

2012, and we have summarized results of molecular analysis of Japanese MBs. Total 236 primary MBs have been subclassified by gene expression profile using the NanoString nCounter system or DNA methylation array, and their single nucleotide mutations and copy number aberrations have been also examined. Mean follow up time was 68.9 months. Proportion of four core subgroups were WNT (16.9%), SHH (25.4%), Group 3 (17.4%) and Group 4 (40.3%), respectively. In cases of less than 3 years old, no WNT have been found and 63.2% cases were SHH. In cases between 3 to 17 years old, Group 4 is the most (47%), and these trends is almost consistent with published references. *TP53* mutations were identified in 23.3% of SHH, and they were significantly poor prognosis. Metastatic or *MYC* gain Group 3 MBs were poor prognosis, while Group 4 MBs with loss of chromosome 11 or whole chromosomal aberration were good prognosis. These findings reveal molecular properties of Japanese MBs and will contribute to develop new therapeutic strategies.

#### MBRS-39. MAP4K4 CONTROLS PRO-INVASIVE SIGNALING IN MEDULLOBLASTOMA CELLS

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The molecular mechanisms contributing to distant dissemination and local recurrence of medulloblastoma, the most common malignant brain tumor in childhood, are poorly understood and no targeted anti-invasion therapies exist till date. We explored regulators and effectors of MAP4K4, a pro-invasive kinase overexpressed in MB and associated with metastatic progression in different solid malignancies. MAP4K4 is upregulated both at mRNA and protein levels in primary pediatric brain tumors compared to normal cerebellum. MAP4K4 is required for growth factor- and irradiation-induced migration and invasion of medulloblastoma cells. It furthermore promotes turnover and activation of the receptor tyrosine kinase c-Met and of the  $\beta 1$  integrin adhesion receptor<sup>1</sup>. To characterize these clinically relevant consequences and to identify druggable targets of MAP4K4 function, we profiled the interactome of MAP4K4 in starved and growth factor stimulated medulloblastoma cells. To systematically address MAP4K4 impact on receptor expression and turnover, we determined the MAP4K4-dependent surface proteome in medulloblastoma cells. We found that MAP4K4 is part of the striatin-interacting phosphatase and kinase (STRIPAK) complex and that STRIPAK component striatin 4 is controlling cell motility and invasiveness in medulloblastoma cells. Invasiveness of medulloblastoma cells is abrogated by a truncation mutant of MAP4K4 lacking the striatin 4 interaction domain. We furthermore found that MAP4K4 mediates growth factor-induced surface expression of solute carriers and immunomodulatory proteins involved in chemoresistance and immune evasion. Thus, our study identified MAP4K4 as a missing link between pro-tumorigenic growth factor signaling and tumor cell functions relevant for disease progression. It may help identifying druggable vulnerabilities in medulloblastoma cells to restrict tumor growth and dissemination. 1. Tripolitioti, D. *et al.*, *Oncotarget* 9, 23220–23236 (2018).

#### MBRS-42. YB-1 - A NOVEL THERAPEUTIC TARGET IN HIGH-RISK MEDULLOBLASTOMA?

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Medulloblastoma relapse occurs in 30–40% of patients and is typically fatal. The emergence of therapy resistant sub-clones likely plays a major role in a large proportion of recurrent medulloblastoma. Y-box binding protein 1 (YB-1) is a multifunctional transcription/translation factor and known onco-protein. Overexpression has been described in numerous cancers, where elevated expression and nuclear accumulation correlates with disease progression, metastasis and drug resistance. Genomic analysis of a large medulloblastoma cohort revealed YB-1 up-regulation across all subgroups of medulloblastoma, where elevated expression correlated with poor survival. Immunohistochemical staining of patient tissue microarrays displayed significant YB-1 expression, with a high proportion (83%) of patients exhibiting nuclear accumulation. High YB-1 expression was also observed at both protein and RNA level across medulloblastoma cell lines, with expression highest in Group 3 and 4. Hence, we hypothesised that YB-1 plays a role in medulloblastoma chemoresistance and progression. Treatment of Group 3 (HDMB-03 and D283MED) and SHH (DAOY) cell lines with vincristine and cisplatin and analysis of cellular localisation by nuclear/cytoplasmic fractionation and immunofluorescence demonstrated that YB-1 undergoes nuclear translocation in response to these standard medulloblastoma chemotherapy agents. Chromatin immunoprecipitation (ChIP) analysis of untreated Group 3 cell lines (D283MED and HDMB-03) demonstrated considerable YB-1 interaction with an inverted CCAAT box in the *ATP-binding cassette subfamily B member 1 (ABCBI)* promoter. RT-PCR analysis of

ABCBI following vincristine and cisplatin treatment revealed differences in transcript expression, indicative of different YB-1 promoter interactions dependent on chemotherapeutic treatment. Our results highlight YB-1 as a novel candidate chemoresistance driver in medulloblastoma.

#### MBRS-43. ELUCIDATING HOW NOVEL EXTRACELLULAR MATRIX SUBTYPES DIFFERENTIALLY IMPACT THE SURVIVAL OF MEDULLOBLASTOMA SUBGROUPS

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Medulloblastoma (MB) is the most common malignant paediatric brain tumour and frequently exhibits metastasis and chemoresistance. MBs are categorised into four molecular subgroups (WNT, Sonic hedgehog, Group 3 and Group 4), each associated with different demographics and clinical features. We have shown that the expression of specific extracellular matrix proteins in the brain tumour microenvironment differ between subgroups. A prime example is laminin (an ECM glycoprotein) the expression of which correlates with good overall survival in the SHH subgroup and poor overall survival in Group 4. Our aim is to determine the cause of this difference in survival. Candidate laminin-responsive-genes (LRGs) were identified using the Cavalli data set and RNA-Seq analysis of MB cells grown on 3D hydrogels with and without laminin. The role of laminin in the regulation of MMPs and the other LRG candidates was investigated by qRT-PCR, western blotting and zymography in 2D and long-term 3D-hydrogel assays. Thus far we have shown that in CHLA-01-R (metastatic Group 4 cell line) three of our LRGs are upregulated in response to laminin in 2D, as well as in preliminary 3D studies. Additionally, we have observed a unique MMP9 secretion profile of SHH cells grown in 3D compared to 2D, suggesting that our 3D assay allows us to observe relevant phenotypes absent in 2D culture. We are now in the process of identifying which of these LRG candidates are involved in metastasis and chemoresistance. This will enable the elucidation of novel therapeutic targets and crucially increase our understanding of MB-microenvironment interactions.

#### MBRS-44. TIME, PATTERN AND OUTCOME OF MEDULLOBLASTOMA RELAPSE ARE ASSOCIATED WITH TUMOUR BIOLOGY AT DIAGNOSIS AND UPFRONT THERAPY: A COHORT STUDY

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Disease relapse occurs in ~30% of children with medulloblastoma, and is fatal in the majority. We sought to establish whether clinico-molecular characteristics at diagnosis are associated with the nature of relapse, subsequent disease-course, and whether these associations could inform clinical management. We surveyed the clinical features of medulloblastoma relapse (time-to-relapse, pattern-of-relapse, time-to-death and overall outcome) in 247 centrally-reviewed patients who relapsed following standard-upfront-therapies. We related these to clinico-molecular features at diagnosis, prognostic factors, and first-line/relapse treatment. Patients who received upfront craniospinal irradiation (CSI-treated) displayed prolonged time-to-relapse compared to CSI naïve patients ( $p < 0.001$ ). Similarly, in CSI naïve patients, CSI at relapse, alongside re-resection and desmoplastic/nodular histology, were associated with long-term survival. In CSI-treated patients, the nature of relapse was subgroup-dependent. Local-nodular relapse patterns were enriched in relapsed-MB<sub>SHH</sub> patients ( $p < 0.001$ ), but a notable proportion (65%) also acquired distant-diffuse disease ( $p = 0.010$ ). MB<sub>Group3</sub> relapsed quickly (median 1.3 years), MB<sub>Group4</sub> slowly (median 2.1 years). Distant-disease was prevalent in MB<sub>Group3</sub> and MB<sub>Group4</sub> relapses (90%) but, in contrast to relapsed-MB<sub>SHH</sub>, nodular and diffuse patterns of distant-disease were observed. Furthermore, nodular disease was associated with a prolonged time-to-death post-relapse ( $p = 0.006$ ). Investigation of second-generation MB<sub>Group3/4</sub> subtypes refined our understanding of heterogeneous relapse characteristics. Subtype VIII had prolonged time-to-relapse; subtype II a rapid time-to-death. Subtypes II/III/VIII developed a significantly higher incidence of distant-disease at relapse, whereas subtypes V/VII did not. The nature of medulloblastoma relapse are biology