Detection of Active Mycobacterium Tuberculosis Infection and Multidrug Resistance by Multiplex Suspension Arrays

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Introduction

Mycobacterium tuberculosis (M.tb) is an aerobic, gram positive, and acid-fast bacillus that leads to the complex disease manifestation of TB. Multidrug resistance (MDR) is a growing concern in endemic countries as a result of poor compliance, length of treatment, and adverse medication effects. Current diagnostic methods for detecting active M.tb infection are slow, unreliable and are not cost effective for high-risk populations. The Khan laboratory is currently developing novel strategies for studying blood based immune-biomarkers as well as intracellular signaling proteins and pathways in cell lysates using high-throughput multiplex microbead immunoassays. The goal of my summer project was aimed at optimizing the detection of active Mycobacterium tuberculosis infection and multidrug resistance by multiplex suspension arrays.

Materials

- Multiplex kits for measuring cytokines, chemokines and growth factors, for use on the Lumines platform (Lumines Corp, Austin, Tx), were obtained from BioRad, Hercules, CA. Assays were performed per manufacturer’s instructions.
- Plasma samples from healthy individuals in the same TB endemic areas were used to compare confirmed TB patient samples.
- Primers targeting variable genomic targets observed in multi-drug resistant M.tb strains were designed from genomic biomarkers for TB-MDR.

Methods

- DNA extracted from sputum samples of TB patients was used to identify TB-MDR through multiplex PCR.
- Multiplex-PCR was used to amplify seven M.tb genes in DNA isolated from sputum samples of TB patients was used to identify multidrug resistance. Multiplex-PCR was used to amplify seven M.tb genes for multi-line drugs (IV). DNA extracted from sputum samples was used to identify MDR-TB. Multiplex-PCR was used to amplify seven M.tb genes in DNA isolated from sputum samples of TB patients was used to identify multidrug resistance.

Results

- Figure 1: Optimization of the plasma antibody multiplex method using microbead suspension arrays to study blood-based biomarkers associated with active TB. P38 and Rv0934 are M.tb specific antigens used in the serodetection of TB in healthy blood plasma (HBP) against plasma from culture confirmed M.tb infected patients.

- Figure 2: First line anti-M.tb. drugs with their corresponding genes identified in MDR-M.tb strains. Multiplex-PCR probes corresponds to the available targets used in the identification of MDR-TB.

- Figure 3: Multiplex-PCR assay detection of genomic targets observed in multidrug resistant M.tb. in comparison to corresponding wild-type genomic targets. H37Rv is a wild-type M.tb strain used to differentiate between the culture confirmed, isolated MDR-M.tb.

Future Directions

- Optimize TB-MDR assay for fresh patient sputum samples.
- Continue to validate and identify more biomarkers for multidrug resistance M.tb.
- Verify results via a field validation study for sensitivity and specificity.
- Ongoing developments for commercialization of blood-based diagnostic assay.

References


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