Technical Profile: DarTG

Project Title

Screening for the first inhibitors of the novel toxin-antitoxin system DarTG

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Goal of research

Infections caused by Mycobacterium tuberculosis and Gram negative bacilli (e.g. E. coli and Klebsiella pneumoniae) are important causes of morbidity and mortality worldwide. The rise of drug resistant pathogenic bacteria makes it imperative that we investigate novel areas of bacterial biology to identify novel drug targets and develop new antimicrobials.

We identified a DarG, a component of DarTG TA system, as a potential drug target for the treatment of pathogens expressing this operon (as explained above). The goal of this project is to develop the first-in-class inhibitors/lead compounds against Mycobacterium tuberculosis DarG and the closely related DarG antitoxins in other pathogens.

Technical Description

Toxin-antitoxin (TA) systems are sets of linked genes that encode a toxin and cognate antitoxin. Many prokaryotes harbour TA systems on their genomes, and these have a variety of important functions including regulation of gene expression and growth, biofilm formation, and the formation of bacterial persisters (Page and Peti, Nat Chem Biol, 2016). Consequently, toxin-antitoxin systems have been suggested as promising targets for inhibitors in treatment of infectious diseases. Recently, we discovered a DarTG, a novel toxin antitoxin (TA) system that operates via reversible ADP-ribosylation of DNA in a number of bacterial organisms (Jankevicius et al, Mol Cell, 2016). ADP-ribosylation is a chemical modification of macromolecules that occurs in all domains of life, and has been extensively studied as a post-translational modification (PTM) of proteins in eukaryotes (Barkauskaite et al, Mol Cell, 2015). We propose to target this system for the development of new antibiotic agents.

ADP-ribosylation is a chemical modification of macromolecules that occurs in all domains of life, and has been extensively studied as a post-translational modification (PTM) of proteins in eukaryotes (Barkauskaite et al, Mol Cell, 2015). ADP-ribosylation occurs via transfer of an ADPribose moiety from NAD+ onto molecular targets, usually proteins, through the activity of ADPribose transferase enzymes. This PTM regulates many critical processes in eukaryotes including gene expression, DNA repair, protein quality control, and cell fate. Within eukaryotes, the final step in removal of ADP-ribosylation is mediated by the hydrolytic activity of macrodomain containing proteins, which reverse this key PTM. Given their significance in human biology and disease, and potential as therapeutic targets (most notable example is targeting ADP-ribosylation systems in cancer treatment), much is known about the activity and structure of eukaryotic ADPribose transferases and macrodomain proteins.

In contrast, remarkably little is known about the mechanisms and roles of ADP-ribosylation and/or its reversal in prokaryotes. Recently we discovered a DarTG, a novel toxin antitoxin (TA) system that operates via reversible ADP-ribosylation of DNA in a number of bacterial organisms (Jankevicius et al, Mol Cell, 2016). TA systems were first described as addiction systems on bacterial plasmids that mediate killing of daughter cells lacking the plasmid. It is now known that many prokaryotes harbour TA systems on their genomes, and these have a variety of important functions including regulation of gene expression and growth, biofilm formation, and the formation of bacterial persisters (Page and Peti, Nat Chem Biol, 2016). Consequently, toxin-antitoxin systems have been suggested as promising targets for inhibitors in treatment of infectious diseases.

TB kills more people than any other single infectious disease with approximately 10 million cases of active disease each year (WHO Global tuberculosis report 2016). Present antimycobacterial drugs require administration as a panel of typically four antibiotics for two months followed by four further months with two drugs. Inappropriate drug regimens and poor adherence have led to the development of multiple drug resistance MDR (resistance to the two most powerful drugs, isoniazid and rifampicin). Over half a million infections per year are now MDR and a growing proportion are extensively drug resistant (XDR, resistant to isoniazid, rifampicin, any fluoroquinolone and any aminoglycoside) (WHO Global tuberculosis report 2016) (Zumla et al, Lancet Respir Med, 2015). Thus it is imperative that new antibiotics are developed with novel targets and shorter treatment times but only two new drugs have been licensed for treatment of tuberculosis in the last 50 years (bedaquiline and delamanid). DarTG encodes a toxin-antitoxin system which occurs in all members of the M.tuberculosis complex and provides control over DNA replication through ADP-ribosylation of ssDNA. The DarG de-ADP-ribosylase is an attractive target for drug development because its activity is essential (see below) for bacterial growth and it is implicated in control of slow bacterial growth rate during persistent infection.

DarTG is found in many gram negative pathogens. Gram negative pathogens including E. coli, Serratia, and Klebiella are recognised as major superbugs which are often resistant to most first line antibiotics. Patients in hospital are particularly susceptible to these bacteria, and resistance against carbapenem antibiotics means that toxic, less effective therapies (such as colistin) are being used to treat patients in many instances.