DNA methylation as well as inferred copy number profiles and correlated these data with clinical parameters. Patients with EPN-PFA, EPN-RELA, and EPN-MYCN tumors showed the worst outcome with 10 year-overall survival rates of 56%, 64%, and a 5 year-overall survival rate of 73%, respectively. EPN-PFA, the most frequent tumor type in our series, occurred in children in >98% of cases, epigenetically split into two major subtypes, and harbored chromosome 1q gains and 6q losses as markers for worse survival (p<0.0001 and p=0.042, respectively). In supratentorial EPN-RELA, a combined loss of CDKN2A/B indicated a worse survival compared to the wildtype (p=0.0004), but the loss of only CDKN2A or CDKN2B did not. Ten out of 169 EPN-RELA were located in the posterior fossa, and Kaplan Meier estimators showed that these tumors relapsed or progressed even earlier than supratentorial cases with a combined loss of CDKN2A/B (p=0.0062). SP-MPE and PF-SE, which are generally regarded as non-aggressive tumors, only had a 10-year progression-free survival of 60% and 63%, respectively, in our series. In ongoing analyses, we are training machine learning algorithms to more accurately predict survival and response to treatments. These methods shall help clinicians make informed decisions regarding treatment options on new patients.

EPEN-05. ADENOSINE RECEPTOR EXPRESSION IN PAEDIATRIC EPENDYMOMA

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PURPOSE: Paediatric ependymoma is associated with dismal outcomes. Whilst understanding of its underlying biology has advanced, there has been little progress in treatment and clinical outcomes. Acting through four G protein-coupled receptors (encoded by ADORA1, ADORA2A, ADORA2B and ADORA3), adenosine is a signalling molecule often present at high levels in tumours. Adenosine signalling can aid tumour proliferation and invasiveness via mechanisms including suppression of tumour-infiltrating immune cells. Adenosine receptors therefore represent a potential therapeutic target in paediatric ependymoma, however neither levels nor patterns of expression have been previously reported. We hypothesised that adenosine receptors would be expressed in paediatric ependymoma and that this expression would vary between molecular subgroups. METHODS: Three publicly available gene expression datasets were analysed for adenosine receptor expression using Kruskal-Wallis, Mann-Whitney U and chi-square tests. RNAscope assays for adenosine receptors and CD68 were then performed on ten full-face ependymoma FFPE sections from posterior fossa A (PFA1 and PFA2) tumours to understand patterns of expression within ependymomas with the highest levels of expression identified by the gene expression datasets. RESULTS: Statistically significant differences were identified between adenosine-related genes across ependymoma subgroups of differing anatomical origin (supratentorial ZFTA-positive versus posterior fossa A and B (PFA/PFB)), with median adenosine-related gene levels generally higher in the PFA subgroup. Particularly, ADORA1, 2A, 2B and 3 gene expression was higher in PFA tumours than other subgroups. Analysis of the ten cases demonstrated measurable expression of all four adenosine receptors by RNAscope and patterns in the distribution and relative levels of expression of the adenosine receptors across PFA1 and PFA2 tumours were described. CONCLUSION: Using two different techniques we demonstrated that adenosine receptors are expressed in paediatric ependymomas. There are significant differences in level of expression between tumour subgroups. Adenosine receptors therefore represent a potential therapeutic target which should be explored further.

EPEN-06. COMPREHENSIVE PROFILING OF MYXOPAPILLARY EPENDYMOMAS IDENTIFIES A DISTINCT MOLECULAR SUBTYPE WITH RELAPSING DISEASE

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Myxopapillary ependymoma (MPE) is a heterogeneous disease regarding histopathology and outcome. The underlying molecular biology is poorly understood, and markers that reliably predict the patients' clinical course are unknown. We assembled a cohort of 185 tumors classified as MPE based on DNA methylation from pediatric, adolescent, and adult patients. Methylation patterns, copy number profiles, and MGMT promoter methylation were analyzed for all tumors, 106 tumors were evaluated histomorphologically, and RNA sequencing was performed for 37 cases. Based on methylation profiling, we defined two subtypes MPE-A and MPEB, and explored associations with epidemiological, clinical, pathological, and molecular characteristics of these tumors. Tumors in the methylation class MPE were histologically diagnosed as WHO grade I (59%), WHO grade II (37%), or WHO grade III tumors (4%). 75/77 analyzed tumors expressed HOXB13, which is a diagnostic feature not detected in other spinal ependymal tumors. Based on DNA methylation, our series split into two subtypes. MPE-A occurred in younger patients (median age 27 vs. 45 years, p=7.3e-05). They were enriched with WHO grade I tumors and associated with papillary morphology and MGMT promoter hypermethylation (all p<0.001). MPE-B included most tumors initially diagnosed as WHO grade II and cases with tanycytic morphology. Copy number alterations were more common in MPE-A. RNA sequencing revealed an enrichment for extracellular matrix and immune system-related signatures in MPE-A. 15/30 MPE-A could not be totally resected compared to 1/58 MPE-B (p=6.3e-08), and progression-free survival was significantly better for MPE-B (p=3.4e-06, 10-year relapse rate 33% vs. 85%). We unraveled the morphological and clinical heterogeneity of MPE by identifying two molecularly distinct subtypes. These subtypes significantly differed in progression-free survival and will likely need different protocols for surveillance and treatment.

EPEN-07. BRAIN-TUMOR COMMUNICATION REVEALS POTENTIAL NEW THERAPEUTIC TARGETS FOR PEDIATRIC EPENDYMOMA

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Pediatric brain tumors (PBTs) represent the most common solid malignancies in children and the leading cause of cancer-related deaths. Despite the fact that survival after a high-grade brain tumor diagnosis is slowly improving, overall survival remains poor compared with most other cancers, thereby highlighting the need for new therapies. This is particularly true for Ependymoma (EPN), the second most common PBT that, despite advances in the understanding of EPN biology, still shows a poor prognosis in approximately 40% of patients. Accumulative evidence reveals that the transcriptome of tumors can be modulated by the host tissue, with brain tumors or brain metastasis derived from extracranial tumors being at the forefront of these studies. Our hypothesis is that understanding the impact of brain-tumor communication may open up new opportunities for therapeutic intervention. To characterize gene expression changes in Ependymoma tumors caused by the tumor microenvironment the transcriptome of patient-derived EPN cells was analyzed comparing in vitro cultured cells, subcutaneous xenografts and orthotopic xenografts. 2734 differentially expressed genes were found after comparing in vitro grown cells versus brain xenografts. Sixty-five of those genes were also differentially expressed when comparing human expression data from aggressive EPN versus the rest of EPN tumours. Among these candidates NTRK2 and CALB2 stand out. Gene depletion phenotype using multiple shRNA showed that the in vivo tumor growth of NTRK2-depleted cells, (but not CALB2) was remarkably reduced compared with control cells. These results suggest that NTRK2 could be a potential new therapeutic target for the treatment of Ependymoma

EPEN-08. SINGLE-CELL TRANSCRIPTOME ANALYSIS DEFINES A TUMOR-SUPPORTIVE MICROENVIRONMENT AND TUMOR-STROMA CROSSTALK IN PEDIATRIC EPENDYMOMA AND MEDULLOBLASTOMA.

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Brain tumors are the leading cause of disease-related death in childhood and strong efforts are required to develop innovative and efficient therapeutic strategies for patients with high-risk disease. Key critical factor of pediatric brain