Mycobacterium abscessus-targeted transcriptional profiling in human samples:
A tool for monitoring treatment response

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Objective

We seek a pathogen-targeted transcriptional profiling method to characterize the physiological state of \textit{Mycobacterium abscessus}, \textit{subspecies abscessus} (M.ab) in clinical samples from patients undergoing treatment of lung disease. Directly monitoring M.ab molecular phenotypes in humans will provide critical insights into why this devastating pathogen becomes refractory to antibiotic treatment and will provide a powerful new approach to monitoring the treatment of individual patients.

The central challenge for transcriptional profiling of mycobacterial pathogens in clinical samples is that pathogen mRNA is present in extremely low abundance relative to either human RNA or pathogen rRNA. Transcriptional profiling via standard RNAseq of total RNA is not feasible because only a tiny proportion of reads are M.ab mRNA. Targeted capture, selective depletion or some alternative method of enriching M.ab mRNA prior to transcriptional profiling is needed to enable phenotyping of M.ab populations in patients but is not currently available.

This is a pressing need because: (1) the phenotypic state of mycobacteria in disease is not recapitulated by current experimental models, (2) new antibiotics for M.ab pulmonary disease are desperately needed and (3) there are currently no accurate markers of treatment response to direct length and intensity of treatment. The proposed project will translate genomic technology into practical clinical tools that will enhance patient care.

Rationale

M.ab causes devastating lung and soft-tissue infections. Treatment requires months of potent and often toxic intravenous antibiotic therapy and in many patients surgical resection of affected lung is required (1). Despite these aggressive measures, less than half (48\%) of pulmonary M.ab patients at our institution achieve relapse-free cure (2). Current clinical markers are insufficient to predict which patients would benefit from intensified regimens or extended treatment. More efficacious antibiotics and accurate markers for clinical monitoring are needed urgently.

Mycobacteria dynamically adapt their physiologic state to environmental stresses. Bacterial phenotypes in human disease are distinct from those observed \textit{in vitro} (3-5). \textit{In vivo} phenotypes are important because antibiotic effectiveness depends on the pathogen’s physiologic state (6, 7). Consequently, \textit{in vitro} antibiotic susceptibility testing does not predict success in killing the M.ab phenotypes present in human disease (8).

Antibiotic treatment results in emergence of bacterial “persister” phenotypes that survive prolonged antibiotic exposure despite genetic drug susceptibility (5, 9-11). Persister bacteria are considered the root cause of treatment failure and relapse. Little is known of the physiologic state of persisters in M.ab pulmonary disease.

We recently completed the first study of drug-tolerant persister phenotypes in a different human mycobacterial disease, tuberculosis (5). Transcriptional profiling of the pathogen \textit{Mycobacterium tuberculosis} revealed adaptations – including down-regulation of growth, metabolism and lipid synthesis and upregulation of drug efflux pumps – that provide enhanced understanding of persistence and identify new drug targets. We are currently testing this same approach as a marker for treatment response in a tuberculosis drug trial. Development of the proposed method would enable expansion of this novel form of treatment monitoring to another important mycobacterial pathogen – M.ab pulmonary disease.
Pathogen-targeted transcriptional profiling in tuberculosis patients was possible using a method developed by collaborators at Stanford University to selectively amplify and quantify low-abundance *M. tuberculosis* mRNA from mixed human samples. After first-strand cDNA synthesis, 2,411 *M. tuberculosis* transcripts underwent controlled PCR pre-amplification using outflanking forward and reverse primers and then were quantified via multiplex qRT-PCR. We do not seek to replicate this method for M.ab because genome-wide coverage for M.ab (4,942 genes) would require design and synthesis of ~25,000 custom primers and probes which would be prohibitively time-consuming and expensive.

We therefore seek a novel method for M.ab-targeted transcriptional profiling in human specimens based on the genomic features of clinical M.ab isolates (12). We envision a method that would selectively enrich M.ab mRNA prior to RNAseq. However, non-RNAseq methods may also be feasible.

Development of such a method would enable clinical studies at National Jewish Health and elsewhere that could shift treatment paradigms. Such a tool could inaugurate a new era in which outcome prediction and treatment decisions are based on molecular markers of a pathogen’s physiologic state.
References


