

fusions and p53 protein showed characteristic cytoplasm positive; Patient3 presented a brand-new ALK-QKI fusion combined with ALK mutation and focal SMARCB1 deletion. All these 3 cases received corresponding targeted therapy and have a good recovery and normal neurologic function till now. **METHOD:** Immunohistochemistry, fluorescent in situ hybridization and whole-transcriptome sequencing. **RESULTS:** Case 18 months, male, right semioval center occupation. Histopathology: High-grade neuroepithelial neoplasm. IHC: GFAP(only few cells+), Olig2(-), S100(+), CD56(+), Syn(-), NSE(focal+), NeuN(-), CD34(-), INI-1(+), Ki67(10%+). Characteristic Molecular Information: EML4-ALK fusion. Follow-up: 15 months, alive. Case 2: 3 years, female, insular lobe occupation. Histopathology: Gliosarcoma. IHC: GFAP(partly+), Olig2(partly+), Vimentin(+), P53(cytoplasm+), pan-TRK(+), Ki67(25%+). Reticular fiber staining showed biphasic tissue pattern with reticulin-rich sarcomatous and reticulin-free gliomatous elements. Characteristic Molecular Information: NTRK1-TP53 and TP53-NTRK1 fusion. Follow-up: 27 months, alive. Case 3: 3 years, male, left parietal occipital lobe occupation. Histopathology: GBM and AT/RT. IHC: GFAP(partly+), Olig2(partly+), INI-1(partly-), BRG1(+), SYN(-), CD34(-), BRAF(-), S100(-), CK(-), H3K27M(-), IDH1(-), P53(40%+), ATRX(+), pan-TRK(-), ALK(+), Ki67(30%+). Characteristic Molecular Information: ALK mutation, ALK-QKI fusion, RAD51C mutation and focal SMARCB1(INI-1) deletion. Follow-up: 14 months, alive. **CONCLUSION:** Infant-type hemispheric glioma is a special kind of glioma, which is particularly suitable for precision-medicine treatment approaches. Their overall survival is good compared with other three pHGGS subtype.

PATH-02. USING DNA METHYLATION PROFILING TO DEFINE TUMOR TYPE AND APPROPRIATE TREATMENT: A CASE REPORT AND REVIEW OF THE LITERATURE OF A RARE CNS CIC ALTERED SARCOMA

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The utilization of methylation profiling is increasingly becoming part of the standard evaluation for high-grade malignancies. CNS primary tumors with CIC alteration are a recently recognized CNS tumor entity identified via DNA methylation profiling of primitive neuroectodermal tumors (PNET). PNETs have historically carried a poor overall prognosis; however, CNS tumors with CIC alteration have rarely been described in the literature and much remains to be discovered regarding the presentation, tumor characteristics, outcomes, and ideal treatment strategy to optimize survival in these patients. We describe the case of a 2-year-old Hispanic female with a CNS primary tumor with CIC alteration. This patient initially presented with a five-week history of fatigue, headaches, emesis, and right-sided muscle weakness and was found to have a right-sided supratentorial mixed solid and cystic mass. The initial diagnosis was consistent with a high-grade neuro-epithelial tumor based on histology and standard CNS tumor genetic markers. Due to poorly defined tumor characteristics, a sample was also sent for DNA methylation profiling. The profiling pattern was consistent with a CNS CIC altered sarcoma thereby significantly altering the treatment plan. Accurate diagnosis is critical in managing patients with cancer. DNA methylation profiling has contributed to diagnostic accuracy in these aggressive tumors; however, it also creates challenges with beginning treatment expeditiously due to the time required for processing and interpretation. This case illustrates the positive utility of DNA methylation to better characterize tumor types and the impact this has on both treatment as well as prognosis.

PATH-03. CLINICALLY TRACTABLE OUTCOME PREDICTION OF GROUP 3/4 MEDULLOBLASTOMA BASED ON TPD52 IMMUNOHISTOCHEMISTRY: A MULTICOHORT STUDY

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BACKGROUND: International consensus and the 2021 WHO classification recognize eight molecular subgroups among Group 3/4 medulloblastoma (representing ~60% of medulloblastoma). However, very few clinical centers worldwide possess the technical capabilities to determine DNA-methylation patterns or other molecular parameters of high-risk for Group 3/4 tumors. As a result, biomarker-driven risk stratification and therapy assignment constitutes a major challenge in medulloblastoma research. Here, we identify an immunohistochemistry (IHC) marker as a clinically tractable method for improved medulloblastoma risk-stratification. **PATIENTS and METHODS:** We bioinformatically analyzed published medulloblastoma transcriptomes and proteomes identifying as a potential biomarker TPD52, whose IHC prognostic value was validated across three Group 3/4 medulloblastoma clinical cohorts (n = 387) treated with conventional therapies. Risk stratification and prediction capability were computed utilizing uni- and multivariate survival analysis. Newly developed risk classifiers including TPD52 IHC were compared to state-of-the-art risk stratification schemes in terms of prediction error, area under the time-dependent receiver operating characteristic (ROC) curves and C-statistic. **RESULTS:** TPD52 IHC percentage positivity represents a significant independent predictor of early relapse and death for Group 3/4 medulloblastoma (HRs between 3.67-26.7 [95% CIs between 1.00-706.23], p = 0.05, 0.017 and 0.0058). Cross-validated survival models incorporating TPD52 IHC with clinical and molecular features (subgroup affiliation, MYC status) outperformed existing disease risk-stratification schemes, and reclassified ~50% of patients into more appropriate risk categories. Finally, interaction effect in multivariate analyses revealed that TPD52 immunopositivity could be a predictive indicator of poor response to chemotherapy (HR 12.66 [95% CI 3.53-45.40], p < 0.0001), suggesting important implication for therapeutic choices. **CONCLUSION:** The current study adds an important asset to improve risk-stratification for Group 3/4 medulloblastoma. Integration of TPD52 IHC in classification algorithms significantly improves outcome prediction and can be rapidly adopted for risk stratification on a global scale, independently of advanced but technically challenging molecular profiling techniques.

PATH-04. ARRAY-BASED GLOBAL DNA METHYLATION PROFILING OF MOUSE BRAIN TUMORS ALLOWS COMPARISON TO HUMAN TUMORS

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The classification of human brain tumors by global DNA methylation profiling has become an essential part of modern integrated neuropathological diagnostics. It has proven to reliably identify known and novel brain tumor (sub-)types that are biologically and clinically distinct. Therefore, this technique has critically improved diagnostic accuracy and risk stratification of brain tumor patients. Although indispensable for the understanding of tumor biology and for preclinical drug trials, the comparison of genetically engineered mouse models to human brain tumors is still difficult. The assessment of tumor morphology only provides an approximate picture, and transcriptomic data from human brain tumors are sparse and suffer from platform-related technical incomparability. Here, we show array-based DNA methylation profiling of well-established murine brain tumors, such as Wnt and Shh medulloblastoma, YAP and RELA ependymoma, ETMR, and AT/RT. Similar to human brain tumors, unbiased clustering methods revealed distinct methylation profiles and mean methylation levels for mouse brain tumor types. Analyses were possible for fresh-frozen as well as for paraffin-embedded tissue, and copy number alterations could be inferred from methylation profiles. Most importantly, first results suggest that interspecies comparisons allow the classification of brain tumors from known or novel mouse models based on the constantly growing spectrum of human brain tumor types and subtypes with hundreds of thousands of available datasets. As an example, upon DNA methylation profiling, cerebellar tumors arising in *Math1-cre::SmoM2Fl/+* mice display the highest similarity to human SHH medulloblastoma when compared to multiple human brain tumor entities including WNT and Non-WNT/Non-SHH medulloblastoma. These results suggest that global DNA methylation profiling may add another very important level of information to the characterization of genetically engineered mouse models.

PATH-05 CHALLENGE AND CLINICAL RELEVANCE OF A NON-MATCHING CLASSIFIER OUTPUT IN GENOME-WIDE DNA METHYLATION ANALYSIS FOR CNS NEOPLASMS IN PEDIATRIC AND ADOLESCENT PATIENTS

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OBJECTIVE: The molecular classification of CNS tumors has revolutionized our understanding of the biological heterogeneity and diversity of tumor subtypes. DNA methylation-based classification allows to discriminate subtypes. Although DNA methylation-based classification can diagnose tumors with high specificity, there are tumors which cannot be classified. We aimed to gain further insight into these challenging cases. **METHODS:** Overall, 21 patients with a CNS tumor that was non-classifiable (confidence score <0.3), or had a low confidence score (<0.9) using the DNA methylation-based classifier version 11b4, were included. Tumors were re-classified using version (v12.3), and clinical data were analyzed. **RESULTS:** A total of 21 pediatric and adolescent brain tumors with a calibrated score below 0.9 in the classifier output were identified. Of these, 11 patients (52.4 %) were assigned to the “no matching methylation class” with a score below 0.3. IDH-wild type glioblastoma (23.8 %), ETMR (14.3 %), and DMG (14.3 %) were the most common histological diagnoses. Surgical and clinicopathological features as well as DNA input and tumor purity did not influence the confidence score. Cases with non-classifiable tumors had a significantly longer time until a decision for adjuvant therapy ($p < 0.01$). Application of the latest classifier version v12.3 led to a re-classification of 9 (42.9 %) cases, of which 5 cases (55.6 %) had an improved calibrated score. **CONCLUSION:** Our study presents unclassifiable cases and the possible clinical impact when waiting for the accurate diagnosis in these challenging cases. Even though DNA methylation profiling significantly contributes to advanced CNS tumor diagnostics, clinicians should be aware of a prolonged interval to treatment initiation, especially for highly malignant brain tumors. Therefore, we would recommend to schedule adjuvant treatment as early as possible, if surgical and histological results are suspicious for this disease.

PATH-06. MOLECULAR SUBGROUPING OF MEDULLOBLASTOMA VIA LOW-DEPTH WHOLE GENOME BISULFITE SEQUENCING

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INTRODUCTION: International consensus recognises four molecular subgroups of medulloblastoma, each with distinct molecular features and clinical outcomes. Assigning molecular subgroup is typically achieved via the Illumina DNA methylation microarray. Given the rapidly-expanding WGS capacity in healthcare institutions, there is an unmet need to develop

platform-independent, sequence-based subgrouping assays. Whole genome bisulfite sequencing (WGBS) enables the assessment of genome-wide methylation status at single-base resolution. To date, its routine application for subgroup assignment has been limited, due to high economic cost and sample input requirements. Currently, no optimised pipeline exists that is tailored to handle samples sequenced at low-pass (i.e., <10x depth). **METHODS:** Two datasets were utilised; 36 newly sequenced low-depth (10x) and 42 publicly available high-depth (30x) WGBS medulloblastoma samples (n=34), alongside cerebellar control samples (n=8), all with matched DNA methylation microarray data. We applied imputation to low-pass WGBS data, assessed inter-platform correlation and identified molecular subgroups by directly integrating WGBS sample data with pre-existing array-trained models. We developed machine learning WGBS-based classifiers and compared performance against microarray. We optimised reference-free aneuploidy detection with low-pass WGBS and assessed concordance with microarray-derived aneuploidy calls. **RESULTS:** We optimised a pipeline for processing, QC, and analysis of low-pass WGBS data, suitable for routine molecular subgrouping and reference-free aneuploidy assessment that achieves 96% sensitivity compared to microarray approaches. A pilot study of the suitability of FFPE was promising, and we demonstrate that WGBS data can be integrated into existing array-trained models with high assignment probabilities. Also, WGBS-derived classifier performance measures exceeded microarray-derived classifiers. **CONCLUSION:** We describe a platform-independent WGBS assay for molecular subgrouping of medulloblastoma. It performs equivalently to array-based methods at increasingly comparable cost (\$400 vs \$580) and provides a proof-of-concept for routine clinical adoption using standard WGS technology. Finally, the full methylome enabled elucidation of additional biological heterogeneity that has hitherto been inaccessible.

PATH-07. DIAGNOSTIC AND THERAPEUTIC VALUE OF CEREBROSPINAL FLUID BIOPSY IN PEDIATRIC BRAIN TUMORS

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BACKGROUND: The application of cerebrospinal fluid (CSF) biopsy in brain tumors is valuable. This paper further explores the diagnostic and therapeutic value of CSF biopsy in pediatric brain tumors. **METHODS:** In this study, children with primary brain tumor aged ≤18 years old in Guangdong Sanjiu Brain Hospital were enrolled. CSF next-generation sequencing (NGS) tests were required for all included patients. The genetic mutations in these children were analyzed. **RESULTS:** A total of 12 pediatric brain tumors who underwent CSF NGS were included in this study. 9 patients were brainstem glioma and 3 were pilocytic astrocytoma. The mutated genes were detected in 67% (8/12) patients by CSF NGS. In brainstem glioma, the rate was 67% (6/9), and the most common genes were H3F3A (5/6, 83%) and TP53 (5/6, 83%). Other detected genes included EGFR, CDK4 and NF2. H3F3A mutation is of great significance for the diagnosis and treatment of brainstem glioma, and CDK4 and EGFR are valuable for the treatment. In pilocytic astrocytoma, the detection rate was 67% (2/3), and the genes included KIAA1549-BRAF fusion, FGFR1 and PTPN11 mutations. KIAA1549-BRAF fusion is of great value in the diagnosis and treatment of pilocytic astrocytoma, and FGFR1 is valuable in the treatment. **CONCLUSIONS:** Fluid biopsy of CSF for pediatric brain tumors may be an important supplement to histological diagnosis, especially when histopathology is not available. The results are significant for the diagnosis and treatment of pediatric brain tumors.

PATH-08. DNA METHYLATION PROFILING IMPROVES ROUTINE DIAGNOSTICS OF PAEDIATRIC CNS TUMOURS: A PROSPECTIVE POPULATION-BASED STUDY

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