Translational profiling and network analysis in aggressive B cell lymphomas

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Constant advances in experimental technology mean successful biological scientists of the future will require a solid understanding of both wet lab techniques and the computational manipulation of big data. This project in the Haematological Malignancies Programme will be jointly supervised between the Hodson and Samarajiwa labs and will provide a rare opportunity for cutting edge training in both functional genomics and computational analysis. There is room for flexibility in terms of the balance between wet lab and computation work, which could be adjusted to suit the interests and training requirements of the candidate.

Project Description

Background and hypotheses:
Strict regulation of gene expression is critical to the proper function of cells and organisms. Its corruption is a prominent feature of cancer. The availability of technologies such as microarray has lead to the widespread use of mRNA abundance as a proxy for gene expression. However, not every mRNA is translated equally and it is now clear that dynamic regulation imposed at the level of translation is a major (possibly the dominant) determinant of final protein expression. Recently developed technology such as ribosome profiling now permits the transcriptome wide quantification of translation(1). It has reinforced the dynamic and extensive changes in translation that occur during cellular differentiation and revealed pathways regulated exclusively at the level of translation. Diffuse large B cell lymphoma is the most common haematological malignancy. Although potentially curable with immunochemotherapy 50% of patients still die during the first 12 months. In an attempt to reveal novel (and potentially targetable) lymphomagenic pathways my lab is using
ribosome profiling and proteomic approaches to identify quantitative and qualitative differences in translation across different subsets of DLBCL. B cell receptor (BCR) and PI-3-Kinase (PI3K) pathways are especially important to the survival of DLBCL and inhibition of these pathways represents one of the most promising approaches for novel treatment(2). The project proposed for this studentship will investigate how the BCR and PI3K signalling pathways influence the lymphoma translome and proteome and the implications this may have for the treatment of patients. The student will become skilled in RNA-sequencing, ribosome profiling and proteomics. In addition they will develop expertise in bioinformatics and systems biology approaches to the integration of large omics data sets.

Hypothesis 1: Signalling pathways downstream of the BCR regulate the lymphoma translome and altered translation contributes to the therapeutic activity of BCR inhibitors.

Hypothesis 2: Feedback regulation at the level of translation contributes to the acquisition of resistance to BCR inhibitors in DLBCL.

Hypothesis 3: Computational networks derived from multilevel expression data in combination with novel methods for analysis of such networks will enable testable biological predictions that are more accurate than conventional networks constructed solely from mRNA abundance data.

Objective 1: Identify the transcriptome-wide changes in translation that occur in DLBCL cells following exposure to therapeutic agents that target B cell receptor signalling. Inhibitors of BCR signalling are proving to be “game-changers” in the treatment of lymphoma. However, predicting which patients will respond remains a challenge that is only partly solved by transcriptional profiling and mutational analysis. It is already established that BCR and PI3K activity regulate the activity of RNA-binding proteins that control the expression of co-ordinated programmes (regulons) of post-transcriptional gene expression(3). However, no study has yet looked at effects of BCR or PI3K inhibition on the lymphoma translome. Doing so will provide a much more complete understanding of mechanisms by which these drugs kill lymphoma cells and will thereby enhance our ability to predict response and understand mechanisms of resistance. This objective will use RNA-seq, Ribosome profiling and proteomic profiling to examine sequential changes at every level of gene expression as cells are exposed to BCR inhibition. I anticipate that this will identify mechanisms of resistance that might be targeted by combination therapy.

Objective 2: Construct computational network models of DLBCL using ribosome profiling data. It is clear that gene expression is not a simple linear concept but rather that the expression of each gene influences the expression of many others in a complex inter-related network. These can be modelled in silico by comparing the mutual information between changes in every expressed gene when compared across multiple data sets(4). Networks will be generated using data driven computational methods and further refined by data integration methodologies. Network topology, connectivity and information flow will be investigated and biologically significant sub-network, pathways and genes will be identified. These network models will be used to identify “nodes” or proteins with particular regulatory importance within any particular network. The accuracy of these predictions is clearly dependent upon the quality of data that is used. Importantly, the molecules interacting within the actual network in the cell are the proteins. Networks have traditionally been constructed from microarray or RNA-seq data, based on measurement of RNA abundance. Data derived from ribosome profiling provides a much more accurate measure of protein expression and may even double the correlation with final protein abundance. This may greatly increase the predictive power of these networks. Data sets used will come from RNA-Seq, ribosome profiling and proteomics data generated in this project and also from other DLBCL projects currently running in my lab. In addition we will incorporate other publicly available genes expression and proteomic
data sets. Much of this aspect of the project will be supervised by Dr Samarajiwa who runs a computational biology group specialised in modelling the gene expression networks that drive malignant change.

**Objective 3:** Functional testing of the predictions made by the network model. Proteins of particular regulatory potential within the *in silico* network will be targeted by shRNA or CRISPR knockdown in the DLBCL cell lines and the effects on the predicted downstream pathways analysed. Experimental techniques used in this objective will include cell culture, retroviral transduction, qRT-PCR, western blotting and flow cytometry. If validated, these regulatory protein nodes may represent important therapeutic targets for the future treatment of lymphoma.

**References**


**Applications**

To apply for this studentship please see [http://www.cambridgecancercentre.org.uk/studentships](http://www.cambridgecancercentre.org.uk/studentships)

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