USE OF POSITIVE SELECTION METHODS FOR DISCOVERY AND IMPROVEMENT OF NITROREDUCTASE ENZYMES FOR CANCER GENE THERAPY

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Bacterial nitroreductases are members of a diverse family of oxidoreductase enzymes that are capable of activating nitroaromatic compounds, including anticancer prodrugs such as CB 1954 and PR-104A. This capability is useful in the anti-cancer gene therapy strategy known as gene-directed enzyme prodrug therapy (GDEPT), which involves the killing of tumour cells through activation of an inert prodrug to its cytotoxic form, following selective delivery of a genetically encoded prodrug-converting enzyme to cancerous tissues. A key limitation in nitroreductase-based GDEPT has been the inability to rapidly and non-invasively determine vector localisation and gene delivery prior to systemic administration of prodrug. To address this we have developed dual-purpose nitroreductases that exhibit the ability to efficiently activate both GDEPT prodrugs and next-generation radioisotope-labelled PET imaging probes, in a manner that renders the probes temporarily cell-entrapped for detection using a PET scanner. This capability places greater control of the therapy in the hands of the clinician, and will facilitate clinical development of this treatment. One key focus has been the engineering of more efficient enzymes using both random and targeted mutagenesis strategies. A complementary strategy has been the discovery of novel nitroreductases through the screening of metagenomic fragments of DNA from the unculturable bacteria present in New Zealand soil. To enable efficient screening of these libraries, we have developed an array of genetic and biochemical tools for the rapid selection of active nitroreductases. Here we have investigated the effectiveness of these different approaches for improving nitroreductase activity, and demonstrate their utility in improving activity with specific target substrates including next- generation prodrugs and PET imaging probes.