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ORIGINAL RESEARCH

Identification of Hub Genes Associated with Diabetes Mellitus and Tuberculosis Using Bioinformatic Analysis

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Purpose: To investigate the potential pathophysiological association between tuberculosis (TB) and diabetes mellitus (DM) using bioinformatic analyses.

Patients and Methods: Gene expression datasets for healthy controls (HCs), TB patients, DM patients, TB+DM patients (GSE114192), and metformin-treated cells (GSE102677) were obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified from pairwise dataset comparisons TB vs HCs and DM vs HCs. DEGs were verified by comparing them to DEGs for TB+DM vs HCs. Enrichment analysis of DEGs common to all three dataset comparisons was conducted using DAVID. The protein–protein interaction (PPI) network was established via STRING and visualised in Cytoscape. Hub genes were identified using the Cytoscape plug-in cytoHubba and then were verified using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Targeted miRNA prediction analysis identified metformin treatment-induced gene expression changes in peripheral blood mononuclear cells.

Results: A total of 422 DEGs were common to all three dataset comparisons. Functional enrichment analysis revealed these DEGs were enriched for functional terms of type I interferon signaling pathway, innate immune response, inflammatory response, and infectious diseases. Ten hub genes identified using PPI network analysis were screened for interactions with metformin target gene *INS* using cytoHubba based on maximal clique centrality (MCC) score. Subsequently, five hub genes were predicted to functionally interact with *INS*, including *STAT1, IFIT3, RSAD2, IFI44L*, and *XAF1*, as verified by RT-qPCR. Meanwhile, seven miRNAs (miR-3680-3p, miR-3059-5p, miR-629-3p, miR-29b-2-5p, miR-514b-5p, miR-4755-5p, miR-4691-3p) were associated with regulation of hub genes. Notably, six hub genes (*STAT1, IFIT3, RSAD2, ISG15, IFI44, IFI6*) were down-regulated in cells exposed to both metformin and *Mycobacterium tuberculosis* antigens.

Conclusion: Network hub genes hold promise as disease status biomarkers and as metformin treatment targets for alleviating TB and DM. This study describes a strategy for exploring pathogenic mechanisms of diseases such as TB and DM.

Keywords: diabetes mellitus, tuberculosis, differentially expressed gene, metformin, bioinformatics

Introduction

Tuberculosis (TB) is an epidemic multifactorial disease caused by *Mycobacterium tuberculosis* (MTB). According to the World Health Organization (WHO), in 2019 there were an estimated 10.0 million new TB cases and 1.4 million TB deaths, including 208,000 deaths among HIV-positive people.[1] TB persists as an important

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infectious disease threat to human health due to ineffective TB prevention and control measures that support continued spread of the disease. Therefore, there is an urgent need to better understand underlying molecular mechanisms that support perpetuation of TB.

Several common metabolic and endocrine diseases, including diabetes mellitus (DM), are increasingly undermining public health around the world.[2] According to a survey from the International Diabetes Federation, dramatically increasing trends in diabetes incidence, if left unchecked, will lead to a worldwide DM patient burden of 629 million people in 2045.[2] Chronic DM illness can cause a series of health issues, including vision, neurological, renal, and vascular complications.[3] Due to the fact that TB and DM are comorbid diseases, the high prevalence of both diseases imposes major barriers to TB elimination.[4]

Accumulating evidence has demonstrated that DM is an independent risk factor for TB occurrence and active disease.[5] In fact, as compared with non-DM patients, DM patients have a 3.59–fold increased risk of active TB.[5] Increased DM patient susceptibility to TB has been attributed to several factors, of which innate and adaptive immune disorders play significant roles.[6] In addition, comorbid DM significantly and adversely impacts TB treatment outcomes by increasing TB patient risks of treatment failure, TB relapse, and death.[7]

Metformin (MET), a time-tested biguanide-based treatment used for the management of type 2 diabetes mellitus (T2DM), has recently been reported to improve TB patient outcomes as well.[8,9] In several studies, MET benefits were linked to its effects on cellular metabolism, immune function, and transcriptional-level expression of genes associated with host innate immune responses to MTB.[10] However, specific pathophysiological mechanisms underlying MET beneficial effects are still unclear.

In this study, we explored specific potential mechanisms underlying MET beneficial effects for TB and DM patients based on screening to detect differentially expressed genes (DEGs) in cells of patients with TB vs healthy controls (HCs) and patients with DM vs HCs. These DEG results were verified by comparing them to DEG obtained for TB+DM vs HCs. Next, DEGs common to TB and DM patient cells were identified and then subjected to functional enrichment analysis followed by protein–protein interaction (PPI) network analysis that generated a PPI network. The PPI network was then used to predict hub genes involved in the pathogenesis of TB complicated by DM that were validated using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Additionally, miRNAs targeting identified hub genes were predicted and then associations between hub genes and genes associated with beneficial MET effects on TB patient outcomes were revealed. The results obtained from this study provide clues revealing details of underlying pathophysiological mechanisms shared by DM and TB.

Patients and Methods

Data Collection

GSE114192[10] and GSE102677[11] datasets were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database,[12] a public database available to researchers. GSE114192, which contained gene expression profiles of 46 TB patients, 52 DM patients, 61 patients with both TB and DM, and 36 HCs, was generated using the GPL18573 platform (Illumina NextSeq 500). GSE102677, which included gene expression profiles of 22 samples, was also generated using the GPL18573 platform (Illumina NextSeq 500). Specifically, in one group, peripheral blood mononuclear cells (PBMCs) collected from 11 healthy donors who were treated with MET (1000 mg twice per day) were cultured in medium in the presence of 5 g of MTB lysate for 4 hours, while in the other group, PBMCs taken from 11 untreated healthy donors were treated with MTB lysate as for the MET-treated group. Detailed information pertaining to these datasets is listed in Table 1. Probes detecting no genes or multiple genes were deleted, while for multiple probes that detect the same gene, the average expression value for all probes was determined.

Table 1 Details of GEO Data

Accession	Platform	Experiment Type	Group	Tissue Type
GSE114192	GPL18573	High throughput sequencing	46 TB, 52DM, 61 TB with DM patients and 36 controls	Whole blood
GSE102677	GPL18573	High throughput sequencing	11 MET+TB treated and 11 TB treated	PBMCs

Abbreviations: DM, diabetes mellitus; GEO, Gene Expression Omnibus; TB, tuberculosis; MET, metformin; PBMCs, peripheral blood mononuclear cells.

Identification of DEGs

NetworkAnalyst (http://www.networkanalyst.ca/),[13] a visual analytics platform for comprehensive gene expression profiling and meta-analysis, was used to identify DEGs. After uploading the readcounts matrix from GSE114192 to NetworkAnalyst, we screened the dataset for DEGs based on comparisons of TB patients vs HCs and DM patients vs HCs, and then verified these results by comparing them to results for the comparison of TB+DM vs HCs. Common DEGs identified were used for further analysis. In this study, default filtering parameters were used and Log2-counts per million was applied for data normalization. DEGs were identified using the EdgeR method and cutoffs for evaluating statistical significance were *P*-value <0.05 and Ilog2 (fold change)I ≥1.

Functional Enrichment Analysis of DEGs

DAVID (https://david.ncifcrf.gov/, version 6.8)[14] is a bioinformatics database that integrates biological data and analytical tools to provide systematic and comprehensive functional annotation information on biological systems. Tools used here to perform functional enrichment analysis of DEGs included Gene Ontology (GO)[15] to obtain function-related terms and Kyoto Encyclopedia of Genes and Genomes (KEGG)[16] to obtain functional pathway-related information. GO terms identified here were used to annotate functions falling under categories of biological processes (BP), cellular component (CC), or molecular function (MF). *P*-value <0.05 was considered statistically significant.

PPI Network Construction and Hub Gene Identification

STRING (https://string-db.org/)[17] is a public online database that can be used to search for known proteins and predict protein–protein interactions, including direct physical interactions between proteins and indirect functional interactions detected through correlation analyses. After common DEGs among different comparison groups were uploaded to STRING's official website, interrelationships between DEGs and STRING database proteins were determined based on a minimum required interaction score set to 0.40. Resulting PPI interaction networks were visualised using Cytoscape (version 3.6.1).[18] CytoHubba (version 0.1),[19] a plug-in of Cytoscape, was used to identify hub genes based on a maximal clique centrality (MCC) algorithm.

The sequence of the target gene of MET was obtained from the Drug Repurposing Hub (https://www.broadinstitute.org/drug-repurposing-hub).[20] Subsequently, we constructed a PPI network comprised MET target genes and interaction protein partners then selected hub genes to further reveal potential MET treatment targets associated with disease mechanisms shared by DM and TB.

Validation of Hub Genes

RT-qPCR assays were performed to verify the reliability of bioinformatics-based prediction results. A total of 40 study participants were recruited from Beijing Chest Hospital, Capital Medical University, including 12 TB patients, 12 T2DM patients, 8 TB patients with T2DM, and 8 healthy controls (HCs) (Table S1). TB patients were diagnosed based on sputum smear or culture, GeneXpert MTB/RIF, and clinical symptoms. DM patients were diagnosed based on American Diabetes Association standards.[21] Participants with cancer or other lung diseases were excluded. Peripheral venous blood was collected from each participant; then, total RNA was extracted from each sample using TRIzol (Invitrogen) according to the manufacturer's instructions. Reverse transcription was conducted using an EasyScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) Kit (TransGen Biotech) with incubations conducted at 42°C for 15 min and then at 85°C for 15 s. Subsequently, RT-qPCR was performed using StarLighter SYBR® Green qPCR Mix (Universal) (Forever Star, China) using an ABI 7500 system (Applied Biosystems). The reaction conditions were as follows: pre-denaturation (95°C for 5 min), 40 cycles of denaturation (94°C for 20 s), annealing and extension (60°C for 34 s). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified to serve as an internal control (Table S2). The $2^{-\Delta\Delta Ct}$ method was utilised to determine relative expression levels. Statistical analysis was performed using Graph Pad Prism 8.0 (Graph Pad Software Inc.). Expression of hub genes was analysed using one-way ANOVA with a Bonferroni correction. *P*-value <0.05 was considered statistically significant.

Prediction of miRNAs and Construction of a Gene–miRNA Interaction Network

Hub genes were selected and analysed using the miRWalk database (http://mirwalk.umm.uni-heidelberg.de/)[22] to predict their targeted miRNAs. The filter setting was set to score >0.90, the target gene binding region was 3′ UTR,

and intersection with other databases was set to miRDB. Results were further processed using Cytoscape.

Analysis of Genes Associated with Metformin Treatment

NetworkAnalyst was applied to analyse the GSE102677 dataset. The gene expression of two groups of samples was included in the analysis. PBMCs in both groups were cultured in medium in the presence of MTB lysate. PBMCs in the MTB group were obtained from healthy participants, while PBMCs for the MET+MTB group were obtained from healthy donors who were treated with metformin. The readcounts matrix was uploaded to NetworkAnalyst, and differential gene expression analysis was conducted using the DESeq2 method after data was normalised. We observed expression changes of the selected hub genes between the two groups. *P*-value <0.05 was considered statistically significant.

Results

Identification of DEGs

In the GSE114192 dataset, 355 up- and 123 down-regulated genes were identified in TB patients as compared with HCs (Figure 1A, Table S3). Meanwhile, 98 up- and 28 down-regulated genes were identified in DM patients as compared with HCs (Figure 1B, Table S4). In addition, 626 up- and 243 down-regulated genes were identified in TB+DM patients as compared with HCs (Figure 1C, Table S5). A total of 350 DEGs (289 up- and 61 down-regulated) were shared by both TB and TB+DM comparison groups, while 72 DEGs (57 up- and 15 down-regulated) were shared by DM and TB+DM comparison groups, as verified using Venn analysis (Figure 1D and E). Next, this final set of 422 verified, overlapping DEGs was subjected to further analysis and included 346 up- and 76 down-regulated genes (Table S6).

Functional Enrichment Analysis of DEGs

We used DAVID to conduct functional enrichment analyses of DEGs. Enriched GO terms associated with DEGs were divided into BP, CC, and MF ontologies. Major BP terms associated with DEGs included type I interferon signaling pathway, innate immune response, and inflammatory response, etc. (Figure 2A). Major CC terms associated with these DEGs included extracellular region, extracellular space, and collagen trimers, etc. (Figure 2B). Finally, MF-associated GO terms were mainly associated with calcium ion binding, oxygen transporter activity, and protein homodimerisation activity, etc. (Figure 2C). With regard to KEGG pathway analysis results, DEGs were mainly enriched for pathways associated with *Staphylococcus aureus* infection, complement and coagulation cascades, and malaria, among others (Figure 2D).

PPI Network Formation

We uploaded 422 DEGs to the STRING online database to generate a PPI network, of which a total of 293 DEGs were extracted for network analysis using an interaction score set to 0.40. The resulting PPI network contained a total of 293 nodes and 1158 edges. Next, based on MCC scores, ten hub genes (*STAT1, IFIT3, RSAD2, IFI44L, GBP1, XAF1, IRF7, ISG15, IFI44*, and *IFI6*) were selected using the plug-in cytoHubba of Cytoscape and were all revealed to have been up-regulated (Table 2, Figure 3A).

Met target genes (*INS, ACACB*, and *PRKAB1*) derived from the Drug Repurposing Hub database were uploaded with the ten abovementioned selected hub genes to the STRING database. Network analysis results revealed that several uploaded genes interacted with *INS* to form a network containing 13 nodes and 53 edges (Figure 3B).

Validation of Hub Gene Expression

RT-qPCR was performed to confirm expression levels of the ten hub genes, leading to elimination of *ISG15* from further analysis, as its expression level was too low to be reliably quantified in each group. Notably, expression of five genes (*STAT1, IFIT3, RSAD2, IFI44L*, and *XAF1*) in TB+DM patients exceeded corresponding expression levels found in HCs, thus confirming our predictions. Notably, no significant expression level differences were found for *STAT1* and *XAF1* in patients with TB only vs HCs or patients with DM only vs HCs. Thus, we speculated that comorbid DM magnified predicted hub gene expression changes occurring in TB patients as compared to HCs (Figure 4).

Further miRNA Prediction and Interaction Gene-miRNA Network Analysis

We uploaded ten hub genes to the miRWalk database and predicted 176 miRNAs targeting eight hub genes. The gene–miRNA interaction network is shown in Figure 5. Seven miRNAs (miR-3680-3p, miR-3059-5p, miR-629-3p, miR-29b-2-5p, miR-514b-5p, miR-4755-5p, and miR-4691-3p) were detected that were associated with high numbers of gene cross-links (≥2) are shown in Table 3.

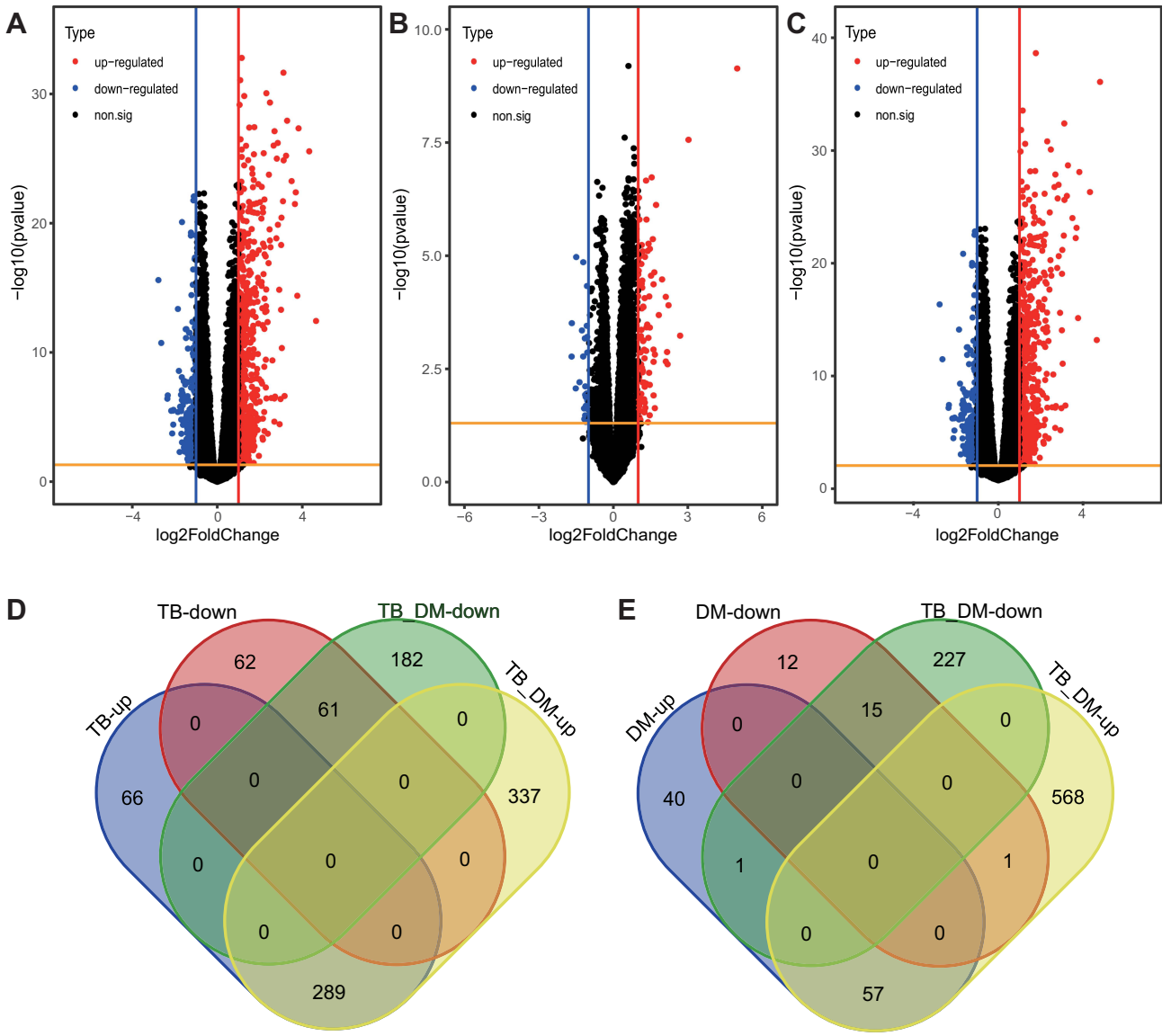

Figure 1 Identification of DEGs in TB, DM, and HC samples. (**A**) 478 significant DEGs detected in TB as compared to HC samples. (**B**) 126 significant DEGs detected in DM as compared to HC samples. (**C**) 869 significant DEGs detected in TB+DM as compared to HC samples. Red dots represent selected up-regulated genes and blue dots represent selected down-regulated genes. Black dots represent genes with no significant inter-group expression differences. (**D**) 350 DEGs shared by both TB and TB+DM groups. (**E**) 72 DEGs shared by both DM and TB+DM groups.

Abbreviations: DEGs, differentially expressed genes; TB, tuberculosis; DM, diabetes mellitus; HC, healthy control.

Association Between Hub Genes and Metformin Treatment

Furthermore, we investigated expression differences of the ten hub genes between the MET+MTB group and MTB group for the GSE102677 dataset. As shown in the hierarchical clustering heat map of these genes (Figure 6A), a total of six hub genes (*STAT1, IFIT3, RSAD2, ISG15, IFI44*, and *IFI6*) were down-regulated after MET treatment ($P < 0.05$), while the other four hub genes were not ($P > 0.05$) (Figure 6B–K).

Discussion

Both tuberculosis and type 2 diabetes mellitus (T2DM) are global epidemic diseases that place a large burden on patients and health services. Many reports have indicated that T2DM is an independent risk factor for TB,[5] but specific underlying mechanisms responsible for this association are not well understood. We therefore conducted a series of bioinformatic analyses aiming to investigate the potential pathophysiological association between TB and T2DM.

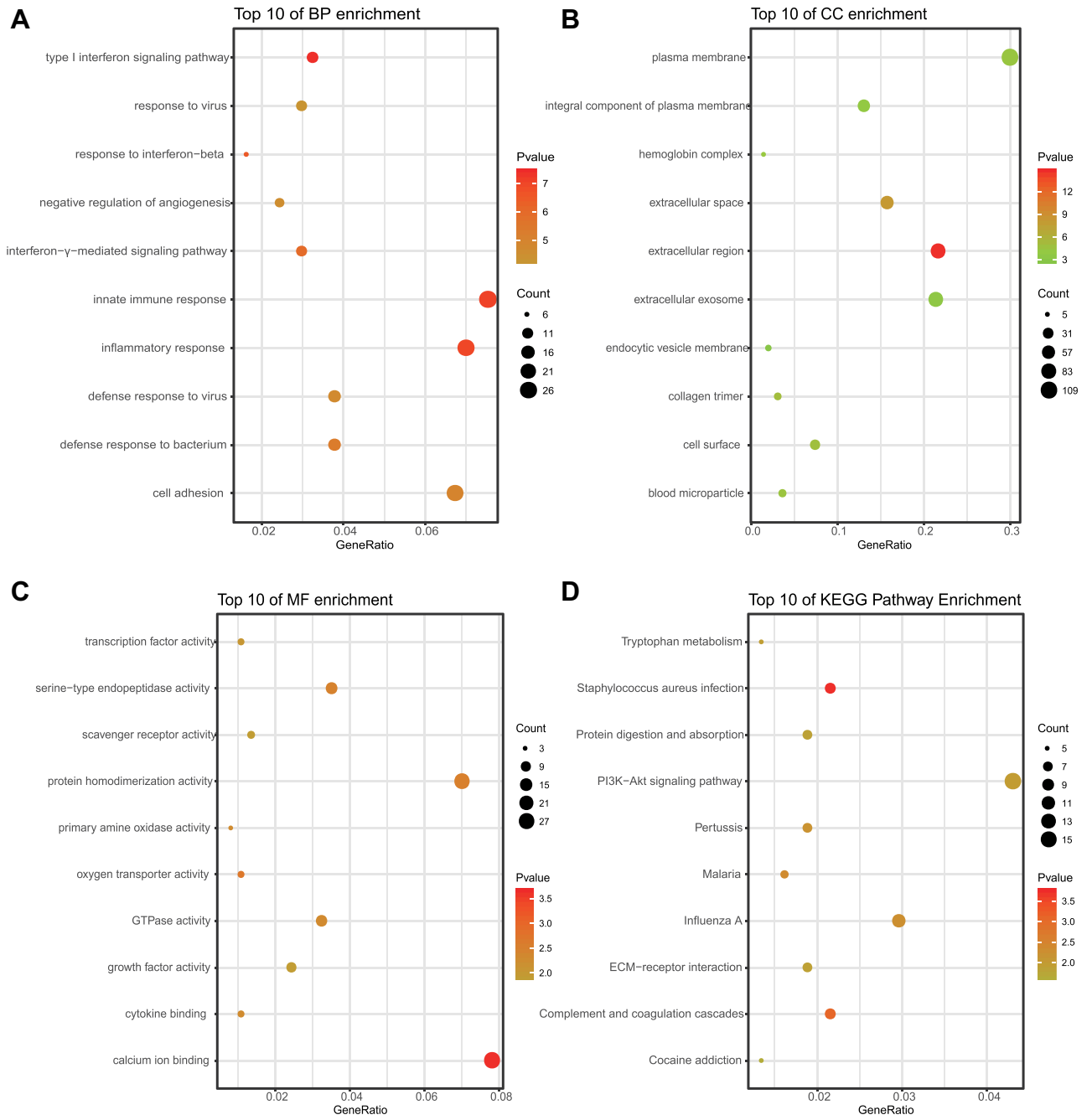

Figure 2 Functional enrichment analysis of common DEGs. (**A**–**D**) represent results of BP, CC, MF, and KEGG pathway analyses of DEGs, respectively.
Abbreviations: DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

In the present study, we screened gene expression datasets and then identified 422 DEGs shared by DM and TB patients that included 346 up- and 76 down-regulated genes. Functional enrichment analysis demonstrated that these DEGs were enriched for functional terms that included type I interferon signaling pathway, innate immune response, inflammatory response, calcium ion binding, oxygen transporter activity, and infectious diseases. Furthermore, analysis of the PPI network based on interactions among STRING database genes and shared DEGs revealed ten hub genes (*STAT1, IFIT3, RSAD2, IFI44L, GBP1, XAF1, IRF7, ISG15, IFI44*, and *IFI6*) that were subsequently identified using the plug-in cytoHubba of Cytoscape based on MCC scores. Of these ten hub genes, five of them (*STAT1, IFIT3, RSAD2, IFI44L*, and *XAF1*) were verified using RT-qPCR.

Table 2 Top 10 Hub Genes

Rank	Name	Full Name	Pathway Involved
1	STAT1	Signal transducer and activator of transcription 1	Influenza A, Toll-like receptor signaling pathway
2	IFIT3	Interferon induced protein with tetratricopeptide repeats 3	/
3	RSAD2	Radical S-adenosyl methionine domain containing 2	Influenza A
4	IFI44L	Interferon induced protein 44 like	/
5	GBP1	Guanylate binding protein 1	/
6	XAF1	XIAP associated factor 1	/
7	IRF7	Interferon regulatory factor 7	Influenza A, RIG-I-like receptor signaling pathway, Toll-like receptor signaling pathway
8	ISG15	ISG15 ubiquitin like modifier	RIG-I-like receptor signaling pathway
9	IFI44	Interferon induced protein 44	/
10	IFI6	Interferon alpha inducible protein 6	/

Notes: Pathways with $P < 0.05$ were considered significantly enriched pathways.

Of the five verified hub genes, *STAT1* has been shown to play an important role in the host immune defence against TB infection, whereby mutation of *STAT1* can lead to susceptibility to MTB infection.[23,24] A previous study revealed that STAT1 protein phosphorylation status was closely related to host resistance to MTB infection, as high levels of phosphorylated STAT1 could drive expression of many pro-apoptotic genes, resulting in anti-tuberculosis effects. However, unphosphorylated STAT1 represses macrophage apoptosis and thus facilitates immune evasion by MTB that supports continued MTB infection.[25] With regard to other STAT1 functions, Kim et al found that STAT1 played critical role in beta-cell death, T-cell immunoregulation, and progression of type 1 diabetes in vivo.[26] Moreover, a more recent study has found that in obese individuals, STAT1 uncouples adipose tissue inflammation from insulin sensitivity.[27] Another hub gene, *XAF1*, was shown in a previous study to encode a strongly pro-apoptotic protein that is induced by IFN exposure with significant value in cancer therapy.[28] In recent years, *XAF1* has been reported to be up-regulated in several TB transcriptomic studies.[29,30] Nevertheless, it is not clear whether this gene participates in TB pathogenesis. We speculate that MTB may induce

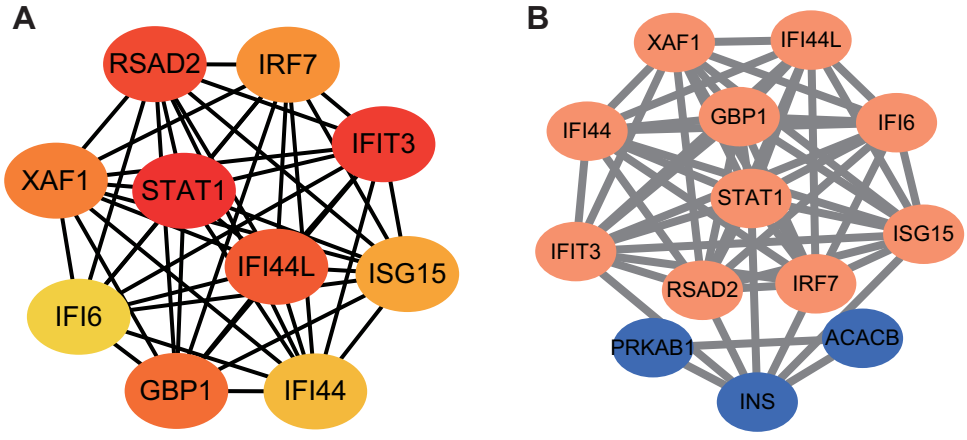

Figure 3 PPI network analysis. (**A**) Ten hub genes were selected using the plug-in cytoHubba of Cytoscape based on MCC scores. (**B**) Interactions between MET target genes and hub genes. Dots represent genes and edges represent functional connections between genes.
Abbreviations: PPI, protein–protein interaction; MCC, maximal clique centrality; MET, metformin.

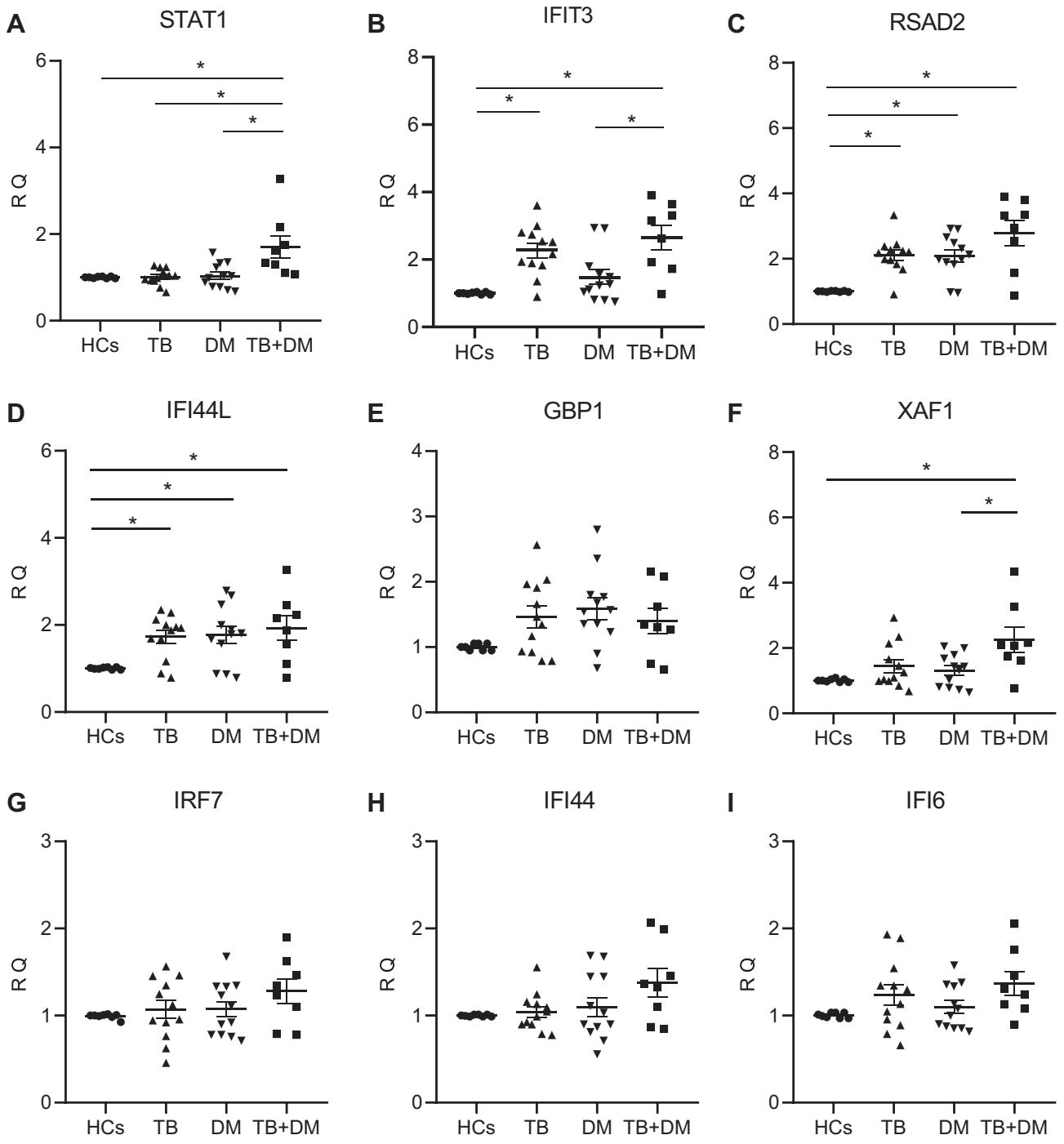

Figure 4 Relative expression of hub genes including (**A**) *STAT1*, (**B**) *IFIT3*, (**C**) *RSAD2*, (**D**) *IFI44L*, (**E**) *GBP1*, (**F**) *XAF1*, (**G**) *IRF7*, (**H**) *IFI44L*, and (**I**) *IFI6* as measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RQ represents relative quantification values; *Represents *P*-value <0.05.

apoptosis of immunocytes by increasing *XAF1* expression, although this speculation awaits experimental confirmation. In another study, metabolic endotoxemia, a disorder associated with T2DM pathogenesis, has been shown to lead to activation of macrophages. Activated macrophages then infiltrate pancreatic islets and produce IFN-β, which induces β-cell apoptosis by increasing *XAF1* expression.[31] Meanwhile, with regard to hub gene *RSAD2*, induction of expression its corresponding protein RSAD2 has been shown to occur in MTB-infected macrophages, with significantly decreased expression observed after six months of isoniazid treatment.[29,30] Consistent with previous studies, we observed up-regulated expression of hub genes

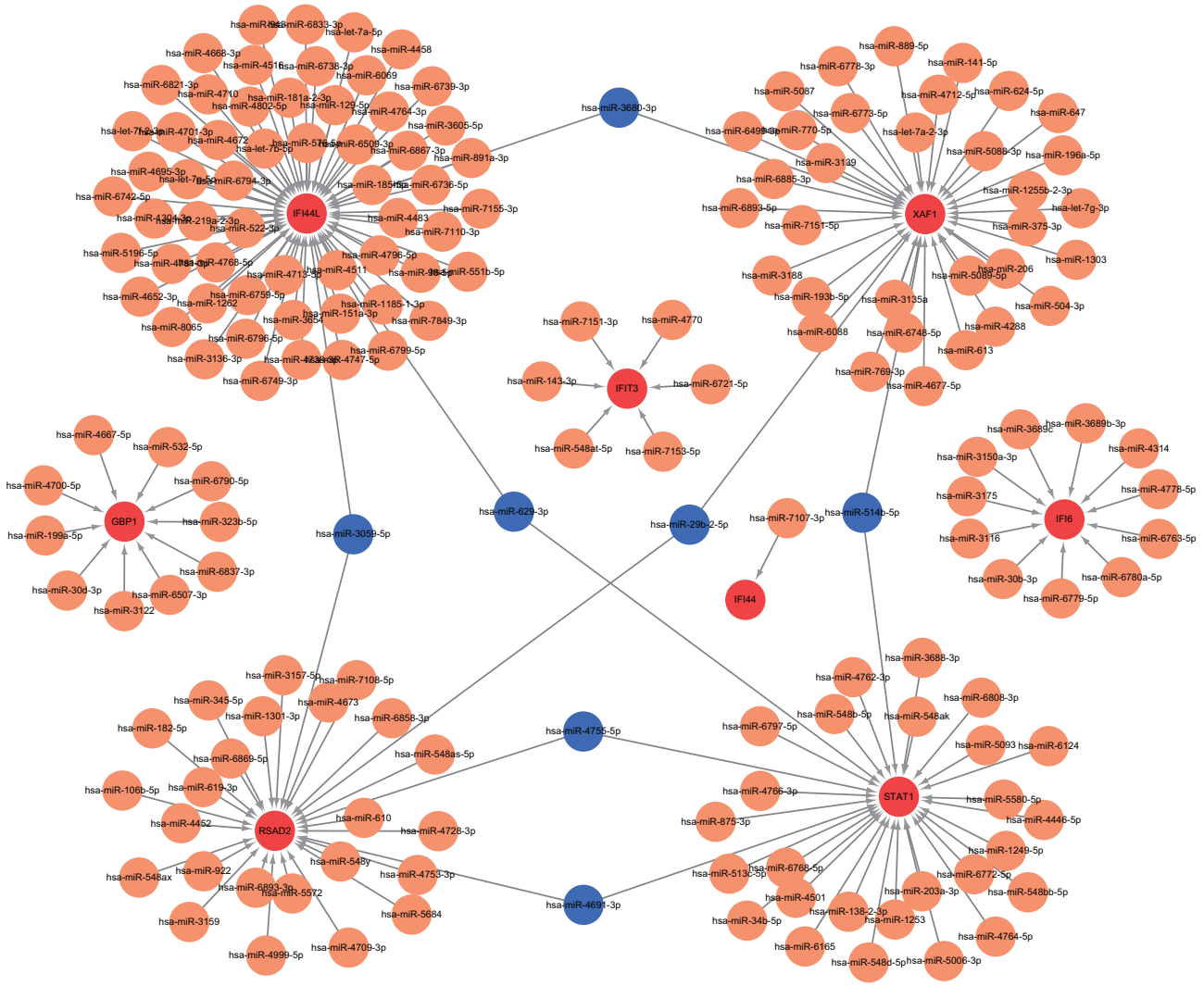

Figure 5 Interaction network between hub genes and their targeted miRNAs. Red dots represent hub genes, orange dots represent miRNAs, and blue dots represent miRNAs targeting two genes simultaneously.

IFIT3[32,33] and *IFI44L*[33,34] after MTB infection. These genes have been shown to be related to IFN-inducible signatures, but their specific roles in resistance to MTB infection have not yet been clarified. Meanwhile, Gupta et al have reported five different hub genes (*CCL2, ELMO1, VEGFA, FOS*, and *TCF7L2*) with major roles in T2DM.[35] The discordance between their results and ours may reflect different sample types, sample sizes, and sample collection and normalization methods. To our knowledge, no study has correlated expression of *RSAD2, IFIT3, IFI44L* genes with DM disease thus far, warranting investigations of their roles in DM pathology in the future. Interestingly, we found no significant difference between expression levels of *STAT1* and *XAF1* in patients with TB or DM only. However, DEG results from dataset comparison TB+DM patients vs HCs exhibited expression level differences that were statistically significant. We, therefore, believe that DM increased the magnitude of hub gene expression changes in TB patients. Although the specific mechanisms of action for this phenomenon have yet to be elucidated, novel

Table 3 miRNA and Its Target Genes

miRNA	Genes Targeted by miRNA	Gene Count
miR-3680-3p	IFI44L, XAF1	2
miR-3059-5p	IFI44L, RSAD2	2
miR-629-3p	IFI44L, STAT1	2
miR-29b-2-5p	XAF1, RSAD2	2
miR-514b-5p	XAF1, STAT1	2
miR-4755-5p	RSAD2, STAT1	2
miR-4691-3p	RSAD2, STAT1	2

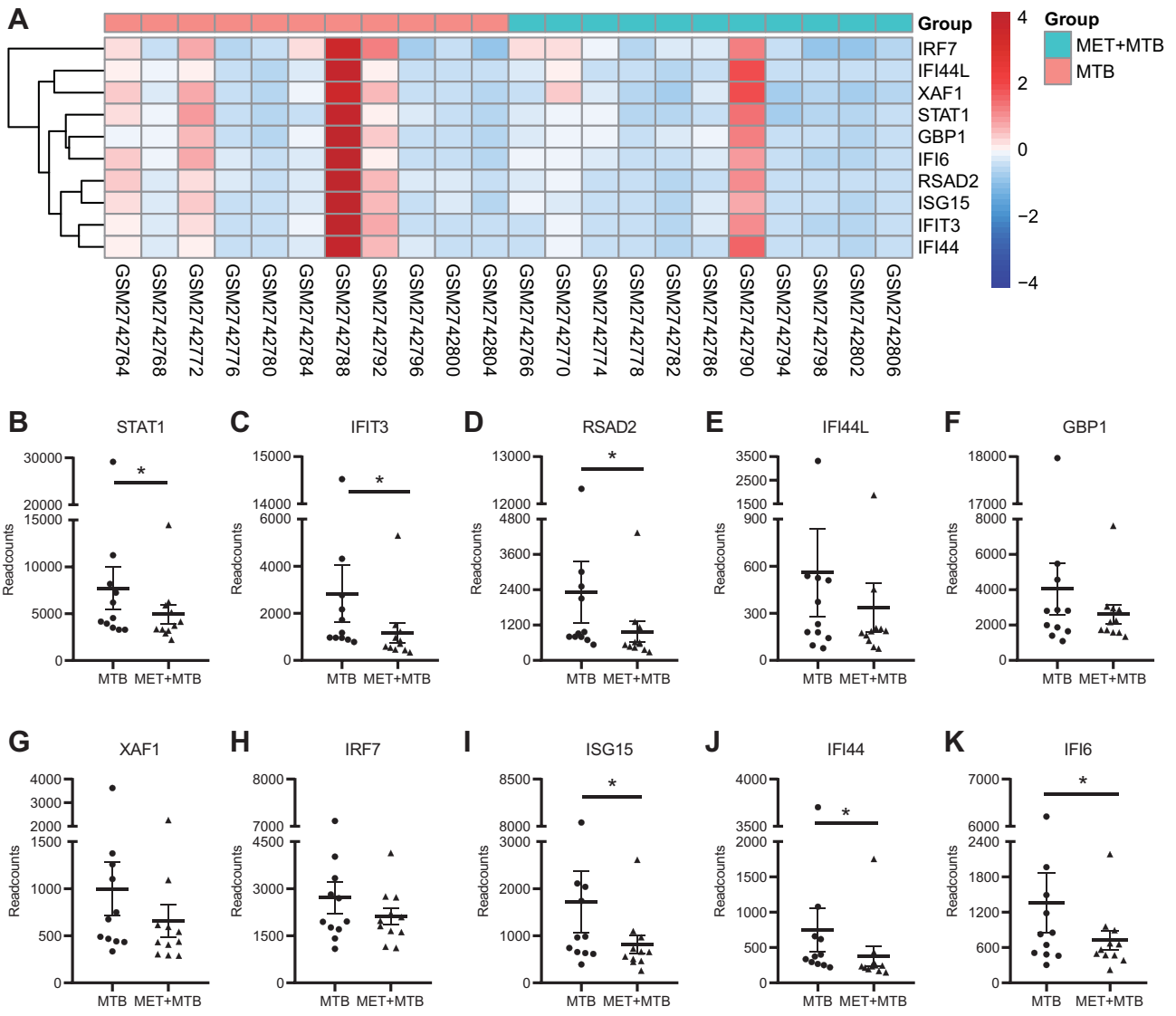

Figure 6 Gene expression changes of ten hub genes after MET treatment of MTB-infected PBMCs. (**A**) Hierarchical clustering heatmap of hub genes of MET+MTB and MTB groups. Red indicates relatively up-regulated gene expression, blue indicates relatively down-regulated gene expression, and white indicates no significant change in gene expression. (**B**–**K**) Representative gene expression profiles of 11 MET+MTB-treated PBMCs and 11 MTB-treated PBMCs. *Represents *P*-value <0.05.
Abbreviations: MET, metformin; MTB, *Mycobacterium tuberculosis*; PBMCs, peripheral blood mononuclear cells.

findings might offer novel insights into the pathogenesis of TB complicated with DM.

Importantly, endogenous, noncoding single-stranded RNAs known as miRNAs can regulate gene expression at the post-transcriptional level to degrade or inhibit translation of target genes.[36] Using the miRWalk database, we predicted 176 miRNAs that were able to target eight hub genes. From the gene–miRNA interaction network, we observed seven miRNAs (miR-3680-3p, miR-3059-5p, miR-629-3p, miR-29b-2-5p, miR-514b-5p, miR-4755-5p, and miR-4691-3p) that targeted at least 2 genes. Therefore, we speculate that these seven miRNAs may play important regulatory roles in disease mechanisms of T2DM with comorbid TB.

Metformin, a first-line medication used for the treatment of T2DM, has been reported to have potential for treating TB. Metformin affects the immune response and inflammation in multiple ways.[9] Its protective effect in TB patients is associated with increased production of mitochondrial reactive oxygen species in host cells and acidification of phagocytes by MTB.[8] Furthermore, the anti-inflammatory effect of MET may be related to the activation of AMP-activated protein kinase (AMPK), a negative regulator of inflammation.[8] In our study, several direct or indirect interactions were detected between MET target genes and selected hub genes. Moreover, our research showed that six hub genes, *STAT1, IFIT3, RSAD2, ISG15, IFI44*, and

IFI6, were down-regulated in cells exposed to MTB and MET treatment, as compared to cells exposed to MTB alone. This result suggests that these genes may participate mechanistically in MET treatment-associated improvement of TB outcomes and provides evidence that MET may have value for TB patient treatment. However, the exact relationship between MET and hub genes is unknown, warranting further examination.

To our knowledge, the current study is the first exploration of the role of ten genes with shared functions, as detected via bioinformatic analysis, in comorbid DM-TB diseases. These results provide useful information that enhances our understanding of DM and TB while guiding future research. Nevertheless, this research study also has limitations. First, the sample size was comparatively small, which resulted in bias to some extent. Second, the 7 miRNAs that were predicted to have a high number of gene interactions were not further validated. Third, mechanisms by which several hub genes participate in pathological processes associated with comorbid TB-DM disease remain unclear. Finally, mechanisms underlying MET treatment benefits to TB patients were not fully elucidated in this study, warranting further research.

Conclusion

In conclusion, we have identified ten hub genes, of which five genes (*STAT1, IFIT3, RSAD2, IFI44L*, and *XAF1*) were verified using RT-qPCR. Importantly, these genes may have value as biomarkers for use in diagnosing and monitoring TB with comorbid DM. Meanwhile, seven miRNAs (miR-3680-3p, miR-3059-5p, miR-629-3p, miR-29b-2-5p, miR-514b-5p, miR-4755-5p, and miR-4691-3p) were identified here that may be involved in regulation of hub genes. In addition, we found six genes (*STAT1, IFIT3, RSAD2, ISG15, IFI44*, and *IFI6*) that were predicted to be targeted by MET. These genes thus may contribute to positive MET-associated treatment outcomes when MET is administered to patients with comorbid TB and DM. These results provide a new experimental foundation on which to further explore pathogenic mechanisms associated with TB and DM. Nevertheless, the specific mechanisms of action of key genes shown here to be associated with comorbid DM and TB require further investigation.

Abbreviations

BP, biological processes; CC, cellular components; DEG, differentially expressed gene; DM, diabetes mellitus; GEO, Gene Expression Omnibus; GO, Gene Ontology; HCs, healthy controls; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCC, maximal clique centrality; MET, metformin; MF, molecular function; MTB, *Mycobacterium tuberculosis*; PBMC, peripheral blood mononuclear cells; PPI, protein–protein interaction; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; T2DM, type 2 diabetes mellitus; TB, tuberculosis; WHO, World Health Organization.

Ethics Approval and Informed Consent

This study was approved by the Ethics committee of Beijing Chest Hospital, Capital Medical University (approval number: YJS-2021-014). The guidelines outlined in the Declaration of Helsinki were followed.

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Disclosure

The authors report no conflicts of interest in this work.

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