esis. Among them, mutations in chromatin modifiers are frequently detected in medulloblastoma, suggesting the importance of alterations in the epigenome in tumor development. However, it remains unclear how epigenomic changes are involved in tumorigenesis. Here, we have used the SHH-group medulloblastoma (MB-SHH) mouse model to understand the epigenomic changes during tumor development and identify new therapeutic targets for medulloblastoma. To this end, we utilized an Atoh1-EGFP:Ptch1+/- mice that specifically label cerebellar granule cell progenitors (GNPs) that are known to be the cellular origin of MB-SHH, preneoplastic (PNCs) and tumor cells with EGFP during MB-SHH formation. Following FACS-based collection of EGFP-positive cells, comparative ATAC-seq analysis revealed that the open chromatin regions dynamically changed during transformation of GNPs into PNCs with enrichment of nuclear factor I (NFI) binding motifs. Cut & Tag analysis on these cells showed that NFI proteins bound chromatin regions that become more accessible during tumorigenesis, suggesting that NFI could play an important role in tumorigenesis after the epigenomic changes. Indeed, CRISPR-engineered in vivo somatic deletion of Nfia and/or Nfib prevented hyperplasia formation, confirming their essential role in tumor initiation. Knockdown of either NFIA or NFIB in patient-derived xenograft models also attenuated tumor growth. Thus, our study has uncovered a novel oncogenic mechanism that contributes to the development of MB-SHH tumors via alterations of accessible chromatin and aberrant DNA binding of NFI transcription factors.

MEDB-16. PERSISTENT RADIOLOGICAL LESIONS AT THE END OF PRIMARY THERAPY IN CHILDHOOD MEDULLOBLASTOMA: RESIDUAL LESION OR ACTIVE RESIDUAL TUMOR?

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BACKGROUND: Magnetic resonance imaging (MRI) of patients with medulloblastoma (MB) often shows persistent residual findings after primary treatment. Criteria for characterizing these lesions and consensus on further therapeutic approaches are not established. MATERIAL AND METHODS: Eighty-four patients ≥4 years with centrally reviewed residual lesions on MRI at the end of primary therapy with initial surgery between 2000 and 2018 were identified. Data were extracted from the German HIT-MED database. RESULTS: Median age at initial diagnosis was 9.3 (4.0-20.8) years. 91.7% were histologically classified as CMB, 7.1% as LC/AMB and 1.2% as DMB. The majority (65.5%) of the evaluated cohort was assigned to molecular subgroup 4, 24.1% to group 3, 6.8% to WNT, 3.4% to SHH. Median follow-up for survivors was 5.96 (1.41-16.67) years. Univariate analysis revealed that patients showing an overall partial response (PR) to primary therapy have a significantly lower risk of progression of residual lesions compared to patients with stable disease (SD) (5-year PFS [PR]: 62.5±7,0; 5-year PFS [SD]: 35.9±12.8; 5-year OS [PR]: 85.6±5.1; 5-year OS [SD]: 54.1±13.7; p=0.02 [PFS], p=0.04 [OS]). Additionally, patients with multiple residual lesions (M+ and R+) were at higher risk of progression (5-year PFS [R+ only]: 72.4±12.0, 5-year PFS [R+/M+]: 22.9±17.9; p=0.02 [PFS]). Further procedures after the end of primary therapy (additional resections, chemotherapy, radiotherapy) did not impact on PFS and OS. These results were confirmed by multivariate Cox regression. For molecular or histological type no significant effect was found, presumably due to small cohort. CONCLUSION: PFS in patients with residual lesions at the end of primary treatment depends on the overall response to primary therapy. Additional procedures do not seem to be superior compared to watch-and-wait strategies. Decisions regarding further therapies should be scrutinized on a case-by-case basis. Further identification of biomarkers is warranted.

MEDB-17. RE-IRRADIATION FOR RECURRENT MEDULLOBLASTOMA IN A MATCHED COHORT: ADVANTAGEOUS ESPECIALLY IN PATIENTS WITHOUT RESECTION

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INTRODUCTION: Radiotherapy with craniospinal irradiation (CSI) is an important part of initial treatment for medulloblastoma in most children. Radiotherapy after recurrence is currently not widely used. This analysis aims to evaluate whether re-irradiation (RT2) may show survival benefits. METHODS: Data for patients with recurrent medulloblastomas from the German HIT-REZ studies was gathered. Patients with RT2 at 1st recurrence were matched by propensity score to an equal number of patients without radiotherapy. Matching variables were sex, initial therapy, time to recurrence, metastatic stage and therapy at 1st recurrence and radiotherapy at subsequent recurrences. The matched cohort was analysed regarding PFS and OS after 1st recurrence. RESULTS: From a cohort of 240 pre-irradiated patients, 106 patients were matched. Patients with RT2 showed improved median PFS [21.0 months (95%-CI: 17.5 – 27.6)] and OS [37.5 months (CI: 30.0 – 59.4)] compared to control patients [(PFS: 12.0 months (CI: 8.1 – 17.7) / OS: 20.1 months (CI: 14.5 – 44.8)]. When stratifying by resection at recurrence (36.8% resected), a survival advantage for RT2 was found in patients without resection in PFS [19.6 (CI: 14.9 – 31.5) vs. 8.0 months (CI: 5.4 – 14.4)] and OS [41.9 (CI: 30.0 – 59.4) vs. 13.3 months (CI: 8.1 – 36.7)]. However, no advantage was found after resection [PFS: 22.5 (CI: 17.5 – 50.4) vs. 19.1 months (CI: 14.1 – 34.3) / OS: 32.3 (CI: 27.6 – NA) vs. 48 months (CI: 23.4 – NA)]. CSI was used in 6 patients without differences in survival to focal RT2. Median PFS after first irradiation was 32.5 months, after RT2 20.9 months. No patients with RT2 were alive past 10 years after 1st recurrence.**CONCLUSION**: Patients with recurrent medulloblastoma show benefits from RT2 in median PFS and OS. However, no advantage for RT2 was found when resection was also applied at recurrence. Cure after treatment with RT2 was not found in our cohort.

MEDB-18. ELONGATION CONTROL OF MRNA TRANSLATION SUPPORTS GROUP 3 MEDULLOBLASTOMA ADAPTATION TO NUTRIENT DEPRIVATION

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Group 3 affiliation and *MYC* genetic amplification are associated with poor life expectancy and substantial morbidity in children suffering from medulloblastoma (MB). However, the high metabolic demand induced by MYC-driven transformation sensitizes MYC-overexpressing MB to cell death under conditions of nutrient deprivation (ND). Additionally, MYC-driven transformation is known to promote mitochondrial oxidative phosphorylation (OXPHOS). We previously reported that eukaryotic Elongation Factor Kinase 2 (eEF2K), the master regulator of mRNA translation elongation, promotes survival of MYC-overexpressing tumors under ND. Interestingly, eEF2K is overexpressed in MYC-driven MB and our preliminary proteomics data highlight large-scale alterations in OXPHOS components affecting eEF2K deficient MB cells. We therefore hypothesized that eEF2K activity is required for the selective translation of mRNAs needed for efficient OXPHOS, and for the progression of MYC-driven MB. We peformed Multiplexed enhanced Protein Dynamic Mass Spectrometry in eEF2K knockdown MYC-overexpressing D425 MB cells to identify mRNAs selectively translated upon eEF2K activation. Messenger RNAs encoding multiple (9 out of 10 detected) components of the mitochondrial OXPHOS pathway are selectively translated upon eEF2K activation. Inactivation of eEF2K by genetic KO leads to the disassembly of electron transport chain (ETC) complexes I-IV without affecting mRNA levels of their respective components. Consistently, *eEF2K* KO MB cells display decreased mitochondrial membrane potential and 20%

increased proton leak thorough the mitochondrial membrane. In addition, eEF2K inactivation results in increased Group 3 MB cell death under ND and doubles survival of MB bearing mice fed with calorie restricted diets ($p< 0.05$). Control of mRNA translation elongation by eEF2K is critical for mitochondrial ETC complex assembly and efficient OXPHOS in MYC-overexpressing MB, likely representing an adaptive response by which MYC-driven MB cells cope with acute metabolic stress. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs as eEF2K activity appears critical under metabolic stress conditions.

MEDB-20. THE OUTCOME OF MEDULLOBLASTOMA PATIENTS IN THE 2010-2018 PERIOD IN CHILDREN'S HOSPITAL ZAGREB

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This study aims to present the key characteristics of the medulloblastoma patients treated in Children's Hospital Zagreb and the University Hospital Center Zagreb in Croatia between 2010-2018 period. Croatia has around 145 newly diagnosed pediatric oncology patients annually, including approximately 30 neurooncology patients. We have conducted the retrospective analysis of the hospital records and have collected data on 32 medulloblastoma patients (9 females, 23 males). At the time of diagnosis, the median age was 5,62 (range 0.85-15.86). Before the treatment commencement, we determined conventional risk factors and stratified our patients into standard and high-risk groups (17 standard risk patients, 15 high risk). Qualification for high-risk included metastatic disease, postoperative local residual disease greater than 1.5 cm2, confirmed myc/nmyc amplification in the tumor tissue, and the large cell/anaplastic tumor subtype (p53 positive). The methods of molecular diagnostics were not available at the time. The patients that received solely postoperative chemotherapy were younger than three years. Children younger than five suffering from desmoplastic tumor subtype also received intraventricular methotrexate (Ommaya). High-dosage chemotherapy with autologous stem cell transplantation failed to treat metastatic infant medulloblastoma (2 patients with a lethal outcome). The rest of the patients received craniospinal irradiation, followed by adjuvant chemotherapy. According to the Kaplan-Meier survival analysis, the 5-year overall survival is 65,6 % (40% in the high-risk group and 88% in the standard-risk group). In addition, 5-year event-free survival is 59,4 % (33% in the high-risk group and 82,4% in the standard-risk group). None of the patients developed a secondary malignant disease during the follow-up. Conventional characteristics that determine standard-risk group affiliation are reliable, leading to a satisfactory treatment outcome. The results of the high-risk group treatment are poor necessitating modification treatment approach within clinical trials.

MEDB-21. SOX2$^+$ CELLS: THE PERPETRATORS OF MEDULLOBLASTOMA RELAPSE

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Pediatric brain tumors are the number one cause of cancer-related death in children, with medulloblastoma being the most common type. While survival in patients with medulloblastoma has dramatically improved since chemotherapy was added to standard of care protocols, still 30% of tumors will recur. As recurrent disease in medulloblastoma patients in considered uniformly lethal, it is key to identify the cells allowing tumor relapse, and their targetable regulators. By analyzing single cell transcriptomic data, we uncovered a population of SOX2 labeled astrocyte like cells resistant to SMO inhibitors in clinical trials. Using SOX2-enriched medulloblastoma cultures, we observed that SOX2$^+$ cells rely on non-canonical GLI signaling to propagate medulloblastoma. Therefore, *in vivo* inhibition of SHH signaling using functionally different GLI inhibitors depleted the SOX2$^+$ cell pool, what led to less aggressive tumors that lacked the ability to further engraft. Stressing the translational relevance of our findings, a clinically relevant GLI inhibitor not only exhausted SOX2$^+$ cells driving tumor relapse, but increased overall survival in mice harboring medulloblastoma. Our results emphasize the importance of using targeted therapies that deplete SOX2$^+$ cells to prevent medulloblastoma recurrence.

MEDB-22. IPSC-DERIVED CEREBELLAR ORGANOID MODEL FOR HEREDITARY GENETIC PREDISPOSITION IN SHH-MEDULLOBLASTOMA

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Medulloblastoma is one of the most common malignant embryonal brain tumors in children. Medulloblastomas of the Sonic Hedgehog (SHH) group arise from excessive proliferation of granule neuron progenitor (GNP) cells during cerebellar development. Genetic predisposition accounts for nearly 40% of all pediatric SHH-medulloblastomas. Recently, *ELP1*, a novel predisposition gene, was shown to be germline mutated in 15% of SHH-medulloblastoma patients. *ELP1* encodes the scaffolding member of the Elongator complex and is required for efficient translation. Heterozygous mutations in *ELP1* have been associated with the neural disorder Familial Dysautonomia, but not cancer. *ELP1*-associated medulloblastomas frequently harbor somatic *PTCH1* co-mutations. It remains unclear how *ELP1* affects the GNP lineage during normal cerebellar development and tumorigenesis in pediatric SHH-medulloblastoma patients. To characterize *ELP1* mutations in the GNP lineage in vitro, we established a cerebellar organoid model from human induced pluripotent stem cells (iPSCs). We genetically inserted an EGFP reporter downstream of the endogenous GNP-specific *ATOH1* locus in control iPSCs and generated cerebellar organoids according to published protocols. Marker gene and protein expression levels confirmed the cerebellar identity of the 3D model. Furthermore, activation of the EGFP reporter in single cells within the organoid highlighted the specification of putative GNPs. Next, we will determine the specific cell state of putative iGNPs and compare to human GNPs identified in our scRNAseq cerebellum atlas. To analyze tumorigenesis through *ELP1* loss, we will introduce patient-specific *ELP1*mutations into *ATOH1-EGFP* iPSCs. Cerebellar organoids derived from *ELP1*-, *PTCH1*-deficient and control iPSCs will serve as models to study GNP proliferation, differentiation, apoptosis and tumor formation. Combining genome editing, in vitro 3D differentiation and functional studies, we will characterize the novel predisposition gene *ELP1*in GNPs during cerebellar development. In addition, we will determine the interplay of *ELP1*and *PTCH1* co-mutations, predisposing SHH-medulloblastoma formation.

MEDB-23. TARGETING EPIGENETIC DYSREGULATION IN MEDULLOBLASTOMA WITH POOR PROGNOSIS

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Medulloblastoma (MB) is the most common paediatric malignant brain tumour and is classified into four distinct molecular subgroups (WNT, SHH, G3 and G4), each of them further subdivided into subtypes with different prognosis and responses to therapy. Deregulation of chromatin modifier genes play an essential role in MB, particularly in the G4 subgroup. A BMI1^{High};CHD7^{Low} molecular signature identifies patients with poor survival within this subgroup. We show that BMI1^{High};CHD7^{Low} sustains MB growth through regulation of MAPK/ERK signalling and via a novel epigenetic regulation of inositol metabolism in both G4 MB cells and patients. These tumours display over-activation of MAPK/ERK signalling, sustaining tumour proliferation, and of AKT/mTOR pathway which leads to energetic rewiring characterised by enhanced glycolytic capacity and reduced mitochondrial function. We demonstrate that inositol administration counteracts this metabolic alteration, impairs proliferation and significantly extends survival in a pre-clinical model. Moreover, inositol synergises with cisplatin, a chemotherapy agent currently used in MB treatment, enhancing its therapeutic effect *in vivo*. Additionally, we identify a synergistic vulnerability of BMI1^{High};CHD7^{Low} MB to a combination treatment with BMI1 and MAPK/ERK inhibitors that overcomes acquired resistance to single-drug therapy. Mechanistically, we observe a CHD7-dependent binding of BMI1 to MAPK-regulated genes underpinning the CHD7-BMI1-MAPK regulatory axis that is critical for the anti-tumour effect of the inhibitors *in vitro* and in a pre-clinical model. Moreover, we demonstrate that the BMI1^{High};CHD7^{Low} molecular signature defines G4 MB patients with an enhanced ERK1-ERK2 phosphorylation activity. Importantly, cerebellar neural stem cells modelling the BMI1^{High};CHD7^{Low} signature are not affected by BMI1 and MAPK/ERK inhibitors and do not show metabolic adaptation hence are resistant to the proposed treatments. In summary, we have identified two actionable vulnerabilities in a pre-clinical setting modelling a molecularly defined group of MB patients, paving the way for the design of signature-matched clinical trials.