Understanding the role of macrophage substrates of *Mycobacterium tuberculosis* secreted Protein Kinase G in the context of pathogenesis

Abstract :

Tuberculosis causing agent Mycobacterium tuberculosis (Mtb), by secreting several virulence factors, engages a plethora of host factors to establish its niche inside macrophages. Protein kinase G (PknG), a eukaryotic-like Serine/Threonine kinase, is one such secreted virulence factor essential for mycobacterial survival within the macrophage. The phosphorylated macrophage substrates of PknG and their subsequent role in mycobacterial pathogenesis remained undeciphered. Using comparative phosphoproteomic screening, we identified a few macrophage substrates that were directly phosphorylated by PknG, of which we sought to characterize the roles of Silencer of death domain (SODD), 26S proteasome non-ATPase regulatory subunit 9 (PSMD9) and Coronin1(Cor1) in context of mycobacterial pathogenesis during early hours of infection. PknG-mediated phosphorylation at threonine 405 residue resulted in irreversible binding of phosphorylated SODD with TNFR1 and thereby prevented the Caspase 8 mediated extrinsic pathway of apoptosis. Another identified substrate PSMD9, upon being phosphorylated by PknG at serine 2 residue, lowers cellular TNFα production and overexpresses Protein A1. Overexpressed Protein A1 was found to sequester pro□apoptotic Bax, Bid and thereby hinder the intrinsic pathway of apoptosis. Moreover, PknG phosphorylated Cor1 induced intracellular rise in cAMP and concomitant Slingshot phosphatase mediated activation of Cofilin1(Cof1) causes depolymerization of F-actin tracts that hinders the mycobacterial phagosome maturation and augments intramacrophage mycobacterial survival. Taken together, this work with mycobacteria□infected wild type, CRISPR/Cas9 mediated substrate knockout macrophages and mice infections for the first time establishes the macrophage substrates of the mycobacteria□secreted kinase PknG. The study also deciphers the role of these substrates in subverting the altruistic death of infected macrophages and hindering phagosome maturation.

Key Words: PknG, Mycobacteria, SODD, PSMD9, Cor1, Phosphorylation, Apoptosis, Phagosome maturation