

Anti-cancer drug Tamoxifen interferes with *Mycobacterium tuberculosis* PhoPR mediated signaling and inhibits mycobacterial growth

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Abstract.

Two-component signaling (TCS) systems empower all bacteria, including intracellular pathogens like *Mycobacterium tuberculosis* (*M. tb*) to regulate key pathways governing growth, physiology and virulence. Amongst all *M. tb* TCS systems, PhoPR and DevRS have been studied extensively for their roles in regulating persistence and virulence. Here, we report that besides its cognate response regulator PhoP, the PhoR sensor kinase displays several non-cognate interactions that augment its role in pathogenesis. We demonstrate that PhoR phosphorylates the DevR response regulator and furthermore, is itself subjected to *O*-phosphorylation by PknK, a Ser/Thr protein kinase (STPK), connecting TCS pathways with “eukaryotic-like” STPK driven phosphosignaling. This intersection of non-canonical regulatory pathways and the coregulation of PhoP and DevR regulons make *M. tb* PhoR a potentially attractive drug target. We rationalized that disruption of PhoPR signaling cascade and the resulting dysregulation may result in decreased virulence of *M. tb*. We tested this hypothesis by performing a high-throughput screen for compounds that inhibit autophosphorylation of PhoR sensor kinase. Screening of pharmacologically active, small molecule libraries yielded 11 potential inhibitors, of which one compound, Tamoxifen was able to attenuate PhoR autophosphorylation at micromolar concentrations *in vitro* and *in vivo*. Tamoxifen not only inhibited growth of *Mycobacterium bovis* BCG in culture but also interrupted PhoPR-mediated downstream signaling. Quantitative expression analysis revealed suppression of target gene, *aprA* under acidic conditions. Our findings highlight TCS sensor kinases as promising drug targets and underscore the applicability of clinically relevant anti-cancer drug tamoxifen as a repurposed anti-TB drug.