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 is a potent regulator of metastatic medulloblastoma. Gene expression studies of these Rabs with non-SSH RING-finger domains indicate their involvement in the progression of metastatic medulloblastoma through the exosome biogenesis pathway. Delineating the role of KBTBD4 mutations in medulloblastoma thus offers significant opportunities to understand its pathogenesis and exploit underpinning mechanisms therapeutically, however their functional consequences remain to be determined. Here, we show that exosome biogenesis pathway where under exosomal mutations in KBTBD4 drive its recognition of neo-substrates for degradation. We observe that KBTBD4 mutants promote the recruitment and ubiquitylation of the REST Co-repressor (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 further subsets of non-mutant tumours, suggesting CoREST alterations as a novel pathogenic 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-SSH medulloblastoma, establishes a novel mode of tumorigenesis through gain-of-function mutations in ubiquitin ligases (nuo-ubiquitin ligase) and identifies both mutant KBTBD4 and CoREST complexes as new drugable targets for improved tumour-specific therapies.

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

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**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 investigated 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 cells and assess their effect 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. Lin28 knockdown and PBK overexpression increases survivin 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 (nominally 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?**

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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 monomeric 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 analysis of these Rabs with non-SSH, 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 tumours. Current 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 MB 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**

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**INTRODUCTION:** Pediatric Medulloblastoma (MB) accounts for approximately 20% of all childhood brain tumors. Molecular subgroups named WNT, SHH, Group 3 and Group 4, exhibit divergent biology, and clinical outcomes. DNA methylation analysis is a robust option to classify pediatric MB groups, 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 133 pediatric MB tumors from 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 (MMC) from Schwalbe et al., 2017. The time from the sample’s centralization to the study result was also collected. **RESULTS:** 25 frozen MB tumor samples from patients at diagnosis were included. 6 were retropositive 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. MMC classified 15 cases (52%) as Group 4, 6 cases (8%) were WNT-activated MBs and 3 (12%) were SHH-activated MBs. Only 1 case (4%) was unclassified by MMC (WNT using IHC). Comparing both methods (IHC and MMC), 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**

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**INTRODUCTION:** Pediatric Medulloblastoma (MB) is a very heterogeneous malignant pediatric brain tumor. Our study’s aim is to better understand how ecDNA containing cells can potentiate malignant growth. EcDNAs 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 MB tumor previously revealed a heterozygous somatic TP53 mutation and two distinct ecDNAs: a 3.2Mbp ampicron comprising 3 regions of chr1 and another 4.5Mbp ampicron comprising 23 segments originating from chr7 and chr17. We then used multimodal analysis to describe the tumor cell types,
gene expression, variant signatures and estimate ecDNA copy number in the medulloblastoma tumor sample. We identified 12 distinct clusters in the human tumor, 3 of which were determined to be normal non-tumor (OSCC1) cells, 7 of which were identified as tumor cells. Enrichment of ecDNA was restricted to only one of these tumor clusters. In addition, we also performed the same multiteme single-cell analyses in an orthotopic xenograft mouse model derived from the PDX tumor. In the PDX cohort, 8 cell clusters were identified, of which 7 were determined to be tumor cells and enriched for ecDNA. Our preliminary results indicate that tumor cells with ecDNA in the human tumor (particularly the ecDNA enriched cluster) almost exclusively account for [OSCC1] the cells in the corresponding PDX, emphasizing the aggressiveness of ecDNA containing cells.

MEDB-67. SUBGROUP SPECIFIC ANALYSIS OF CELLULAR METABOLISM IN MEDULLOBLASTOMA

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INTRODUCTION: Molecular subgrouping of Medulloblastoma (MB) has expanded our understanding of its biology and the impact on clinical parameters. However, detailed analysis of intra- and intertumoral heterogeneity on a metabolic level is currently lacking. Within this study, we aimed at improving our understanding of metabolic heterogeneity between the MB subgroups, between samples within these subgroups and how these differences affect prognosis. METHODS: We analyzed metabolic characteristics of four MB cohorts covering 1,804 samples in total. In 911 samples (ICGC and SJMB03 cohort), we explored metabolic patterns on RNA level, in two subgroups (ICGC and G3/G4 samples from the HIT cohort, n=1,035) we examined genetic alterations on DNA level. Furthermore, single-cell RNA sequencing data of six samples were used to explore intratumoral metabolic heterogeneity. Intra- and intertumoral heterogeneity was correlated to clinical data. RESULTS: Using publicly available gene signatures, we discovered significant differences in metabolic gene expression comparing established MB subgroups. Three metabolically distinct clusters of G3/G4 samples could be defined by unsupervised analyses in two independent cohorts. We were able to confirm our finding of intertumoral metabolic differences on single-cell RNA level. Additionally, our analysis revealed the possibility of sample-specific metabolic features. On DNA level, we identified regulatory genes with known role in MB development to be predominantly associated with lipids and fatty acids metabolism. After all, lipid metabolism is of great interest for future therapeutic options in the future.

MEDB-68. ANALYSIS OF TELOMERES LENGTH AND ALTERNATIVE LENGTHENING OF TELOMERES (ALT) IN MOLECULAR SUBGROUPS OF INFANT MEDULLOBLASTOMA

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We investigated the association between the molecular profile and telomere length in a infant medulloblastoma (iMB) cohort, retrospectively studied. Activation of telomeres maintenance mechanisms was examined to determine whether the senescence escape triggered by telomere-elongation mechanisms could explain the aggressiveness of some iMB belonging to the same molecular subgroup. Interestingly, several telomerase- and ALT-targeted therapies have recently been tested on pediatric cancers and might represent a promising strategy for the future treatment of aggressive tumors. We performed ALT-positive iMB from young MB patients (age ≤ 3); IHC, FISH, and an Illumina 850K methylation profile were used to identify molecular subgroups. Telomere length was measured using Telo-quantitative FISH, and image analysis was performed using TFL-Telo software. Three distinct telomere intensity categories (low (L), medium (M), and high (H)) were identified by comparing neoplastic- to endothelial-cell signals in each sample. ATRX loss and TERTp mutation/methylation were investigated using IHC and Sanger sequencing/methylation-specific PCR. SHH-MBs accounted for 39%; MYC-MBs accounted for 4%; iMBs were accounted for 41%; 72% of all iMBs were detected. ALT was found to be activated in 10% of iMBs and was not exclusive to any molecular subgroup, implying that it could be a potential mechanism associated with aggressive behaviour in a subset of the iMB. Promising results from other published studies have been found in the distribution among the iMB molecular subgroups: SHH iMBs had a higher frequency of High (H) telomeres length (85%) than NON-SHH/NON-WNT iMBs (p=0.046), which were more frequently associated with MeSHH iMBs. CONCLUSIONS: ALT activation in infant MBs (10%) could be a novel target for risk-stratification and personalized therapy. It may be useful to examine ALT as a potential predictor of aggressiveness and as a promising novel therapeutic approach for a subset of these tumors in the diagnostic workup.

MEDB-69. CLINICAL AND MOLECULAR META-ANALYSIS OF THREE MAJOR MEDULLOBLASTOMA CLINICAL TRIALS (ACNS0331, SJMB03, ACNS0332) UNCOVERS NOVEL STRATEGIES TO IMPROVE RISK-STRATIFIED THERAPY

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BACKGROUND: Given the vast molecular heterogeneity present within medulloblastoma (MB) and considerable differences in therapy, we performed a meta-analysis of the three largest recent published clinical trials (ACNS0331, SJMB03, ACNS0332) comprising 898 children with newly-diagnosed MB to shape future therapy. METHODS: Molecular subgroups, subtypes, and copy number variations were uniformly provided. In the ACNS0331 cohort, we compared outcomes of patients with non-metastatic (M0), non-residual (R0), non-anaplastic MB treated with QPC (HD) craniospinal irradiation (CSI); (2) ACNS0331_SDCS - patients with M0R0 non-anaplastic MB treated with standard-dose (SD) CSI; (3) SJMB03_SDCS - patients with M0R0 non-anaplastic MB treated with SD CSI; (4) SJMB03_HDCS - patients with metastatic (M+), MB treated with high-dose (HD) CSI; (5) ACNS0332_HDCS - patients with M+ MB treated with HDCS; (6) ACNS0332_HDCS_Carbo - patients with M+ MB treated with HDCSI and carboplatin. RESULTS: 803 (WNT=123, SHH=122, G3=189, G4=367) of 898 patients formed the cohort. No significant difference was observed between the event-free survival (EFS) from ACNS0331_SDCS and SJMB03_SDCS, or SJMB03_HDCS and ACNS0332_HDCS when analyzed as a whole or by subgroup. ACNS0331_LDCS outcome was inferior to the combined ACNS0331_SDCS + SJMB03_SDCS cohorts (p<0.001) and in G3 (p=0.016), ACNS0332_HDCS and SJMB03_HDCS only in G3/G4 subtype III (p=0.045). Additional molecular risk factor analysis identified M0R0 G3/G4 subtype VII and SHH without risk factors as very low risk (>90% EFS) and M0R0 G3/G4 subtype III as high risk (<40% EFS). CONCLUSION: The comparable results observed across trials presents a welcome opportunity to reduce toxicity by eliminating excessive doses of chemotherapy (i.e. vincristine, cisplatin, and cyclophosphamide) from therapy. Furthermore, these results support molecularly driven risk classification as the means for a better, more-refined, treatment stratification.

MEDB-70. METABOLISM MEDIATED RADIATION RESISTANCE IN MYC-DRIVEN MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most prevalent malignant brain tumor in children and demonstrates a high level of heterogeneity. Treatment for MB includes chemotherapy and radiation often resulting in long-term morbidity. MYC-driven MB, are high-risk tumors with poor long-term survival and increased susceptibility to develop recurrent tumors. Recurrent MB is far more aggressive with limited treatment options leading to a 3-year survival rate of 50%. Within this context, we performed single-cell RNA sequencing of irradiated MB xenograft tumors. We identified an overall enhancement of metabolic activity in radiation-resistant cells. We further observed enhanced wild-type IDH1 and IDH2 expression in two clusters, which coincide with hypoxia and Nestin expression, marking a stem-cell like niche. Stem-like cancer cells are notorious for their expression, marking a stem-cell like niche. Stem-like cancer cells are notorious for their