granule neuron precursors (CGNPs) undergo proliferation. Analysis of presymptomatic mutant mice showed proliferative defects and retained cells in the EGL, suggesting that the tumors may arise from CGNPs. However, targeting a subset of CGNPs using Math1-creER^{T2} did not lead to MB development, suggesting that an earlier EGL precursor may be required for tumorigenesis. Analysis of tumor transcriptome and MB subtype-specific genes and markers show that Dicer tumors most resemble extremely high risk p53-mutated SHH MB. Small RNA and mRNA sequencing analyses showed downregulation of microRNAs and dysregulation of its targets such as N-Myc. These studies demonstrate a role for microRNAs in MB development and show a fully penetrant genetic mouse model of highly metastatic MB.

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OMIC-01. THE LANDSCAPE OF EXTRACHROMOSOMAL CIRCULAR DNA IN MEDULLOBLASTOMA SUBGROUPS

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Extrachromosomal circular DNA (ecDNA) is an important driver of particularly aggressive human cancers. However, the prevalence of ecDNA, and its role in tumor development and progression in the different molecular subgroups of medulloblastoma (MB), remain unknown. To answer these questions, we have assembled a multi-institutional retrospective cohort of 472 MB patients with available whole genome sequencing (WGS) data, drawing from three cancer genomic data repositories and covering all MB subgroups (WNT, SHH, Group 3 and Group 4). Using recent computational methods to detect and reconstruct ecDNA, we find ecDNA in 66 patients (14%) and observe that the presence of ecDNA is associated with significantly poorer outcomes. By subgroup, ecDNA was found in 0/24 WNT (0%), 22/109 SHH (20%), 15/107 Group 3 (14%) and 20/181 Group 4 (11%) patients. Affected genomic loci harbor up to hundredfold amplification of oncogenes including MYC, MYCN, TERT, and other novel putative oncogenes. We further analyzed 24 patient-derived xenograft (PDX) and four cell line models of MB tumors. ecDNA was substantially more frequent in patient-derived models (17 of 29, 59%) than in our patient cohort. To elucidate the functional regulatory landscapes of ecDNAs in MB, we generated transcriptional (RNA-seq), accessible chromatin (ATAC-seq), and chromatin interaction (Hi-C) profiles of 6 MB tumor samples. In each case, we identify regulatory interactions that cross fusion breakpoints on the ecDNA, representing potential "enhancer rewiring" events which may contribute to transcriptional activation of co-amplified oncogenes. To test this hypothesis, we are currently conducting in-vitro CRISPRi screens targeting regulatory regions on the ecDNA of a MB cell line to determine whether these enhancers promote proliferation. In summary, our study analyzes the frequency, diversity and functional relevance of ecDNA across MB subgroups and provides strong justification for continued mechanistic studies of ecDNA in MB with the potential to uncover new therapeutic approaches.

OMIC-02. COGNITIVE DEFICITS AND ALTERED FUNCTIONAL BRAIN NETWORK ORGANIZATION IN PEDIATRIC BRAIN TUMOR PATIENTS

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Pediatric brain tumor survivors experience significant cognitive sequelae from their diagnosis and treatment. The exact mechanisms of cognitive injury are poorly understood, and validated predictors of long-term cognitive outcome are lacking. Large-scale, distributed brain systems provide a window into brain organization and function that may yield insight into these mechanisms and outcomes. We evaluated functional network architecture, cognitive performance, and brain-behavior relationships in pediatric brain tumor patients. Patients ages 8–18 years old with diagnosis of a brain tumor underwent awake resting state functional Magnetic Resonance Imaging during regularly scheduled clinical visits and were tested with the National Institutes of Health Toolbox Cognition Battery. Age- and sex-matched typically developing children were used as controls. We observed that functional network organization was significantly altered in patients compared to controls ($p < 0.001$), with the integrity of the dorsal attention network particularly affected ($p < 0.0001$). Moreover, patients demonstrated significant impairments in multiple domains of cognitive performance, including attention ($p < 0.0001$). Finally, a significant amount of variance (R squared = 0.52, F = 3.2, $p < 0.05$) of age-adjusted total composite scores from the Toolbox was explained by changes in segregation between the dorsal attention and default mode networks. Our results suggest that changes in functional network organization may provide insight into long-term changes in cognitive function in pediatric brain tumor patients.

OMIC-03. TRANSLATIONAL CONTROL IN MYC AND MYCN MEDULLOBLASTOMA

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Medulloblastoma has been extensively characterized at the genomic and transcriptional levels, but little is known about how alterations in translational control underlie tumor development. Myc and Mycn are often deregulated in medulloblastoma and play important roles in tumor initiation, maintenance and progression. Although both proteins have similar structures and are functionally redundant in hindbrain development, their amplification in cerebellar granule neural precursor cells leads to different medulloblastoma subtypes. In this project we are employing ribosome profiling on mouse medulloblastoma tumors generated from granule neural precursor cells with enforced expression of Myc or Mycn. Ribosome-protected mRNA sequencing allows us to quantitatively assess the specific transcripts regulated at the level of translation, identify translation regulatory sequences within the mammalian transcriptome, and understand genotype-to-phenotype processes. We discovered that Myc- and Mycn-driven tumors exhibit many more changes at the translational rather than at the transcriptional level. In particular, we found that Mycn-driven medulloblastoma upregulates the translation of Myc target genes, while mRNA levels of those genes show no difference between Myc- and Mycn-driven tumors. Furthermore, we find that the most significant translationally upregulated Myc target genes in the Mycn tumors are transcripts that encode ribosome biogenesis factors. We will further study the role of Myc and Mycn on translational regulation of the medulloblastoma transcriptome using our xenograft model of human iPSC-derived neuroepithelial stem cells overexpressing Myc or Mycn. Our goal is to understand the regulatory function of the translational landscape in Myc- and Mycn-driven medulloblastoma and to decipher the oncogenic signaling cascades leading to different medulloblastoma subtypes.

OMIC-04. IDENTIFICATION AND CHARACTERIZATION OF CIRCULATING RNAS (CODING AND NONCODING) AND METABOLITES IN CEREBROSPINAL FLUID IN MEDULLOBLASTOMA PATIENTS

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Medulloblastoma (MB) is the most common malignant brain tumor in children, and monitoring patients for treatment response and recurrence can be challenging with available current technologies in neuro-imaging and performing a biopsy to confirm response or recurrence carries risks, whereas cerebrospinal fluid (CSF) can be obtained with a little invasiveness. MB has altered cellular metabolism due to changes in gene expression, therefore, we hypothesized that any changes in MB cells lead to changes in cell-free transcripts and metabolites in CSF. To test this, we applied RNA-sequencing and mass spectrometry to analyze transcripts and metabolites including lipid in CSF from patients with different sub-groups of MB tumors (i.e., WNT, SHH, G3/4, G4, and unknown) and compared them to non-cancerous CSF. Tumor and sub-group specific transcriptomic and metabolic signatures were shown by unsupervised hierarchical clustering facilitating tumor type differentiation. By comparison with previously published tumor tissue RNA-seq data, we were able to identify a group of upregulated molecular signatures in both tumor tissue and CSF. We also identified a group of lipids that differentiate each MB sub-group from normal CSF, and Pathway analysis confirmed alterations in multiple metabolic pathways. Finally, we attempted to integrate RNA-seq data with lipidomics data, and results depict that the combinatorial analysis of CSF RNAs and metabolites can be useful in diagnosing and monitoring patients with MB tumors. (This research was conducted using samples made available by The Children's Brain Tumor Network.)

OMIC-05. PHOSPHOPROTEOMIC ANALYSIS IDENTIFIES SUBGROUP ENRICHED PATHWAYS AND KINASE SIGNATURES IN MEDULLOBLASTOMA

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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 radiohistologic 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.