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The Identification of Phosphatases and Phosphatase Inhibitors for Antibacterial Intervention

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With the emergence of multidrug-resistant bacteria, it is imperative to develop novel intervention strategies. Current antibiotics target biochemical pathways in pathogens rather than the host. In the past we identified PKB/Akt1 and PKB/Akt1 inhibitors that prevent intracellular growth of pathogens like Salmonella and Mycobacterium tuberculosis. If kinases are involved in intracellular survival of pathogens, phosphatases are likely to be involved as well. We therefore investigated if phosphatases are involved in the survival of intracellular pathogens by siRNA screening of the human phosphatome with high throughput FACS. This screen revealed several phosphatases selectively inhibiting or increasing intracellular growth of Salmonella. The phosphatases increasing intracellular growth upon knockdown are almost all proteins that normally inactivate PKB/Akt. Upon knockdown these phosphatases PKB/Akt1 stays active, confirming the importance of activated PKB/Akt for survival of the pathogen. However inhibition of intracellular growth is our goal as opposed to stimulation. Several phosphatases involved in the down regulation of MAP kinase signalling were identified that upon knockdown decreased intracellular growth of Salmonella. These phosphatases are potential drug targets to treat infections with intracellular pathogens. Therefore a phosphatase inhibitor library was synthesized and screened for inhibition of intracellular growth with high throughput FACS. Several compounds were identified that reduced intracellular Salmonella growth. Whether these compounds inhibited the phosphatases identified in the siRNA screen was our next question. We therefore produced several recombinant phosphatase domains of the proteins identified in our siRNA screen and tested the selected compounds for their potential to inhibit these phosphatases. Several of the compounds were potent inhibitors of our identified phosphatases in the nanomolar range. This genetics and chemical genetics approach thus identifies novel phosphatase inhibitors for antibacterial intervention strategies and corresponding host targets.

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