DISCOVERING AND ENGINEERING NOVEL PRODRUG ACTIVATING AND DETOXIFYING ENZYMES TO IMPROVE TARGETED CELL ABLATION

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The ablation of specific cells in regenerative species has been used to model degenerative diseases and cellular regeneration. Zebrafish (*Danio rerio*) are a particularly useful organism for targeted cellular ablation studies due to their ability to regenerate tissues including the fin, heart, and eye. A prominent ablation technique involves the expression of prodrug-converting enzymes in target tissues driven by cell-type specific promoters. Nitroreductases, which can convert the prodrug metronidazole to a toxic and cell-entrapped form, are widely used for this purpose. However, many tissues cannot be defined effectively by a single tissue-specific promoter. A desirable alternative scenario is to only target a subset of cells within a group that express a particular promoter. We propose that if non-target cells can be distinguished from target cells by a second promoter, then it may be possible to defend them by introducing metronidazole resistance genes under transcriptional control of that second promoter (Fig. 1). The aim of this project has been to discover transferrable metronidazole resistance genes using a functional metagenomic approach. For this, bespoke high-expression metagenome libraries have been generated in *Escherichia coli* using total DNA extracted from soil and gut metagenomes. Plating these libraries on metronidazole-containing media has enabled positive selection for metronidazole resistance elements. Resistance genes have then been engineered using directed evolution to improve their metronidazole-protective activity. Parallel avenues of research are also being undertaken to improve targeted cellular ablation in zebrafish, including testing of a split nitroreductase system to achieve ablation of cell types defined by two distinct promoters, and a multiplex nitroreductase-prodrug system to enable selective targeting of multiple cell/tissue types in a single organism.

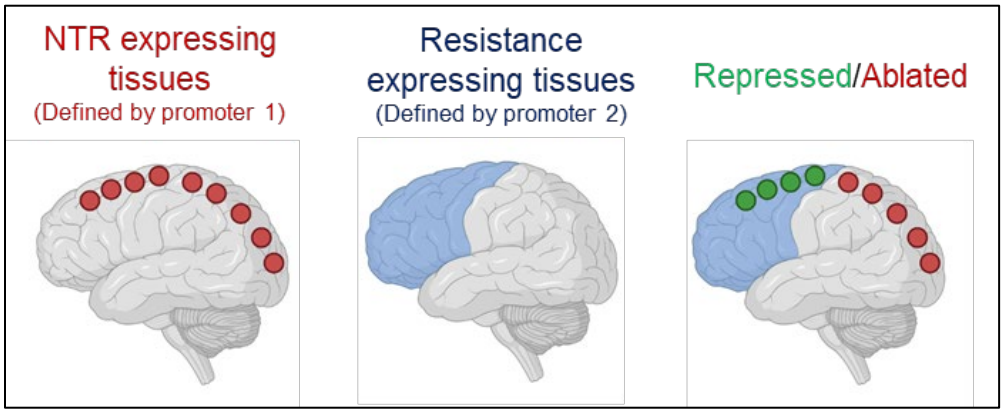

Figure 1: Graphical representation of the proposed metronidazole resistance-based cell ablation repressor system.