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Ependymomas encompass multiple, clinically relevant tumor types based on localization, genetic alterations, and epigenetic and transcriptomic profiles. Tumors belonging to the methylation class of spinal ependymoma (SP-EPN) represent the most common intramedullary neoplasms in children and adults. However, molecular data of SP-EPN are scarce, and clear treatment recommendations are lacking. The only known recurrent genetic events in SP-EPN are loss of chromosome 22q and *NF2* mutations. Yet, it remains unclear whether SP-EPN with germline or sporadic *NF2* mutations or with *NF2* wild type status differ clinically or molecularly. To provide a comprehensive molecular profile of SP-EPN, we integrated epigenetic, genomic, transcriptomic, and histological analyses of up to 237 cases. Clustering of methylation data revealed two distinct molecular SP-EPN subtypes. The distribution of *NF2* mutated cases differed significantly across these subtypes ($p < 0.0001$): The vast majority of tumors harboring either a previously known *NF2* germline mutation or a sporadic mutation were assigned to subtypes A, whereas subtype B tumors mainly contained *NF2* wild type sequences. In addition, subtype A tumors showed a lower frequency of MGMT promoter methylation ($p = 0.018$) and contained almost all pediatric patients of the cohort. Whole-exome sequencing (30 cases) identified numerous mutations in *NF2* wild type and mutated tumors. Mutated genes in *NF2* wild type tumors were enriched for genes associated with cell cycle and cytoskeleton. RNA sequencing revealed two distinct transcriptional groups with upregulation of proliferative genes in one group and upregulation of cilial genes in the other group. The molecular subtypes displayed subtle, but significant differences in the appearance of histopathological characteristics, such as surfaces, inflammation, and hyalinized vessels. Investigation of clinical parameters is ongoing and will complete the picture of SP-EPN heterogeneity as an important basis for future clinical decision-making.

EPEN-28. ONCOGENIC DEPENDENCY OF PEDIATRIC EPENDYMOMAS ON EXTRACELLULAR VESICLE PATHWAYS

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INTRODUCTION: The majority of pediatric ependymoma (EPN) comprise either supratentorial EPN characterized by ZFTA-fusions (ST-EPN-ZFTA) or posterior fossa group A EPN (PF-EPN-A), for both of which only limited therapeutic options are available. Because pediatric EPNs have a relatively low mutational burden, identification and characterization of tumor-associated pathways and molecular processes are of critical importance to reveal potential therapeutic targets. Data from previous transcriptional studies and a cross-species in vivo screen implied aberrant vesicular pathways in ST-EPN-ZFTA, prompting further investigation of their putative role in EPN pathogenesis. METHODS: We investigated EPN group-specific differences in extracellular vesicle (EV) biogenesis pathways in human EPN transcriptome and proteome datasets. In addition, we characterized isolated EPN EVs by mass spectrometry. EPN-specific EV cargo was further investigated by immunofluorescence staining and western blotting. This enhanced understanding of EPN vesicular signaling allowed for a pre-selection of inhibitors targeting specific EV biogenesis pathways. *In vitro* proliferation and invasion assays as well as *in vivo* treatment studies were performed on EPN model systems. RESULTS: Integration of multi-omic data from both EPN tissues and EPN-EV-associated proteome led to the identification of ST-EPN-ZFTA-specific EV populations. We could spatially map specific EV markers to the perivascular niche that primarily harbors undifferentiated ST-EPN-ZFTA cell populations. Targeting EV biogenesis pathways by inhibiting factors of the lipid metabolism reduced the abundance of released EVs resulting in altered growth behavior and decreased invasion of tumor cells *in vitro*. I*n vivo* validation of EV release inhibitors in an orthotopic ST-EPN-ZFTA PDX model significantly reduced tumor growth and increased survival. OUTLOOK: In summary, we have leveraged ST-EPN-ZFTA-specific EV pathways as a potential therapeutic vulnerability. Further mechanistic investigations on EPN EV biogenesis, release, or uptake are expected to improve our understanding of the cross-talk between tumor cells and cells of the microenvironment and may lead to potential new therapeutic avenues.

EPEN-29. SPATIAL TRANSCRIPTOMIC ANALYSIS OF EPENDYMOMA IMPLICATES UNRESOLVED WOUND HEALING AS A DRIVER OF TUMOR PROGRESSION

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Ependymoma is a childhood brain tumor that remains incurable in approximately 50 percent of cases, most commonly in posterior fossa subgroup A (PFA). Uncovering how heterogeneous cell types within the tumor microenvironment (TME) interact is crucial to a complete understanding of PFA disease progression. The underlying cellular components of the PFA TME have been revealed by single-cell RNAseq (scRNAseq), identifying divergent epithelial differentiation and epithelial-mesenchymal transition (EMT) lineages. Here, we utilize spatial transcriptomics (Visium) of 14 PFA samples, integrated with scRNAseq, to chart neoplastic and immune cell architecture, with a higher resolution of cellular heterogeneity than scRNAseq alone. At a gross level, all PFA were primarily comprised of neoplastic epithelial and mesenchymal transcriptomic spatial zones, each containing a diversity of hierarchical cellular stages. In all samples we revealed spatially and transcriptomically-distinct mesenchymal zone-associated subclusters, including a quiescent undifferentiated progenitor-like subpopulation and clusters with characteristics of early and late stage EMT. Two early stage EMT clusters were distinguished by signatures of either myeloid cell interaction or hypoxia, and both were demonstrated to be EMT-initiating processes in in-vitro PFA experimental models. Myeloid cell interaction is the predominant initiating stage of EMT in PFA, occurring in zones that are spatially distinct from hypoxia induced EMT. Other mesenchymal clusters represent later EMT stages characterized by wound repair and tissue remodeling. Increased proliferation was a general characteristic of epithelial zone clusters, which included a second undifferentiated progenitor-like population that showed a particularly high mitotic rate and was associated with histologically hypercellular areas. Given the biological parallels with normal wound healing, we propose that mesenchymal and epithelial zones interact to create a cycle of persistent tissue damage response and mitogenic re-epithelialization signals. Unresolved wound repair is therefore a potential driver of PFA progression, a new concept that could provide novel targets for effective therapeutic intervention.

EPEN-30. 5FU WITH RADIATION FOLLOWED BY MAINTENANCE OF 5FU AND ATRA SIGNIFICANTLY IMPROVES SURVIVAL OF 1Q+/6Q- PFA EPENDYMOMA XENOGRAFT MODELS

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In a screen of over 100 FDA approved drugs on PFA 1q+ EPN cells, 5-fluorouracil (5FU) and All-Trans-Retinoic Acid (ATRA) were identified as