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Metastatic disease at initial presentation of intracranial ependymoma is an uncommon occurrence with only rare reports of survival and is reportedly more prevalent in the youngest of children. Clinical and molecular characteristics associated with metastatic presentation, their prognostic implications, as well as optimal treatment options for such patients, have not been identified. CASE REPORT: A seven months old child presented with posterior fossa anaplastic ependymoma; following sub-total resection of primary tumor, a spine MRI revealed leptomeningeal dissemination along the cervical spinal cord and nerve roots of the cauda equina. The patient was successfully treated with five cycles of intensive induction chemotherapy (as per Head Start with high-dose methotrexate) followed by three sequential cycles of marrow-ablative chemotherapy and autologous hematopoietic progenitor cell rescue (AuHPCR) without irradiation; he is currently without evidence of the disease now 60 months following initial diagnosis. MOLECULAR/GENOMIC RESULTS: The patient was enrolled on a patient-centric comprehensive molecular profiling protocol, which included paired tumor-normal whole-exome sequencing, RNA sequencing of the disease-involved tissue, and DNA methylation classification. The genomic profile of the tumor was relatively unremarkable, revealing only a terminal gain of chromosome 3p and a terminal deletion of chromosome 22q, suggestive of an unbalanced translocation. Using RNA sequencing, we identified a novel *SPECC1L-RAF1* gene fusion. The tumor harbors unique transcriptomic and DNA methylation profiles, failing to discretely classify with well-established ependymoma subgroups. CONCLUSION: Use of genomic profiling techniques provides meaningful information for disease characterization allowing for further expansion of the molecular spectrum associated with malignant disease.

EPEN-18. CROSS-SPECIES GENOMICS IDENTIFIES *GLI2* AS AN ONCOGENE OF *C11ORF95* FUSION-POSITIVE SUPRATENTORIAL EPENDYMOMA

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The majority of supratentorial ependymomas (ST-EPN) are driven by fusions between *RELA* and a zinc finger containing gene, *C11orf95*. Apart from fusions to the Hippo effector *YAP1,* which affects a small group of infant patients, the oncogenic mechanism of remaining ST-EPNs is unclear. Aiming at refining the molecular classification of ST-EPNs, we analyzed methylation profiles, RNA and DNA sequencing results as well as clinical data in a cohort of 617 ST-EPNs. Unsupervised clustering analysis of DNA methylation data revealed four distinct clusters that formed in addition to the known molecular groups ST-EPN-RELA and –YAP1. Tumors within these additional clusters were characterized by fusions of *C11orf95* to numerous fusion partners different from *RELA,* e.g. *MAML2*, *MAML3*, *NCOA2* and *SS18*, suggesting a general role of *C11orf95* in tumorigenesis of ST-EPN. Transforming capacity of newly identified fusion genes was validated using an electroporation-based *in vivo* gene transfer technology. All fusion genes were sufficient to drive malignant transformation in the cerebral cortex of mice 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 *C11orf95,* were essential to exert tumorigenesis. When exploring genes commonly upregulated in *C11orf95* fusion-expressing tumors of human and murine origin, the Sonic Hedgehog effector gene *Gli2* was identified as a promising downstream target. Subsequent co-expression of *C11orf95:RELA* and a dominant negative form of *Gli2* indeed hampered tumorigenesis. We thus propose *GLI2* as a potential therapeutic downstream target of *C11orf95* fusion-dependent oncogenic signaling in ST-EPN.

EPEN-20. EZHIP/CATACOMB COOPERATES WITH PDGF-A AND P53 LOSS TO GENERATE A GENETICALLY ENGINEERED MOUSE MODEL FOR POSTERIOR FOSSA A EPENDYMOMA

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BACKGROUND: PFA ependymoma is a pediatric brain tumor with only 30% long-term survival. Recently a gene called *CXORF67/EZHIP/CATACOMB* (henceforward: *CATACOMB*) was found to be overexpressed in PFA ependymoma. CATACOMB's mechanism of action has been found to be analogous to that of the H3K27M mutation as its expression reduces H3K27me3 via inhibition of PRC2 catalytic activity. METHODS: We infected NESTIN- or GFAP-expressing neonatal hindbrain progenitors with wild-type *CATACOMB* or a loss of function (LOF) point mutant (M406K), alone, with PDGFA, and with and without p53 deletion. RESULTS: CATACOMB overexpression alone or with p53 loss was insufficient to induce tumorigenesis. CATACOMB overexpression with PDGFA and p53 loss was sufficient to induce tumorigenesis using either the LOF mutant (M406K) or the wild-type CATACOMB in both cells-of-origin. The histology appeared more ependymoma-like when CATACOMB was expressed in GFAP-expressing progenitors. Median survival for the model initiated in NESTIN progenitors was 99.5 days for the CATACOMB mutant (n=26) group and 61 days for the CATACOMB wild-type (n=28; log-rank test p=0.0033). Median survival for the model initiated in GFAP progenitors were 144 days for the CATACOMB mutant (n=19) group and 65 days for the CATACOMB wild-type (n=21; log-rank test is P<0.0013). Immunohistochemistry for H3K27me3 demonstrated that CATACOMB wild-type tumors had reduced H3K27me3 compared to CATACOMB mutant tumors. CONCLUSIONS: Disrupting CATACOMB inhibitory activity toward PRC2 significantly increases survival in mice in both models, suggesting this activity plays a critical role in accelerating tumorigenesis. Ependymoma-like histology was more commonly observed in the model initiated in the GFAP-expressing progenitors.

EPEN-21. IMPAIRED NEURONAL-GLIAL FATE SPECIFICATION IN PEDIATRIC EPENDYMOMA REVEALED BY SINGLE-CELL RNA-SEQ

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