Genetically engineered mouse models and cross-species transcriptomics have provided mounting evidence of discrete, subgroup-specific developmental origins. Likewise, murine single-cell transcriptional atlases of cerebellar development have recently provided further clues into MB subgroup origins, particularly for poorly defined Group 3 and Group 4-MB. However, initial studies were underpowered to characterize rare populations and lacked robust validation, resulting in incomplete findings. Herein, we leveraged a large harmonized murine cerebellar atlas, targeted lineage enrichment, and integrative multi-omic strategies to deeply dissect MB origins. Isolation of spatially and temporally discrete developmental trajectories of key glutamatergic lineages born out of the murine upper rhombic lip provided an enhanced reference for mapping MB subgroup origins, especially for Group 3 and Group 4-MB. However, human-specific anatomic and cellular complexity, particularly within the rhombic lip germinal zone complicated murine-derived inferences. Further tumor-normal integrations using a novel single-cell atlas of the human fetal cerebellum, companioned with laser-capture micro-dissected transcriptional and epigenetic datasets, reinforced developmental insights extracted from candidate murine cerebellar lineages. Characterization of compartment-specific transcriptional signatures identified in the human upper rhombic lip implicated convergent cellular correlates of Group 3 and Group 4-MB, suggestive of a common developmental trajectory underlying their ancestry. Systematic imaging review and 3D summarization of a large clinical trial series of patient tumors, coupled with our advanced insights into developmental signatures, substantiated subgroup-specific tumor location patterns observed at diagnosis. Together, our results strongly implicate a common lineage trajectory of the upper rhombic lip as the probable origin of Group 3 and Group 4-MB. These important findings provide unprecedented opportunities to explore context-dependent mechanisms of MB pathogenesis and will foster generation of improved preclinical models that more faithfully recapitulate tumor biology.

MEDB-79. MYC-DRIVEN UPREGULATION OF THE *DE NOVO* SERINE AND GLYCINE PATHWAY IS A NOVEL THERAPEUTIC TARGET FOR GROUP 3 MYC-AMPLIFIED MEDULLOBLASTOMA

Magretta Adiamah[1], Janet C. Lindsey[1], Florence Burté[1], Sarah Kohe[2], Alaide Morcavallo[3], Helen Blair[1], Rebecca M. Hill[1], Mankaran Singh[1], Stephen Crosier[1], Tong Zhang[4], Oliver Maddocks[4], Andrew Peet[2], Louis Chesler[3], Ian Hickson[1], Ross Maxwell[1], Steven C. Clifford[1]; [1]Newcastle University Centre for Cancer, Newcastle University, Newcastle, United Kingdom. [2]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom. [3]Division of Clinical Studies, Institute of Cancer Research, London, United Kingdom. [4]Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

Despite advances in the molecular sub-classification and risk-stratification of medulloblastoma (MB), a subset of tumours remain refractory to current multimodal therapies. Group 3 (MB_{Group3}) patients represent around 25% of MBs, and amplification and elevated expression of *MYC* in this group correlates with dismal clinical outcomes. Since direct targeting of MYC remains elusive, understanding and exploiting metabolic dependencies in MYC-amplified MB_{Group3} may reveal novel therapeutic opportunities. We engineered three independent regulable *MYC*-amplified MB_{Group3} cell-based models, each harbouring doxycycline-inducible anti-MYC shRNAs (two independent species) or a non-silencing shRNA control. In all three models, MYC knockdown (KD) revealed persistent MYC-dependent cancer phenotypes, reduction in proliferation and cell cycle progression. We utilised ^{1}H high-resolution magic angle spectroscopy (HRMAS) and stable isotope-resolved metabolomics to assess changes in intracellular metabolites and pathway dynamics when *MYC* expression was modulated. Profiling revealed consistent MYC-dependent changes in metabolite concentrations across models. Notably, glycine was consistently accumulated following *MYC* KD suggesting altered pathway dynamics. ^{13}C-glucose tracing further revealed a reduction in glucose-derived serine and glycine (*de novo* synthesis) following MYC KD which was attributable to lower expression of PHGDH, the rate-limiting enzyme of this pathway. Furthermore, in human primary tumours, elevated expression of PHGDH was associated with *MYC* amplification and poorer survival outcomes. MYC expressing cells showed greater sensitivity to pharmacological inhibition of PHGDH compared to MYC KD (MB_{Group3}) and MB_{SHH} subgroup cell lines *in vitro*. Critically, targeting PHGDH *in vivo*, using MYC-dependent xenografts and genetically engineered mouse models, consistently slowed tumour progression and increased survival. In summary, metabolic profiling has uncovered MYC-dependent metabolic alterations and revealed the *de novo* serine/glycine synthesis pathway as a novel and clinically relevant therapeutic target in *MYC*-amplified MB_{Group3}. Together, these findings reveal metabolic vulnerabilities of *MYC*-amplified MB_{Group3} which represent novel therapeutic opportunities for this poor-prognosis disease group.

MEDB-80. CDK8 PROMOTES STEMNESS OF MYC-DRIVEN MEDULLOBLASTOMA

Dong Wang[1], Bethany Veo[1], Angela Pierce[1], Sujatha Venkataraman[1], Rajeev Vibhakar[2]; [1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA. [2]University of Colorado Anschutz Medical Campus, Aurora, `CO, USA

Cyclin-dependent kinase 8 (CDK8) belongs to the transcription-related cyclin dependent protein kinase family. CDK8 and cyclin C associate with the mediator complex to regulate gene transcription. Although CDK8 has been shown to be implicated in the malignancy of several types of cancer, its functional role and mechanism in medulloblastoma remains largely unknown. Here, we demonstrate how CDK8 plays an essential role in maintaining stemness and tumorigenicity in medulloblastoma stem cell. CDK8 inhibition suppresses stem cell associated signaling in medulloblastoma cells and inhibits tumor cell self-renewal. Additionally, CDK8 is amplified in MYC-driven medulloblastoma, is positively correlated with c-MYC expression in human medulloblastoma specimens and associates with poor survival in patients. Using cut&run assay, we found CDK8 associates with MED1 to activate transcription of MYC target genes. CDK8 attributes to MYC-driven transcriptional programs mediating DNA repair. Pharmaceutic inhibitors and genetic depletion result in cessation of tumor growth in xenograft mouse models and increase in apoptosis and DNA damage. Collectively, our studies establish the selective inhibition of CDK8 inhibition as a viable therapeutic strategy in MYC-driven medulloblastoma.

MEDB-81. COMBINED INHIBITION OF CDK11 AND EZH2 RESULTS IN REGRESSION OF MYC-AMPLIFIED MEDULLOBLASTOMA

Dong Wang, Bethany Veo, Angela Pierce, Sujatha Venkataraman, Rajeev Vibhakar; University of Colorado Anschutz Medical Campus, Aurora, CO, USA

We explored an shRNA library screen on 20 cyclin-dependent kinases to establish cyclin-dependent kinase 11 (CDK11) as a critical mediator in MYC-driven medulloblastoma. The effect and molecular mechanism of CDK11 in the proliferation and growth of medulloblastoma were investigated in vitro. Pharmaceutic inhibitors and genetic depletion of CDK11 resulted in cessation of tumor growth in xenograft mouse models. Through combination chemical screening, we identified that 5-FU enhanced the apoptosis which induced by inhibition of CDK11 in medulloblastoma cells. In addition, we found CDK11 is a significant candidate kinase participating in the negative control of Wnt/b-catenin signaling. Down-regulation of CDK11 led to the accumulation of Wnt/β-catenin signaling receptor complexes through activation of transmembrane Frizzled (FZD) receptors which is suppressed by H3K27Me3. RNASeq and cut&run revealed that Cdk11 and mediator associated Cdk8 kinase regulate a common set of genes. Lack of Cdk8 and Cdk11 impaired Ezh2 recruitment and the establishment of histone H3 lysine 27 tri-methylation. We concluded that combined EZH2 and CDK8/CDK11 inhibitors treatment concurrently activated Wnt signaling may be an effective treatment for Group 3 medulloblastoma.

MEDB-82. EXPLORING CELL-CELL COMMUNICATION NETWORKS IN MEDULLOBLASTOMA USING SINGLE-CELL GENOMICS

Lisa Gabler[1,2], Johannes Gojo[3], Kyle S. Smith[4], Laure Bihannic[4], Mariella G. Filbin[1,2], Paul A. Northcott[4], Walter Berger[5], Volker Hovestadt[1,6]; [1]Department of Pediatric Oncology, Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA. [2]Broad Institute of Harvard and MIT, Cambridge, MA, USA. [3]Department of Pediatrics and Adolescent Medicine and Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna, Austria. [4]Department of Developmental Neurobiology, St Jude Children's Research Hospital, Memphis, TN, USA. [5]Center for Cancer Research and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria. [6]Broad Institute of Harvard and MIT, Boston, MA, USA

Medulloblastoma is a high-risk embryonal brain tumor arising in the cerebellum. Genomic profiling has revealed a striking molecular heterogeneity between medulloblastoma patients, yet treatment regimens are mostly uniform. Many children with medulloblastoma die from their disease and surviving patients often face severe long-term side effects, highlighting an urgent need for more effective treatment options. We and others have recently identified pronounced intra-tumoral heterogeneity and defined cellular hierarchies within medulloblastoma tumors. The functional role of these cellular hierarchies remains unknown. We now hypothesize the existence of an inter-cellular communication network that is maintained by receptor/ligand interactions. To test our hypothesis, we use our medulloblastoma single-cell RNA sequencing dataset of 25 patients, as well as bulk RNA sequencing, DNA methylation array, and genome sequencing data across molecular subtypes. Single-cell RNA sequencing data are analyzed to dissect cell compartments characterized by high expression of potentially oncogenic receptors and their respective ligands. Consequently, cell type-specific roles in auto- or paracrine signal transduction within the cellular community are explored. We further investigate downstream oncogenic signaling pathways by approximating transcription factor activity and explore genetic and epigenetic