Coexpression network analysis-based identification of critical genes and regulatory factors driving inter-individual variation in response to *Mycobacterium tuberculosis* infection.

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INTRODUCTION

Why?

- Tuberculosis (TB) the second leading infectious killer after COVID-19 and above HIV and AIDS [1].
- Inter-individual variation in M.tb infection presents both challenges and opportunities in understanding, diagnosing, treating, and preventing the disease [1].
- However, the substantial inter-person variability in response to a single M.tb strain in the lungs remains poorly understood.

What?

- Examine M.tb-induced dysregulated genes in human alveolar macrophages (HAMs) cells to identify potential therapeutic targets.

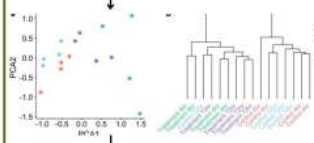
How?

- RNA sequencing data from interactions of a virulent M.tb strain (H37Rv) with freshly isolated HAMs from 28 healthy adult donors (GSE189996) and 11 healthy donors (GSE223863) over 72 hours were pooled for weighted gene coexpression network analysis (WGCNA) to reveal dysregulates biomarkers.

We aim to reveal dysregulated molecular signatures including potential biomarkers, correlated regulatory motifs (RMs), and transcription factors (TFs) driving inter-individual variation associated with M.tb infection

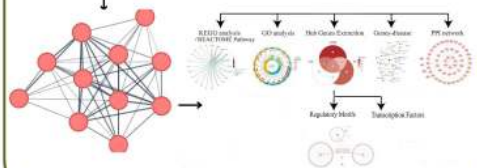
METHODS

Collect RNA-seq Dataset from NCBI GEO (GSE189996 and GSE223863)



Data Quality Check (Hierarchical Clustering & Principal component Analysis, Batch effect correction, outliers removed)

WGCNA Construction (Identify gene modules)



Identification of Hub Genes (Export to Cytoscape)

RESULTS

- RNA-Seq counts of infected and control HAMs samples were downloaded, with 10 control and two infected samples identified as outliers and removed from downstream analyses.
- WGCNA visualized the unmerged and merged module structures representing distinct and biologically meaningful gene groups of final clusters (Fig. 1).
- Heatmap of module-traits correlation highlighted the strength and significance of six identified modules of genes with major biological events following M.tb infection (Fig. 2).
- Coexpression analysis of inter-individual variation in response to M.tb infection significantly ($p<0.05$) implicated matrix metallopeptidase 14 (MMP14), TNF superfamily member 13 (TNFSF13), signaling lymphocytic activation molecule family member 7 (SLAMF7), and neutrophil cytosolic factor 1 (NCF1) as hub genes mediating this response.
- RMs analysis revealed binding sites for TFs critical for gene regulation. These included short DNA sequences representing promoter and enhancer elements, with binding sites for TF families such as C2H2 zinc finger factors, homeodomain factors, T-box factors, and high-mobility group (HMG) domain factors. (Table 1).

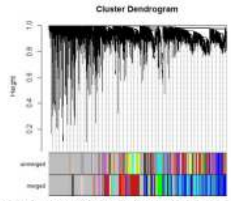


Figure 1: Gene clusters (modules) of MP12/ZH548 inoculated Homo sapiens co-expression network.

Figure 2: Module-trait correlation

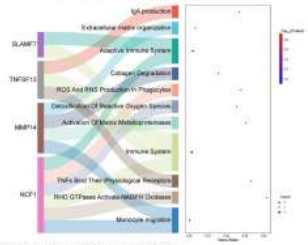


Figure 3: Pathway enrichment analysis

Table 1: Consensus regulatory motifs in each of the enriched modules.

Module	Consensus Motif
Blue	
Greeen	

DISCUSSION

- MMP14 plays a crucial role in monocyte migration and tuberculosis (TB) pathogenesis. Its mRNA levels are significantly elevated in TB patients, correlating with lung infiltration [1]. It is essential for collagen degradation, and neutralizing MMP14 activity reduced M.tb-dependent monocyte migration by 44%, highlighting its importance in granuloma formation and disease progression in TB [2].
- TNFSF13, or APRIL, regulates IgA production and immune responses during M.tb infection. TNFSF13 is crucial for producing antibodies like IgA, IgG, and IgM highlighting its role in immunity against M. tuberculosis in the central nervous system [3].
- Rho GTPases, particularly Rac1 and Rac2, are crucial for activating NADPH oxidases like NOX2, which are essential for microbicidal activity during phagocytosis. GTP-bound Rac interacts with NCF2 (p67phox) and NCF1 (p47phox), both of which are key components in assembling the NOX2 complex. NCF1 acts as an organizer, facilitating the translocation of other regulatory proteins to the membrane, enabling superoxide production, a critical aspect of the immune response against mycobacterial infections [4].
- HMG domain factors are involved in chromatin remodeling, transcriptional regulation, and protein-protein interactions suggesting that they could potentially play a role in the host response to M.tb infection or in the pathogen's gene regulation. STAT1 signaling on the other hand is crucial in the host immune response to M.tb, especially in isoniazid-resistant strains.

CONCLUSION/FUTURE PERSPECTIVES

- This study highlights these elements as central to TB pathogenesis, offering insights into the variability in disease response that could inform targeted and personalized TB therapies and vaccine strategies.
- Future research is recommended to elucidate the potential interaction between NCF1 and MMP14 in regulating monocyte migration and extracellular matrix remodeling in tuberculosis.
- Further investigation is needed into TNFSF13's complex role in modulating IgA and overall immunity during tuberculosis, particularly its differential effects in various tissues and conditions.
- Exploring the specific interactions between Rho GTPases, NCF1, and other NADPH oxidase components in the context of tuberculosis infection will enhance our understanding of their roles in immune responses and antimicrobial defense.

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