

ABSTRACT

Mycobacterium tuberculosis (*M.tb*), the causative agent of tuberculosis (TB), is a major threat to human health worldwide. Ten percent of *M.tb* genome codes for unique family of PE/PPE/PE_PGRS proteins present exclusively in genus *Mycobacterium*. PE_PGRS proteins, a subset of this family has been found exclusively in the pathogenic strains of mycobacterium. Functions of most of these proteins are unexplored. Despite the reductive evolution of *M.tb* the PE/PPE/PE_PGRS family has expanded over time. Identifying the plausible roles of these proteins during the course of *M.tb* infection may help in the better understanding of pathogenesis of TB. Proteomic analysis revealed the presence of a hypothetical protein, Rv0297 (PE_PGRS5), in lung granulomas 90 days post infection. However, the biological role of Rv0297 remains unidentified. The work described in the present thesis presents the functional significance of PGRS domain of Rv0297 (Rv0297PGRS) in virulence and pathogenesis of *M.tb*.

Intrinsically disordered regions and putative Endoplasmic Reticulum (ER) localization signals have been found in Rv0297PGRS. The Rv0297PGRS domain aids in ER localization of the protein as shown by infecting macrophage cells with *M. tb* *H₃₇Rv* and by overexpressing the protein by transfection in macrophage cells followed by stimulation of the Unfolded Protein Response (UPR). The UPR activation leads to disruption of intracellular calcium homeostasis along with enhanced nitric oxide and reactive oxygen species production. The subsequent activation of the effector caspase-8 caused apoptosis of macrophages. The ER stress mediated apoptosis in macrophages by Rv0297PGRS has been found to be Toll-like receptor 4 dependent.

Tandem repeats sequence search analysis revealed that PGRS domain of Rv0297 consists of 15 Ca²⁺-binding motifs GGXGXD/NXUX that form parallel beta helix, which serve as a possible calcium binding sites. Molecular Dynamics simulations and fluorescence spectroscopy revealed Ca²⁺ dependent stabilization of Rv0297PGRS. Moreover, Rv0297PGRS enhanced TLR4 surface expression of macrophages in the presence of Ca²⁺ and leads to enhanced production of nitric oxide from macrophages in a calcium dependent manner.

M.bovis BCG_PE_PGRS5 mutant was found to be enriched in acidified phagosomes in a high-throughput study. The functional significance of Rv0297 in bacterial persistence and macrophage function modulation was also investigated. Ca²⁺ is an important secondary messenger involved in the pathogenesis of TB, mainly in phagolysosomal acidification. Rv0297PGRS affects calcium homeostasis of macrophages followed by impedance of phagolysosomal acidification process. Rv0297 has also been shown to be involved in rescuing the bacterium from oxidative and hypoxic stress employed by macrophages and augmented survivability of the recombinant bacterium. These results highlight the functional significance of this protein in *M.tb* virulence mechanism.

The results presented in this thesis implicate a hitherto-unknown role of the PGRS domain of the Rv0297 in ER stress-mediated cell death through TLR4. The fact that this protein gets expressed at later stages of lung granulomas during *M.tb* infection suggests that the bacterium possibly employs Rv0297 as its survival and dissemination strategy *via* inhibition of phagosomal acidification and apoptotic

mechanism. These findings will lead to a better understanding the pathogenic potential PE_PGRS proteins that can be targeted for therapeutic interventions.