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Data in Brief

Transcriptional profiling of *Mycobacterium tuberculosis* replicating in the human type II alveolar epithelial cell line, A549

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ABSTRACT

Alveolar epithelial cells outnumber alveolar macrophages by ~500 fold and increasing evidence suggests that *Mycobacterium tuberculosis* may replicate dramatically in these cells during the initial weeks of infecting the lung (Wolf et al., 2008 [1]; Ryndak et al., 2015 [2]). Here, we report in experimental detail the transcriptional profiling of *M. tuberculosis* replicating at 72 h post-infection in the human type II alveolar epithelial cell line, A549, as compared to *M. tuberculosis* growing logarithmically in laboratory broth culture (Ryndak et al., 2015 [2]). All resulting transcriptional profiling data was deposited to the Gene Expression Omnibus (GEO) database under the accession number GSE58466.

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Specifications	
Organism/cell line/tissue	*Mycobacterium tuberculosis* H37Rv
Sex	N/A
Sequencer or array type	*Mycobacterium tuberculosis* whole genome oligo-based spotted DNA microarray generated by Center for Applied Genomics (Public Health Research Institute, Newark, NJ)
Data format	Raw GPR files and normalized log ratio (test/reference) data
Experimental factors	Intracellular *Mycobacterium tuberculosis* H37Rv recovered from A549 cell line at 3 days post-infection vs. *Mycobacterium tuberculosis* H37Rv growing logarithmically in 7H9 laboratory broth.
Experimental features	This microarray study was performed to determine *Mycobacterium tuberculosis* transcriptional adaptation to the intra-type II alveolar epithelial environment.
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58466.

2. Experimental design, materials and methods

2.1. Growth conditions of experimental and reference bacteria

Alveolar epithelial cells may provide a permissive niche for the inhaled *Mycobacterium tuberculosis* to replicate dramatically and acquire a disseminative phenotype [1,2]. To obtain *M. tuberculosis* replicating in human type II alveolar epithelial cells, the cell line, A549 (ATCC; CCL-185), was cultured to confluence in ~5–7 T225 tissue culture flasks per biological replicate and infected with a multiplicity of infection of 5:1 (bacteria:cell) of logarithmically growing *M. tuberculosis* H37Rv as described previously [2]. At 72 h post-infection, cells were lysed and bacteria isolated as described previously [2]. *M. tuberculosis* H37Rv growing logarithmically in Middlebrook 7H9 broth containing final concentrations glycerol (0.2%), Tween 80 (0.05%) and ADC supplement (10%) was used as the reference bacteria. Briefly, 10 ml supplemented Middlebrook 7H9 media was inoculated with a 0.5 ml frozen aliquot of bacteria (OD600 = 0.7 at time of storage) yielding a starting culture of OD600 = 0.035. The culture was incubated at 37 °C with shaking (110 rpm) for 7–9 days and harvested at an OD600 of 0.7–0.9 (mid to late log phase) [3]. The bacterial pellets (both experimental and reference) were immediately resuspended in 1 ml of TRI reagent (Molecular Research Center) with polyacryl carrier (Molecular Research Center) added (1:100), transferred to sterile bead beater tubes containing 150–200 μl of 0.1 mm zirconia beads and placed in −80 °C until further use.

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2.2. RNA extraction and amplification

Total RNA was extracted using a previously described protocol [4]. Therefore, bead beater tubes containing the bacteria pellets in TRI/polyacryl carrier were thawed on ice then subjected to bead beating 3 times for 1 min with 2 min on ice between pulses. Beads were briefly allowed to settle and supernatant transferred to sterile DNAse- and RNAse-free Eppendorf tubes (used throughout). An additional 100 μl of TRI/polyacryl carrier was added to the remaining beads and vortexed. After settling, the supernatant was added to the previously collected supernatant and allowed to set for 10 min at room temperature (RT) followed by 10 min centrifugation at 12,000 ×*g*, 4 °C. Supernatant was transferred to a new tube and 100 μl BCP Phase Separation reagent (Molecular Research Center) added then shaken vigorously for 15 s. After 10 min settling at RT, the phases were separated by centrifugation at 12,000 ×*g* for 15 min at 4 °C. About 75% of the upper phase was transferred to a new tube. To the remaining phases in the original tube, 500 μl of TRI and 50 μl BCP were added and shaken vigorously for 15 s, let set 10 min at RT, and centrifuged as before. The upper phase was removed and combined with the previously collected upper phase. An equal volume (~700 μl) isopropanol was added, mixed by gentle inversions, and let set 10 min at RT to precipitate the RNA. Precipitated RNA was collected by centrifugation at 12,000 ×*g* for 8 min at 4 °C. The pellet was then washed with 1 ml 75% ethanol (in DEPC-water), i.e., vortexed 10 s and centrifuged at 7500 ×*g* for 5 min at 4 °C. The ethanol was removed and pellet air-dried. Experimental and reference RNA were each suspended in 630 μl nuclease-free water by vortexing and divided 2 × 315 μl. One tube was placed immediately at −80 °C for future use and one tube DNAse-treated using TURBO DNAse (Ambion/Life Technologies) as follows. DNAse buffer (36 μl of 10×) was added and 58.5 μl aliquots were divided to six tubes to which 1.5 μl DNAse was added to each. DNA degradation was carried out in a 37 °C water bath for 1 h 15 min. The volume in each tube was adjusted to 100 μl with nuclease-free water. RNA was purified using the RNeasy MinElute Cleanup Kit (QIAGEN). To combine and purify the RNA aliquots, the six DNAse-treated RNAs were passaged over the same purification column after addition of RLT buffer and 100% ethanol in the RNeasy MinElute protocol. Purified RNA was eluted with 14 μl nuclease-free water. If necessary (as determined by PCR), RNA was DNAse-treated a second time to rid contaminating DNA and purified once again. RNA quality and quantity were verified by Agilent Bioanalyzer 2100, and RNA integrity numbers for each experimental RNA sample F1, F2, and 2F, were 8.7, 8.6, and 7.7, respectively, and 9.5 for reference RNA. To ensure adequate amounts of RNA for the microarray study, an RNA amplification strategy using the MessageAmp II-Bacteria RNA Amplification kit (Ambion/Life Technologies) was performed on ~100 ng of all RNA samples, experimental and reference, as per the kit protocol [2,3,5,6].

2.3. cDNA preparation and labeling

Amplified RNA (aRNA), experimental and reference, were adjusted to 3 μg in 8 μl (diluted with nuclease-free water if necessary) each and divided to two PCR tubes. To each tube of 4 μl, 1 μl of Random Primers (0.5 μg) (Promega) was added and incubated at RT for 5 min. The aRNA was then reverse transcribed by adding 5 μl of a reverse transcription mixture with concentrations of First Strand Buffer (1×), dithiothreitol (10 mM), and Superscript III reverse transcriptase (10 U/μl) from Life Technologies, plus dNTP mix (500 μM each) and RNasin (2 U/μl) (Promega) to each tube and subjected to thermocycling 37 °C 20 min, 42 °C 20 min, 50 °C 10 min, 55 °C 10 min, 65 °C 15 min. One microliter (1 U) of RNase H (Epicenter) was added to each PCR tube and returned to thermocycler at 37 °C 30 min then 95 °C 2 min to degrade enzymes then immediately cooled to 4 °C. The resulting 10 μl of cDNA was mixed with 90 μl Klenow Mixture in a darkened room using components of the BioPrime DNA Labeling System Kit (Invitrogen) Random Primer Solution (final 1×) and Klenow fragment (final 1 U/μl), along with a lowT dNTP mix (final 200 μM each dATP, dCTP, dGTP, 50 μM dTTP), and either cyanine 3-dUTP (Cy3) dye or cyanine 5-dUTP (Cy5) dye (final 30 μM) (PerkinElmer). Tubes in rack were wrapped in foil and placed in 37 °C hybridization oven overnight. Labeling was stopped at ~16 h by adding 10 μl Stop Buffer (BioPrime DNA Labeling System Kit; Invitrogen).

2.4. Hybridization and washing

The *M. tuberculosis* microarray chips used in this study contained 70-mer oligonucleotides representing all open reading frames annotated in the *M. tuberculosis* H37Rv genome sequence and were obtained from the Center for Applied Genomics (Public Health Research Institute; Newark, NJ) [4]. These microarray slides were prehybridized in a glass slide-staining dish containing freshly prepared Prehybridization solution (106 ml autoclaved distilled water, 3 g BSA, 1.2 ml 10% SDS) preheated to 42 °C in a water bath. Microarray slides were submerged in the Prehybridization solution and hybridized for 1 h at 42 °C. Meanwhile, to prepare the labeled probes for hybridization, Cy5- and Cy3-labeled cDNA probes generated from aRNA obtained from *M. tuberculosis* H37Rv grown in each A549-grown biological replicate was mixed with the alternately labeled reference cDNA probe prior to purification with Microcon YM10 filter (Millipore). Filter cartridges were first primed by adding 400 μl tris-EDTA buffer pH 8 (TE) (Ambion) and centrifuging 10,000 ×*g* for 10 min. Labeled cDNA mixtures were added to individual cartridges and centrifuged at 10,000 ×*g* until ~25 μl remained. To each cartridge, 200 μl TE was added to the concentrated labeled mixtures and centrifuged 10,000 ×*g* until ~5.5 μl remained in the cartridge. The cartridge was inverted and briefly centrifuged to collect the concentrated sample in a new tube. To each 5 μl of labeled sample the following were added in order: 2.5 μl formamide (Sigma), 0.5 μl (10 mg/ml) tRNA (Invitrogen), 1 μl 20× SSC (Ambion), and 1 μl 1% SDS (Ambion). The final 10 μl was heated at 98 °C for 2 min in a thermocycler then placed on ice until application to the microarray slides. Prior to applying the labeled probe mixtures, the prehybridized slides were placed into a slide holder and washed by vigorous up and down submersion in a slide-washing tray containing DEPC-treated water for 2 min followed by 2 min of vigorous washing in isopropanol. The slides were transferred to 50 ml Falcon tubes (array side to the top and kimwipes in the cone to collect moisture) and centrifuged immediately to dry at 600 rpm for 3 min. The prehybridized array slides were placed in DIGILAB HybChambers and 10 μl sample applied to the center of each printed array. A clean 22 × 22 mm glass cover slip (Corning) was placed over the sample on each array avoiding bubbles. Under each end of each slide 20 μl of nuclease-free water was pipetted to maintain humidity during hybridization. The hybridization chambers were screwed tightly shut and submerged in a leveled 50 °C water bath overnight. The hybridized microarray slides were submerged in 2× SSC/0.1% SDS with gentle motions to remove the cover slips then washed with vigorous shaking in 1× SSC/0.05% SDS for 2 min followed by two sequential 2 min vigorous washes in 0.06× SSC. To dry, the slides were placed in 50 ml conical tubes with clean kimwipes in the bottom of the tubes and centrifuged at 200 ×*g* for 5 min. Note that all steps involving Cy3 and Cy5 dyes were performed in darkened conditions.

2.5. Microarray data analysis

For one biological sample, four technical replicates were used (two dye flips) and for the other biological sample, two technical replicates were used (one dye flip) totaling six microarrays. The microarrays were scanned and processed with an Axon 4000B scanner and GenePix Pro 6.1 software, respectively. The chips were normalized by the print-tip Lowess method, and the Cy5/Cy3 intensity ratios determined for each gene [7]. The intensity ratio data obtained from microarray chips was used to perform Significance Analysis of Microarrays (SAM) with MultiArray Viewer Software on the TMEV website for determination of differentially expressed genes of *M. tuberculosis* grown in A549 cells

compared to 7H9 broth grown *M. tuberculosis* [8]. Among genes identified by SAM, only genes that showed a >2 fold change at a false discovery rate of <2% were considered significantly differentially expressed.

3. Discussion

In this study, we investigated the transcriptional adaptation of *M. tuberculosis* during infection of the human type II alveolar epithelial cell line, A549. Using laboratory broth-cultured, logarithmically growing *M. tuberculosis* as the reference, 186 genes were upregulated and 75 downregulated [2]. Among upregulated genes, those involved in aerobic respiration, energy production, protein synthesis, and ESAT-6-like encoding genes were statistically enriched. Among downregulated genes, genes of the DevR (DosR) regulon, as well as hypoxia-induced genes and genes involved in nitrate reduction and transport (non-aerobic respiration) were statistically enriched. Similar microarray studies have been performed by others to determine the *M. tuberculosis* transcriptional adaptation to the intra-phagosomal environment of both naïve and activated macrophages [4,9]. Importantly, the *M. tuberculosis* transcriptome during intra-alveolar epithelial cell residence and intra-macrophage residence indicates adaptation to the former by dramatic replication and growth and to the latter by transition to dormancy [2].

Conflict of interest

The authors have no conflicts of interest to report.

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