lease assay, we identified eight subpopulations of functional myeloid cells. One subpopulation significantly secreted IL-8, which represented the hypoxia subpopulation based on IL-8 gene expression in the scRNAseq dataset. This data suggests the tumor necrosis resulting in the development of MEC tumor subpopulation is driving the immune suppressive myeloid phenotype in PFA1 tumors through polarization of myeloid cells to the hypoxia subpopulation. Further studies are needed to determine how these myeloid cells interact with the lymphocyte subpopulations and whether they contribute to the progression of PFA1 EPN.

EPEN-08. THE TREM1 POSITIVE HYPOXIC MYELOID SUBPOPULATION IN POSTERIOR FOSSA EPENDYMOMA

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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 PFA1 tumors. This subpopulation, denoted as hypoxia myeloid subpopulation, is defined by genes 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 within this hypoxia myeloid subpopulation. 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 PFA EPN samples using TREM1 to characterize and identify the location of the hypoxia myeloid cells. The TREM1 positive cells have an ambiguous cytomorphology reminiscent of a monocyte with modest cytoplasm and 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 suggests that the TREM1+ cells are derived from the bone marrow and that they may be associated with the mesenchymal tumor population (MEC), which we have previously described as being enriched in PFA1 tumors and localizing to perinecrotic zones. This is supported by parallel IHC analysis of subpopulation-specific markers in the same cohort of PFA EPN which showed the highest TREM1 correlation was with CAIX, a marker of MEC. In PFA 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 subpopulation and neoplastic cells over the disease course.

EPEN-09. SUPER ENHANCER GENES AS MOLECULAR TARGETS IN C11ORF95-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, Pajtler, 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 *C11ORF95-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, BUB1B, PHF5A and MYC*. Importantly, we identified many subtype specific dependencies in both *C11ORF95-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 ependymoma lines were also identified as pan-cancer dependencies or 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.

EPEN-10. UNRAVELLING THE TUMOR IMMUNE MICROENVIRONMENT OF POSTERIOR FOSSA A EPENDYMOMAS 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 of the findings, immunohistochemistry 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. Connecting gene expression to bulk sequence data validated the findings. In addition, immunohistochemical analysis confirmed that protein expression for some of the findings. Conclusion: The TIME varies in ependymomas based on the location of the tumor. Moreover, the immune-related expression profiles indicated that PF-A ependymomas 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.

EPEN-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 (UEC) 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 PFA 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 proportion was significantly changed at recurrence. However, separation of PFA into subtypes PFA1 and PFA2 revealed an increase in the proportion of the cilia-differentiated EPN cell subpopulation is more frequent event in PFA1 (15/24), and rare in PFA2 (2/11). Changes in other 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 transcriptomic based-subgroup assignment, whereas PFA2 proportions remained largely stable.