outcome among the histo-molecular subgroups in Chinese children. METHODS: A total of 93 patients of MB who underwent surgical treatment at Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine from January 2006 to December 2015. The clinical profile, treatment, and survival patterns are discussed. The relevant statistical analysis was done using SPSS software, version 21.0. RESULTS: At the most recent follow-up date, 15 (16.1%) had been lost to follow-up , 78 cases were tested for RNA-seq, of which 54 cases were successful, and 24 cases failed the quality control due to sample quality problems.The male-to-female ratio was 2.4:1, and the patients' mean age at diagnosis was 5 years (ranged from 4 months to 11 years).The estimated 5-year overall survival and progression-free survival, based on Kaplan-Meier analysis, were 55.1% ± 5.6% and 40.9% ± 5.7%.Multivariate survival analysis showed that progression -free survival were significantly affected by extent of surgery (P=0.026) and postoperative radiotherapy(P < 0.001).Preoperative metastasis (P=0.032) and postoperative radiotherapy (P=0.001) had a significant influence on overall survival. While , the molecular subtypes showed no statistically significant differences in 5-year PFS and 5-year OS. **CONCLUSION**: Extent of resection of MB and postoperative radiotherapy were the important clinical prognostic factors for survival. In the future, we will continue to expand cases and study the clinical characteristics and prognostic risk factors of different molecular types of medulloblastoma in Chinese children,and to improve the survival rate of children with medulloblastoma.

MEDB-06. SPATIAL TRANSCRIPTOMIC ANALYSIS OF SONIC HEDGEHOG MEDULLOBLASTOMA IDENTIFIES THAT LOSS OF HETEROGENEITY AND INDUCED DIFFERENTIATION UNDERLIES THE RESPONSE TO CDK4/6 INHIBITION

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Medulloblastoma (MB) is a malignant tumour of the cerebellum which can be classified into four major subgroups on the basis of gene expression and genomic features. Single cell transcriptome studies have defined the cellular states underlying each MB subgroup, however the spatial organisation of these diverse cell states and how this impacts response to therapy remains to be determined. Here, we used spatially resolved transcriptomics to define the cellular diversity within a sonic hedgehog (SHH) patient-derived model of MB and identify how cells specific to a transcriptional state or spatial location are pivotal in responses to treatment with the CDK4/6 inhibitor, Palbociclib. We distinguish neoplastic and non-neoplastic cells within tumours and from the surrounding cerebellar tissue, further refining pathological annotation. We identify a regional response to Palbociclib, with reduced proliferation and induced neuronal differentiation in the majority of the tumours. Additionally, we resolve in cellular resolution a distinct tumour "interface" where the tumour contacts neighbouring mouse brain consisting of abundant astrocytes and microglia and continues to proliferate despite Palbociclib treatment. Our data highlight the power of this approach to characterise the response of a tumour to targeted therapy and provide further insights into the molecular and cellular basis underlying the response and resistance to CDK4/6 inhibitors in SHH MB

MEDB-07. LONG-TERM MEDICAL AND FUNCTIONAL OUTCOMES OF MEDULLOBLASTOMA SURVIVORS: A POPULATION-BASED, MATCHED COHORT STUDY

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BACKGROUND: Most medulloblastoma survivors suffer from late treatment-related sequelae. There are no population-based studies examining such late effects in a dedicated cohort of medulloblastoma survivors. METHODS: Using a provincial pediatric cancer registry, all 5+ year medulloblastoma survivors diagnosed between 1987-2015 in Ontario, Canada were identified and matched to cancer-free population controls based on age, sex, and geographical location. Cases were followed from the index date (five years from latest of diagnosis, or relapse or subsequent malignancy prior to age 18 years) until December 31, 2020 or censorship (death, or relapse or new cancer after age 18 years). Clinical data were linked to administrative health databases to estimate cumulative incidences and cause-specific hazard ratios (HR) of mortality, hospitalizations, strokes, hearing loss requiring a hearing aid, and receipt of homecare services between cohorts, accounting for matching and competing risks. RESULTS: We identified 230 cases [65.7% female; median diagnostic age: 7y, interquartile range (IQR) 4-10; median attained age: 24y, IQR 18-31] and 1150 controls. One hundred eighty-seven (81.3%) received craniospinal irradiation. Ten-year survival probability after index was 92.4% in cases and 99.4% in controls (HR 21.5, 95% CI 9.8-54.0). Cases were at higher risk for hospitalizations (HR 3.4, 95% CI 2.7-4.3), stroke (HR 45.6, 95% CI 12.8-289.8), hearing loss (HR 96.3, 95% CI 39.7-317.3), and requiring homecare services (HR 7.9, 95% CI 5.8-10.9). By 10 years after index, 4.8% (95% CI 2.2-9.0) of survivors had experienced a stroke compared to 0.1% (95% CI 0.01-0.7) of controls. CONCLUSIONS: Survivors of childhood medulloblastoma experienced an increased risk of mortality and serious morbidity compared to population controls. Consideration for mitigation strategies or early interventions in preventing neurovascular sequelae and hearing loss is warranted, as are dedicated supports for survivors.

MEDB-08. INHIBITION OF DIFFERENT MITOTIC TARGETS DEMONSTRATED DISTINCT DNA DAMAGE AND CELL DEATH RESPONSE IN P53-MUTANT MEDULLOBLASTOMA

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BACKGROUND: In normal cells, cell cycle is tightly regulated by mitotic proteins to ensure smooth transition through each phase of cell division. Here, we examine two proteins – KIF11, a mitotic kinesin, responsible for assembly and maintenance of mitotic spindle during mitosis; and MELK, a serine/threonine kinase, essential for mitotic progression. Cancer cells can upregulate MELK and KIF11 to promote uncontrolled cell division and protect the cells from apoptotic cell death, leading to tumorigenesis. AIMS: We investigated the response of p53-mutant medulloblastoma (MB) by inhibiting KIF11 and MELK separately to study the effects on cell cycle progression and cell death mechanisms. RESULTS: Cell proliferation was suppressed by inhibition of either KIF11 or MELK in MB, independent of p53-mutant status. Regardless of p53-mutant status, inhibiting KIF11 induced cell cycle arrest at G2/M. In contrast, inhibiting MELK (IC_{50} dose) induced more prominent G2/M arrest in p53-mutant cells compared to p53-wildtype cells. In p53-mutant MB, arrested cells during MELK inhibition subsequently underwent apoptotic cell death at 24h and 48h. With KIF11 inhibition, p53-mutant cells at 24h were already in necrotic stage. p53-mutant cells reached necrotic stage in a shorter time with KIF11 inhibition than MELK inhibition. On immunoblotting, independent of p53-mutant status, KIF11 inhibition produces more significant increase in DNA damage marker and c-PARP indicative of apoptosis, compared to MELK inhibition. Treatment with KIF11 or MELK inhibitor increased p53 protein expression in p53-wildtype (normal stress response). However, in p53-mutant cells, p53 protein expression decreased post-KIF11-inhibition, but remained unchanged post-MELK inhibition. In-vivo, inhibiting KIF11 was less tolerable in a patient-derived orthotopic xenograft model with p53-mutation. CONCLUSION: Inhibition of either mitotic target KIF11 or MELK, can induce anti-proliferative effects in MB. In p53-mutant MB, DNA damage and cell death response with KIF11-inhibition are more marked.

MEDB-09. UNRAVELING THE ROLE OF UNFOLDED PROTEIN RESPONSE IN MEDULLOBLASTOMA CANCER STEM CELLS

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Medulloblastoma (MB) is the most common malignant childhood brain tumor. The current clinical approach consists of multimodal strategies with

debilitating long-term effects and risk of tumor recurrence. Medulloblastoma stem cells (MBSCs) are a fraction of tumor cells with high proliferation potential and the capability to adapt to adverse/restrictive conditions in tumor milieu thus driving the refractoriness to conventional therapies. Recently, high basal levels of Unfolded Protein Response (UPR) molecules have been found in tumors of different tissue-origin and are correlated with poor prognosis and low patient survival. However, little is known about the role of UPR in MB. We investigated the expression and activation of UPR players in MBSCs. Human group 3 MB (G3MB) cell lines, specifically CHLA-01, D283- and D341-Med, were grown in Vitamin A and/or FBS or in stem selective medium (B27™) for 72 h before collection. Cells were fixed, stained with proper primary antibodies and images were acquired by confocal microscopy. The analysis of the transcription factors ATF-4 and CHOP revealed their elevated nuclear expression and co-localization, which resulted to be more marked in G3MB stem-like cells than in the differentiated ones. Also the ATF-6 branch was investigated, in differentiating conditions D283 and D341-Med showed a greater activation of ATF-6, represented by its nuclear localization, in respect to stem cells, while CHLA-01 did not show differences. Conversely XBP1, the transcription factor downstream IRE1 signaling, was not expressed in the three cell lines. Lastly, a Kaplan-Meier analysis on MB patients showed a worse prognosis with a shorter survival rate of patients expressing high ATF4 transcript levels. Our results reveal, even in resting conditions, preferential activation of the PERK branch in G3MB cells grown in stem-like condition suggesting that ATF-4 might be a promising therapeutic and prognostic factor to specifically target the stem compartment in aggressive MB.

MEDB-10. COMPARING PEDIATRIC MEDULLOBLASTOMA WITH AND WITHOUT SPINAL METASTASIS

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AIM: To compare pediatric medulloblastoma with and without spinal metastasis METHODOLOGY: Pediatric medulloblastoma cases from 1999 to 2021 were retrospectively reviewed in this Institutional Review Board approved study. Imaging reports, presence of spinal drop metastases at diagnosis, degree of tumor excision, treatment given and survival status were captured. RESULTS: Brain and spine imaging at diagnosis was available in 54 medulloblastoma patients with no drop metastasis and in 7 with drop metastasis. Largest tumor dimension at presentation is 4.54 ± 0.94 cm with those with drop metastasis, similar to the 4.43 ± 0.94 cm in those without drop metastasis (p = 0.79). For the 54 medulloblastomas with no drop metastasis, 44 (81%) were completely excised, 9 (17%) partially excised and there was no follow up for 1. For the 7 medulloblastomas with drop metastasis, 3 (43%) of the primary tumours were completely excised, 3 (43%) partially excised and there was no follow up for 1. Post operative chemo/radiotherapy was given to 48 of the 54 with no drop metastasis, not given for 1 with no information available for 5. Chemo/radiotherapy was given to 6 of the 7 with drop metastasis with no information available for 1. At 1 year follow up of the 54 with no spinal drop metastasis at diagnosis , 42 remain tumour free, 3 have tumour, 4 are deceased and 5 are lost to follow up. At 1 year follow up of the 7 with drop metastasis, 2 are free of tumour, 2 have tumour and 3 are lost to follow up. Higher percentage of medulloblastomas without drop metastasis are completely excised (p<0.01). No significant difference between postoperative chemotherapy/radiation rates between groups CONCLUSION: Most medulloblastomas do not have spinal drop metastasis at diagnosis and complete excision is more frequently in those without drop metastasis.

MEDB-11. MYC OVEREXPRESSION AND SMARCA4 LOSS IN CEREBELLAR GRANULE CELL PRECURSORS COOPERATE TO DRIVE MEDULLOBLASTOMA FORMATION IN MICE

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Group 3 medulloblastoma is one of the most aggressive types of childhood brain tumors. Roughly 30 % of cases carry genetic alterations in *MYC*, *SMARCA4* or both genes combined. While overexpression of MYC has previously been shown to drive medulloblastoma formation in mice, the functional significance of *SMARCA4* mutations and their suitability as a therapeutic target remain largely unclear. To address this issue, we combined an overexpression of MYC with a loss of SMARCA4 in cerebellar granule cell precursors. Cells were isolated from 7-day-old *Math1-creERT2::Smarca4fl/fl* pups after tamoxifen-induced loss of SMARCA4. Subsequently, MYC overexpression was achieved by lentiviral transduction, and transduced cells were transplanted into immunodeficient *CD1nu/nu* recipient mice. Preliminary results show tumor formation in 5/19 transplanted mice (26 %) after 6 months. SMARCA4 loss in all tumor cells was confirmed both immunohistochemically and on a genetic level and suggests a dependency of tumor growth on SMARCA4 loss. In a next step, additional cohorts will clarify if tumor development is accelerated by or even dependent on the loss of SMARCA4 in our model. Additionally, the neoplastic potential of tumor cells will be verified with the aid of secondary recipient mice. To evaluate to what extent the generated tumors are comparable to human Group 3 medulloblastomas, tumors will be extensively analyzed on a morphological, transcriptional, and epigenetic level. Altogether, we hope to establish a suitable mouse model for *SMARCA4* mutated Group 3 medulloblastoma that will help to elucidate the role of SMARCA4 in tumor development and to identify new therapeutic targets.

MEDB-12. SEVERE DEVELOPMENTAL ABNORMALITIES AND PROLIFERATIVE CEREBELLAR LESIONS INDUCED BY COMBINED ACTIVITY OF WNT SIGNALLING AND LOSS OF SMARCA4

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Almost all medulloblastomas (MB) of the WNT subgroup are characterized by hotspot mutations in *CTNNB1*, and mouse models have convincingly demonstrated the tumor-initiating role of these mutations as well as the tumor origin in the dorsal brain stem. Around 20 % of WNT MB additionally carry *SMARCA4*mutations, but the functional role of these alterations is mostly unknown. We therefore amended previously described *Blbp-cre::Ctnnb1(ex3)Fl/+* mice by the introduction of a floxed *Smarca4* allele. In contrast to existing literature, Blbp-cre::Ctnnb1(ex3)$^{Fl/+}$ mice had a maximum life span of only 17 days, even after breeding into two different genetic backgrounds (C57BL/6J and 129S2/Sv). The mice displayed a severe developmental phenotype including a thinned cerebral cortex, hydrocephalus, missing cerebellar foliation and layering as well as non-proliferative cell accumulations in brain stem and cerebellum. An additional homozygous loss of SMARCA4 even resulted in prenatal death for most mice and caused big proliferative lesions in the cerebellum at embryonal day 14.5. These lesions appear to originate from SOX2-positive progenitor cells in the cerebellar ventricular zone. In a next experiment, cells isolated from this region will be characterized *in vitro* and will be transplanted orthotopically to evaluate their neoplastic potential *in vivo*. Altogether, we hope to elucidate how a loss of SMARCA4 and mutations of *Ctnnb1* cooperate during hindbrain development and tumor formation within this region.

MEDB-13. NEUROCOGNITIVE AND RADIOLOGICAL FOLLOW-UP OF CHILDREN UNDER 5 YEARS OF AGE TREATED FOR MEDULLOBLASTOMA ACCORDING TO THE HIT-SKK PROTOCOL

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BACKGROUND: HIT-SKK protocol is used for the treatment of low risk medulloblastomas in young children with the aim of eliminating cranial irradiation and its long-term side effects, in particular neuropsychological (NP) sequelae. This therapy includes IV and intraventricular (ITV) methotrexate (MTX) potentially responsible for leukoencephalopathy (LE) and neurocognitive disorders.The objectives are to describe the risk factors and