ENGINEERING BACTERIAL NITROREDUCTASES FOR ANTICANCER GENE THERAPY AND TARGETED CELL ABLATION

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Bacterial nitroreductases are members of a diverse family of oxidoreductase enzymes that can catalyse the bioreductive activation of nitroaromatic compounds, including anti-cancer and antibiotic prodrugs. Nitroreductases have diverse applications in medicine and research, including anti-cancer gene therapy and targeted ablation of nitroreductase-expressing cells in transgenic zebrafish to model degenerative diseases. Research in these fields to date has focused almost exclusively on the canonical nitroreductase NfsB from *Escherichia coli* (NfsB_Ec), which is a relatively inefficient choice for most applications. By exploring alternative nitroreductase candidates from a variety of bacterial species, in concert with enzyme engineering to fine-tune specific activities, we have generated improved prodrug-activating enzymes. The nitroreductase NfsB from *Vibrio vulnificus* (NfsB_Vv) has been central to our efforts, and following solution of its crystal structure, was selected as a scaffold for directed evolution via site-saturation mutagenesis. By applying library screening strategies that involved rounds of both positive and negative selection, several mutants that displayed improved activity with a promising next-generation cancer prodrug were identified. In parallel work, an engineered NfsB_Vv variant from the same library was found to be substantially improved in activation of the antibiotic prodrug metronidazole, which is widely used for targeted cell ablation in transgenic zebrafish. Current methods of ablation employing NfsB_Ec require high, near lethal concentrations of metronidazole to achieve total ablation of nitroreductase-expressing cells. A transgenic zebrafish line expressing a lead NfsB_Vv variant was generated and we found we could achieve robust ablation of nitroreductase-expressing cells at a 100-fold reduced metronidazole concentration compared to the NfsB_Ec line (0.1 mM challenge for 24 hours vs 10 mM challenge for 48 hours respectively). The identification of these superior nitroreductase variants offers improved tools for researchers aiming to achieve targeted cell ablation in either a cancer therapy or degenerative disease-modelling context.