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

Nourhan Abdelfattah[1], Sivaraman Natarajan[2], David Baskin[1], and Kyuson Yun[1]; [1]Houston Methodist Research Institute, Houston, TX, USA, [2]The Jackson Laboratory, The Jackson Laboratory Headquarters in Bar Harbor, Maine Bar Harbor, ME, USA

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

Amir Arabzade[1], Yanhua Zhao[2], Srinidhi Varadharajan[2], Hsiao-Chi Chen[2], Kelsey Bertrand[2], Benjamin Deneen[2], and Stephen Mack[2]; [1]Rice University, Houston, TX, USA, [2]Baylor College of Medicine, Houston, TX, USA

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

Austin Stuckert[1,2], Amir Arabzade[3,1], Yanhua Zhao[1], Hsiao-Chi Chen[1], and Stephen Mack[1,2]; [1]Baylor College of Medicine, Houston, TX, USA, [2]Texas Children's Hospital, Houston, TX, USA, [3]Rice University, Houston, TX, USA

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

Tuyu Zheng[1], David R. Ghasemi[1], Konstantin Okonechnikov[1], Andrey Korshunov[2,3], Kendra K. Maaß[1,4], Martin Sill[1], Johannes Gojo[1,5], Ulrich Schüller[6,7], Till Milde[8,4], Stephen C. Mack[9,10], Richard J Gilbertson, [11,12], Andreas von Deimling[2,3], David T.W. Jones[13], Marcel Kool[1,14], Stefan M. Pfister[1,4], Felix Sahm[15,3], Daisuke Kawauchi[1,16], and Kristian W. Pajtler[1,4]; [1]Hopp Children's Cancer Center Heidelberg (KiTZ) and Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), German Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany, [3]Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany, [4]Department of Pediatric Oncology, Hematology, and Immunology, University Hospital Heidelberg, Heidelberg, Germany, [5]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria, [6]Research Institute, Children's Cancer Center Hamburg, Hamburg, Germany, [7]nstitute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [8]Hopp Children's Cancer Center Heidelberg (KiTZ) and CCU Pediatric Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [9]Department of Pediatrics, Division of Pediatric Hematology and Oncology, Texas Children's Cancer and Hematology Centers, Dan L. Duncan Cancer Center, Houston, TX, USA, [10]Therapeutic Innovation Center at BCM, Houston, TX, USA, [11]Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge, UK, [12]Department of Oncology, University of Cambridge, Hutchison/MRC Research Centre, Box 197, Cambridge Biomedical Campus, Cambridge, UK, [13]Hopp Children's Cancer Center Heidelberg (KiTZ) and Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, [14]Princess Máxima Centre for Paediatric Oncology, Utrecht, Netherlands, [15]Hopp Children's Cancer Center Heidelberg (KiTZ) and Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [16]Department of Bicochemistry and Celllar Biology, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

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-