BACKGROUND: Cell-free DNA (cfDNA) profiling has been shown to carry utility as a clinically relevant biomarker in a variety of cancers, but studies in pediatric brain tumors, including medulloblastoma, are scarce. We hereby evaluated the actionability of profiling cfDNA from cerebrospinal fluid (CSF) based on a multi-institutional cohort of children with medulloblastoma. METHODS: 103 children aged ≥ 3 years with medulloblastoma harboring chromosomal aneuploidy enrolled on two prospective therapeutic trials were included. cfDNA was extracted from CSF obtained longitudinally, and profiles by low-coverage whole-genome sequencing (IcWGS) for annotating copy-number variants (CNVs). cfDNA-derived CNVs were compared against patient-matched primary tumor-derived CNVs and correlated with outcome. cfDNA profiles at diagnosis and relapse were compared to evaluate tumor evolution. RESULTS: Tumor-derived somatic CNVs were detected in 72% of baseline cfDNA samples, with higher detection rate in samples from patients with metastatic disease than those without (90% vs. 50%, p<0.001). Longitudinal profiling of cfDNA revealed dynamic CNV changes over time, and was significantly associated with inferior PFS (log-rank p<0.001 for both time-points). Comparison of CNV profiles from cfDNA at baseline and relapse revealed molecular divergence in a subset of patients. CONCLUSION: cfDNA-derived CNVs in CSF collected during chemotherapy and at the end of therapy were consistent with CNVs at relapse detected by IcWGS. These results carry major implications and support the incorporation of cfDNA profiling in upcoming medulloblastoma protocols for more sensitive and accurate disease monitoring and personalization of treatment.

MBRS-21. CLINICAL AGGRESSIVENESS OF TP53-WILD TYPE SONIC HEDGEHOG MEDULLOBLASTOMA WITH MYCN AMPLIFICATION
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Clinical implication of MYCN amplification in sonic hedgehog (SHH) medulloblastoma may still be controversial due to the frequent co-occurrence with TP53 mutation, which is one of the poorest prognostic factors among the subgroup. We described two cases of TP53-wild type SHH medulloblastoma with MYCN amplification, showing dismal clinical course with rapid disseminated relapse just after the end of treatment. CASE 1: A 7-year-old boy developed a non-metastatic cerebellar tumor. Pathology of the tumor was consistent with classic medulloblastoma. The patient received radiation treatment that involved reduced-dose (18 Gy) craniospinal irradiation (CSI), local irradiation, and chemotherapy. However, sudden respiratory arrest developed due to massive intracranial disseminated relapse 9 months after the initial surgery. CASE 2: A 6-year-old boy presented a large mass in his 4th ventricle without dissemination. He diagnosed with large cell anaplastic medulloblastoma and underwent craniospinal irradiation (24 Gy of CSI and local irradiation) and chemotherapy, followed by high-dose chemotherapy. However, dissemination through neuroaxis occurred 9 months after the diagnosis. Methylation data of the cases was entered into a recently published classifier and both tumors were classified as “medulloblastoma, subclass SHH A (children and adult)”4. Copy number analysis demonstrated MYCN amplification in both cases. TP53 mutation analysis from exon 2 to 10 in both cases indicated wild type. The cases remind us of the clinical aggressiveness of SHH medulloblastoma with MYCN amplification, even if there is no TP53 mutation. The tumor should be treated with the most intensified treatment.

MBRS-22. SIGNIFICANCE OF RNF213 IN TUMORGENICITY OF MEDULLOBLASTOMA
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RNF213 gene, initially identified as a disease-causing gene for moyamoya cerebrovascular disease, has recently been recognized as a key regulator of tumor growth. The gene is known to be associated with WNT signaling, lipid metabolism, angiogenesis and genomic instability. The purpose of this study was to investigate the association of RNF213 in tumorigenicity of medulloblastoma. Incidence of medulloblastoma and histopathological findings were compared among ptc1+/−, ptc1−/−, rnf213+/+ and ptc1−/−, rnf213−/− mice. Knockout of rnf213 in ptc1−/− transgenic mouse model increased the incidence of spontaneous generation of medulloblastoma from 19.8% (ptc1−/−) to 76.5% (ptc1+/−, ptc1−/−, rnf213+/+ at 9 months (p<0.001). Heterozygous knockout was equivalent to homozygous knockout. Haploinsufficiency of rnf213 seems to be associated with tumorigenicity in medulloblastoma. Molecular mechanism of medulloblastoma generation needs to be further investigated.

MBRS-23. SIGNIFICANCE OF MI-R33 IN GENERATION AND PROGRESSION OF MEDULLOBLASTOMA
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Lipid metabolism has been shown to be associated with tumorigenesis in various malignancies. The purpose of this study was to investigate the association of miR-33, a key regulator of lipid metabolism, in tumorigenicity and progression of medulloblastoma. miR-33 is an isotype of miR-33 in rodents although miR-33b is also detected in human. Incidence of medulloblastoma and histopathological findings were compared between ptc1−/− major/mice and ptc1−/−, rnf213−/− mice. Effect of miR-33 deregulation by cordycepin was tested in DAOY medulloblastoma cells both in vitro and in vivo. Knockout of miR-33a in ptc1−/− transgenic mouse model increased the incidence of spontaneous generation of medulloblastoma from 19.8% to 49.5% (p<0.001) at 10 months. Cordycepin, which regulates miR-33b, prevented tumor growth in DAOY human medulloblastoma cell line, but the effect was not evident in an orthotopic mouse medulloblastoma model. Although miR-33 seems to be an important regulator of medulloblastoma, treatment efficacy of cordycepin was not enough. Combination treatment with immunotherapy or cytotoxic treatment needs to be tested to show survival benefit in preclinical models.

MBRS-24. FUNCTIONAL CHARACTERIZATION OF IKKBA/ELP1 AS A NOVEL SHH MEDULLOBLASTOMA PREDISPOSITION GENE
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Medulloblastoma (MB), a common malignant pediatric brain tumor, comprises at least four distinct molecular entities: WNT, SHH, Group 3, and Group 4. SHH-MB is driven by aberrant activation of the Sonic hedgehog (SHH) pathway in granule neuron progenitors (GNPs) and is associated with hereditary cancer predisposition syndromes including Li-Fraumeni Syndrome and Turcot’s syndrome. Mice with RNF213 knock-out exhibit tumor growth in vivo. In contrast, knock-out of ELP1 in human SHH-MBs cell lines increases tumor growth in vitro and in vivo. We recently identified germline loss of function (LoF) mutations affecting IKBKA/ELP1, the primary scaffolding subunit of the Elongator complex in a subset of SHH-MB patients. Germline ELP1 mutations account for ~15% of all pediatric SHH-MBs and position ELP1 as the most prevalent hereditary predisposition gene in MB. We genetically engineered ELP1 LoF in mouse GNPs to determine ELP1 function in cerebellar development and SHH-MB. Results of both mechanistic and phenotypic studies demonstrate that ELP1 is immunomodulatory and controls some pausing and protein aggregation, reinforcing the critical role of ELP1 in translational elongation and protein homeostasis. Further, we generated new transgenic mouse models mimicking germline ELP1 LoF mutations observed in SHH-MB patients. ELP1−/− mice exhibit purkinje cell...
degeneration and an increased DNA damage response. These mice are currently being evaluated for their capacity to recapitulate ELPI-1-associated SHH-MB. Additional analyses carried out on SHH-MB patient-derived xenografts revealed that ELPI-1 mutant medulloblastoma cells specifically exhibit defects in tRNA biogenesis. Therefore, the function of ELPI-1 as a translational regulator is severely impaired in ELPI-1 mutant SHH-MBs. Our ongoing molecular and functional studies will provide important insights into the most common MB predisposition gene and will lay the foundation for future preclinical studies.

MBRS-26. CDK7 MEDIATED TRANSCRIPTIONAL PROCESSIVITY OF DNA REPAIR NETWORKS REGULATES SENSITIVITY TO RADIATION IN MYC DRIVEN MEDULLOBLASTOMA
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My- driven medulloblastoma remains a major therapeutic challenge due to frequent metastasis and a poor 5-year survival rate. Myc overexpression results in transcriptional dysregulation, proliferation, and survival of malignant cells. Therefore, targeted therapeutic strategies in Myc- amplified medulloblastoma are a priority. To identify key transcriptional targets in Myc- amplified medulloblastoma, we performed a CRISPR-Cas9 essentiality screen targeting 1140 genes annotated as the druggable genome. The cyclin-dependent kinase, CDK7, was identified as a top candidate. CDK7 phosphorylates the c-terminal domain of RNA polymerase II (RNA Pol II), facilitating transcriptional initiation and elongation. We subjected Myc- amplified cells treated with CDK7 inhibitors to whole transcriptomic analysis. The resultant data revealed gene networks mediating DNA repair were functionally repressed. Consistent with this data, ChIP-sequencing showed the most significant reduction in RNA Pol II and Myc promoter occupancy within a subset of DNA repair genes including BRCA2 and RAD51 but not across the whole genome. These data suggest that inhibition of CDK7 mechanistically limits Myc driven transcriptional processivity of DNA repair networks. Further, evaluation of genes mediating DNA repair showed a muted response to DNA damage and increased cell death with CDK7 inhibition. We next evaluated Myc- amplified MB cell response to ionizing radiation in vitro and in vivo with CDK7 inhibition. Inhibition of CDK7 abrogated radiation sensitivity of the MB cells by potentiating DNA damage. Further, cotreatment produced decreased MRI T2 tumor volumes and enhanced survival benefit in orthotopic PDX xenografted mice compared to radiation alone. Our studies establish a mechanism for selective inhibition of Myc-driven MB by CDK7 inhibition combined with radiation as a viable therapeutic strategy for Myc-amplified medulloblastoma.

MBRS-27. EXOSOMES CARRY DISTINCT MIRNAS THAT DRIVE MEDULLOBLASTOMA PROGRESSION
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INTRODUCTION: Extracellular vesicles (EVs) represent an ideal source of functional biomarkers due to their role in intercellular communication and their ability to protect cargo, including RNA, from degradation. The most investigated EVs are exosomes, nanovesicles secreted by all cell types and able to cross the blood-brain-barrier. Here we characterised the RNA of exosomes isolated from medulloblastoma cell lines with the aim of investigating exosomal RNA cargo as potential functional biomarkers for medulloblastoma. METHODS: Exosomes derived from a panel of matched (original tumour and metastasis) medulloblastoma cell lines were isolated and characterised by NanoSight, electron microscopy, western blotting and Nanoscale flow cytometry. Exosomal miRNA and mRNA from our matched cell lines and foetal neuronal stem cells, which were used as a normal control, were analysed by RNA-sequencing technology. RESULTS: Based on hierarchical clustering, malignant derived exosomes were distinctly separated from normal control exosomes. miRNA profiling revealed several established oncomiRs identified in our malignant derived exosomes compared to control samples. Using interaction pathway analysis, we identified 5 miRNAs implicated in migration, proliferation, cellular adhesion and tumour growth. The use of exosomal biomarkers as markers of metastatic disease could be an innovative and powerful non-invasive tool.

MBRS-28. EXOSOMES DRIVE MEDULLOBLASTOMA METASTASIS IN A MMP2 AND EMMPRIN DEPENDENT MANNER
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INTRODUCTION: Recurrent/metastatic medulloblastoma (MB) is a devastating disease with an abysmal prognosis of less than 10% 5-year survival. The secretion of extracellular vesicles (EVs) has emerged as a pivotal mediator for communication in the tumour microenvironment during metastasis. The most investigated EVs are exosomes, nanovesicles secreted by all cell types and able to cross the blood-brain-barrier. Matrix metalloproteinases (MMPs) are enzymes secreted by tumour cells that can potentiate their dissemination by modification of the extracellular matrix. We hypothesise that exosomal MMP2 and its inducer EMMPRIN could enhance metastasis of MB. METHODS: Proliferation, invasion and migration assays were used to evaluate the phenotypic behaviour of primary cell lines pre-treated with metastatic tumour cell-derived exosomes. Gelatin zymography and western blotting were performed to confirm MMP2 functional activity in cell lines and exosomes. Nanoscale flow cytometry was used to measure surface exosomal EMMPRIN levels. Exosomal MMP2 and EMMPRIN were modulated at the RNA level. RESULTS: Numbers of exosomes and migratory behaviour of primary cell lines were increased in exosomes derived from parental MB and metastatic cell lines also conferred increased migration on poorly migratory foetal neuronal stem cells. CONCLUSION: Together this data suggests that exosomal MMP2 and EMMPRIN may promote medulloblastoma metastasis and supports analysis of exosomal MMP2 and EMMPRIN levels in patient cerebral spinal fluid samples.

MBRS-29. PROSPECTIVE MOLECULAR PROFILING IN PEDIATRIC MEDULLOBLASTOMA PATIENTS ENROLLED ON THE “HEAD START 4” PROTOCOL
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Medulloblastoma is the most common malignant embryonal brain tumor in children with only modest improvements in outcomes achieved over the last decades. The implementation of irradiation-avoidant molecular trials by the “Head Start” consortium, have demonstrated improved cure rates along with enhanced quality of life. Simultaneously, the classification of medulloblastomas has undergone a dramatic shift as molecular testing has made it possible to divide these diseases into distinct subtypes. Currently, the WHO recognizes four medulloblastoma molecular subgroups; however it remains unclear how patients within these subgroups respond to modern irradiation-avoiding therapies. This study aims to demonstrate the feasibility of prospective molecular profiling in medulloblastoma patients enrolled on the “Head Start 4” trial. Whole-exome sequencing (SureSelect Human All Exon V6+cosmic) and DNA methylation (illumina epic array) profiling were performed on 10 paired tumor/blood samples and 4 tumor samples, respectively. High-quality mutational and copy number data were produced for each of the 10 subjects demonstrating well-described gene mutations (suFU) and chromosomal losses (9q and 10q). Four subjects had methylation profiling which successfully separated them into the WHO subgroups (two SHH and two Group 3). These data showed the feasibility of prospective high-dimensional mutational and DNA methylation analysis using “Head Start 4” patients. Future work will focus on finalizing these profiling efforts, enabling the development of models that predict response to irradiation-avoiding treatment and, in general, a better understanding of the molecular mechanisms underlying treatment resistance and tumor progression, leading to more personalized approaches to treating children with medulloblastoma.

MBRS-31. COMBINING IRRADIATION AND ANTI-C4D7 TO ENHANCE THE TREATMENT OF GROUP 3 MEDULLOBLASTOMA
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Medulloblastoma (MB) is the most common malignant primary paediatric brain tumor. The Group 3 molecular subgroup of Medulloblastoma