SIONS: We found that pre-treatment NLR was an independent prognostic factor for recurrence or metastasis of medulloblastoma after treatment. In combination with NRL and clinical factors, nomogram has a good prediction of PFS in patients with medulloblastoma after radiotherapy. It has the potential to facilitate more precise risk stratification to guide personalized treatment of medulloblastoma.

MEDB-59. A DRAFT ATLAS OF MEDULLOBLASTOMA CELLULAR EVOLUTION UNDER THERAPY

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How standard care shapes the cellular composition of recurrent medulloblastoma (MB), if therapy selects for specific tumor or immune cell types, is unknown. We report the pilot phase of our ongoing effort to profile human longitudinal MB specimens via single-cell transcriptomics and epigenetics. We profiled 11 diagnostic and eight recurrent specimens from 19 subjects via single-nucleus RNA sequencing (snRNA-seq), and four subjects via single-nucleus assay for transposase-accessible chromatin. Specimens from select subjects were also profiled to assess genome-wide enhancer activity via single-nucleus cleavage-under-targets and tagmentation. We found an upregulation of the DNA-damage response, RNA translation, WNT and NOTCH signaling in recurrent specimens. The percentages of stem-like cells increased by over two-fold at recurrence. We found that microglia and oligodendrocyte-lineage cells were the most abundant non-malignant tumor-associated cell types, representing 2%-10% of cells profiled. Microglia abundances were relatively stable across molecular subtypes, and when comparing primary to recurrent tumors. There was a moderate, but statistically significant, increase in oligodendrocyte abundance in SSH and WNT tumors, compared to Group 3/4 tumors. We compared gene expression in tumor cells with public snRNA-seq from developing human cerebella (PCW 9-21). Combined Group-3/4 cell analysis supports a common lineage hierarchy, with an enrichment for unipolar brush-cell and Purkinje-cell phenotypes found in Group-4 tumors. All Group-3/4 cases contained cycling cells expressing markers of PAX2+ interneuron progenitors, most cycling cells had this phenotype. All specimens contained populations of non-cycling granule-cell progenitor-like cells. We performed single-cell co-expression receptor/ligand analysis to infer paracrine signaling between tumor and non-malignant cell types. This identified both tumor cells and microglia as sources of growth factors, pro-inflammatory cytokines, and pro-apoptotic ligands. Non-malignant oligodendrocyte-lineage cells uniquely expressed IL6-family cytokines, pleiotrophin, and class-III semaphorins. These studies shed light on the cellular heterogeneity of MB and the effect of standard therapy in shaping composition at recurrence.

MEDB-60. MEDULLOBLASTOMA WITH EXTENSIVE NODULARITY MIMICS CEREBELLAR DEVELOPMENT AND DIFFERENTIATES ALONG THE GRANULAR PRECURSOR LINEAGE

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BACKGROUND: Medulloblastoma with extensive nodularity (MBEN) represents a rare type of cerebellar tumors of infancy comprising two histologically distinct components that differ in cell differentiation and mitotic activity. Whereas some children suffering from MBEN experience disease recurrence, MBEN can also spontaneously differentiate and discontinue to grow. The underlying mechanisms of this variable biological behavior may offer insight into how embryonal tumors develop. METHODS: Fresh frozen and FFPE tumor tissue from nine MBEN-patients was subjected to multi-omics characterization including bulk sequencing, microdissection followed by RNA sequencing, single nucleus RNA-sequencing using the 10X Genomics- and SMART Seq. V2-protocols and spatial transcriptomics via RNAscope. RESULTS: All cases were molecularly classified as Sonic Hedgehog (SHH)-MB, and harbored somatic mutations within the SHH-pathway. After quality control, a total of ~30.000 cells were subjected to downstream analysis. Several non-malignant cell types, such as glial cells, were identified. In accordance with previous studies, we found only sparse immune infiltration. Unsupervised clustering identified cell clusters that differed in differentiation state and represented a continuum from embryonal-like cells with SHH-upregulation over intermediate cell states, to neuronal-like, postmitotic cells. Mapping to a single nucleus sequencing atlas of cerebellar development indicated that tumor cells reflected various stages of normally developing cerebellar granular precursors. Interestingly, one cluster of malignant cells with tumor-specific copy number alterations showed both transcriptomic features of astrocytes and embryonal cells. Using spatial transcriptomics, we were able to correlate different clusters of MBEN cells with distinct histologic MBEN compartments, with astrocyte-like tumor cells being located in the internodular compartment and in close proximity to mitotically active cancer cells. CONCLUSION: MBEN is formed by a continuum of malignant cell differentiation along the granular precursor lineage, with a subset of cells developing into cells that may represent tumor astrocytes. This differentiation process is reflected in the bicompartmental structure of MBEN.

MEDB-61. GENETIC ALTERATIONS OF *TP53* AND *OTX2* INDICATE INCREASED RISK OF RELAPSE IN WNT MEDULLOBLASTOMAS

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PURPOSE: This genetic analysis of WNT-activated medulloblastomas (WNT-MBs) aimed to re-evaluate the prognostic impact of *TP53* mutations and to identify specific chromosomal aberrations as possible prognostic markers in a retrospective cohort of patients treated according to the protocols of the HIT medulloblastoma studies. PATIENTS AND METHODS: In a cohort of 191 patients with WNT-MBs, mutations in *CTNNB1*, *APC*, and *TP53* were analyzed by Sanger and/or NGS panel sequencing. Chromosomal copy number aberrations (CNAs) were assessed by high-resolution, genome-wide molecular inversion probe technology (MIP), SNP6 array, and/or 850k methylation bead-array hybridization. Complete clinical data were available from 120 patients. RESULTS: Patients with WNT-MBs had a female predominance (1.4:1) and a median age of 13 years (range 3-69 years). *CTNNB1* mutations were present in 92.2% of the samples, *APC* mutations in 6.8%. One *CTNNB1* wildtype tumor gained WNT-activation due to a homozygous deletion of *FBXW7*. Monosomy 6 was present in 78.6%, but more frequent in children compared to adults. 16.1% of the tumor samples showed *TP53* mutations, of those 60% with nuclear positivity for the p53 protein. A loss of heterozygosity at the *TP53* locus on chromosome 17p13.1 was found in 40.7% (11/27) of *TP53* mutant tumor samples and in 18.5% of the whole cohort (24/130 cases). Patients with tumors harboring *TP53* mutations showed significant worse progression-free survival (PFS; p=0.001), but not overall survival (OS) and were enriched for chromosomes 17p (p=0.001), 10, and 13 losses. Gains of the *OTX2* locus on chromosome 14q were also associated with poor PFS and OS (p=0.017 resp. p=0.006). Multivariate Cox regression analysis identified both genetic alterations as independent prognostic markers for PFS and OS. CONCLUSION: For ongoing and future de-escalation trials for patients with WNT medulloblastomas, we recommend to exclude patients with tumors carrying *TP53* mutations or *OTX2* gains.

MEDB-62. DISEASE-ASSOCIATED KBTBD4 MUTATIONS IN MEDULLOBLASTOMA ELICIT NEOMORPHIC UBIQUITYLATION ACTIVITY TO PROMOTE COREST DEGRADATION

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Genomic studies in medulloblastoma have identified distinct disease subgroups: wnt/wingless (WNT), sonic hedgehog (SHH), and non-WNT/non-SHH, comprising group 3 and group 4. Alterations in WNT and SHH signalling form the pathogenetic basis for their subgroups, whereas those for non-WNT/non-SHH tumours remain largely elusive. Recent analyses have revealed recurrent in-frame insertions in the E3 ubiquitin ligase adaptor Kelch Repeat and BTB Domain Containing

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

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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 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 Group3 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 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

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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

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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,