Research Square

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Differential lncRNA and mRNA expression profiles in peripheral blood mononuclear cells of mild and severe influenza-associated pneumonia patients

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Abstract

This study was to compare the Long noncoding RNAs (lncRNA) and messenger RNA (mRNA) expression profiles, their related biological functions, and pathways in the peripheral blood mononuclear cells (PBMC) of patients with mild and severe influenza associated pneumonia (IAP). Initially,10 mild and 10 severe IAP patients were enrolled. RNA samples were extracted from the PBMCs of those patients and comprehensive lncRNA and mRNA profiles were examined using Clariom™ D microarrays. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) analysis were used to predict the function and signaling pathways affected by the differentially expressed mRNA. LncRNA-mRNA interaction network was performed to explore the synergistic effect of differentially expressed lncRNAs and their function-related mRNAs. Real-time polymerase chain reaction (RT-PCR) was carried out to verify the microarray results. A total of 40 upregulated and 40 downregulated lncRNAs and 120 upregulated and 60 downregulated mRNAs were identified using microarray analysis. A total of 23 GO items were significantly associated with related differential expressed mRNA. KEGG pathway analysis demonstrated that nuclear factor-κB (NF-κB) signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway and tumor necrosis factor (TNF) signaling pathway were significantly related to those differentially expressed mRNAs. LncRNA-mRNA network analysis identified several key lncRNAs and mRNAs including NONHSAT105043, SBDSP1, CA5BP1, DDX3X, and IL-4R. Altogether, our data signify that the differently expressed lncRNAs and mRNAs identified in the current study as well as the related signaling pathways may provide systematic information for understanding the pathogenesis of IAP.

1. Introduction

Influenza viruses cause worldwide outbreaks and seasonal pandemics and pose serious risks to the health of human beings[1]. Influenza- associated pneumonia (IAP) is the main complication and leading death cause of influenza infection[2, 3].

Innate immune responses play critical roles in the pathogenesis of influenza infection and are associated with the severity and outcome of the disease[4, 5]. Comprehensively understanding the fundamental mechanisms orchestrating innate immune responses during IAV infection is of great importance.

Long noncoding RNAs (lncRNAs) are a class of RNAs with a length greater than 200 nucleotides. LncRNA can participate in multiple biological processes and disease progression by directly regulating proteins or indirectly regulating the target genes of related mRNAs[6, 7].

It has been documented in vitro studies that dysregulation of several lncRNAs is involved in influenza infection. For example, Chen Y and colleagues found that overexpression of lncRNA RDUR could suppress Influenza A virus(IAV)replication by upregulating the expression of several vital antiviral molecules including interferons and interferon-stimulated genes [8]. Wang J and colleagues revealed that the level of lncRNA-PAAN specifically increased upon infection of IAV. Silencing lncRNA-PAAN could significantly decrease IAV replication through impairing the activity of viral RNA-dependent RNA polymerase [9]. However,

few reports have investigated the changes of expression profiles of lncRNAs in influenza patients, especially in severe IAP patients.

The aim of the study was to explore the expression profiles of lncRNA and mRNA in the peripheral blood mononuclear cells (PBMC) of patients with mild and severe influenza pneumonia. Furthermore, bioinformatics analyses were performed to predict the possible roles of differentially expressed lncRNAs and mRNAs in the pathogenesis of IAP.

2. Materials And Methods

2.1 Clinical data

Ten mild and 10 severe influenza pneumonia patients were prospectively recruited from the Department of infectious diseases and clinical microbiology, Beijing Chao-Yang Hospital (Beijing, China), between December 2018 and March 2019. The enrollment criteria were : fulfilling the Chinese Thoracic Society criteria for community-acquired pneumonia (CAP)[10]; having a positive respiratory sample for influenza virus by the reverse transcriptase polymerase chain reaction (RT-PCR) method; within 7 days of symptom onset[2]. Participants were excluded if they were: with confirmed secondary bacterial or viral infection; with chronic liver and/or kidney dysfunction; with autoimmune disease; with malignant diseases. Demographic, clinical, and diagnostic data of the patients were recorded on admission.

The disease severity was assessed by baseline CURB-65 score (confusion, urea level, respiratory rate, blood pressure, and age > 65 years). Mild IAP was defined as CURB-65 score of 0–1 and severe IAP was defined as CURB-65 scores of 3–5 [11].

This study was approved by the Ethical Committee of Beijing Chao-Yang Hospital. Written informed consents were obtained from all participants.

2.2 Isolation of PBMCs

Peripheral venous blood samples (5 mL) were obtained in BD vacutainer heparin blood collection tubes. PBMCs isolation was performed by density gradient centrifugation on Ficoll-Paque (Sigma, USA) according to the manufacturer's protocol and stored at -80 ℃ until RNA extraction.

2.3 RNA isolation and quality control

Total RNA from PBMC was extracted using Trizol reagent according to the manufacturer's protocol (Life Technologies, Carlsbad, CA, USA) and purified with RNeasy mini kit (Qiagen, Valencia, CA, USA). Quantity and purity of RNA samples were evaluated by Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific).

2.4 Microarray analysis

The lncRNA and mRNA expression profiling was measured by Clariom™ D solution for Human (Affymetrix GeneChip, Santa Clara, CA), which contained 55,900 noncoding RNAs and 57,500 coding genes based on

the RefSeq (https://ncbi.nlm.nih.gov/refseq/), Ensemble (http://ensemblgenomes.org/), lncRNAwiki (http://lncrna.big.ac.cn/) and NONCODE (http://www.noncode.org/) databases. The raw data were normalized by the software TAC (Transcriptome Analysis Console; Vension:4.0.1) with Robust Multichip Analysis (RMA) algorithm. The sample preparation and microarray hybridization were performed according to the manufacturer's protocol.

The microarray work was performed by Zhongkangbo Biotech, Inc., (Beijing, China). Fold change (FC) was adopted to analyze the statistical significance of the microarray results. FC ≥ 2 or ≤ -2 and $P < 0.05$ were considered the critical values for designating differentially expressed lncRNAs and mRNAs. A volcano plot was used to assess and visualize all differentially expressed genes and heatmap was used to perform hierarchical clustering analysis using The R package.

2.5 GO and KEGG analysis

Gene ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG, www.genome.ad.jp/kegg) pathway analyses were performed based on the correlated mRNAs. The statistical criteria in the screen of statistically significant GO and KEGG terms were $P < 0.05$ and false discovery rate(FDR)<0.05.

2.6 LncRNA-mRNA co-expression network

LncRNA-mRNA interaction networks were constructed to identify the functions of the differentially regulated lncRNAs and mRNAs in mild IAP patients compared to the severe group. Pearson's correlation coefficient (PCC) was calculated for each pair of lncRNAs and mRNAs, and the strongest correlated genes (PCC ≥ 0.85 or≤-0.85; $P < 0.05$) were presented in the co-expression network, which was visualized using Cytoscape software (The Cytoscape Consortium, San Diego, CA, USA).

2.7 Validation by quantitative RT-PCR Validation

Selected lncRNAs expression levels were measured using RT-PCR by ABI 7500 fast Real time PCR System (Applied Biosystems, CA, USA). The relative quantities of lncRNAs were calculated by the $2 - \Delta\Delta Ct$ formula, and all samples were measured in triplicate. The gene expression levels were normalized to that of GAPDH in cDNA samples.

2.9 Statistical analysis

Continuous variables with normal distribution were expressed as the mean with standard deviation (SD) and comparisons between groups were performed with Student's *t-test* (two-sample two-tailed comparison). Continuous variables with nonparametric distribution were expressed as medians with interquartile range and comparisons between groups were performed with the Mann-Whitney test. The *P* value < 0.05 was considered to indicate a statistically significant difference. All statistical data were analyzed by SPSS v20.0 software (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Clinical data

The age of mild IAP patients was 62.0 (34.5 ~ 69.8) years old, including 4 males; the age of severe IAP patients was 50.0 (37.0 ~ 60.0) years old; all were males. Two patients of the mild group were infected with influenza B virus (not subtyped), the other 18 patients were infected with influenza A virus (not subtyped). The baseline characteristics of those patients are summarized in Table 1.

Table 1
Basic information of enrolled IAP patients

Variables	Mild group(n = 10)	Severe group (n = 10)	*P* value
Age (years)	62 (34. 5-64.75)	50 (37–60)	0.405
Sex			
Male	4	10	0.011
BMI	22.95 ± 3.21	24.93 ± 4.70	0.282
Influenza A/B infection	8/2	10/0	0.474
WBC, 10^9/L	5.75 (3.75–7.17)	6.36 (5.93–10.73)	0.112
Lymphocytes, 10^9/L	0.85(0.71–1.12)	0.87(0.65–1.01)	0.791
CRP (mg/dL)	10.04 ± 5.94	14.62 ± 7.65	0.189
Procalcitonin (ng/mL)	0.24(0.07–4.18)	0.57(0.26–20.5)	0.218
BUN (μmol/L)	4.59 ± 1.43	5.04 ± 1.44	0.498
AST (U/L)	25.5 (22.5-33.75)	59 (36-86.25)	0.001
Non-invasive ventilation(n)	0	7	0.003
Invasive ventilation (n)	0	2	0.474
ICU admission (n)	0	3	0.211
Death (n)	0	3	0.211
AST: aspartate aminotransferase. BMI: body mass index. BUN: blood urea nitrogen.			
CRP: c-reactive protein. IAP: influenza-associated pneumonia. ICU: intensive care unit. WBC: white blood cell.			

3.2 Differentially expressed lncRNAs and mRNAs

The lncRNA and mRNA expression profiles of the in the PBMC of mild and severe IAP patients were compared based on the microarray data. Based on the cut-off criteria (fold change value > 2 or <-2, $P < 0.05$), 80 differentially expressed lncRNAs were identified, of which 40 were upregulated and 40 were

downregulated in mild IAP patients compared with severe patients. Then, a total of 180 differentially expressed mRNAs were identified, of which120 were upregulated and 60 were downregulated in the mild group.

The top ten upregulated and downregulated lncRNAs and mRNAs were listed in Table 2 and Table 3, respectively. As showed in Table 2, the most significantly upregulated and downregulated lncRNA was XIST (FC value: 160.898) and lnc-PRYP4-3:1 (FC value: -407.315). As showed in Table 3, the most significantly upregulated and downregulated mRNA was CD83 (FC value: 9.254) and RPS4Y1 (FC value: -17.63). Volcano plot and hierarchical clustering were performed to present the differential expression of lncRNAs and mRNAs between mild and severe IAP patients (Figure.1A-D).

Table 2
Top 10 upregulated and downregulated lncRNAs screened by microarray analysis (mild IAP patients compared with severe IAP patients)

No	LncRNA Symbol	FC value	*P* Value	Description
1	XIST	160.898	0.001	X inactive specific transcript
2	TSIX	4.79	0.006	TSIX transcript, XIST antisense RNA
3	NR_110028	3.837	0.003	Homo sapiens long intergenic non-protein coding RNA 1215 (LINC01215)
4	NONHSAT137711	3.758	0.040	Non-coding transcript identified by NONCODE
5	CA5BP1	3.605	0.017	carbonic anhydrase VB pseudogene 1
6	NONHSAT040399	3.58	0.025	Non-coding transcript identified by NONCODE
7	RPS4XP3	3.095	0.048	Ribosomal protein S4X pseudogene 3
8	NR_037600	3.031	0.002	Homo sapiens long intergenic non-protein coding RNA 1578 (LINC01578)
9	NONHSAT090394	2.99	0.025	Non-coding transcript identified by NONCODE
10	NONHSAT105043	2.868	0.036	Non-coding transcript identified by NONCODE
11	lnc-PRYP4-3:1	-407.315	0.001	Transcript Originally Identified by LNCipedia, alternate transcript name: NONHSAT139812
12	lnc-PRYP4-2:1	-324.034	0.001	Transcript Originally Identified by LNCipedia, alternate transcript name: NONHSAT139811
13	lnc-PRYP4-4:1	-186.108	0.003	Transcript Originally Identified by LNCipedia, alternate transcript name: NONHSAT139813
14	IGHV4-55	-7.111	0.004	Immunoglobulin heavy variable 4–55
15	CH17-212P11.4	-6.964	0.007	Alternate transcript name: ENSG00000282600
16	IGHV3-47	-6.543	0.013	Immunoglobulin heavy variable 3–47
17	IGKV1-13	-5.464	0.024	Immunoglobulin kappa variable 1–13
18	RP11-1166P10.8	-4.823	0.009	Alternate transcript name: ENSG00000223931
19	IGHV3OR16-10	-4.823	0.009	Immunoglobulin heavy variable 3/OR16-10
20	ENST00000420327.1	-4.627	0.005	N/A
* IAP: influenza-associated pneumonia; FC: fold change				

N/A: not applicable

Table 3
Top 10 upregulated and downregulated mRNAs screened by microarray analysis (mild IAP patients compared with severe IAP patients)

No	mRNA Symbol	FC value	*P* Value	Description
1	CD83	9.254	0.0005	CD83 molecule
2	IL1β	6.681	0.006	interleukin 1 beta
3	SLFN5	5.134	0.038	schlafen family member 5
4	TC2N	4.228	0.026	tandem C2 domains, nuclear
5	CD69	4.199	0.041	erythrocyte membrane protein band 4.1
6	EPB41	4.199	0.026	CD69 molecule
7	YTHDC1	4.141	0.016	YTH domain containing 1
8	ZNF101	4.028	0.014	zinc finger protein 101
9	NELL2	3.864	0.035	neural EGFL like 2
10	BIRC3	3.758	0.020	baculoviral IAP repeat containing 3
11	RPS4Y1	-17.63	0.002	ribosomal protein S4, Y-linked 1
12	IL1R2	-17.268	0.010	interleukin 1 receptor, type II
13	EIF1AY	-15.455	0.001	eukaryotic translation initiation factor 1A, Y-linked
14	DEFA1B	-14.621	0.016	defensin, alpha 1B
15	DEFA1	-14.621	0.016	defensin, alpha 1
16	DDX3Y	-14.221	0.008	DEAD (Asp-Glu-Ala-Asp) box helicase 3, Y-linked
17	DEFA3	-12.295	0.019	defensin, alpha 3, neutrophil-specific
18	MMP8	-12.126	< 0.001	matrix metallopeptidase 8
19	S100A12	-6.869	0.033	S100 calcium binding protein A12
20	LTF	-6.543	< 0.001	lactotransferrin

IAP: influenza-associated pneumonia; FC: fold change

3.2 GO and Kyoto KEGG pathway analysis

According to the criteria of $P < 0.05$ and FDR < 0.05, 13 GO terms associated with upregulated genes and 10 GO terms associated with down-regulated genes were identified. The results of GO analysis were illustrated in Fig. 2. All 23 GO terms were categorized into three functional groups: biological process (BP), molecular function (MF), and cellular component (CC).

The BP terms included innate immune response in mucosa (GO:0002227), transcription, DNA-templated (GO:0006351), regulation of transcription, DNA-templated (GO:0006355), etc.

The CC terms included extracellular region (GO:0005576), extracellular space (GO:0005615), nucleus (GO:0005634), etc.

The MF terms included RNA polymerase II core promoter proximal region sequence-specific DNA binding (GO:0000978), metal ion binding (GO:0046872), transcription factor activity, sequence-specific DNA binding (GO:0003700), etc.

Our KEGG pathway enrichment analysis showed that these upregulated mRNAs were closely related to three signaling pathways including the NF-kappa B signaling pathway(hsa:04064), MAPK signaling pathway(hsa:04010) and TNF signaling pathway(hsa:04668), according to the criteria of $P < 0.01$ and FDR < 0.05 (Table 4).

Those differentially downregulated mRNAs in the mild group were mainly enriched in pathways such as transcriptional misregulation in cancers(hsa05202) and one carbon pool by folate (hsa00670), but none of them met the screen criteria.

Table 4
KEGG enrichment analysis results

Pathway ID	Pathway Name	Number of mRNA involved	Enrichment score	*P* value	FDR
hsa04064	NF-κB signaling pathway	10	5.509	< 0.001	0.002
hsa04010	MAPK signaling pathway	16	3.284	< 0.001	0.002
hsa04668	TNF signaling pathway	9	4.282	< 0.001	0.016
FDR: false discovery rate.					

NF-κB: nuclear factor kappa B.

MAPK: mitogen-activated protein kinase.

TNF: tumor necrosis factor.

3.3 Analysis of lncRNA-mRNA co-expression network

A total of 12 lncRNA s and 52 mRNAs were selected to construct the co-expression network as showed in Fig. 3. (A). The lncRNA-mRNA network included 111 positive pairs and 2 negative pairs. As can be observed in Fig. 4-A, the majority of differentially expressed genes were upregulated genes. Only 2 downregulated mRNAs were listed in the lncRNAs-mRNAs network, including EIF1AY and RPS4Y1. Both were negatively regulated by lncRNA XIST. Of note, lncRNA NONHSAT105043(degree = 24), SBDSP1(degree = 23), and

CA5BP1 (degree = 12) were in the central position of the network and connected with most of the mRNAs. Representative co-expression networks of NONHSAT 105043, SBDSP1, and CA5BP1 with the target genes are shown in Fig. 3. (B), (C), and (D).

3.4 Quantitative RT-PCR Validation

We selected 2 upregulated lncRNAs (XIST, and CA5BP1) and 2 downregulated lncRNAs (ENST00000420327.1 and CH17212P11.4) for quantitative RT-PCR validation in the isolated RNA samples (n = 20). The primers used for RT-PCR were listed in Table 4. As showed in Fig. 4, the level of XIST and CA5BP1 were significantly higher in mild patients, which was consistent with microarray data. Although the level of ENST00000420327.1 and CH17212P11.4 were higher in severe group, the difference was not statistically significant.

Table 5
LncRNA gene primers for polymerase chain reaction

Target Gene	Forward Primer (5′ – 3′)	Reverse Primer (3′ – 5′)	FC value
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG	N/A/
XIST	TGGATAGAGGACCCAAGCGA	CAAGACTGGCCCAGGCATAA	160.898
CA5BP1	CCCAAAAGCACTGGGTTCTG	GATGCAGATGCGGGTGTAGT	3.605
CH17-212P11.4	CTGGGGGAGGCTTGGTAAAG	GTGAATCGGCCCTTCACAGA	-6.964
ENST00000420327.1	ACAACCACTCAAGGTCTGCAA	TGGCAGTGTAAGTATGGCACA	-5.464
FC: fold change; N/A: not applicable.			

Discussion

In this study, we demonstrated that there were notable changes in the expression profiles of lncRNA and mRNA in PBMCs of mild and severe IAP patients.

At present, there is only little data available on lncRNAs related to influenza infection. This study was designed to increase our knowledge of this relation. The study revealed that there were 80 lncRNAs (40 upregulated and 40 downregulated) and 180 mRNAs (120 upregulated and 60 downregulated) differentially expressed in mild IAP patients compared with severe IAP patients.

Among those dysregulated lncRNAs, some of them have been reported in human diseases. For example, the lncRNA CA5BP1 have a protective role in the pathogenesis of acute myocardial infarction[12] and high levels of lncRNA SBDSP1 were associated with unfavorable prognosis of colorectal cancer [13]. However, none of these lncRNAs have been reported to be involved in influenza infection.

From the microarray profile, it was interesting to note that compared with the severe group, a significant proportion of differentially up-regulated mRNAs in mild IAP patients have been proved to play protective roles in influenza or other virus infections. For example, CD 83 has been proved to play a protective role

against influenza A virus(IAV) infection by activating the innate and acquired antiviral immune response[14]. Interleukin-1 beta (IL-1β)[15] is a key component of the cytokine storm evoked by influenza infection [16] and could recruit CD4(+) T cells to the site of infection and improve survival during influenza virus infection [17]. Schlafen family member 5 (SLFN5) has been proved to play an antiviral role in herpes simplex virus 1(HSV-1) infection by binding viral DNA and suppressing viral transcription[18].

In the top ten down-regulated mRNAs, matrix metallopeptidase 8 (MMP8)[19] and S100 calcium binding protein A12 (S100A12)[20] have been proved to be biomarkers that correlate with disease severity and poor outcome in influenza and COVID-19 patients, respectively. The results of our study are consistent with the above-mentioned research, which also indirectly demonstrated that our research results are credible.

KEGG analysis identified only three significantly altered pathways between mild and severe IAP patients, including NF-κB signaling pathway, MAPK signaling pathway, and TNF signaling pathway. These pathways have been demonstrated to make great contributions to the pathogenesis of influenza infection[21]. For example, NF-κB signaling pathway is a critical regulator that control the expression of various antiviral cytokines, such as interferon-α (IFN-α) and interferon-β (IFN-β) upon influenza virus infection [22, 23]. TNF-α expression plays essential role via regulating the CD8(+) T cell mediated immune response to influenza infection [24, 25]. MAPK family members could regulates the production of antiviral chemokines and cytokines such as TNF-α, monocyte chemotactic protein-1(MCP-1), interleukin (IL)-6 and IL -8[26, 27]. Except for the direct antiviral effect on influenza infection, both MAPK and TNF signaling pathways are involved in the process of the activation of NF-κB signaling pathway[28–30] .

As the function of the majority of lncRNAs remains unclear, the lncRNA-mRNA interaction network was used to predict the function of differentially expressed lncRNAs. A total of 16 lncRNAs and 64 mRNAs were identified as the hub genes. Five of the top 10 up-regulated lncRNAs were listed in the lncRNA-mRNA network, including XIST, NR_110028, NONHSAT137711, NR_037600, CA5BP1 and NONHSAT105043. Among those including mRNAs, DDX3X[31], IL4R[32], MAL[33], CDR2[34], and CAMK4[35] were previously reported in influenza infection. For example, the host protein DEAD box helicase 3 X-linked ,also known as DDX3X, has been proved to be an antiviral protein during influenza infection by coordinating the activation of the nucleotide-binding oligomerization domain-like receptor with a pyrin domain 3 (NLRP3) inflammasome, assembly of stress granules, and activation of type I interferon (IFN) signaling[31]. IL-4R is a key member of IL-4 signaling, which is essential for the B cell response during influenza infection. Miyauchi K and colleagues found that IL-4R-deficient B cells showed less proliferation and a marked reduction of dual-specific B cells in germinal center, which lead to the reduction of the protective antibodies [32].Although not reported in influenza infection, existing evidence showed that SLFN5[18] and RPS4Y1[36] was involved in the pathogenesis of HPV and RSV infection, respectively.

On the other hand, previous studies have demonstrated that PIK3R1[37], BIRC3[38] and PLCG1[39] are all important regulators of NF-κB signaling pathway, meanwhile BIRC3[40] and PIK3R1[41] are pivotal mediators in the regulation of TNF signaling pathway.

Taken together, we can speculate that hub lncRNAs such as NONHSAT105043 might contribute to the pathogenesis of influenza infection via interaction with hub mRNAs, such as DDX3X[31] and IL4R. It was subsequently indicated that those hub lncRNAs might play crucial roles in influenza infection via regulating NF-κB, TNF, and MAPK signaling pathway expression. Furthermore, several interactions between hub lncRNAs and mRNAs, including lncRNA NONHSAT105043-DDX3X, CA5BP1-DDX3X, and CA5BP1-IL-4R, deserve further investigation in the future studies.

Limitation

There are some limitations to the interpretation of this study. First, only a small number of patients were enrolled. Therefore, the differences we identified between mild and severe IAP patients need to be further validated by larger scale studies. Secondly, the quantitative RT-PCR Validation results were only partly consistent with the microarray data in the current study, which may be attributable to the limited patient's number. Thirdly, the sex ratio in our study is imbalanced. Most of the rerolled patients were male, especially in the severe group.

Conclusion

In the present study, we explored the expression profiles and functional networks of lncRNAs and mRNAs in the PBMC of patients with mild and severe influenza pneumonia. We identified several key lncRNAs/mRNAs and related pathways that might play essential roles in the pathogenesis of influenza-associated pneumonia. These findings provide potential targets and novel insights into the mechanisms of severe influenza infection.

Declarations

Conflict of interest.: The authors declare that they have no conflict of interest.

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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:

Li Gu conceived and supervised the study. Yudong Yin performed the bioinformatics analysis and was a major contributor in writing the manuscript. Hong Shen, Ming Wei, Zhenjia Liu performed the RNA sample retraction and RT-PCR test. Di Cao and Shenghu Feng performed the enrollment job and data collection. Chunxia Yang prepared the figures and edited the manuscript. All authors read and approved the final manuscript.

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Figures

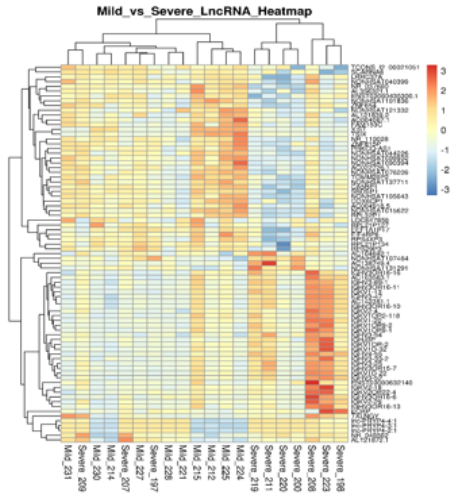
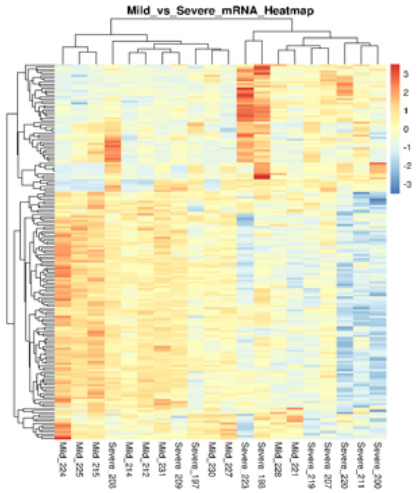


Figure 1. (A)



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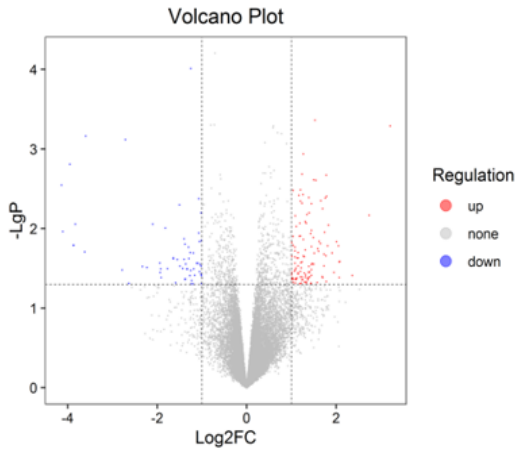

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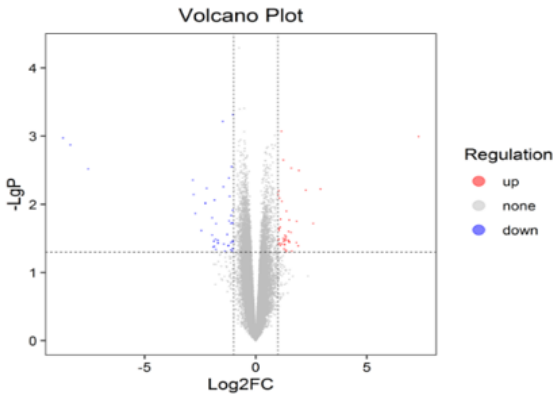

Figure 1. (D)

Figure 1

(A) Hierarchical clustering shows a distinguishable lncRNA expression profile between the mild and severe IAP groups and homogeneity within the groups. (B) Hierarchical clustering shows a distinguishable mRNA expression profile between the two groups and homogeneity within groups; red represents the upregulated genes and blue represents the downregulated genes. The relative lncRNAs and mRNAs expression are

depicted according to the color scale. Figure 1. (C) Volcano plot of lncRNA expression profile, Figure 1. (D): Volcano plot of mRNA expression profile.

The red and blue points indicate differentially upregulated and downregulated genes, respectively.

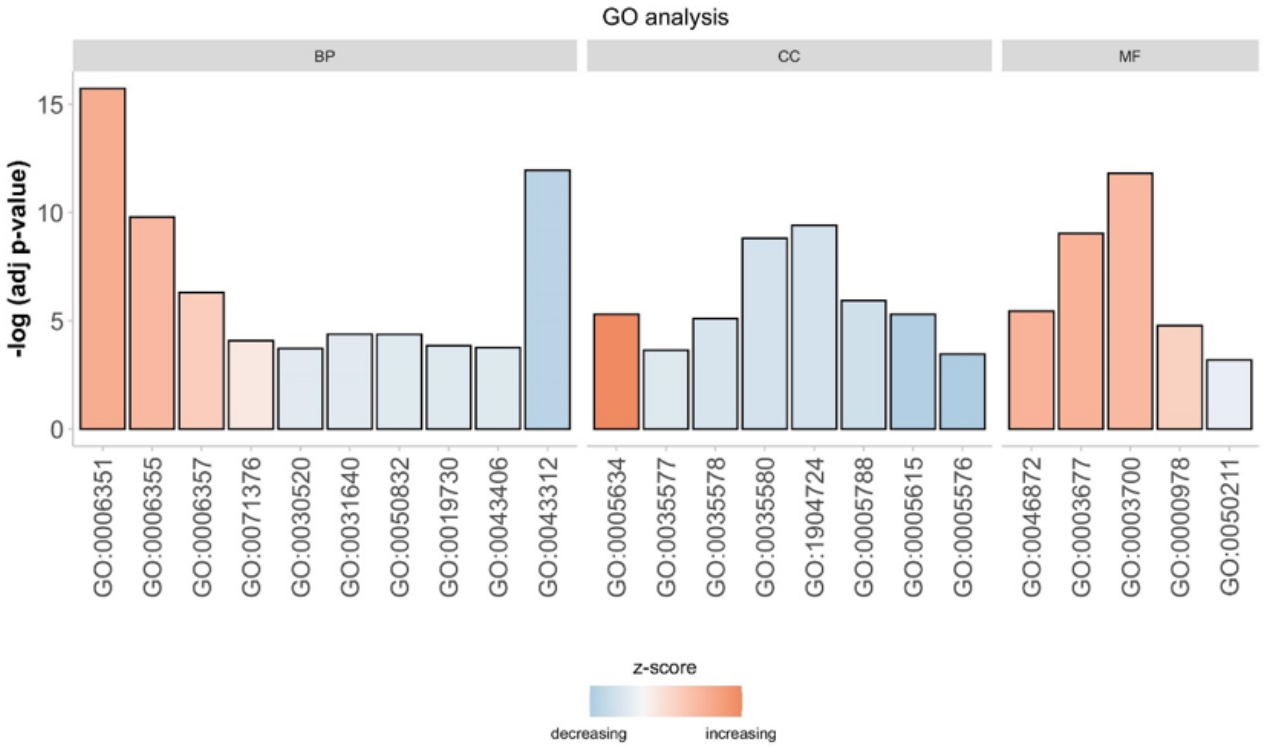

Figure 2

Gene ontology annotation. The significantly enriched GO terms in each category, MF: molecular function; CC: cellular component; BP: biological process. Red column represented upregulated gene related GO terms and the blue column represented downregulated gene related GO terms. Red represents upregulated gene enriched GO terms and grey represents downregulated gene enriched GO terms. Darker color represents a higher level of enrichment, and the lighter color represents a lower level of enrichment.

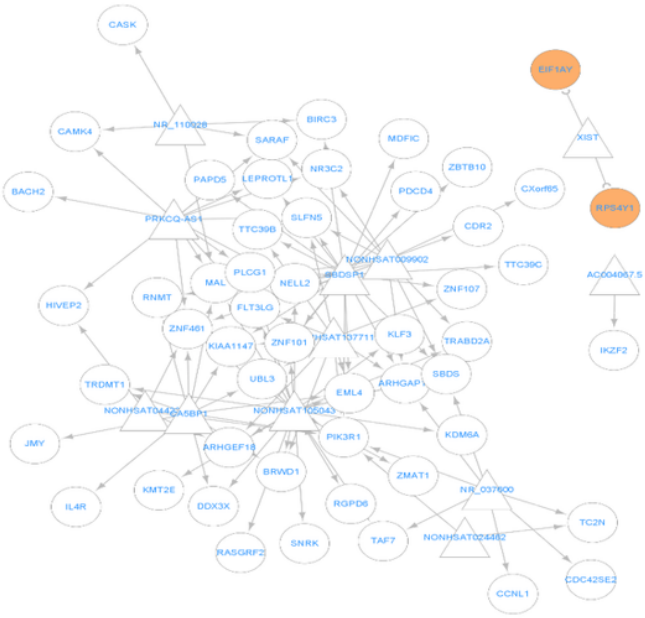

Figure 3. (A)

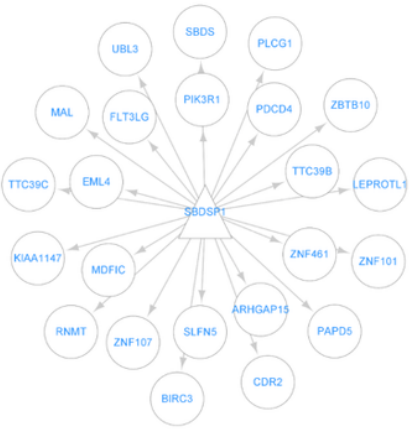

Figure 3. (C)

Figure 3. (B)

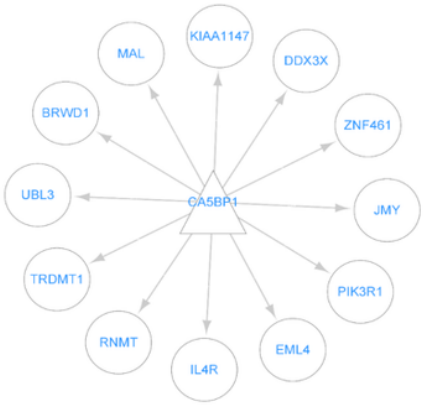

Figure 3. (D)

Figure 3

(A): LncRNA-mRNA interaction network of differentially expressed genes. Figure 3. (B): Network between lncRNA NONHSAT 105043 and its target mRNAs; Figure 3. (C): Network between lncRNA SBDSP1 and its target mRNAs; Figure 3. (D): Network between lncRNA CA5BP1 and its target mRNAs. The white nodes and orange nodes represent the upregulated and downregulated mRNAs, respectively. The white and orange triangles represent the up-regulated and downregulated lncRNAs, respectively. The solid arrow represents the positive regulation effect. The open half circles represented the negative regulation effect.

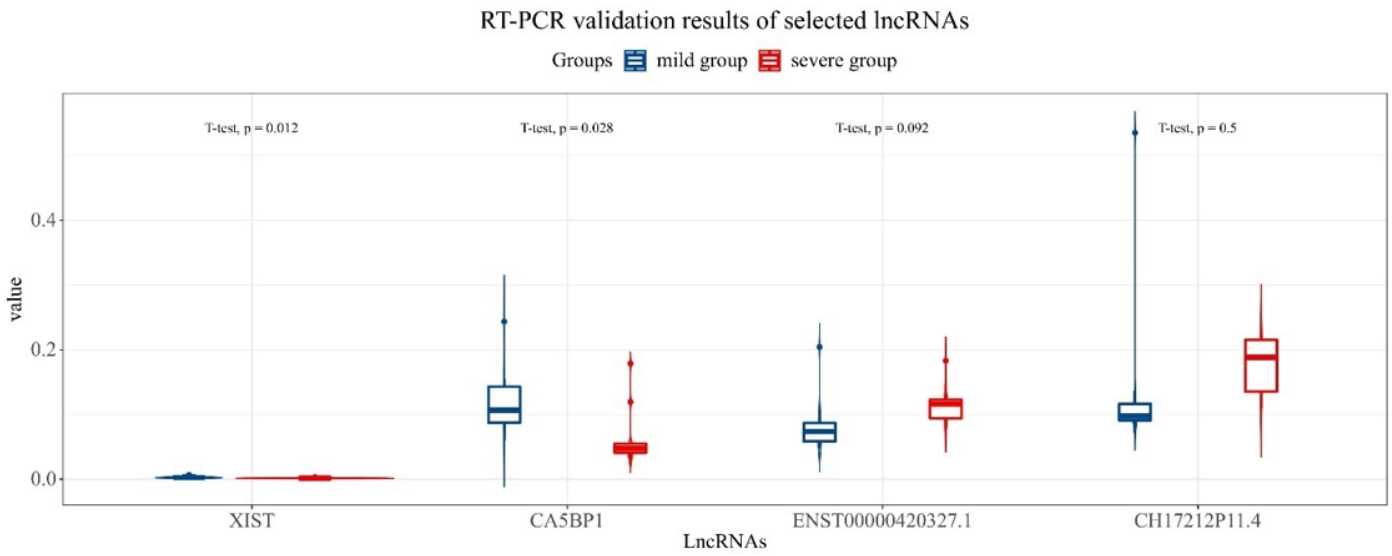

Figure 4

Relative expression levels of selected lncRNAs in mild and severe IAP patients determined by RT-PCR. The expression levels of lncRNA were normalized using GAPDH as an internal control. The heights of the columns in the chart represent the mean fold changes in expression for each of these lncRNAs.