Medulloblastoma regulatory circuitries reveal subgroup-specific cellular origins

Charles Y. Lin[1]*, Serap Erkek[2,3]*, Daisuke Kawauchi[3], Alexander J. Federation[1], Rhamy Zeid[1], Marc Zapatka[4], Thomas Risch[5], Hans-Jörg Warnatz[5], Barbara Worst[3], Sebastian M. Waszak[2], David T.W. Jones[3], Marcel Kool[3], Volker Hovestadt[4], Ivo Buchhalter[6], Laura Sieber[3], Pascal Johann[3], Stefan Gröschel[7], Marina Ryzhova[8], Andrey Korshunov[9], Vyacheslav Amstislavskiy[5], Hans Lehrach[5], Marie-Laure Yaspo[5], Roland Eils[6,10], Peter Lichter[4], Jan O. Korbel[2], Stefan M. Pfister[3,11#], James E. Bradner[1#] and Paul A. Northcott[3,12#]

[1]Dana Farber Cancer Institute (DFCI), Medical Oncology, Boston, MA, USA
[2]European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany
[3]German Cancer Research Center (DKFZ), Division of Pediatric Neurooncology, Heidelberg, Germany
[4]German Cancer Research Center (DKFZ), Division of Molecular Genetics, Heidelberg, Germany
[5]Max Planck Institute for Molecular Genetics, Department of Vertebrate Genomics, Berlin, Germany
[6]German Cancer Research Center (DKFZ), Division of Theoretical Bioinformatics, Heidelberg, Germany
[7]NCT Heidelberg, Department of Translational Oncology, Heidelberg, Germany
[8]NN Burdenko Neurosurgical Institute, Department of Neuropathology, Moscow, Russia
[9]Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), and Department of Neuropathology University Hospital, Heidelberg, Germany
[10]Institute of Pharmacy and Molecular Biotechnology and BioQuant, University of Heidelberg
[11]University of Heidelberg, Department of Pediatrics, Heidelberg, Germany
[12]St. Jude Children's Research Hospital, Developmental Neurobiology, Memphis, TN, USA

*These authors contributed equally to this study
[#]Co-senior authorship

Medulloblastoma is a highly malignant paediatric brain tumour, often inflicting devastating consequences on the developing child. Genomic studies have revealed four transcriptionally distinct molecular subgroups with divergent biology and clinical behaviour. An understanding of the regulatory circuitry governing the transcriptional landscapes of medulloblastoma subgroups, and how this relates to their respective developmental origins, is currently lacking. Using H3K27ac and BRD4 ChIP-Seq, coupled with tissue-matched DNA methylation and transcriptome data, we describe the active *cis*-regulatory landscape across 28 primary medulloblastoma specimens. Analysis of differentially regulated enhancers and super-enhancers reinforced inter-subgroup heterogeneity and revealed clinically relevant insights into oncogenic TGFβ signaling in Group 3. Computational reconstruction of core regulatory circuitry identified a master set of transcription factors responsible for subgroup divergence and revealed candidate cellular origins for Group 4. The integrated analysis of *cis*-regulatory elements in primary human tumour samples reveals insights into *cis*-regulatory architecture, unrecognized dependencies, and cellular origins.

Medulloblastoma is a highly malignant paediatric brain tumour classified into four biologically and clinically distinct molecular subgroups[1-3]. Transcriptional diversity underlying WNT, SHH, Group 3, and Group 4 subgroup medulloblastoma is partially explained by active and discriminatory signaling pathways, such as the Wingless/WNT and Sonic hedgehog/SHH developmental cascades inherent to WNT and SHH medulloblastoma, respectively. Recent next-generation sequencing studies of medulloblastoma have defined recurrently mutated genes and pathways, the proportion of cases affected by such alterations, and their respective subgroup distribution[4-8]. Recurrent targeting of genes involved in chromatin modification has been the most consistent theme to emerge from these studies[4,9,10], strongly suggesting epigenetic deregulation of developmental cell state as a critical step during medulloblastoma pathogenesis. However, this hypothesis has yet to be substantiated and knowledge pertaining to how the medulloblastoma epigenome influences subgroup-specific transcriptional programs remains in its infancy. Underscoring the need for new subgroup-specific therapeutic insights, the present clinical approach to medulloblastoma involves invasive surgery, cranio-spinal radiation and cytotoxicity, together associated with profound morbidity in the developing child.

Here, we describe the medulloblastoma active *cis*-regulatory enhancer landscape across a series of 28 fresh-frozen, treatment-naïve tissue samples and three cultured cell lines, to our knowledge representing the largest such dataset for any single cancer entity. Our data reveal dramatic divergence between primary tumour and tumour cell line material and uncover considerable *cis*-regulatory element heterogeneity between subgroups of the disease that would be overlooked and unsubstantiated in series limited to just a few cases. Clinically relevant medulloblastoma subgroups are principally defined based on their underlying transcriptional profiles. Here, differentially regulated medulloblastoma enhancers and large clustered super enhancers (SEs) are shown to recapitulate these subgroups, and importantly extend our understanding of this disease to inferences regarding cell specification and actionable tumour dependencies, as evidenced by subgroup-specific SE regulation of TGFβ pathway components.

Biological themes and signaling networks extracted from transcriptional data have served as the primary source of annotation for medulloblastoma subgroups, with WNT and SHH subgroups characterized by activation of their respective signaling pathways, and Group 3 and Group 4 recognized for their GABAergic and glutamatergic expression phenotypes, respectively. Although these data provide a functional and phenotypic annotation of medulloblastoma, they fail to articulate the cell of origin and developmental identity of individual subgroups. Using a reverse analysis of the medulloblastoma chromatin landscape starting at the level of differentially-regulated enhancers and SEs, we have reconstructed a model of the core regulatory circuitry inherent to medulloblastoma subgroups, and inferred master transcriptional regulator transcription factors (TFs) responsible for subgroup-specific divergence. The majority of these master regulator TFs were not previously implicated in medulloblastoma developmental biology, nor were they visible amongst transcriptionally-derived gene sets dominated by aberrant signaling and overwhelming phenotypic signatures. Through tracing the spatiotemporal activity of a subset of Group 4 master TFs, these studies identified DCN of the cerebellar NTZ, or plausibly their earlier precursors originating from the rhombic lip, as putative cells-of-origin for this large subgroup of patients.

The ability to implicate cellular origins of Group 4 medulloblastoma and other cancers has broad implications[11]. Numerous cancers, especially those of the immune

compartment are treated through targeting of the lineage (*e.g.* anti-B cell therapies)[12,13]. As Group 4 medulloblastoma is believed to originate from rhombic lip precursor populations that normally exist ephemerally during development, targeting the aberrant persistence of tumour cells from these lineages may represent a novel therapeutic strategy. Moreover, elucidation of core regulatory circuitry implicates upstream signaling dependent regulators of master TFs, their co-activators, and their downstream effectors as potential, rational subgroup-specific therapeutic targets. These insights demonstrate the critical importance of epigenetic analyses of primary tumours as opposed to cell line model systems and highlight the broad utility of core regulatory circuitry inference especially in poorly characterized and clinically diverse malignancies.

1 Northcott, P. A., Dubuc, A. M., Pfister, S. & Taylor, M. D. Molecular subgroups of medulloblastoma. *Expert review of neurotherapeutics* **12**, 871-884, doi:10.1586/ern.12.66 (2012).

2 Northcott, P. A., Korshunov, A., Pfister, S. M. & Taylor, M. D. The clinical implications of medulloblastoma subgroups. *Nature reviews. Neurology* **8**, 340-351, doi:10.1038/nrneurol.2012.78 (2012).

3 Taylor, M. D. *et al.* Molecular subgroups of medulloblastoma: the current consensus. *Acta neuropathologica* **123**, 465-472, doi:10.1007/s00401-011-0922-z (2012).

4 Northcott, P. A. *et al.* Medulloblastomics: the end of the beginning. *Nature reviews. Cancer* **12**, 818-834, doi:10.1038/nrc3410 (2012).

5 Jones, D. T. *et al.* Dissecting the genomic complexity underlying medulloblastoma. *Nature* **488**, 100-105, doi:10.1038/nature11284 (2012).

6 Kool, M. *et al.* Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. *Cancer cell* **25**, 393-405, doi:10.1016/j.ccr.2014.02.004 (2014).

7 Robinson, G. *et al.* Novel mutations target distinct subgroups of medulloblastoma. *Nature* **488**, 43-48, doi:10.1038/nature11213 (2012).

8 Pugh, T. J. *et al.* Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* **488**, 106-110, doi:10.1038/nature11329 (2012).

9 Batora, N. V. *et al.* Transitioning from genotypes to epigenotypes: why the time has come for medulloblastoma epigenomics. *Neuroscience* **264**, 171-185, doi:10.1016/j.neuroscience.2013.07.030 (2014).

10 Jones, D. T., Northcott, P. A., Kool, M. & Pfister, S. M. The role of chromatin remodeling in medulloblastoma. *Brain pathology* **23**, 193-199, doi:10.1111/bpa.12019 (2013).

11 Gilbertson, R. J. Mapping cancer origins. *Cell* **145**, 25-29, doi:10.1016/j.cell.2011.03.019 (2011).

12 Byrd, J. C. *et al.* Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *The New England journal of medicine* **369**, 32-42, doi:10.1056/NEJMoa1215637 (2013).

13 Hale, G. *et al.* Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* **2**, 1394-1399 (1988).