

College PhD Studentship in Cancer Research

Studentship Number: MBSP/10/03

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Project Title: Mechanisms of action of small molecule BCL6 antagonists: linking structure to function

Project description:

BCL6 is an oncogene and transcriptional repressor that drives proliferation and prevents terminal differentiation of normal germinal centre B-cells. It is constitutively expressed due to chromosomal translocations or mutations in regulatory elements in 30 to 40% of human diffuse large B-cell lymphoma (DLBCL). Over-expression of BCL6 in transgenic mice is sufficient to produce lymphomas resembling DLBCL. Abolition of BCL6 function by a peptide, which prevents the BTB/POZ domain of BCL6 from recruiting the SMRT/NCOR co-repressor proteins, kills Burkitt's lymphoma and DLBCL cell lines. Thus BCL6 is a promising and validated drug target.

In collaboration with Dr Paul Ko Ferrigno (Leeds) we have developed a number of small compound inhibitors of BCL6 based on binding of a peptide aptamer sequence (1) to a surface on the BTB/POZ domain of BCL6. Like the peptide, these compounds also kill Burkitt's lymphoma and DLBCL cell lines. Currently further compounds are being developed which will feed into the project.

In another line of work we (SW and Dr Andy Porter, Imperial College London) have created, by homologous recombination, a B-cell line (DG75) lacking endogenous BCL6, but possessing tetracycline inducible BCL6 (DG75^{DBCL6}). When BCL6 is turned off the growth rate of the cell line falls dramatically before the cells die.

We propose to use structural biology approaches to experimentally determine the mode of binding of our novel inhibitors to the BTB/POZ domain of BCL6. We will use this information to design higher affinity compounds and to understand the exact mechanism of inhibition. We will combine these studies with cell-based assays to explore the effect of these compounds on co-repressor recruitment and effects on Burkitt's lymphoma and DLBCL cell lines.

There are several crystal structures of the BCL6 BTB/POZ domain and therefore we expect that co-crystallisation with these compounds should be straightforward. Furthermore, although the BTB/POZ domain dimer is around 240 amino acids, it has a globular structure that we anticipate will give tractable NMR spectra to allow us to use HSQC titrations to characterise binding.

Specific Aims:

1. To express & purify the BCL6 BTB/POZ domain
2. To crystallise and determine the structure of the domain in complex with inhibitors
3. To use ¹⁵N labelled protein and NMR spectroscopy to characterise inhibitor binding
4. To complement these studies with various cell-based assays. We will measure the effects of small compound inhibitors on cell growth, apoptosis (annexin V and PI binding) and differentiation of B- and non-B-cell lines. Differentiation will be measured by expression of Blimp-1 and XBP-1 mRNA by Taqman real time PCR (both required for terminal B-cell differentiation). We will also combine structural and functional approaches using our DG75^{DBCL6} cell line. In the presence of wild-type BCL6 the cells is expected to be susceptible to the effects of the small molecule inhibitor. However, making mutations, based on the structural studies, that abolish binding, are anticipated to prevent the effects of the inhibitor.

Ref: Chattopadhyay A, Tate SA, Beswick RW, Wagner SD, Ko Ferrigno P. A peptide aptamer to antagonize BCL-6 function. Oncogene 2006;25: 2223-2233.