

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 Ptc; 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 Ptc;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 native 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, *C11orf95-RELA* (CR^{fus}) dictates a neoplastic transcriptional program and binds to unique sites across the genome enriched with *Plagl1* 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 *Plagl1* 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 *C11orf95-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 native-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-κB 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 *SSI8*, 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-