Determining the role and potential therapeutic targeting of aberrant fatty acid metabolism in lymphoma

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Principal supervisor's CRUK CC theme: Haematological Malignancies Programme

Department for student registration: Stem Cell Institute
Department or institute where research will take place: Department of Haematology, Jeffrey Cheah Biomedical Centre

Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

Aims:
1) To determine the aberrant role of fatty acid metabolism in malignant lymphoma.
2) To characterise the role of the candidate protein Bin1 in this process.

Diffuse large B cell lymphomas (DLBCL) are aggressive malignancies of germinal centre or post-germinal centre B cells. Commonly recurrent mutations found in DLBCL are inactivating mutations of the CREBBP (Creb-binding protein) gene. CREBBP is a lysine acetyltransferase which can acetylate both histone and non-histone proteins and plays an important role as a transcriptional co-activator, non-histone protein lysine-acetyltransferase and a scaffolding protein that nucleates multifunctional protein complexes.

We have successfully modelled DLBCL utilising Crebbp knock-out mice (Horton et al, Nature Cell Biology, 2017). Using scRNA-Seq, we have profiled tumours from this model along an evolutionary disease continuum, identifying multiple aberrant metabolic pathways, with fatty acid (FA) metabolism being the most prominent. FAs serve essential roles as important components of cellular membranes, as second messengers in multiple signalling pathways and as a vital substrate for mitochondrial Ox Phos. scRNA profiling of Crebbp-/- tumours has identified several potentially critical candidate target genes, including Bin1, a known inhibitor of MYC transcription factors, which are key lymphoma oncogenes. Preliminary data demonstrate that Bin1, a direct transcriptional target of CREBBP, also plays a role in fatty acid metabolism and previous studies in other contexts have implicated its role in lipid droplet formation and endocytosis.

This project will investigate the mechanisms underlying the metabolic reprogramming of CREBBP mutant/deficient DLBCL following downregulation of Bin1 expression, using a variety of resources, (Crebbp KO mouse model, panels of human lymphoma cell-lines, including those isogenic for a CREBBP mutation engineered by CRISPR-Cas9).

MRes experimental plan:

This project will benefit from an impressive list of existing genetic (sc and bulk RNA-Seq data for human and murine models), genomic (ChIP-Seq for CREBBP/Crebbp and H3K27Ac), proteomic (acetyl-proteomics in CREBBP WT-mutant isogenic cell lines) and metabolic datasets (metabolic flux analysis by Seahorse assay and stable and isotope-labelled metabolomic datasets by

LC/MS) in lymphoma and normal or isogenic control cells as orthogonal control datasets.

Preliminary functional data demonstrates that inducible restoration of Bin1 expression abrogates cellular growth in DLBCL cell lines. These findings are in keeping with our working hypothesis of a role for Bin1 in aberrant metabolism within malignant lymphomas. This rotation will further investigate this hypothesis, aiming to determine the mechanisms whereby restoration of Bin1 alters lymphoma cell growth, initially analysing alterations in the transcriptional and metabolic states evident upon Bin1 overexpression, using RNA-Seq and Seahorse analysis. Additional experiments, and dependent upon time and progress, will utilise a variety of tools, including confocal microscopy, flow cytometry, Seahorse extracellular flux assays and western blotting to provide further molecular granularity, investigating alterations in lipogenesis, lipid uptake, lipid storage (in lipid droplets) and break down of lipids by lipophagy or lipolysis in Crebbp-/- murine and CREBBP mutant human cells. The long-term aim of this project is to determine whether lipid metabolism is a targetable vulnerability in DLBCL, playing to the pre-clinical expertise within the lab in identifying actionable novel therapies within haematological malignancies.

PhD project outline:

Extended Aims:
1) To mechanistically determine the aberrant role of fatty acid metabolism in malignant lymphoma.
2) To characterise Bin1 loss and identify other candidate target genes downstream of CREBBP that mediate the aberrant metabolic state in CREBBP mutant lymphomas.
3) To identify if FA metabolism is a targetable vulnerability in malignant lymphoma.

For further background please see rotation outline above.

PhD experimental plan:

Leading on from the experimental plan executed for the research rotation, the PhD project would involve a much more in-depth and multi-disciplinary study, performing further complementary metabolomic, functional and therapeutic experiments. Initially data-mining of the extensive datasets available for our model systems would seek to identify other CREBBP targets that drive aberrant metabolism for further study and these would be investigated in parallel with Bin1.

Further experiments would include completion of the additional experiments described above (thus providing further molecular granularity; investigating alterations in lipogenesis, lipid uptake, lipid storage - in lipid droplets- and break down of lipids by lipophagy or lipolysis in Crebbp-/- murine and CREBBP mutant human cells). These experiments will provide an excellent technical training and would utilise a variety of experimental tools, including confocal microscopy, flow cytometry, Seahorse extracellular flux assays and western blotting. Moreover, in collaboration with investigators from the Institute of Metabolic Science, we would combine this with lipidomic profiling by mass spectroscopy to provide detailed analysis of the altered lipid status within the lymphoma cells. We anticipate that integration of the characterisation of these putative targets and the altered transcriptional and lipid states that they generate, in combination with available genome-wide CRISPR screens within CREBBP mutant and WT DLBCL (unpublished data), will identify potentially actionable therapeutic strategies. Our lab are expert in pre-clinical validation of targets and molecules in haematological malignancies (Dawson et al, Nature, 2011; Gallipoli et al, Blood, 2018; Anderson et al, Blood, 2022) and following in vitro validation of potential candidate targets across cell line and primary models, we would aim to take a limited number of these candidates into in vivo experiments, prioritising targets with extant and clinically actionable inhibitors.

Main techniques:

Proteomics
Mass spectroscopy
Flow cytometry
Imaging
CRISPR-Cas9 modification
Mouse modelling and pre-clinical drug trials

Key references:

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