ROLE OF REGULATORY POLYMORPHISMS IN ETHIONAMIDE RESISTANCE IN *Mycobacterium tuberculosis*

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Abstract

The treatment of tuberculosis (TB) caused by multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) is still a global challenge. A second-line anti-TB drug, ethionamide (ETH), abrogates the synthesis of mycolic acids, essential components of the *M. tuberculosis* cell wall, conferring bactericidal properties to this drug. ETH is a prodrug that is activated by the enzyme EthA, whose gene is regulated by the adjacent TetR repressor, EthR. Polymorphisms were found in the *ethA-ethR* promoter region and *ethR* in clinical isolates and in the literature. However, except for the c-11t promoter mutation, their roles in resistance have not been tested. Inactivation of *ethR* results in ETH hypersusceptibility (due to increased *ethA* expression) and *ethR* overexpression leads to ETH resistance (due to decreased *ethA* expression). Thus, mutations in *ethR* may affect *ethA* expression levels in clinical isolates, with consequences to ETH resistance. During our work, we showed through mobility shift assays that the EthR-F110L mutant, identified in a clinical isolate, binds to the *ethA-ethR* intergenic region more readily than the *wt* regulator, indicating a role for this mutation in ETH resistance. It has previously been shown that F110 is located in the EthR hydrophobic ligand binding pocket and as such is in a functionally relevant position. Some molecules, such as BDM41906, have been developed to mimic the effect of the EthR ligand. These molecules act as boosters for the activation of ETH because they cause conformational changes in the protein that diminish its interaction with DNA. In our current project, recombinant strains of *M. smegmatis*, a model for *M. tuberculosis*, containing 5 mutations in *ethR* or in the *ethA-ethR* intergenic region, identified in clinical isolates in our study and in the literature, will be generated. These regions will be cloned in an integrative plasmid such that the *mCherry* gene, which encodes a fluorescent protein, will act as a surrogate marker for *ethA* expression. Thus, once the plasmid is introduced into *M. smegmatis*, fluorescence levels will be a quantitative indicator of *ethA* transcription. Our experiments with the mutant and *wt* recombinant *M. smegmatis* strains for the *ethA-R-ethR* locus will be carried out in order to understand the role of *ethR* mutations in: *ethA* transcription, through fluorescence levels; clinical resistance to ETH, via DSTs; and the impact of BDM41906 booster on DNA binding by EthR-F110L and EthR *wt* through gel shift assays.