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ORIGINAL RESEARCH

The role of genome-scale leukocyte long noncoding RNA in identifying acute aortic dissection

Tongyao Li[1,2,†], Yiwu Zhou[1,†], Dongze Li[1], Zhi Zeng[1], Shu Zhang[1,*]

[1]Department of Emergency Medicine, Emergency Medical Laboratory, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China
[2]West China Medical School, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China

***Correspondence**
zhangs@wchscu.cn
(Shu Zhang)

[†] These authors contributed equally.

Abstract

Acute aortic dissection (AAD) is a disease with intima rupture and explosive inflammatory reaction. In this study, we detected the expression pattern of genome-scale leukocyte long noncoding RNAs (lncRNAs) in patients with AAD and explored the diagnostic efficacy and inflammatory mechanism. The expression levels of lncRNAs and mRNAs in 5 patients with AAD and 5 control cases were detected by microarray technology and were verified in 20 AAD cases and 10 control individuals. A total of 540 up- and 1078 down-regulated lncRNAs were significantly expressed between the two groups. Based on microarray analysis, confirmation through quantitative real-time polymerase chain reaction, and criteria for clinical relevance, three lncRNAs *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* were identified as potentially important diagnostic lncRNAs of AAD. Receiver operating characteristic curve (ROC curve) analysis showed that the area under curve (AUC) of *ENST00000593848.1* was 0.790 (neutrophils, $p < 0.05$) and 0.725 (monocytes, $p < 0.05$). The corresponding target gene of *ENST00000593848.1* was *solute carrier family 8 member A1* (*SLC8A1*), which encodes a sodium calcium exchanger. Compared with transfection of no-load siRNA group, the Ca^{2+} levels, interleukin-6 (IL-6) levels, mRNA levels of extracellular regulated kinase (ERK) 1/2, and the activity of P-NF-κB p65 in THP-1 cells were increased after transfection of si-lncRNA (SILNC) ($p < 0.05$). Together, these results indicate that the three different lncRNAs *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* may play an anti-inflammatory role in patients with AAD, and *ENST00000593848.1* is a promising candidate to further explore as a potential diagnostic and prognostic factor as well as for developing novel therapeutic strategies.

Keywords

Acute aortic dissection; Long non-coding RNA; Diagnosis; Microarray; Anti-inflammation

1. Introduction

Acute aortic dissection (AAD) refers to the local rupture of the aortic intima under the effect of the artery itself or external force. The arterial blood enters the middle layer of the arterial wall through the tear of the intima, and under the effect of the arterial blood pressure, it is pulled along the longitudinal axis of the artery near or far [1]. AAD shows characteristics of sudden, rapid development, and acute death, and thereby seriously threatens the life of patients [2]. The mortality rate of type A AAD in the early stage is more than 60%, and the rate increases by 1–2% per hour in the first 24 hours after initial presentation [3]. Early diagnosis and effective treatment are key to improving the prognosis of patients with AAD. At present, the diagnosis of AAD depends on computed tomographic angiography (CTA), and its treatment depends on surgery [2]. However, CTA examination is not a routine examination, as it has the disadvantages of limited use and high cost, and there is almost no effective drug treatment to control the progression of AAD. Therefore, exploring the intrinsic pathophysiological mechanism of AAD and thereby identifying and developing new diagnoses and treatment strategies are expected to improve the prognosis of patients [4].

The direct manifestation of AAD is vascular intimal injury, with systemic and serious inflammatory reactions during its occurrence and development [5]. It is reported that the level of plasma leukocyte is significantly related to the prognosis of AAD patients [6, 7]. Inflammatory factors (such as interleukin) secreted by leukocytes can infiltrate the aortic wall, degrade extracellular matrix and elastin, resulting in thinning of the vascular wall, and interact with platelets, tissue factors, and fibrin, which has a decisive impact on vascular disease [8, 9].

Long non-coding RNAs (lncRNAs) is a class of RNA se-

quences with a nucleotide (nt) length greater than 200 nt. It can regulate the expression of genes through epigenetic, transcriptional process, and post transcriptional regulation, and shows stable expression levels in plasma [10]. Previous studies have reported that lncRNAs are involved in the pathophysiological changes associated with heart failure, atherosclerosis, and cardio cerebrovascular diseases [11–15], and lncRNAs in leukocytes have shown a good diagnostic efficacy in myocardial infarction [16]. However, the association between lncRNAs in leukocytes and AAD has not been reported. In this study, the expression pattern of lncRNAs in leukocytes of patients with AAD was evaluated, and the diagnostic power and inflammatory pathophysiological mechanism of the lncRNAs were explored.

2. Materials and Methods

2.1 Design and population

From September 1, 2016 to November 30, 2016, 25 AAD patients and 15 healthy volunteers were included in the prospective study. Inclusion criteria were: age $\geq$18 years, onset time $\leq$2 weeks, and patients were diagnosed with AAD by CTA. Exclusion criteria were: pregnant women, patients with AAD caused by trauma, and AAD combined with infection or blood system, immune system or cancer and other diseases. There were 5 cases in the AAD group and 5 cases in the healthy group in initial experiments, and further experiments to verify role of differentially expressed lncRNAs were conducted with another independent group comprising of 20 cases in the AAD group and 10 cases in the healthy group.

Experimental groups included blank control group: without any intervention factors; LPS group: Lipopolysaccharide (LPS, 1 μg/mL); target *SILNC-2-1* group: transfection of *SILNC-2-1* + LPS (1 μg/mL); target *SILNC-2-2* group: transfection of *SILNC-2-2* + LPS (1 μg/mL); *SINC* control group: transfection of no-load siRNA + LPS (1 μg/mL).

2.2 Data and collection

Characteristics of patients and healthy volunteers were collected, including age, gender, vital signs, previous disease history, treatment plan, and drug information. The laboratory results of AAD patients and healthy volunteers were recorded, including levels of leukocytes, neutrophils, monocytes, interleukin-6 (IL-6), c-reactive protein (CRP), and D-dimer.

2.3 Collection of leukocytes and subtypes

Peripheral venous blood (6 mL) of AAD patients and healthy volunteers were collected and packed into anticoagulant ethylene diamine tetraacetic acid (EDTA) tubes (Invitrogen, CA, USA). The red blood cells were lysed and centrifuged to obtain the white plaque cell layer (white blood cells); after adding trizol reagent, it was frozen at −80 °C for the subsequent isolation of leukocyte subtypes. The separation solution (Tianjin Haoyang biological products Technology Co., Ltd., Tianjin, China) was added to extract the neutrophils and monocytes. Finally, the monocytes were purified by differential attachment method. After the cells were collected, trizol reagent was added and the samples were frozen at −80 °C.

2.4 Extraction, labeling, and hybridization of RNA

Total RNA was extracted from leukocytes and their subtypes by trizol reagent (Invitrogen, CA, USA) and purified by mirVana™ miRNA Isolation Kit (AM1561) according to the manufacturer's instructions. The OD260/280 reading was determined using a spectrophotometer (NanoDrop nd-1000) to determine the purity and concentration of RNA. The integrity of RNA was determined by 1% formaldehyde denaturing gel electrophoresis. Samples were labeled and array hybridized according to the gene expression analysis specification of monochromatic microarray (Agilent technique).

2.5 Microarray imaging and data analysis

The purified RNA was screened using Agilent lncRNAs expression microarray V4.0 (Agilent, Santa Clara, CA, USA). The chip scanner was used to scan the cleaned chip and obtain the hybrid picture, and the Agilent feature extraction (V10.7) software (Agilent, Santa Clara, CA, USA) was used to analyze the hybrid images and extract the data. Agilent gene printing software (Agilent, Santa Clara, CA, USA) was used to normalize the data and analyze the differences between groups, and thereby identify the lncRNAs with statistical differences. The differentially expressed genes were identified by random variance model, and p-value was calculated by paired t test. Up-regulated and down-regulated genes with fold change $\geq$2.0 and $p \leq 0.05$ were considered as significant genes.

2.6 Functions and pathways analysis

GO (Gene Ontology) is an international standard classification system of gene function, including biological process, molecular function and cell composition. Through GOseq software (http://www.bioconductor.org/), according to the distribution relationship of differential genes in GO classification, enrichment analysis of differential genes was carried out. KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis is a functional analysis that maps genes to KEGG pathway. According to KEGG pathway analysis (http://www.genome.jp/kegg/), the main pathway of the differential genes was determined. $P < 0.05$, was considered to indicate statistical difference between GO and pathway enrichment; the lower the p-value, the more obvious the enrichment difference [17].

2.7 Quantitative multi-polymerase chain reaction (qRT-PCR)

Differential gene expression between two groups was verified using qRT-PCR. According to the high-throughput screening, the difference multiple is large, the original expression amount is more than 300, and its regulatory signal pathway can be used as a candidate target gene in bioinformatics analysis. The total RNA was separated with trizol reagent and then reverse transcribed into cDNA using RNeasy Mini kit17 (Takara, Shiga, Japan) according to the manufacturer's protocol. Primer 5.0

TABLE 1. Primer RNA sequence of qRT-PCR.

	Primer sequence (5′ to 3′)
Probe	
ENST00000430893.1-F	ACTGTGATTCCCCAGGTGATG
ENST00000430893.1-R	GCTTCTTATTGTCTGCTCACTCCTT
ENST00000593848.5-F	ATACTAGATGCTGGGCTCAAGACA
ENST00000593848.5-R	ACCCTCTCTCCCAGCTTCCA
ENST00000536517.1-F	TTGATCACCTTGACTGAAGCA
ENST00000536517.1-R	TGTCTGGAAAATGCCATCTG
β-actin forward primer	CTGGAACGGTGAAGGTGACA
β-actin reverse primer	CGGCCACATTGTGAACTTTG
mRNA	
β-actin forward primer	AGGT CATCACCATT GGCAATGAGC
β-actin reverse primer	AGCACTGTGTTGGCGTACAGGTCT
LNC2 forward primer	TGTCCACAGCCAAGGGAAATAGC
LNC2 reverse primer	GCAGGTTCTGGGTGGTTAGTTGG
SLC8A1 forward primer	CCTTGTGGTTGGGACTAACAG
SLC8A1 reverse primer	CCCAGCCATTCCAGTATTCAG
ERK1/2 forward primer	CAAGAAAATCAGCCCCTTTGAG
ERK1/2 reverse primer	AAGATCTGTTTCCATGAGGTCC

SLC8A1—solute carrier family 8 member A1, ERK—extracellular regulated kinase.

was used to design the primers for each lncRNAs, and BLAST (NCBI) was used to ensure the uniqueness of the amplified products. The *β-actin* (CST, Danvers, MA, USA) gene was selected as the endogenous reference gene. In the same way, the lncRNAs and its target genes were amplified (Table 1).

2.8 Cell culture and transfection

THP-1 cells (purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institute of Life Sciences, Chinese Academy of Sciences, Shanghai, China) were cultured and sub-cultured in the complete cell culture medium with 10% fetal bovine serum. THP-1 cells (8×10^5) were inoculated into 24-well plates, and then the 1ug plasmid containing siRNA-lnc was transfected into the THP-1 cells by Life Technologies (Carlsbad, CA, USA) to inhibit the expression of our candidate lncRNA *ENST00000593848.1*. After transfection, lipopolysaccharide (LPS; 1 μg/mL; Sigma, St. Louis, MO, USA) was added to the cells, and the cell suspension was collected after 4 hours of culture for subsequent protein and mRNA detection.

2.9 Western blot

The total protein extracted from THP-1 cells was subjected to 10% sodium dodecyl sulfate-polyacrylamide Sigma-Aldrich, St. Louis, MO, USA) gel electrophoresis, and the separated proteins were transferred to PVDF (polyvinylidene fluoride) membrane (Minipore, Shanghai, China). The membrane was then incubated in 5% skim milk for 2 hours at room temperature, followed by incubation with primary antibody at 4 °C overnight. The membrane was then washed in PBS and incubated with appropriate secondary antibody (1:3000) for 2 hours at room temperature. All protein bands were detected by enhanced chemiluminescence Kit (Pierce, Rockford, IL, USA), and β-actin was used as the internal reference protein for group analysis.

2.10 Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-6, CRP, and matrix metalloprotein 9 (MMP-9) in the supernatant of THP-1 cell culture medium and intracellular concentrations of Ca^{2+} were measured according to the instructions of the ELISA kit (CUSABIO, Wuhan, China).

2.11 Data analysis

All data are expressed as mean $\pm$ standard deviation. The data comparison between two groups was carried out by an independent sample t test. Comparison of more than two groups as done by one-way analysis of variance. The comparison between two groups adopted the least significant difference method. The area under receiver operating characteristic (ROC) curve (AUC) was compared for different lncRNAs to judge their diagnostic efficacy for AAD. All data were analyzed using SPSS19.0. (IBM, Armonk, NY, USA) and a $p < 0.05$ was considered to be statistically significant.

3. Results

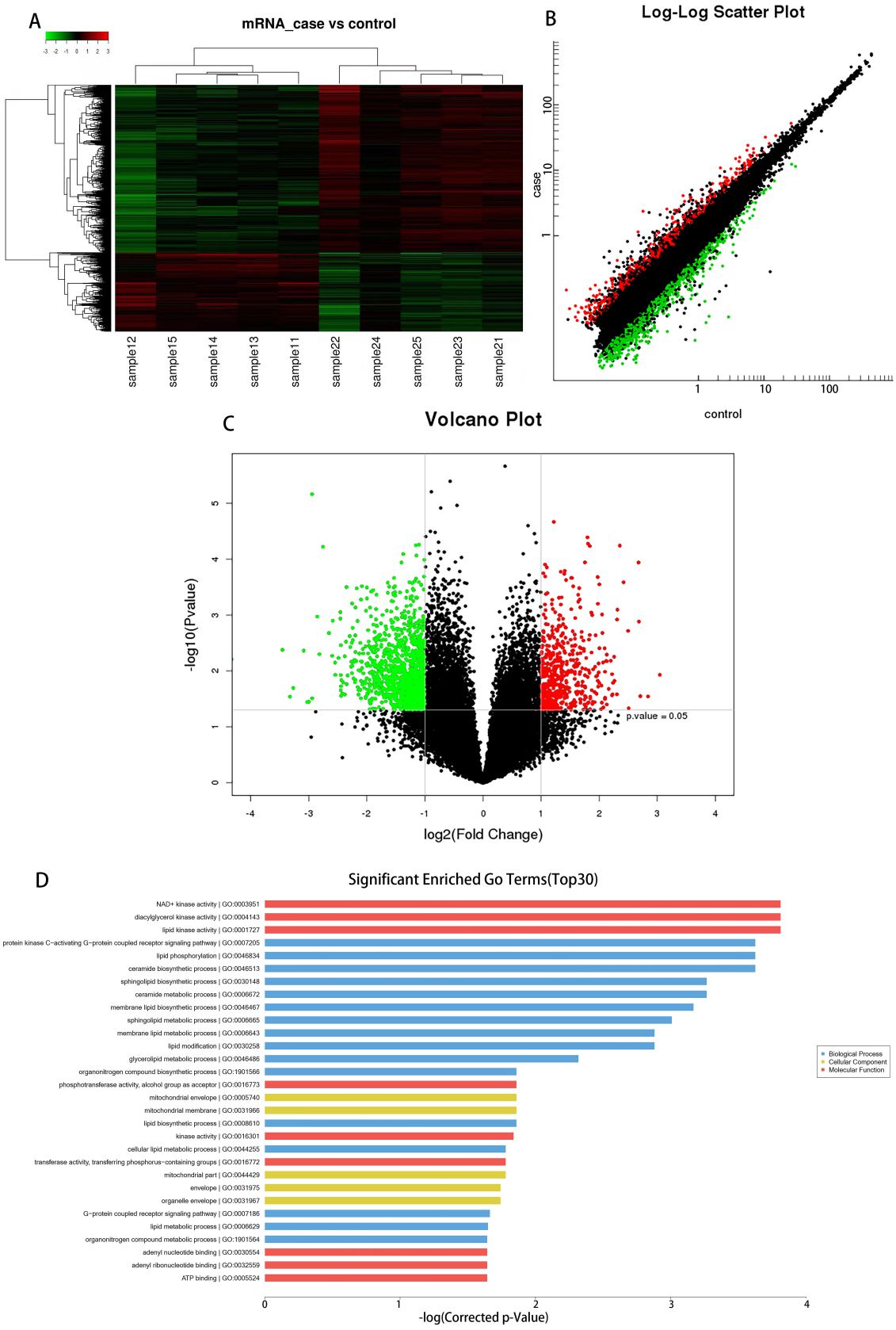

FIGURE 1. Differential expression of lncRNAs in patients with acute aortic dissection (AAD) and control individuals. Hierarchical clustering analysis of 1618 lncRNAs that were differentially expressed in the two groups. (A) Expression values are represented in red and green, indicating expression above and below the median expression value in AAD patients (sample21, sample22, sample23, sample24, sample25) or control individuals (sample11, sample12, sample13, sample14, sample15), respectively. (B) Scatter plot of differential lncRNAs expression. X-axis: N-U, Y-axis: AMI-U. (C) Volcano plot of differential lncRNAs expression. X-axis: log2 fold change; Y-axis: $-1 \times$ log10 (corrected *p*-value) for each probes. (D) Biological functions of lncRNAs in leukocytes of AAD patients by GO pathway enrichment analysis.

TABLE 2. Basic characteristics of patients with AAD and healthy control group.

Variable	AAD (n = 5)	Control (n = 5)	*p*
Age, year	56 ± 3	56 ± 9	-
Male, n (%)	4 (100)	4 (100)	-
Stanford type			
Stanford A, n (%)	3 (60)	0	-
Stanford B, n (%)	2 (40)	0	-
Smoke, n (%)	5 (100)	5 (100)	-
Hypertension, n (%)	4 (80)	4 (80)	-
Hyperlipidemia, n (%)	0 (0)	0 (0)	-
Diabetes, n (%)	0 (0)	0 (0)	-
Marfan syndrome, n (%)	0 (0)	0 (0)	-
Leukocyte count, (10^9/L)	11.86 ± 3.37	5.98 ± 1.42	0.025
Neutrophils Percentage, (%)	81.26 ± 8.56	55.40 ± 9.33	0.002
Monocyte percentage, (%)	9.53 ± 1.11	6.18 ± 0.26	0.034
IL-6, mg/L	168.66 ± 99.17	0 (0 ± 3.3)	0.028
CRP, mg/L	113.75 ± 37.75	2.41 ± 1.84	0.021
D-dimer, mg/L	9.57 ± 4.69	0.24 ± 0.10	0.007

AAD—Acute aortic dissection, CRP—c-reactive protein.

3.1 Characteristics of patients and healthy controls

The lncRNA expression profile was examined in 5 patients with AAD and 5 healthy controls. The average age of the patients was 56 years old, and 60% and 40% of the patients displayed type A and type B AAD, respectively. There was no difference in age, hypertension, diabetes, and smoking behavior between the patient and control groups ($p > 0.05$, Table 2).

3.2 Expression profile of leukocyte lncRNAs

The expression of plasma lncRNAs and mRNA was examined in the patient and control groups using the human crystal core® lncRNA mRNA expression profile chip (the chip analysis was completed by Beijing Bo'ao company, Beijing, China). The microarray results showed that there was a significant difference in the expression profile of lncRNAs in leukocytes between the patient and control groups. A total of 26,235 lncRNAs were detected by microarray, including 540 up-regulated lncRNAs (>2-fold, $p < 0.05$) and 1078 down-regulated lncRNAs. Cluster analysis was carried out for the differentially expressed lncRNAs between the two groups. The respective expression values in the patient and control groups are expressed as the median. The fold change of each lncRNA and the corresponding *p*-value were mapped and subjected to GO and KEGG analysis to analyze the biological functions of the lncRNAs in leukocytes of the AAD patients (Fig. 1).

3.3 Network interpretation of the co-expression of lncRNAs and mRNA

To verify the results of co-expression between lncRNAs and mRNA, the co-expression network was analyzed. The data of different lncRNAs and mRNA genes were combined to calculate the correlation between each gene in the two sets of data, and the correlation coefficient and corresponding *p*-values were obtained. The *p*-value indicates the reliability of measuring the correlation between genes. Based on the similarity of gene expression, the possible interaction between the genes was analyzed, and the degree of interaction was quantified; degree = 10 represents that there are 10 genes related to the gene, and a larger degree reflects a higher number of genes interactions [18]. Based on the degree of each gene in the gene synergy network, the key genes in the network were obtained (Table 3).

3.4 Verification of chip results

Based on the above information, combined with the needs of clinical research, the possible biomarkers were selected from 174 up-regulated lncRNAs, and the conditions were defined as: average basic intensity >8, multiple of difference >2, $p \leq 0.005$, and lncRNA co-expressed with mRNA (AAD group: degree ≥10; control group: degree ≥8). After screening, only three lncRNAs met these criteria: *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1*. To further identify the origin of the three lncRNAs, neutrophils and monocytes from another group of independent population (20 patients with AAD and 10 controls) were isolated, and the expression of the three lncRNAs was examined by qRT-PCR. The expression of *ENST00000430893.1*, *ENST0000536517.1*, and *ENST0000593848.1* in neutrophils of AAD patients was 0.98 times ($p = 0.703$), 0.60 times ($p < 0.001$) and 1.58 times ($p = 0.010$) higher than that of the control group, respectively. The expression levels of *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* in mononuclear cells of AAD patients

TABLE 3. LncRNA and mRNA co-expression network analysis (degree ≥10).

Gene	Degree		Regulation	Type	Fold	*p*
ENST00000430893.1	12	p36791_v4	Down	LncRNA	2.47	0.004
ENST00000536517.1	11	p3447	Down	LncRNA	3.15	0.005
ENST00000545508.1	10	p3446	Down	LncRNA	3.2	0.014
ENST00000589556.1	10	p8716	Down	LncRNA	3.17	0.002
ENST00000433933.1	9	p10197	Down	LncRNA	2.65	0.014
ENST00000593848.1	8	p34791 v4	Up	LncRNA	2.64	0.004
ENST00000596307.1	8	p11741	down	LncRNA	4.12	0.02
DNHD1	11		Down	mRNA	-	-
TMEM19	11		Down	mRNA	-	-
ADAM23	10		Down	mRNA	-	-
HERC2P4	9		Down	mRNA	-	-
C3orf18	8		Down	mRNA	-	-
HTATIP2	8		Up	mRNA	-	-
LIN7A	8		Up	mRNA	-	-

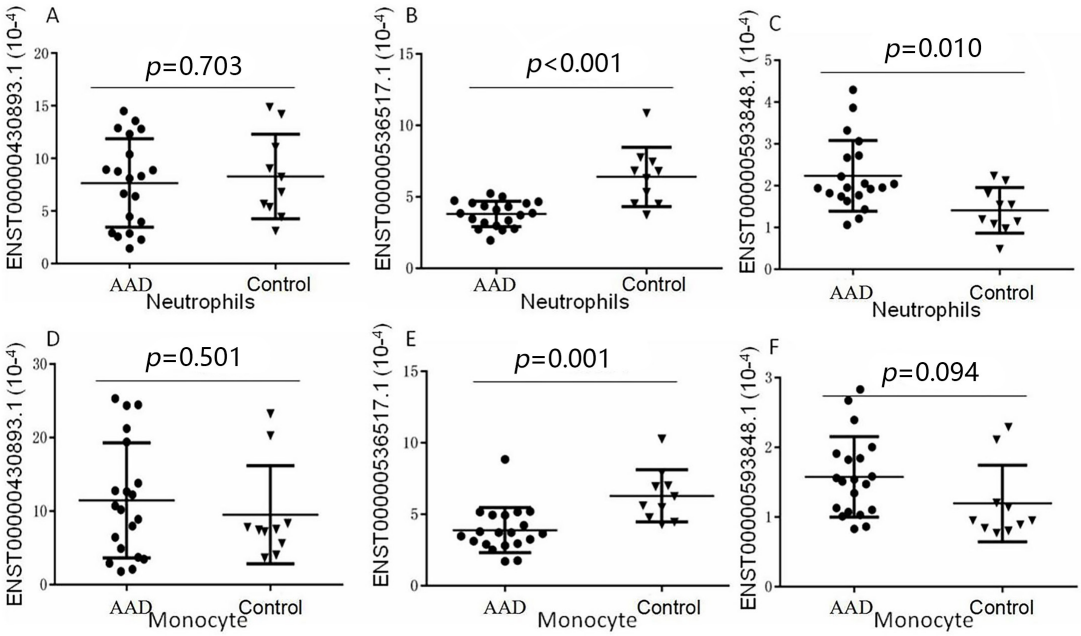

FIGURE 2. Real-time fluorescence quantitative multi-polymerase chain reaction to verify the chip results. AAD, acute aortic dissection; Control, healthy volunteers.

were 1.21 times (p = 0.501), 0.62 times (p = 0.001) and 1.32 times (p = 0.094) higher than those of the control group, respectively (Fig. 2).

3.5 The diagnostic value of lncRNAs for AAD

In ROC curve analysis, the AUC of *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* in neutrophils was 0.440 (p = 0.598), 0.885 (p = 0.001), and 0.790 (p = 0.011); the AUC of *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* in monocytes was 0.581 (p = 0.481), 0.880 (p = 0.001), and 0.725 (p = 0.048), respectively (Fig. 3).

3.6 Expression of inflammatory factors

The expression of lnc2 was detected 48 hours after the transfection of *SINC, SILNC2-1*, and *SILNC2-2*. The results showed that both *SILNC2-1* and *SILNC2-2* could effectively reduce the expression of lnc2 in THP-1 cells compared with that in the *SINC* group ($p < 0.05$). Compared with the *SINC* group, *SILNC2-1* and *SILNC2* groups showed increased Ca^{2+} concentration in THP-1 cells after LPS induction ($p < 0.05$); *SILNC2-2* group showed significantly increased *IL-6* secreted by THP-1 cells after LPS induction ($p < 0.05$) (Fig. 4). However, the *SILNC2-1* and *SILNC2-2* groups did not show significantly increased CRP secretion by THP-1 cells after LPS induction.

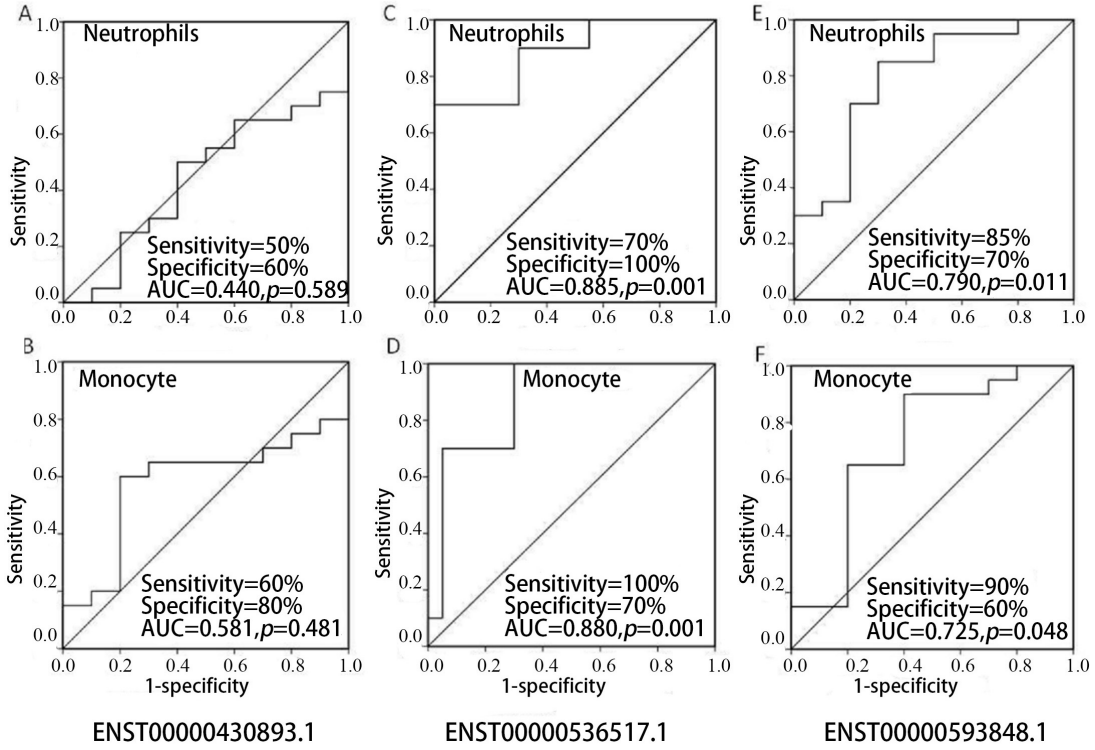

FIGURE 3. The diagnostic value of lncRNAs of leukocytes for acute aortic dissection (AAD) by receiver operating characteristic (ROC) curve.

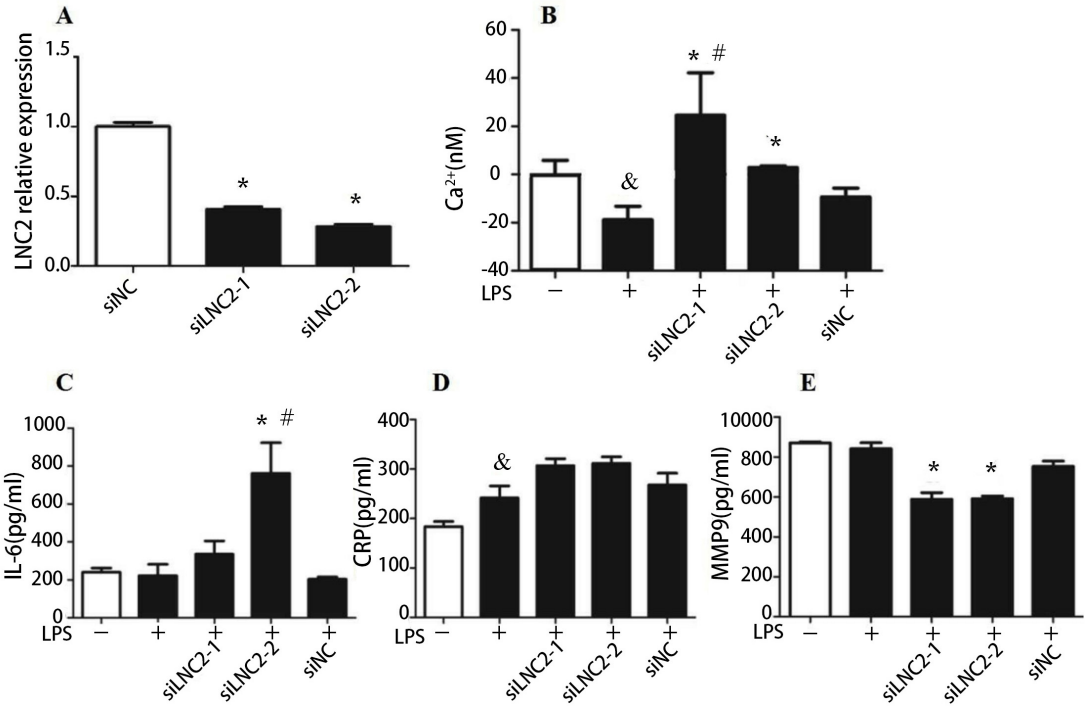

FIGURE 4. The expression of lnc2 was detected 48 hours after the transfection of *SINC*, *SILNC2-1*, and *SILNC2-2*. Intracellular Ca^{2+} concentration and the concentration of IL-6, CRP, MMP9 of cell culture medium in each group were tested by ELISA kit. (A) The expression of LNC2 was detected at 48 hours after the transfection of *SINC*, *SILNC2-1* and *SILNC2-2*. (B) Intracellular Ca^{2+} concentration of each group were tested by ELISA kit. (C, D and E). The concentration of *IL-6*, CRP, MMP9 in the supernatant of cell culture medium of each group were tested by ELISA kit. &$p < 0.05$ compared with blank control group; #$p < 0.05$ compared with group of LPS; *$p < 0.05$ compared with SINC.

3.7 Target genes and possible signaling pathways

Compared with the control group, the LPS group showed a significantly down-regulated level of *solute carrier family 8 member A1* (*SLC8A1*) mRNA in THP-1 cells ($p < 0.05$) (Fig. 5). Compared with *SINC* group, both *SILNC2-1* and

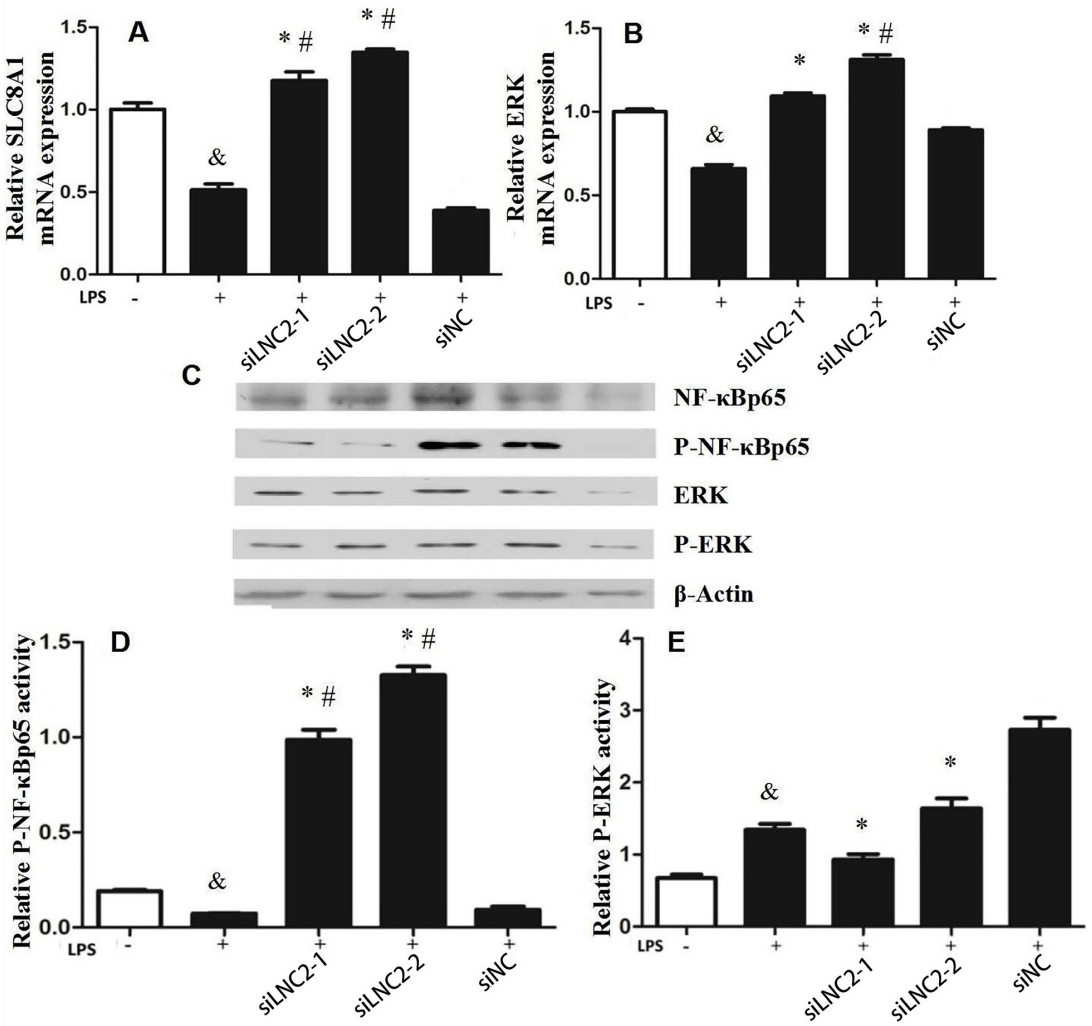

FIGURE 5. Relative *SLC8A1* and *ERK* mRNA expression level and differential expression of target protein signaling pathway related proteins in each group. (A and B) relative *SLC8A1* and *ERK* mRNA expression level were tested by real-time fluorescence quantitative multi-polymerase chain reaction. (C, D and E) expression of target protein signaling pathway related proteins in each group of cells by Western Blot. &$p < 0.05$ compared with blank control group; #$p < 0.05$ compared with group of LPS; *$p < 0.05$ compared with *SINC*.

SILNC2-2 groups showed significantly upregulated levels of *SLC8A1* and *ERK1/2* mRNA in THP-1 cells after LPS induction ($p < 0.05$). Compared with *SINC* group, both *SILNC2-1* and *SILNC2-2* groups showed significant activation of the NF-κB/p65 signaling pathway in THP-1 cells after LPS induction ($p < 0.05$). Compared with the control group, the LPS group showed increased p-ERK1/2 activity in THP-1 cells ($p < 0.05$).

4. Discussion

AAD is a serious disease, because early diagnosis is difficult and there is no efficient therapy for patients with AAD [2]. Therefore, exploring new biomarkers and studying the underlying pathophysiological mechanism may provide better diagnosis and treatment strategies for AAD. In this study, gene chip technology was used to study the whole genome expression patterns of lncRNAs and mRNA in leukocytes of 5 AAD patients and 5 healthy volunteers. We found 174 up-regulated lncRNAs in leukocytes of AAD patients compared with those in healthy controls. These lncRNAs were screened based on the needs of clinical research and conditions; only three lncRNAs, namely *ENST000000430893.1, ENST00000536517.1*, and *ENST00000593848.1* met these screening criteria. We further verified the results in 20 patients with AAD and 10 healthy volunteers. The results showed that these lncRNAs were stably expressed in leukocytes, mainly in neutrophils and monocytes.

Previous studies using Agilent microarray analysis have identified a large number of lncRNAs that show significant differential expression in the plasma of patients with cardiovascular disease [19–21]. Recent studies found that lncRNAs also show differential expression in monocytes of patients with myocardial infarction, among which lncRNAs H19, *metastasis associated in lung denocarcinoma transcript 1* (*MALAT1*) and myocardial infarction association transcript (MIAT) have strong early diagnostic value for acute myocardial infarction [16]. This indicates that lncRNAs may be a new and promising direction for biomarkers in the future. The current study also indicated that the differential expression of lncRNAs in neutrophils may have a high

diagnostic potential for AAD. Therefore, we used ROC curve analysis to test this hypothesis. The results showed that the AUC of AAD was 0.885 (neutrophils) and 0.880 (monocytes) in case of *ENST00000536517.1*, and 0.790 (neutrophils) and 0.725 (monocytes) in case of *ENST00000593848.1*, suggesting that these lncRNAs may be an early diagnostic biomarker for AAD; however, this should be verified using a larger clinical cohort.

lncRNAs are of value not only for early diagnosis in AAD, but also for providing insights into the intrinsic pathophysiological mechanism of the disease. Hence, our findings are expected to provide novel strategies for disease treatment and improve the prognosis of critical patients in the future. In this study, based on the degree of differential expression in patients with AAD and healthy patients, along with the expression observed in leukocytes, predicted target genes, and AUC analysis results for early diagnosis, we chose *ENST00000593848.1* for further investigation of the role of lncRNAs in the inflammatory response of AAD and possible underlying mechanisms. Firstly, we used the lncRNA database, and identified the target gene of *ENST00000593848.1* as *SLC8A1*, which is mainly responsible for encoding a sodium calcium exchanger, and regulating the intracellular and extracellular calcium concentration through Na^+ influx and Ca^{2+} efflux [22]. Our data showed that inhibition of *ENST00000593848.1* significantly increased *SLC8A1* expression and LPS-induced Ca^{2+} concentration in THP-1 cells compared with those in the control group. Previous studies have found that calcium influx is a necessary condition for activation of an inflammatory cell, including neutrophils, monocytes, and macrophages [23]. After the immune cells receive stimulation, the increase in intracellular calcium concentration stimulates the proliferation of inflammatory cells and the release of inflammatory factors such as tumor necrosis factor α (TNF-α), IL-6, interleukin-8 (IL-8), and granulocyte macrophage colony stimulating factor (GM-CSF) [24]. However, it remains unclear if serum inflammatory markers may reflect clinical stability, and its kinetics lag behind clinical presentation [24]. Our data showed a significant increase in IL-6 and CRP concentrations in the *SILNC2-1* and *SILNC2-2* groups. Therefore, *ENST00000593848.1* in leukocytes in AAD patients may play an anti-inflammatory role by inhibiting calcium influx.

Previous studies have shown that Ca^{2+} can activate *ERK* by activating the Ras signaling pathway and upregulating nuclear transcription factors, such as NF-κB and p65 [25, 26]. NF-κB is an important regulatory factor in inflammatory responses. Many factors in various stages of immune inflammatory response are regulated by NF-κB, including TNF-α, IL-1β, IL-2, IL-6, IL-8, interleukin-12 (IL-12), Inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX 2), chemokines, adhesion molecules, and colony stimulating factor [27, 28]. The results showed that inhibition of *ENST00000593848.1* could increase ERK expression activity and upregulate NF-κB activity. Therefore, the inhibitory effect of *ENST00000593848.1* may be mediated by inhibition of the NF-κB signaling pathway.

This study has its limitations. Importantly, it is a single-center study with a small sample size which is unable to improve statistical efficacy. And because of the small sample size, the relationship between lncRNAs and adverse events of AAD is not clear. Our results should be validated among patients from different regions and populations in future large-scale, multicenter research. In addition, we didn't dynamically capture biomarker changes and cannot dynamically analyze the link between lncRNA and the development of AAD. Furthermore, no animal experiments were conducted in this study to verify whether *ENST00000593848.1* inhibitor can regulate the inflammation of the aortic vessel wall in AAD model, and whether it can reduce the morbidity and mortality of AAD. Future research should investigate this hypothesis.

5. Conclusion

There are many lncRNAs that show differential expression profiles in leukocytes of AAD patients and healthy people, among which lncRNAs *ENST00000430893.1*, *ENST00000536517.1*, and *ENST00000593848.1* have the potential to be used as biomarkers for early diagnosis or prognosis of AAD. In the model of monocyte macrophage inflammation induced by LPS, inhibition of the lncRNA *ENST00000593848.1* aggravated the inflammation, suggesting that this lncRNA may play an anti-inflammatory role in patients with AAD, and that the increased expression of *ENST00000593848.1* in leukocytes of patients with AAD may be due to negative feedback regulatory mechanisms during the inflammatory response.

AUTHOR CONTRIBUTIONS

TYL, YWZ, SZ—conception and design; TYL, YWZ—data curation; TYL, TYL, YWZ, SZ—methodology; TYL, YWZ—writing original draft; TYL, YWZ, DZL, ZZ, SZ—manuscript proofing and final approval of the manuscript, all authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Human Ethical Committee of West China Hospital of Sichuan University and the China Ethics Committee of Registering Clinical Trial (ChiECRCT-20150063). All study subjects provided written informed consent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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