysis demonstrated that the differentially upregulated lncRNAs are associated with cell differentiation, homeostasis, cell growth and angiogenesis. Kyoto Encyclopedia of Gene and Genomes pathway analysis demonstrated that the differentially downregulated lncRNAs are mainly associated with arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy and dilated cardiomyopathy. To reduce the lncRNAs for further investigation and to enrich those potentially involved in TAD, a total of 16 candidate lncRNAs with a significant expression (fold change >4, P<0.01) were selected, that were associated with an annotated protein-coding gene through the GO term and scientific literatures. Then a set of significantly expressed lncRNAs [purinergic receptor P2X7 (P2RX7), hypoxia inducing factor (HIF)-1A-AS2, AX746823, RP11-69I8.3 and RP11-536K7.5] and the corresponding mRNAs (P2RX7, cyclin dependent kinase inhibitor 2B, HIF-1A, runt-related transcription factor 1, connective tissue growth factor and interleukin 2 receptor a chain] were confirmed using RT-qPCR. The present study revealed that the expression profiles of lncRNAs and mRNAs in aorta tissues from TAD were significantly altered. These results may provide important insights into the pathogenesis of TAD disease.

Introduction

Thoracic aortic dissection (TAD) is a highly lethal cardiovascular disease, which is characterized by the separation of thoracic aortic medial layer along the length of the vessel (1). Typical TAD begins with the sudden initial tear in the aortic intima, and intraluminal pulsatile blood enters into the medial layer through an intimal tear, resulting in rapid aortic dilation and rupture. In spite of the improvement of diagnostic and therapeutic techniques over the years, the overall mortality of TAD remains high (2). The understanding of the pathogenesis of this serious illness may produce a better outcome in the future. Many studies have been performed to explore the pathogenesis of TAD. Most studies were mainly focused on the genetic diversity, clinical pathology, and hemodynamics. However, the potential molecular mechanism of TAD remains unclear.

Long non-coding RNAs (lncRNAs) are defined as more than 200 nt in length and found to regulate protein-coding gene expression at both the transcriptional and post-transcriptional levels. Studies showed that lncRNAs play critical roles in cardiovascular physio-pathological processes (3). For example, lncRNA Braveheart (Bvht) was associated with cardiovascular development (4). Upregulated myocardial infarction-associated transcript 1 (Mirt1) and Mirt2 can promote cardiac contractile function and decrease left ventricular remodeling (5). Myosin heavy chain-associated RNA transcript (Mhrt) can protect the

Correspondence to: Dr Yang Li, Department of General Surgery, Beijing Yuho Rehabilitation Hospital of Integrated Chinese and Western Medicine, 15 Yongding Road, Haidian, Beijing 100039, P.R. China
E-mail: juxieyu2017@sina.com

*Contributed equally

heart from pathological cardiac hypertrophy (6). Although an increasing number of lncRNAs have been characterized, the role of lncRNAs in TAD has not been investigated. A recent study revealed that the overexpressed lncRNA HIF1 alpha-antisense RNA 1 (HIF1A-AS1) in the thoraco-abdominal aortic aneurysm (TAAA) promoted the proliferation and apoptosis of vascular smooth muscle cells (VSMCs), which may contribute to the pathogenesis of TAAA (7). Wang et al (8) reported that the interaction between BRG1 and HIF1A-AS1 may be involved in the pathogenesis of thoracic aortic aneurysms. Since TAA and TAD possesses similar pathological basis, these results suggested the potential role of lncRNAs in the pathogenesis of TAD. However, until now, there was no study on the expression profile of lncRNAs in TAD.

In the present study, we demonstrated expression profile of lncRNAs between TAD and normal thoracic aorta (NTA) using third-generation lncRNA microarray techniques. These results will help provide further insight into the pathogenesis of TAD.

Materials and methods

Acquisition of clinical specimens. Ascending aorta specimens were obtained from TAD patients undergoing surgical repair (TAD group, n=6; mean age, 51.4±13.4 years) at Fuwai Hospital and organ donors without aortic diseases (NTA group, n=6; mean age, 49.6±12.6 years). No significant difference in the age was found between TAD and NTA (P>0.05). All patients were confirmed to have acute Stanford type I aortic dissection within 14 days of the symptom onset before surgery. All subjects have not any history of Marfan syndrome, bicuspid aortic valve, or any other connective tissue disease. The clinical characteristics of patients and donors were in Table I. The study protocol was approved by the international review board of Beijing Yuho Rehabilitation Hospital, (Beijing, China). Written informed consent was obtained from each of the patients. Aortic media tissues were sectioned into smaller sizes and briefly stored at -80°C until RNA extraction.

RNA isolation and lncRNA microarray analysis. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the manufacturer's instruction. For the global profiling of human lncRNAs and protein-coding transcripts, we utilized the third-generation lncRNA microarray (Arraystar v3.0; KangChen, Shanghai, China), which contains more than 30,000 capture probes, covering all lncRNAs from authoritative databases (UCSC Knowngenes, RefSeq and Ensembl) and their coding proteins. The microarray hybridization was performed based on manufacturer's instruction. Quantile normalization and subsequent data analysis was performed using GeneSpring GX 11.5.1 software (Agilent Technologies, Inc., Santa Clara, CA, USA).

Validation by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). To confirm the microarray data, expressions of selected lncRNA and coding proteins were tested using RT-qPCR with the GoTaq qPCR Master Mix (Promega Corporation, Madison, WI, USA) on an Mx3005P real-time PCR System (Agilent Technologies, Inc.). The primers used for RT-qPCR were listed in Table II. Each RNA sample was

Table I. Clinical characteristics.

Variables	NTA (n=6)	TAD (n=6)
Age (years)	49.6±12.6	51.4±13.4
Males/females	6/0	6/0
Hypertension	2 (33.3%)	3 (50.0%)
Atherosclerosis	1 (16.7%)	2 (33.3%)
Smoking	2 (33.3%)	3 (50.0%)
Aortic size (mm)	31.4±9.7	63.4±15.2

Data are presented as the mean ± standard deviation, as the n number of individuals or as n (%). NTA, normal thoracic aorta; TAD, thoracic aortic dissection.

evaluated in triplicate. Gene expression results were analyzed with the $2^{-\Delta\Delta Cq}$ method and normalized to GAPDH expression.

Gene ontology (GO) and pathway analysis. The GO analysis (www.geneontology.org) and Kyoto Encyclopedia of Gene and Genomes database (KEGG, www.genome.jp/kegg) were used to analyze the main function and pathway of differentially expressed lncRNAs and mRNAs.

LncRNA and mRNA interaction network analysis. MetaCoreTM software (Auto Expand Algorithm) was used to analyze the regulatory network of differentially expressed genes associated with lncRNAs.

Statistical analysis. All statistical data were analyzed by SPSS v20.0 software (IBM Corp., Armonk, NY, USA). Data were presented as the mean ± standard deviation of at least 3 independent experiments. The results were analyzed by analysis of variance with a Bonferroni post hoc test, or by Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

LncRNA microarray profiling. Arraystar lncRNA Microarray (v3.0), which can detect about 30,586 lncRNAs and 26,109 coding transcripts, was used for the comprehensive analysis of core genes associated with the pathogenesis of TAD. Using third-generation lncRNA microarray, we detected a total of 25,348 lncRNAs and 24,852 mRNAs. The statistical information of lncRNAs was shown in Fig. 1A. Furthermore, the differentially expressed lncRNAs and mRNAs were screened as per the fold-change and P-value. The screening criteria for differentially expressed lncRNAs and mRNAs is fold-change ≥2 and P-value <0.01. Subsequently, we have used log2 (fold-change) as the X-axis and log10 (P-value) as the Y-axis to show the distribution of lncRNAs and mRNAs in Volcano plot (Fig. 1B and C). In total, 765 differentially expressed lncRNA were identified between TAD and NTA, including 289 up-regulated and 476 down-regulated (fold-change ≥2, P<0.01). Through microarray, we found 619 differentially expressed mRNA (fold-change ≥2, P<0.01). Among mRNAs, 265 were up-regulated and 354 were down-regulated in TAD compared with NTA.

Table II. Primers used in the present study.

A, lncRNA

Gene name	Forward (5'-3')	Reverse (3'-5')
CERKL	TGTAAAGTTGAATAAAGCGG	TGCCTTGGGCTGTCGTAG
RP11-395B7.4	AGGACTTGGAACTATGGG	AAGGGAGAATTAAAGGCTA
RP11-887P2.1	TGTGCCTGGCTGCCTTAG	TAGCCGGAGGGTTAGGGAT
lncP2RX7	GGGGGTTGAATGTGAGATGA	GTTAGGGAATGTTCTGAAGG
CDKN2B-AS1	AGATGCAGAGGACATGCACGG	GGCAAGGCAAGTGAGGAG
RP11-796E2.4	CGCCTGTAATCCCAGCAC	TCCGGGTTCACGCCATTC
HIF1A-AS2	TTCAACCCTGCTTCCTGG	TGCTACCTTCTGTCCTGG
HOXD-AS2	CCTCAGTACATAAACTTCCTAA	CTTTCACCTTCTCACAGG
AC007254.3	ACGACCAGGCTTACGGAG	TCATTTCCCGTGTTGGGC
OGFR-AS1	ACTGCCCTTGTTAATGTCA	TGGTCGTATTGTTCTTGC
AX746823	TCGATTTCGTCAAGCATT	GAGGGCAGAAAGATCACA
RP11-69I8.3	GAAGTCAGAAATACTGTGGGTA	CAAATGTGTGTTCGTGGG
RP11-317J10.2	CTCCAAACTGTCTCACCC	AAAACCACCGTTACCAAA
RP11-318K15.2	GCTCTTACCGCTGGGTGT	CCTTGGGGAGGGAAGTTAG
RP11-536K7.5	CACAGTAGAGGCGAAACC	CAGGGAATAAGTGGACAGAT
NPPA-AS1	GTGAAGAAAGACTTCAT	GTAGGGAAGGCAGTAGGGTGGAG

B, mRNAs

Gene name	Forward (5'-3')	Reverse (3'-5')
ITGA4	TGGACAGCTAGAATTGGT	GTAGCTCTTAGATCCGGTG
MUC12	CATGTACGTCTGCTATCCAGG	CTCCTTAGTCATACCACGATG
SOCS2	TATAGGTGCAGGAGATGGTTC	GCTGTTATGGGTGAAACTCTG
P2RX7	GGCGTCGTGGTGAATGATG	CCTAACTTGGCGTCCTATGC
CDKN2B	TCAACGCTCGATTCTATTGTGG	TCAACGAGATTCTGATTGTGGG
BTG1	GGGGGCGGATACTAATCCC	CACGACAAAAGCGTACTCTGT
HIF1A	TCTCTGACGGCTATCCAAAGAA	GCAGCAGTTCTGGGTTAGTTG
HOXD3	CTTGGAGGACGGCGATTA	CCGTCACGAACTTCTTCGT
SLC8A1	GCTAGAAGCCGTGACGGCT	CTGACGGTACTCTAGTGGAG
OGFR	TCGTCGTCTAGACATTA	CTCTAGTGGGTGACTC
RUNX1	GACGGCTGTCTATCCAAA	GAAAGACGTCTCTTC
CTGF	AAGGTCAAGCTAGACTG	GGAGACTAAGGCAGTG
CA2	ATTGCTTATGAGTGAACGCC	AGGCATAGGTAGGCAGAG
LYN	GGTGGTGAAGATTAATCGC	ATGGGACATTTAAGGCAATC
IL2RA	GGAAGTAATACAATAGA	GCTGTTAACAACGTAAACTC
NPPA	AGCATGTAGGAGAGTTAACA	TAGAGGGGACCTTACAAG
GAPDH	GCCGTCTAGACCTGACCT	AGGATTGTCGCGTGGTGG

Expression signatures of dysregulated lncRNAs between TAD and NTA. The distribution of relationship types, the sequence length and chromosomes of differentially expressed lncRNA are shown in Fig. 2. For those up-regulated lncRNAs, there were 156 intergenic, 75 natural antisense, 69 exon sense-overlapping, 57 intronic antisense, 30 intron sense-overlapping and 26 bidirectional lncRNAs. For those down-regulated lncRNAs, there were 329 intergenic, 105 natural antisense, 99 intronic antisense, 39 exon sense-overlapping, 19 bidirectional, and 11 intron sense-overlapping lncRNAs (Fig. 2A). The lncRNAs were mainly concentrated between 400 and 800 bp in length (Fig. 2B). The chromosome distribution showed the up-regulated and down-regulated lncRNAs were located at various chromosomes, respectively (Fig. 2C).

GO and pathway analysis. GO analysis indicated that the functions of up-regulated mRNAs were involved in a variety of biological processes, including VSMC development and vascular homeostasis, such as in cell growth (GO:0001558), cell differentiation (GO:0045597), homeostatic process
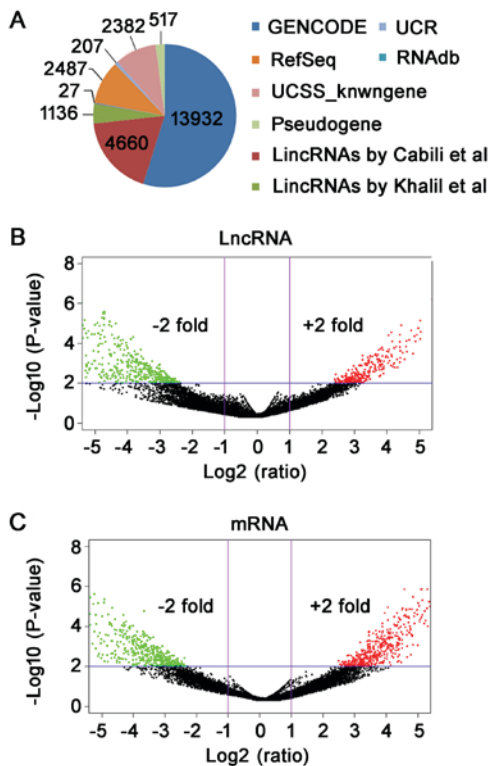
Figure 1. Differential expression of lncRNAs between TAD and NTA by microarray analysis. (A) A pie chart presenting the relative numbers of lncRNAs from the majority of authoritative databases. Volcano plots were constructed using fold-change and P-values to present the distribution of (B) lncRNAs and (C) mRNAs, respectively. The vertical lines represent P<0.05 differences, and the horizontal lines correspond to 2.0-fold up and down. The red and green points in the plot represent the differentially expressed genes with statistical significance. TAD, thoracic aortic dissection; NTA, normal thoracic aorta, LncRNA, long non-coding RNA.

(GO:0032844) and angiogenesis (GO:0045766; Fig. 3A). Meanwhile, the functions of down-regulated mRNAs were mainly involved in cell-cell signaling (GO:0007267), transcription regulation (GO:0045893), RNA metabolism (GO:0051254), and gene expression (GO:0010628; Fig. 3B). KEGG pathway analysis indicated that 31 pathways were down-regulated and 24 pathways were up-regulated. Most of up-regulated pathways were enriched in Jak-STAT signaling pathway (KEGG: hsa04630), chemokine signaling pathway (KEGG: hsa04062), PPAR (KEGG: hsa03320) signaling pathway and B cell receptor signaling pathway (KEGG: hsa04662), suggested that up-regulated pathways in TAD were closely associated with signal transduction (Fig. 3C). Moreover, down-regulated pathways were enriched in calcium signaling pathway (KEGG: hsa04020), arrhythmogenic right ventricular cardiomyopathy (ARVC) (KEGG: hsa05412), hypertrophic cardiomyopathy (HCM) (KEGG: hsa05410) and dilated cardiomyopathy (KEGG: hsa05414; Fig. 3D).

Selection of core genes in TAD. To reduce the lncRNAs for further investigation and to enrich those potentially involved in TAD, we first selected candidate lncRNAs with a significant expression (fold-change >4, P<0.01) that were associated with an annotated protein-coding gene through the GO term enrichment and scientific literatures. After functional annotation and enrichment analysis, we found: i) 8 protein-coding

genes (BTG1; CA2; CDKN2B; HIF1A; IL2RA; LYN; RUNX1; HOXD3) are associated with cell differentiation; ii) 5 genes (CA2, HIF1A, IL2RA, LYN, P2RX7) are related to homeostasis; iii) 6) genes (CTGF, SOCS2, NPPA, MUC12, OGFR, BTG1) are correlated with cell growth; iv) 3 genes (BTG1, HIF1A, RUNX1) have positive regulation on angiogenesis and v) 2 genes (ITGA4, SLC8A1) are associated with cardiac disease. Finally, 16 lncRNAs associated with important genes were selected as candidates for the pathogenesis of TAD (Table III).

Regulatory network analysis. To further study relationship between coding genes, regulatory network analysis was performed by MetaCoreTM software to show network objects of 16 most promising lncRNA candidates (Fig. 4). Network objects that were associated with 16 candidate lncRNAs were listed Table IV. Similarly, 16 mRNA candidates (ITGA4, MUC12, SOCS2, P2RX7, CDKN2B, BTG1, HIF1A, HOXD3, SLC8A1, OGFR, RUNX1, CTGF, CA2, LYN, IL2RA and NPPA) also played an important role in the regulatory network. Additionally, more genes and signal pathways were involved in this network, suggested regulatory mechanisms of lncRNAs and mRNAs are complex in the pathogenesis of TAD.

Validation of lncRNA and mRNA candidates by RT-qPCR. Validation of lncRNAs candidates by RT-qPCR was shown in Fig. 5A. The detection of the expression level of selected 16 lncRNAs demonstrated a good consistency with the microarray results. Among them, lncP2RX7, HIF1A-AS2, AX746823, RP11-69I8.3 and RP11-536K7.5 increased dramatically in the TAD group compared with the NTA group (P<0.01, respectively). Validation of 16 mRNAs candidates by RT-qPCR was shown in Fig. 5B. Among mRNAs, P2RX7, CDKN2B, HIF-1A, RUNX1, CTGF and IL2RA increased significantly in the TAD group compared with the NTA group (P<0.01, respectively). These genes may play critical roles in the pathogenesis of TAD.

Discussion

TAD is a life-threatening vascular disease that involves the separation of the layers within the aortic wall. Its formation, progression, and rupture cannot be reliably prevented by pharmacological therapies because the molecular mechanisms of the pathogenesis are still currently unclear (9). LncRNAs are a newly discovered class of non-coding RNAs, and they have important functions in regulating a variety of physio-pathological processes at transcriptional and post-transcriptional levels (10). Comprehensive comparison study of lncRNA expression profiles may help to identify candidate genes for the pathogenesis of TAD. In this study, the microarray that contains more than 30,000 known human lncRNAs and coding genes was utilized to detect fold-changes in the expressions of lncRNAs and mRNAs in TAD compared with those of NTA. After detailed analysis of data, we identified 765 differentially expressed lncRNAs, in which 289 were up-regulated and 476 were down-regulated. In 619 differentially expressed mRNA, 265 mRNAs were up-regulated and 354 mRNAs were down-regulated. Through bioinformatics approaches of GO, pathways and network analysis, 16 lncRNAs and their
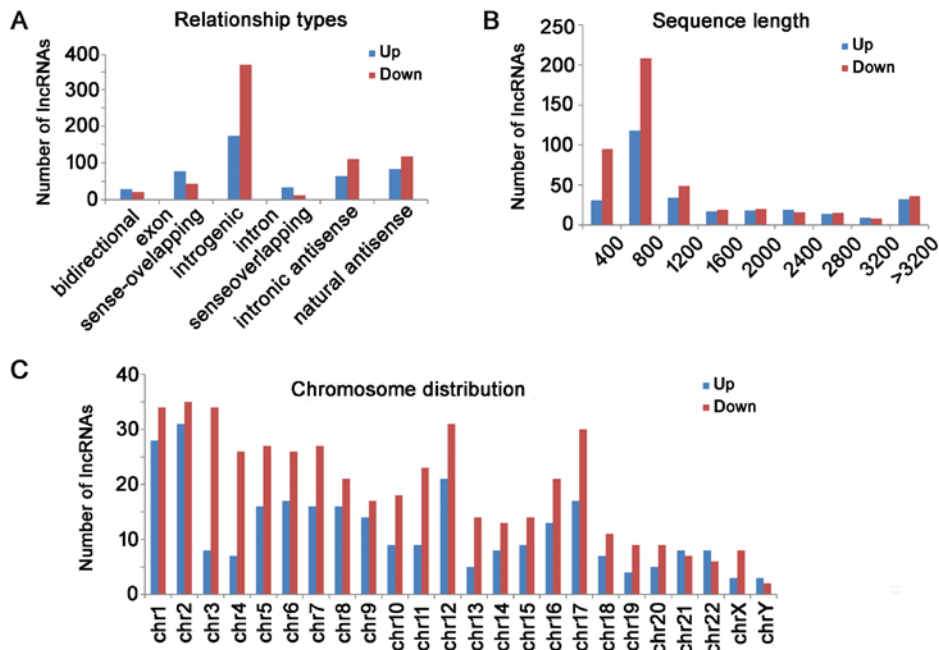
Figure 2. Differential lncRNAs were classified according to their distribution. (A) For those upregulated lncRNAs, there were 156 intergenic, 75 natural antisense, 69 exon sense-overlapping, 57 intronic antisense, 30 intron sense-overlapping and 26 bidirectional lncRNAs. For those downregulated lncRNAs, there were 329 intergenic, 105 natural antisense, 99 intronic antisense, 39 exon sense-overlapping, 19 bidirectional and 11 intron sense-overlapping lncRNAs. (B) Length distribution of the dysregulated lncRNAs. The lncRNAs were mainly between 400 and 1200 bp in length. (C) The chromosome distribution indicated the numbers of upregulated and downregulated lncRNAs locations in various chromosomes. LncRNA, long non-coding RNA; Chr, chromosome.
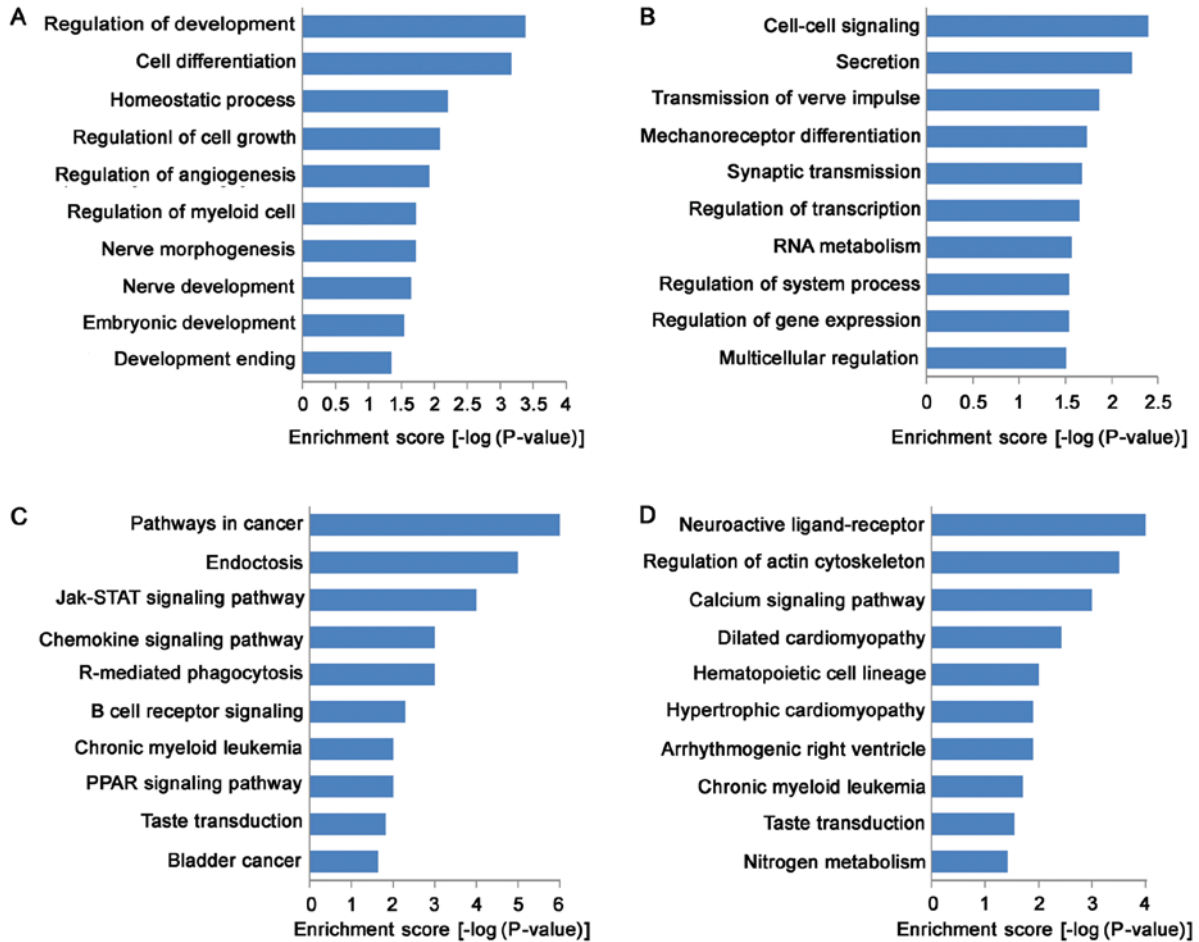

Figure 3. GO and pathway analysis. The top 10 GO terms that were associated with the coding gene function of (A) upregulated lncRNAs and (B) downregulated lncRNAs are listed. The top 10 pathways that were associated with the coding gene of (C) upregulated lncRNAs and (D) downregulated lncRNAs are listed. GO, gene ontology; lncRNA, long non-coding RNA.

Table III. Details of the 16 candidate lncRNAs in thoracic aortic dissection.

Gene symbol	Regulation	RNA length	Chromosome	Strand	Relationship	mRNA	TAD/NTA	P-value
CERKL	Down	3,030	Chr2	-	Natural antisense	ITGA4	0.04	0.005
RP11-395B7.4	Up	372	Chr7	-	Natural antisense	MUC12	8.58	0.014
RP11-887P2.1	Up	3,325	Chr12	-	Intronic antisense	SOCS2	7.75	0.017
lncP2RX7	Up	3,604	Chr12	+	Exon sense-overlapping	P2RX7	12.48	0.009
CDKN2B-AS1	Up	1,469	Chr9	+	Intronic antisense	CDKN2B	10.92	<0.001
RP11-796E2.4	Down	1,898	Chr12	+	Natural antisense	BTG1	5.49	0.003
HIF1A-AS2	Up	2,051	Chr14	-	Natural antisense	HIF1A	12.67	<0.001
HOXD-AS2	Up	692	Chr2	-	Natural antisense	HOXD3	6.63	0.012
AC007254.3	Down	344	Chr2	+	Intronic antisense	SLC8A1	0.01	0.008
OGFR-AS1	Down	668	Chr20	-	Intronic antisense	OGFR	11.00	<0.001
AX746823	Up	2,943	Chr21	-	Intron sense-overlapping	RUNX1	9.80	0.023
RP11-69I8.3	Up	495	Chr6	+	Natural antisense	CTGF	10.29	0.019
RP11-317J10.2	Up	430	Chr8	-	Bidirectional	CA2	13.79	<0.001
RP11-318K15.2	Up	651	Chr8	+	Intron sense-overlapping	LYN	13.34	0.004
RP11-536K7.5	Up	480	Chr10	+	Natural antisense	IL2RA	21.43	0.006
NPPA-AS1	Up	660	Chr1	+	Natural antisense	NPPA	6.51	<0.001

TAD, thoracic aortic dissection; NTA, normal thoracic aorta; Chr, chromosome.

Table IV. Network objects associated with 16 lncRNA candidates.

No.	Tag	Gene	Network object
1	NM_001122607	RUNX1 (Runt-related transcription factor 1)	AML1 RUNX1
2	NM_006172	NPPA (Natriuretic peptide A)	ANP
3	NM_001731	BTG1 (B-cell translocation gene 1)	BTG1
4	NM_000067	CA2 (Carbonic anhydrase II)	Carbonic anhydrase II
5	NM_001901	CTGF (Connective tissue growth factor)	CTGF IGFBP7/8
6	NM_181054	HIF1A (Hypoxia inducible factor 1, α subunit)	HIF1A
7	NM_000417	IL2RA (Interleukin 2 receptor α)	IL-2R α chains IL2RA
8	NM_000885	ITGA4 (Integrin, α 4)	ITGA4
9	NM_002350	LYN (LYN proto-oncogene, Src family tyrosine kinase)	Lyn
10	NM_001164462	MUC12 (Mucin 12, cell surface associated')	Mucin 12
11	NM_001112802	SLC8A1 (Solute carrier family 8 (sodium/calcium exchanger), member 1)	NCX1
12	NM_007346	OGFR (Opioid growth factor receptor)	OGFR
13	NM_078487	CDKN2B (Cyclin-dependent kinase inhibitor 2B)	p15
14	NM_002562	P2RX7 (Purinergic receptor P2X, ligand gated ion channel, 7)	P2X7
15	NM_003877	SOCS2 (Suppressor of cytokine signaling 2)	SOCS2
16	NM_006898	HOXD3 (Homeobox D3)	HOXD3

coding genes were selected as candidates for the pathogenesis of TAD. These candidates were verified by RT-qPCR. Results showed that 5 lncRNAs (lncP2RX7, HIF1A-AS2, AX746823, RP11-69I8.3 and RP11-536K7.5) and 6 mRNAs (P2RX7, CDKN2B, HIF-1A, RUNX1, CTGF and IL2RA) were significant expressed in dissected thoracic aortas, suggested they may be core genes and play critical roles in the pathogenesis of TAD.

So far, exploratory studies in the cardiovascular setting have identified several lncRNAs associated cardiovascular diseases (11). For example, lncRNA Bvht was identified as a key regulator of cardiovascular commitment from nascent mesoderm, suggested the potential implication in cardiovascular development (12). Both Mirt1 and Mirt2 lncRNAs were up-regulated in a mouse model of myocardial infarction. Increased expression of both Mirt1 and Mirt2 can promote cardiac contractile function and decrease left ventricular remodeling (5). The H19 lncRNA is a novel negative regulator of cardiomyocyte hypertrophy (13). A cardiac specific
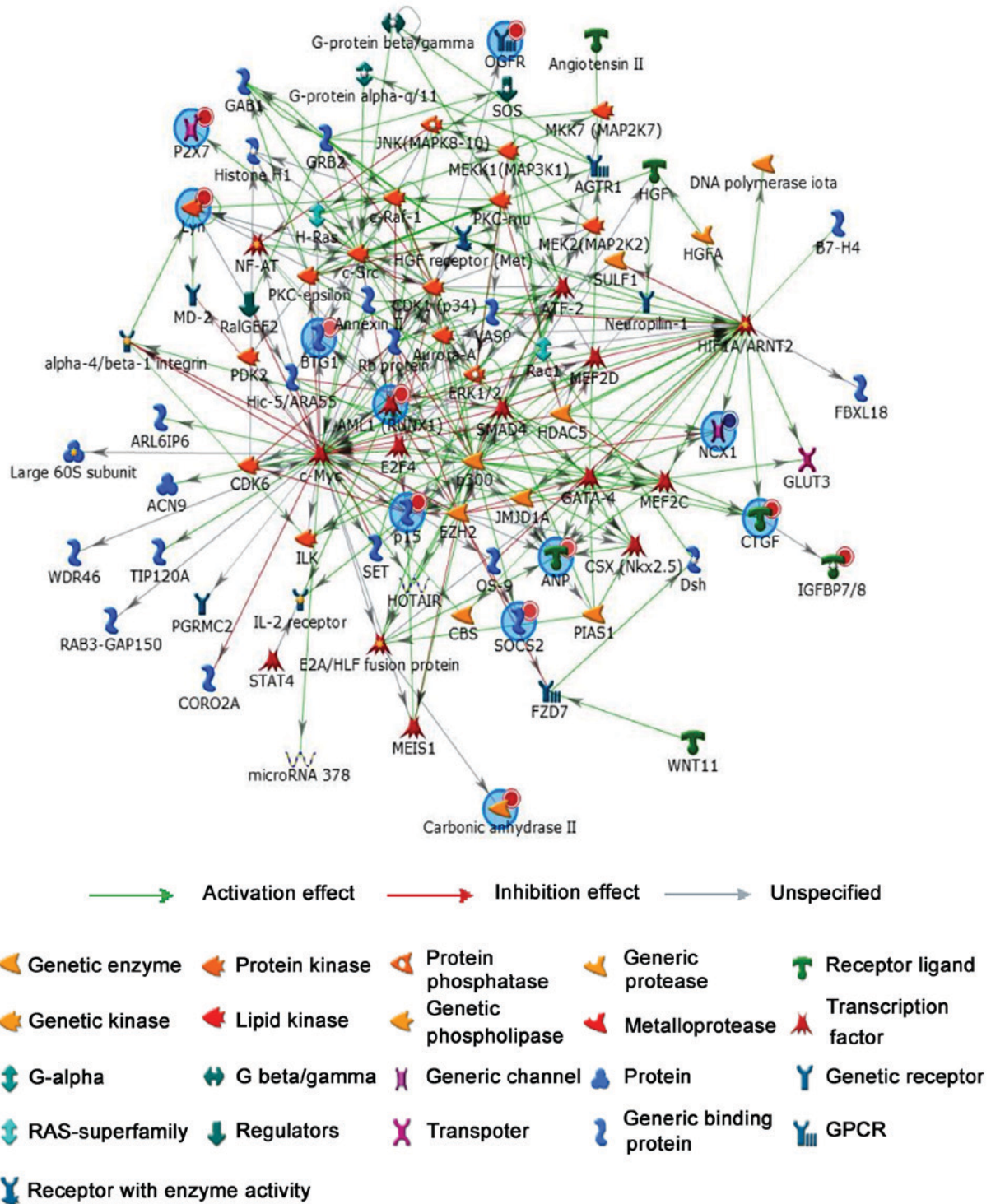
Figure 4. Regulatory network analysis was performed to present the network objects of 16 lncRNA candidates. LncRNA, long non-coding RNA.

lncRNA Mhrt has been demonstrated to protect the heart from pathological cardiac hypertrophy (6). However, specific studies looking at lncRNAs in TAD are still lacking. Recent studies showed some exciting reports on lncRNAs in aortic aneurysm. The inhibition of lncRNA HIF1A-AS1 in VSMCs suppressed cell apoptosis and enhanced cell proliferation, which may participate in the pathogenesis of thoraco-abdominal aorta aneurysm (TAAA) (7,14). BRG1 expression is increased in TAA and regulates proliferation and apoptosis of VSMCs through the HIF1A-AS1 (6). These results may

shed light on important functions of lncRNAs in TAD. Using third-generation lncRNA microarray, we revealed differential expression profiles of lncRNAs between TAD and NTA. The fact that some lncRNAs were differentially expressed in the developing or diseased heart provides a strong indication for their involvement in cardiac physio-pathology (15-17). Our results may provide important insights into the pathogenesis of TAD disease.

GO term and KEGG pathway analyses were utilized to gain insight into the function of differentially expressed genes (18).
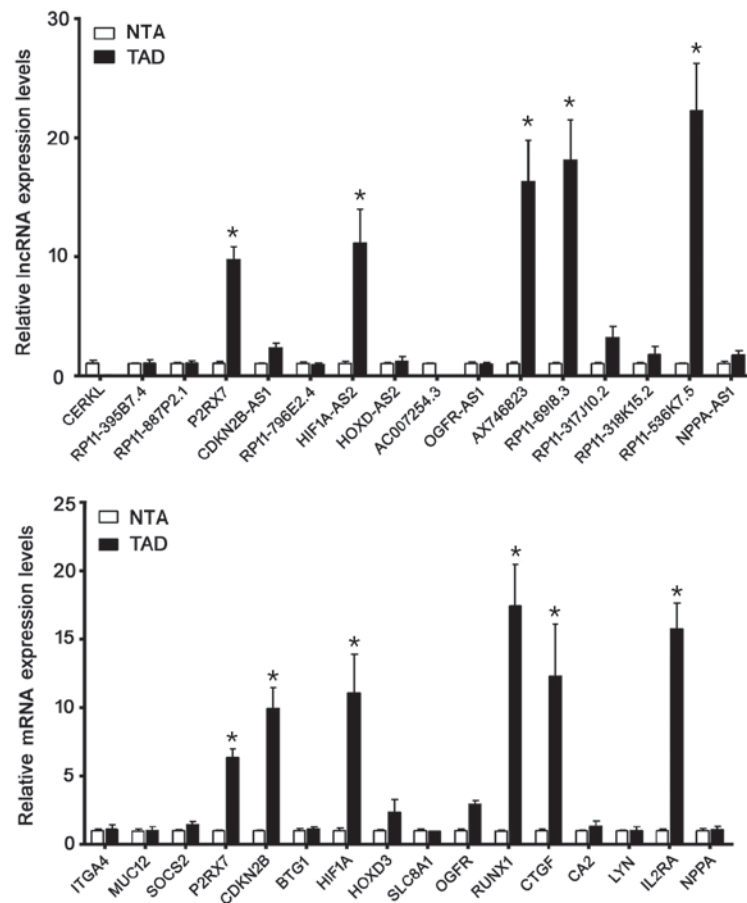
Figure 5. Candidate genes were validated by reverse transcription-quantitative polymerase chain reaction. Among the lncRNAs, lncP2RX7, HIF1A-AS2, AX746823, RP11-69I8.3 and RP11-536K7.5 increased markedly in the TAD group when compared with the control group. Among the mRNAs, P2RX7, CDKN2B, HIF-1A, RUNX1, CTGF and IL2RA increased markedly in the TAD group when compared with the NTA group. *$P<0.05$ vs. NTA. LncRNA, long non-coding RNA; P2RX7, purinergic receptor P2X7; HIF, hypoxia inducing factor; TAD, thoracic aortic dissection; CDKN2B, cyclin dependent kinase inhibitor 2B; RUNX1, runt-related transcription factor 1; CTGF, connective tissue growth factor; IL2RA, interleukin 2 receptor a chain; NTA, normal thoracic aorta.

GO analysis indicated that the functions of dysregulated mRNAs were involved in a variety of biological processes, including VSMC development and vascular homeostasis. These both processes were closely associated with the formation and development of TAD. The aortic media is mainly composed of VSMCs, which are the source of extracellular matrix (ECM) proteins such as collagen and elastin. Medial degeneration is the main histopatholocgic characteristics of dissected thoracic aorta (18). GO results may imply the therapeutic potential of TAD by modulating lncRNAs. Moreover, KEGG pathway analysis indicated that 31 pathways were down-regulated and 24 pathways were up-regulated. Most of up-regulated pathways were enriched in Jak-STAT, chemokine, PPAR and B cell receptor signaling pathways, suggested that up-regulated pathways in TAD were closely associated with signal transduction. In addition, down-regulated pathways were mainly enriched in ARVC, HCM and dilated cardiomyopathy, indicated that these diseases may have a common pathogenic mechanism. To reduce the lncRNAs for further investigation and to find those potentially involved in TAD, we selected 16 lncRNAs as candidate genes for TAD through the significant expression (fold-change >4, P<0.01) and GO term enrichment. Regulatory network analysis demonstrated complex regulatory mechanisms of 16

mRNAs in the pathogenesis of TAD. Microarray results of lncRNAs and mRNAs was validated by RT-qPCR. Among lncRNAs, lncP2RX7, HIF1A-AS2, AX746823, RP11-69I8.3 and RP11-536K7.5 increased dramatically in the TAD group compared with the NTA group (P<0.01, respectively). With the exception of HIF1A-AS2 reported in TAAA, other lncRNAs were first found in the dissected thoracic aorta. According to protein-coding genes, lncP2RX7, AX746823, RP11-69I8.3 and RP11-536K7.5 were related to the activation of nuclear receptor (P2RX7), nuclear transcription (RUNX1), connective tissue development (CTGF) and inflammation (IL2RA). These genes may play important roles in the pathogenesis of TAD. Whether therapeutic modulation of these lncRNAs could decrease TAD development and whether this effect translates into improved survival after TAD remains to be determined.

PCR results showed that P2RX7, CDKN2B, HIF-1A, RUNX1, CTGF and IL2RA were significant expressed in dissected thoracic aortas, suggested they may be core genes and play critical roles in the pathogenesis of TAD. To date, HIF-1A has been involved in the proliferation, migration and morphological changes of VSMCs. Mechanically, hypoxia promoted the expression of HIF-1A by PI3K-AKT pathway in human aortic SMCs; HIF-1A further suppressed the

expressions of AEG-1, a-SMA and SM22a, and promoted osteopontin (OPN) expression. Functionally, HIF-1A inhibited the proliferation and migration of human aortic SMCs (19). Moreover, CTGF is a matricellular protein expressed in the vascular wall, which regulates diverse cellular functions. Ungvari et al (20) showed that the expression of CTGF was increased in the abdominal aorta of ApoE$^{-/-}$ mice and in the adventitial region of the abdominal aorta in human AAA. CTGF is principally regulated at the level of transcription and is induced by mechanical stresses and a number of cytokines and growth factors, including TGF-β (21). Additionally, used vascular injury models, Leeper et al (22) found that CDKN2B knockout mice displayed reduced neointimal lesions and developed larger aortic aneurysms. In situ and in vitro studies suggested that these effects were attributable to increased smooth muscle cell apoptosis (23).

Besides, P2X7 receptor activation is the initial event leading to vascular dysfunction following lipopolysaccharide (LPS) treatment. Activation of P2X7 receptor amplifies LPS-induced hyporeactivity in mouse endothelium-intact aorta, which is associated with IL-1β-mediated release of nitric oxide by iNOS (24). And then, Pahl et al (25) reported that mRNA and protein expression of transcription factor RUNX1 in human abdominal aortic aneurysm. Furthermore, an integral-membrane protein, soluble IL2RA has been isolated and determined to result from extracellular proteolysis. This suggested the pathogenesis and development of human TAD may be related to inflammation of the local environment in the aorta (26). However, the molecular mechanisms of these genes are not completely understood in TAD.

lncRNAs typically show tissue-specific and vascular disease-specific patterns of expression (27,28). Give this specificity, lncRNAs may be favorable biomarkers than current coding proteins for TAD. In addtion, lncRNAs are functional molecules, thus their expressions may be a better indicator of disease states. Because of the limited sample size, the screening biomarker had some limitations, hoping to expand the sample size in the future. Future studies would also be needed to reveal whether P2RX7, CDKN2B, HIF-1A, RUNX1, CTGF and IL2RA expressions were associated to TAD and which cells expressed these proteins in TAD lesion.

This is first report about the differentially expressed lncRNA profiles between TAD and NTA. These results may provide important insights into the pathogenesis of TAD disease. Expanding our understanding about differential expression profiles of lncRNAs will assist into novel diagnostics and therapeutics, which will ultimately improve outcomes for patients with TAD.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and NY conceived and designed the experiments. XZ and XB conducted RNA sequencing and analysis. GQ and MZ performed the remaining experiments. HL and DL conducted data analysis. YL and NY produced the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the international review board of Beijing Yuho Rehabilitation Hospital, (Beijing, China). Written informed consent was obtained from each of the patients.

Consent for publication

Written informed consent was obtained from each of the patients.

Competing interests

The authors declare that they have no competing interests.

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