Overcoming intrinsic multidrug resistance to discover and expand the repertoire of antibiotics active against mycobacterial pathogens

Main results and findings

Part I  Ligation of mycolic acids to structural components of the mycobacterial cell wall generates a hydrophobic, impermeable barrier that provides resistance to toxic compounds such as antibiotics. Secreted proteins FbpA, FbpB and FbpC attach mycolic acids to arabinogalactan, generating mycolic acid methyl esters (MAME) or trehalose, generating α,α’-trehalose dimycolate (TDM; also called cord factor). Our studies of *Mycobacterium smegmatis* showed that:
- disruption of *fbpA* did not affect MAME levels but resulted in a 45% reduction of TDM.
- the *fbpA* mutant displayed increased sensitivity to both front-line tuberculosis-targeted drugs as well as other broad-spectrum antibiotics widely used for antibacterial chemotherapy.
- the irregular, hydrophobic surface of wild-type *M. smegmatis* colonies became hydrophilic and smooth in the mutant.
- while expression of *M. smegmatis* *fbpA* restored defects of the mutant, heterologous expression of the *Mycobacterium tuberculosis* *fbpA* gene was less effective.
- a single mutation in the *M. smegmatis* FbpA esterase domain inactivated its ability to provide antibiotic resistance.

To conclude, these data show that production of TDM by FbpA is essential for the intrinsic antibiotic resistance and normal colonial morphology of some mycobacteria and support the concept that FbpA-specific inhibitors, alone or in combination with other antibiotics, could provide an effective treatment for tuberculosis and other mycobacterial diseases.

Part II  Here a system is described that coordinates resistance to drugs that have penetrated the envelope, allowing mycobacteria to tolerate diverse classes of antibiotics that inhibit cytoplasmic targets. This system depends on *whiB7*, and its characterisation revealed that:
- *whiB7* is a gene that pathogenic *Mycobacterium* shares with *Streptomyces* (phylogenetically related genus known as the source of diverse antibiotics).
- in *M. tuberculosis*, *whiB7* is induced by subinhibitory concentrations of antibiotics (erythromycin, tetracycline and streptomycin) and by exposure to fatty acids that pathogenic *Mycobacterium* species may accumulate internally or encounter within eukaryotic hosts during infection.
- *whiB7* null mutants (*Streptomyces* and *Mycobacterium*) are hypersusceptible to antibiotics in vitro.
- *M. tuberculosis* is also antibiotic sensitive within a monocyte model system.
– gene expression profiling analyses demonstrate that \textit{whiB7} transcription determines drug resistance by activating expression of a regulon including genes involved in ribosomal protection and antibiotic efflux.

To conclude, components of the \textit{whiB7} system may serve as attractive targets for the identification of inhibitors that render \textit{M. tuberculosis} or multidrug-resistant derivatives more antibiotic-sensitive.

**Part III** Characterisation of WhiB7 protein (first step for the design of WhiB7 inhibitors) revealed that:
– this transcriptional regulator has (like other members of the WhiB family) a specific tryptophan/glycine-rich region and four conserved cysteine residues; in addition, it has a DNA-binding domain (AT-hook) at its carboxy terminus.
– soluble forms of a number of WhiB family proteins were obtained by overexpression and labelled by the isotopes $^{15}$N and $^{13}$C for NMR analysis.
– when reconstituted under anaerobic, reducing conditions by iron(II) and sulphide, all proteins formed a complex with an oxygen-sensitive iron-sulphur cluster (FeS cluster).
– the NMR spectra were obtained showing that these proteins were largely unstructured both in apo and reconstituted forms.

To conclude, WhiB-like proteins, without a further binding partner, are in solution largely unstructured both in their apo and reconstituted form with a FeS cluster.

**Publications of the NRP 49 project**

Nguyen LS, Jensen PR, et al.

\textbf{WhiB7 dependent multidrug resistance in \textit{Mycobacterium}.}


Nguyen L, Thompson CJ.

\textbf{Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm.}


Nguyen L, Chinnapapagari S, Thompson CJ.

\textbf{FbpA-dependent biosynthesis of trehalose dimycolate is required for the intrinsic multidrug resistance, cell wall structure, and colonial morphology of \textit{Mycobacterium smegmatis}.}


\textbf{Ancestral antibiotic resistance in \textit{Mycobacterium tuberculosis}.}


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