**BBSRC MIBTP Studentship Project**

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| **Project Title** | Structural-guided PROTAC targeting of BMX to modulate apoptotic sensitivity in disease |
| **Project Summary** | |
| What determines at the molecular level whether a cell lives or dies? Regulation of the cellular life–death switch is essential in healthy cells for normal foetal development and for the clearance of damaged cells. Aberrant regulation of cell death has been implicated in numerous human diseases, including AIDS, degenerative diseases, autoimmune diseases and particularly cancer, resulting in an unmet need to understand the fundamental mechanisms which exist in cells to regulate apoptosis. Commitment to apoptosis and regulation of the cell life-death switch is dependent largely on protein-protein interactions and the formation of multi-protein complexes, yet the regulation of these complexes is still not fully elucidated.  One crucial member of the apoptosis machinery is BAK. Once activated, BAK permeabilises the mitochondrial membrane, releasing factors which commit a cell to death. BH3 mimetics have been developed, which inhibit anti-apoptotic BCL-2 proteins allowing activation of BAK and BAX. Some of these agents now have FDA approval, but they have on-target toxicity resulting in severe limitations to their use in clinical settings (1).  The unmet need is still for a drug that promotes apoptosis, without significant side effects.  In ground-breaking work, we identified the first and obligatory step in BAK activation (2), which is regulated by tyrosine kinase BMX. BMX phosphorylates BAK on residue Y108, and locks BAK in an inactive conformation making it insensitive to activators, blocking apoptosis. Removal of BMX protein via RNA interference has been shown to sensitize cells to apoptotic cell death (2).  Our therapeutic hypothesis therefore is that removal of BMX will reverse the apoptotic block observed in apoptosis resistance cells and result in increased levels of de-phosphorylated BAK, re-sensitising cells to a wide range of chemotherapeutic drugs, radiation therapies and other existing cancer treatments. This innovative approach will enable a therapeutic window to be created, which can be exploited to increase cell killing with reduced doses of current therapies. Not only making these existing agents more efficacious, but has the potential to reduce side effects and increase quality of life during therapy.  PROteolysis Targeting Chimeras (PROTACs) regulate protein function by degrading target proteins instead of inhibiting them. The removal of the protein via proteolysis can have many advantages over small molecule inhibition, such as enhanced selectivity for the target protein. This project will used a structure-guided approach to design, synthesis and test PROTACs against BMX to potentiate apoptotic cell death in cancer cells.  AIM 1: Utilise structural biology techniques including X-ray crystallography and NMR to determine the structure of tyrosine kinase BMX and use this and previously determined structures of individual domains of BMX to design and synthesise PROTAC molecules  AIM2: Characterise binding of the PROTAC molecules to BMX both in vitro using structural techniques (x-ray crystallography and NMR) and biophysical methods to detect and analysed binding to recombinant proteins  AIM3: Characterise the PROTAC molecules in cell-based models. Initially the molecules will be characterised in paired cell line model +/- BMX overexpression to determine the effect on cell growth, sensitivity to induction of apoptotic cell death and cellular morphology. These studies would then be extended to a panel of normal and cancer prostate cell lines to determine if a therapeutic window can be developed in these cell lines with the combination of PROTAC molecules in combination with chemotherapeutics routinely used to treat prostate cancer.  Techniques that will be undertaken during the project   * Synthetic organic chemistry/ Medicinal chemistry * Recombinant protein expression and purification * Biophysical techniques including …… * Cell culture * Flow cytometry (FACS) analysis * Cell survival and proliferation assays   BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology | |
| **References** | |
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