PATH-23. ADULT SPINAL CORD ARSTOBLASTOMA WITH EWSR1-BEND2 FUSION
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The most recurrent fusion of CNS high-grade neuroepithelial tumor with MN1 alteration [HGNET-MN1] is EWSR1-BEND2. 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. Here, we report a 36-year-old man with a spinal cord astroblastoma with EWSR1-BEND2 fusion. The patient presented with back pain, gait disorder and dysphasia 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, intracellular fibrous, and mitotic. Immunohistochemical staining showed positive for Olig2, GFAP, EMA, SSTR2, EWSR1, but negative for p53, BRAF/VEGF. The tumor was diagnosed as astroblastoma, and was classified as HGNET-MN1 by the DKFZ methylation classifier. However, the MN1 alteration was not detected by fluorescence in situ hybridization, instead EWSR1 and BEND2 alternation which suggested EWSR1-BEND2 fusion. After radical radiation therapy of 34 Gy/30fr with bevacizumab and temozolomide, the alternations which suggested EWSR1-BEND2 fusion were detected. After perfusion with MN1 alternation (HGNET-MN1) is a neoplastic entity. Our results suggest that the use of EWSR1-BEND2 fusion is a useful molecular marker for the diagnosis of astroblastoma.

PATH-24. MOLECULAR CLASSIFICATION OF HIGH RISK INFANT RESIDUAL TUMOR REDUCED THE SIZE AND HIS SYMPTOMS IMPROVED. THIS CASE PROVIDED EVIDENCE THAT THE USE OF INTENSIVE RADIATION SPARING TREATMENT IN THE ACNS0334 PHASE III TRIAL, 91 CONSENTED PATIENTS <36 MONTHS OLD WITH THE ABOVE DIAGNOSIS WERE RANDOMIZED TO INTENSIVE INDUCTION CHEMOTHERAPY WITH OR WITHOUT MESTHOTREXATE FOLLOWED BY CONSERVATIVE STUDY. 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 INFANT BRAIN TUMORS, Yamanishi, 2019. 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平行 clinical analysis will yield important markers of risk stratification and predictors of treatment response.

PATH-25. GENOME-WIDE METHYLATION ANALYSIS CAN SEGREGATE RADIATION-INDUCED GLOBLASTOMA FROM LATE RECURRENCE OF MEDULLOBLASTOMAS
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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. HNTRC gene analysis revealed recurrent tumors in both cases. In the first patient, 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 cord, suggests the 9 years after the treatment and developed a new 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 profiles and copy number abnormalities between primary and recurrent tumors of case 1, the analysis revealed that the recurrent tumor of case 2 was different 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-27. MUTATION DETECTION USING PLASMA CELL-FREE DNA IN CHILDREN WITH CENTRAL NERVOUS SYSTEM TUMORS

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BACKGROUND: The role of plasma cell-free DNA (cfDNA) as a communication between primary and metastatic tumors has been well described for solid tumors outside the central nervous system (CNS). However, the presence of a blood-brain barrier complicates the application of plasma cfDNA analysis for patients with CNS malignancies. METHODS: cfDNA was extracted from plasma of pediatric patients with CNS tumors utilizing a QIAamp® MinElute® kit and quantitated with Qubit 2.0 fluorometer. Extensive genomic testing, including targeted DNA and RNA solid tumor panels, exome and transcriptome sequencing, as well as copy number array, was performed on matched tumor samples as part of the KidsCanSeq study. An Archer® Reveal cfDNA28 NGS kit was then used for assessing the sensitivity of detecting tumor-specific mutations in the plasma of these patients. RESULTS: A median of 10.7ng cfDNA/mL plasma (Interquartile range: 6.4 – 15.3) was extracted from 78 patients at time of study enrollment. Longitudinal samples from 24 patients exhibited a median yield of 7.7ng cfDNA/mL plasma (IQR: 5.9 – 9.1). An initial cohort of 6 patients was identified with 7 somatic variants covered by the Archer® Reveal kit. Four of seven mutations identified in matched tumor specimens were detected in patient plasma at variant allele frequencies ranging from 0.2–1%. CONCLUSIONS: While challenging, detection of cfDNA in the plasma of pediatric patients with CNS tumors is possible and is being explored in a larger patient cohort along with pilot studies investigating cerebrospinal fluid as an additional source for tumor-specific cfDNA.

PATH-28. MOLECULAR DIAGNOSIS FOR CENTRAL DIAGNOSIS OF BRAIN TUMORS FROM 2016 TO 2019—A REPORT FROM THE JAPAN CHILDREN’S CANCER GROUP (JCCG)

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PATH-29. HIGH FREQUENCY OF CLINICALLY-RELEVANT TUMOR VARIANTS DETECTED BY MOLECULAR TESTING OF HIGH-RISK PEDIATRIC CNS TUMORS—PRELIMINARY FINDINGS FROM THE TEXAS KIDSCANSEQ STUDY

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BACKGROUND: DNA and RNA-based tumor sequencing tests have the potential to guide the clinical management of children with CNS tumors. However, data describing the utility of these tests is limited. METHODS: Children with high-risk or recurrent CNS tumors are included in the diverse cohort of patients enrolling in the KidsCanSeq study from six Texas sites. DNA and RNA from FFPE tumor is subjected to targeted sequencing using a 124-gene mutation panel and a 81-gene fusion panel. Tumor capture transcriptome sequencing, exome sequencing, and copy number array (as well as germline panel and exome testing) are also performed. Tumor variants are classified using AMP/ASCO/CAP consensus guidelines. RESULTS: A total of 74 children with high-risk/recurrent CNS tumors enrolled as of 1/28/20. Targeted tumor DNA and RNA panel testing was completed for 57 patients with varied diagnoses. At least one tumor variant with strong or potential clinical significance was identified in 43 of 57 (75%) tumors, with therapeutic significance in 20 of 57 (35%) tumors. The 38 therapeutically-relevant variants most frequently affected MAPK signaling (BRAF x9, EGFR x3, FGFR2, FGFR3, KRAS, NF1, NTRK2) and the AKT/mTOR pathway (PIK3CA x3, PTEN x2, mTOR x1, TSC1, PIK3R1). Most had not been detected by prior targeted diagnostic testing (27/38, 71%). CONCLUSION: Integrated DNA and RNA-based panel testing identified variants with potential to impact clinical decision-making in a majority of children with high-risk/recurrent CNS tumors. The comparative performance of panel testing vs. exome/capture/transcriptome/array will be evaluated in the KidsCanSeq study cohort.

PATH-30. EXOSOMES AS A SOURCE OF PLASMA CTDNA TO IDENTIFY POINT MUTATIONS IN PEDIATRIC GLIOMA PATIENTS

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Pediatric brain tumor entities harbor a variety of gene fusions. While other molecular parameters like somatic mutations and copy number alterations have become pivotal for brain tumor diagnostics, gene fusions are less well covered by routine methods due to their complexity. Through the application of plasma cfDNA analysis for patients with CNS malignancies, we in addition provide an overview of the detection accuracy of different methods, including breakpoint detection in DNA methylation array data and fusion gene detection in DNA panel sequencing data. Our data show that RNA sequencing has great diagnostic as well as therapeutic value by clinically detecting relevant alterations.

INTRODUCTION: Since 2016, the Japan Children’s Cancer Group (JCCG) has established a nationwide network that prospectively provides pathologic review and molecular analysis. METHODS: Patients who were diagnosed with brain tumors between ages 0 and 29 were enrolled. The central office at National Center for Child Health and Development served as a hub for the hospitals involved and institutions conducting pathologic and molecular analysis, and managed the patients clinical information and tumor samples. Histopathology of all cases were centrally reviewed. Routine non-NGS based analyses were conducted based on histological diagnosis and included pyrosequencing for glioma-associated hot spot mutations and PFA/PFB classification for epidermoidy, RT-PCR for RELA fusion and BRAF fusion, and nanostring for subgrouping medulloblastoma. In selected cases, methylation analysis, RNA sequencing and exon sequencing of 93 genes were performed in selected cases. RESULTS: In total, 985 cases were registered to this study in four years. Frozen samples were collected from approximately 80% of cases. The number increased from 132 in 2016 to 326 in 2019. They include glioma (n=268), medulloblastoma (n=161), epidermoidy (n=103), germ cell tumor (n=93), ATRT (n=29) and others. In 55% of the glioma cases, at least one abnormality was detected by the routine analysis. The detailed analysis for atypical cases identified targetable alternations. DISCUSSION: This nationwide central diagnostic system has now been well established. Current issues and future prospective of the system will be discussed.