

Transcription factor regulatory network for early lung immune response to tuberculosis in mice

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Abstract. Numerous transcription factors (TFs) have been suggested to have a role in *Mycobacterium tuberculosis* infection; however, the TFs involved in the early immune response of lung cells remains to be fully elucidated. The present study aimed to identify TFs which may have a role in the early immune response to tuberculosis and the gene regulatory networks in which they are involved. Gene expression data obtained from microarray analysis of the early lung immune response to tuberculosis (Gene Expression Omnibus; accession no. GSE23014) was integrated with data for TF binding sites and protein-protein interactions in order to construct a TF regulatory network. The role of TFs in protein complexes, active modules, topology of the network and regulation of immune processes were investigated. The results demonstrated that the constructed gene regulatory network harbored 1,270 differentially expressed (DE) genes with 4,070 regulatory and protein-protein interactions. In addition, it was revealed that 17 DE TFs were involved in the positive regulation of numerous immunological and biological processes, including T cell activation, T cell proliferation and tuberculosis-associated gene expression, in the constructed regulatory network. Signal transducer and activator of transcription 4, interferon regulatory factor 8, spleen focus-forming virus proviral integration 1, enhancer of zeste homolog 2 and kruppel-like factor 4 were predicted to be the primary TFs regulating the DE genes during early lung infection by *M. tuberculosis*, as determined through various analyses of the gene regulatory network. In conclusion, the present study identified novel TFs

involved in the early response to *M. tuberculosis* infection, which may enhance current understanding of the molecular mechanism underlying tuberculosis infection and introduced potential targets for novel tuberculosis therapies.

Introduction

Tuberculosis is a disease caused by the intracellular bacterium *Mycobacterium tuberculosis*, which has a high mortality rate (1). CD⁺ T lymphocytes have been reported to have a central role in the defense against *M. tuberculosis* and T helper 1-type (Th1) T cell-mediated immunity is the primary response during tuberculosis (2-4). A previous study reported that *M. tuberculosis* infection in the absence of α - β T lymphocytes caused mortality in mice within 48 days (5). Protective immunity against *M. tuberculosis* is predominantly regulated by transcription factors (TFs).

Numerous TFs have been reported to be involved in the immune response to tuberculosis. Protective immunity against *M. tuberculosis* infection was reported to be under regulation of the TF signal transducer and activator of transcription (Stat) 4 (6). Nuclear factor of activated T cells p (NFATp) and tumor necrosis factor (TNF) were suggested to regulate the inflammatory response, while mice deficient for NFATp had an increased mortality rate following the development of tuberculosis (7). BXH2 mice deficient for the *Irf8*^{294c} allele succumbed to the disease due to uncontrolled growth of *M. tuberculosis* (8). Expression of spleen focus-forming virus proviral integration 1 (Sfpil) TF was demonstrated to be increased following 28 days of *M. tuberculosis* infection (9). Significant overlap between the binding sites of interferon regulatory factor 8 (Irf8) and Sfpil was observed using chromatin immunoprecipitation microarray (ChIP-chip) analysis of tuberculosis infection (10). Th1 cells have a crucial role in defense against tuberculosis. V-rel avian reticuloendotheliosis viral oncogene homolog (Rel) B (RelB) TF is essential for differentiation of Th1 cells and its deficiency was reported to induce defects in the differentiation of these cells (11). In addition to RelB, Stat4 and Stat1 were demonstrated to be involved in the development of Th1 cells in response to infection (12). Therefore, investigating these individual TFs may elucidate numerous underlying processes in tuberculosis; however, it

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may not provide an overview of gene regulation during tuberculosis.

Construction of gene regulatory networks in tuberculosis, which include genes and TFs, provide a novel opportunity for understanding the dynamic of molecular processes involved in the disease. Such networks have been constructed for *M. tuberculosis*. A previous study reviewed and constructed a gene regulatory network for *M. tuberculosis* genes involved in persistence in order to provide insight into the molecular mechanisms involved in persistency (13). In addition, a comprehensive network of infection-associated processes in human macrophages following *M. tuberculosis* infection was constructed (14) as well as a host intracellular network for the regulation of *M. tuberculosis* survival (15). However, there has not yet been a regulatory network constructed for the TFs involved in the early lung immune response to *M. tuberculosis* infection.

In the present study, a network was constructed of the genes that were differentially expressed (DE) specifically in the lung cells in response to tuberculosis. TF binding sites, protein-protein interactions and expression data were integrated in order to construct the gene regulatory network. Network analyses using system biology tools were used to determine the most prominent TFs involved in early lung immune responses to tuberculosis.

Materials and methods

Microarray availability and preprocessing. Raw data for early lung infection with *M. tuberculosis* were obtained from the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) server database (accession no. GSE23014). These data contributed by Kang *et al* (16), contained time course (0, 12, 15 and 21 days) expression data for infection of C57BL/6 mice with the H37Rv strain of *M. tuberculosis*. Microarray samples were divided into three groups for the comparison and identification of DE genes during the early days of lung response to tuberculosis: Group 1, data from 12 days post infection was compared with that of day 0; group 2, comparison of days 12 and 15 post infection; and group 3, comparison of day 15 with day 21 post infection.

A Robust Multi-array Averaging (RMA) algorithm was used for normalization of raw data (17). DE genes were identified using a two-sample Student's t-test algorithm. These algorithms were each performed using Flexarray software v1.6.2 (18). A fold change of 1.5 was set as the threshold criteria for identifying DE genes. Group III data was used for all subsequent analysis.

Functional clustering DE genes. In order to determine the enrichment process during each comparison, the Databases for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/>) bioinformatics tool was used. Clusters with enrichment scores of >1.3 were regarded to be meaningful clusters in the functional clustering analysis (19).

TFs involved in regulating DE genes. In order to distinguish regulators of DE genes in each comparison, data was submitted to the ChIP Enrichment Analysis (ChEA; <http://amp.pharm.mssm.edu/lib/chea.jsp>) database (20). This

database analyzed 458,471 potential regulatory interactions based on ChEA from 221 publications. In addition, the TFactS database (<http://www.tfacts.org/>) was used, which integrates data from experimentally validated TFs/targets regulatory interactions. This database consists of data from 6,401 regulatory interactions for 343 TFs which regulate 2,720 genes from the following databases: PAZAR, Transcriptional Regulatory Element Database, Nuclear Factor I Regulome Database and Transcription Regulatory Regions Database, as well as literature. TFactS was used to compare submitted DE gene data from the present study with validated target genes available in its catalog in order to identify the regulatory genes in the submitted datasets (21). TFs were limited based on their P-value (<0.05) and altered expression (≥ 1.5 fold change).

TFs protein-protein interactions. The Biological General Repository for Interaction Datasets (BioGRID; <http://thebiogrid.org/>) database was used to extract valid protein-protein interactions information for DE TFs (22). In addition to BioGRID, protein-protein interaction data was obtained from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; <http://string-db.org/>) database, which accommodates physical (validated and predicted) and functional interactions. Interactions with a confidence score >0.4 (medium confidence) were incorporated into the networks (23).

The protein interactions obtained from BioGRID and STRING were mapped to the expression data in order to identify only meaningful interactions. These interactions contributed to the construction of the TFs regulatory network and TFs protein-protein interaction network.

Network construction and ontology. In order to construct regulatory networks, TF/target interactions, TFs protein-protein interactions and expression data were integrated and visualized using Cytoscape v3.0.2 (24). The enriched immune system processes were investigated in the constructed network using the ClueGO v1.8 plugin for Cytoscape. A two-sided hypergeometric statistical test and Bonferroni correction were used for P-value correction. In addition, ClueGO was used to determine processes affected by the DE TFs based on Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>) pathway and biological process analyses (25).

Centrality analysis of regulatory network. Central genes in the regulatory network were identified using a CentiScaPe v2.0 plugin for Cytoscape. Three centrality indexes were used on a directed regulatory network: Degree, betweenness and stress. Degree is considered to be the simplest index for topological analysis; the number of adjacent connected nodes to a given node (x) reveal the degree of this node. In the directed network constructed, out-degree rather than in-degree was considered. Nodes with a high degree are considered the hubs of the network. In addition to degree, stress and betweenness were used as centrality indexes to find hub TFs. These two indexes provide complementary results from analysis of the central genes (26).

Protein complexes and active modules. Protein-protein interactions obtained for DE TFs from BioGRID and STRING were used to construct a protein interaction network. This network was used to identify protein complexes which may

be involve in the response of lung cells to early infection of tuberculosis. These protein complexes were identified using MCODE v1.4.1 plugin for Cytoscape (27).

Integrated regulatory networks were composed of expression data and interactions. Parts of this network demonstrated increased activity compared with other parts based on expression, these are referred to as active modules. These modules were explored in Cytoscape using the JActiveModules v1.8 tool (28). To identify active modules in constructed regulatory networks using JActiveModules, the expression values were converted to P-values. JActiveModules find active modules using the loaded P-values.

Summary of methods. Overall, the most crucial DE TFs involve in early lung immune response to tuberculosis were identified based on several analyses, including presence in protein complexes, contribution in active modules, centrality as well as role in regulation of significant processes and tuberculosis-associated genes. These analyses were divided into three parts: Involvement in protein complexes and active modules; centrality analysis; and regulation of the most enriched processes. DE TFs that were present in ≥ 2 out of 3 of these analyses were assumed to be important factors in early lung immune response to tuberculosis.

Results

Gene expression during different stages of early lung responses to tuberculosis. Gene expression data obtained from early lung immune response to tuberculosis was divided into three distinct groups. By comparing the expression profiles at day 0 and 12 post infection (Group I), 240 DE genes were identified, while 153 and 2,105 DE genes were detected when the gene expression profiles were compared between day 12 and 15 (Group II) and between day 15 and 21 (Group III), respectively. As the number of DE genes obtained from group III was higher than those of the two other groups, the third comparison was selected to establish the regulatory network of TFs in lungs during infection by *M. tuberculosis*.

Functional clustering analysis of DE genes obtained from the comparison of day 15 and 21 post *M. tuberculosis* infection resulted in the identification of 113 meaningful clusters with an accepted enrichment score of >1.3 . The presence of immune system-associated terms, including those for signaling, defense response, inflammatory response and T cell activation, indicated the involvement of the immune system during the early response of lung cells infected by *M. tuberculosis*.

Regulators of DE genes. ChEA and TFactS databases revealed the involvement of 17 DE TFs in the regulation of early responses, including comesodermin, v-ets avian erythroblastosis virus E26 oncogene homolog, enhancer of zeste homolog 2 (Ezh2), Irf8, jun B proto-oncogene, kruppel-like factor 4 (Klf4), myeloblastosis-related protein B (Mybl2), nuclear receptor subfamily 3 group C member 1, Rel, RelB, Sfp11, sex determining region Y-box 17, Stat1, Stat2, Stat4, T-cell acute lymphocytic leukemia 1 (Tal1) and thyrotroph embryonic factor. These DE TFs contribute to the regulation of 1,253 out of 2,105 DE genes; however, TFs were

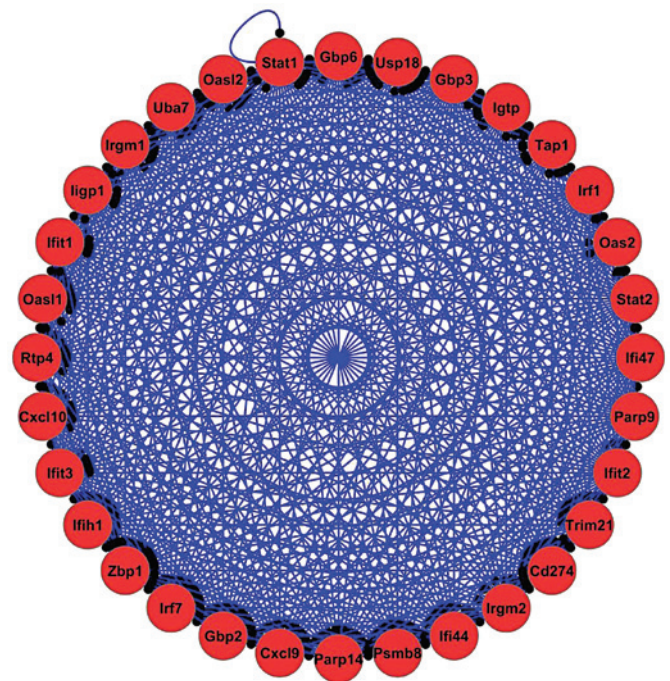


Figure 1. Protein complex with highest score in the TFs protein-protein interaction network. Red nodes represent upregulated proteins and blue lines indicate protein-protein interactions. Black dots indicate the direction of interactions.

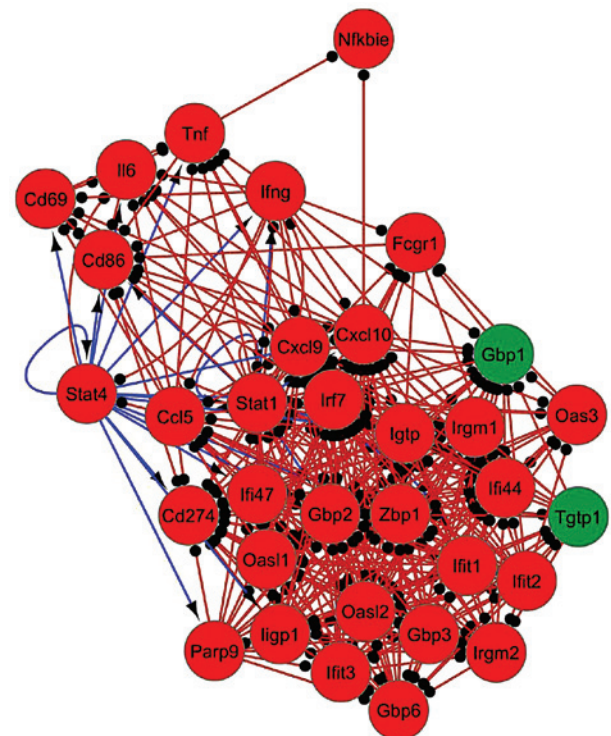


Figure 2. Top active modules extracted from the gene regulatory network. Red and green nodes indicate up- and downregulated modules, respectively. Blue and red lines represent protein-DNA and protein-protein interactions, respectively. Arrows and dots indicate the direction of protein-DNA and protein-protein interactions, respectively.

not identified for the remainder of the DE genes submitted. Following protein-protein interaction analysis of these TFs, a gene regulatory network was constructed, including all

Table I. Ranking of TFs based on analyses of three centrality indexes.

Symbol	TF name	Centrality indexes			Mean	Rank
		Degree	Stress	Betweenness		
Sfp1	Spleen focus-forming virus proviral integration 1	1	1	1	1.00	1
Stat4	Stat4	5	2	2	3.00	2
Ezh2	Enhancer of zeste homolog 2	4	3	4	3.66	3
Mybl2	Myeloblastosis-related protein B	7	4	3	4.66	4
Irf8	Interferon regulatory factor 8	8	5	5	6.00	5
Sox17	Sex determining region Y-box 17	6	7	8	7.00	6
Eomes	Eomesodermin	10	8	6	8.00	7
Stat1	Stat1	12	6	7	8.33	8
Klf4	Kruppel-like factor 4	2	13	13	9.33	9
Nr3c1	Nuclear receptor subfamily 3 group C member 1	11	9	9	9.66	10
Stat2	Stat2	13	11	11	11.66	11
Junb	Jun B proto-oncogene	15	10	10	11.66	11
Tal1	T-cell acute lymphocytic leukemia 1	3	17	17	12.33	12
Rel	Rel	14	12	12	12.66	13
Erg	V-ets avian erythroblastosis virus E26 oncogene homolog	9	16	16	13.66	14
RelB	RelB	15	14	14	14.33	15
Tef	Thyrotroph embryonic factor	16	15	15	15.33	16

TF, transcription factor; Stat, signal transducer and activator of transcription; Rel, v-rel avian reticuloendotheliosis viral oncogene homolog.

seventeen TFs and 1,270 DE genes, which were revealed to be connected by 4070 interactions.

TFs involved in protein complexes and modules. In order to determine the involvement of TFs in protein complexes, the MCODE tool for Cytoscape was used. A total of 6 complexes were identified in early lung immune response to tuberculosis with a score of ≥ 3 . The top protein complex (score 30.516 based on the reference of MCODE plugin of Cytoscape) contained 32 DE genes with 474 linking interactions (Fig. 1). Biological process analysis of this protein complex indicated that the innate immune response was the top term, as determined using P-values and the number of DE genes. The DE TFs Ezh2, Irf8, Klf4, Rel, RelB, Stat1 and Stat2 were identified to be present in the protein complexes identifies.

The active modules of the regulatory network were analyzed in order to provide an in-depth view of the activity of the network's components. TFs involved in these modules are likely to be more important compared with other TFs. Based on the biological process analysis of the top active modules, the biological processes which were most affected by these TFs were as follows: Response to cytokine stimulus, response to viruses and the innate immune response. The top active module was composed of 33 DE genes with 348 interactions (Fig. 2); DE genes in this module were primarily regulated by the TFs Stat4 and Stat1. The top 5 modules were investigated for the presence of DE TFs and the results revealed that the TFs Stat4, Stat2, Stat1, Rel and RelB were detected in

≥ 1 of the these modules. Of note, Stat4 and Stat1 were present in all of 5 modules. These results therefore demonstrated the central role of Stat4 and Stat1 in active modules in the early lung response to tuberculosis infection. Overall, the TFs Ezh2, Irf8, Klf4, Rel, RelB, Stat1, Stat2 and Stat4 were identified to be involved in protein complexes and core active modules.

Central DE TFs in the regulatory network. Centrality analysis using the CentiScaPe plugin in Cytoscape highlighted the importance of TFs in the network topology and regulation of the whole network (Table I). Three parameters were considered in analyzing the network: Degree, betweenness and stress. According to degree, Sfp1, Klf4, Tal1, Ezh2 and Stat4 were the DE TFs with highest interactions in the constructed gene regulatory network during early infection of the lung with tuberculosis. Rankings for stress and betweenness revealed that Sfp1, Stat4, Ezh2, Mybl2 and Irf8 were the top 5 central genes in gene regulatory network. The overall ranking of the DE TFs, according to the mean ranking of the three parameters, identified Sfp1, Stat4, Ezh2, Mybl2 and Irf8 as the top regulatory TFs in the constructed gene regulatory network.

TFs involvement in the regulation of immune system processes. In order to determine the most important TFs in the regulation of immune system processes, gene regulatory network ontology was used to identify the affected immune processes and their regulators (Table II). According to the P-values of affected processes in immune system analysis, the results demonstrated

Table II. Enriched immune system processes in the gene regulatory network for early lung response to tuberculosis infection.

No.	Gene ontology term	No. genes	%	P-value	Genes
1	Positive regulation of T cell activation	42	38.53	1.85E-04	<i>Ada, Aif1, Blm, Ccl2, Ccl5, Ccr2, Cd28, Cd4, Cd5, Cd83, Corol1a, H2-Aa, H2-Ab1, H2-DMa, Hes1, Ifng, Igfbp2, Irf1, Il12a, Il12b, Il12rb1, Il1b, Il2, Il21, Il2ra, Il6, Il7r, Irfl, Itgal, Jak3, Lck, Malt1, Nckap1l, Pdcd1lg2, Prkcq, Ptprc, Sash3, Spn, Thyl, Tnfrsf11, Vcam1, Zap70</i>
2	Positive regulation of T cell proliferation	26	43.33	4.08E-04	<i>Aif1, Blm, Ccl5, Ccr2, Cd28, Corol1a, Hes1, Ifng, Igfbp2, Il12a, Il12b, Il12rb1, Il1b, Il2, Il21, Il2ra, Itgal, Jak3, Nckap1l, Pdcd1lg2, Prkcq, Ptprc, Sash3, Spn, Vcam1, Zap70</i>
3	Mature B cell differentiation	7	77.77	7.58E-04	<i>Ada, Bcl3, Malt1, Plcg2, Pou2f2, Ptk2b, Tnfrsf3</i>
4	Positive regulation of isotype switching to IgG isotypes	6	85.71	8.13E-04	<i>Cd28, Cd40, Ifng, Il2, Ptprc, Tbx21</i>
5	Antigen processing and presentation of exogenous peptide antigen via major histocompatibility complex class II	9	64.28	1.00E-03	<i>Fcgr2b, H2-Aa, H2-Ab1, H2-DMa, H2-DMb2, H2-Eb1, H2-Oa, Ifi30, Unc93b1</i>
6	Regulation of isotype switching to IgG isotypes	7	70.00	2.00E-03	<i>Cd28, Cd40, Ifng, Il2, Il27ra, Ptprc, Tbx21</i>
7	Regulation of T cell activation	55	32.73	2.10E-03	<i>Ada, Aif1, Blm, Ccl2, Ccl5, Ccr2, Cd274, Cd28, Cd4, Cd5, Cd83, Corol1a, Ctla4, Ctmb1, H2-Aa, H2-Ab1, H2-DMa, H2-Oa, Hes1, Ido1, Ifng, Igfbp2, Irf1, Il12a, Il12b, Il12rb1, Il1b, Il2, Il21, Il2ra, Il6, Il7r, Irfl, Itgal, Jak3, Lag3, Lat, Lck, Malt1, Nckap1l, Nfkbid, Nrarp, Pdcd1lg2, Pde5a, Prkcq, Ptprc, Ptprn6, Ptprc, Sash3, Sit1, Spn, Thyl, Tnfrsf11, Vcam1, Zap70</i>
8	Positive regulation of lymphocyte activation	49	32.66	3.70E-03	<i>Ada, Aif1, Blm, Ccl2, Ccl5, Ccr2, Cd28, Cd4, Cd40, Cd5, Cd83, Cdkn1a, Corol1a, H2-Aa, H2-Ab1, H2-DMa, Hes1, Ifng, Igfbp2, Irf1, Il12a, Il12b, Il12rb1, Il15ra, Il1b, Il2, Il21, Il2ra, Il6, Il7r, Inpp5d, Irfl, Itgal, Jak3, Lck, Malt1, Myd88, Nckap1l, Pdcd1lg2, Prkcq, Ptprc, Sash3, Spn, Tbx21, Thyl, Tnfrsf4, Tnfrsf11, Vcam1, Zap70</i>
9	Regulation of lymphocyte proliferation	45	33.33	4.50E-03	<i>Ada, Aif1, Blm, Ccl5, Ccr2, Cd274, Cd28, Cd40, Cdkn1a, Corol1a, Ctla4, Ctmb1, Fcgr2b, Hes1, Ido1, Ifng, Igfbp2, Irf1, Il12a, Il12b, Il12rb1, Il1b, Il2, Il21, Il2ra, Inpp5d, Irfl, Itgal, Jak3, Lst1, Myd88, Nckap1l, Pdcd1lg2, Pde5a, Prkcq, Ptprn22, Ptprn6, Ptprc, Sash3, Sox11, Spn, Tnfrsf4, Vcam1, Zap70</i>
10	Cellular response to interferon- γ	15	44.11	6.10E-03	<i>Aif1, Arg2, Ass1, Ccl2, Ccl5, Gbp1, Gbp2, Gbp3, H2-Ab1, Il12b, Il12rb1, Irfl, Jak2, Nos2, Stat1</i>

IgG, immunoglobulin G; %, Fraction of immune system processes, which were affected during early lung immune response to tuberculosis infection..

that the positive regulation of T cell activation and proliferation were the most enriched process. In the constructed regulatory gene network the most important regulatory DE TFs (based on number of regulatory interactions) for T cell activation included Sfp1, Stat4, Tal1, Rel and Klf4. Positive regulation of T cell proliferation contained 26 DE genes that were primarily regulated by Sfp1, Stat4, Tal1, Rel, Irf8, Stat1, Klf4 and Mybl2 DE TFs in the network. The regulators identified as the top five TFs involved in the positive regulation of T cell proliferation term, some of which have the same number of targets, for example Stat4 and Tal1 both regulate nine DE genes in this term. In addition to these processes, DE genes associated with tuberculosis were investigated based on KEGG pathway analysis of the gene regulatory network. A total of 38 DE genes involved in tuberculosis were found to be present in the regulatory network constructed in the preset study. In addition, Sfp1, Stat4, Tal1, Irf8, Klf4 and Stat1 were determined to be the top 5 DE TFs involved in regulation of Tuberculosis-associated DE genes in early lung immune response to tuberculosis. Overall, 4 DE TFs were identified (Sfp1, Stat4, Tal1 and Klf4) which were actively involved in the regulation of the following three processes: Positive regulation of T cell activation, positive regulation of T cell proliferation and tuberculosis-associated gene expression.

In conclusion, according to the results of the analyses of TF involvement in protein complexes and active modules, centrality and the regulation of most enriched processes, it was revealed that the top DE TFs involved in the regulation of the early lung response to tuberculosis were Stat4, Irf8, Sfp1, Ezh2 and Klf4.

Discussion

The present study identified possible novel TFs involved in the regulation of early lung immune response to tuberculosis in mice and their roles. Numerous TFs and their regulated processes in this constructed regulatory network were revealed to overlap with known regulators and affected processes during tuberculosis.

Stat4 has a crucial role in the development of the protective response against *M. tuberculosis* infection in mice (6). CD4⁺ T cells deficient for Stat4 were reported to be unable to differentiate into Th1 cells during tuberculosis (29,30). T cell receptors (TCR) induce activation of CD4⁺ T cells when they encounter antigens presented by antigen presenting cells (29). Activated T cells produce interleukin (IL)-2 and express IL-12 receptors in response to this signal. IL-12 receptors are activated on encountering IL-12, which is produced and secreted by macrophages and CD8 α ⁺ dendritic cells; in addition, the activated IL-12 receptor subsequently activates Stat4, which results in the initiation of Th1 differentiation from CD4⁺ T cells during *M. tuberculosis* infection (29). During tuberculosis infection, Stat4 upregulation following IL-12 stimulation in bronchoalveolar cells was reported to lead to increased expression of interferon γ (Inf γ) (31).

Previous studies have suggested that Irf8 was critical for the differentiation of myeloid cells and defense against intracellular microbes (8,10). A study by Marquis *et al* (8) reported that uncontrolled *M. tuberculosis* growth occurred in the spleen, liver and lungs of BXH2 mice with a defective *IRF8*^{R294C} allele

and induced premature mortality (8). Irf8 is a crucial regulator of the immune responses mediated by Th1 cells and is involved in regulation of toll-like signaling (32). A previous study reported that high overlap between the binding sites of Irf8 and Sfp1 was observed in tuberculosis infection, as determined using ChIP-chip data analysis (10). Sfp1 was demonstrated to be involved in the development of mature macrophages as well as B and T cells (33). This regulator primarily affected the efficiency of T cell progenitors fate commitment and/or their differentiation (34). In addition Sfp1 was reported to have a role in the generation of cytokine expression patterns in T helper 2-type (Th2) cells (35).

Ezh2 is a methyltransferase component of polycomb repressive complex (PRC2) (36). Low expression of Ezh2 was reported in mature T cells; however, following antigen recognition through the TCR and activation of T cells Ezh2 expression was rapidly increased (36). Expression of cytokines during the development of Th1 and Th2 cells was predominantly regulated by PRC2 members (37). For example, Inf γ expression was downregulated in Th1 cells following *Ezh2* mRNA knock-down (37). IL-4 and IL-13 are Th2-specific cytokines, the expression levels of which were reported to be down-regulated in Th1 cells through the methyltransferase activity of Ezh2 (38,39).

Mice with T cell knock-out of the *Klf4* gene were used to investigate its roles in the development and differentiation of T cell. Klf4 was reported to be highly expressed in mature T cells; however, it was demonstrated that upon T cell activation, Klf4 expression was downregulated (40). Another study on the T cell activation network revealed the role of Klf4 in the regulation of transcription in tuberculosis (41).

Collectively, the present study aimed to identify novel TFs and dissect their roles in the concept of gene regulatory network during early lung immune response to tuberculosis. This analysis led to identification of 17 DE TFs involved in regulation of numerous immunological and biological processes, including T cell activation, T cell proliferation and tuberculosis-associated gene expression. Constructed network analysis revealed Stat4, Irf8, Sfp1, Ezh2 and Klf4 as master regulators of early lung response to tuberculosis. Identification of these master TFs extend the current understanding of the underlying molecular mechanisms and may be useful as candidate novel targets for novel tuberculosis therapies.

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