Abstracts

inert negative form of GL2 indeed hampered tumorigenesis. Targeting GL2 with arsenic trioxide caused extended survival of tumor-bearing animals, indicating GL2 as a critical regulator of ZFATA fusion-positive tumorigenesis as well as a potential therapeutic vulnerability in these tumors.

EPEN-04. SIOP Ependymoma I: FINAL RESULTS, LONG TERM FOLLOW-UP: MOLECULAR ANALYSIS AND RESULTS OF THE TRIAL COHORT: A BIOMECA CONSORTIUM STUDY

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Introduction: Surgery and radiotherapy are established childhood ependymoma treatments. The efficacy of chemotherapy has been debated. We report final results of the SIOP Ependymoma I trial, with 12-year follow-up, in the context of a post-hoc analysis of more recently described biomarkers. Aims and Methods: The trial assessed event free (EFS) and overall survival (OS) of patients aged three to 21 years with non-metastatic intracranial ependymoma, treated with a staged management strategy targeting maximum local control. The study also assessed: the response rate (RR) of subtotally resected (STR) disease to vincristine, etoposide and cyclophosphamide, and surgical grade. Children with disease that did not receive resection (GTR) received radiotherapy of 54 Gy in 30 fractions over six weeks, whilst those with STR received VEC before radiotherapy. We retrospectively assessed methylation and 1q status alongside bETRT, RELA, Tenascin C, H3K27me3 and pAKT expression. Results: Between 1999 and 2007, 89 participants were enrolled, 15 were excluded with metastatic (n=4) or non-ependymoma tumors (n=11) leaving a final cohort of 74. Five- and ten-year EFS was 49.5% and 46.7%, OS was 69.3% and 60.5%. 1q gain was associated with poorer EFS (p=0.002, HR=3.30, 95% CI 1.49–6.10). bETRT expression was associated with worse five-year EFS (20.0% vs 83.3%, p=0.014, HR=5.8). GTR was achieved in 33/74 (44.6%) and associated with improved EFS (p=0.006, HR=2.81, 95% confidence interval 1.35–5.84). There was an improvement in GTR rates in the latter half of the trial (1999-2002 35.3%, 2003-2007 56.8%). Despite the protocol, 12 participants with STR did not receive chemotherapy. However, chemotherapy RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the RRT criteria defined in the trial. Participants with STR did not receive chemotherapy. However, chemotherapy was associated with improved EFS. There was an improvement in GTR rates in the latter half of the trial. Chemotherapy does not appear to add significant benefit to children with STR. We also confirmed associations between 1q gain, RELA expression and outcome.

EPEN-05. MUTATIONAL ANALYSIS OF THE C11ORF95 DOMAIN AND SINGLE-CELL RNA-SEQ PROFILE OF A MOUSE MODEL OF SUPRATENTORIAL EPENDYMOMA

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We used a recently developed mouse model to better understand the cellular and molecular determinants of tumors driven by the oncogenic fusion protein C11orf95-RELA. Our approach makes use of in utero electroporation and a binary transposase system to introduce human C11orf95-RELA sequence, wild type and mutant forms, into neural progenitors. We used single cell RNA-seq to profile the cellular constituents within the resulting sequence, wild type and mutant forms, into neural progenitors. We used single cell RNA-seq to profile the cellular constituents within the resulting tumors in mice. We find that approximately 70% of the cells in the tumors display expression of the oncogenic C11orf95-RELA and these non-ependymoma expressing cells are a combination of different non-tumor cell types, including significant numbers of T-cells, and macrophages. The C11orf95-RELA expressing tumor cells have a unique transcriptomic profile that includes a point mutation analysis of the C11orf95 domain. We found that mutations were distinguished by upregulation of non-NF-kB pathways involved in cell proliferation, cell fate determination, and immune activation. We hypothesized that the C11orf95 domain may function to bring RELA transcriptional activation to inappropriate non-NF-kB targets, and we therefore performed a point mutation analysis of the C11orf95 domain. We found that mutations in either of the cysteines or histidines that make up a possible zinc finger domain in C11orf95 eliminate the ability of the fusion to induce tumors. In cell lines, these loss-of-function point mutants still trafficked to nuclei, and activated NF-kB pathways. We are currently using RNAseq and CRISPR loss of function to identify genes downstream of C11orh95-RELA that are required for tumorigenesis.

EPEN-06. CELL ECOSYSTEM AND SIGNALING PATHWAYS OF PRIMARY AND METASTATIC PEDIATRIC POSTERIOR FOSSA EPENDYMOMA

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Childhood ependymoma is a cancer of the nervous system with a chronic relapsing pattern. In children, 90% of ependymomas occur intracranially where prognosis is grim. Standard care for this disease includes surgical resection followed by radiation. Despite several clinical trials, adjuvant chemotherapies have yet to extend patient survival, highlighting a need for more effective treatments. In this study, we found that tumors have been stratified into nine molecular subgroups based on their DNA methylation profile. The most prevalent and aggressive pediatric subgroup is known as posterior fossa ependymoma type A (PFA) which represents approximately 60% of pediatric cases and has a 5-year progression-free survival rate of 30%. Whole genome sequencing studies have revealed that PFA tumors rarely harbor recurrent mutations. To inform the potential development of new treatment options for this disease, we sought to identify specific mechanisms leading to the tumorigenic, progression, and metastasis of PFA tumors. By means of single-nuclei RNA-seq and an array of computational methods, we show that the expression profile of PFA tumor cells recapitulate the developmental lineage of radial glia in neurogenic niches, and is consistent with an origin in LGR+ stem cells and a pro-inflammatory environment. In addition, our analysis reveals the abundance of a mesenchymal cell population expressing TGF-β signaling, reactive glia, and hypoxia-related genes in distal metastases from PFA tumors. Taken together, our results uncover the cell ecosystem of pediatric posterior fossa ependymoma and identify WNT/T-catenin and TGF-β signaling as candidate drivers of tumorigenesis for this cancer.

EPEN-07. SINGLE-CELL RNA SEQUENCING IDENTIFIES A UNIQUE MYELOID SUBPOPULATION ASSOCIATED WITH MESAENCHYMAL TUMOR SUBPOPULATION IN POOR OUTCOME PEDIATRIC EPENDYMOMA

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We have previously shown immune gene phenotype variations between posterior fossa ependymoma subgroups. PFA1 tumors chronically secrete IL-6, which induces secretion of myeloid cell IL-8 and pushes the infiltrating myeloid cells to an immune suppressive function. In contrast, PFA2 tumors have a more immune activated phenotype associated with a better prognosis. The objective of this study was to use single-cell(s) RNAseq to descriptively characterize the infiltrating myeloid cells. We analyzed approximately 8500 cells from 21 PFA patient samples. Using advanced machine learning, we identified eight myeloid cell subpopulations with unique gene expression profiles. Interestingly, only one population was significantly enriched in PFA1 tumors. This subpopulation, denoted as the hydropys myeloid subpopulation, was defined by genes associated with phox activation and response to oxygen levels. These myeloid cells also share similar gene expression profile to a mesenchymal tumor subpopulation (MEC) enriched in PFA1 and associated with poor outcome in EPN patients. This tumor subpopulation was the only population expressing IL-6. Using immunohistochemistry, we found the hypoxia myeloid located in regions of tumor necrosis and perivascular niches. The MEC cells were also more abundant in these regions. In an independent single-cell cytokine re-
We have previously shown the importance of immune factors in posterior fossa ependymoma (PF EPN). Recently, we found eight transcriptionally unique subpopulations of myeloid cells infiltrating PF EPN with one population particularly enriched in PF tumors. This subpopulation, defined as a marker of the myeloid infiltration, is named TREM1+ and is associated with angiogenesis, hypoxia response, wound healing, cell migration, neutrophil activation, and response to oxygen levels. TREM1 (Triggering receptor expressed on myeloid cells 1) was found to be expressed almost exclusively in these hypoxia myeloid cells. Significantly, TREM1 encodes for a receptor belonging to the immunoglobulin superfamily that is expressed on myeloid cells, and stimulates neutrophil and monocyte inflammatory responses. However, single-cell RNAseq give little data suggesting location of cells within the tumor microenvironment. We performed immunohistochemistry (IHC) on our bank of ~90 FFPE PF EPN samples using TREM1 to characterize and identify the location of the hypoxia myeloid cells. The TREM1+ cells have an ambiguous cytomorphology reminiscent of a monocyte with modest cytopyctosis or a mono-lobated nucleus. IHC also showed that TREM1+ myeloid cells are largely localized to the interface of necrosis and viable tissue, most frequently in a perivascular and intravascular distribution. The latter finding suggested that the TREM1+ cells are derived from the bone marrow and that they may be associated with the mesenchymal tumor population (MEC), which have previously described as being enriched in PF EPN tumors and localizing to perinecrotic zones. This is supported by parallel IHC analysis of subpopulation-specific markers in the same cohort of PF EPN which showed the highest TREM1 correlation was with CAIX, a marker of MEC. In PF A matched primary/recurrent pairs, the proportion of TREM1+ cells were increased at recurrence in the majority of cases, suggesting an evolving interaction between this TREM1+ hypoxia myeloid subset and neoplastic cells over the disease course.

EPN-08. THE TREM1 POSITIVE HYPOXIC MYELOID SUBPOPULATION IN POSTERIOR FOSSA PFA EPENDYMOMA
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Background: Ependymomas account for 8–10% of pediatric brain tumors, and the standard therapy of surgery and radiation has not changed for the past two decades. Characterization of the tumor immune microenvironment (TIME) is of great importance in order to find better therapies. However, the TIME of ependymomas is still not defined. In this retrospective observational study we aimed to unravel the TIME of ependymomas at mRNA and protein expression levels. Methods: Ependymoma samples from two locations were selected: Posterior Fossa (PF-A, n=8), and supratentorial (ST, n=5). Targeted gene expression profile using the PanCancer immune profile panel of NanoString technology was performed. Data were analyzed using the nSolver software. In addition, 8 samples were subjected to RNA bulk sequencing, and the sequenced data were connected to the expression data of the same samples. To validate some marker enrichment, immunohistochemistry (IHC) was performed. Results: Unsupervised hierarchical clustering showed that PF-A ependymomas can be divided into two groups based on the expression of their immune-related genes. PF-A that showed high immune-expression clustered closely to the ST Ependymomas. Significant expressions of genes related to “antigen processing” and “adhesion” pathways were found in the immune-active groups. On the contrary, the PF-A that had low expressions of immune-related genes showed a high expression of BMI1 that has a prognostic and therapeutic value in some tumors. PF-A tumors can be divided into two groups: immune-active and immune-not active PF-A. The prognostic and therapeutic values of the immune activity of PF-A should be further studied.

EPN-09. SUPER ENHANCER GENES AS MOLECULAR TARGETS IN C11ORP95-RELA FUSION EPENDYMOMA
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Genomic sequencing has driven precision-based oncology therapy; however, genetic drivers remain unknown or non-targetable for many malignancies, demanding alternative approaches to identify therapeutic leads. Ependymomas comprise histologically similar tumor entities driven by distinct molecular mechanisms, such as fusion oncoproteins, genome-wide chromosomal instability, or disruption of DNA methylation patterns. Despite these differences, ependymomas resist chemotherapy and lack available targeted agents for clinical trial development. Based on our previous findings, we hypothesized that mapping chromatin landscapes and super enhancers (SE) could uncover transcriptional dependencies as targets for therapy (Mack, Paijler, Chavez et al., Nature, 2018). To functionally test the requirement of these SE genes for ependymoma cellular growth, we designed a pooled RNA interference screen against 267 SE associated genes. Our screen was performed in one C11ORP95-RELA fusion model and two PF-EPN-A models as controls in biological triplicates. As an indication that our screen was successful, positive controls scored among lead hits including KIF11, RUB1B, PHF5A and MYC. Importantly, we identified many sub-type specific dependencies in both C11ORP95-RELA fusion and PF-EPN-A models, thus revealing novel pathways that potentially govern subgroup-specific ependymoma cell growth. Further, several candidates detected across all models were also identified in cancer datasets of glioma/glioblastoma specific essential genes from the DepMap Cancer Dependency Gene Resource. Our findings reveal novel targets and pathways that are essential for ependymoma cell growth, which may provide new insight into therapeutic strategies against these aggressive brain tumors.

EPN-10. UNRAVELLING THE TUMOR IMMUNE MICROENVIRONMENT OF POSTERIOR FOSSA A EPENDYMOMA ON RNA AND PROTEIN EXPRESSION LEVELS
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Background: Ependymomas account for 8–10% of pediatric brain tumors, and the standard therapy of surgery and radiation has not changed for the past two decades. Characterization of the tumor immune microenvironment (TIME) is of great importance in order to find better therapies. However, the TIME of ependymomas is still not defined. In this retrospective observational study we aimed to unravel the TIME of ependymomas at mRNA and protein expression levels. Methods: Ependymoma samples from two locations were selected: Posterior Fossa (PF-A, n=8), and supratentorial (ST, n=5). Targeted gene expression profile using the PanCancer immune profile panel of NanoString technology was performed. Data were analyzed using the nSolver software. In addition, 8 samples were subjected to RNA bulk sequencing, and the sequenced data were connected to the expression data of the same samples. To validate some marker enrichment, immunohistochemistry (IHC) was performed. Results: Unsupervised hierarchical clustering showed that PF-A ependymomas can be divided into two groups based on the expression of their immune-related genes. PF-A that showed high immune-expression clustered closely to the ST Ependymomas. Significant expressions of genes related to “antigen processing” and “adhesion” pathways were found in the immune-active groups. On the contrary, the PF-A that had low expressions of immune-related genes showed a high expression of BMI1 that has a prognostic and therapeutic value in some tumors. PF-A tumors can be divided into two groups: immune-active and immune-not active PF-A. The prognostic and therapeutic values of the immune activity of PF-A should be further studied.

EPN-11. TUMOR DIFFERENTIATION IMPACTS THE BIOLOGY OF RECURRENCE IN CHILDHOOD POSTERIOR FOSSA EPENDYMOMA
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Ependymoma (EPN) of childhood is curable in only 50% of cases, with recurrences in the remainder that are refractory to treatment. In recent years significant advances have been made in understanding the molecular and cellular biology of EPN. Recent studies show that PFA subgroup EPN are comprised of multiple neoplastic subpopulations that show undifferentiated, differentiated and mesenchymal characteristics. These studies focused on tumor at presentation, with recurrent EPN being less well understood. In the present longitudinal study we examine changes in neoplastic cell heterogeneity in serial presentations of PFA EPN using deconvolution (Cibersort) of bulk RNAseq data. Analysis of a cohort of 48 PFA EPN presenting at Children's Colorado showed survival and PFA1/PFA2 subtype assignment was associated with the proportion of individual neoplastic subpopulations as determined by deconvolution. Tumors that subsequently regrew had a significantly higher estimated proportion of undifferentiated EPN cells (UCE) at presentation, than those that were non-recurrent after 5 years follow-up. This outcome association potentially age related, as UEC proportions are significantly higher in EPN arising in children < 1 year old who have a particularly poor prognosis. Changes in PFA neoplastic subpopulations at recurrence was performed in two cohorts of patients from Children's Colorado (n=23) and Nottingham, UK (n=15). As a whole, no subpopulation proportions were significantly higher in PFA arising in children < 1 year old who have a particular poor prognosis. Changes in PFA neoplastic subpopulations at recurrence were smaller and only seen in PFA1, both UEC and mesenchymal subpopulations being lower at recurrence. In summary, only PFA1 showed dynamic changes in neoplastic subpopulation proportions at recurrence, with potential impacts on transcriptional based-subgroup assignment, whereas PFA2 proportions remained largely stable.