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Medulloblastoma (MB) is classified into four molecular subgroups: wingless (WNT), sonic hedgehog (SHH), Group 3 (G3) and Group 4 (G4), each with different molecular profiles and patient outcomes. Subgroup heterogeneity and low mutational burdens have hindered the identification of actionable therapeutic targets, especially in G3 MB which has a particularly poor prognosis. Therefore, we took a (phospho)-proteomics approach to identify active pathways and potential therapeutic opportunities in twenty orthotopic patient-derived xenograft (PDX) models of MB comprising SHH, G3 and G4 subtypes. Through our enrichment analysis, we identified processes and pathways specifically upregulated in each MB subgroup. We also utilized neural network derived kinase-substrate predictions and kinase activity scores inferred by a heuristic machine learning algorithm to further characterize phosphosignaling activity. We found that MB PDX models recapitulate many features of primary MB tumors including two distinct proteomic subtypes of G3. G3a was enriched for transcription, translation and MYC target genes while G3b was enriched for axon guidance and neurotrophin signaling pathways. Notably, both G3a and G3b contained higher abundance of mitochondrial proteins, suggesting altered tumor metabolism in G3 MB. SHH PDXs displayed increased NFκB and JNK-MAPK signaling. Group 4 MBs most closely resembled differentiated neuronal cells and were enriched for PKC and AMPK signaling as well as DNA repair pathways. In conclusion, we have provided a comprehensive proteomic and phosphoproteomic characterization of commonly studied MB PDX models and revealed new insights into subgroup enriched pathways and kinase activity in MB.

#### OMIC-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. The current gold-standard for subgroup assignment is DNA methylation microarray. There is an unmet need to develop platform-independent subgrouping assays which are both non-proprietary and compatible with rapidly-expanding WGS capacity in healthcare. Whole Genome Bisulfite Sequencing (WGBS) enables the assessment of genome-wide methylation status at single-base resolution. Previously, WGBS adoption has been limited by cost and sample quality/quantity requirements. Its application for routine detection of medulloblastoma subgroups has not previously been reported. **Methodology:** Two datasets were utilised; 36 newly-sequenced low-depth (10x coverage) and 34 publicly-available high-depth (30x) WGBS medulloblastomas, all with matched DNA methylation microarray data. We compared platform concordance and identified molecular subgroups. Machine-learning WGBS-based subgroup classifiers were optimised and compared between platforms. Aneuploidy and mutation detection using WGBS was optimised and compared to microarray-derived estimates where possible. Finally, comprehensive subgroup-specific DNA methylation signatures were identified. **Results:** We optimised a pipeline for processing, quality control and analysis of low-depth WGBS data, suitable for routine molecular subgrouping and aneuploidy assessment. We demonstrated the suitability of fresh-frozen and FFPE DNA for WGBS, and, using downsampling, showed that subgroup calling is robust at coverages as low as 2x. We identified differentially methylated regions that, due to poor representation, could not be detected using methylation microarrays. Molecular subgroups of medulloblastoma assigned using WGBS were concordant with array-based definitions, and WGBS-derived classifier performance measures exceeded microarray-derived classifiers. **Conclusion:** We describe a platform-independent assay for molecular subgrouping of medulloblastoma using WGBS. It performs equivalently to current array-based methods at comparable cost (\$405 vs \$596) and provides a proof-of-concept for its routine clinical adoption using standard WGS technology. Finally, the full methylome enabled elucidation of additional biological heterogeneity that has hitherto been inaccessible.

#### OMIC-07. FEASIBILITY AND UTILITY OF EPIGENOMIC PROFILING FOR CHILDHOOD CNS TUMORS IN HONG KONG

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Genome-wide DNA methylation profiling has emerged as an important diagnostic tool that complements histopathology for CNS tumors in children and adults. Literature describing its application in Asian countries is nonetheless limited. Herein, we report the feasibility and utility of adopting such platform for children diagnosed with CNS tumors in Hong Kong. A multi-institutional cohort (n=94, 97% of Chinese ethnicity) with CNS embryonal or high grade neuroepithelial tumors (HGNET) diagnosed in Hong Kong from 1996–2020 was assembled based on tissue availability. DNA was extracted from FFPE tumor material (median 301ng, range 13–1000ng), bisulfite converted and profiled with the Infinium Methylation EPIC BeadChip kit. Raw data were analyzed on the German Cancer Research Center MNP 2.0 classifier and through unsupervised dimensionality-reduction analysis (t-SNE) referencing a published CNS tumor reference dataset (GSE90496). The radio-histologic diagnosis included medulloblastoma (n=65), ATRT (n=9), pineal parenchymal tumors (n=7), ETMR (n=5), CNS-PNET (n=4) and other embryonal tumors/HGNETs (n=4). Methylation class could be assigned based on results from MNP 2.0 (calibrated score ≥ 0.9) in 62 patients (66%, including 2 clustering with control) and t-SNE in 22 (23%), while no-match was encountered in 10 (11%). Methylation-based analysis allowed confirmation of diagnosis and assignment of molecular subgroup in 64 patients (68%), confirmation of histologic diagnosis alone in 5 (5%) and resulted in revision/reassignment of diagnosis in 13 (14%). Among medulloblastoma samples that were assigned molecular tumor classes (n=57), 8 clustered with WNT-activated medulloblastoma, 13 with SHH-activated medulloblastoma, 10 with Group 3 medulloblastoma, 21 with Group 4 medulloblastoma, and 5 with non-medulloblastoma entities (high-grade gliomas=3, ETMR=1, ATRT=1). In conclusion, epigenomic profiling allowed refinement of disease classification for pediatric CNS tumors. Availability of such methodology in Asia sets the stage for international collaborations in molecularly-driven trials.

#### OMIC-08. COMPOUND HETEROZYGOSITY OF POLE AND PMS2

LEADS TO CMMRD-LIKE PHENOTYPE- "POLYNCH" SYNDROME  
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Mono-allelic germline pathogenic variants (PV) in one of the mismatch repair (MMR) system genes cause Lynch syndrome, associated mainly with colon and endometrial cancer in adults. Germline PVs in DNA polymerase epsilon (*POLE*) are associated with a dominantly inherited syndrome which confers risk for polyposis and colon cancer. Brain tumors have been described as part of Lynch syndrome and *POLE* associated syndrome, mostly in adults. Constitutional mismatch repair deficiency (CMMRD) is caused by bi-allelic mutations in the MMR genes, associated with multiple café au lait macules (CAMs) and high incidence of pediatric cancer, including brain tumors. Both MMRD and *POLE* associated tumors have high tumor mutation burden (TMB), however, microsatellite status is usually unstable in MMR tumors, and stable in *POLE*. Germline *POLE* and CMMRD tumors have different mutational signatures, as is signature of MMR tumors with secondary somatic *POLE*. We describe a 4.5 y/o male who presented with a grossly metastatic SHH-activated, TP53-wildtype desmoplastic medulloblastoma. Physical examination was noted for CAMs. Family history was positive for a heterozygous *POLE* variant with variable clinical manifestations. Immunohistochemistry of the tumor showed loss of nuclear expression of the MMR gene *PMS2*, specifically in tumor cells. Analysis showed exceptionally high TMB (up to 276 Mut/Mb) and both the MMR and the *POLE* signatures. Germline analysis detected the familial *POLE* variant as well as a *de novo* heterozygous *PMS2* PV. The phenotype of the patient together with the tumor's features, led us to classify this case as a CMMRD-like. The patient had a partial response to intensive chemotherapy and is currently on immunotherapy without radiation. Collectively, our data suggest that heterozygous simultaneous germline mutations in MMR and polymerase genes can lead to novel "POLYNCH syndrome" that manifests with an ultra-hypermutant aggressive tumor and requires appropriate treatment and surveillance.