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4 (KBTBD4) in cases of group 3 and group 4 medulloblastoma. Critically, group 3 and 4 tumours with KBTBD4 mutations typically lack other gene-specific alterations, such as MYC amplification, indicating KBTBD4 insertion mutations as the primary genetic driver of these malignancies. Delineating the role of KBTBD4 mutations in medulloblastoma thus offers significant opportunities to understand its pathogenesis and exploit underpinning mechanisms therapeutically, however their function is currently unknown. Here, we show a novel mechanism in cancer pathogenesis whereby indel mutations in KBTBD4 drive its recognition of neo-substrates for degradation. We observe that KBTBD4 mutants promote the recruitment and ubiquitylation of the REST Corepressor (CoREST), which forms a complex to modulate chromatin accessibility and transcriptional programmes. The degradation of CoREST promoted by KBTBD4 mutations diverts epigenetic programmes inducing significant alterations in transcription to promote increased stemness of cancer cells. Transcriptional analysis of >200 human group 3 and 4 medulloblastomas by RNA-seq, highlights the presence of CoREST and stem-like signatures in tumours with KBTBD4 mutations, which extend to a further subset of non-mutant tumours, suggesting CoREST alterations as a novel pathogenetic mechanism of wide relevance in group 3 and 4. Our findings uncover KBTBD4 mutation as a novel driver of epigenetic reprogramming in non-WNT/non-SHH medulloblastoma, establishes a novel mode of tumorigenesis through gain-of-function mutations in ubiquitin ligases (neo-substrate recruitment) and identifies both mutant KBTBD4 and CoREST complexes as new druggable targets for improved tumour-specific therapies.

MEDB-63. DECIPHERING THE ROLE OF LIN28B IN GROUP 3 MEDULLOBLASTOMA

Shubin Shahab^{1,2}, Jessica Sun¹, Robert Schnepf¹, Tobey MacDonald^{1,2}, Anna Kenney¹; ¹Emory University, Atlanta, GA, USA. ²Aflac Cancer and Blood Disorders Center. Children's Healthcare of Atlanta, Atlanta, GA, USA

BACKGROUND: Children with Group 3 medulloblastoma (MB) have a very poor long-term outcome and many do not survive beyond 5 years. Several drivers for Group 3 MB have been identified but none have resulted in targeted therapy to date. LIN28B is a stem cell factor that is upregulated in Group 3 medulloblastoma and is associated with worse survival. Here we investigate the role of the LIN28B pathway in Group 3 MB development. Pharmacologic inhibition of the LIN28B pathway is feasible and may provide a unique opportunity to target this tumor. **METHODS:** Using LIN28B knockdown and overexpression in G3 MB cells we test LIN28B's effect on proliferation, self-renewal and metastasis. Similarly, we used shRNAs targeting PBK and demonstrate a similar effect on G3 MB growth. We also investigate the role of let-7 as a target of LIN28B by introducing let-7 mimetics and overexpression vectors into MB cells. Finally, we use a LIN28 inhibitor 1632 and a PBK inhibitor HITOPK032 to treat G3 MB cell lines and then assess their impact on proliferation and apoptosis. **RESULTS:** We find that down-regulation of LIN28B or PBK using shRNA results in significant reduction in cell proliferation. In contrast overexpression of LIN28B increases Group 3 cell proliferation and tumor sphere formation. LIN28B knockdown also significantly ($p < 0.01$) increases survival in mice with orthotopic Group 3 tumors. The LIN28 inhibitor 1632 also leads to significant reduction in G3 MB growth through decreased cell cycle entry and increased apoptosis. In addition, HITOPK032 also demonstrates significant reduction in Group 3 MB cell proliferation at low (nanomolar to low micromolar) concentration. **CONCLUSIONS:** Our study establishes a critical role for the LIN28B-let-7-PBK pathway in Group 3 MB and provides encouraging preliminary preclinical results for drugs that target this pathway.

MEDB-64. ARE RAB GTPASES METASTATIC DRIVERS IN METASTATIC MEDULLOBLASTOMA?

Susannah Entwistle, Hannah K. Jackson, Ian Kerr, Beth Coyle, Alistair Hume; University of Nottingham, Nottingham, United Kingdom

Medulloblastoma is the most common malignant paediatric brain cancer with poorer prognosis related to the onset of metastasis. It has four molecular subgroups; Wingless (WNT), Sonic Hedgehog (SHH), group 3 and group 4, of which group 3 is the most likely to be metastatic and is therefore associated with the poorest prognosis. Exosomes are small membrane-bound extracellular vesicles of endosomal origin which contain a variety of cargo including RNA and proteins. Increased exosome release is connected with disease progression and metastasis in multiple cancers. Rabs are a family of small GTPases (70 in humans) which regulate vesicle trafficking. Several Rabs are known to regulate exosome biogenesis and secretion and may thereby contribute to cancer progression. The role of Rabs in metastatic medulloblastoma is unclear. We aim to explore whether Rabs contribute to the progression of metastatic medulloblastoma through the exosome biogenesis and secretion pathways. Through analysis of literature, databases

such as ExoCarta.org, the R2: Genomics analysis and visualisation platform, and mRNA content of medulloblastoma exosomes, five novel Rab candidates were identified that may contribute to disease progression in group 3 medulloblastoma. Gene expression of these Rabs was then verified across SHH, group 3 and group 4 patient-derived cell lines using RT-qPCR, with candidate Rab expression confirmed in the three subgroups. Presence of Rab mRNA has also been found in exosomes derived from group 3 and group 4 patients, with an enrichment in group 3 exosomes. Current and future work aims to determine the potential roles of Rabs in medulloblastoma pathogenesis, and to determine whether Rabs contribute to increased exosome biogenesis which drives metastasis or are metastatic drivers in medulloblastoma themselves. Therefore, experiments to characterise Rab candidate protein expression within cells and assess their function after knockdown are necessary and timely.

MEDB-65. MOLECULAR SUBCLASSIFICATION OF A NATIONAL COHORT OF PEDIATRIC MEDULLOBLASTOMA BASED ON METHYLATION PROFILE

Lide Alaña¹, Idoia Martin-Guerrero², Aurora Navajas^{1,3}, Laura Zaldumbide⁴, Lorena Mosteiro⁴, Edward C. Schwalbe^{5,6}, Debbie Hicks⁵, Steven C. Clifford⁵, Miguel García-Ariza^{1,3}; ¹Pediatric Oncology, Biocruces Health Research Institute, Barakaldo, Spain. ²Department of Genetics, Physic Anthropology and Animal Physiology, University of the Basque Country, Bilbao, Spain. ³Pediatric Hematology and Oncology Unit, Cruces University Hospital, Barakaldo, Spain. ⁴Department of Pathology, Cruces University Hospital, Barakaldo, Spain. ⁵Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle upon Tyne, United Kingdom. ⁶Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom

INTRODUCTION: Pediatric Medulloblastoma (MB) accounts for approximately 20% of all childhood brain tumors. Molecular subgroups namely WNT, SHH, Group 3 and Group 4, exhibit divergent biology, and clinical outcomes. DNA methylation analysis is a robust option to classify pediatric MB into molecular subgroups, which allows the optimization of diagnosis and stratification of the treatment. We review the first experience of molecular subclassification carried out at the national level in our country. **METHODS:** Multi-center centralized prospective and retrospective study of frozen tumor samples at diagnosis from pediatric MB patients diagnosed in Spanish hospitals, from April 2021 to December 2021. A registry was created with histology review, immunohistochemical (IHC) subgrouping, and a molecular subgrouping based on the Minimal Methylation Classifier (MIMIC) from Schwalbe et al., 2017. The time from the sample centralized reception to the study result was also collected. **RESULTS:** 25 frozen MB tumor samples from patients at diagnosis were included. 6 were retrospective and 19 prospective. IHC classified 19 cases (76%) as non-WNT/non-SHH MBs, 3 (12%) as WNT-activated and 3 (12%) as SHH-activated. MIMIC classified, in the non-WNT/non-SHH, 6 tumors (24%) as Group 3 and 13 (52%) as Group 4. 2 cases (8%) were WNT-activated MBs and 3 (12%) were SHH-activated MBs. Only 1 case (4%) was unclassified by MIMIC (WNT using IHC). Comparing both methods (IHC and MIMIC), diagnosis agreed in 96% of cases. The response time ranged from 5 to 10 days. **CONCLUSIONS:** DNA methylation profiling has proven to be a robust and quick option to classify MB into subgroups and it correlates with the IHC diagnosis. This tool was successfully implemented in our national routine diagnosis, enabling a reliable and rapid molecular subgrouping classification.

MEDB-66. INVESTIGATING INTRA-TUMORAL HETEROGENEITY OF EXTRACHROMOSOMAL DNA IN SHH MEDULLOBLASTOMA

Owen Chapman¹, Sunita Sridhar^{2,1}, Robert J. Wechsler-Reya³, Lukas Chavez¹, Jill P. Mesirov¹; ¹University of California San Diego, San Diego, California, USA. ²Rady Children's Hospital, San Diego, California, USA. ³Sanford Burnham Prebys Medical Discovery Institute, San Diego, California, USA

Extrachromosomal circular DNA (ecDNA) is an important driver of aggressive cancers, including medulloblastoma (MB), the most common malignant pediatric brain tumor. Our study's aim is to better understand how ecDNA containing cells can potentiate malignant growth. EcDNA's role in the development of treatment resistance and association with poor outcomes is hypothesized to arise from its contribution to intra-tumoral heterogeneity and its potential to promote oncogene dependency switching. To analyze the intra-tumoral distribution of ecDNA, we have now simultaneously analyzed the accessible chromatin and gene expression in single cells of a SHH medulloblastoma (MB) patient using multiome single-cell ATAC-seq and gene expression (10X Genomics). Whole genome sequencing (WGS) of this tumor previously revealed a heterozygous somatic TP53 mutation and two distinct ecDNAs: a 3.2Mbp amplicon comprising 3 regions of chr1 and another 4.5Mbp amplicon comprising 23 segments originating from chr7 and chr17. We then used multimodal analysis to describe the tumor cell types,