ASCL1 and its interactors in neuroblastoma differentiation

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Principal supervisor's CRUK CC theme:

- Cell and Molecular Biology Programme
- Paediatric Cancer Programme
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Department for student registration: Cambridge Stem Cell Institute, School of Clinical Medicine
Department or institute where research will take place: Jeffrey Cheah Biomedical Centre, Cambridge Stem Cell Institute

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

MRes project outline:

Background
The cell cycle and differentiation are co-ordinated during development via the action of cyclin-dependent kinases. This ensures that cells either stably maintain stemness/progenitor identity or adopt a mature state. It is crucial to understand this co-ordination to both control stem cell differentiation in normal development and homeostasis and understand how this can go awry in cancer.

Using diverse experimental systems including embryonic stem cell culture and Xenopus frog embryos, we have uncovered a direct mechanism whereby CDKs phosphorylate and regulate a key class of transcription factors, bHLH proneural proteins, which act as master regulators of differentiation in multiple tissues. We find that mutant forms of these proneural proteins that cannot be phosphorylated by CDKs are hyperactive at driving cell differentiation.

Dephosphorylation of these proneural proteins by kinase inhibitors is therefore a promising therapy in several tumour types including the paediatric cancer, neuroblastoma. Neuroblastoma arises from precursors of the peripheral nervous system, is the most common extracranial tumour of childhood and ASCL1 in part of the core transcriptional circuit driving neuroblastoma cell identity. We find activating ASCL1 in neuroblastoma cells by blocking its cyclin-dependent kinase-mediated phosphorylation inhibits proliferation and drives differentiation of tumour cells.

Aims and Objectives
We aim to understand how blocking dephosphorylation of ASCL1 promotes its differentiation-specific transcriptional programme. Using “qPLEX-RIME”, a quantitative mass spectrometry-based technique to find proteins that interact on chromatin, we have identified co-factors binding to DNA-bound ASCL1 in multiple neuroblastoma cell lines. We aim to understand the mechanisms by which they regulate ASCL1 function and how this can be exploited to drive tumour differentiation.

MRes experimental plan:

In this project, we will use neuroblastoma cell lines to explore the functional consequences of the interplay between ASCL1 and one or two of the identified chromatin-associated factors, investigating their role in neuroblastoma cell proliferation and differentiation.

We have seen that ASCL1 has several interactors that may be affecting its function in neuroblastoma. Depending on the interests of the student, we will choose one or two of these interacting proteins identified in our RIME experiments, confirm their association by co-immunoprecipitation and/or a microscopy-based proximity ligation assay. We will then use our ASCL1 CRISPR knockout cells to determine whether ASCL1 is required for factor association with chromatin. We will also knock down the interactor expression with siRNA and determine the effect on chromatin binding by ASCL1 and ASCL1-mediated differentiation using morphological and qPCR-based assays. The main techniques used will include cell culture, western blotting, co-immunoprecipitation, siRNA knockdown, microscopy and qRT-PCR.

PhD project outline:

Hypothesis
The goal of the Philpott lab is to exploit an understanding of normal neuroblast developmental control to restart differentiation in neuroblastoma cells by targeting a key transcriptional network both genetically and with drugs. Neuroblastoma is unique in that a subset of patients undergo spontaneous regression, making differentiation therapy an attractive therapeutic avenue. The proneural transcription factor ASCL1 is part of the core transcriptional network driving an adrenergic cell fate in neuroblastoma and has a number of interactors that may be affecting its function in controlling proliferation and differentiation. We hypothesise that by modifying the levels of these factors, we can influence ASCL1's role in controlling differentiation of neuroblastoma cells.

Aims and Objectives
Depending on the interests of the student and the progress of the MRes rotation project, the student will continue investigation of the chosen ASCL1 interactors in relation to proliferation/differentiation of neuroblastoma. The project will validate promising interactors with ASCL1 and go on to confirm their functional role in ASCL1-induced differentiation, using gain- and loss-of-function studies involving CRISPR and transgene overexpression, or targeted drugs where appropriate. Dependent on initial findings, the student will be able to further explore the role of these interactions in additional neuroblastoma cell lines and new culture systems, including serum-free and 3D cultures. Sufficient autonomy will be given to explore the molecular underpinnings balancing proliferation vs. differentiation in neuroblastoma, and how this understanding can guide strategies to improve differentiation therapies.
PhD experimental plan:
The PhD project we continue to use neuroblastoma cell lines to explore the functional consequences of the interplay between ASCL1 and identified chromatin-associated interactors in neuroblastoma cell proliferation and differentiation. Functional assays such as a qRT-PCR, co-immunoprecipitation, microscopy-based proximity-ligation assays, CRISPR/Cas9 gene deletion, transgene overexpression and fluorescence microscopy will be used to assess this. In addition, there will be opportunity for the student to use "wet lab" and bioinformatic techniques to perform genome-wide analyses of transcription factor binding and gene expression. The role of these interactions will also be explored in alternative neuroblastoma culture systems, including serum-free and 3D cultures. Beyond this, scope will be given to expand the project in line with findings and the student's research interests.

Main techniques:

The main techniques will build upon those used during the MRes project including: cell culture, western blotting, co-immunoprecipitation, siRNA knockdown, transgene overexpression, CRISPR/Cas9 gene deletion, fluorescence microscopy and qRT-PCR. There will also be an opportunity for the student to perform genome-wide analyses and train in bioinformatics techniques, if desired.

Key references:

Woods LM et al; Elevated ASCL1 activity creates de novo regulatory elements associated with neuronal differentiation BMC Genomics 2022. PMID: 35366798
Ali FR et al; Dephosphorylation of the proneural transcription factor ASCL1 re-engages a latent post-mitotic differentiation program in neuroblastoma Mol Cancer Res 2020. PMID: 33046535
Papachristou EK et al; A quantitative mass spectrometry-based approach to monitor the dynamics of endogenous chromatin-associated protein complexes Nature Comms 2018. PMID: 29899353