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SHORT COMMUNICATION

A host blood transcriptional signature differentiates multi-drug/rifampin-resistant tuberculosis (MDR/RR-TB) from drug susceptible tuberculosis: a pilot study

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Abstract

Background Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* is one of the top thirteen causes of death worldwide. The major challenge to control TB is the emergence of drug-resistant tuberculosis (DR-TB); specifically, multi-drug resistant TB which are resistant to the most potent drugs; rifampin and isoniazid. Owing to the inconsistencies of the current diagnostic methods, a single test cannot identify the whole spectrum of DR-TB associated mutations. Recently, host blood transcriptomics has gained attention as a promising technique that develops disease-specific RNA signatures/biomarkers. However, studies on host transcriptomics infected with DR-TB is limited. Herein, we intended to identify genes/pathways that are differentially expressed in multi-drug/rifampin resistant TB (MDR/RR-TB) in contrast to drug susceptible TB.

Method and results We conducted blood RNA sequencing of 10 pulmonary TB patients (4; drug susceptible and 6; DR-TB) and 55 genes that were differentially expressed in MDR/RR-TB from drug-susceptible/mono-resistant TB were identified. CD300LD, MYL9, VAMP5, CARD17, CLEC2B, GBP6, BATF2, ETV7, IFI27 and FCGR1CP were found to be upregulated in MDR/RR-TB in all comparisons, among which CLEC2B and CD300LD were not previously linked to TB. In comparison pathway analysis, interferon alpha/gamma response was upregulated while Wnt/beta catenin signaling, lysosome, microtubule nucleation and notch signaling were downregulated.

Conclusion Up/down-regulation of immunity related genes/pathways speculate the collective effect of hosts' attempt to fight against continuously multiplying DR-TB bacteria and the bacterial factors to fight against the host defense. The identified genes/pathways could act as MDR/RR-TB biomarkers, hence, further research on their clinical use should be encouraged.

Keywords Gene signature · Drug resistance · Transcriptome · Tuberculosis · Biomarkers

Introduction

Over the last decade, Tuberculosis (TB) remained the leading cause of global mortality due to a single infectious agent [1]. The emergence of drug resistant TB (DR-TB), particularly, multidrug-resistant (MDR-TB: TB resistant to isoniazid (INH) and Rifampin (RIF)), has become a challenge in combating TB worldwide. In 2020, around 10 million people were diagnosed with TB, 1.5 million people died and among those tested for drug resistance, 132 222 cases were multi-drug resistant/ RIF resistant TB (MDR/RR-TB) [1]. Current TB diagnosis methods such as culture-based techniques and nucleic acid amplification test (NAAT) based methods (ex: GeneXpert system (Cepheid, USA)) have limitations such as low turnaround time (TAT) and false negative detection. For

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instance, the line probe assay (Hain Lifescience, Germany) detects only specific mutations and Xpert MTB/RIF may give a positive result even in the presence of a silent mutation [2]. Therefore, with current methods, any strain resistant due to a not reported or uncommon mutation would still be recorded as a false negative and any strain with a silent mutation would be recorded as false positive. In this light, whole genome sequencing would seem to be a promising diagnostic method. However, with time, new mutations may emerge in bacteria, which would require the constant need of updated/validated databases of drug resistance associated mutations. Furthermore, there are mechanisms other than mutations such as reduced cell wall permeability and efflux pumps that can cause drug resistance [3]. Hence, none of the currently accepted methods, can cover the whole spectrum of drug resistance associated mutations/mechanisms in one single test. Thus, development of new fast, sensitive, and reliable DR-TB diagnostic methods is a timely requirement.

Host blood transcriptome analysis has gained popularity over the last few years in identifying disease-related gene signatures with high resolution and low TAT [4]. Blood biomarkers are preferred over other types due to less invasiveness of the sample acquisition. Several studies have generated transcriptional profiles as potential biomarkers for identifying active TB vs latent TB, disease progression and treatment outcome [5, 6]. One bio archive study had identified several DEGs expressed in THP1 cells which were infected with resistant (INH and RIF mono-resistant) and sensitive strains of *Mycobacterium tuberculosis* (MTB) [7]. However, there is limited information on host blood transcriptome in DR-TB vs drug susceptible TB. Hence, this study was aimed to identify blood transcriptomic differences between DR-TB and drug susceptible TB patients as a pioneer attempt to identify potential DR-TB biomarkers.

Materials and methods

Sample population and ethics

Ethical approval was obtained from the ethical review committee at the general teaching hospital, Kandy, Sri Lanka. All the patients agreed to the study protocol and provided written informed consent prior to recruitment. Drug resistance status of each patient was known as per prior in-house drug susceptibility testing (DST) and whole genome sequencing of the respective MTB isolates (NCBI; accession numbers SAMN18650575-84) (DST and WGS methodology; online resource file 1: Tables S1–S3 and Fig. S1). Accordingly, the study population included a total of 10 (4 multi-drug resistant/rifampin resistant TB (MDR/RR-TB), 2 mono-resistant and 4 drug susceptible TB) HIV-negative pulmonary TB (PTB) patients diagnosed in Sri Lanka. Primary diagnosis of the recruited patients was conducted using conventional sputum microscopy and GeneXpert (MTB/RIF) assay.

RNA sequencing and bioinformatics analysis

Prior to the treatment initiation, whole blood was collected from the corresponding patients, into an RNA stable buffer, and RNA was extracted using the phenol–chloroform method [8]. The extracted RNA's quality and quantity were assessed using agarose gel electrophoresis and a Quantifluor RNA system (Promega, USA). RNA was subjected to sequencing on the Illumina NovaSeq platform using 150 bp paired end reads at a commercial service provider, Macrogen Inc., Korea. All sequences were deposited in NCBI; accession numbers are SAMN18651952-61 (Online resource file 1Table S2).

Each sequence file was checked for quality using FastQC- v0.11.9 and pre-processed using fastp- v0.23.2. Human genome GRCh38 from ENSEMBL was used to generate reference genome index files. Alignment of sequences with the reference genome was performed using HISAT2- v2.2.1, and the outputs were used to generate count tables using FeatureCounts- v2.0.1. The count files were used for differential expression analysis using DESEQ2- v2.0.1. Derived differentially expressed gene (DEG) panels for each contrast were compared with published gene signatures. Morpheus software from broad institute was used for heatmap generation. The DEGs were uploaded to the STRING-DB database to construct the protein–protein interaction network. For preliminary gene enrichment analysis, DEGs were uploaded to Metascape- v3.5, an online resource for gene annotation and analysis [9]. For further analysis, the output files were used in fgsea- v1.8.0 and EGSEA- v1.20.0 platforms using Broad`s MSigDB gene sets as references. The gene sets across all the contrast groups analyzed were used as inputs for comparison analysis in EGSEA with reference to gene sets; h Hallmark Signatures (5.2, 07 March 2017), c5 GO Gene Sets (5.2, 07 March 2017), GeneSetDB Pathway (15 January 2016), GeneSetDB Gene Regulation (15 January 2016), GeneSetDB Gene Ontology (15 January 2016), KEGG Pathways (07 March 2017).

Results

RNA sequencing identified a panel of DEGs

RNA sequencing identified 55 statistically significant DEGs ($p < 0.05$) in MDR/RR-TB in contrast to all other drug susceptible and mono-resistant TB patients amongst which 28 were of $p < 0.01$ statistical significance (Fig. 1a; Table 1). The C-type lectin family member, CD300LD showed the highest fold change ($\log_2(FC) = 2.7576$) followed by the high

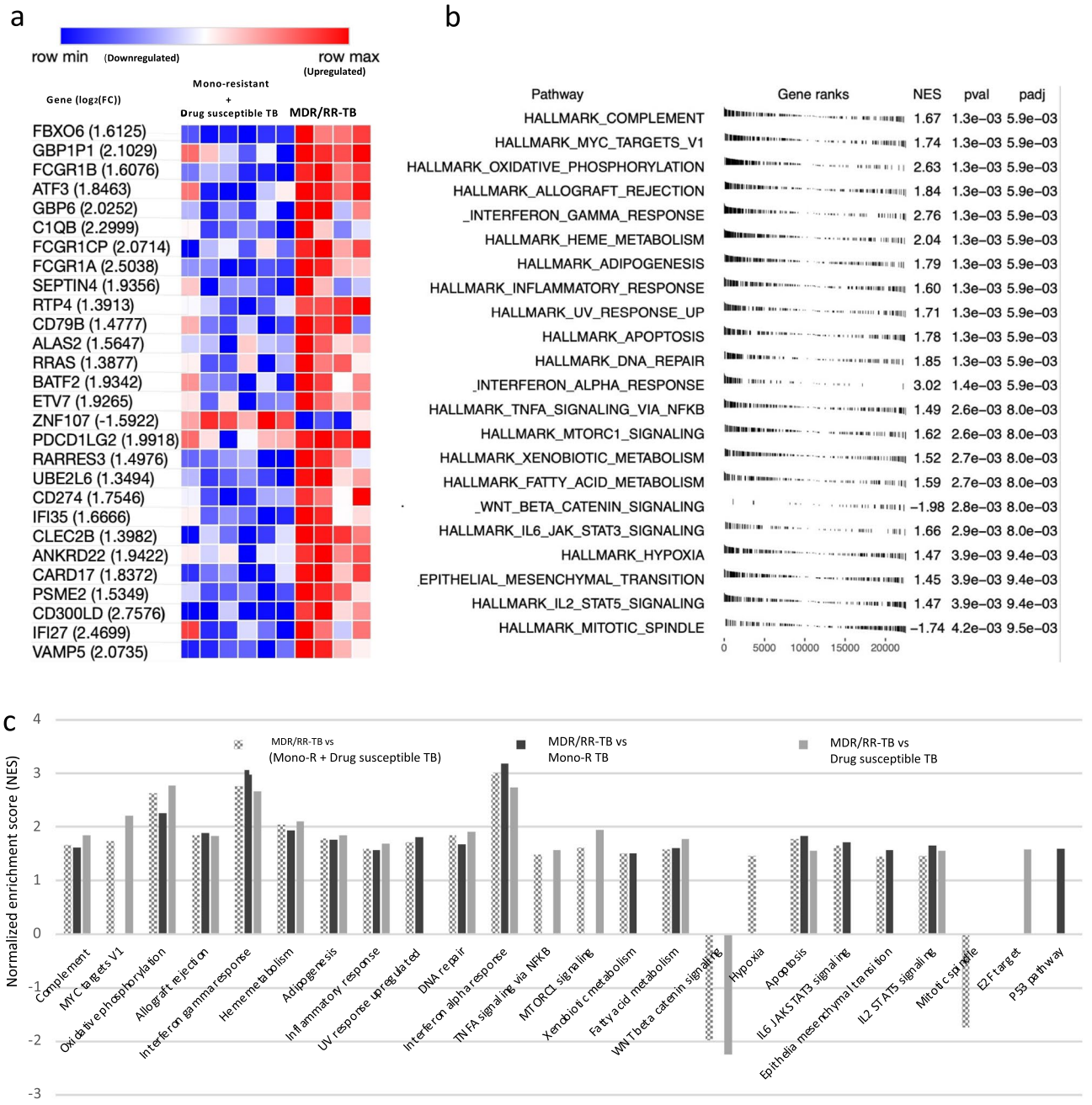

Fig. 1 **a** RNA sequencing identified differentially expressed genes in multi-Drug resistant/Rifampin resistant tuberculosis (MDR/RR-TB) in contrast to all other patients-Mono-resistant (mono-R) + drug susceptible tuberculosis. Heat map displays 28 differentially expressed genes (P-adj < 0.01, log2(FC) > 1.3); one downregulated and twenty-seven upregulated genes (generated using Morpheus (https://software.broadinstitute.org/morpheus **b** fgsea analysis of differentially expressed genes in MDR/RR-TB in contrast to all other patients-Mono-resistant + drug susceptible tuberculosis (p-adj < 0.01) **c** fgsea gene enrichment analysis: Normalized enrichment score (NES) of all the pathways that were significantly enriched at least in one contrast group (p < 0.01, NES > 1.4)

affinity immunoglobulin gamma Fc receptor I, FCGR1A ($\log_2$(FC) = 2.5038) and interferon alpha-inducible protein 27, IFI27 ($\log_2$(FC) = 2.4699). All the remaining statistically significant DEGs showed a $\log_2$(FC) value above 1.16. ZNF107, a Zinc finger protein coding gene was the only downregulated gene with an adjusted *p* value below 0.01 (*p-adj* = 4.79E-03, $\log_2$(FC) = − 1.5922). Majority of the genes were functionally immune related and nearly half of them showed protein–protein interactions in their associated functions; specifically, interferon gamma/alpha signaling, antigen processing and presentation and programmed cell death (Online resource file 1 Fig. S2). Among the DEGs,

Table 1 Differentially expressed genes $p < 0.05$ (n = 55) in multi-drug resistant/rifampin resistant tuberculosis (MDR/RR-TB) patients in contrast to all other patients- Mono-resistant + drug-susceptible tuberculosis

Gene ID	log2(FC)	*p-value*	*p-adj*	Description
FCGR1A[a]	2.50373	8.55E-12	1.27E-07	High affinity immunoglobulin gamma Fc receptor I
VAMP5[a, b]	2.07347	1.05E-09	7.83E-06	Vesicle-associated membrane protein 5
IFI27[a]	2.46988	2.52E-09	1.25E-05	Interferon alpha-inducible protein 27, mitochondrial
FBXO6[a]	1.61252	4.82E-09	1.79E-05	F-box only protein 6
CD300LD	2.75755	1.14E-08	3.39E-05	Cd300 molecule like family member d
FCGR1CP	2.07135	3.51E-08	8.68E-05	Fc Fragment of igg Receptor Ic, Pseudogene
FCGR1B[a]	1.60755	1.35E-07	2.86E-04	High affinity immunoglobulin gamma Fc receptor IB
PSME2[a]	1.53486	1.57E-07	2.92E-04	Proteasome activator complex subunit 2
C1QB[a]	2.29985	3.32E-07	5.47E-04	Complement C1q subcomponent subunit B
CARD17[a]	1.83723	5.84E-07	8.68E-04	Caspase recruitment domain-containing protein 17
ANKRD22[a, b]	1.94223	7.66E-07	1.03E-03	Ankyrin repeat domain-containing protein 22
CLEC2B	1.39824	1.10E-06	1.36E-03	C-type lectin domain family 2 member B
IFI35[a]	1.66658	1.90E-06	2.17E-03	Interferon-induced 35 kda protein
CD274[a, b]	1.75461	2.20E-06	2.33E-03	Programmed cell death 1 ligand 1
UBE2L6[a, b]	1.34938	2.92E-06	2.88E-03	Ubiquitin/ISG15-conjugating enzyme E2 L6
RARRES3[a]	1.49764	4.17E-06	3.87E-03	Phospholipase A and acyltransferase 4
GBP1P1[a]	2.10287	4.86E-06	4.01E-03	Guanylate binding protein 1, interferon-inducible pseudogene 1
GBP6[a]	2.02524	4.73E-06	4.01E-03	Guanylate-binding protein 6
PDCD1LG2[a]	1.99184	5.59E-06	4.36E-03	Programmed cell death 1 ligand 2
ZNF107[a]	− 1.59215	6.45E-06	4.79E-03	Zinc finger protein 107
ETV7[a]	1.92650	7.67E-06	5.43E-03	Transcription factor ETV7
BATF2[a, b]	1.93419	8.51E-06	5.76E-03	Basic leucine zipper transcriptional factor ATF-like 2
RRAS	1.38771	1.13E-05	7.29E-03	Ras-related protein R-Ras
ALAS2[a]	1.56468	1.35E-05	8.35E-03	5-aminolevulinate synthase, erythroid-specific, mitochondrial
CD79B[a]	1.47765	1.43E-05	8.51E-03	B-cell antigen receptor complex-associated protein beta chain
RTP4[a]	1.39134	1.52E-05	8.65E-03	Receptor-transporting protein 4
SEPTIN4[a]	1.93559	1.67E-05	9.16E-03	Septin-4, Filament-forming cytoskeletal gtpase
ATF3[a, b]	1.84626	1.75E-05	9.27E-03	Activating transcription factor 3 (stress induced)
SIRPB2	− 1.34109	2.36E-05	1.21E-02	Signal regulatory protein beta 2
DHRS9[a]	1.34690	2.51E-05	1.24E-02	Dehydrogenase/reductase 9
OAS1[a, b]	1.58431	2.75E-05	1.32E-02	2′-5′-oligoadenylate synthetase 1
FFAR2[b]	1.35512	3.67E-05	1.70E-02	Free fatty acid receptor 2
SDSL	1.61967	3.94E-05	1.77E-02	Serine dehydratase like
EDIL3	1.95787	5.12E-05	2.23E-02	EGF like repeats and discoidin domains 3
IFI6[b]	1.50053	5.47E-05	2.32E-02	Interferon alpha inducible protein 6
TREML1[b]	1.23028	5.84E-05	2.35E-02	Triggering receptor expressed on myeloid cells like 1
Lnc-LY96-2	1.94817	5.85E-05	2.35E-02	A novel transcript affiliated with the lncrna class
GOLGA8B	− 1.29030	7.27E-05	2.84E-02	Golgin A8 family member B
RHOC	1.25396	8.14E-05	3.08E-02	Ras homolog family member C
PSMB9[b]	1.25997	8.29E-05	3.07E-02	Proteasome 20S subunit beta 9
CNN1	1.75834	8.85E-05	3.21E-02	Calponin 1
KIR3DL1	− 1.75744	1.01E-04	3.58E-02	Killer cell immunoglobulin like receptor
Lnc COPG13	1.84875	1.04E-04	3.59E-02	Uncharacterized RNA gene affiliated with lncrna class
HLA-DQB1	1.31813	1.06E-04	3.59E-02	Major histocompatibility complex, class II, DQ beta 1
MT-TA	1.46257	1.09E-04	3.59E-02	Mitochondrially encoded trna-Ala
AC026333.3	1.86762	1.13E-04	3.64E-02	A novel transcript affiliated with the lncrna class
GBP4[b]	1.31886	1.20E-04	3.65E-02	Guanylate binding protein 4
AFF3[b]	1.34395	1.25E-04	3.65E-02	AF4/FMR2 family member 3
LY6E	1.53773	1.20E-04	3.65E-02	Lymphocyte antigen 6 family member E

Table 1 (continued)

Gene ID	log2(FC)	*p-value*	*p-adj*	Description
MS4A4E	− 1.74161	1.16E-04	3.65E-02	Membrane spanning 4-domains A4E
TCN2[b]	1.32006	1.25E-04	3.65E-02	Transcobalamin 2
PPP1R14A	1.41985	1.42E-04	4.06E-02	Protein phosphatase 1 regulatory inhibitor subunit 14A
CARMIL3	− 1.83197	1.48E-04	4.16E-02	Capping protein regulator and myosin 1 linker 3
LINC01232	1.16118	1.72E-04	4.73E-02	Long intergenic non-protein coding rna 1232
APOL1[b]	1.26490	1.78E-04	4.82E-02	Apolipoprotein L1

* The differentially expressed genes of statistical significance $p < 0.01$ are in bold

[a]Genes that have been previously reported from DS-TB associated studies

[b]Genes that have been previously reported from both DR-TB associated studies and DS-TB associated studies (Supplementary file 2)

The genes of statistical significance of $p < 0.01$ are in bold (n = 28, one downregulated, 27 upregulated)

there were three immunoglobulin gamma Fc receptor-1 related genes: FCGR1A, FCGR1B and FCGR1CP, and two interferon related genes; IFI27 and IFI35 (Table 1; Fig. 1a).

DEG analysis was also conducted for other contrast groups and respectively, 39 (10), 22 (11), 4 (1) and 7 (6) genes were statistically significant $p < 0.05$ ($p < 0.01$) in groups: MDR/RR-TB vs drug susceptible TB, MDR/RR-TB vs mono-resistant TB, all DR-TB (MDR/RR-TB + mono-resistant TB) vs drug susceptible TB and mono-resistant TB vs drug susceptible TB (Online resource file 1 Table S4, Fig. S3, Online resource file 2). Significantly, CD300LD was differentially expressed in all three contrast groups: MDR/RR-TB vs mono-resistant TB, MDR/RR-TB vs drug susceptible TB and MDR/RR-TB vs (mono-resistant and drug susceptible TB) with a $\log_2$(FC) value above 2.4. In contrast to drug susceptible TB, the highest differential expression was shown by PSPHP1 in mono-resistant TB ($\log_2$(FC) = − 3.50, *p-adj* = 3.75E-08), RNF182 in MDR/RR-TB ($\log_2$(FC) = 2.92, *p-adj* = 4.01E-04) and OR56A7P in all DR-TB isolates ($\log_2$(FC) = 2.32, *p-adj* = 9.59E-05).

MDR/RR-TB patients displayed differential expression of immune related pathways

As per Metascape, DEGs were significantly enriched in adaptive immune system/response, and interferon signaling ($p < 0.01$, minimum count = 3 and an enrichment factor > 1.5) (Online resource file 1 Figs. S2, S3). Fgsea gene enrichment analysis identified 22 significantly enriched pathways in MDR/RR-TB in contrast to drug susceptible and mono-resistant TB patients (Fig. 1c, d). Highest enrichment scores among upregulated pathways were shown by hallmark gene sets of oxidative phosphorylation, and interferon (INF) (both INF-β and INF-γ) response. Only two pathways were downregulated, Wnt/beta-catenin pathway and mitotic spindle with normalized enrichment scores of − 1.9761 and − 1.7404, respectively. The observations were consistent with fgsea enrichment of other contrast groups (Online resource file 3).

The significance of the gene sets of all contrast groups was investigated using EGSEA comparison analysis. The top two significantly enriched (downregulated) KEGG pathways were lysosome (average $\log_2$(FC) = − 0.91, significance = 34.91) (Fig. 2a, c) and notch signaling (average $\log_2$(FC) = − 1.01, significance = 27.54) (Fig. 2a, b) pathways. As per the enrichment analysis on hallmark signature gene sets, Wnt/beta catenin signaling, and mitotic spindle displayed the highest significance values. Details on other enriched gene sets are as in online resource file 3.

Discussion

To the best of our knowledge, this is the first study that presents a panel of genes that might have potential clinical applicability to differentiate MDR/RR-TB patients from a cohort of patients infected with either drug susceptible or mono-resistant TB isolates. There are five key findings. The study identified 55 DEGs (of which 28 genes had $p < 0.01$ value) that were differentially expressed in MDR/RR-TB in contrast to mono-resistant/drug susceptible TB. 10 genes (CD300LD, MYL9, VAMP5, CARD17, CLEC2B, GBP6, BATF2, ETV7, IFI27 and FCGR1CP) which were common in more than one contrast group can be posited as those with more potential to be considered as future MDR/RR-TB biomarkers. In the 28 DEG panel, two genes that were not previously linked to TB; CD300LD, and CLEC2B were encountered. Even though the majority of DEGs were consistent to the previously established active-TB signatures, all the DEGs showed a fold change of at least 2.5 × from drug susceptible active-TB. As per the pathway analysis, interferon alpha and gamma response were highly upregulated in MDR/RR-TB while Wnt/beta catenin signaling, lysosome pathway, microtubule nucleation and notch signaling were downregulated in comparison analysis.

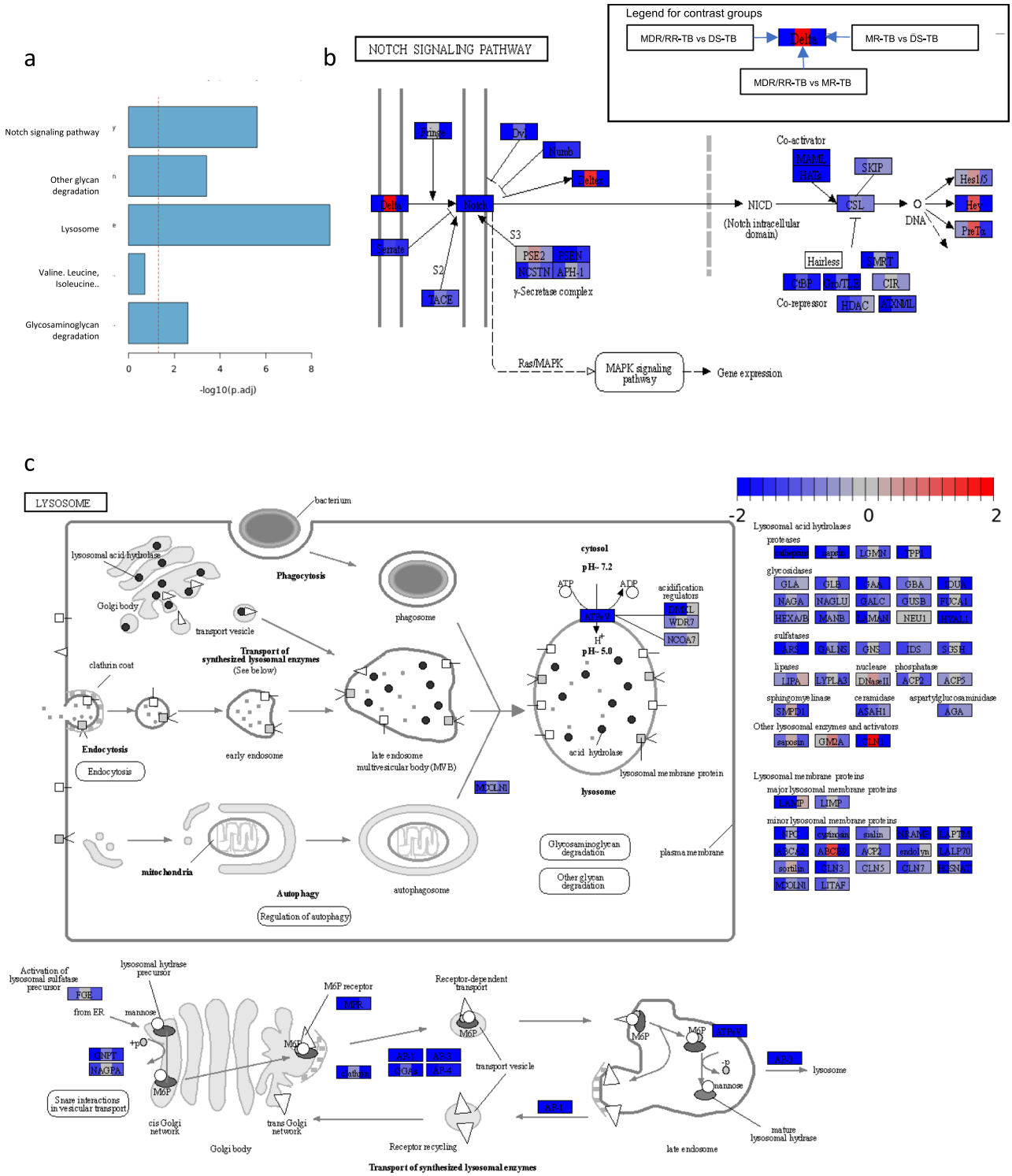

Fig. 2 **a** KEGG pathways sorted by median rank scores (dotted line represents the p value boundary 0.05). **b** Enriched notch signaling pathway **c** Enriched lysosome pathway as per KEGG comparison analysis in EGSEA galaxy platform. The legend displays the contrast groups considered for EGSEA comparison: multi-drug resistant/Rifampin resistant tuberculosis (MDR/RR-TB) vs drug susceptible tuberculosis (DS-TB), MDR/RR-TB vs mono-resistant tuberculosis (MR-TB), MR-TB vs DS-TB. Each colored box represents a gene and is divided into three sections to display the relative magnitude and direction of the fold change of the gene under each experimental contrast (see legend)

This is the first study to directly link the two genes, CD300LD and CLEC2B to TB and MDR/RR-TB. CD300LD is a member of the immunoglobulin receptors` superfamily of which the roles in immune system are yet to be discovered. CD300LD is reported to code for a cellular receptor of murine norovirus, and in humans, its expression is linked to viral replication [10, 11]. Generally, the proteins of the CD300 family are known to perform immune regulatory and inhibitory functions in association with DAP and FCGR receptors [12]. As per Metascape, TREML1, a cell surface receptor (involved in innate and adaptive immune response) which interacts with CD300LD is also significantly upregulated in MDR/RR-TB [13]. Hence, upregulation of CD300LD along with TREML1 and FCGR associated genes (FCGR1CP, FCGR1A and 1B) could be entirely multi-drug resistance driven. CLEC2B (AICL), belongs to the C-type lectin/lectin-like superfamily which is known to have roles in inflammation and immune response. CLEC2B encodes a ligand that activates NKp80, a receptor that is associated with the development and maturity of natural killer cells [14, 15]. In another study, CLEC2B is linked to ferroptosis, an important host immune response that promotes cell death in TB which is triggered due to the accumulation of lipid peroxides when MTB infects macrophages [16]. Ferroptosis related genes were previously studied elsewhere as biomarkers for TB, as in SOCS1, however, not CLEC2B [17].

Majority of the identified DEGs in this study were known to be active TB markers or potential markers (FCGR1A, VAMP5, IFI27, FBXO6, FCGR1B, PSME2, C1QB, CARD17, ANKRD22, IFI35, CD274, UBE2L6, GBP6, PDCD1LG2, ETV7, BATF2, ALAS2, CD79B, SEPTIN4, ATF3, and RTP4), however, the higher fold change values observed in this study shows that their differential expression is due to the infection by MDR/RR-TB [5, 6]. Enriched gene ontology biological processes of the DEGs consistent with previous TB signatures were assessed and it was seen that DEGs associated with interferon signaling and adaptive immune system are preferentially associated with MDR/RR-TB in comparison to mono-resistant/drug susceptible TB (Online resource file 1 Fig. S3). For example, the DEG panel includes three immunoglobulin gamma Fc receptor-1 related genes (FCGR1A, FCGR1B and FCGR1CP) and since the role of FCGR1 is to induce phagocytosis in macrophages, it could be argued that the host immune system attempts to fight the continued presence of drug resistant bacteria by overexpressing FCGR1 genes [18]. This is further supported by the high IFN-γ enrichment scores observed in fgsea analysis as the expression of FCGR1 genes is induced by IFN-γ. With reference to ZNF107, the only downregulated gene in the signature, one study reported that it is upregulated in PTB, and another study reported that it is downregulated in HIV/TB patients suffering from immune reconstitution inflammatory syndrome, however none of the patients were HIV positive [19, 20]. Since interferon stimulating genes (ISGs) and ZNF genes have a functional interconnection, the later study hypothesized that ISGs were upregulated as a compensation to the low ZNF levels [20]. It can be posited that the upregulated interferon pathway genes and downregulated ZNF observed in this study could be due to a similar compensation effect. The attempt of host to defend the drug resistant strains is further supported by the overexpression of other DEGs identified in the study. For instance, UBE2L6, which is linked to type-1 interferon response in TB and ATF3, which is linked regulation of macrophage related genes were upregulated as was seen in the previous study on INH and RIF resistant THP1 cells [7].

Gene enrichment analysis identified important host immune pathways up/downregulated in response to drug resistant MTB infection. Upregulation of INF-γ and INF-β were significant and demarcates the continued need of driving the immune cells to the site of infection (INF-β) and activation of macrophages (INF-γ) to fight the higher loads of multiplying drug resistant MTB [21]. Another significant observation was the downregulation of Wnt/beta-catenin signaling in all drug-resistant patients in agreement to previous findings that the pathway is downregulated with elevated severity of infection. It's hypothesized that when the severity increases, TB lesions deteriorate resulting an impairment of some Wnt proteins in T cells, however, the mechanism is unknown [22, 23]. Further studies on links of Wnt proteins to TB pathogenesis should be encouraged as they can be potential severity/drug resistance biomarkers. EGSEA analysis resulted controversial results with respect to notch signaling and lysosomes [24]. Since both pathways are involved in fighting TB (lysosomes-phagocytosis and notch signaling-T cell mediated immunity), it is expected to see upregulation in drug-resistant groups. Further, the notch1 regulatory and notch-HLH transcription pathways were also significantly downregulated in the study. Since notch signaling and Wnt/beta-catenin pathway has parallel links, perhaps, downregulation of Wnt proteins led to the downregulation of notch signaling [25]. The mechanisms underlying lysosomal downregulation needs to be explored. Since microtubules play a role in transportation of lysosomes, the downregulation of microtubule nucleation observed in this study, further supports this observation. Perhaps, this could be an effect due to mycobacterial factors developed in MDR/RR-TB that can resist lysosomal phagocytosis.

The study had limitations. The sample size was small, and the results would be more supportive if the groups had more representatives. However, given the practicality, the sample number represents one third of the drug resistant cases (and all culture positive MDR/RR-TB cases) identified from Kandy and Welisara, Sri Lanka during the study period. Nonetheless, instead of laboratory infected cell lines, we were able to bioinformatically analyze the host

transcriptomes of WGS confirmed drug resistant cases and the outputs of each analysis method were confirmed with two/more similar methods for further support. We believe that as a pioneer attempt in the field of host blood transcriptomic biomarkers of MDR/RR-TB, the study outcome revealed significant genes/pathways in MDR/RR-TB and opened new directions for further studies.

Conclusion

In conclusion, the identified DEGs can be of clinical importance as future biomarkers to differentiate MDR/RR-TB patients from drug susceptible/mono-resistant patients. The highly upregulated DEGs and downregulated pathways indicate that the outcome is a collective effect of host immune responses in face of multiplying MDR/RR-TB bacteria and the bacterial factors present in MDR/RR-TB to overcome the host immune responses. Underlying mechanisms needs to be explored further to understand their links to TB pathogenesis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-023-08307-6.

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Author contributions DM-A contributed to the study conception, and design. Material preparation, sample collection, data collection, analysis, and the preparation of the first draft of the manuscript was performed by PM. The study was supervised by SR, DM, BG and DM-A. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Ethical approval was obtained from the ethical review committee at the general teaching hospital, Kandy, Sri Lanka. All the patients agreed to the study protocol and provided written informed consent prior to recruitment. This study was performed in line with the principles of the Declaration of Helsinki.

References

1. World Health Organization (WHO) (2021) Global tuberculosis report-2021 https://www.who.int/publications/i/item/9789240037021 Accessed 15 May 2021
2. Koch A, Cox H, Mizrahi V (2018) Drug-resistant Tuberculosis: challenges and opportunities for diagnosis and treatment. Curr Opin Pharmacol 42:7–15. https://doi.org/10.1016/j.coph.2018.05.013
3. Ghajavand H, Kamakoli KM, Khanipour S et al (2019) Scrutinizing the drug resistance mechanism of multi- and extensively-drug resistant Mycobacterium tuberculosis: mutations versus efflux pumps. Antimicrob Resist Infect Control 8:70. https://doi.org/10.1186/s13756-019-0516-4
4. Hardy-Sosa A, León-Arcia K, Llibre-Guerra JJ et al (2022) Diagnostic accuracy of blood-based biomarker panels: a systematic review. Front. Aging Neurosci 14:683689. https://doi.org/10.3389/fnagi.2022.683689
5. Berry MPR, Graham CM, McNab FW et al (2010) An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466(7309):973–977. https://doi.org/10.1038/nature09247
6. Bloom CI, Graham CM, Berry MPR et al (2013) Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS One 8(8):e70630. https://doi.org/10.1371/journal.pone.0070630
7. Tazi L, Wang P, Fornage M (2020) Differential host gene signatures in response to Mycobacterium Tuberculosis Infection. biorxiv. https://doi.org/10.1101/2020.02.19.955203
8. Chomczynski P, Sacchi N (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nat Protoc 1(2):581–585. https://doi.org/10.1038/nprot.2006.83
9. Zhou Y, Zhou B, Pache L et al (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 10:1523. https://doi.org/10.1038/s41467-019-09234-6
10. Haga K, Fujimoto A, Takai-Todaka R et al (2016) Functional receptor molecules CD300lf and CD300ld within the CD300 family enable murine noroviruses to infect cells. Proc Natl Acad Sci USA 113(41):E6248–E6255. https://doi.org/10.1073/pnas.1605575113
11. Orchard RC, Wilen CB, Doench JG et al (2016) Discovery of a proteinaceous cellular receptor for a norovirus. Science 353(6302):933–936. https://doi.org/10.1126/science.aaf1220
12. Borrego F (2013) The CD300 molecules: an emerging family of regulators of the immune system. Blood 14 121(11):1951–60. https://doi.org/10.1182/blood-2012-09-435057
13. Bleharski JR, Kiessler V, Buonsanti C et al (2003) A role for triggering receptor expressed on myeloid cells-1 in host defense during the early-induced and adaptive phases of the immune response. J Immunol 1 170(7):3812–8. https://doi.org/10.4049/jimmunol.170.7.3812
14. Freud AG, Keller KA, Scoville SD et al (2016) NKp80 defines a critical step during human natural killer cell development. Cell Rep 16(2):379–391. https://doi.org/10.1016/j.celrep.2016.05.095
15. Spreu J, Kuttruff S, Stejfova V, Dennehy KM, Schittek B, Steinle A (2010) Interaction of C-type lectin-like receptors NKp65 and KACL facilitates dedicated immune recognition of human keratinocytes. Proc Natl Acad Sci U S A 107(11):5100–5105. https://doi.org/10.1073/pnas.0913108107
16. Meunier E, Neyrolles O (2019) Die another way: ferroptosis drives tuberculosis pathology. J Exp Med 216(3):471–473. https://doi.org/10.1084/jem.20190038
17. Liang T, Chen J, Xu GY et al (2022) Ferroptosis-related gene SOCS1, a marker for Tuberculosis diagnosis and treatment, involves in macrophage polarization and facilitates bone destruction in Tuberculosis. Tuberculosis 132:102140. https://doi.org/10.1016/j.tube.2021.102140
18. Satproedprai N, Wichukchinda N, Suphankong S et al (2015) Diagnostic value of blood gene expression signatures in active

tuberculosis in Thais: a pilot study. Genes Immun 16:253–260. https://doi.org/10.1038/gene.2015.4
19. Zhao M, Di X, Jin X et al (2020) Identification of biomarkers for Sarcoidosis and Tuberculosis of the Lung using systematic and integrated analysis. Med Sci Monit 26:e925438-1-e925438-12. https://doi.org/10.12659/MSM.925438
20. Ma J, Zhao F, Su W et al (2018) Zinc finger and interferon-stimulated genes play a vital role in TB-IRIS following HAART in AIDS. Per Med. https://doi.org/10.2217/pme-2017-0084
21. Cavalcanti YVN, Brelaz MCA, de Andrade Lemoine Neves JK, Ferraz JC, Pereira VRA (2012) Role of TNF-alpha, IFN-gamma, and IL-10 in the development of pulmonary Tuberculosis. Pulm Med 2012:745483. https://doi.org/10.1155/2012/745483
22. Fan L, Shen H, Huang H, Rui Yang LY (2017) Impairment of Wnt/β-catenin signaling in blood cells of patients with severe cavitary pulmonary tuberculosis. PLoS One 12(3):e0172549. https://doi.org/10.1371/journal.pone.0172549
23. Pitabut N, Mahasirimongkol S, Yanai H et al (2011) Decreased plasma granulysin and increased interferon-gamma concentrations in patients with newly diagnosed and relapsed tuberculosis. Microbiol Immunol 55:565–573
24. Bermicka JR, Lincoln PM, Allen RM, Kunkel SL, Schaller MA (2021) Elevated Notch ligands in serum are associated with HIV/TB coinfection. J Clin Tuberc Other Mycobact Dis 24:100258. https://doi.org/10.1111/j.1348-0421.2011.00348.x
25. Gao J, Fan L, Zhao L et al (2021) The interaction of Notch and Wnt signaling pathways in vertebrate regeneration. Cell Regen 10:11. https://doi.org/10.1186/s13619-020-00072-2