Here, we have used real R-loops sequencing data sets, including ETMR DRIP-seq data, and the bioinformatic tool QmRLFS-finder (Wongsurawat et al. 2012), which can predict R-loops forming sequences (RLFSs) based on DNA sequence composition. By performing bioinformatic analyses of DRIP-seq data sets and integrating these with RLFSs from human reference genome simulations, we aim to analyze how reliable the different methods are to measure and predict R-loops and what role they have in cancer genomes. In ETMR, R-loops signals from DRIP-seq data were enriched around transcriptional start sites (TSS), comparable to R-loops distribution and enrichment in other published cell models. However, the overlap with RLFSs was limited, indicating that more validations with real DRIP-seq data from more ETMRs and other pediatric cancers are needed to validate the different methods. Taken together, until now, we developed methods to (semi-)quantify R-loops by combining real sequencing data and computational simulation approaches to investigate R-loops distribution in cancer genomes and to investigate whether R-loops are associated with breakpoints or other genetic aberrations in pediatric cancer entities.

TBIO-06. STUDY OF CELL FREE DNA EXTRACTED FROM CSF ENABLES MOLECULAR CLASSIFICATION OF EMBRYONAL BRAIN TUMORS

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Liquid biopsies are revolutionary tools to detect tumour-specific genetic alterations in body fluids. Here, we assess whether the circulating tumor DNA (ctDNA) in Cerebral Spinal Fluid (CSF) could be used for the tumor genetic profiling of pediatric embryonal brain tumors (EBT). Cell free DNA extracted from CSF from 4-5 lumbar puncture droplets from patients with Medulloblastoma (n=18), ATRT (n=3), ETMR(n=1), FOXR2 EBT (n=2) or other pediatric EBT (n=1) (cfDNA mean quantity 48 ng/ml ; range 5.6 - 442 ng/ml) and matched genomic DNA from primary tumors were sequenced by WES (Illumina 100PE) using Nimblegen Medexome Capture. SNVs/mutations were called using GATK-UnifiedGenotyper, GATK-HaplotypeCaller and Samtools. Copy Number profiles were generated with CNVkits. 10/13 cfDNA WES yielded satisfactory depth (>10x). A mean of 466 (range 93-945) SNVs were detected in the primary tumor and 474 (range 18-922) in the CSF. A mean of 416 (range 18-872) commons SNVs were observed between the cfDNA and the primary tumor, comprising classical medulloblastoma genes such as SMO or MLL2. Interestingly, several SNVs were observed either in the tumor only (mean 50 ; range 3-115) or in CSF only (mean 58 ; range 0-148) suggesting a clonal heterogeneity. For 5 cases, Copy Number profiles were also available, allowing the detection of MYCN amplification or 19q13 miRNA cluster amplification. Altogether, we demonstrate the feasibility of WES on CSF with a low input of ctDNA. These results may pave the way for new tumor monitoring tools.

TBIO-07. PEDIATRIC TUMOR CLASSIFICATION THROUGH GENOME-WIDE METHYLATION PROFILING OF EXTRACELLULAR VESICLE DNA

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BACKGROUND: Genome-wide methylation profiling reliably classifies pediatric central nervous system (CNS) tumors. Extracellular vesicles (EVs) are released by pediatric CNS tumor cells (pCC) and contain high molecular weight tumor DNA, rendering EVs a potential biomarker source to identify tumor subgroups, stratify patients and monitor therapy by liquid biopsy. We investigated, whether the DNA in pCC-derived EVs reflects genome-wide tumor methylation profiles and allows tumor subtype classification. Currently, the tests are being expanded to include blood samples (n=80 patients). METHODS: DNA was isolated from EVs secreted by pediatric CNS tumor cells (pCC) as well as from the shortly cultured tumor cells and from the original tumor samples (n=4 patients). Pediatric Fibroblasts and EVs derived thereof were used as a non-tumorous control. EVs were classified by nanoparticle analysis (NTA), immunoblotting, imaging flow cytometry (IFCM and electron microscopy. Genome-wide DNA methylation profiling was performed using an 850k Illumina EPIC array and results were classified according to the DKFZ brain tumor classifier and further analysed by t-SNE and Copy number alteration analysis (CNA). RESULTS: The size range of pCC-derived EVs was 120-150 nm, as measured by NTA. The majority of secreted EVs exhibited high expression of common EV markers (i.e. CD9, CD63 and CD81), as characterized by IFCM. Genome-wide DNA methylation profiling of pCC-derived EVs correctly identified the methylation class of the original tumor (i.e. pilocytic astrocytoma, medulloblastoma). In addition, t-SNE analysis and copy number alterations matched the pattern of the parental pCC and original tumor samples. CONCLUSION: EV DNA faithfully reflects the tumor methylation class and copy number alterations present in the parental cells and the original tumor. Methylation profiling of circulating tumor EV DNA could become a useful tool to detect and classify pediatric CNS tumors.

TBIO-08. THE MOLECULAR BASIS FOR RATIONAL TARGETING OF FGFR-DRIVEN GROWTH AND INVASIVENESS IN PEDIATRIC BRAIN TUMORS

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The oncogenic activation of receptor tyrosine kinases (RTK) promotes growth, survival and dissemination in pediatric tumors including glioma, ependymoma and medulloblastoma (MB). Direct targeting of either the RTK or of downstream kinases can effectively block tumor promoting pathway functions. However, emergence of resistance is common. We hypothesized that alternative interference strategies that target protein-protein interactions (PPIs) instead of enzymatic activities could overcome the emergence of resistance. We characterized the molecular interactions downstream of the FGFR that regulate relevant growth and invasion-promoting mechanisms in MB cells, to identify potentially druggable PPIs. We found that the FRS2 protein is an essential up-stream effector of FGFR signaling towards invasiveness. Using a proteomics approach, we furthermore identified the Striatin 3 protein as a novel oncogenic effector of the FGFR pathway downstream of FRS2, as it integrates antagonistic growth and invasion signals downstream of FGFR. Mechanistically, Striatin 3 interacts with the Ser/Thr kinase MAP4K4, couples it to the protein phosphatase 2A, and thereby inactivates growth repressing activities of MAP4K4. In parallel, Striatin 3 enables MAP4K4-mediated phosphorylation of PKC-theta and VASP, which combined are necessary to promote tissue invasion. To selectively repress pro-invasive FGFR functions, we identified and functionally validated small molecule ligands of FRS2, that prevent FRS2 activation and downstream signaling. We demonstrate efficacy of these compounds in inhibiting invasion and growth promoting activities *in vitro* and *in vivo*, and identified potential off-target activities of the ligand using a proteome-wide interaction analysis. We propose inhibition of FRS2 by a small molecular PTB domain ligand as a strategy to repress FGF signaling in FGFR-driven tumors. The development of this ligand, and the de novo design of functional analogs thereof bear promise for further pre-clinical evaluation of these structures as anti-growth promoting and anti-metastatic therapeutics applicable to FGFR-driven tumors.

TBIO-09. EZHIP IS NOT EXPRESSED WITHIN THE DEVELOPING MOUSE OR HUMAN BRAIN

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The protein EZHIP, first described as part of the genetic signature of group A posterior fossa ependymoma (PFA), functions in a manner analogous to the onco-histone H3K27M, itself first described in diffuse midline glioma (DMG). These two proteins modulate activity of the Polycomb repressive complex 2 (PRC2), resulting in global loss of H3K27 trimethylation. Thus, both PFA and DMG are characterized by global loss of the H3K27me3 mark, and PRC2 modulation is critical to oncogenesis of these tumors. However, the precise mechanism(s) of EZHIP and H3K27M-induced tumorigenesis is not known. One possibility is that EZHIP is expressed within the developing brain where it acts to modulate cellular development. In turn, EZHIP overexpression or H3K27M mutation could co-opt this developmental program to drive tumorigenesis. Relatedly, whether EZHIP is expressed in the cell of origin for PFA or DMG is not known. In order to evaluate these hypotheses, we defined the landscape of EZHIP expression in both murine and human developing brain. Leveraging single cell RNA sequencing databases, we show that EZHIP is not expressed within the developing mouse brain, a finding that is consistent across separate datasets. Similarly, EZHIP is not expressed in the developing murine cerebellum, where the cell of origin for PFA is located. Furthermore, we examined bulk RNA sequencing datasets of the developing human brain. Similar to our