The molecular mechanisms of 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 potential. Here, we report solid malignancies with normal copy number aberrations and nuclear 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 receptor downregulation of SHH in syrnostioma kind-1 and of the β1 integrin adhesion receptor 1. 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 MB 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 stratin-interacting phosphatase and kinase (STRIPAK) complex and that STRIPAK component stratin 4 is controlling cell motility and invasiveness in medulloblastoma cells. Invasiveness of medulloblastoma cells is abrogated by a truncation mutant of MAP4K4 lacking the stratin 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. Tripolitsioti, D. et al., Oncotarget 9, 23220–23236 (2018).

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 oncoprotein. 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 paraffin tissue microarrays displayed significant YB-1 expression, with a high proportion (83%) of patients exhibiting nuclear accumulation. 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 (DA0Y) cell lines with vincristine and cisplatin and analysis of cellular localisation by nuclear/cyttoplasmic fluorescence 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 (ABCB1) promoter. RT-PCR analysis of ABCB1 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.

Medulloblastoma (MB) is the most common malignant paediatric brain tumor 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 MB tumor microenvironment 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 seen, whereas in over 13.2% were cases were SHH. In cases between 3 to 17 years old, Group 4 is the most (47%), and these trends are almost consistent with published studies. 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 alteration were good prognosis. These findings reveal molecular properties of Japanese MBs and will contribute to develop new therapeutic strategies.

Medulloblastoma 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/re-relapse treatment. Patients who received upfront craniospinal irradiation (CSI-treated) displayed prolonged time-to-relapse compared to CSI naive patients (p<0.001). Similarly, in CSI naive patients, CSI at relapse, alongside re-resection and desmoplastic/nodular histology of associated with long-term survival. In CSI naive patients, the nature of relapse was subgroup-dependent. Local nodular relapse patterns were enriched in relapsed-MB_SHH patients (p<0.001), but a notable proportion (65%) also acquired distant-diffuse disease (p=0.010). MBs_Grp3/4 relapsed quickly (median 1–3 years), MBs_Grp4 slowly (median 2.1 years). Distant-disease was prevalent in MBs_Grp1 and MBs_Grp2 re- lapses (90%) but, in contrast to relapsed-MB_SHH, nodular and diffuse patterns of distant-disease were observed. Furthermore, nodular disease was associated with a prolonged overall survival (p<0.001). Investigation of second-generation MB_SHH subtypes refined our understanding of heterogeneous relapse characteristics. Subtype VIII had prolonged time-to-relapse; subtype II a rapid time-to-death. Subtypes I/II/VIII developed a significantly higher incidence of distant-disease at relapse, whereas subtypes V/VII did not. The nature of medulloblastoma relapse are biology
and therapy-dependent, providing immediate translational opportunities for improved disease management through biology-directed surveillance, post-repair classification and risk-stratified selection of second-line treatment.

MBRS-45. TWIST1 AND ABCB1 ARE FUNCTIONAL DETERMINANTS OF METASTASIS IN MEDULLOBLASTOMA

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Paediatric medulloblastomas (MB) are frequently metastatic, resulting in a poor prognosis for the patient. Of the four MB subgroups, group 3 patients present with the highest rates of metastasis and worst outcomes. The mechanisms behind the metastatic process are poorly understood, limiting our ability to develop novel therapeutic treatments. We hypothesised that the epithelial-mesenchymal transition (EMT) transcription factor TWIST1 and the multidrug efflux pump ABCB1 (ATP-binding cassette subfamily B member 1) synergistically drive MB metastasis. TWIST1 protein expression was analysed in patient tissue microarrays by immunohistochemistry. High TWIST1 expression was associated with metastatic patients (p=0.041). Physical and functional interactions between TWIST1 and ABCB1 were investigated using in vitro systems. Low-depth whole genome with long-read single-molecule nanopore sequencing efficient for rapid, cheaper, and reliable subgrouping of clinical MB samples. The gold standard of MB is the MYC-driven group 3 MB, which is the molecular subgroup that is associated with shorter survival and conversely, differentiated subpopulation that is associated with longer survival. This scRNAseq dataset also afforded the opportunity to study neoplastic subpopulations, including photoreceptor subpopulation cells are more abundant in GP3-alpha. In both our present study builds on the findings of existing studies, providing further characterization of conserved neoplastic subpopulations, including indistinguishable from genetically engineered mouse (GEM) models.

MBRS-46. CHARTING NEOPLASTIC AND IMMUNE CELL HETEROGENEITY IN HUMAN AND GEM MODELS OF MEDULLOBLASTOMA USING SCRNASEQ

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We explored cellular heterogeneity in medulloblastoma using single-cell RNA sequencing (scRNAseq), immunohistochemistry and deconvolution of bulk transcriptomic data. Over 45,000 cells from 31 patients from all four subgroups of medulloblastoma (WNT, SHH, 9G8, 1P4 and 1G3A) were clustered using Harmony alignment to identify conserved subpopulations. Each subgroup contained subpopulations exhibiting mitotic, undifferentiated and neuronal differentiated transcript profiles, corroborating the MAGIC transcriptomic cohort data showed that neoplastic subgroups are associated with major and minor subgroup subdivisions, for example, photoreceptor subpopulation cells are more abundant in GP3-alpha. In both WNT and G3A, high-chromatin state differentiated subpopulations are associated with shorter survival and conversely, differentiated subpopulation is associated with longer survival. This scRNAseq dataset also afforded unique insights into the immune landscape of medulloblastoma, and revealed disordered myeloid subpopulation that was restricted to SHH medulloblastoma. Additionally, we performed scRNAseq on 16,000 cells from genetically engineered mouse (GEM) models of GP3 and SHH medulloblastoma. These models showed a level of fidelity with corresponding human subgroup-specific neoplastic and immune subpopulations.

Collectively, our findings advance our understanding of the neoplastic and immune landscape of the main medulloblastoma subgroups in both humans and GEM models.

MBRS-47. RAPID MOLECULAR SUBGROUPING OF MEDULLOBLASTOMA BASED ON DNA METHYLATION BY NANOPORE SEQUENCING

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Medulloblastoma (MB) can be classified into four molecular subgroups (WNT group, SHH group, group 3, and group 4). The gold standard of assignment of molecular subgroup through DNA methylation profiling uses Illumina EPIC arrays. However, this tool has some shortcomings in terms of cost and timing, in order to get the results soon enough for clinical use. We present an alternative DNA methylation assay based on nanopore sequencing efficient for rapid, cheaper, and reliable subgrouping of clinical MB samples. Using bioinformatics analysis and ChIP sequencing, additional TWIST1 downstream targets are now being identified and compared across the metastatic cell lines (ONS-76, D283MED and HD-MB03). This data will provide a deeper insight into the pathways associated with MB metastases, enabling personalised treatment approaches for patients with metastatic disease.

MBRS-48. IDENTIFICATION OF NOVEL THERAPEUTIC APPROACHES FOR MYC-DRIVEN MEDULLOBLASTOMA USING DRUG SCREENING

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We conducted a high-throughput drug screening to identify novel compounds showing efficiency in group 3 MB using both clinically established inhibitors (n=196) and clinically-applicable compounds (n=464). More than 20 compounds demonstrated a significantly higher anti-tumour effect in MYC-driven (n=7) compared to MYC-driven (n=4) MB cell models. Among these compounds, Navitoclax and a proteasome inhibitor showed the strongest effect in inducing cell cycle arrest and apoptosis in MYC-driven MB cell models. Furthermore, we show that Navitoclax, an orally bioavailable and blood-brain barrier passive anti-cancer drug, inhibits specifically Bcl-xL proteins. In line, we found a significant correlation between Bcl-xL and MYC nuclear level in 763 primary MB patient samples (Data source: “R2 https://r2.er/i.amc.nl”). In addition, Navitoclax and Clofarabine have been tested in cells obtained from MB patient-derived-xenografts, which confirmed their specific anti-myeloid effect in MYC-driven MB. In summary, our approach has identified promising new drugs that significantly reduce cell viability in MYC-driven compared to MYC-driven MB cell models. Our findings point to novel therapeutic vulnerabilities for MB that need to be further validated in vitro and vivo.