ABSTRACT

Identification and characterization of interacting partners of Erp protein of M. tuberculosis H37Rv with Macrophage proteins

Exported repetitive protein (Erp), also known as P36, Pirg and Rv3810 is an extracellular/secreted protein of *M. tuberculosis* and has been shown to be a crucial virulence determinant. Erp protein is highly upregulated cell envelop gene under nutrient starvation condition. Disruption of *erp* gene from *M. tuberculosis* and *M. bovis BCG* resulted in a marked attenuation of virulence whereas no differences in growth characteristics were observed in axenic media between wild-type, mutant and complemented H37Rv and BCG strains (Berthet *et al.*, 1998). Attenuation of the growth of *erp*⁻ *M. tuberculosis* only in the infected macrophage and not *in vitro*, suggests that the Erp protein somehow modulates the environment within the host macrophages. Despite many efforts, the biological roles of this virulence factor during host infection remain elusive.

In order to gain insight into the function of the secretory Erp protein within the host, we have explored interacting partners of Erp from mouse macrophage library. The Erp protein devoid of its signal sequence (ΔssErp) was used as a "bait" to fish out the "prey" protein encoded by mouse macrophage cDNA library using yeast two hybrid assay. After analysis, homology search and sequencing, nine putative interacting host partners of ΔssErp were identified. One of the identified putative interacting partners of ΔssErp was LDHA. ΔssErp and LDHA interact directly with each other under *in vitro,* as demonstrated by GST pull down assays and also under *in vivo* conditions as shown by Co-immunoprecipitations. The deletion of PGLTS, COOH and SRC domains of Erp protein has no effect on the interaction with LDHA whereas deletion of amino terminus abrogates its interaction with LDHA. This result is consistent with our finding that ΔssErp resides in the cytoplasm whereas the ΔNH2Erp localizes to the nucleas. Furthermore, a doublet of ΔssErp was observed in the immunorecipitates and in the lysates of cells upon over-expression, suggesting posttranslational modification(s) of ΔssErp protein in the mammalian cells. Also, increased expression levels of LDHA were observed when it was co-transfected along with ΔssErp. Unlike ΔssErp of *M. tuberculosis*, the ΔssErp protein from non-pathogenic *M. smegmatis* does not interacts with LDHA and thus render this interaction specific to pathogenic strain. Confocal studies revealed that both Erp and ΔssErp localized in the cytoplasm of CHOK1 and THP1 macrophage whereas ΔNH2Erp localized predominantly in the nucleus. The Erp

and ΔssErp protein co-localized with LDHA in the cytoplasm whereas ΔNH2Erp being nuclear in localization did not co-localize with LDHA. *In-vitro* activity assay revealed 7 fold increase in the enzymatic activity of GST-LDHA after treatment with purified recombinant ΔssErp protein whereas no significant change in the activity was observed after treatment with purified recombinant ΔNH2Erp protein. Moreover, three fold increase in LDHA activity of THP1 was observed after treatment with purified ΔssErp whereas ΔNH2Erp had no significant effect. Further, LDHA, SPT2 and Erp appeared to indicate that they are associated in a common pathway related to metabolism of sphingosine.

In total, we found that Erp protein of *M. tuberculosis* interacts with LDHA and modulates its activity. Further studies are required to understand the mechanism involved in the Erp mediated upregulation of LDHA activity and to understand the significance of the Erp and LDHA interaction in the intracellular survival of *M. tuberculosis* within the host. Also, the post translational modification(s) of Erp in the mammalian system needs to be identified and their role in the regulation of the interactions and modulation of host cell biology needs to be explored further.