Introduction

Every year, there are approximately 9,000 new cases of Small Cell Lung Cancer (SCLC) in the UK and despite standard treatment, there is a low overall survival, approximately 7% at 5 years ("Lung Cancer…", 2023). This is due to the development of drug resistance in the malignant cells which results from phosphorylated hnRNPA1 binding to XIAP and Bcl-xl leading to anti-apoptotic, survival signals. UP1, the N-terminal DNA/RNA binding domain of hnRNPA1, is one therapeutic target that has been identified. Locating compounds that can bind to UP1 and selectively inhibit the binding of UP1 to XIAP and Bcl-xl is significant in overcoming mechanisms of drug resistance. It is expected that when utilised together with standard chemotherapy treatments, antitumor responses should be attained and overall survival should increase.

Aims of the project

The project is intended to optimise the conditions for small molecule soaking in UP1 microcrystals. UP1 is a model system for drug screening in microcrystals. It has been shown that X-ray diffraction can be recorded with similar quality from UP1 crystals and microcrystals. Soaking in microcrystals also comes with many advantages compared to large crystals, as it allows lower concentrations of ligand and shorter incubation times, therefore minimizing damage to the target protein.

Methods

UP1 protein production

Competent *E. coli* BL21 cells were transformed using UP1 wildtype plasmids. The *E. coli*, now containing a lac operon, was then incubated before being signalled using IPTG to express proteins. The cells were then incubated overnight for optimal protein production.

Immobilized metal affinity chromatography

To purify the expressed protein, it was concentrated and then purified using a nickel column.
The recombinant protein contained a His-tag which was bound to the nickel through electrostatic attraction. Purification was then completed through elution with imidazole.

Ion exchange chromatography

Purifying the protein with further specificity was conducted through an ion exchange column. To solve the issue of slight protein contamination, an ion exchange purification was done with two MES-based buffers, one without NaCl and one containing NaCl.

Crystallisation of UP1 microcrystals

There were numerous attempts to crystallise UP1 into microcrystals to try and optimise small molecule soaking conditions. This consisted of using buffers with different ratios of UP1 to crystallisation buffers, using buffers with varying concentrations of Polyethylene glycol 4000 (PEG) and 2-Methyl-2,4-pentanediol (MPD), using different pHs and using varying concentrations of protein.

Fixed target serial crystallography

This technique is the collection of data from several microcrystals and the merging of that data. It utilises a silicon chip that allows 25,600 potential positions to record data from in 10 minutes (Diamond Light Source).

Results and Discussions

During the summer studentship, the crystallisation of UP1 into microcrystals was successful. However, when attempting to perform small molecule soaking in the microcrystals at the Diamond Light Source synchrotron, we were unable to properly recreate UP1 microcrystals which resulted in an excess of

protein precipitate. While at the I24 beamline, we failed to obtain any results of significance regarding the soaking of small molecules as the x-ray crystallography of the microcrystals had no relevant hits.

Although we were not able to complete our overall aim, we did manage to optimise the process of protein expression and crystallisation. The conditions that we found were optimal for the production of microcrystals were either the 10ul of UP1 with 10ul of 20% (PEG and MPD) buffer or the 16.7ul UP1 with 13.3 ul 18% (PEG and MPD) buffer, both with Tris at pH8.5 and 10mg/ml UP1 concentration. We also found that 22% (PEG and MPD) buffer also worked, however, made crystals that were too big for our purposes.

Future directions

The research done could be taken further if the crystallisation is successful when performing X-ray crystallography, as the optimisation of small molecule soaking could be done and researched further. Along with this, it would aid in discovering and optimising a therapeutic target for Small Cell Lung Cancer by reducing the role that hnRNPA1 plays in the illness as we could see what small molecules can bind to it with high affinity.

Departures from original plan

When purifying the protein, it didn't purify as we planned and as such we had to express the proteins again from the start midway through the project. Along with this, we had numerous attempts of crystallising the protein resulting in a clear solution without any crystals. We eventually managed to crystalise our protein, realising that something in the crystallisation buffer was at fault as we used an older buffer that managed to work. We had to constantly adapt and adjust our conditions as we faced various setbacks with crystallising. However, there was not enough time to redo the final part of our project and complete the overarching goal.

Value of studentship

Working with Dr. Prischi, I learned many skills in the lab, such as cell transformations, culturing, protein expression, SDS-PAGE electrophoresis, chemically competent cell creation and chromatography. I have also improved other skills that can be applied to other areas of my life, including time management (from having many time-dependent steps in our experiments) and communication from working with my supervisor. By participating in this project and completing it, I have been able to experience what the field of research would be like for myself. This has further motivated and strengthened my resolve to pursue a career as an academic. The studentship also optimised crystallisation conditions and provided information for future research.

References

Diamond Light Source. "SSX at I24." *SSX at I24 - - Diamond Light Source*, Diamond Light Source, www.diamond.ac.uk/Instruments/Mx/I24/I24-serial.html. Accessed 17 Sept. 2023.

"Lung Cancer - Small Cell - Statistics." *Cancer.Net*, American Society of Clinical Oncology (ASCO), 20 Mar. 2023, www.cancer.net/cancer-types/lung-cancer-small-cell/statistics.