CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**How does chromatin deregulation underpin a specific programme of neoplastic transformation in paediatric gliomas?**
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Second supervisor if applicable	
Programme	Neurological and Brain Cancers
Supervisor's Email	Mp915@cam.ac.uk
Laboratory Location	

Project Outline	**Aims and objectives** Brain tumours are the leading cause of cancer-related mortality in children. For paediatric high-grade gliomas (pHGGs) in particular, there are no effective treatments. Tumours are either not amenable to surgical resection, or invariably develop resistance to conventional therapy and recur. Heterozygous driver mutations in the histone 3 (H3) variant H3.3 are present in 40% of pHGGs and 80% of diffuse midline gliomas (DMGs), occurring at lysine 27 (K27M) and glycine 34 (G34R/V) (1). These pHGG-specific mutations disrupt DNA methylation and H3 lysine trimethylation at K27 or K36 genome-wide (2). However, the underlying biology that translates this global epigenomic deregulation into a specific programme of neoplastic transformation remains unknown. Recently, I established the first *in vivo* models of mutant histone-driven pHGG by delivering driver mutations into neural stem cells using *in utero* electroporation of transposons and CRISPR vectors (3). These models molecularly and histologically recapitulate human tumours and are 100% penetrant. Using this approach, we will now investigate how deregulated chromatin remodelling instructs glioma initiation and maintenance in H3.3-mutant pHGGs, and identify candidate molecules important in these processes.
Experimental plan	This project will investigate the roles for Trithorax and Polycomb group proteins in K27M-driven pHGGs (4). You will clone lentiviral vectors designed to interrupt the function of chromatin writers and validate their efficacy in a mouse neuroblastoma cell line (Neuro2a) using lentiviral transduction, qPCR and biochemistry. Following validation, you will deliver these vectors into neural stem cells and K27M tumour cells *in vitro* and assess their effects on proliferation and differentiation using FACS and confocal immunofluorescence microscopy. You will also compare their effects on histone posttranslational modifications at key genes involved in neurodevelopment, the cell cycle and tumour suppression via chromatin immunoprecipitation and qPCR. If continuing on for a PhD project, you will validate your *in vitro* data *in vivo*, using transposon and CRISPR *in utero* electroporation-based models of K27M-driven pHGG. You will liaise with myself, a postdoctoral associate and a research technician for the genomics analyses, molecular biology, biochemistry, cell culture and *in vivo* animal work required to complete this project.

Main Techniques	• Molecular biology • Lentivirus production • Cell culture • Immunofluorescence • Confocal and epifluorescence microscopy • Chromatin immunoprecipitation • qPCR • FACS • *In utero* electroporation • Animal handling • Perfusion fixation • Brain sectioning
Key References	1. Sturm D, Bender S, Jones DT, Lichter P, Grill J, Becher O, Hawkins C, Majewski J, Jones C, Costello JF, Iavarone A, Aldape K, Brennan CW, Jabado N, Pfister SM. Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge. Nat Rev Cancer. 2014;14(2):92-107. 2. Lewis PW, Müller MM, Koletsky MS, Cordero F, Lin S, Banaszynski L, Garcia B, Muir TW, Becher OJ, Allis CD. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Science. 2013;340:857-61. 3. Pathania M, De Jay N, Maestro N, Harutyunyan AS, Nitarska J, Pahlavan P, Henderson S, Mikael LG, Richard-Londt A, Zhang Y, Costa JR, Hebert S, Khazaei S, Ibrahim NS, Herrero J, Riccio A, Albrecht S, Ketteler R, Brandner S, Kleinman CL, Jabado N, Salomoni P. H3.3K27M Cooperates with Trp53 Loss and PDGFRA Gain in Mouse Embryonic Neural Progenitor Cells to Induce Invasive High-Grade Gliomas. Cancer cell. 2017;32(5):684-700 e9. 4. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. Exp Mol Med. 2017;49(4):e324.