Abstract

Tuberculosis is one of the ten leading causes of death and ranks above HIV/AIDS as a major cause of death from infectious diseases. This deadly menace is effectuated by *Mycobacterium tuberculosis* (Mtb), an adamant intracellular pathogen. Although enormous research is being conducted globally to eradicate this disease, the varied efforts being made are jeopardized by the commendable ability of pathogen to evade the host defense strategies. A major challenge facing the world today is the ensuing resistance in Mtb to the currently used anti-TB drugs. Development of an effective treatment for TB relies on revisiting the knowledge of virulence factors and strategies employed by the mycobacterium.

An important virulence associated protein of Mtb is **Enhanced Intracellular Survival** (Eis) which has been named so based on its ability to enhance the survival of *Mycobacterium smegmatis* inside macrophages post infection. Eis protein is a GNAT family acetyltransferase which has been shown to acetylate aminoglycoside antibiotics. The acetylation and the resultant inactivation of kanamycin by Eis accounts for kanamycin resistance among the clinical isolates of Mtb. MtbEis (RvEis) is a cytosolic and cell wall associated protein which is released into the host cell cytosol upon infection. Detection of anti-Eis antibodies in the sera from pulmonary TB patients provided cues to the immune modulatory role of Eis. Eis disturbs the balance of T_H1 and T_H2 type immune response and supresses the production of IL-4 and TNF-α while stimulating the production of IL-10 and IFN-ϒ. Eis has been shown to suppress host cell autophagy, production of proinflammatory cytokines and generation of Reactive Oxygen Species (ROS). The suppression of JNK activated pro-inflammatory cytokines and Reactive Oxygen Species production is achieved by RvEis mediated acetylation and activation of JNK specific phosphatase, Dual specificity phosphatase 17 (DUSP16)/ Mitogen activated protein kinase phophatase-7 (MKP-7). Induction of IL-10 gene expression by RvEis dependent acetylation of Histone H3 protein in IL-10 promoter, leading to the increased recruitment of STAT3 and SP1 transcription factors, results in the suppression of autophagy. RvEis is a hexamer composed of two trimers and only hexameric assembly of RvEis possesses the acetyltransferase activity. *M. smegmatis* (Msm), a non pathogenic surrogate of Mtb, also expresses Eis protein (MsEis) which has been shown to acetylate aminoglycosides with the same catalytic efficiency as RvEis.

The indispensable contribution of Eis in Mtb infection involving the recruitment of host proteins encouraged an investigation to explore novel roles of Eis protein in the host. To accomplish this, we screened for the interacting partners of RvEis protein in the host

macrophages using Yeast Two-Hybrid (Y2H) approach. RvEis was used as bait and the mouse macrophage cDNA library was used as prey for Y2H screening. Y2H resulted in identification of mouse Dihydrofolate Reductase (mDHFR) as an interacting partner of RvEis protein. The interaction of RvEis and DHFR was confirmed *in vivo* by co-immunoprecipitation (Co-IP) assay and *in vitro* using GST Pull down assay. The study of interactions of RvEis with domain deletion mutants of DHFR disclosed the participation of C-terminal domain of DHFR protein in mediating its interaction with RvEis. The Co-IP experiments revealed the interaction of ectopically expressed RvEis with endogenous DHFR and the absence of DHFR interaction with MsEis confirmed the specificity of this interaction for pathogenic mycobacteria.

Confocal microscopy was used to demonstrate colocalization of the two proteins inside THP1 macrophages. Colocalization studies also highlighted a punctate localization pattern exhibited by ectopically expressed RvEis protein inside macrophages. Immunostaining of HeLa cells expressing RvEis revealed these punctaes to be associated with host cell mitochondria. We also found that RvEis interacts and colocalizes with DHFR inside the mitochondria. Inhibition of expression of mitochondrial isoform of DHFR, DHFRL1, in the infected macrophages resulted in an increased host cell autophagy and a decreased intracellular survival of Mtb. *In vitro* acetylation assays and mass spectrometric analysis established that RvEis acetylates m DHFR at K158 and K177 residues. In contrast, MsEis was inefficient in acetylating mDHFR.

The above findings open up new domains of research into the function of RvEis and DHFRL1 proteins. Mitochondrial localization of RvEis is a new addition to the study of mycobacterial Eis protein. Identification of acetylation as a novel post translational modification of DHFR promotes further investigations into the acetylation mediated regulation of DHFR expression and function. The only reported function of DHFRL1 is its contribution in the mitochondrial one carbon metabolism. Interestingly, our study demonstrates a previously unexplored role of DHFRL1 in Mtb pathogenesis. These facts highlight the significance of the current study besides developing a scope for future investigations.