populations within the geographically constricted Visium data, including SHH-C2, a population located in histologic nodules, the predominant neuronal-differentiated population SHH-C1, and progenitor populations (SHH-B1 and B2). In addition, we were able to visualize clusters not detectable by scRNAseq – a cluster lining nodules with expression of vascular endothelium marker, reticulin and M2-macrophage genes, and a novel DNA-repair cluster. In addition, Visium data permits the spatial constraint of proliferating cells, which is frequently problematic in scRNAseq, as dividing cells cluster independently. The proliferation is highest in the SHH-B2 minor progenitor population, absent in the SHH-C1 major differentiated population, and is moderate in other population including the SHH-C2 nodules. Group 3 and 4 medulloblastoma are more complex but show preliminary corroboration with scRNAseq data. In summary, Visium allows us to map subpopulations identified by scRNAseq to tumor architecture more definitively and rapidly than IHC. These novel insights advance our understanding of medulloblastoma, a critical step in improving treatment options for children with this disease.

MEDB-45. FUNCTIONAL GENOMICS IDENTIFIES EPIGENETIC REGULATORS AS NOVEL THERAPEUTIC TARGETS FOR SONIC HEDGEHOG MEDULLOBLASTOMA

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Medulloblastoma (MB) is among the most common malignant childhood brain tumors that comprises a group of four molecularly distinct diseases. A significant proportion of these tumors is characterized by aberrant activation of the canonical sonic hedgehog (SHH) signaling pathway. Although small-molecule inhibitors targeting Smoothened (SMO) have proven a promising treatment approach for SHH-MB subgroup, primary or acquired resistance impedes its clinical efficacy. Therefore, novel targeted approaches are urgently needed to improve therapeutic strategies for this tumor entity. Here, we conducted a genome-wide CRISPR/Cas9 knockout screen in a murine and a human SHH-MB cell line, SMB21 and DAOY, respectively, in order to decipher tumor-specific genetic dependencies. Our data demonstrate that SMB21 cells highly depend on positive regulators of the SHH pathway, such as Smo and Gli1 for their survival, as opposed to DAOY cells, suggesting that the latter does not represent a faithful model of SHH-MB. Members of the epigenetic machinery such as Dnmt1 and Smarca5 scored strongly as SMB21-context specific essentialities. Pharmacologically, we show that DNMT1 inhibition is efficacious at clinically relevant concentrations against SMO inhibitor- sensitive, as well as resistant SHH-MB cell lines, indicating novel therapeutic avenues for SHH-MB. By performing RNA sequencing of SMB21 cells, we identified early and late changes in global gene expression induced by DNMT1 inhibition, including decreased expression of mediators of SHH signaling, such as Gli1 and Gli2. Of note, gene set enrichment analysis revealed that DNMT1 inhibition downregulates top gene sets associated with cell cycle progression, corroborating the screening results that Dnmt1 is essential for SMB21 proliferation. Further global DNA methylation profiling in SMB cells will help to define the molecular basis of sensitivity to DNMT1 inhibitors in SHH-MB. Summarizing, our data highlight the potential of inhibitors targeting epigenetic regulators in SMO inhibitor- sensitive and resistant MB for more efficacious treatment options.

MEDB-46. ONC201 AFFECTS GROUP 3 MEDULLOBLASTOMA GROWTH BY IMPAIRING CANCER STEM CELLS

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Cancer stem cells (CSCs) represent a sub-population of cancer cells capable of proliferating and generating heterogeneous cancer cell types. Acquisition of stemness features may represent a strong advantage for neoplastic cells to promote tumorigenesis and progression, driving resistance to conventional therapy and promoting disease relapse. CSCs have been discovered and isolated in major pediatric brain tumors, including medulloblastoma (MB), the most common solid malignancy in childhood. The unfolded protein response (UPR) represents an adaptation mechanism to metabolic obstacles in CSCs, able to increase tumor aggressiveness. The initial activation of the UPR is cytoprotective but the acute activation led to cell death. We found that UPR is active in MB stem cells (MBSC) and particularly in group 3 (G3). ONC201 is an imipridone compound that activates p53-independent apoptosis causing changes in gene expression similar to those caused by UPR. Here, we aim to test the in vitro efficacy of ONC201 on G3 MBSC. We selected 4 G3 MBSC (D341-Med, D283-Med, Med411, and CHLA-01-Med), for the in vitro study. Cells were choosen for their "fidelity" to the MB subgroup through the analysis of global methylation profiling, were grown in stemness conditions and expressed stemness markers at high levels. We investigated the efficacy of ONC201 treatment on CSC features, by evaluating cell viability, cell death, protein synthesis, self-renewal, and cell cycle. ONC201 treatment on G3 MB cells led to an upregulation of ATF4, a key molecule of the UPR, and the induction was stronger in MB cultured in a "stem-like" medium. Moreover, in the most MBSC analyzed, ONC201 was effective against CSCs whether by reduced cell viability, protein synthesis, and self-renewal. We also observed a trend of increased cell death. Our results suggest that ONC201 is potentially effective in treating G3 MB by compromising the stem cell compartment, and thus deserving further investigations.

MEDB-47. CD4+ T CELLS RESTRICT MEDULLOBLASTOMA GROWTH AND DISSEMINATION

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The immune system serves as a powerful defense not only against pathogens and parasites but also against neoplastic cells. Emerging immunotherapies that boost the activity of tumor-reactive immune cells or counteract immune suppressive mechanisms have shown promising effects in certain cancer types. However, the success of immunotherapy for brain tumors has been limited, highlighting the need for a better understanding of the immune microenvironment. Our preliminary studies have shown that T cells critically affect tumor growth in mouse models of the pediatric brain tumor medulloblastoma. In particular, depletion of CD4+ T cells results in more aggressive growth of medulloblastoma cells and allows these cells to metastasize to the spinal cord. To test whether CD4+ T cells can recognize and attack tumor cells directly, we generated MHC class II knockout tumors. Surprisingly, depletion of CD4+ cells still enhanced tumor growth and metastasis. These results suggest that CD4+ T cells regulate medulloblastoma growth independently of MHC II on tumor cells. We hypothesized that CD4+ T cell may not directly kill tumor cells but recruit and activate another effector immune cell type that eliminates tumor cells. As CD4+ T cells have a well-studied helper function for CD8+ T cells, we examined whether their anti-tumoral function relies on the activation of cytotoxic CD8+ T cells. The depletion of CD4+ T cells still resulted in advanced growth of MHC class I-deficient, and thus CD8+ T cell resistant, tumor cells indicating that CD4+ T cells counteract tumor growth in a CD8+ T cell-independent manner. Ongoing studies are aimed at elucidating the mechanisms by which CD4+ T cells regulate medulloblastoma growth, including the antigen-presenting cells that activate them and the effector cells responsible for killing tumor cells. These studies will advance our understanding of the immune microenvironment in medulloblastoma and allow us to design more effective therapies.

MEDB-48. INFANT MEDULLOBLASTOMA - SHH SUBTYPE – WITH RESIDUAL DISEASE. TO TREAT OR NOT TO TREAT

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Management of infant medulloblastoma remains a challenge. Front-line chemotherapy can successfully avoid radiation in low-risk infant medulloblastoma. Patients that do relapse can be salvaged long-term with radiotherapy. We report 4 cases of infants with medulloblastoma treated with chemotherapy (HIT2000 protocol) with residual or progressive disease. RESULTS: Four cases of infant medulloblastoma, all MBEN/nodular desmoplastic SHH type B, p53 WT, no MYC / MYCN amplification. CASE 1: 16 month old girl, metastatic lesions in the cerebellum and meningeal enhancement. Germline SUFU mutation. After 3 cycles of chemotherapy MRI showed more enhancement of the residual disease.

To inform management, second look surgery was performed. Pathology showed fibrous tissue only, no malignant cells. The child continues to be treated as per HIT2000. CASE 2: 5 month old girl, metastatic lesions in the cerebellum. Germline SUFU mutation. 2 months after end of treatment, MRI demonstrated progression of cerebellar lesion. Surgical resection was performed, pathology showed differentiated mature neuronal tissue. No further treatment; remains in remission 1 year after suspected progression. CASE 3: 27 month old boy, metastatic lesions in cerebellum. Germline SUFU mutation. 1 month post-completion of treatment progressive prominent nodules along the cerebellum and cerebellar leptomeningeal enhancement. Biopsy not feasible so close MRI surveillance was initiated. MRI remains stable 1 year after suspected progression. CASE 4: 30 months old boy, non-metastatic disease. Complete resection. No germline mutation. End-of-treatment MRI showed subtle new intraspinal leptomeningeal deposits and a suspicious left optic tract nodule, subsequent MRI 8 weeks later showed clear progressive disease. Unfortunately, the child died before radiotherapy could be delivered. CONCLUSION: Salvage radiotherapy for infants with medulloblastoma who progress following chemotherapy treatment can be life-saving but risk significant cognitive impairment. Differentiation of medulloblastoma following radio/chemotherapy has been reported. We recommend considering tissue confirmation prior to embarking on further treatment for suspected relapse.

MEDB-49. RELAPSED SHH MEDULLOBLASTOMAS IN YOUNG CHILDREN. ARE THERE ALTERNATIVES TO FULL-DOSE CRANIOSPINAL IRRADIATION?

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BACKGROUND/RATIONAL: Following initial irradiation sparing therapy, many young children with relapsed medulloblastoma can be salvaged with craniospinal irradiation (CSI). However, the interval to relapse is short and neurocognitive sequelae remain a major concern. The contribution of molecular subgrouping may help refine indications and modalities of salvage strategies in this population. METHOD: From a cohort of 151 young children with molecularly characterized relapsed medulloblastoma, subset analysis of the SHH medulloblastoma was conducted to describe the practice of salvage radiotherapy and associated post-relapse survival (PRS). RESULTS: Sixty-seven SHH medulloblastoma patients (46 M0; 54 GTR; 11 non-ND/MBEN) received salvage therapy with curative intent. Before relapse, 54 (80.6%) received conventional chemotherapy (CC), 13 (19.4%) high-dose chemotherapy (HDC), while seven had additional focal radiotherapy (fRT). Median time to relapse was 11.1 months (range 3.8-41.0) and 43.3% were localized. Thirty patients (16 localized relapse) underwent surgery. Forty-seven (71.2%) received salvage radiotherapy (20 with CC; 10 with HDC; 15 alone, two unknown). CSI and fRT accounted for 82% and 18% respectively. CSI median dose was 36Gy (range 18-39Gy). Ten patients (eight with localized relapse) received CSI doses ≤23.4Gy. Nineteen patients (28.8%) did not receive any radiotherapy (nine HDC; 10 CC only). Radiotherapy was associated with better 3-year PRS (73.0% versus 36.1%; p=0.001). All patients treated with CSI ≤ 23.4Gy were alive at median follow-up of 69 months(24-142). Six of nine patients treated with HDC without irradiation were alive at last follow-up. Sixty-three percent of patients received reduced dose CSI(≤23.4Gy), fRT, or no radiotherapy, and their PRS did not significantly differ from those who received CSI ≥ 30.6Gy (p = 0.54). CONCLUSION: While salvage CSI provided PRS benefit in this SHH medulloblastoma cohort, we report the use of reduced salvage radiotherapy and irradiation avoidance in 63% of the patients, with 60% alive at last follow-up.

MEDB-50. ASSESSMENT OF CELLULAR RADIOSENSITIVITY AND DNA REPAIR IN MEDULLOBLASTOMA CELL LINES AND PATIENT-DERIVDED XENOGRAFT SLICE CULTURES

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Medulloblastoma (WHO grade 4) is the most common malignant brain tumor of childhood. Despite the high importance of radiotherapy for disease control, the mechanisms underlying response and resistance to radiotherapy are incompletely understood. Therefore, we assessed the radiosensitivity and DNA repair capacity of medulloblastoma cell lines in-vitro and of patient-derived xenograft (PDX) models ex-vivo. Cell survival after irradiation of seven medulloblastoma cell lines displaying different subgroups was assessed via colony formation assay (DAOY, UW228, UW473, SJMM4, ONS-76, HDMB-03, D283). The ONS-76 and the mouse SJMM4 cell line were the most radioresistant strains (surviving fraction after 6 Gy (SF6): 0.33 and 0.31, respectively), followed by UW473, UW 228 and DAOY cells (SF6 0.16-0.21). The non-WNT/non-SHH-activated cell lines HDMB-03 and D283 cells demonstrated profoundly higher cellular radiosensitivity (SF6 <0.05). Analysis of residual (24h after irradiation) DNA double-strand breaks (DSB) as assessed by co-localized γH2AX/53BP1-foci demonstrated a significant correlation between DSB repair capacity and cellular survival. To use a more reliable pre-clinical model for medulloblastoma, we further examined DNA repair foci in ex-vivo irradiated slice cultures of PDX models MED-113 (SHH) and NCH2194 & HT028 (Gr. 3). Immunofluorescence analyses of frozen sections demonstrated non-hypoxic (pimonidazole-negative) and proliferating (EdU-positive) cells at the outer rim of the tumor slices. Two hours after irradiation all three PDX models showed a strong increase in 53BP1-foci, clearly indicating DNA damage induction. Most radiation-induced DSB were repaired after 24h. In a first radiosensitization approach, we treated the HT028 model with the PARP inhibitor olaparib (1μM ± 2Gy irradiation). Twenty-four hours after treatment the sample displayed a strong increase in the amount and size of 53BP1-foci, indicating compromised DNA repair. Further in-vitro and ex-vivo investigations with the aim to predict individual radiosensitivity and effective radiosensitization strategies are ongoing.