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Introduction: KIF11, a mitotic kinesin, is a component responsible for assembly and maintenance of mitotic spindle during mitosis. Tumor cells can upregulate KIF11. Inhibition of KIF11 results monopolar spindle formation, resulting in monoastral mitosis in cells. This activates the spindle assembly checkpoint, cells are arrested and prevented from entering cell cycle, resulting in cell death via apoptosis or necrosis, cell division with aneuploidy or mitotic slippage without division into tetraploid G1 phase. Methods: We hypothesized that the effect of KIF11 inhibition on medulloblastoma (MB) is dependent of its p53 mutational status. Results: Our findings on Hoechst staining demonstrated a small molecule inhibitor of KIF11 which induced apoptosis in p53-wildtype MB cells at 48h ($p<0.0001$), was able to trigger mitotic catastrophe ($p = 0.0010$) in p53-mutant MB cells at 24h and subsequent necrosis ($p=0.0039$) at 48h. KIF11 inhibitor exerted anti-proliferative effects on five MB cell lines at nanomolar concentration range, independent of its p53 mutational status. Cells treated with KIF11 inhibitor were arrested in G2/M phase. Apoptosis was observed on Annexin V flow cytometry 24h after treatment, followed by necrosis after 48h in p53-wildtype cells. In contrast, treated p53-mutant cells underwent necrosis at 24h. Differences in cell death mechanisms upon KIF11 inhibition was confirmed on immunoblotting by upregulated p53 expression and presence of cleaved-PARP and DNA-damage marker in p53-wildtype cells, indicative of apoptosis. While inhibition of KIF11 and increased p53 expression were observed only after 48h, cleaved-PARP expression was detected as early as 24h in p53-wildtype, suggesting KIF11-independent, cleaved-PARP-mediated cell death at 24h. In contrast, treated p53-mutant cells showed decreased p53 expression and absence of cleaved-PARP and DNA-damage marker after 24h. Conclusions: Our results suggest that when mitotic arrest is induced, p53-mutant MB cells undergo mitotic catastrophe and necrosis while p53-wildtype MB cells predominantly undergo apoptosis.

EMBR-24. YB1 IS CRITICAL FOR MEDULLOBLASTOMA TUMOR MAINTENANCE AND DNA REPAIR FOLLOWING THERAPEUTIC INTERVENTION

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Medulloblastoma (MB) is the most common pediatric central nervous system malignancy. Although the current standard of care leads to ~70% patient survival, the therapies are highly toxic, leading to life-long side effects, and recurrence due to therapeutic resistance is fatal. We sought to investigate mediators of radiation response in mouse models for the Sonic hedgehog (SHH) subgroup MB as well as human cell lines. We previously identified Y-box binding protein 1 (YB1) as a downstream effector of YAP-mediated MB radiation resistance. YB1 is a crucial, yet understudied, protein highly expressed across all 4 subgroups of MB. Through its DNA- and RNA-binding cold shock domain, YB1 mediates both transcriptional and translational changes important for tumor maintenance and therapeutic response. We show that following ionizing radiation, YB1 mediates DNA repair through PARP and that PARP inhibition abrogates YB1-mediated DNA repair in cells overexpressing YB1. Additionally, through its inhibitory effects on p53, YB1 is capable of mediating anti-apoptotic effects in response to genotoxic insult. By targeting YB1 with short hairpin RNA, we show that cells are more amenable to ionizing radiation induced double strand breaks. Additionally, we utilize RNA binding protein immunoprecipitation sequencing to investigate post transcriptional regulation of RNAs bound by YB1. We show that YB1 binds numerous transcripts critical for the identity of early cerebellar progenitor cells, the putative cell of origin for SHH subgroup tumors, in addition to transcripts important for cell cycling and migration.

EMBR-25. GENOME-WIDE GENETIC AND EPIGENETIC ASSESSMENT OF GROUP 4 MEDULLOBLASTOMA FOR IMPROVED, BIOMARKER DRIVEN, PROGNOSTICATION AND RISK-STRATIFICATION

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Introduction: Medulloblastoma (MB) is the most common malignant brain tumour in children. The most frequent molecular subgroup, Group 4 (MB_{Grp4}) accounts for ~35/40% of cases, however it has the least understood underlying biology. Clinical outcomes are heterogeneous in MB_{Grp4} and are not accounted for by established clinico-pathological risk factors. There is now a requirement for a comprehensive study of MB_{Grp4}, considering established clinico-pathological features and novel molecular biomarkers to enhance risk-stratification and identify novel therapeutic targets. Methods: A clinically-annotated, retrospective MB_{Grp4} discovery cohort (n = 420) was generated from UK CCLG institutions, collaborating European centres and SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 clinical trials. Contemporary, multi-omics profiling was performed. Focal and arm level copy number aberrations (CNAs) were determined from molecular inversion probe (MIP) or DNA methylation array which additionally provided next generation non-WNT/non-SHH (Grp3/Grp4) subtype classifications. Targeted next-generation DNA sequencing was performed to overlay the mutational landscape. Survival modelling was carried out with patients >3 years old who received craniospinal irradiation. Results: MB_{Grp4} subtypes were assigned to 88% of tumours with available data. Subtype VIII was strongly associated with i17q ($p<0.0001$). The favourable-risk cytogenetic signature (2 or 3 of; chromosome 7 gain, chromosome 8 loss and/or chromosome 11 loss) associated with both subtypes VI and VII ($p<0.0001$). *MYCN* amplifications were strongly associated with subtype V ($p<0.0001$) in addition to 16q loss ($p<0.0001$). The high-risk CNA group was enriched for mutations in genes involved in chromatin remodelling ($p<0.0001$). Risk factors were identified from multivariate survival modelling. Subtype and CNA groups contributed to improved risk-stratification models that outperformed current clinical schemes. Conclusion: Comprehensive genetic and epigenetic profiling in this large retrospective cohort has improved our understanding of the molecular and clinical heterogeneity within MB_{Grp4}. Incorporation of molecular biomarkers improved risk-stratification for MB_{Grp4}.

EMBR-27. NEOPLASTIC AND IMMUNE SINGLE CELL TRANSCRIPTOMICS DEFINE SUBGROUP-SPECIFIC INTRA-TUMORAL HETEROGENEITY OF CHILDHOOD MEDULLOBLASTOMA

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Medulloblastoma (MB) is a heterogeneous disease in which neoplastic cells and associated immune cells contribute to disease progression. To better understand cellular heterogeneity in MB we used single-cell RNA sequencing, immunohistochemistry and deconvolution of transcriptomic data to profile neoplastic and immune populations in childhood MB samples and MB genetically engineered mouse models (GEMM). Neoplastic cells clustered primarily according to individual sample of origin which is in part due to the effect of chromosomal copy number gains and losses. Harmony alignment of single cell transcriptomic data revealed novel MB subgroup/subtype-associated subpopulations that recapitulate neurodevelopmental processes and are associated with clinical outcomes. This includes photoreceptor-like cells and glutamatergic lineage unipolar brush cells in both GP3 and GP4 subgroups of MB, and a SHH subgroup nodule-associated neuronally-differentiated cell subpopulation. We definitively chart the spectrum of MB immune cell infiltrates, which reveals unexpected degree of myeloid cell diversity. Myeloid subpopulations include subgroup/subtype-associated developmentally-related neuron-pruning as well as antigen presenting myeloid cells. Human MB cellular diversity is recapitulated in subgroup-specific MB GEMM, supporting the fidelity of these models. These findings provide a clearer understanding of both the neoplastic and immune cell heterogeneity in MB and how these impact subgroup/subtype classification and clinical outcome.