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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

inhibitors of EPN cell line growth. We performed in-vitro cell growth assays combining increasing doses of radiation and 5FU and found a significant synergistic effect on cell growth and apoptosis in 1q+ PFA EPN cell lines. Further growth attenuation was seen when ATRA was added 48 hours following radiation and 5FU treatment. This led us to development of preclinical studies in the 1q+ PFA orthotopic xenograft models MAF-811_XF and MAF-928_XF. In the initial cohort, tumors were allowed to establish prior to treatment start confirmed by MRI. In both MAF-811 and MAF-928, chemotherapy improved survival compared to no treatment. As consistent with standard of care, radiation significantly improved survival (p=0.0016) but there was no added benefit to combining 5FU or 5FU+ATRA with radiation. A second cohort was treated using the same treatment approach, however radiation and 5FU were started with minimal to no visible tumors by MRI. Interestingly, we found a significant increase in survival between vehicle control and combination 5FU+ATRA (HR 5.121, 95% CI: 0.2506, 2.409, p=0.048) in MAF-811 mice. However, again with radiation, there was no significant change in survival with only a single cycle of 5FU+ATRA. This led to continued maintenance of 5FU+ATRA cycles of 6 weeks with 2 weeks off for 4 cycles post radiation in mice with minimal tumor. When 5FU with radiation is followed by 5FU+ATRA and is continued in mice with minimal disease, survival significantly improved when compared to radiation alone (HR 9.020, 95% CI: 1.933 to 42.09, p=0.007). These studies highlight the importance of chemotherapy in minimal disease and is the rationale for a Phase I/II study in relapsed PFA EPN and in upfront 1q+ PFA EPN.

EPEN-31. DEVELOPMENTAL AND ONCOGENIC TRANSCRIPTION FACTOR CIRCUITS AS DEPENDENCIES IN EPENDYMOMA

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Brain tumors are the most common cause of cancer death in children. ZFTA-RELA gene fusion is one the most potent drivers of cancer and is sufficient to induce tumors when expressed during brain development. ZFTA-RELA (denoted ZRFUS) fusion is the most frequent events that occurs in an aggressive childhood brain tumor called ependymoma (> 70% of cases). ZFTA recruits RELA to novel DNA binding sites and is necessary to activate ependymoma oncogene transcription. There are currently no targetable treatments for ependymoma, thus studying the mechanisms that regulate ZRFUS oncogenic programs may yield opportunities to develop effective therapies. To study proteins that regulate gene expression programs in brain cancer, the Mack lab and others have comprehensively characterized the active chromatin landscapes of several adult and pediatric brain cancers. This genome-wide analysis has identified highly active TFs, termed core regulatory circuit (CRC) TFs that govern gene expression programs such as MYC, GLI2, SOX2, and OLIG1/2, previously described in brain tumors such as glioblastoma and medulloblastoma. Critically, a glial cell fate specification TF, SOX9, showed the highest levels of activity in ependymoma. A functional RNA interference screen of CRC TFs prioritized SOX9 as the top cancer dependency gene required for ZRFUS ependymoma cell proliferation. To study ZRFUS ependymoma, we developed one of the first genetic mouse models of the disease, and show in preliminary data, that SOX9 knockout abolishes tumor initiation. Surprisingly, SOX9 KO has no impact on tumor initiation in an aggressive glioma model, suggesting tumor-specific contributions of SOX9. This concept is supported by our data that shows SOX9 co-recruitment to a vast majority of ZRFUS binding sites in the genome. Our data supports that SOX9 regulates ZFTA-RELA target cistrome; presenting a potential pathway that may be explored for therapeutic benefit.

EPEN-32. LEVERAGING CELL SURFACE TARGETS AS THERAPEUTIC VULNERABILITIES FOR PEDIATRIC EPENDYMOMA

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Brain tumors are the leading cause of cancer-associated death in children. Ependymoma, an aggressive type of childhood brain tumor, is currently treated with surgery and radiotherapy. Ependymomas are a molecularly heterogeneous group of tumors driven by distinct genetic and epigenetic alterations. In children, 90% of ependymomas arise intracranially, with two thirds occurring in the posterior fossa (PF) and one third in the supratentorial brain (ST). PF ependymomas are divided into at least two groups termed, PFA and PFB, with PFA tumors associated with poor clinical outcomes. Over 70% of ST ependymoma are characterized by an oncogenic fusion between ZFTA and RELA and shown in some cohorts to have poor clinical outcome, particularly in the context of CDKN2A tumor suppressor gene loss. A major challenge in identifying therapies against ependymoma, has been the paucity of genetic abnormalities available for targeting. PFA ependymomas harbor largely balanced genomes with no recurrent CNVs, fusions, or somatic mutations that are amenable to pharmacologic inhibition. ZFTA-RELA ependymoma while representing a clear disease driver, functions as a transcription factor and lacks clear binding surfaces available for direct inhibition using small molecules. Therefore, alternative approaches are needed to identify new targets and effective therapies in ependymoma to be evaluated in pre-clinical models. In both human ependymoma cell culture lines and PDX models, we demonstrate that a multi-omic approach is promising for cell surface target discovery, and further, focused cell surface profiling can identify lead targets that can be rapidly translated for CAR T-cell therapy.

EPIDEMIOLOGY

EPID-01. DIFFERENCES IN FIRST-LINE TREATMENT BUT COMPARABLE SURVIVAL OUTCOMES FOR PEDIATRIC BRAINSTEM AND NON-BRAINSTEM HIGH-GRADE GLIOMAS IN THE NETHERLANDS – A POPULATION-BASED STUDY

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INTRODUCTION: Pediatric high-grade gliomas (pHGG) are among the most devastating childhood cancers. Due to their limited treatment options and tumor biology, brainstem (BS) pHGG are considered to have worse survival outcomes compared to non-brainstem (NBS) pHGG. METHODS: Detailed clinical data were gathered by trained registrars for all children diagnosed with a pHGG (including radiologically diagnosed brainstem tumors) in the Netherlands for the period 2003-2017. Tumors were grouped into BS and NBS tumors according to the ICD-O-3 topography codes. Differences in treatment characteristics were tested with the Chi-squared, Fisher exact or Mann-Whitney-Wilcoxon test. Median survival time was determined by Kaplan-Meier method. Trends and survival differences were tested with Cox Proportional-Hazards Models. RESULTS: In total, 276 pHGG patients (BS n=166, NBS n=110) were diagnosed during 2003-2017. Differences in first line treatment were found for neurosurgery (25% of BS versus 95% of NBS patients, p<0.001) and systemic therapy (20% of BS versus 70% of NBS, p<0.001). Notable, 10% of BS patients received temozolomide compared to 55% of NBS patients (p<0.001). No significant difference was found for first-line radiotherapy. However, total cumulative dose and number of fractions differed significantly (BS: median 44.8 Gy and 16 fractions; NBS: median 57.4 Gy and 30 fractions, both p<0.001), reflecting hypofractionation regimens in BS pHGG. Survival remained stable over time for both BS (p=0.9) and NBS (p=0.3). Median survival time was comparable between BS (9.7 months) and NBS patients (9.8 months, p=0.6).**CONCLUSION:** Despite differences in treatment characteristics we found comparable survival outcomes for BS and NBS pHGG. It remains unclear why survival for both BS and NBS pHGG in this retrospective population-based study is substantially inferior to published data. If the underlying reasons can be found in differences in treatment characteristics, data type (hospital-based versus population-based) or incompleteness of non-microscopically verified cases, is subject to further research.

EPID-02. SURVIVAL EXPERIENCE IN PEDIATRIC PATIENTS OF CENTRAL NERVOUS SYSTEM (CNS) TUMOUR IN CANADA

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Established in 2016, the Brain Tumour Registry of Canada Surveillance Research Collaboration aims to address the lack of detailed information on CNS tumours in Canada. Using Canadian Cancer Registry (CCR) data with linked vital status, we present survival estimates for all primary CNS tumours (excluding Quebec) among pediatric patients (age 0-14). Pediatric patients diagnosed with primary CNS tumours during 2010-2017 were included. Vital status was obtained by Statistics Canada through linkage to the Canadian Vital Statistics Database and the income tax returns file, with a cut-off date of December 31, 2017. We used the Pohar-Perme method to estimate the net survival rate (NSR) through the period approach. International Classification of Diseases for Oncology (3rd edition) site/histology codes were grouped into 25 histological categories, irrespective of tumour behaviour, according to the schema developed by the Central Brain Tumor Registry of the United States. Of 1725 pediatric CNS tumours, the 1-, 2- and 5-year NSR are 0.89 (95%CI 0.87-0.90), 0.84 (95%CI 0.81-0.86) and 0.80 (95%CI 0.78-0.82), respectively. All non-malignant CNS tumours have a median survival over 8 years. The 5-year NSR range from 0.90 (95%CI 0.47-0.99) for unique astrocytoma variants, 0.95 for tumour of sellar region (95%CI 0.85-0.98) and germ cell tumours, cysts and heterotopias (95%CI 0.67-0.99), to 1.0 for choroid plexus tumours, tumours of cranial and spinal nerves, and meningioma. For malignant CNS tumours, NSR vary greatly depending on histology grouping. 5-year NSR from lowest to highest are glioblastoma (0.10, 95%CI 0.03-0.23), anaplastic astrocytoma (0.19, 95%CI 0.05-0.40), glioma not otherwise specified (0.54, 95%CI 0.46-0.62), embryonal tumours (0.72, 95%CI 0.65-0.79), diffuse astrocytoma (0.74, 95%CI 0.59-0.85), ependymal tumours (0.78, 95%CI 0.67-0.87), germ