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STUDY OF THE EFFECT OF DIFFERENT miRNA ON GENE EXPRESSION PROFILE DURING *Mycobacterium tuberculosis* INFECTION USING BIOINFORMATICS TOOLS

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Abstract

Tuberculosis (TB) is one of the most common diseases worldwide. *Mycobacterium tuberculosis* (Mtb) is the main causal agent of tuberculosis. Changes in gene expression are very important for normal biological functions. Changes in gene expression have adverse effects and cause many diseases. Tb is one of them. Gene expression is controlled by many small noncoding miRNAs. In this study, we identified relationships between miRNAs and differentially expressed genes in tuberculosis by using bioinformatics tools. Dataset GSE51029 was downloaded from the GEO database to check for expression. DEGs in the microarray dataset were identified by a bioinformatics tool, namely GEO2R. Enrichment analysis of DEGs was done through DAVID. The PPI network was constructed through the Cytoscape of the DEGs. Hub-genes were identified by using *cyto-Hubba* extracted from the PPI network. The gene's interaction network of miRNA-hub was constructed to evaluate the miRNAs interacting with hub genes. A total of 285 DEGs were identified. Of those, 82 genes were downregulated and 202 genes were upregulated. Enrichment analysis of DEGs showed their involvement in cell surface receptors, regulation of proteins, signaling pathways, cytoplasmic endoplasmic reticulum, endoplasmic, nuclear membrane, transcription coactivator activity, mRNA binding, protein kinase binding, and deacetylase binding. KEGG analysis presented that identified DEGs were involved in signaling pathways and other diseases. The ten genes closely related to miRNA have also been identified. This study shows that identified DEGs, pathways, and miRNAs that closely interact with hub genes are involved in causing tuberculosis. Their clinical evaluation at an early stage may provide important targets for the treatment of tuberculosis.

INTRODUCTION

Tuberculosis (TB), which is a long-known bacterial infectious disease, is considered the main cause of mortality and morbidity worldwide. It has been a neglected disease in both developing and industrialized countries [1]. Every year, 2 million people die as a result of tuberculosis, and 8 million new cases are reported worldwide [2]. TB is directly related to lifestyle, malnutrition, and spreads quickly among undernourished people [3]. *Mycobacterium tuberculosis* (Mtb) is the main causal agent of tuberculosis. It belongs to the genus Mycobacterium [4], which consists of many genetically homogenous mycobacteria species. *M. bovisand*, *M. canetti*, and *M. aficanum* are human-adopted MTB pathogens, and animal-adopted Mycobacterium pathogens such as *M. microtiand*, *M. pinnipedii*, and *M. caprae* have also been found to infect humans [5]. Mtb transmitted through the respiratory route causes disease in many other organs, but the most common is pulmonary tuberculosis, which rarely becomes active. Active tuberculosis happens

due to a small infection and a lack of initiation of an immune response [6].

Gene regulation is very important for biological functions in healthy and diseased people. Irregular changes in the expression of genes have adverse effects and lead to the disease. In this study, we selected 18 samples from a dataset on TB for bioinformatics analysis and identified DEGs, important pathways and miRNAs that change the expression of genes during Mtb [7]. MiRNAs are endogenous, small noncoding RNAs that influence gene expression through post-transcriptional events, act as gene regulators, and can inhibit gene expression [8].

Many studies have reported that in eukaryotic cells, cellular immune responses are controlled by miRNAs, and modulation of miRNAs is associated with biological responses. It is an important factor implemented by bacterial pathogens living within host cells [9]. MiRNAs bind to the UTR (untranslated region) of protein coding mRNA targets as a result of translational inhibition or transcriptional degradation [10]. In the human genome, more than 2000 genes encode functional miRNAs, predicting that one-half of proteins are encoded by miRNA regulation [11]. Multiple miRNAs may be controlled by one mRNA, and multiple genes may be suppressed by a single miRNA. Thus, new therapeutic targets and a new class of diagnostic markers may be identified by disease associated miRNAs [12-14].

A few recent studies have described mammalian miRNA regulation against bacterial infection. In mycobacterial infection, immune and inflammatory pathways are disturbed, which are regulated by miRNA like immune cell activation needs miRNA with miR-9, miR-21, miR-155, miR-125, and miR-146a. For example, during Mtb infection in human alveoli, miR-125 inhibits TNF biosynthesis [15].

During mycobacterial infection, miR-146a promotes mycobacterial existence by controlling the production of nitric oxide via tumor necrosis factor at the post transcriptional level [16].

In this analysis, we identified relationships between miRNAs and DEGs in TB by using bioinformatics tools. This study shows that identified DEGs, pathways, and miRNA that closely interact with hub genes are involved in tuberculosis. Clinical evaluation at an early stage provides important targets for treatment of tuberculosis. However, further biological experiments are essential to identify the function of genes associated with tuberculosis**.**

MATERIALS AND METHODS

Data Preparation and Identification of DEGs

We used to get the dataset with the ID GSE51029 [17]. At the time point of 6 hours, 18 samples were taken from the dataset categorized as infected (9 samples) and uninfected (9 samples) at the time point. The samples were then subjected to differential expression analysis through GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), which is a built-in tool in the GEO database. Differentially expressed genes (DEGs) were filtered using threshold criteria of an *adjusted p-value* 0.05.

Enrichment Analysis of DEGs

The identified DEGs were then subjected to functional enrichment analysis through DAVID (https://david.ncifcrf.gov/), a bioinformatics resource [18]. A functional enrichment analysis with cutoff criteria of counts (number of genes involved) > 5 and a *p-value* 0.05 was performed to evaluate the functional enrichment analysis.

Construction of a Protein-Protein Interaction (PPI) Network

DEGs were deployed to Cytoscape [19] using its plugin for the STRING database, namely stringApp [20].

Hub Genes and Clusters' Extraction from the PPI Network

The PPI network was then subjected to the *cytoHubba* [21] plugin in Cytoscape to extract the candidate hub genes. The top 10 genes on the basis of their degrees of extraction were also Clusters' extraction from the PPI network was performed through MCODE [22], a plugin in Cytoscape. The parameters were used as default ("degree 2," "Node score cutoff: 0.2", "K-core: 2," "Maximum depth from Seed: 100").

KEGG Pathway Analysis of Hub Genes

Enricher, a web-based tool, was used to look at the KEGG pathway analysis of hub genes.

miRNA and Hub Gene Interaction Network

A miRNA-hub gene interaction network was constructed to evaluate the miRNAs that may have a strong influence on the hub genes. The tool used for this purpose was miRNet (https://www.mirnet.ca/) [23], which is a web-based tool. The threshold criteria for this purpose was degree = 2.

RESULTS

DEG Identification in Tuberculosis

For analysis of gene expression in TB, a microarray dataset from GEO under the following ID: GSE51029 was processed through the GEO2R tool. Box plots of the normalized samples are represented in Figure 1. We filtered out 285 DEGs from the raw data by applying a filter with an *adjusted p. value* of 0.05. The result shows that 202 genes were upregulated while 82 genes were downregulated in

tuberculosis tissues. Figure 2 represents the volcano plot of the filtered DEGs. Blue shows downregulation, red shows upregulation of genes, and black shows non-significance genes.

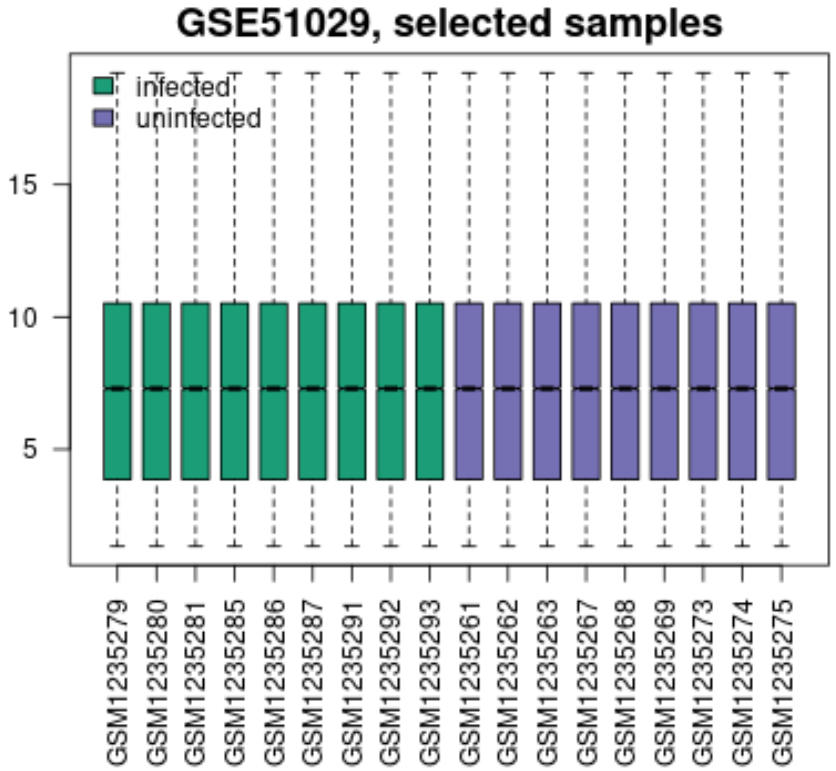

Figure 1. Box plots of the samples after normalization

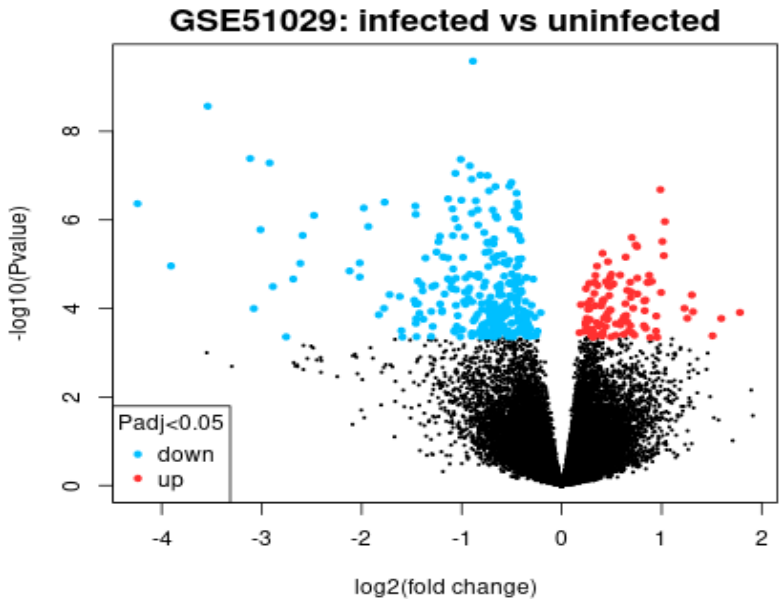

Figure 2. Volcano plot of DEGs in tuberculosis l. Different colors show differential gene expression in tuberculosis

Enrichment Analysis of DEGs

Biological annotation of DEGs in tuberculosis disease was performed by the online bioinformatics tool DAVI D. Functional enrichment of DEGs was performed under a threshold criterion of *p-value* 0.05. Functional annotation of DEGs consisted of three functional groups, including "cellular components" (CC), "biological processes" (BP) and "molecular function" (MF).

In the BP category, the DEGs were mainly found enriched in cellular response, signaling pathways, regulation of protein, innate immune response, and apoptotic processes (Figure 3). DEGs were found to be involved in cytoplasm, endoplasmic reticulum, endoplasmic reticulum membrane, nuclear membrane, ribonucleoprotein complex, and nuclear speck in the CC category (Figure 4).MF category, DEGs were significantly enriched in transcription coactivator activity, histone deacetylase binding, protein binding, mRNA binding, and protein kinase binding (Figure 5).

Furthermore, KEGG pathway analysis showed that DEGs were involved in mineral absorption, insulin resistance, longevity regulating pathway–worm, TNF signaling pathway, Measles, Toxoplasmosis, NF-kappa B signaling pathway, Hepatitis C, Epstein-Barr virus infection, Influenza A, atherosclerosis, and human immunodeficiency virus 1 infection, and, these pathways are involved in tuberculosis (Figure 6).

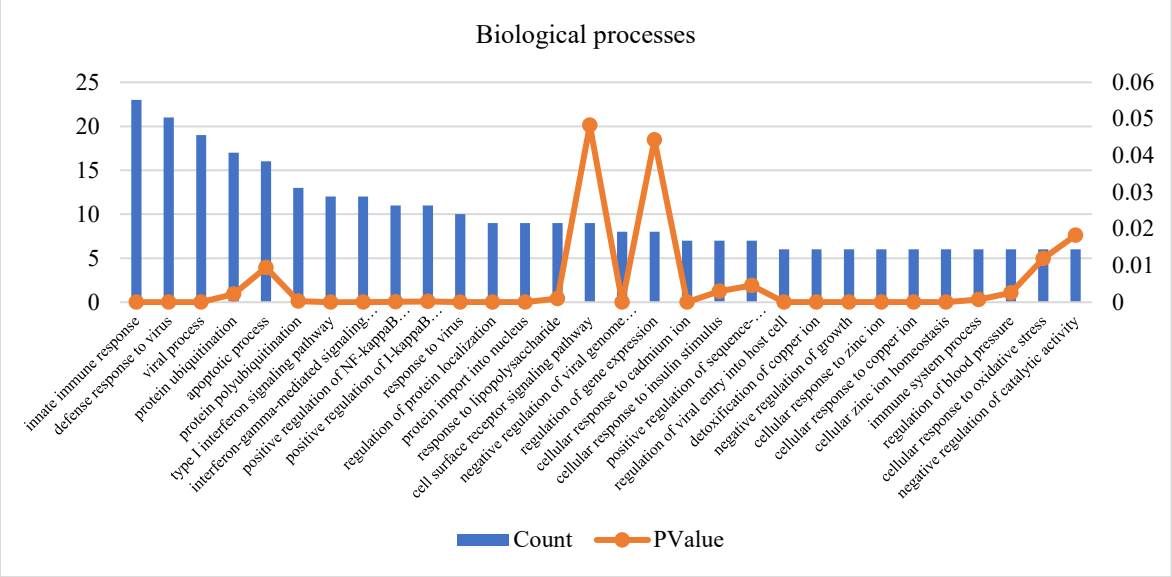

Figure 3. GO terms enrichment analysis of DEGs in biological processes

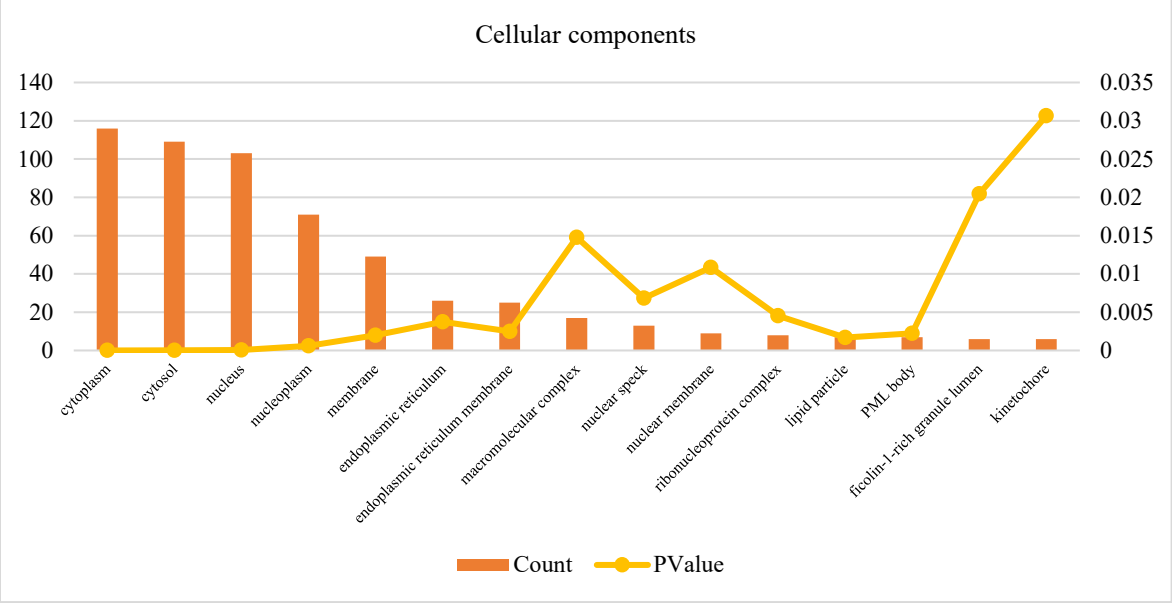

Figure 4. GO terms enrichment analysis of DEGs in cellular components

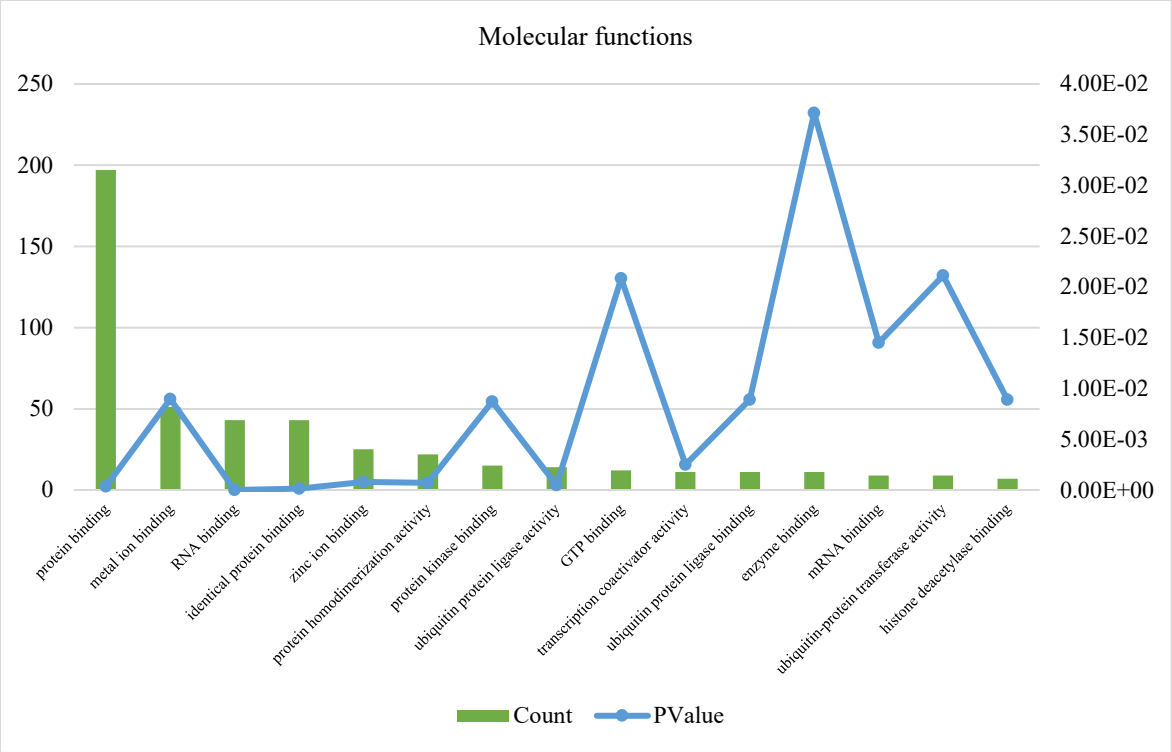

Figure 5. GO terms enrichment analysis of DEGs in molecular functions

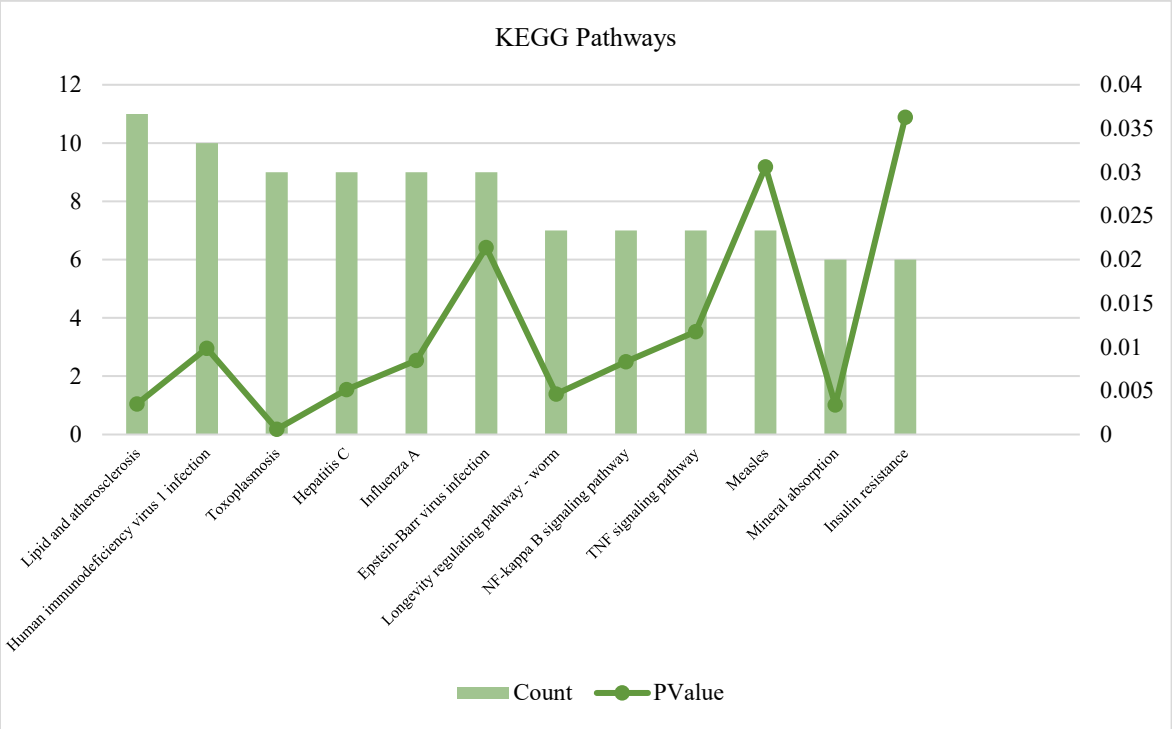

Figure 6. KEGG pathway analysis of DEGs

Building a PPI Network

The PPI network consists of 271 nodes and 493 edges. The cut-off value for the confidence score was 0.40. After removing partially connected nodes, a complex network of DEGs was constructed. The final PPI network was constructed with 490 edges and 194 nodes (Figure 7). Red-colored nodes show up and green-colored nodes show downregulated genes.

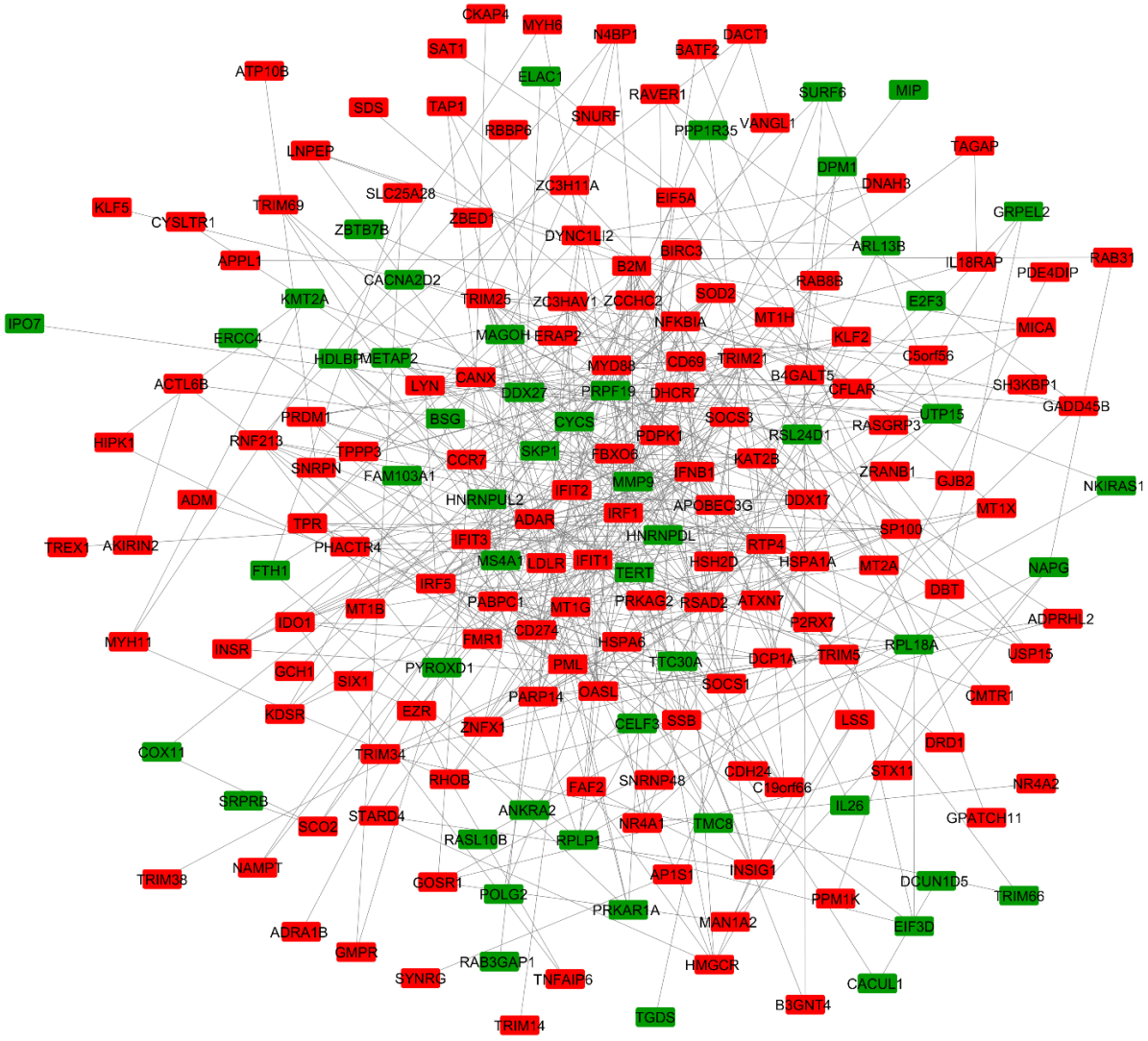

Figure 7. PPI network of differential expression genes in tuberculosis

Hub Genes and Clusters' Extraction from the PPI Network

Hub genes describe those genes with greater association in the PPI network. Greater connectivity defines that the top 10% connectivity rank means that closely connective genes were chosen. Hub genes were selected on the basis of the highest connectivity (degree), which was extracted from the string network. The most significant 10 genes showing important interactions with genes are NFKBIA, IFIT2, RSAD2, ADAR, IFNB1, IRF1, IFIT3, MYD88, OASL, and IFIT1 (Figure 8). Figure 9 shows the network of hub genes. Circles denote genes, lines denote the interaction of proteins among genes, and within the circle represents the protein structure. The line color shows the interaction between the proteins.

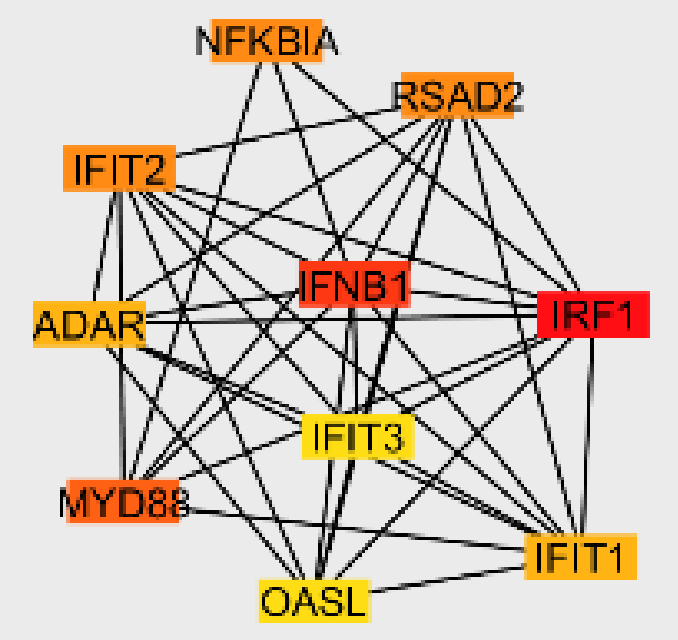

Figure 8. Interconnection of 10 genes, darker color shows that high degree score

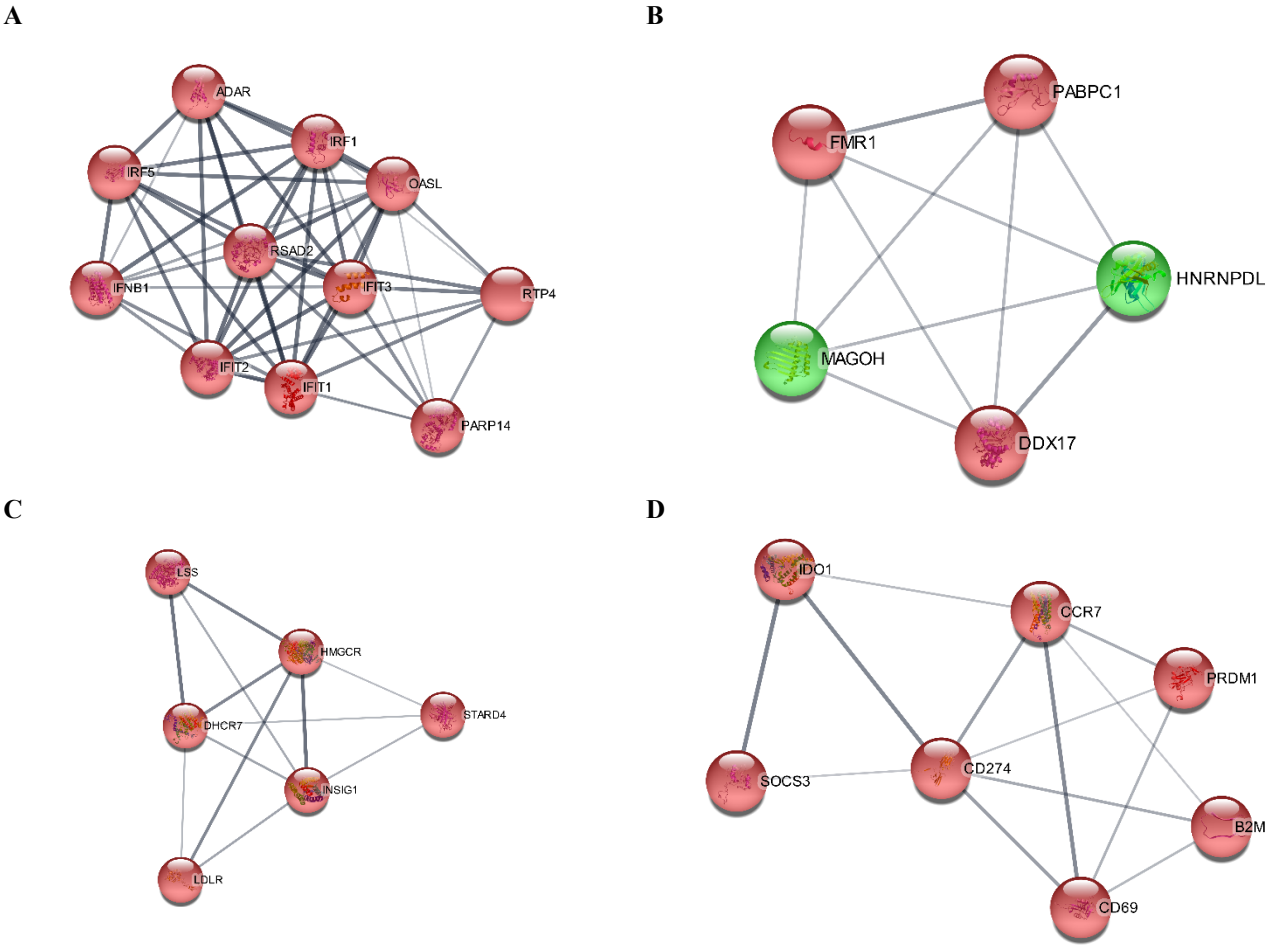

Figure 9. Figure sections A, B, C, D representing modules from PPI network

KEGG Pathway Analysis of Hub Genes

Hub genes were found to be involved in a variety of biological pathways, including RNA binding, signaling pathways, and membrane transport. Hub genes enrichment analysis shows that DEGs enrich in toll-like signaling pathways, cytosolic DNA sensing pathways and in different disease pathways like influenza A, measles, hepatitis C, and the TNF signaling pathway.

Hub Genes and miRNAs Interact to form a Network of Hub Genes and miRNAs

miRNA and hub gene interactions are shown in (Figure 10). Circles represent genes and boxes represent miRNA. Greater node size represents a large number of interactions with miRNA.

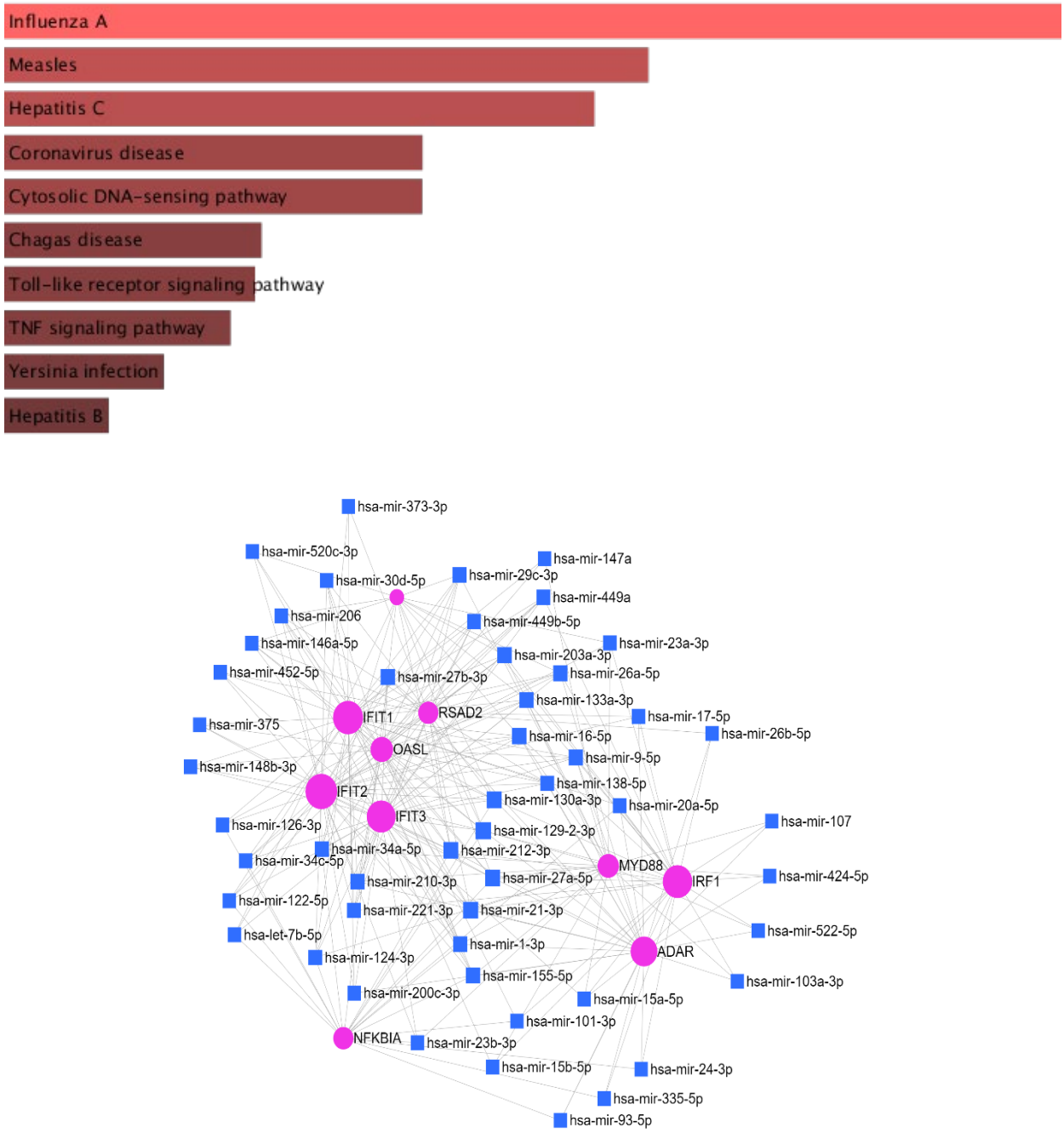

Figure 10. Interaction between hub gene and miRNA in tuberculosis

DISCUSSION

In the past few decades, many studies have highlighted the importance of tuberculosis infection at the RNA level. Tuberculosis is a bacterial infection disease that kills millions of people each year. Tuberculosis is caused by *Mycobacterium tuberculosis* and is transmitted by respiratory routes. Gene regulation is very important in healthy and diseased people for biological function. miRNAs are also involved in controlling the functions of genes. A miRNA is a small noncoding RNA and is approximately 20–22 nucleotides long. Post transcriptional regulation of many proteins is done by miRNAs, which play an important role as regulators in biological processes [24, 25]. miRNA plays a key role in many infectious diseases as a modulator of the host immune system. Previous studies show that miRNAs play an important role in tuberculosis infection by either promoting pathogens' immune evasion or increasing the immune response. MiRNAs are involved in biological processes like apoptosis and autophagy during tuberculosis infection, providing an opportunity to analyze miRNAs as a therapeutic tool against tuberculosis infection [26].

In the present study, through bioinformatics analysis, we identified DEGs in tuberculosis disease. For the analysis of DEGs of tuberculosis, we used datasets under the following ID: GSE51029. The results represent 285 DEGs, including 202 upregulated genes and 82 down-regulated genes. The top 10 most significant upregulated genes are ACTL6B, ADAR, ADM, ADPRHL2, ADRA1B, AKIRIN2, ANKRA2, ANP32D, AP1S1 and APOBEC3G. The top 10 most significant downregulated genes are RBM43, REC8, RHOB, RIF1, RIN2, RNF213, RPAIN, RPL18A, RPLP1, RSAD2 and RSL24D1. Further, 285 DEGs were classified into three groups by gene ontology (GO) enrichment analysis or functional annotation like cellular component, molecular function, and biological process. The DEG enrichment analysis was constructed by the KEGG pathway to determine protein interaction.

In biological processes, DEGs are mainly enriched in cellular response (KAT2B, RAB31, PDPK1), cell surface receptor signaling pathway (P2RX7, CD274, CYSLTR1), regulation of protein (TRIM5, TPR, TRIM14), innate immune response (LYN, AKIRIN2, APOBEC3G), and apoptotic processes. Cellular component DEGs are significantly involved in the cytoplasm (RIF1, FMR1, MT1L), endoplasmic reticulum (SLC27A1, INSIG1, FAF2), endoplasmic reticulum membrane (INSIG1, ATP10B, HMGCR), nuclear membrane (NR4A1, GCH1, RIF1), ribonucleoprotein complex (DDX17, SSB, APOBEC3G) and nuclear speck (DDX17, ARHGAP18, PRPF19). For molecular function, DEGs are significantly enriched in histone deacetylase binding (KAT2B, PRDM1, ZBTB7B), mRNA binding (SSB, RAVER1, ZC3H11A), protein binding (P100, USP15, SLC27A1), transcription coactivator activity and protein kinase binding (PDPK1, PRKAG2, RHOB).

Furthermore, KEGG analysis showed that DEGs were involved in mineral absorption, insulin resistance, TNF signaling pathway, longevity regulating pathway, NF-kappa B signaling pathway, measles, toxoplasmosis, influenza A, immunodeficiency virus 1 infection, Epstein-Barr virus infection, and hepatitis C atherosclerosis, Suppression type I IFN signaling tries to protect the Mtb-specific IFN- response and strengthens lung immunopathology. Standard sequence analysis, tissue immunoblotting staining, and adoptive transfer research suggests that type I IFN signaling stimulated by viral infection could indeed inhibit CXCL9/10 creation in myeloid cells, inhibiting lungs movement of Epitope CD4+ T cells [27]. These pathways are involved in tuberculosis. IFIT1, IFIT2, IFIT3, and IRF1 are the most frequently interacting hub genes. A large number of miRNAs interact with these genes, including miR-21-3p, miR-148b-3p, miR-27-5p, miR-155-5p, miR-122-5p, miR-34c-5p, and miR-520c-5p. These DEGs, pathways and miRNAs will provide important information for future research and point out those pathways that play an important role in tuberculosis infection.

CONCLUSION

In the present study, through bioinformatics analysis, we identified DEGs in tuberculosis disease. For analysis of DEGs in tuberculosis datasets, the following ID: GSE51029 was used: The results show that 285 DEGs, including 202 upregulated genes and 82 down-regulated genes, might be potential biomarkers in Tb. Top upregulated genes are ACTL6B, ADAR, ADM, ADPRHL2, ADRA1, and the top downregulated genes are RBM43, REC8, RHOB, RIF1, and RIN2 which could be related to the occurrence of Tb. In biological processes, DEGs are mainly enriched in cell surface receptor signaling pathways (P2RX7, CD274, CYSLTR1), cellular component DEGs are significantly involved in the cytoplasm (RIF1, FMR1, MT1L), endoplasmic reticulum (SLC27A1, INSIG1, FAF2), and molecular function DEGs are significantly enriched in histone deacetylase binding (KAT2B, PRDM1, ZBTB7B), mRNA binding (SSB, RAVER1, ZC3H11A), and protein binding (P100, USP15, SLC27A1). IFIT1, IFIT2, IFIT3, and IRF1 are the most frequently interacting hub genes. A large number of miRNAs, such as miR-148b-3p and miR-21-3p in tuberculosis DEGs, interact with these genes. These pathways will provide important information for future research and point out those pathways that play an important role in tuberculosis infection. miRNAs are considered diagnostic biomarkers for many diseases, including tuberculosis. This study shows that identifying DEGs, pathways, and miRNA that closely interact with hub genes that are involved in tuberculosis provides important targets for tuberculosis treatment. These identified genes will provide useful information for future research and highlight those pathways which play an important role in the occurrence of tuberculosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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