

Eukaryotic-like Protein Kinases as Targets for the Development of Compounds to Combat *Mycobacterium tuberculosis*

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Abstract

Mycobacterium tuberculosis, the causative agent of tuberculosis is one of the most successful pathogens known today. An important virulence factors for pathogenic mycobacteria was recently identified to be a eukaryotic-like serine/threonine protein kinase, termed protein kinase G. Specific inhibitors of protein kinase G activity lead to a rapid degradation of mycobacteria inside host cells. We are currently investigating the cellular mechanisms that are targeted by protein kinase G, in order to better understand mycobacterial pathogenicity.

Introduction

The hallmark of pathogenic mycobacteria is their ability to block delivery to lysosomes, thereby surviving within the macrophage phagosome. In recent years, several molecules were shown to play a role in the modulation of phagosome-lysosome fusion. In addition to cell-wall lipids, mycobacterial proteins are also involved in the arrest of phagosome maturation (Pethe et al., 2004; Walburger et al., 2004). A comprehensive screen revealed several proteins potentially involved in this process. Disruption of these proteins led to defects in preventing acidification of phagosome and attenuation of

intracellular survival. An independent search for mycobacterial proteins involved in signal transduction reactions with a potential to modulate phagosome-lysosome fusion suggested that mycobacterial kinase(s) might be involved in modulating phagosome-lysosome fusion. Treatment of *Mycobacterium bovis* BCG (Bacillus Calmette-Guerin) with a eukaryotic protein kinase C α (PKC α) inhibitor prior to infection of macrophages leads to an immediate transport of the mycobacteria to lysosomes suggesting the involvement of a PKC α -like kinase activity in blocking lysosomal delivery. Interestingly, eleven different eukaryotic-like protein kinases are encoded by pathogenic mycobacteria (Av-Gay and Everett, 2000; Cole et al., 1998), and a Clustal-W alignment of these kinases with mammalian protein kinase C α revealed a closest homology to mycobacterial protein kinase G (PknG). Whereas most of the mycobacterial kinases are predicted to be transmembrane proteins with an intracellular kinase domain, two of these, protein kinase G and protein kinase K lack such a transmembrane segment. Interestingly, protein kinase G is retained in the downsized genome of the obligate intracellular pathogen *M. leprae* that carries the minimum set of mycobacterial genes necessary for intracellular survival. This suggests that protein kinase G encodes an important function for mycobacterial survival in host (Cole et al., 2001).

Protein Kinase G and Mycobacterial Survival

To analyze a role for protein kinase G in mycobacterial survival inside host cells, protein kinase G was disrupted in *M. bovis* BCG using phage-mediated homologous recombination. The resulting *M. bovis* BCG *pknG*-deleted mutant was immediately transferred to lysosomes and the mutant mycobacteria were unable to survive inside

macrophages (Walburger et al., 2004). Subcellular localization studies suggest that protein kinase G is present in the phagosomal lumen as well as in the cytosol of infected macrophages suggesting that pathogenic mycobacteria actively secrete protein kinase G. These data suggest that protein kinase G is a mycobacterial virulence factor that is delivered into the cytosol of host cells and may interfere with the regulation of phagosome-lysosome transfer.

Notably, protein kinase G lacks signal sequences required for its translocation via the classical Sec secretion system. However, many pathogenic microbes deliver virulence factors into host cells by specialized secretion mechanisms, such as the type III secretion systems (Cornelis, 2002) well as conjugation systems (Cascales and Christie, 2003). Also pathogenic mycobacteria possess specialized secretion systems required for their virulence, such as proteins encoded in the RD1 (Region of Deletion in *M. bovis* BCG) region of the *M. tuberculosis* genome. These proteins encode a secretion apparatus that is essential for the secretion of major T-cell antigenic proteins, ESAT-6 and CFP-10, which have no detectable signal sequence (Pym et al., 2003; Stanley et al., 2003). Whereas the RD1 region is absent in the genome of *M. bovis* BCG, five RD1-homologous regions are distributed among different loci in the genome of *M. tuberculosis* (Gey Van Pittius et al., 2001). Whether or not protein kinase G is secreted via an RD1-like secretion machinery remains to be investigated.

Inhibition of Protein Kinase G

The activity of protein kinase G to block lysosomal delivery suggests that this kinase might be a valuable target in the development of compounds that could induce

mycobacterial death inside macrophages. A screen for protein kinase G inhibitors identified a tetrahydrobenzothiophene, which specifically inhibits the kinase activity of protein kinase G. When added to infected macrophages, this compound induces the fusion of phagosomes to lysosomes and mediates killing of mycobacteria inside macrophages (Walburger et al., 2004). One possible advantage of targeting protein kinase G is that this will allow the macrophage to carry out its natural antibacterial activity of redirecting intracellularly surviving mycobacteria to lysosomes. Also, by targeting a secreted molecule such as protein kinase G, transport of antimycobacterial compounds through the extremely impermeable mycobacterial cell wall can be circumvented, greatly improving the accessibility of the compounds to their target.

The downstream targets of protein kinase G are currently unknown. It is however interesting to note that mammalian protein kinase $C\alpha$, besides being itself associated with phagosomal membranes, modulates the association of p57, the human homolog of coronin 1/TACO, to phagosomes (Ferrari et al., 1999; Gatfield et al., 2005; Itoh et al., 2002). Given the importance of protein kinase $C\alpha$ in membrane trafficking events and its possible involvement in phagosome maturation, protein kinase G may modulate signaling pathways involved in phagolysosome biogenesis by competing with host protein kinase $C\alpha$ in binding to its substrate(s).

Conclusion

[Recent work has revealed molecules being actively secreted by pathogenic mycobacteria within macrophages to exert phagosomal maturation.](#) In a search for

mycobacterial factors that allow these pathogens to avoid lysosomal destruction, a eukaryotic-like serine/threonine kinase, termed protein kinase G (PknG), was discovered. Protein kinase G inhibits maturation of the mycobacterial phagosome, thereby allowing pathogenic mycobacteria to survive within macrophage phagosomes (Walburger et al., 2004). In macrophages, mycobacterial mutants lacking PknG are immediately transferred to lysosomes and destroyed whereas they [are viable outside the host cells](#) (Nguyen et al., 2005). PknG is secreted within the macrophage cytosol and its activity is inhibited by specific kinase inhibitors opening the prospect of developing anti-mycobacterial drugs that promote the innate bactericidal activity of the macrophages rather than directly attacking mycobacterial targets *per se*. [By targeting](#) a secreted protein such as [PknG the](#) poor transport of antibiotics through the extremely impermeable mycobacterial cell wall [could be circumvented](#), thereby [greatly improving the accessibility of the](#) [drugs to their](#) [target](#)

Supprimé : Targeting

Supprimé : PknG also circumvents

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Supprimé : compounds to their target.*

This abstract is based on: (Nguyen and Pieters, 2005)

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