gene expression, variant signatures and estimate ecDNA copy number in the medulloblastoma tumor sample. We identified 12 distinct clusters in the human tumor, 5 of which were determined to be normal non-tumor [OSC1] cells, as identified by specific cell type markers, and 7 of which were determined to be tumor cells. Enrichment of ecDNA was restricted to only one of these tumor clusters. In addition, we also performed the same multiome single-cell analyses in an orthotopic xenograft mouse model derived from this SHH MB patient tumor. In the PDX, 17 clusters were identified, all of which 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 [OSC2] the cells in the corresponding PDX, emphasizing the aggressiveness of ecDNA containing cells.

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

Viktoria Funke[1], Carolin Walter[1,2], Viktoria Melcher[1], Lanying Wei[2], Sarah Sandmann[2], Julian Varghese[2], Natalie Jäger[3,4], Thomas K. Albert[1], Ulrich Schüller[5,6], Kornelius Kerl[1]; [1]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany. [2]Institute of Medical Informatics, University of Münster, Münster, Germany. [3]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany. [4]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [5]Research Institute Children's Cancer Center, Hamburg, Germany. [6]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

INTRODUCTION: Molecular subgrouping of Medulloblastoma (MB) has expanded our understanding of its biology and the impact on clinical parameters. However, detailed analysis of inter- and intratumoral 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 MAGIC cohort), we explored metabolic programs on RNA level. In two cohorts (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. Inter- and intratumoral heterogeneity were 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 lipid metabolic processes. After all, lipid metabolism and metabolism of nucleotides in MB have prognostic value and correlate with the outcome of patients. CONCLUSION: Our data highlight the importance of metabolic properties in MB. We show the distinct metabolic signatures are clinically relevant and, thus, might provide opportunities for novel target-directed therapeutic options in the future.

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

Francesca Romana Buttarelli[1], Simone Minasi[1], Maria Luisa Garrè[2], Maura Massimino[3], Veronica Biassoni[3], Tobias Goschzik[4], Torsten Pietsch[4], Felice Giangaspero[1]; [1]Department of Radiologic,Oncologic and Anatomopathological Sciences, Sapienza Universita, Roma, Italy. [2]Neuroncology Unit, IRCCS- Istituto Giannina Gaslini, Genova, Italy. [3]Pediatric Oncology Unit, Fondazione IRCCS-Istituto Nazionale dei Tumori, Milano, Italy. [4]Department of Neuropathology, University of Bonn Medical Center,, Bonn, Germany

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 analyzed to determine whether the senescence escape triggered by telomere-elongation mechanisms could explain the aggressivity 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 telomerase- or ALT-positive iMB. We analyzed a cohort of 50 FFPE tissues 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 59% of our cohort, while Group3/4-MBs accounted for 41%; no WNT-MBs 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 iMBs. Promising results have been found in the telomere length 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 Medium (M) telomeres length. 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 aggressive behaviour 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

Kyle S Smith[1], Catherine A Billups[1], Sandeep K Dhanda[1], Laure Bihannic[1], Aksana Vasilyeva[1], Yimei Li[1], Jeff M Michalski[2], James M Olsen[3], Sarah Leary[3], Maryam Fouladi[4], Amar Gajjar[1], Arzu Onar[1], Paul A Northcott[1], Giles W Robinson[1]; [1]St Jude Children's Research Hospital, Memphis, TN, USA. [2]Washington University School of Medicine, St Louis, MO, USA. [3]Fred Hutchinson Cancer Research Center, Seattle, WA, USA. [4]Nationwide Children's Hospital, Columbus, OH, USA

BACKGROUND: Given the vast molecular heterogeneity present within medulloblastoma (MB) and considerable differences in therapy, we performed a meta-analysis of three large, recently published, prospective 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 procured from DNA methylation profiles and mutations from next-generation sequencing. Patients were stratified into six clinically homogeneous groups for cross-trial comparisons: (1) ACNS0331_LDCSI - patients with non-metastatic (M0), non-residual (R0), non-anaplastic MB treated with low-dose (LD) craniospinal irradiation(CSI); (2) ACNS0331_SDCSI - patients with M0R0 non-anaplastic MB treated with standard-dose(SD) CSI; (3) SJMB03_SDCSI - patients with M0R0 non-anaplastic MB treated with SDCSI; (4) SJMB03_HDCSI - patients with metastatic (M+) MB treated with high-dose (HD) CSI; (5) ACNS0332_HDCSI - patients with M+ MB treated with HDCSI; (6) ACNS0332_HDCSI_Carbo - patients with M+ MB treated with HDCSI and carboplatin. RESULTS: 803 (WNT=125, 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_SDCSI and SJMB03_SDCSI or from SJMB03_HDCSI and ACNS0332_HDCSI when analyzed as a whole or by subgroup. ACNS0331_LDCSI outcome was inferior to the combined ACNS0331_SDCSI + SJMB03_SDCSI cohorts (p<0.001) and in G3 (p=0.030). ACNS0332_HDCSI_Carbo EFS was superior to ACNS0332_HDCSI + SJMB03_HDCSI only in G3/G4_subtype III (p=0.045). Additional molecular risk factor analysis identified M0R0 G3/G4_subtype VII and SHH without high-risk features 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

Bethany Veo[1], Andrew Goodspeed[1], Eric Prince[1], Sujatha Venkataraman[1], Rajeev Vibhakar[1,2]; [1]University of Colorado, Aurora, CO, USA. [2]Childrens Hospital Colorado, Aurora, CO, USA

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 5-year survival rate of 12%. To understand what drives MYC-amplified MB relapse 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 observe 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 notori-