PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Exploiting lineage-specific dependencies in paediatric-type diffuse high-grade glioma.

Supervisory team

Primary Supervisor:	Chris Jones
Associate Supervisor(s):	Rebecca Rogers
Secondary Supervisor:	Sally George

Divisional affiliation

Primary Division:	Cancer Biology
Primary Team:	Glioma
Site:	Sutton

Project background

Paediatric-type diffuse high-grade glioma (PDHGG) is an umbrella term for a collection of related, but biologically and clinically distinct brain tumours primarily affecting children and young adults, characterized by its diffuse infiltration and poor prognosis, with a median survival of only 9-18 months. The latest edition of the WHO CNS Tumour Classification schema recognised four major subtypes, with around half harbouring somatic mutations in histone H3 genes. These have defining molecular alterations and clinical phenotypes – diffuse midline glioma with H3K27 alterations (DMG-H3K27) and diffuse hemispheric glioma with G34R/V mutations (DHG-H3G34). Although similar histologically, these two subtypes have been recognised to arise from highly distinct developmental origins – DMG-H3K27 from an oligodendroglial (oligodendrocyte precursor cell-like-derived) and DHG-H3G34 from an interneuronal (radial glia / neuronal precursor cell-liked derived) lineage. The malignant cells which comprise these tumours may present in a variety of cell states along these trajectories, recapitulating developmental hierarchies; from stem-like precursors at the apex through to more differentiated oligodendrocyte (OC)-like cells or early interneuron (eIN)-like cells, respectively. These lineage-specific cell states are defined by normally developmentally-restricted transcription factors that represent novel and highly specific dependencies identified through CRISPR dependency screens. As an example, in DHG-H3G34 we have identified the CDK6 as a key dependency associated with self-renewal and proliferation of the most immature cell state; upon selective inhibition/depletion of CDK6 cells become more differentiated along the interneuronal lineage. In DMG-H3K27, cholesterol biosynthesis represents a selective dependency under stem cell conditions which enrich for OPC-like cells; upon differentiation into an astrocyte (AC)-like cell state, this is removed by an increased production of cholesterol by the malignant cells, mimicking the role of normal astrocytes.

However these cell states are highly plastic, and little is known how they are maintained, what controls cell state transitions, and how the microenvironment may modulate this.

Project aims

We hypothesise that PDHGG subtypes harbour selective targetable dependencies that are related to their distinct lineage of origin. Focusing on H3-mutant glioma subtypes, we aim to systematically map the ability of these cells to transition through lineage-restricted cell states in complex model systems and in response to lineage-guided interventions.

- We will characterise the cell state specific chromatin organisation in DMG-H3K27 and DHG-H3G34 through single cell multi-omics
- We will model cell state trajectories *in vitro* in complex co-culture and self-assembling organoid models in response to targeting of lineage-specific dependencies
- We will develop cell identity reporter assays for screening of novel therapeutic approaches to target cell state plasticity

Research proposal

PDHGG, especially the H3-mutant subtypes, have a dire clinical outcome and represent a major unmet need. As treatments derived from traditional target identification approaches have underperformed in the clinic, we propose a more advanced approach to identify novel avenues for intervention based upon a thorough understanding of the evolutionary dynamics of cell state transitions within lineage of origin of these tumours, both tumour cell intrinsic and in response to cues from the normal microenvironment.

- Characterisation of cell state specific chromatin accessibility in DMG-H3K27 and DHG-H3G34

  We have to-date collated a panel of >100 patient-derived PDHGG cell models, which have been fully profiled at the bulk level by DNA methylation array and DNA and RNA sequencing, and are currently being profiled by CUT&Tag for key histone modifications. In order to define the core regulatory circuits underpinning the distinct cell state hierarchies observed in DMG-H3K27 (oligodendroglial) and DHG-H3G34 (interneuronal), we will perform single cell combined RNA and ATAC sequencing (10x Multiome) to assess cell state-specific chromatin accessibility. This will be combined with Droplet-based Paired-Tag, which combines single cell RNA-seq and CUT&Tag to allow for simultaneous profiling of gene expression and histone modifications in the same cell (10x Chromium). Performed first in cells grown under standard glioma stem cell conditions, this necessarily enriches for cell states at the apex of the proposed hierarchies (OPC-like, RG/NPC-like). In order to explore the more differentiated cell states along each of these lineages, we will further carry out our single cell multi-omic profiling in cells grown under distinct culture conditions (eg serum, driving AC-like differentiation), and after isolation by FACS using lineage and cell state-specific surface markers derived from profiling of both cell lines and patient tumours. The inherent plasticity and tumorigenicity of the enriched cell states will further be assessed through multi-omic characterisation of expanded populations *in vi*tro, and orthotopic engraftment in mice. This will provide an unprecedented atlas of the molecular underpinnings of the epigenetic plasticity of cell states in each H3-mutant glioma subtype, as well as cellular resources for further functional assays.

- Modelling cell state trajectories in complex co-culture and self-assembling organoid models

  In order to build a more complete picture of how lineage-specific cell states are modulated by the tumour microenvironment, we will develop stepwise co-culture systems of tumour cells paired with

differentially-labelled region-specific normal human astrocytes, microglia, T cells and endothelial cells. These will be utilised first in dual culture systems to explore the specific interactions of each cell type with the malignant DMG-H3K27 and DHG-H3G34 cells, but will also be combined and allowed to form self-assembling multicellular 3D organoids that are amenable to interventional experiments using high-content imaging approaches. These platforms (Incucyte, Celigo) will be used to explore the cell state transitions observed in the presence of normal cells during normal organoid growth, but also in response to lineage / cell state-targeting via genetic or pharmacological means. This will be unravelled using a novel barcoding library for lineage tracing which we have previously employed for drug resistance monitoring (CloneSifter),which takes advantage of the compatibility of the Crop-seq derived vectors with the 10X platform in order to simultaneously carry out scRNA-seq alongside DNA sequencing of the barcodes. We will validate these associations in the untreated setting by carrying out spatial transcriptomics on patient biopsy / diagnostic samples using a bespoke 480-gene panel (10x Xenium) from patients prospectively profiled through our local neurosurgical centres and ongoing clinical trial cohorts (BIOMEDE2.0, CONNECT-TarGeT); the latter may also afford access to post-treatment samples of relevance of cell state modulation (e.g. ONC201 in DMG-H3K27, ribociclib in DHG-G34).

- Reporter assays for targeting cell state plasticity

With the ultimate goal of identifying novel therapies based upon a modulation of lineage-specific cell states, we will derive an initial list of candidate targets by exploiting existing high-throughput screening and bulk multi-omic profiling data of H3-mutant glioma generated by ourselves as well as large scale initiatives such as the Childhood Cancer Model Atlas (CCMA). We will apply machine learning tools being developed by our mathematical biologist Haider Tari to identify tumour subtype-specific dependencies associated with genes which mark specific cell states using our previously collated model and patient sample single cell and bulk datasets. The dependencies identified will be validated in our panel of DMG-H3K27 and DHG-H3G34 cells for which we will develop a cell identity reporter assays based upon a fluorescent read-out of precise markers linked to appropriate cell states refined in the previous aims. Screens will be thus be run with differentiation status as a read-out with the goal of identifying modulators of cell state transitions which could lead to the development of novel therapies, alone or in combination with conventional treatments. Future collaborative work could involve testing extant agents in vivo using our repository of orthotopic patient-derived xenograft (PDX) models, or new drug discovery projects internally as part of our Centre for Children and Young Peoples Cancer, or externally via the C-Further initiative.

Literature references

Jones C, (2017) “Pediatric high grade glioma: biologically and clinically in need of new thinking” Neuro-Oncol 19(2):153-161

Liu, I., R. et al. The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location. Nat Genet 54, 1881–1894 (2022).

Liu I, et al (2024) “GABAergic neuronal lineage development determines clinically actionable targets in diffuse hemispheric glioma, H3G34-mutant” Cancer Cell 42(9):1528-1548

Mackay, A., et al., Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. Cancer Cell, 2017. 32(4): p. 520-537 e5.

Mbah, N.E., Myers, A.L., Sajjakulnukit, P. et al. Therapeutic targeting of differentiation-state dependent metabolic vulnerabilities in diffuse midline glioma. Nat Commun 15, 8983 (2024)

Upreti, M., Petrosyan, A., Thornton, M.E. et al. Multicellular tumor-stromal interactions recapitulate aspects of therapeutic response and human oncogenic signaling in a 3D disease model for H3K27M-altered DIPG. Oncogene (2025).

Xie, Y., Z. et al. Droplet-based single-cell joint profiling of histone modifications and transcriptomes. Nat Struct Mol Biol 30, 1428–1433 (2023).

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:

BSc in relevant biological science

Intended learning outcomes:

- Knowledge of the underlying biology of PDHGG
- Detailed understanding of PDHGG origins and cell states
- Ability to design and carry our complex molecular cell biology and multi-omic experiments in patient-derived glioma models
- Skills in critical thinking, experimental design and interpretation
  Training in appropriate bioinformatic analyses

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science