EMBR-33. YAP1 FUNCTION IN SHH MEDULLOBLASTOMA PROGRESSION AND IMMUNE EVASION

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Our incomplete understanding of the key players in Medulloblastoma (MB) development and progression, and their roles in modulating highly Immune desert-like microenvironment in MBs present major hurdles in successfully applying existing therapies and developing new therapies for MBs. Here, we demonstrate that Yap1 acts as a critical modulator of SHH MB (fSmoM2; GFAPcre (SG) and Ptch;p53 SHH-MB mouse models) progression and immune evasion. Yap1 genetic deletion in SG mice significantly extends survival and normalizes brain development by increasing neuronal differentiation. Both bulk and single-cell RNA sequencing analyses show that Yap1 deleted tumors contain cells with more differentiated molecular signatures similar to late CGNPs and differentiating neurons, and less stem-like cells, compared to SG tumors. Additionally, integrated analyses of ChiPseq, RNAseq, and scRNAq data suggest that Yap1 directly binds to the Super enhancer region containing Sox2 and promotes Sox2 expression in SHH MB cells. We postulate that Yap1 expression is maintained or re-activated in SHH MB cells to generate long-term self-renewing tumor cells. Consistently, Yap1-deleted SHH MB or Verteporfin (a small molecule inhibitor of Yap1) -treated Ptch;p53 MB cells lose self-renewal ability in vitro. Furthermore, we hypothesize that a molecular mechanism underlying this stemness promoting function is mediated through Sox2 expression. Intriguingly, Yap1 deletion in SG MBs is accompanied by a significant change in the immune microenvironment, when compared to age-matched SG MBs. There is a significant increase in the number of bone marrow-derived immune cells (including cytotoxic T-cells, neutrophils, and macrophages). RNAseq analysis of rescued tumors shows marked enrichment of interferon-gamma response genes and pro-inflammatory cytokines. This study highlights Yap1 as a crucial mediator of MB progression and a molecular regulator of inflammatory immune cell infiltration into SHH MBs. Consequently, our work paves the way for improving immunotherapy treatments in brain malignancies.

EPENDYMOMA

EPEN-01. C11ORF95-RELA DICTATES ONCOGENIC TRANSCRIPTIONAL PROGRAMS TO DRIVE AGGRESSIVE SUPRATENTORIAL EPENDYMOMA

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Over 60% of supratentorial (ST) ependymomas harbor a gene fusion between *C11orf95*, an uncharacterized gene, and RELA (also known as p65), a main component of the NF-κB family of transcription factors. While its sufficiency to drive tumor has been established, the mechanism of tumorigenesis remains elusive. To tackle this question, we developed a natively forming mouse tumor model using *in utero electroporation* (IUE) of the embryonic mouse brain and performed integrative epigenomic and transcriptomic mapping. Our findings indicate that in addition to direct canonical NF-κB pathway activation, C11*orf95-RELA* (CR^{fus}) dictates a neoplastic transcriptional program and binds to unique sites across the genome enriched with Plagl family transcription factor motifs. CR^{fus} modulates the transcriptional landscape by recruiting transcription co-activators (Brd4, EP300, Cbp, Pol2) which are amenable to pharmacologic inhibition. Downstream CR^{fus} target genes converge on developmental programs marked by Plagl family of transcription factors and activate neoplastic programs enriched in Mapk, focal adhesion, and gene imprinting networks, many of which contain previously unreported therapeutic leads in C11*orf95-RELA* ependymoma.

EPEN-02. FUNCTION AND DEPENDENCY OF NF-KB ACTIVITY IN C11ORF95-RELA FUSION EPENDYMOMA

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Introduction: Ependymoma is an aggressive type of pediatric brain tumor resistant to chemotherapy, with treatment to date limited to surgical resection and radiation. Thus, identification and validation of molecular targets that can translate into clinical trials in ependymoma is desperately needed to improve patient outcomes. Over 70% of supratentorial ependymoma are driven by an oncogenic fusion between C11orf95 and Rela (denoted CR^{FUS}). CR^{FUS} expression initiates ependymoma development in mice by potentially acting as an oncogenic transcription factor and disrupting gene expression programs. We hypothesized that specific CR^{FUS} interacting proteins are required for tumor formation and could represent lead therapeutic targets. Methods: To study CR^{FUS} ependymoma, a natively-forming tumor model of CR^{FUS} generated by in utero electroporation of the developing mouse brain was utilized. Tumor cells were isolated and then subjected to nuclear Rapid Immunoprecipitation and Mass Spectrometry Analysis of Endogenous Proteins (RIME) of HA-tagged CR^{FUS} protein. Immunoprecipitation and Western Blot (IP-WB) were utilized to probe for leading protein interactions. Results: We identified NF-kB proteins consistent with canonical Rela mediated transcription (NFKB1 and NFKB2) as well as novel protein interactomes that converged on RNA splicing and translational regulation. In addition, we identified a large series of novel chromatin-binding proteins as candidates potentially required for CR^{FUS} mediated tumorigenesis. Conclusions: Further study is ongoing to validate key CR^{FUS} protein interaction dependency on tumor development. ChIP-Seq (chromatin immunoprecipitation with massively parallel DNA sequencing) and CUT&RUN (cleavage under target and release using nuclease) assays have been employed to further analyze the functional role of canonical Rela pathway members. By interrogating these mechanisms, novel therapeutic targets and pathways may be identified in parallel with dissecting the molecular basis of CR^{FUS} driven ependymoma.

EPEN-03. ZFTA/C11ORF95 FUSIONS DRIVE SUPRATENTORIAL EPENDYMOMA VIA SHARED ONCOGENIC MECHANISMS

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The majority of supratentorial ependymomas (ST-EPN) are driven by fusion genes between *RELA* and zinc finger translocation associated, *ZFTA*, previously named *C11orf95*. Apart from fusions with a portion of the Hippo effector *YAP1*, which affects a small group of infant patients, the oncogenic mechanism of remaining ST-EPNs remains unclear. Aiming at refining the molecular classification of ST-EPNs, we have analyzed methylation profiles, RNA and DNA sequencing results as well as clinical data in a cohort of 613 ST-EPNs. An unbiased approach revealed distinct methylation clusters composed of tumors with ependymal but also various other histological features containing alternative translocations that shared *ZFTA* as a partner gene. Tumors within these additional clusters were characterized by fusions of *ZFTA* to numerous fusion partners different from *RELA,* e.g. *MAML2*, *MAML3*, *NCOA2* and *SS18*, implying a general role of *ZFTA* in tumorigenesis of ST-EPN. Indeed, the transforming capacity of newly identified fusion genes was validated using an electroporation-based *in vivo* gene transfer technology in mice. All fusion genes themselves were sufficient to drive malignant transformation in the developing cerebral cortex and resulting tumors faithfully recapitulated molecular characteristics of their human counterparts. We found that both, the partner gene and the zinc finger DNA binding domain of ZFTA, were essential to exert tumorigenesis. Together with two additional studies, we performed a comprehensive analysis across datasets to derive a 93 gene signature of ZFTA-RELA-driven tumors, in which the Sonic Hedgehog effector gene *GLI2* was identified as a promising downstream target. Subsequent co-expression of *ZFTA:RELA* and a dom-

inant negative form of *Gli2* indeed hampered tumorigenesis. Targeting GLI2 with arsenic trioxide caused extended survival of tumor-bearing animals, indicating GLI2 as a critical regulator of ZFTA fusion-positive tumorigenesis as well as a potential therapeutic vulnerability in these tumors.

EPEN-04. SIOP EPENDYMOMA I: FINAL RESULTS, LONG TERM FOLLOW-UP AND MOLECULAR ANALYSIS 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 (VEC); and surgical operability. Children with gross total resection (GTR) received radiotherapy of 54 Gy in 30 daily fractions over six weeks, whilst those with STR received VEC before radiotherapy. We retrospectively assessed methylation and 1q status alongside *hTERT*, 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 tumours (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=3.00, 95%CI 1.49–6.10). *hTERT* 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 32.4% versus 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 pre-specified RR of 45% in children over three years with STR intracranial ependymoma. However, cases of inaccurate stratification at treating centres highlights the need for rapid central review. We also confirmed associations between 1q gain, *hTERT* 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 tumors in mice. We find that approximately 70% of the cells in the tumors do not express the oncogene C11orf95-RELA and these non-oncogene expressing cells are a combination of different non-tumor cell cell-types, including significant numbers of T-cells, and macrophages. The C11orf95-RELA expressing tumor cells have a unique transcriptomic profile that includes both astrocytic and neural progenitor marker genes, and is distinct from glioblastoma transcriptomic profiles. Since C11orf95-RELA is believed to function through a combination of both activation of NF-κB response genes by constitutive activation of RELA, and genes not activated by NF-κB, we assessed the expression of NF-κB response genes across the populations of cells in the tumor. Interestingly, when tumor cells highly expressing C11orf95-RELA were analyzed further, the subclusters identified 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-κB 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-κB pathways. We are currently using RNAseq and CRISPR loss-of function to identify genes downstream of C11orf95-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 central nervous system with a chronic relapsing pattern. In children, 90% of ependymal tumors 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 treatment options. Ependymal 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 decipher the specific mechanisms leading to the tumorigenesis, 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 lineages 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 gliosis, 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/β-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 MESENCHYMAL 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(sc) 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 subpopulation was significantly enriched in PFA1 tumors. This subpopulation, denoted as the hypoxia myeloid subpopulation, was defined by genes associated with angiogenesis, response to hypoxia, wound healing, cell migration, neutrophil 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-