

Diagnostic Value of DHX58 - IRF7 Involved in RIG-I-Like Receptor Signaling Pathway in Tuberculosis

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Abstract

Background: Tuberculosis (TB) is an infectious disease that endangers human health. This study set out to search for key genes and related pathways in dendritic cell (DC) from TB patients to reveal the potential molecular mechanisms and identify potential biomarkers for TB.

Method: DC data GSE34151 related to TB was downloaded from GEO data sets for analysis. Differentially expressed genes (DEGs) were obtained by employing an online tool, GEO2R. PPI network of DEGs and gene module were visualized and calculated applying STRING and Cytoscape, respectively. GO analysis and KEGG pathway were utilized to annotate the functions of DEGs. ROC curve analysis was applied to screen the most diagnostic hub genes associated with TB. Furthermore, their expression was validated in blood data (GSE83456), which were further compared to the T-spot·TB test.

Results: A total of 290 DEGs were screened, which were significantly enriched in Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, and RIG-I-like receptor signaling pathways. Among them, 27 candidate hub genes with the most significant cluster in PPI were enriched in RIG-I-like receptor signaling pathway, which has been known to be associated with TB. We further found that the top 10 hub genes(DHX58, ISG20, IRF1, IRF7 and RSAD2, etc) showed high performance for TB diagnosis, among which both DHX58 and IRF7 were enriched in the RIG-I-like receptor signaling pathway. Moreover, DHX58 and IRF7 were also increased in blood, which were consistent with that in dendritic cell. Interestingly, DHX58 and IRF7 display higher diagnostic efficacy than T-spot·TB test.

Conclusion: In this study, we revealed that both DHX58 and IRF7 with high diagnostic value in blood and dendritic cells, potentially involved in RIG-I-like receptor signaling pathway, may serve as a marker for TB diagnosis.

Background

Tuberculosis (TB) is a chronic and highly communicable disease caused by *Mycobacterium tuberculosis* (Mtb). The main clinical manifestations are persistent cough, emaciation, and hemoptysis[1]. Mtb can infect multiple organs and tissues, involving the intestine, spine, and lymph. Among these, pulmonary infection appears most frequently[2]. Epidemiologically, TB still remains as a concerns in public health emergencies. Roughly 2 million of the human population suffer from Mtb and is responsible for about 1.5 million deaths annually[3]. Initiation of TB infection is related to its virulence size, number, and host immune response. Both the adaptive and innate immune systems of the organism immune system are involved in TB killing. Unlike adaptive immunity, innate immunity provides a non-specific response to defense against bacterial invasion. Its response pattern and intensity are not altered by frequent contact with pathogens. The Immune cells play an important role in innate immunity. Mtb is an acid-fast stain-positive facultative intracellular bacterium that caused body cellular immunity of the host[4]. Inherent immune cells mainly include macrophages and dendritic cells (DC), which play important roles in warding off the bacterium's invasion. The “fight” between Mtb and host has a long history, when host has evolved

intricate strategies to prevent Mtb, which evaded host immune killing by oxidative stress, apoptosis, and autophagy[5]. Rising evidence suggests that DC plays an equally important role as macrophages in Mtb infection. However, the relationship between DC and Mtb is not fully understood. Therefore, systematic knowledge on the immune response mechanism of Mtb to host DC infection is of great importance for diagnosing and treating TB[6]. Several recent studies indicated that the transcriptional profile of active TB (ATB) reflects the disease status and have found that ATB can be clearly distinguished from healthy individuals by their transcriptional status[5]. In this study, we analyzed and compared raw gene expression data from healthy individuals and TB patients using bioinformatics methods to provide potential candidates for TB diagnostic biomarkers at the molecular level.

Materials And Methods

Data collection and inclusion criteria

mRNA expression data was downloaded from Gene Expression Omnibus (GEO) database(available online: <http://www.ncbi.nlm.nih.gov/geo/>) by the following keywords "tuberculosis," "Homo sapiens" and "RNA." DCs were from TB patients with none anti-TB therapy), respectively. Sufficient information was available for analysis.

Microarray data

In this study, microarray data GSE34151 were based on GPL10558 Platforms[Illumina HumanHT-12 V4.0 expression beadchip], which is composed of 130 patients with TB and 129 HC. This study's expression data was performed in Light of GEO's publication guidelines and data access.

Identify differentially expressed mRNA

GEO2R is an effective interactive web tool which can identify DEGs in different groups from the GEO. In the GSE34151 expression profile, we set P-value <0.05 and $|\log_{2}FC| > 2$ as cutoff criteria to screen DEGs[7].

Selection of modules in the protein-protein interaction network

STRING, a search tool for retrieval of interacting genes which can construct PPI network in DEGs, analysis of the interactive functions between proteins can shed new insights into the disease occurrence and development. Furthermore, the PPI network was obtained and visualized using Cytoscape version 3.7.2, based on selection criteria degree cut-off = 2, hair cut, node density cut off = 0.1, node score cut-off = 0.2, k-core = 2, and max. depth = 100. Next, MCODE was utilized to identify the hub gene in the most important modules[8]. Finally, the heatmaps and volcano plots of the hub gene were performed and visualized by the SangerBox tool(<http://sangerbox.com/Tool>).

Functional enrichment analysis of DEGs

Gene ontology (GO) analysis was applied for gene biological function analysis and annotation, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was performed to annotate the gene pathways, both analyses were available in the DAVID (<https://david.ncifcrf.gov/>) database. The database provides the data on gene mRNA biological information, which extracts bioinformatics information of genes and proteins. P-value ≤ 0.05 was regarded as significant. Furthermore, we wanted to know how many pathways were play a role in the screened hub genes. Hence we constructed a pathway analysis of the most significant modules of the hub gene.

ROC curve analysis

The ROC curve of the most significant cluster 1 hub genes in the PPI network was used to GraphPad Prism 8.3.0 software. The larger the area under the ROC curve (AUC) indicated that the hub genes were able to more efficiently distinguish TB from HC. Furthermore, to verify whether the expression profile of the selected genes was consistent with DC in blood and whether it has a high diagnostic value. We downloaded GSE83456 dataset from GEO web, this dataset comprised 61 HC and 97 ATB. Finally, we screened the most potential candidate genes for comparison with T-spot·TB.

T-spot·TB test

A total of 97 participants included 58 TB patients, and 39 controls were recruited from the Weifang No.2 Peoples Hospital. The diagnosis of patients with TB was based on sputum smear, Mtb culture, or clinical symptoms. Meanwhile, participants who suffer from diabetes, HIV, and HBV were excluded. All subjects were informed consent prior to their enrolment in this work. An 5ml heparinized blood sample was collected from each subject. Peripheral blood mononuclear cells (PBMCs) were separated from participants to make cell suspension with a concentration of $2.5 \times 10^5/\text{mL}$. The T-spot·TB (Oxford Immunotec, Abingdon, UK) test was performed strictly according to the instructions. Briefly, $100\mu\text{l}$ ($2.5 \times 10^5/\text{ml}$) of cell suspension was added to the T-spot assay plate and incubated with positive, negative control and specific antigens ESAT-6 and CFP-10, respectively. Plate was a 37°C , 5% CO_2 incubator for 20 h. Then, plate was washed 4 times with phosphate buffer solution(PBS) and $50\mu\text{l}$ secondary antibodies were allowed to react for 1 h at $2-8^\circ\text{C}$. Afterwards, $50\mu\text{l}$ of chromogen was added and treatment of 5 min with kept from light. The reaction was terminated with distilled water. Finally, the number of spots was counted in each well.

Results

Identification of TB DEGs

A total of 290 DEGs were screened out from GSE34151 dataset, which contained 229 up-regulated and 61 down-regulated genes. The top 10 significantly deregulated genes were presented in Table 1, including IDO1, ISG20, LAD1, EBI3, CCR7, IFI44L, MIR155HG, RGS1, CD86, G0S2, DUOX1, CSF1R, CDH1, FZD2, C1orf162, PALD1, NDRG2, EVI2B, MGAT4A, CHN2.

Table 1. The top 10 up-regulated and down-regulated DEGs

mRNA ID	Up/Down	LogFC	Adj. <i>P</i> -val	<i>P</i> -value
IDO1	Up	5.62	2.77E-177	2.34E-181
ISG20	Up	5.43	1.77E-189	3.74E-194
LAD1	Up	5.06	4.36E-180	2.77E-184
EBI3	Up	4.99	9.91E-155	1.89E-158
CCR7	Up	4.84	4.68E-138	2.87E-141
IFI44L	Up	4.62	1.94E-117	4.68E-120
MIR155HG	Up	4.55	1.72E-148	5.10E-152
RGS1	Up	4.29	6.45E-132	6.83E-135
CD86	Up	4.17	8.48E-124	1.36E-126
G0S2	Up	3.97	3.15E-158	4.00E-162
DUOX1	Down	-2.01	2.33E-84	3.01E-86
CSF1R	Down	-2.02	1.81E-61	6.08E-63
CDH1	Down	-2.03	2.41E-32	2.34E-33
FZD2	Down	-2.04	6.88E-97	5.17E-99
C1orf162	Down	-2.05	2.19E-50	1.14E-51
PALD1	Down	-2.06	4.00E-104	1.98E-106
NDRG2	Down	-2.06	5.63E-57	2.25E-58
EVI2B	Down	-2.07	4.15E-78	7.12E-80
MGAT4A	Down	-2.08	5.40E-95	4.38E-97
CHN2	Down	-2.08	6.78E-93	6.13E-95

Mine of hub genes based on PPI networks

To further mined the genes associated with TB, we constructed PPI network of DEGs and visualized using STRING database and Cytoscape software respectively(Fig.1A). We then screened 7 modules in the PPI network by the MCODE. The most significant module consists of 27 nodes, including IFI44L, MX1, OASL, HERC5, IRF1, RSAD2, IFI27, IFIT2, OAS1, MX2, HERC6, IRF7, IFITM1, OAS2, CXCL10, IFI6, IFITM2, IFI44,

IFITM3, GBP1, ISG20, IFIT3, OAS3, IFIT1, ISG15, USP18, DHX58, all of which are increased (Table 2). The Volcano plot and heatmap of hub genes are displayed in Figure.1B and 1C, respectively.

Table 2. Hub genes of cluster 1

SUID	MCODE_Cluster	MCODE_Score	MCODE_Node_Status	Gene-Name
368	Cluster 1	21.59384615	Clustered	IFI44L
112	Cluster 1	21.59384615	Clustered	MX1
113	Cluster 1	21.59384615	Clustered	OASL
118	Cluster 1	21.92028986	Clustered	HERC5
248	Cluster 1	22.00000000	Clustered	IRF1
120	Cluster 1	21.59384615	Clustered	RSAD2
185	Cluster 1	21.70666667	Clustered	IFI27
122	Cluster 1	21.59384615	Clustered	IFIT2
124	Cluster 1	21.59384615	Clustered	OAS1
130	Cluster 1	21.59384615	Clustered	MX2
262	Cluster 1	21.92028986	Clustered	HERC6
134	Cluster 1	21.59384615	Clustered	IRF7
200	Cluster 1	21.59384615	Clustered	IFITM1
137	Cluster 1	21.59384615	Clustered	OAS2
153	Cluster 1	21.59384615	Clustered	CXCL10
155	Cluster 1	21.59384615	Clustered	IFI6
220	Cluster 1	21.00000000	Clustered	IFITM2
349	Cluster 1	21.59384615	Clustered	IFI44
221	Cluster 1	22.00000000	Clustered	IFITM3
285	Cluster 1	21.59384615	Clustered	GBP1
226	Cluster 1	21.59384615	Clustered	ISG20
100	Cluster 1	21.59384615	Clustered	IFIT3
164	Cluster 1	21.59384615	Clustered	OAS3
101	Cluster 1	21.59384615	Clustered	IFIT1
103	Cluster 1	21.59384615	Clustered	ISG15
104	Cluster 1	21.59384615	Clustered	USP18
492	Cluster 1	22.00000000	Seed	DHX58

Pathway enrichment and biological functions analysis of the DEGs

The 290 DEGs were uploaded to the DAVID 6.8 online tool for pathway enrichment and functional clustering analyses, P-value < 0.05 were represented as significant terms. In regards to biological processes, DEGs were enriched in defense response to virus, type I interferon signaling pathway, and inflammatory response; in regards to Molecular Function, DEGs were enriched in chemokine activity, 2'-5'-oligoadenylate synthetase activity, and cytokine binding; in regards to cellular component, DEGs were enriched in the external side of plasma membrane, cytosol/extracellular space and in regards to KEGG pathway enrichment, DEGs were enriched in Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, TNF signaling pathway (Fig.2A). In addition, we further conducted KEGG pathway enrichment analysis on 27 hub genes, we found enrichment of 27 hub genes belonging to the Influenza A, Hepatitis C and RIG-I-like receptor signaling pathway (Fig.2B). Among these, the RIG-I-like receptor signaling pathway was correlated well with the TB infection (Fig.3).

Assess the diagnostic value of hub genes by ROC curve

To screen the hub genes clinical value, we constructed ROC curves of 27 hub genes by using the GraphPad Prism 8.3.0. Among these, the AUC values of each hub gene were ≥ 0.500 . The top 10 hub genes DHX58, ISG20, IRF1, IRF7, RSAD2, IFI44, IFI44L, USP18, IFITM1, OAS3 with higher diagnostic efficacy were list in Table 3. Among them, we found DHX58 and IRF7 were strongly correlated with the RIG-I-like receptor signaling pathway. Thus, we picked DHX58 and IRF7 out as target genes. We then verified the expression of DHX58 and IRF7 in blood using GSE38456 and found that DHX58 and IRF7 were up-regulated ($P \leq 0.01$). We constructed ROC curves to compare the screened genes DHX58 and IRF7 in combination with specific antigen A (ESAT-6) and specific antigen B (CFP-10) in T-spot-TB (Fig.4). the results revealed that DHX58+IRF7 (AUC=0.9277, 95%CI:0.8917-0.9637, $P \leq 0.01$) diagnosis potency was significantly higher than ESAT-6+CFP-10 (AUC=0.7657, 95%CI:0.6984-0.8329, $P \leq 0.01$). In addition, the sensitivity and specificity values were significantly higher in the DHX58+IRF7 (Sensitivity=88.14, 95%CI:81.07%-92.80%; Specificity=86.67, 95%CI:78.13%-92.21%) compared to the ESAT-6+CFP-10 (Sensitivity=78.21, 95%CI:67.84%-85.92%; Specificity=66.96, 95%CI:57.82%-74.99%). Furthermore, we compared single DHX58 and IRF7 with single ESAT-6 and CFP-10 in T-spot-TB were constructed using ROC curve (Table.4). the result showed the single DHX58 and IRF7 diagnostic capacity were significantly higher than single ESAT-6 and CFP-10.

Table 3. ROC curve analysis of the top 10 hub genes in DC

Items	Performance					
	Sensitivity %	95% CI	Specificity %	95% CI	AUC	95% CI
ISG20	100.00	97.13% - 100.00%	100.00	97.11% - 100.00%	1.0000	1.0000 - 1.0000
DHX58	100.00	97.13% - 100.00%	100.00	97.11% - 100.00%	1.0000	1.0000 - 1.0000
IRF1	99.23	95.77% - 99.96%	100.00	97.11% - 100.00%	0.9999	0.9996 - 1.0000
IRF7	98.46	94.56% - 99.73%	99.22	95.74% - 99.96%	0.9989	0.9972 - 1.0000
IFI44L	96.92	92.36% - 98.80%	99.22	95.74% - 99.96%	0.9983	0.9961 - 1.0000
RSAD2	100.00	97.13% - 100.00%	99.22	95.74% - 99.96%	0.9976	0.9929 - 1.0000
USP18	97.69	93.43% - 99.37%	98.45	94.52% - 99.72%	0.9965	0.9915 - 1.0000
IFITM1	96.15	91.31% - 98.35%	99.22	95.74% - 99.96%	0.9963	0.9925 - 1.0000
OAS3	90.77	84.56% - 94.64%	100.00	97.11% - 100.00%	0.9948	0.9902 - 0.9993
HERC5	97.69	93.43% - 99.37%	97.67	93.39% - 99.37%	0.9930	0.9857 - 1.0000

Table 4. Diagnostic performance of single DHX58, IRF7, ESAT-6, and CFP-10 in patients with TB

Items	Performance					
	Sensitivity, %	95% CI	Specificity, %	95% CI	AUC	95% CI
DHX58	84.44	71.22%-92.25%	86.89	76.20%-93.20%	0.8893	0.8224-0.9561
IRF7	91.11	79.27%-96.49%	94.74	85.63%-98.57%	0.9637	0.9310-0.9964
ESAT-6	79.49	64.47%-89.22%	71.43	58.52%-81.58%	0.8017	0.7134-0.8901
CFP-10	76.92	61.66%-87.35%	62.50	49.41%-73.99%	0.7305	0.6293-0.8318

Notes: all the single DHX58, IRF7, ESAT-6, and CFP-10 p -value were ≤ 0.01 .

Discussion

Tuberculosis is a multifactorial and tightly regulated process which involves the various immune interactions of Host-pathogen. In the present study, a total of 290 DEGs were screened out in dendritic cells (DCs) from TB patients, which contained 229 up-regulated and 61 down-regulated genes. Then, we established the PPI network and found the most significant clusters of hub genes. Meanwhile, pathway enrichment analysis revealed which the hub genes were enriched in RIG-I-like receptor signaling pathway, which was known as TB-associated pathway. According to the ROC curve analysis results, we further found that the top 10 hub genes with high diagnostic value closely related to the RIG-I-like receptor signaling pathway were DHX58 and IRF7. Therefore, we chose DHX58 and IRF7 for further analysis.

Some structures on the surface of the pathogen were evolutionarily conserved and can be shared by many microbes but lacked in the host, i.e. PAMP[9]. In addition, PRR included TLR, NLR, CLR, and RLR were broad present in the host immune cells that can recognize PAMP[10,11]. Macrophages and DC, which activate innate immunity and the related-killing mechanism to kill bacteria through PRR recognize PAMP has been found in recent years. Once Mtb infects the host DC, the DC express a large number of PRRs that respond to the stimulus signal and activate the DC to convert to antigen-presenting cells (APC) [12,13]. In this study, we found that DHX58 and IRF7 in DC were closely associated with the RIG-I-like receptor signaling pathway. We would like to know how DHX58 and IRF7 perform its role in TB? However, RIG-I was in an inactive conformation through its repressor domain (RD) interacted with RNA helicase protein. Evelyn Dixit et al. found that RIG-I and MDA5 together with DHX58 constitute the RLR family and they had a common features that contains DExD/H-box ATPase[14,15]. When the host was infected, RIG-I and MDA5 could activate the RIG-I-like receptor signaling pathway through recognition of pathogenic RNAs. However, DHX58 lacks a CARD domain that can not compete with RIG-I and MDA5 receptors for binding RNA and stimulated IFN transcription, thus acts as a negative regulator in RNA helicase[15,16]. Although DHX58 lacks a CARD, however, DHX58 can regulate RIG-I and MDA5 activated signal pathway transduction activated inflammatory cytokines, and NF- κ B induced TNF- α and IL-12, which in turn regulated antibacterial immunity[17,18]. IL-12 induces Th1 cells, which turn on cellular immunity and limit Mtb. Activated Th1 cells release IFN- γ to stimulate macrophages to release RNI and ROS against Mtb, and TNF- α can induce granuloma formation and thus limit Mtb activity[18]. Furthermore, DHX58 was nominal association with the C-termini of IPS-1 that can recruit IKK ϵ to phosphorylate. DHX58 regulated the activity of RIG-I, MDA5 and downstream mitochondria-related factors, demonstrated the importance of DHX58 as a key regulator in stimulated IFN synthesis and excretion. Furthermore, IRF7 was also involved in the release of host type 1 interferon. When the body was stimulated, the signals reach the RIG-I and MDA5, RIG-I and MDA5 were bound to the IFN- β promoter stimulator 1 (IPS-1) via their N-terminal CARD domains, next the TRAF3, TBK1, and IKK-Related Kinase Signaling caused phosphorylation of IRF3, IRF7 and induction of IFN[19–21]. IFN α also induces the formation of Th1 immunity and exerts anti-Mtb activity. In addition, Sato et al. demonstrated that IFN- α/β was not induced in cells with loss of IRF3 and IRF7 expression[22]. This also indicated that IRF7 plays an important role in type I interferons that were

an integral component of host defense system. Macrophage was well known to be an important target cell of TB and plays an important role in TB pathogenesis. Next, through analysis of the data GSE17477 submitted by Pine R et al. in the GEO database, interestingly, we found that DHX58 and IRF7 were also up-regulated genes in macrophages and enriched in the RIG-I-like receptor signaling pathway. This predicts that DHX58 and IRF7 may played the same role in Macrophages as in DC and conformed our results were compatible with the previous findings. Although both types of cells can release IL-12 and IFN- α to activate Th1 cells and TNF- α to induce granuloma formation, macrophages do not release IL-12 and IFN- α nearly as much as DC[23,24], in contrast, macrophages exerted a higher function than DC in terms of TNF- α .

We validated the expression of DHX58 and IRF7 in blood and used them as potential markers for the diagnosis of TB by analyzing GSE83456. We found that DHX58 and IRF7 were also up-regulated in blood, and it could significantly distinguish the TB patients from HC. Moreover, To further test the ability of DHX58 and IRF7 to diagnose TB, we performed a comparative analysis of their diagnostic performance with T-spot·TB trial. T-spot·TB is a clinical test that detected TB-specific antigens, which stimulated specific T cells to released IFN- γ . These antigens included ESAT-6 and CFP-10, these antigens were successfully applied in T-lymphocyte assays to detect TB infection[25,26]. Zhu et al. demonstrated that the sensitivity and specificity of T-spot·TB for the diagnosis of TB group were 85.64% and 66.04%[27], respectively, compared with the control group. However, Ma et al. reported that the sensitivity and specificity of T-spot·TB were 78.4% and 59.0%–93.0%, respectively[28–31]. In this study, we found that combined DHX58 and IRF7 were significantly higher than ESAT-6 and CFP-10 in diagnostic performance. In addition, single the sensitivity and specificity of ESAT-6 and CFP-10 were inferior to DHX58 and IRF7. This also proves that DHX58 and IRF7 have great potential as novel diagnostic markers for TB.

Conclusions

In summary, By combined genome-wide analysis in DC, we found RIG-I-like receptor signaling pathway was involved in the pathogenesis of TB, which plays an important role in host immune defense through the induction of type I interferons. We also found that the most significant hub genes, DHX58 and IRF7, were enriched in this pathway and had high diagnostic value. In addition, we validated DHX58 and IRF7 in blood and compared them with T-spot·TB. We found that the diagnostic efficacy of DHX58 and IRF7 both single or in combination was higher than that of T-spot·TB. Finally, we salient finding that DHX58 and IRF7 had significant diagnostic value in both DC and blood, which could be identified as potential candidates for TB biomarkers.

Declarations

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Availability of data and materials

The material supporting the conclusion of this review has been included within the article.

Authors' contributions

Zheng-jun Yi and Yu-rong Fu designed and concepted this study; Chong-hui Li drafted this manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Competing Interests

The authors declare no competing interests in this work.

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Figures

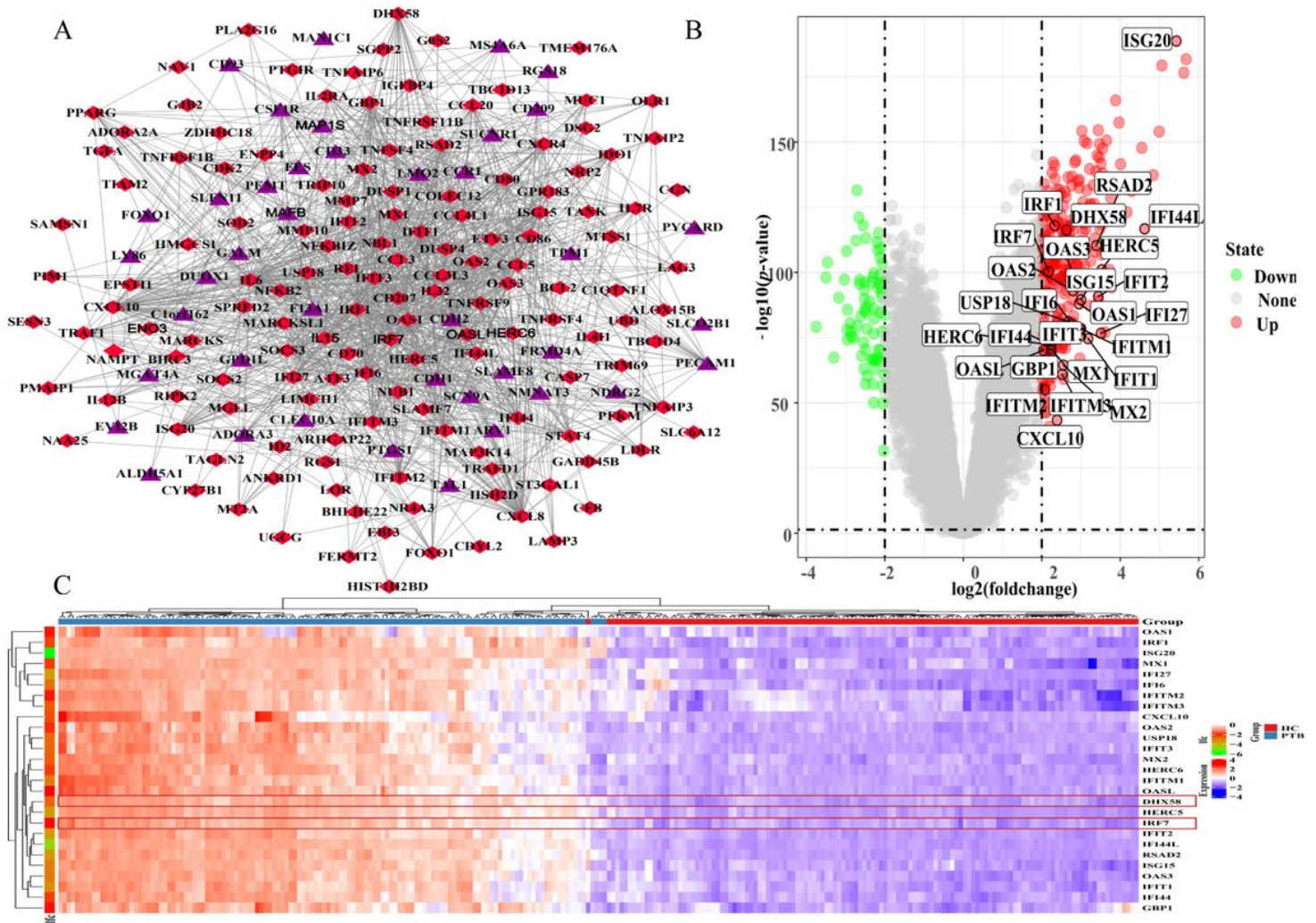
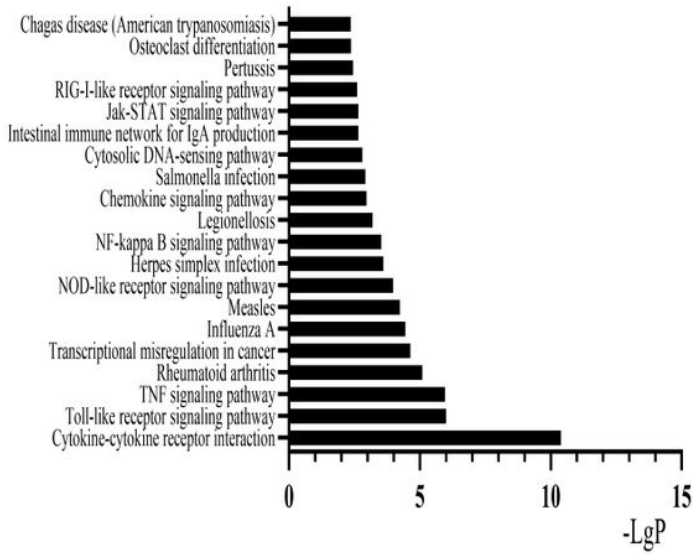


Figure 1

PPI network, volcano map, and heat map of hub genes. (A) PPI network of DEGs, Red diamonds indicate up-regulated genes, purple triangles indicate down-regulated genes. (B) volcano map of 27 hub genes, the x-axis represents the $\log_2\text{FC}$, and the Y-axis represents $-\log_{10}(P\text{-value})$, Red means up-regulated genes, brown means undifferentiated genes, and green for down-regulated genes. (C) heat map of 27 hub genes, Red means high relative expression, and Purple means low expression.

A



B

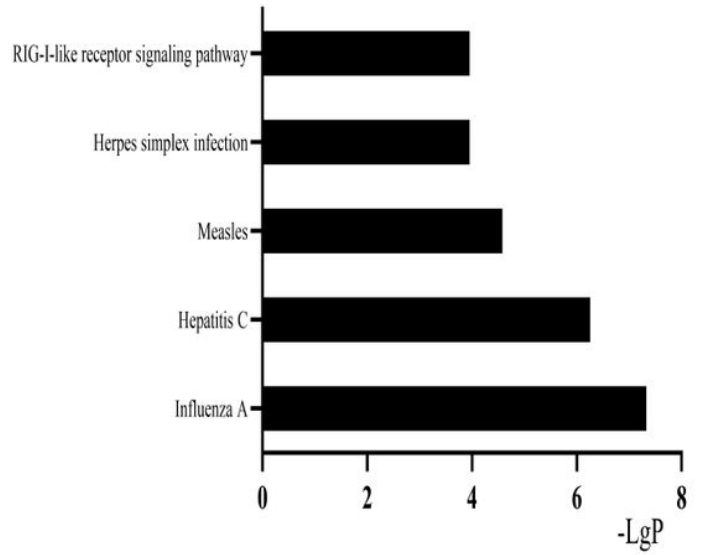


Figure 2

KEGG pathway enrichment of DEGs and hub genes. (A) KEGG pathway enrichment of DEGs. (B) KEGG pathway enrichment of hub genes.

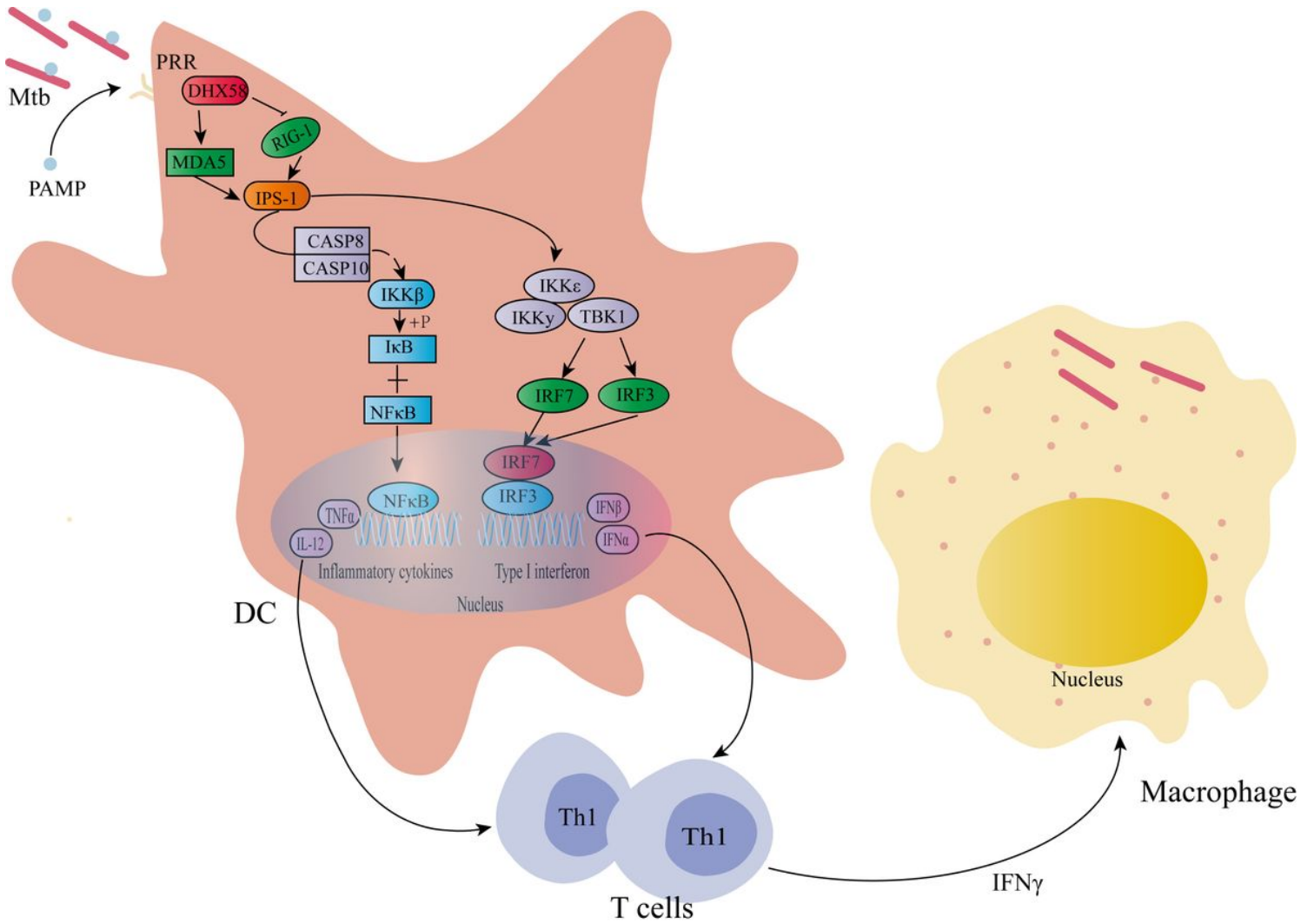
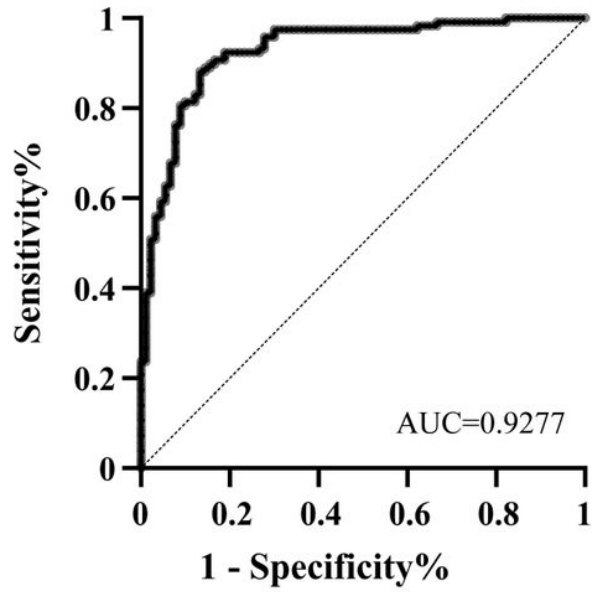


Figure 3

Schematics of the RIG-I-like receptor signaling pathway. PAMP of Mtb binds to PRR of dendritic cells and initiates RIG-I-like receptor signaling pathway, releasing IL-12 and IFN- α , activating Th1 cells to release IFN- γ and stimulating macrophages for antibacterial immunity. On the other hand, activated TNF- α can limit the propagation of Mtb by forming granulomas.

A



B

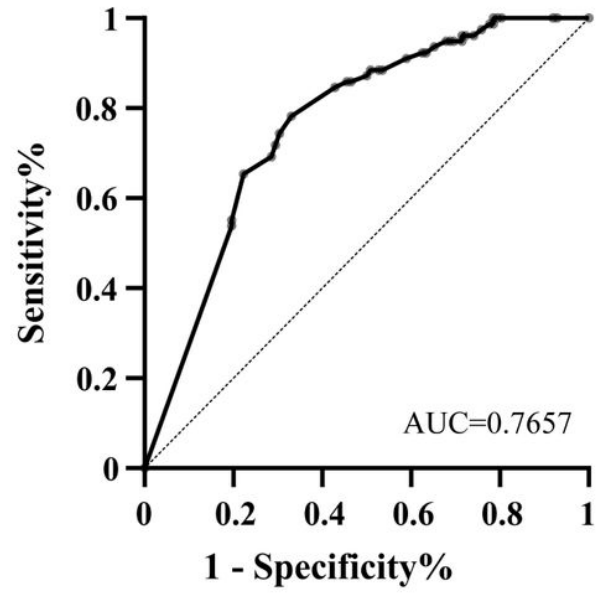


Figure 4

The ROC curve analysis of DHX58+IRF7 and ESAT-6+CFP-10. (A) ROC Curve of DHX58+IRF7 in Patients with Tuberculosis. (B) ROC Curve of ESAT-6+CFP-10 in Patients with Tuberculosis.