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BACKGROUND: Pediatric ependymoma is a heterogenous disease. Subgroup-specific clinical information on prospectively treated patients will help to improve treatment stratification. METHODS: Within the population based, prospective, multicenter E-HIT-trial (2001–2011) patients with localized ependymoma confirmed by neuropathological central-review, received hyperfractionated local radiotherapy (68Gy, 2x1Gy/day) followed by chemotherapy (stratum-A), or chemotherapy followed by local radiotherapy (54Gy, 1.8Gy/day) (children < 4years, stratum-B), or age-adapted radiotherapy with pre-/post-irradiation chemotherapy (residual tumor, diagnosis after 2005, stratum-C). Retrospective classification of DNA-methylation was available for n=164 E-HIT-trial participants, and n=80 patients with comparable treatment and prospective registration in the subsequent HIT-interim-registry (2012–2014). FINDINGS: For 291 E-HIT-trial patients, 5-year progression-free (PFS) and overall survival (OS) were 61±3%, and 81±2%. Five-year PFS/OS after complete resection were 71±4% and 87±3% in stratum-A (n=127), and 64±5% and 86±4% in stratum-B (n=86). Outcome was poor after incomplete resection, irrespective of treatment-stratum (n=78, 5-year PFS/OS: 43±6%, 68±5%). In the pooled trial- and registry-cohort, there were 152 patients with PF-EPN-A (5-year PFS/OS: 44±4%, 77±4%), 40 of them with 1q-gain (5-year PFS/OS: 28±7%, 66±8%), 21 with PF-EPN-B (5-year PFS/OS: 90±7%, 100%), 59 with ST-EPN-RELA (5-year PFS/OS: 63±7%, 87±5%), and 4 with ST-EPN-YAP1 (2 progression/relapse, no death). CONCLUSION: Outcome differed between molecular subgroups and insufficient survival rates were achieved for patients with PF-EPN-A with 1q-gain, despite combined radio- and chemotherapy treatment. Treatment reduction in the context of a clinical trial may be considered for PF-EPN-B.

EPEN-41. C11ORF95-RELA FUSION REGULATES ABERRANT GENE EXPRESSION THROUGH THE UNIQUE GENOMIC BINDING SITES FOR EPENDYMOMA FORMATION

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A majority of supratentorial ependymoma is associated with recurrent C11orf95-RELA fusion ($RELA^{FUS}$). The presence of RELA as one component of the $RELA^{FUS}$ leads to the suggestion that NF-kB activity is involved in the ependymoma formation, thus being a viable therapeutic target in these tumors. However, the oncogenic role of another C11orf95 component in the tumorigenesis is not still determined. In this study, to clarify the molecular mechanism underlying tumorigenesis of $RELA^{FUS}$, we performed $RELA^{FUS}$-ChIP-Seq analysis in cultured cells expressing the $RELA^{FUS}$ protein. Genomic profiling of $RELA^{FUS}$ binding sites pinpointed the transcriptional target genes directly regulated by $RELA^{FUS}$. We then identified a unique DNA binding motif of the $RELA^{FUS}$ different from the canonical NF-kB motif in de novo motif discovery analysis. Significant responsiveness of $RELA^{FUS}$ but not RELA to the motif was confirmed in the reporter assay. An N-terminal portion of C11orf95 was sufficient to localize in the nucleus and recognizes the unique motif. Interestingly, the $RELA^{FUS}$ peaks concomitant with the unique motif were identified around the transcription start site in the $RELA^{FUS}$ target genes as previously reported. These observations suggested that C11orf95 might have served as a key determinant for the DNA binding sites of $RELA^{FUS}$, thereby induced aberrant gene expression necessary for ependymoma formation. Our results will give insights into the development of new ependymoma therapy.

EPEN-42. MOLECULAR PROFILING REVEALS DISTINCT SUBGROUPS OF PEDIATRIC SPINAL EPENDYMOMA

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Paediatric spinal ependymomas are important, albeit uncommon, malignant central nervous system tumours. Unlike adults, children with these tumours are likely to experience a more aggressive disease course, with a higher rate of local failure and a higher rate of metastases. The clinical and molecular factors underlying these differences remain poorly characterized. We analyzed spinal ependymoma (SEPN) tumour samples from 27 paediatric patients (female: 11, male: 15; age range: 4–18 years) using genome-wide DNA methylation profiling, copy-number analysis, as well as transcriptome profiling. Using DNA methylation profiles, two distinct unsupervised consensus-clustering approaches, hierarchical clustering and non-negative matrix factorization reliably identified two subgroups. These subgroups were designated as Myxopapillary ependymomas (SP-MPE) and spinal ependymomas (SP-EPN) based on the online Classifier tool (MNP2.0). The genome-wide copy-number analysis showed differences in numbers and pattern of copy-number alterations between these groups. The gain of chromosome 20 (39%) followed by loss of chromosomes 6 (28%), 10 (28%), and 13 (28%) were detected in the SP-MPE group, whereas loss of chromosome 22 was frequent (60%) in the SP-EPN group. Transcriptomics analysis showed that genes associated with oxidative phosphorylation, TCA cycle components, electron transport, and Interferon-gamma production characterize the SP-MPE group whereas potassium ion import and regulation of astrocyte differentiation characterize the SP-EPN group. Western blot analysis validated the increased protein expression of oxidative phosphorylation complexes in SP-MPE. With this study, we provide a foundation for further molecular characterization of pediatric SEPN subgroups. Our results suggest that mitochondrial oxidative phosphorylation may drive the regulation of energy metabolism of SP-MPE tumours.

EPEN-43. TARGETING INTRA-TUMOUR HETEROGENEITY IN PAEDIATRIC EPENDYMOMA: AN INTEGRATED OMICS STUDY TOWARDS PATIENT-TAILORED THERAPY

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Ependymoma (EPN) is the second most common malignant paediatric brain tumour, which despite extensive genomic sequencing, no novel therapeutic options have been discovered. Multi-omics are anticipated to reveal dysregulated pathways that may be predictive of patient-specific biomarkers. Given the close association between gene expression, active biochemical signaling and metabolism, there is an unmet scientific challenge to determine whether EPN gene expression correlates with aberrant metabolic pathways, thus presenting therapeutic vulnerabilities. We first compared two distinct subgroups of EPN, PF-A and ST-RELA, identifying 115 metabolites and 1580 upregulated genes between the two subgroups, therefore validating previously reported genetic clustering of these two subtypes. We next integrated transcriptomics and metabolomics, comparing 28 intra-tumour tissue regions from eight primary PF-A EPN patients. Polar metabolites and RNA were simultaneously extracted from the same population of cells. RNAseq identified dysregulated genes and liquid chromatography-mass spectrometry (LC-MS) detected 98 significantly altered metabolites between 18 multi-regions, the majority mapping onto the arginine and proline pathways. Integration of genes and metabolites using pathway-based network analysis revealed 124 aberrant gene-metabolite interactions between intra-tumour regions, with large numbers occurring in the glucogenesis and glycine metabolic pathways in 6/8 patients. These may represent ubiquitous and therapeutically relevant metabolic pathways critical for EPN survival. Additionally, patients presented at least one unique intra-tumour genomic-metabolomic interaction, applicable for patient-tailored therapy. This is the first exploration of EPN multi-omic integration and intra-tumour heterogeneity. Selected drug targets predicated on aberrant gene-metabolite networks will be validated in multi-region patient-derived cell lines and orthotopic models using repurposed therapeutics.