in the clinical laboratory, the performance characteristics of different supervised classification models has not been directly compared. We developed 3 methods using methylation profiles to classify CNS tumors: an exact bootstrap k-nearest neighbor (kNN), a multi-layer perceptron neural net (NN), and a random forest classifier (RF). We trained these methods on the publicly available CNS tumor reference cohort (GSE90496) with 2,801 profiles and 91 classes. We evaluated the performance of these methods by leave-out-25% cross-validation. The relative performance of these methods were evaluated in terms of accuracy, precision, and recall for class or class family. The kNN, RF, and NN classifier had an estimate error rate of 10.74%, 4.01%, and 1.89%, respectively for class prediction and an error rate for family prediction of 5.97%, 0.90%, and 0.6%, respectively. At perfect recall for class assignment, the RF and kNN had a precision of 0.96 and 0.89 while the NN reached 0.98. For family assignment, the precision for the three classifiers was almost 1.0 with recall of nearly 0.8. At the recall rate of 1.0, the precision dropped to 0.94, 0.991 and 0.994 for kNN, RF, and NN, respectively. Overall, the NN showed improved performance metrics compared to the kNN and RF in CNS tumor classification for both class and class family assignment.

PATH-23. ADULT SPINAL CORD ASTROBLASTOMA WITH EWSR1-BEND2 FUSION

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The most recurrent fusion of CNS high-grade neuroepithelial tumor with MN1alteration(HGNET-MN1) is *MN1- BEN Domain Containing 2*(BEND2) fusion. Recently, there was a report of a 3-month-old boy with spinal astroblastoma, classified as CNS HGNET-MN1 by DKFZ methylation classification but positive for EWSR1-BEND2 fusion(Yamasaki, 2019). Here, we report a 36-year old man with a spinal cord astroblastoma with EWSR1 alternation. The patient presented with back pain, gait disorder and dysesthesia in lower extremities and trunk was referred to our hospital. MRI showed intramedullary tumor in Th3-5 level, displaying low-intensity on T1 weighted image, high-intensity on T2 weighted image, and homogeneous gadolinium enhancement. Partial removal was performed with the laminectomy. The tumor extended to extramedullary and its boundary was unclear. Histological examinations showed the epithelium-like tumor cells with eosinophilic cytoplasm with high cellularity palisade, intracellar fibrosis, and mitosis. Immunohistochemical staining showed positive for Olig2, GFAP, EMA, SSTR2, S-100, but negative for p53, PgRAE1/AE3. The tumor was diagnosed as astroblastoma, and was classified as HGNET-MN1 by the DKFZ methylation classifier. However, the MN1 alternation was not detected by fluorescence in situ hybridization, instead EWSR1 and BEND2 alternations which suggested EWSR1-BEND2 fusion were detected. After radiation therapy of 54Gy/30fr with bevacizumab and temozolomide, the residual tumor reduced the size and his symptoms improved. This case provides evidence that *EWSR1-BEND2* fusion is recurrent in HGNET-MN1 and, as previously reported, suggests the importance of BEND2 in this entity. These two cases suggested that it may be the BEND2 alteration that biologically defines the HGNET-MN1 subclass rather than MN1.

PATH-24. MOLECULAR CLASSIFICATION OF HIGH RISK INFANT EMBRYONAL BRAIN TUMORS ENROLLED IN THE ACNS0334 TRIAL: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP

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Young children with embryonal brain tumors including medulloblastoma (MB), supratentorial primitive neuro-ectodermal tumor, or pineoblastoma have historically been considered high-risk patients with poor outcomes despite the use of intensive radiation-sparing treatment. In the ACNS0334 phase III trial, 91 consented children <36 months old with the above diagnoses were randomized to intensive induction chemotherapy with or without methotrexate followed by consolidation with stem cell rescue. Here we present the results of a centralized integrated molecular analysis including global methylation profiling (65/91), and whole exome sequencing of tumor (46/91) and germline (35/91) DNA. Unsupervised clustering analyses of methylation profiles using multiple orthogonal methods against a reference dataset of 1200 pediatric brain tumors, revealed known and new molecular entities. For tumors diagnosed as MB on central pathology review, 7.3% (3/41) had a non-MB molecular diagnosis (2 embryonal tumor with multiple rosettes/ETMR, 1 group MYC pineoblastoma), with the remainder as MB Group SHH (11/41), Group3 (25/41), and Group4 (2/41). Among histologic non-MBs, 3/24 (12.5%) were molecular entities not intended for trial inclusion (1 each for ATRT, pleomorphic xanthoastrocytoma, and high-grade glioma). ETMR, historically considered a rare entity, was molecularly identified in a significant proportion (14/65; 21.5%) of samples. Among MB-SHH, we detected deleterious PTCH1 mutations in 6/9 tumors but none among 5 germline samples tested; a germline SUFU frameshift mutation with tumor LOH was also observed in MB-SHH. Correlation of these and other molecular features to the parallel clinical analysis will yield important markers of risk stratification and predictors of treatment response.

PATH-25. GENOME-WIDE METHYLATION ANALYSIS CAN SEGREGATE RADIATION-INDUCED GLIOBLASTOMA FROM LATE RECURRENCE OF MEDULLOBLATOMAS

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It could be difficult to diagnose recurrent medulloblastoma with conventional diagnostic tools because other lesions mimic relapse of the tumor from both a morphological and radiological standpoint, particularly when it happens late. We report two medulloblastoma cases, both of which seemed to develop late-recurrence more than 5 years from the initial surgery. Genome-wide methylation analysis revealed that one of the recurrent tumors was in fact a radiation-induced glioblastoma. The first patient was a 6-year-old female patient who developed a posterior fossa tumor. The pathological diagnosis was medulloblastoma with focal desmoplasia. She was in complete remission for 9 years after the treatment but developed an intradural lesion in her thoracic spine. The lesion was biopsied and pathologically confirmed as recurrence of the tumor. The second patient was a female patient who developed non-metastatic medulloblastoma at the age of 10. She suffered local recurrence 5 years after the diagnosis. Biopsy was performed, and the pathological diagnosis was relapse of the tumor. We performed unsupervised hierarchical clustering of the methylation data from our cases and reference data. In contrast to consistency of methylation profiling and copy number abnormalities between primary and recurrent tumors of case 1, the analysis revealed that the recurrent tumor of case 2 was distinct from medulloblastomas and clustered with "IDH-wild type glioblastomas", which suggested that the recurrent tumor was radiation-induced glioblastoma. This report highlights the clinical utility of molecular genetic/epigenetic approach to confirm diagnosis of brain tumor recurrence.

PATH-26. RNA SEQUENCING OF FORMALIN-FIXED PARAFFIN-EMBEDDED SPECIMENS IN DIAGNOSTIC ROUTINE IDENTIFIES CLINICALLY RELEVANT GENE FUSIONS

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