ImmunoTools *special* Award 2015

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Comparative profiling of multiple cytokine/chemokine responses; single cell analysis of RNA translation in primary human leukaemia cells

The microenvironment plays a key role in promoting the growth and survival of malignant B cells. The factors involved in this microenvironment support are complex and this project will profile the effects of multiple microenvironment-derived cytokines and chemokines on primary human leukaemic cells. The project is closely allied to on-going research in the host laboratory which will provide an excellent, supportive environment for the work. However, it will allow me to expand my research into an exciting new area and will provide important novel insight into the biology of human lymphoma and leukaemia.

The project will focus on chronic lymphocytic leukaemia (CLL) the most common B-cell cancer in the western world. In this disease, stimulation within the lymph node microenvironment drives proliferation and survival of malignant cells. Following stimulation, cells exit the lymph nodes and accumulate to high numbers in the circulation where they are readily available for study. The project will use primary leukaemic cells isolated directly from the blood of CLL patients to avoid potential artefacts associated with the establishment and long-term culture of human B-cell lines.

One key player in the stimulation of CLL cells *in vivo* is antigen, acting via the cell surface B-cell receptor (BCR). My host laboratory has played a leading role in understanding how antigen stimulation promotes survival and proliferation of CLL cells, and how it influences clinical behaviour.[1] This is a critical area since new drugs targeted against BCR-associated kinases, such as the BTK inhibitor ibrutinib, are revolutionising treatment for B-cell cancers.

My own key contribution has been to demonstrate that stimulation of the BCR on CLL cells increases RNA translation, both in cultured cells and in lymph nodes of

patients.[2] This response was most prominent in samples from poor prognosis subsets of disease. Increased RNA translation plays a central role in cancers via global effects to support increased cell growth, as well as upregulation of specific oncoproteins which are often subject to tight translational control. Indeed, by analysis of polysomes, I was able to show that BCR stimulation increased translation of RNA encoding MYC, a critical oncoprotein for B-cell malignancies that is expressed in CLL cells in the lymph nodes. These responses were inhibited by ibrutinib, suggesting that inhibition of RNA translation may contribute to clinical responses to this drug.

Given the complex nature of the lymph node microenvironment, it will be critical to identify other factors which can promote RNA translation in CLL cells, or modulate response to BCR stimulation. The project will therefore compare the effects of a broad panel of human chemokines and cytokines (alone and with BCR stimulation) on RNA translation in primary CLL cells. The study will be performed using samples (n=20) from both good and poor prognosis forms of disease, already characterised within our tissue bank. This will allow us to determine whether responses differ between clinically important subsets of disease. RNA translation will be measured using a novel flow cytometry analysis which allows quantitation of responses on a single cell basis.[3] I have adapted this assay for use with CLL samples and inclusion of antibodies against cell surface markers will allow me to separately quantify effects of stimulation on RNA translation in CLL cells, as well as residual non-malignant T and B cells, and to probe potential variation of responses with the malignant clone.

At the end of the study we will have completed the first comparative profiling of effects of multiple human cytokines and chemokines on RNA translation in primary human leukaemia samples.