How do ACVR1/ALK2 mutations cause childhood brainstem tumours?

Elizabeth Brown[1], Gillian Farnie[1,2], Alex Bullock[1]

1 Structural Genomics Consortium, 2 Nuffield Department of Orthopaedics, Rheumatology and Muscoskeletal Sciences

Diffuse intrinsic pontine glioma (DIPG) is a paediatric brain stem tumour characterised by infiltrative growth. Diagnosis occurs on average at 6 years of age and the median overall survival time is 9-15 months[1]. Survival has not improved in decades due to the impossibility of complete surgical resection, only temporary response to radiotherapy, and a lack of an effective targeted therapies[2].

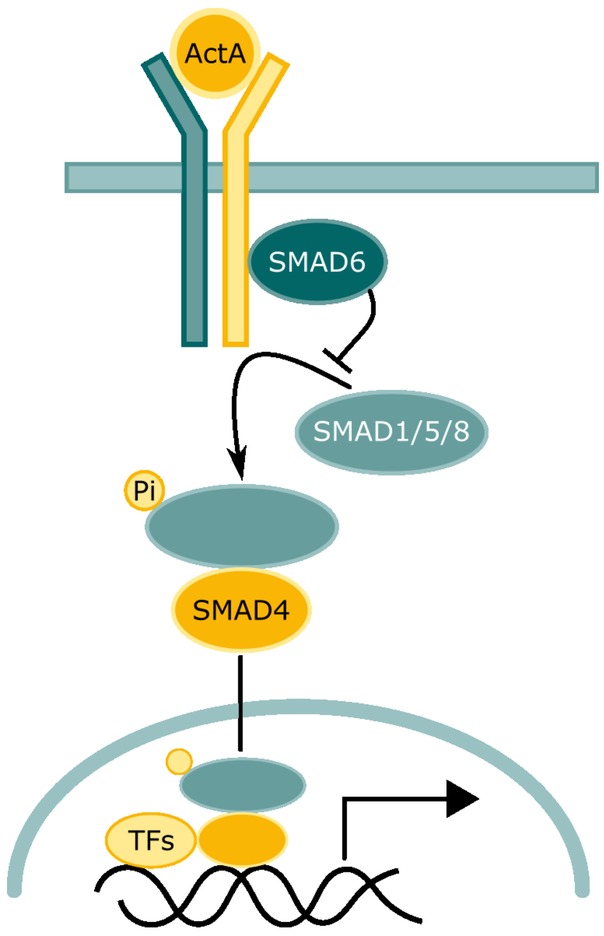
20% of DIPG cases carry missense ACVR1/ALK2 mutations[3], which lead to increased activation of BMP signalling, as well as responsiveness to activin A, in addition to the normal BMP ligands[4]. Thus, the ALK2/BMP signalling pathway is a promising new target for DIPG therapeutics.

Project aims

Development of stereotactic biopsy techniques and controlled autopsy protocols have allowed the generation of patient derived DIPG cell lines. I will use these cell lines to investigate:

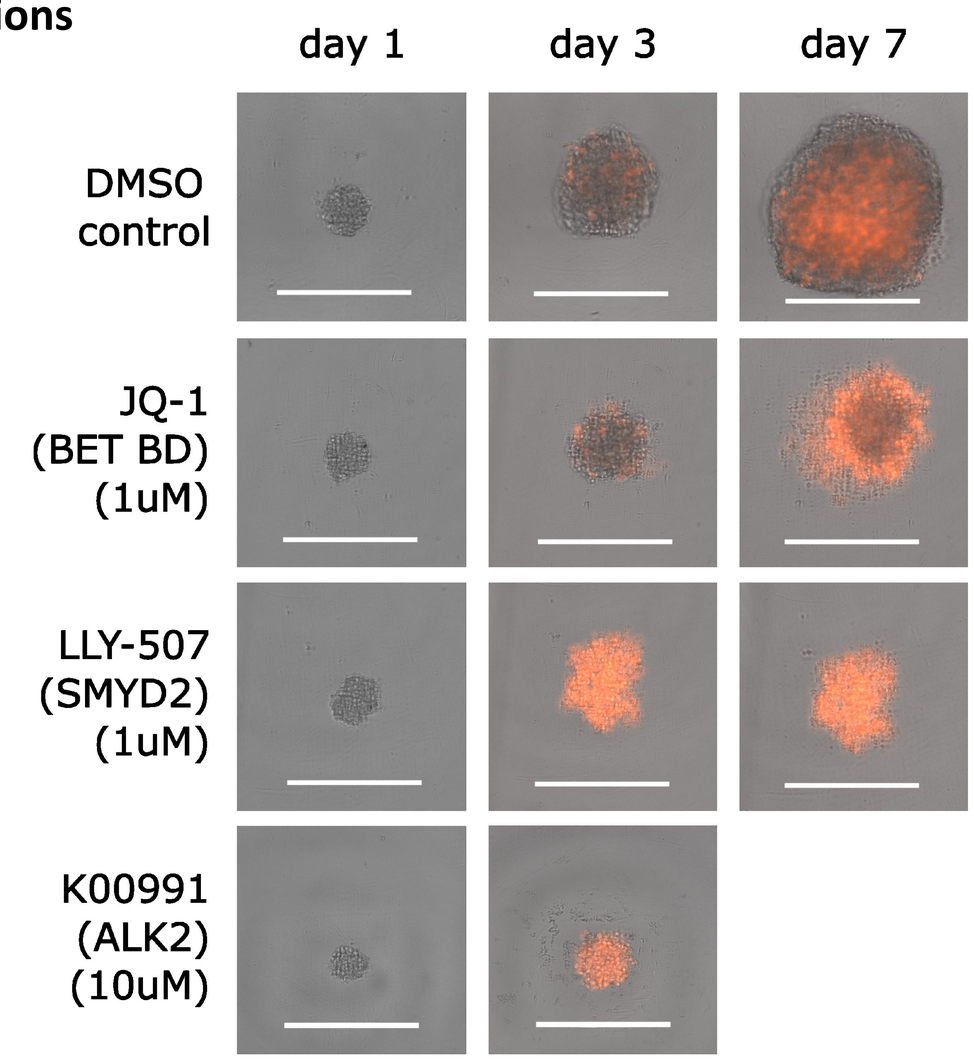
- Changes in intracellular signalling caused by the mutation
- The link between ACVR1/ALK2 and histone H3K27M mutations
- Efficacy of SGC synthesised ALK2 inhibitors

ACVR1/ALK2 mutant signalling



The link to histone mutations

Almost all DIPG cases contain histone H3.3 or H3.1 K27M mutations[5] indicating the importance of epigenetic regulation in this cancer and over the aberrant ALK2/BMP signalling pathway. I aim to screen the effects of a comprehensive panel of epigenetic probes[6] on DIPG cell line viability and migration, and BMP signalling responsiveness.

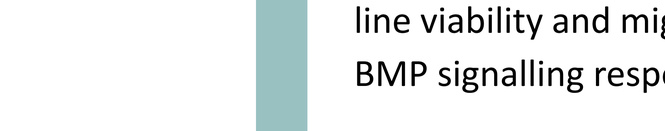


Treatment of DIPG tumour spheres with a test panel of epigenetic probes

Are DIPG cells dependent on the ACVR1 mutation?

Knockdown of ALK2 in DIPG cell lines reduces cell viability[(unpublished)], but this does not indicate whether WT ACVR1 or mutant ACVR1 is the necessary gene. I will disentangle these possibilities using the CRISPR-Cas9 technique to introduce a tetracycline dependent knock-down cassette in the mutant allele only of DIPG cell lines.

First transformation:



Second transformation:

1. Mackay A, Burford A, Carvalho D, et al. Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell*. 2017;0(0):1-18. doi:10.1016/j.ccell.2017.08.017.

2. Jones C, Perryman L, Hargrave D. Paediatric and adult malignant glioma: close relatives or distant cousins? *Nat Rev Clin Oncol*. 2012;9(7):400-413. doi:10.1038/nrclinonc.2012.87.

3. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet*. 2014;46(5):451-456. doi:10.1038/ng.2936.

4. Hatsell SJ, Idone V, Wolken DMA, et al. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Sci Transl Med*. 2015;7(303):303ra137. doi:10.1126/scitranslmed.aac4358.

5. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet*. 2012;44(3):251-253. doi:10.1038/ng.1102.

6. Ackloo S, Brown PJ, Müller S. Chemical probes targeting epigenetic proteins: Applications beyond oncology. *Epigenetics*. 2017;12(5):378-400. doi:10.1080/15592294.2017.1279371.

open notebook

As part of the Structural Genomics Consortium’s extreme open science initiative most of my work will be pre-published on the Zenodo database and https://opennotebook.thesgc.org/

By publishing data and working protocols as quickly as possible we aim to reduce redundant work and accelerate research progress

The Brain Tumour Charity

Jo’s friends