Aberrant expression of long non-coding RNAs in peripheral blood mononuclear cells response to tuberculosis in children

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Abstract

We aimed to identify long non-coding RNAs (lncRNAs) aberrantly expressed in peripheral blood mononuclear cells (PBMCs) triggered by active tuberculosis (ATB), latent tuberculosis infection (LTBI), and healthy controls (HC). We examined lncRNAs expression in PBMCs isolated from children with ATB and LTBI, and from HC using RNA sequencing. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to explore the biological processes and signaling pathways of aberrantly expressed mRNAs. A total of 348 and 205 lncRNAs were differentially expressed in the ATB and LTBI groups, respectively, compared to the HC group. Compared to the LTBI group, 125 lncRNAs were differentially expressed in the ATB group. Compared to the HC group, 2317 mRNAs were differentially expressed in the ATB group, and 1093 mRNAs were differentially expressed in the LTBI group. Compared to the LTBI group, 2328 mRNAs were differentially expressed in the ATB group. The upregulated mRNAs were mainly enriched in neutrophil activation, neutrophil-mediated biological processes, and positive regulation of immune response in tuberculosis (TB), whereas the downregulated mRNAs were enriched in signaling pathways and structural processes, such as the Wnt signaling pathway and rDNA heterochromatin assembly. This is the first study on the differential expression of lncRNAs in PBMCs of children with TB. We identified significant differences in the expression profiles of lncRNAs and mRNAs in the PBMCs of children with ATB, LTBI, and HC, which has important implications for exploring lncRNAs as novel biomarkers for the diagnosis of TB. In addition, further experimental identification and validation of lncRNA roles could help elucidate the underlying mechanisms of *Mycobacterium tuberculosis* infection in children.

Abbreviations: ATB = active tuberculosis, ceRNA = competitive endogenous RNA, GO = gene ontology, HC = healthy controls, KEGG = Kyoto Encyclopedia of Genes and Genomes, lncRNAs = long non-coding RNAs, LTBI = latent tuberculosis infection, miRNAs = microRNAs, *MTB* = *Mycobacterium tuberculosis*, NEAT1 = Nuclear Paraspeckle Assembly Transcript 1, PBMCs = peripheral blood mononuclear cells, TB = tuberculosis, XIST = X-inactive specific transcript.

Keywords: children, lncRNA, mRNA, PBMC, tuberculosis

1. Introduction

Approximately 7.1 million new cases of tuberculosis (TB) were confirmed in 2020 worldwide, which is less than that reported in 2019 because of the COVID-19 pandemic.[1] However, 1.3 million patients with TB died of TB in 2020, which is more than that reported in 2019. China is among the top 20 high-burden TB countries worldwide.[1] Approximately 11% of all TB cases occur in children.[1] Because children have a weaker immune system than adults, they suffer more seriously from *Mycobacterium tuberculosis* (*MTB*) infection, with fewer typical clinical manifestations and signs, which lead to delayed and ineffective treatment, eventually leading to death.[2,3]

Currently, the gold standard for the diagnosis of TB is sputum smear and *MTB* culture.[4] However, *MTB* culture is time-consuming and has low sensitivity,[4] and the diagnosis of TB remains a worldwide challenge. Therefore, it is necessary to develop rapid and accurate molecular biological detection methods to diagnose TB earlier to improve its prognosis and reduce its mortality.

Based on their length, non-coding RNAs can be divided into short non-coding RNAs (<200 nucleotides) and long non-coding RNA (lncRNAs, >200 nucleotides).[5,6] Many studies have shown that lncRNAs play important roles in the occurrence and development of various diseases, including tumors, cardiovascular diseases, nervous system diseases, and infectious diseases.[7–11] Previous studies have also confirmed that the expression of lncRNAs in T cells, B cells, and macrophages is closely related to the onset of TB.[12,13]

To date, many studies have explored the expression profiles and important roles of lncRNAs in TB, which could be new molecular diagnostic markers for TB.[14,15] Wang et al reported that lnc-CD244 is an epigenetic regulator of interferon (IFN)-γ and tumor necrosis factor (TNF)-α released by CD8 + T cells and can influence the immune response of CD8 + T cells in ATB.[16] LIU Z.-Z et al showed that lncRNA SNHG15is highly expressed in patients with spinal TB and promotes the occurrence of TB.[17] Low expression of lncRNA SNHG15 can inhibit the RANK/RANKL pathway in osteoclasts and inhibit the secretion of inflammatory cytokines, such as IL-6 and TNF-α. It is difficult to diagnose TB early in children. Our study aimed to investigate the differential profiles of lncRNAs in children between TB, latent tuberculosis infection (LTBI), and healthy controls (HC) groups in peripheral blood mononuclear cells (PBMCs) using RNA sequencing and bioinformatic analysis. This is the first study on the differential expression analysis of lncRNAs in *MTB* infection in children in vivo using high-throughput sequencing technologies, which provides novel information in the exploration of new molecular diagnostic biomarkers for TB in children.

2. Materials and Methods

2.1. PBMC extraction and RNA extraction

The advice of 2 clinical experts and the diagnostic standards of TB in the Nelson Textbook of Pediatrics (the 21st ed)[18] were followed, and we selected 4 ATB, 4 LTBI, and 4 HC children (the characteristics are shown in Table 1). ATB, LTBI, and HC patients from the West China Second Hospital, Sichuan University were enrolled in the study. The diagnosis of LTBI was based on a positive tuberculin skin test or T-spot results; however, there were no positive symptoms, signs, or imaging manifestations. None of the children had a history of HIV or other infectious diseases and none had used immunosuppressants for nearly 6 months.

Ficoll density gradient centrifugation was used to extracted PBMCs (Isolation Kit, Solarbio, China). TRIzol reagent (Invitrogen, Carlsbad, CA) was used to extract total RNA. This study was approved by the Ethics Committee of West China Second Hospital, Sichuan University, and was conducted in accordance with the Declaration of Helsinki. All the participants provided informed consent.

2.2. RNA sequencing and differentially expressed lncRNAs analysis

A NanoDrop 2000 UV spectrophotometer (Thermo Fisher Scientific) was used to determine the concentration and purity of

total RNA. The acceptable OD260/280 ratio ranges from 1.8 to 2.1. Total RNA concentration was 50 to 200 ng/μL. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples with an RNA integrity >7 were used for subsequent processing. After quality testing, the rRNA was removed from the total RNA using reagents. We constructed the cDNA library and performed RNA sequencing using a Novaseq 6000 (Illumina).

Paired-end short reads were aligned to the mouse reference genome GRCh38 using HISAT2 (v.2.2.1). Stringtie2 (v.2.2.1) was used to assemble and quantify parameters. The read counts of the transcripts were extracted using a Python script (prepDE.py3). The DESeq DataSet function from the matrix of the R package DESeq2 (v.1.26.0) created a DESeqDataSet object, which was then normalized through the function rlog using the parameter "blind = FALSE." Principal component analysis and sample distances were calculated using DESeq2 (v.1.26.0), and differential analysis was performed using the DESeq function; the cutoff was set as |log2 fold change (FC)| > 2 and p.adjust < 0.05, after adjustment using Benjamini and Hochberg's approach. Hierarchical clustering analysis based on centered Pearson correlation was performed for all the differentially expressed mRNAs through the heatmap (v.1.0.12). ggplot2 (v.3.3.5) was used to construct volcano plots to show the differences. Differentially expressed lncRNAs were identified using the DESeq function, with thresholds of |log2 FC| > 2 and p.adjust < 0.05.

2.3. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis

Bioinformatic analysis of the differentially expressed genes was performed to obtain enriched GO terms (http://www.geneontology.org)[19] and KEGG pathways (padj < 0.05). R software was used to conduct visible analysis of the RNA sequencing data. FC > 2 and *P* value <.05 were considered the criteria for differential expression.

2.4. Statistical analysis

The data are presented as the mean ± standard deviation. ANOVA or Fisher's accuracy test was used for statistical analysis. *P* < .05 indicated that the difference was considered statistically significant.

3. Results

3.1. Population characteristic

There are no significant differences in age and sex (*P* > .05) among the ATB, LTBI, and HC groups in this study, and the details are shown in Table 1.

3.2. Significant analysis of differential expressed lncRNAs in different groups

Samples from the ATB, LTBI, and HC groups were compared side by side. A principal component analysis plot was used to visualize the distributions of the ATB, LTBI, and HC groups (Fig. 1). As shown in Figure 1, PC1 represented 13% of the variance, and 13% of the variance for PC2. Volcano plots (Fig. 2) and hierarchical clusters (Fig. 3) show the gene expression patterns. In Figure 2, each graph shows the results of the differentially expressed genes, where the horizontal coordinate denotes the FC and the vertical coordinate represents the significance of the differences. The horizontal and vertical gray dashed lines represent thresholds (p.adjust < 0.05, |FC| > 2). Clustering analysis (Fig. 3) of ATB and the samples

Table 1

Characteristics of ATB patients, LTBI and healthy control subjects.

	ATB	LTBI	HC
Age (yrs)	6.75 ± 1.71	3.5 ± 0.58	4.75 ± 2.87
Gender (male/female)	4/0	4/0	3/1

A *P* value <.05 indicates statistical significance.

ATB = active tuberculosis, HC = healthy controls, LTBI = latent tuberculosis infection.
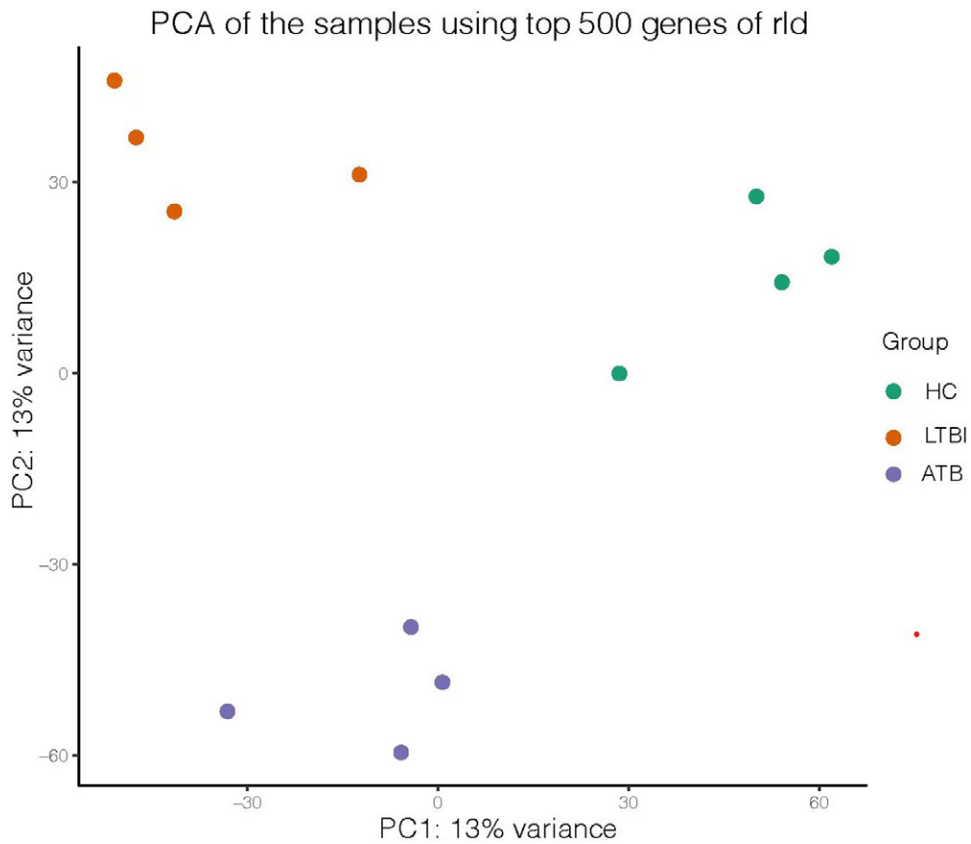
Figure 1. Distances between samples in the ATB group, LTBI group and HC group. The distance analysis of the samples from the 3 groups is shown in Figure 1. Principal component analysis (PCA) plot with colors indicating the ATB group (purple), LTBI group (red) and HC group samples (green). ATB = active tuberculosis, HC = healthy controls, LTBI = latent tuberculosis infection.

Figure 2. Volcano plot of samples from the ATB group, LTBI group and HC group. The lncRNA variants between samples from the ATB group, LTBI group and HC group are shown in Figure 2. The horizontal axis represents fold change and the vertical axis represents the *P* value after correction. (A) ATB group and HC group. (B) LTBI group and HC group. (C) ATB group and LTBI group. ATB = active tuberculosis, HC = healthy controls, LTBI = latent tuberculosis infection.

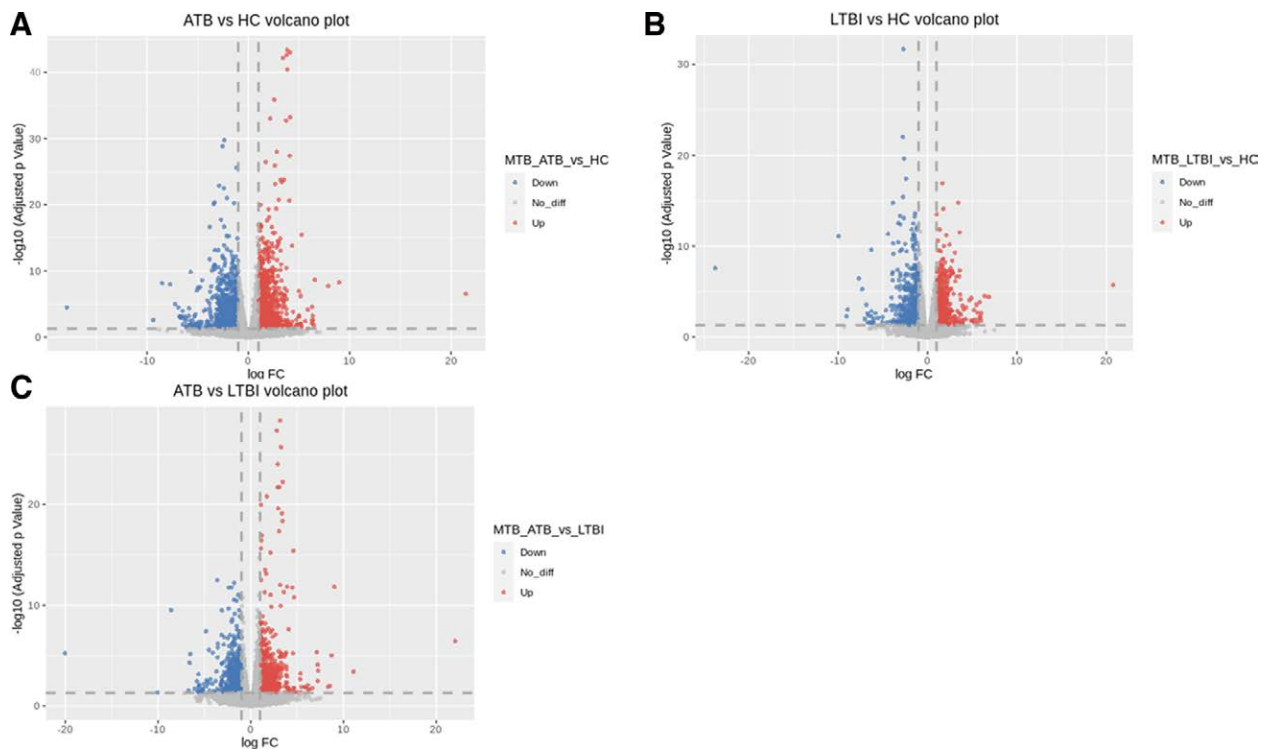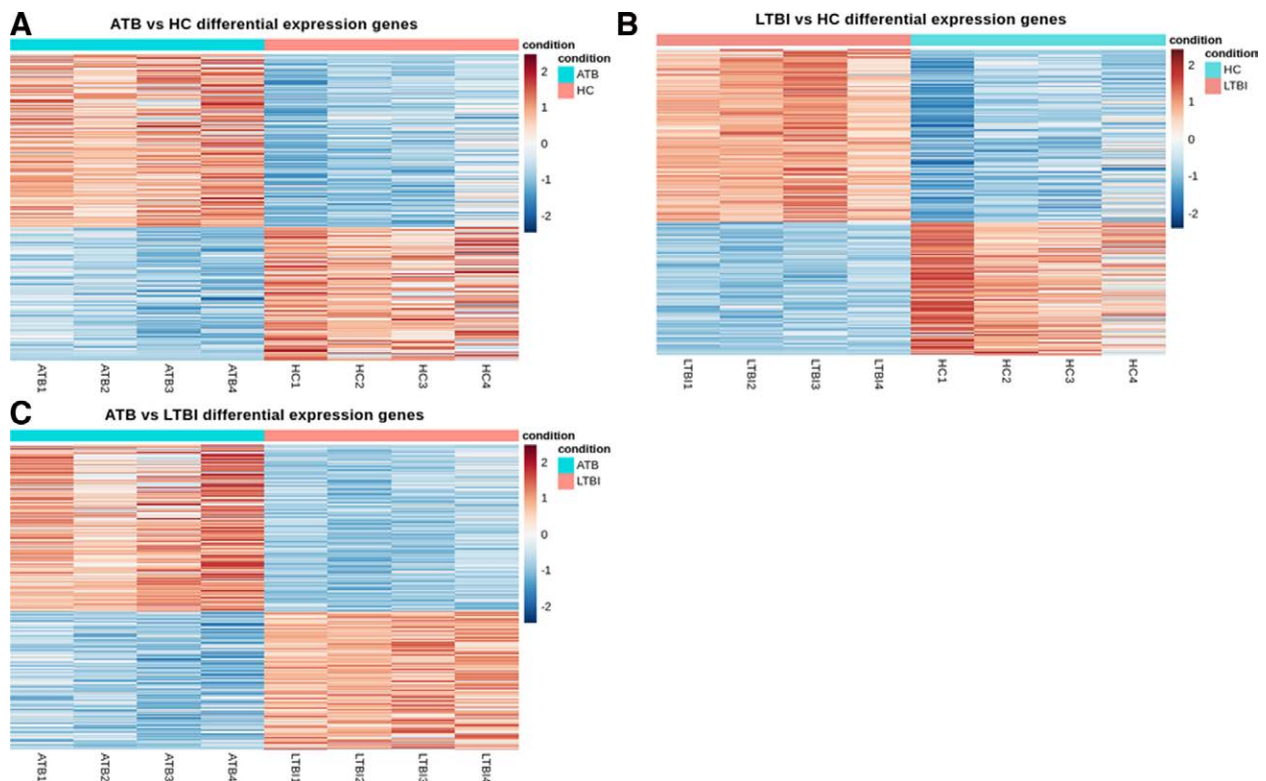**Figure 3.** Heatmap of differentially expressed genes between ATB, LTBI and HC. Clustering analysis of ATB, LTBI and HC groups samples reveals that up-regulated and down-regulated lncRNAs exhibit ploidy changes. The color gradient indicates gene expression abundance, with red indicating up-regulated genes and blue indicating down-regulation of genes. (A) ATB and HC. (B) LTBI and HC. (C) ATB and LTBI. ATB = active tuberculosis, HC = healthy controls, lncRNA = long non-coding RNAs, LTBI = latent tuberculosis infection.

from the LTBI and HC groups revealed that upregulated and downregulated lncRNAs exhibited changes in ploidy. The top 5 differentially expressed lncRNAs in each group are shown in Table 2. The top 5 differentially expressed mRNAs are listed in Table 3.

3.3. Analysis of differentially expressed lncRNAs

Genes with similar expression patterns in the hierarchical clustering analysis were categorized together, indicating that these genes may have similar biological functions. The differences in lncRNA expression patterns of different groups were characterized by a hierarchical clustering heatmap to observe the gene expression of each group. As shown in Figure 3, ATB versus HC, ATB versus LTBI, and LTBI versus HC are clustered correctly to their own groups.

After lncRNA differential expression analysis, 348 lncRNAs were differentially expressed (220 upregulated and 128 downregulated) in the ATB group compared to the HC group, screening at the threshold of p.adjust < 0.05 and | FC | > 2, representing 51.33% of the total lncRNAs tested. The most upregulated lncRNA was ENST00000636967, followed by ENST00000425497, ENST00000423719, ENST00000429829, ENST00000604411, and ENST00000439423. The top 5 upregulated and downregulated lncRNAs are listed in Table 2.

Comparing the ATB and LTBI groups revealed that 125 lncRNAs were differentially expressed (43 upregulated and 82 downregulated), accounting for 18.44% of the total lncRNAs tested. The most upregulated lncRNAs were ENST00000586348, ENST00000596996, and ENST00000625598, and the most downregulated lncRNA was ENST00000439423, followed by ENST00000604411 and ENST00000616315. Additionally, comparing the LTBI and HC groups revealed that 205 lncRNAs were differentially expressed (172 upregulated and 33 downregulated), accounting for 30.23% of all lncRNAs tested. The most upregulated lncRNA was ENST00000636967, followed by ENST00000414008, ENST00000425497, ENST00000429829, ENST00000586348, and ENST00000604411.

Compared to the HC group, 2317 mRNAs (1304 upregulated, 1013 downregulated) were differentially expressed in the ATB group, and 1093 (616 upregulated and 477 downregulated) in the LTBI group. Compared to the LTBI group, 2328 mRNAs (1271 upregulated and 1057 downregulated) were differentially expressed in the ATB group (FDR < 0.05). These results indicate that lncRNAs and mRNAs in PBMCs were altered during TB infection. However, most lncRNAs and mRNAs in diseases remain poorly understood.

3.4. KEGG and GO enrichment analysis of differentially expressed mRNAs

To further investigate the biological processes involved in the differentially expressed mRNAs, we used the biological processes in the GO database as well as KEGG pathway analysis. The database was used to annotate the differentially expressed lncRNAs in the ATB versus HC, ATB versus LTBI, and LTBI versus HC groups.

The results of the ATB versus HC group (Fig. 4A) showed that many up-regulated mRNAs were significantly enriched, which is related to immune function or inflammatory response, such as neutrophil activation involved in the immune response, neutrophil degranulation, and positive regulation of cytokine production. The downregulated genes were significantly enriched in rDNA heterochromatin assembly (Fig. 5A). KEGG pathway analysis showed that many upregulated genes were enriched in disease-associated and signaling pathways, such as prion

Table 2

Differential expression of lncRNAs between each groups.

	UP-Transcript ID	Name	log2FoldChange	Down-Transcript ID	Name	log2FoldChange
ATB-HC	ENST00000636967	novel	19.71823104	ENST00000429829	XIST	-30
	ENST00000425497	SMIM25	9.23719227	ENST00000604411	TSIX	-27.59241694
	ENST00000423719	LINC00484	8.890240309	ENST00000439423	novel	-22.22586335
	ENST00000471299	novel	8.06676611	ENST00000553207	LINC01619	-7.72088949
	ENST00000436710	LINC01503	8.045261948	ENST00000586185	LINC02081	-7.52572772
LTBI-HC	ENST00000636967	novel	19.88874109	ENST00000429829	XIST	-30
	ENST00000414008	HCG27	11.33724081	ENST00000586348	novel	-25.48526597
	ENST00000425497	SMIM25	8.623807932	ENST00000604411	TSIX	-10.26420179
	ENST00000471299	novel	7.970533782	ENST00000418001	novel	-7.558921777
	ENST00000531076	novel	7.589882276	ENST00000559402	novel	-6.021368149
ATB-LTBI	ENST00000586348	novel	26.75147808	ENST00000439423	novel	-22.37968033
	ENST00000596996	novel	6.676823666	ENST00000604411	TSIX	-17.32821515
	ENST00000625598	novel	6.645743859	ENST00000616315	NEAT1	-6.996666712
	ENST00000631211	novel	6.607530326	ENST00000638682	LINC02001	-6.876675321
	ENST00000617117	novel	6.352019004	ENST00000634796	LINC02381	-6.49011017

lncRNA = long non-coding RNAs.

Table 3

Differential expression of mRNAs between each groups.

	UP-Gene ID	Symbol ID	log2FoldChange	Down-Gene ID	Symbol	log2FoldChange
ATB-HC	ENSG00000196826	AC008758.1	21.47436938	ENSG00000142541	RPL13A	-1.000658622
	ENSG00000272414	FAM47E-STBD1	8.951070062	ENSG00000106123	EPHB6	-1.001832081
	ENSG00000011201	ANOS1	7.891424269	ENSG00000083812	ZNF324	-1.001876837
	ENSG00000214435	AS3MT	6.571328179	ENSG00000225663	MCRIP1	-1.002589789
	ENSG00000170439	METTL7B	6.434148847	ENSG00000136270	TBRG4	-1.002622133
LTBI-HC	ENSG00000196826	AC008758.1	20.76315495	ENSG00000171132	PRKCE	-1.000243016
	ENSG00000272414	FAM47E-STBD1	6.840011732	ENSG00000020633	RUNX3	-1.000271605
	ENSG00000135373	EHF	6.397292936	ENSG00000152484	USP12	-1.004826838
	ENSG00000187546	AGMO	6.003592496	ENSG00000122068	FYTTD1	-1.00597169
	ENSG00000163646	CLRN1	6.00325188	ENSG00000164674	SYTL3	-1.007668846
ATB-LTBI	ENSG00000285446	Z84488.2	22.03252742	ENSG00000172006	ZNF554	-0.586175518
	ENSG00000269711	AC008763.3	11.07154854	ENSG00000188566	NDOR1	-0.586880611
	ENSG00000214435	AS3MT	9.029306644	ENSG00000070759	TESK2	-0.589295216
	ENSG00000230453	ANKRD18B	8.71397766	ENSG00000103064	SLC7A6	-0.589586827
	ENSG00000166211	SPIC	8.559447592	ENSG00000196998	WDR45	-0.590223845

disease, *Salmonella* infection, the NOD-like receptor signaling pathway, and neutrophil extracellular trap formation (Fig. 6A). The downregulated genes were enriched in signaling pathways such as the Wnt signaling pathway, alcoholism, and the Hippo signaling pathway (Fig. 7A).

Results from the LTBI and HC groups (Fig. 4B) showed that upregulated lncRNAs were significantly enriched mainly in immune response or immune function processes, such as neutrophil degranulation, neutrophil activation involved in immune response, positive regulation of cytokine production, cell chemotaxis, and regulation of the inflammatory response. The downregulated mRNAs were enriched in the regulation of cell-cell adhesion, T-cell activation, leukocyte cell-cell adhesion, and positive regulation of leukocyte activation (Fig. 5B). KEGG results showed that the upregulated lncRNAs were mainly involved in the NOD-like receptor signaling pathway, osteoclast differentiation, and viral protein interaction with cytokines and cytokine receptors (Fig. 6B). The downregulated lncRNAs were enriched in cytokine-cytokine receptor interactions and the intestinal immune network for IgA production (Fig. 7B).

The results of the ATB and LTBI groups (Fig. 4C) showed that there were many differentially expressed mRNAs that were significantly enriched in relation to the inflammatory response, such as neutrophil-mediated immunity, neutrophil activation, neutrophil degranulation, and neutrophil activation involved in the immune response. Downregulated mRNAs were enriched in structural assembly, such as heterochromatin, rDNA heterochromatin, and DNA replication-dependent nucleosome assembly (Fig. 5C). Similarly, KEGG results showed that many upregulated genes were enriched in prion disease, regulation of the actin cytoskeleton, and endocytosis (Fig. 6C). The downregulated genes were enriched in alcoholism and neutrophil extracellular trap formation (Fig. 7C).

4. Discussion

Using RNA sequencing and in-depth data profiling, we found that many lncRNAs and mRNAs were differentially expressed in *MTB*-infected PBMCs in vivo. The data showed that there were more upregulated lncRNAs and mRNAs than downregulated lncRNAs and mRNAs, and the number of upregulated lncRNAs and mRNAs was higher in the ATB group than in the LTBI group, which indicates that TB infection is mainly regulated by the activation of certain pathways in PBMCs, and some pathways are further activated in the development of LTBI into ATB. This finding provides some directive for research related to TB therapy. Compared to the HC group, the lncRNAs expression profiles in patients with ATB and LTBI were differentially expressed, indicating that lncRNAs may play important roles in *MTB* infection. Compared to the HC group, the number of differentially expressed lncRNAs and mRNAs in ATB was higher than that in LTBI in PBMCs, which is similar to a previous study.[20] This suggests that lncRNAs may play an important role in the development of LTBI to ATB. Moreover, ENST00000636967,
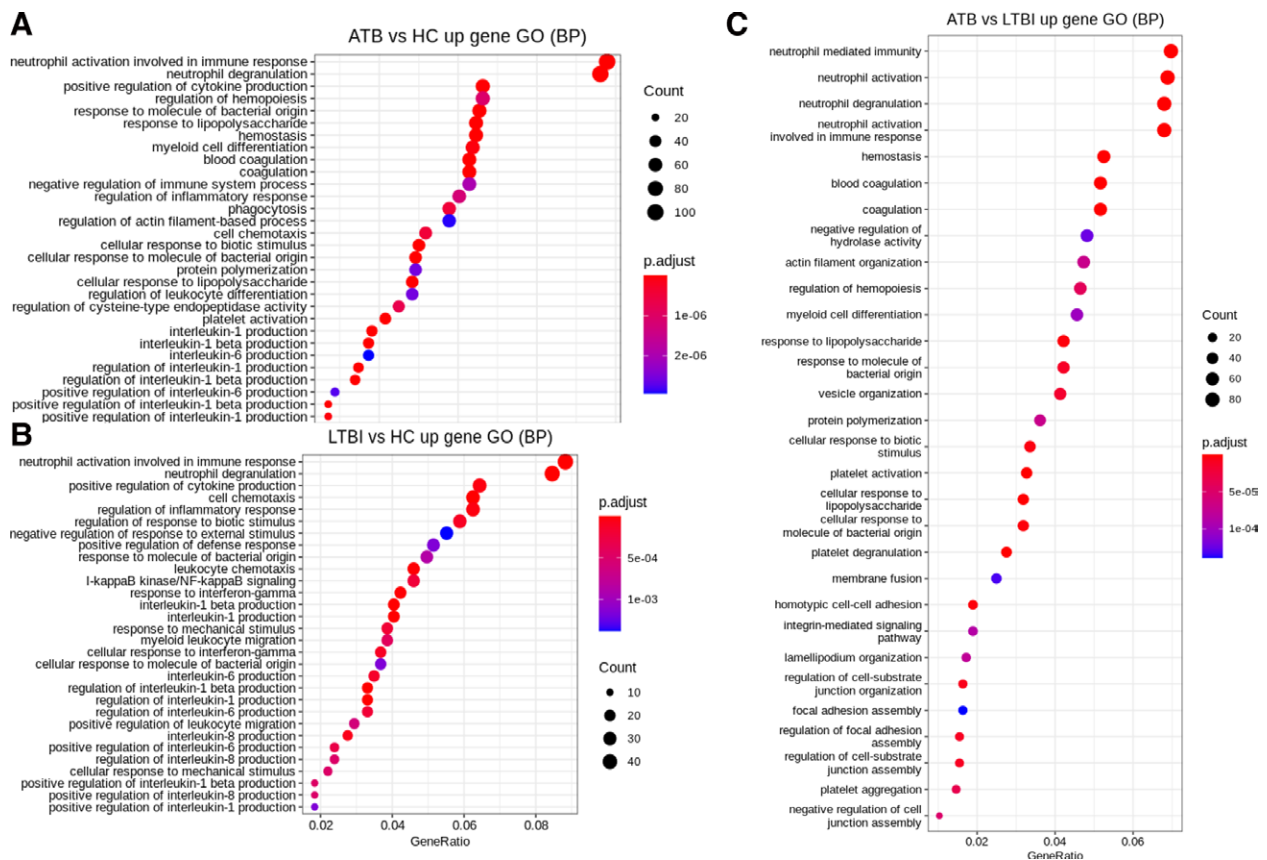
Figure 4. GO analysis of up-regulated mRNAs. (A)ATB versus HC; (B) LTBI versus HC; (C) ATB versus LTBI. ATB = active tuberculosis, GO = gene ontology, HC = healthy controls, LTBI = latent tuberculosis infection.

Figure 5. GO analysis of down-regulated mRNAs. (A) ATB versus HC; (B) LTBI versus HC; (C) ATB versus LTBI. ATB = active tuberculosis, GO = gene ontology, HC = healthy controls, LTBI = latent tuberculosis infection.
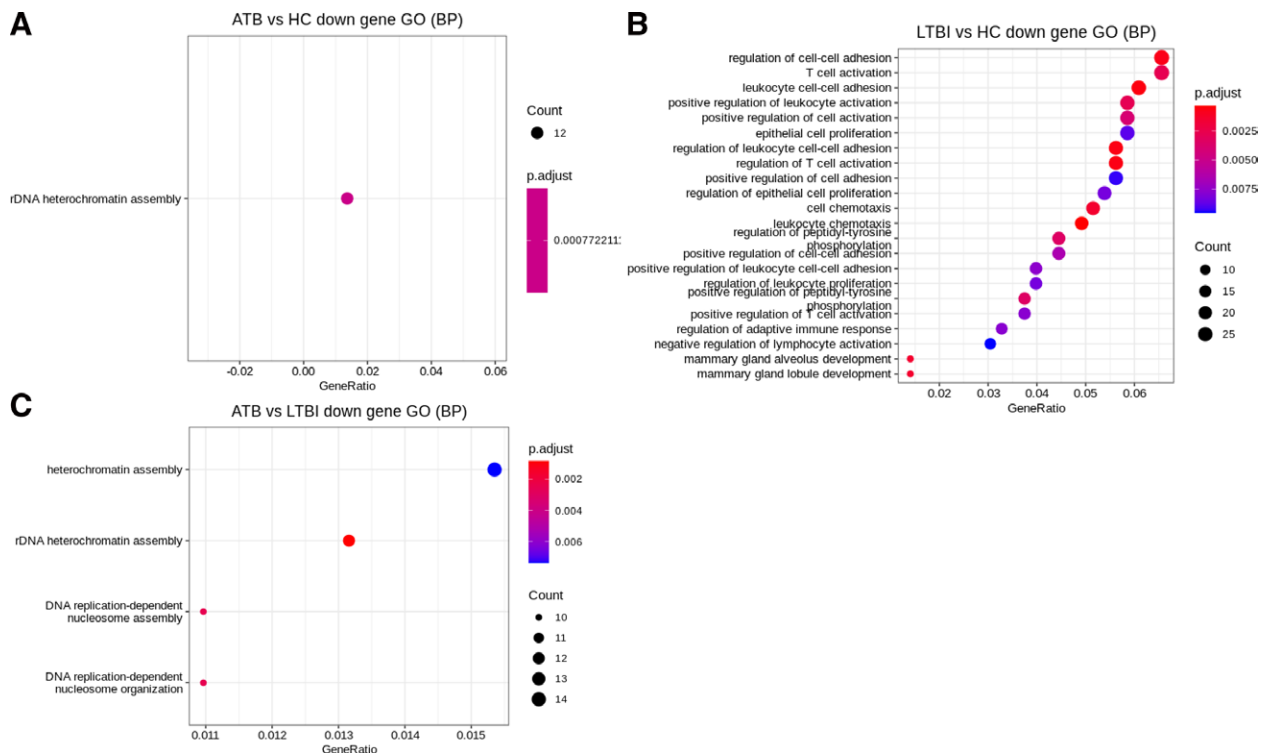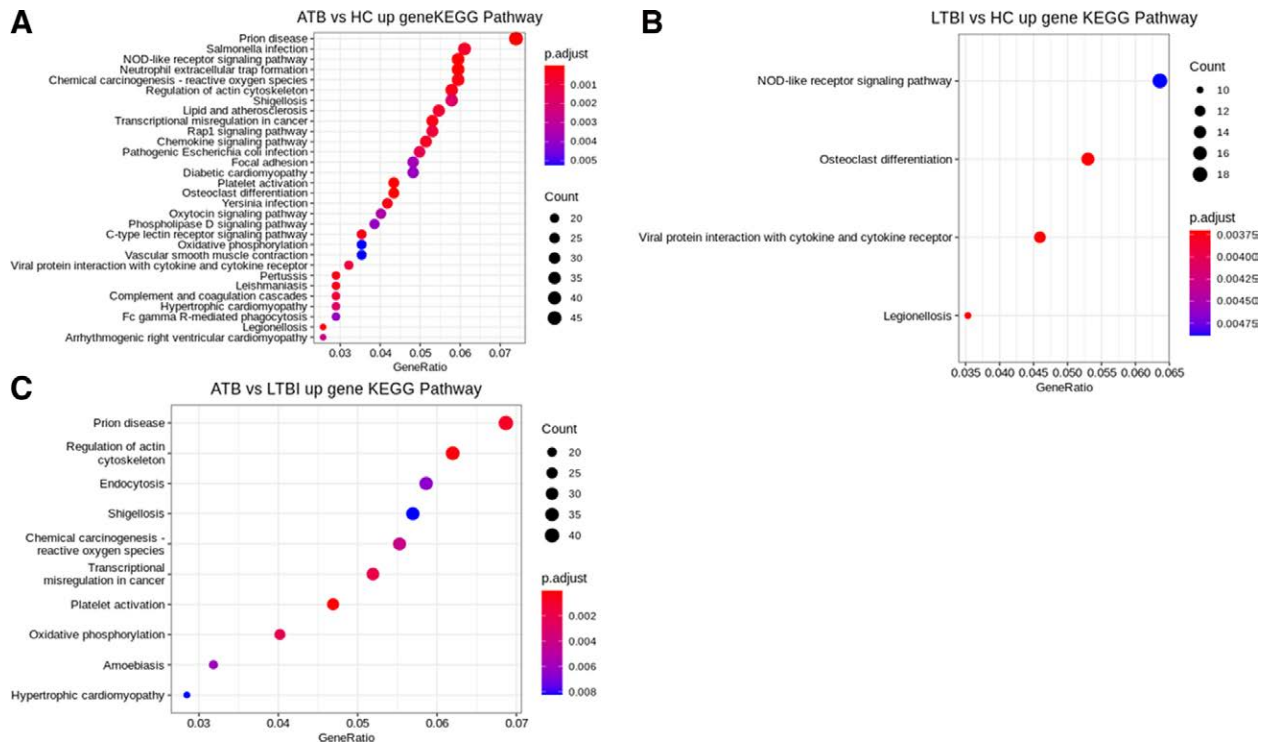
Figure 6. KEGG analysis of up-regulated mRNAs. (A) ATB versus HC; (B) LTBI versus HC; (C) ATB versus LTBI. ATB = active tuberculosis, HC = healthy controls, KEGG = Kyoto Encyclopedia of Genes and Genomes, LTBI = latent tuberculosis infection.

Figure 7. KEGG analysis of down-regulated mRNAs. (A) ATB versus HC; (B) LTBI versus HC; (C) ATB versus LTBI. ATB = active tuberculosis, HC = healthy controls, KEGG = Kyoto Encyclopedia of Genes and Genomes, LTBI = latent tuberculosis infection.
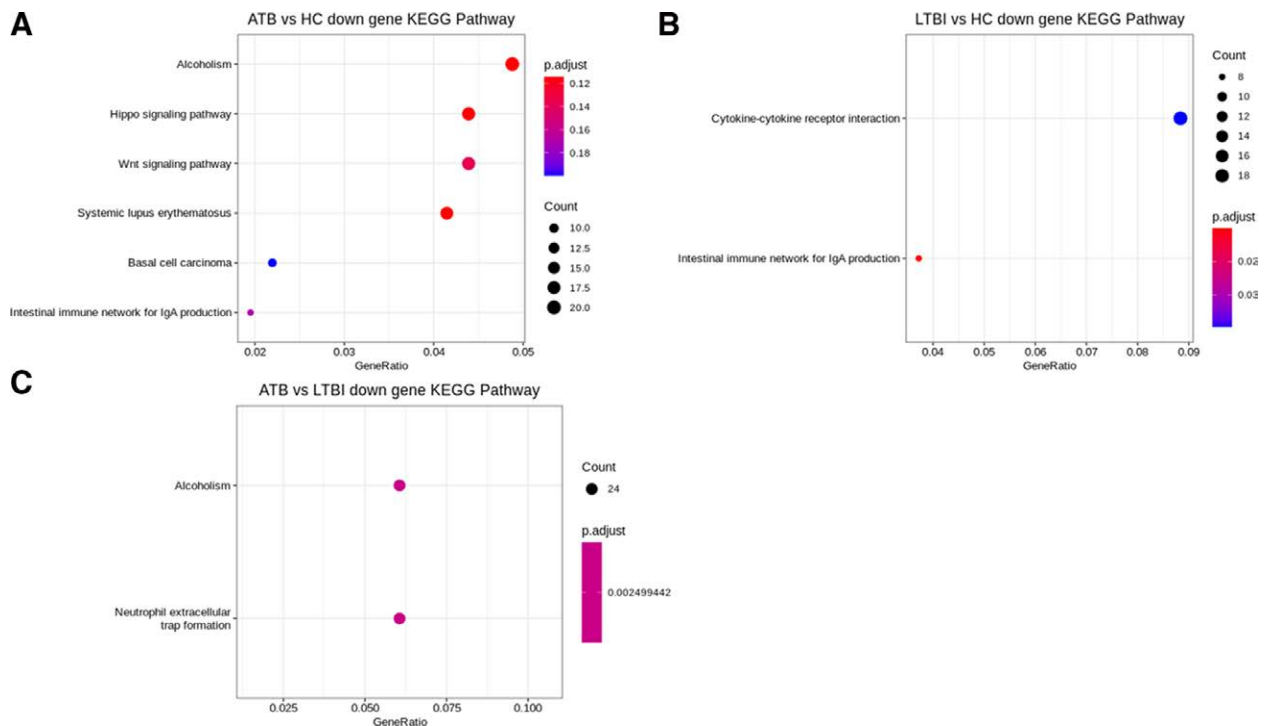
ENST00000471299, and ENST00000425497 were upregulated in both the ATB and LTBI groups, but to a greater extent in the ATB group, indicating that ATB and LTBI have similar regulatory mechanisms and that these lncRNAs could be used as diagnostic markers in TB infection. To date, their potential functions in TB remain unknown. Further research is needed to explore their immune functions in *MTB* infection.

LncRNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) was significantly higher in PBMCs from patients with LTBI than in patients with ATB in our study. A previous study[21]

showed that the expression level of NEAT1 was significantly increased in a model of extracellular *MTB* infection. When NEAT1 was knocked down, the level of IL-4 decreased, indicating that NEAT1 is associated with *MTB* infection and has potential value in the outcome of TB treatment. In our study, NEAT1 promoted the development of ATB from LTBI. The effect of lncRNA NEAT1 on *MTB* infection requires further exploration.

LncRNA TSIX is an antisense lncRNA of lncRNA X-inactive specific transcript (XIST), which is involved in the inactivation of the X chromosome.[22] Previous studies have shown that the downregulation of lncRNA TSIX leads to the ectopic expression of lncRNA XIST.[23] According to Weakley et al, TSIX appears to be differentially expressed in ovarian cancer and inhibits XIST expression in breast and ovarian cancers.[24] In this study, lncRNA TSIX and lncRNA XIST were downregulated in both the ATB and LTBI groups, and TSIX was downregulated to a greater extent in the ATB group (Table 2). LncRNAs XIST and TSIX may have potential functions in *MTB* infection immunity. TSIX was lower in the ATB group, indicating that it may play an important role in the development of ATB from LTBI and targeting TSIX therapy may inhibit the development of ATB from LTBI. LncRNAs XIST and TSIX may provide new directives regarding the regulatory mechanism in TB.

Most lncRNAs have not been reported to function in TB; however, some studies have been conducted on other diseases. HCG27-201 may be a diagnostic biomarker for T2DM, with a sensitivity of 64.6% and specificity of 79.8%.[25] AC079949 and LINC01619 may be used as prognostic biomarkers for esophageal and ovarian cancers, respectively.[26,27] AC073172 may influence the occurrence and development of polycystic ovarian syndrome by influencing the Toll-like receptor signaling pathway; however, no study has demonstrated its function in TB.[28] LINC01503 increases expression in squamous cell carcinoma cells, which could promote squamous cell carcinoma cell proliferation, migration, invasion, and growth.[29] Meisam Jafarzadeh found that lncRNA LINC02381 as a competitive endogenous RNA (ceRNA) inhibits gastric cancer progression through regulating the Wnt signaling pathway by competitively binding to miR-21, miR-590, and miR-27a.[30] LINC02381 may be a promising therapeutic target for gastric cancer. Another study stated that SMIM25 may act in the rehabilitation mechanisms of RSV infection through cytokine-cytokine receptor interactions.[31] Wang et al indicated that SMIM25 could be used as a potential diagnostic biomarker for inflammatory bowel disease.[32] All of these studies have shown that lncRNAs have important functions in various diseases. Further research is needed to explore their roles in TB.

GO enrichment and KEGG pathway analysis were performed to preliminarily explore the possible metabolic pathways involved in *MTB* infection. The results showed that the upregulated mRNAs were mainly enriched in neutrophil activation, neutrophil-mediated biological processes, and positive regulation of the immune response in TB. CXCL1 is a neutrophil chemokine that is at the core of IL-1β production during *MTB* infection.[33] This indicates that neutrophils play an important role in *MTB* infection. After *MTB* infection, neutrophil-related gene-mediated pathways are activated and the corresponding inflammatory factors are released to promote the occurrence and development of TB. The NOD-like receptor pathway is enriched by upregulated genes in both ATB and LTBI patients. Evidence from previous studies has shown that NOD2 is an intracellular receptor likely to regulate the immune response of TB by promoting an inflammatory response.[34] LncRNAs may affect the signaling pathway in macrophages by affecting the activity of NOD2.[15] The cytokine-cytokine receptor interaction pathway was enriched by the downregulated genes in the LTBI group. A previous study showed that increased cytokine responses are associated with the occurrence of TB.[35] This pathway may be involved in the occurrence and development of

TB. Taken together, these studies suggest that neutrophil-related gene-mediated pathways play important roles in *MTB* infection, and additional mechanisms need to be explored.

However, little is known about the mechanism of lncRNAs in TB, and the most important explanation is the competitive ceRNA hypothesis[36], proposed by Salmena et al.[37] This hypothesis states that some lncRNAs can regulate mRNAs by competing for shared microRNAs(miRNAs).[37] An increasing number of studies have demonstrated that lncRNAs can serve as ceRNA.[38] Li et al found that lncRNA PCED1B-AS1 showed low expression in patients diagnosed with ATB.[39] LncRNA PCED1B-AS1 reduced monocyte apoptosis and increased autophagy by regulating miR-155 expression. The lncRNA-miRNA-mRNA regulatory network requires further exploration. This may be the next step in our research.

This study has some limitations. Diagnostic accuracy tests have not been performed to further determine the diagnostic value of lncRNAs in TB. The molecular mechanisms underlying *MTB* infection should be further explored. Whether lncRNAs can be used as diagnostic markers in TB is still under investigation, and this study provides a directive for related research. Further research is planned in several directions: establishing diagnostic models to detect the diagnostic value of lncRNAs in children with TB. Pneumonia and TB are often difficult to distinguish; therefore, patients with pneumonia should be included in the control group. The value of differentially expressed lncRNAs in differentiating TB from pneumonia should be further explored. Animal or cell experiments should be conducted to further verify the role of lncRNAs in the occurrence and development of TB and to explore the mechanism of lncRNAs in TB.

5. Conclusions

A previous study has found that miRNAs play important roles in the occurrence and development of TB and could be diagnostic markers for TB in children.[40] Limited studies have reported the lncRNAs expression profiles during *MTB* infection in PBMCs. This is the first analysis of differential lncRNAs expression in children with *MTB* infection in vivo. In this study, lncRNAs showed a very limited role in PBMCs associated with *MTB* infection; however, they are, at least partly, associated with the dysregulation of PBMCs and may eventually participate in the pathogenesis of TB. The differentially expressed lncRNAs identified in our study may provide new answers to the dysfunction of PBMCs and mechanisms of TB. In summary, this study is still in the exploratory stage and it is difficult to draw an exact conclusion about the detailed functions of lncRNAs in *MTB* infection. Undoubtedly, we identified significant differences in the expression profiles of lncRNAs and mRNAs in PBMCs of children with ATB and LTBI, which has important implications for exploring lncRNAs as novel biomarkers for the diagnosis of TB. Moreover, further experimental identification and validation of lncRNAs roles could help elucidate the underlying mechanisms of *MTB* infection in children.

Author contributions

Xiaoling Zhong designed the study, conducted the experiments, and wrote the manuscript. Min Shu and Jing-Wen Lin designed the study and reviewed the manuscript. Shikun Lei made the data analysis and wrote the manuscript. Min Ren made the data process.

Conceptualization: Min Ren.
Data curation: Shikun Lei.
Methodology: Shikun Lei.
Software: Shikun Lei.
Writing – original draft: Xiaoling Zhong.
Writing – review & editing: Min Shu, Jing-Wen Lin.

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