The Open Pediatric Cancer Project

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In Brief

Highlights

Summary

Keywords

Introduction

Results

Discussion

Acknowledgments

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Eric Wafula	Formal analysis, Software
Sangeeta Shukla	Data curation, Formal analysis, Investigation, Methodology, Software, Writing – Original draft, Writing - Review and editing
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Christopher Blackden	Resources
Jennifer L. Mason	Supervision
Saksham Phul	Data curation, Methodology, Formal analysis
Miguel A. Brown	Data curation, Methodology, Formal analysis
Alex Sickler	Methodology, Formal analysis
Adam C. Resnick	Conceptualization, Funding acquisition, Resources, Supervision
Jo Lynne Rokita^	Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Writing – Original draft, Writing - Review and editing

Author	Contributions
Kelsey Keith	Software, Writing - original draft, API, Formal Analysis, Data Curation, Visualization

Declarations of Interest

Figure Titles and Legends

Table Titles and Legends

OPENPEDCAN METHODS

RESOURCE AVAILABILITY

Lead contact

Requests for access to OpenPedCan raw data and/or specimens may be directed to, and will be fulfilled by Jo Lynne Rokita (rokita@chop.edu).

Materials availability

This study did not create new, unique reagents.

Data and code availability

Within OpenPedCan (OPC), we harmonized, aggregated, and analyzed data from multiple sources. We harmonized data from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET cohort) Initiative, an NCI-funded collection of disease-specific projects that seeks to identify the genomic changes of pediatric cancers [1]. We included already harmonized neuroblastoma samples from the Gabriella Miller Kids First (GMKF cohort) Pediatric Research Program, a large-scale effort to accelerate research and gene discovery in pediatric cancers and structural birth defects [2]. Additionally, we re-harmonized all samples from the Open Pediatric Brain Tumor Atlas (OpenPBTA, PBTA cohort), an open science initiative led by Alex's Lemonade Stand Foundation Childhood Cancer Data Lab and the Center for Data-Driven Discovery (D3B) at the Children's Hospital of Philadelphia (CHOP), which genomically characterized pediatric brain tumor data from the Children's Brain Tumor Network (CBTN), and the Pacific Pediatric Neuro-oncology Consortium (PNOC) [3,4]. Building on the work of OpenPBTA, OPC added the PBTA X01 data [5], the Chordoma Foundation data [6/], and the MI-ONCOSEQ Study [7], donated to CBTN by the University of Michigan, to the PBTA cohort. Finally, OPC includes the Children's Hospital of Philadelphia (CHOP) P30 Panel data generated by CHOP's Division of Genomic Diagnostics (DGD cohort) which includes fusion panel data [3]. In addition to pediatric cancer data, OpenPedCan contains adult data from large science consortiums as references. For normal gene expression, GTEx [8] was used, and for comparison with adult cancers, The Cancer Genome Atlas (TCGA) [9] was included.

Merged summary files for OpenPedCan v12 are openly accessible in <u>CAVATICA</u> or via download-data.sh script in the https://github.com/PediatricOpenTargets/OpenPedCan-analysis repository. Cancer group summary data are visible within the NCI's pediatric Molecular Targets Platform and cohort, cancer group, and individual data are visible within PedcBioPortal

OpenPedCan analysis modules were developed within OpenPBTA [4], modified based on OpenPBTA, or newly created and can be found within the following publicly available repositories. OpenPBTA module analyses can be found at https://github.com/AlexsLemonade/OpenPBTA-analysis. OpenPedCan module analyses can be found at

https://github.com/PediatricOpenTargets/OpenPedCan-analysis. OpenPedCan api code can be found at https://github.com/PediatricOpenTargets/OpenPedCan-api.

All original code was developed within the following modules in the OpenPedCan analyses repository as listed below. Links to the modules are available here, and within each module is a detailed README that describes the purpose and intended usage of the scripts, along with pointers to the results from the data those scripts process.

chromosomal-instability cnv-frequencies collapse-rnaseq compare-gistic copy number consensus call create-subset-files data-pre-release-qc efo-mondo-mapping filter-mtp-tables focal-cn-file-preparation fusion-frequencies fusion-summary fusion filtering gene-set-enrichment-analysis gene match immune-deconv independent-samples long-format-table-utils methylation-preprocessing methylation-summary molecular-subtyping-ATRT molecular-subtyping-CRANIO molecular-subtyping-EPN molecular-subtyping-EWS molecular-subtyping-HGG molecular-subtyping-LGAT molecular-subtyping-MB molecular-subtyping-NBL molecular-subtyping-chordoma molecular-subtyping-embryonal molecular-subtyping-integrate molecular-subtyping-neurocytoma molecular-subtyping-pathology mtp-annotations mtp-tables-qc-checks mutational-signatures pedcbio-cnv-prepare pedcbio-sample-name pedot-table-column-display-order-name rna-seq-expression-summary-stats rnaseq-batch-correct run-gistic snv-frequencies tmb-calculation tp53 nf1 score tumor-gtex-plots tumor-normal-differential-expression

Software versions are documented in **Table XX**.

Data releases

We maintained a data release folder on Amazon S3, downloadable directly from S3 or our open-access CAVATICA project, with merged files for each analysis (See data and code availability section). As we produced new results that we expected to be used across multiple analyses, or identified data issues, we created new data releases in a versioned manner.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

METHOD DETAILS

Nucleic acids extraction and library preparation

Data generation

DNA WGS Alignment

Please refer to the OpenPBTA manuscript for details [4].

Quality Control of Sequencing Data

Please refer to the OpenPBTA manuscript for details [4].

SNP calling for B-allele Frequency (BAF) generation

Please refer to the OpenPBTA manuscript for details [4].

Somatic Mutation Calling

SNV and indel calling

Please refer to the OpenPBTA manuscript for details [4].

VCF annotation and MAF creation

Gather SNV and INDEL Hotspots

Consensus SNV Calling

Somatic Copy Number Variant Calling (WGS samples only)

Consensus CNV Calling

Somatic Structural Variant Calling (WGS samples only)

Please refer to the OpenPBTA manuscript for details [4].

Methylation Analysis

Methylation array preprocessing

We preprocessed raw Illumina 450K and EPIC 850K Infinium Human Methylation Bead Array intensities using the array preprocessing methods implemented in the minfi Bioconductor package [10]. We utilized either preprocessFunnorm when an array dataset had both tumor and normal samples or multiple OpenPedcan-defined cancer_groups and preprocessQuantile when an array dataset had only tumor samples from a single OpenPedcan-defined cancer_group to estimate usable methylation measurements (beta-values and m-values) and copy number (cn-values). Some Illumina Infinium array probes targeting CpG loci contain single-nucleotide polymorphisms (SNPs) near or within the probe [11], which could affect DNA methylation measurements [12]. As the minfi preprocessing workflow recommends, we dropped probes containing common SNPs in dbSNP (minor allele frequency > 1%) at the CpG interrogation or the single nucleotide extensions.

Details of methylation array preprocessing are available in the <u>OpenPedCan methylation-preprocessing module</u>.

Methylation beta-values summaries

We comprehensively summarized gene-level and isoform-level metrics for the methylation betavalues estimated by array preprocessing to provide insight into the variations in overall genomic DNA methylation levels observed across different pediatric tumors by computing CpG probe-level summary metrics in each cancer group within a cohort, including 1) beta-values quantiles, 2) gene expression (TPM) and methylation (beta-values) correlation, 3) TPM median expression, and 4) transcript representation - a proxy for percent isoform expression in a gene. In addition, each CpG probe was annotated with a gene feature to identify the genomic regions likely involved in regulating gene expression.

Details of the analysis are available in the OpenPedCan methylation-summary module.

Methylation sample classification

We ran the <u>dkfz's brain classifier version 12.5</u>, a comprehensive DNA methylation-based classification of CNS tumors across all entities and age groups [13]. Unprocessed IDAT-files from the <u>Children's Brain Tumor Network (CBTN)</u> Infinium Human Methylation EPIC (850k) BeadChip arrays were used as input and the following information was compiled into the histologies.tsv file: dkfz_v12_methylation_subclass (predicted methylation subtype), dkfz_v12_methylation_subclass_score (classification score), dkfz_v12_methylation_mgmt_status (*MGMT* methylation status), and dkfz_v12_methylation_mgmt_estimated (estimated *MGMT* methylation fraction).

Gene Expression

Abundance Estimation

Gene Expression Matrices with Unique HUGO Symbols

Gene Expression Summary Statistics

We generated RNA-Seq gene expression (TPM) summary statistics for independent tumor samples from the combined OpenPedCan gene expression matrices, including cancers from pediatric cohorts (PBTA, GMKF, and TARGET) and adult cancers from the TCGA cohort. We grouped selected samples into two groups containing samples from a cancer group in either each cohort or all cohorts, and calculated TPM means, standard deviations, gene-wise z-scores, group-wise z-scores, and ranks for each group as described in the OpenPedCan rna-seq-expression-summary-stats module in detail. The resulting gene-wise and group-wise summary statistics tables were annotated with EFO and MONDO disease codes associated with the cancer groups.

Gene fusion detection

QUANTIFICATION AND STATISTICAL ANALYSIS

Focal Copy Number Calling (focal-cn-file-preparation analysis module)

Please refer to the OpenPBTA manuscript for details on assignment of copy number status values to CNV segments, cytobands, and genes [4]. We applied criteria to resolve instances of multiple conflicting status calls for the same gene and sample, which are described in detail in the <u>focal-cn-file-preparation</u> module. Briefly, we prioritized 1) non-neutral status calls, 2) calls made from dominant segments with respect to gene overlap, and 3) amplification and deep deletion status calls over gain and loss calls, respectively, when selecting a dominant status call per gene and sample. These methods resolved >99% of duplicated gene-level status calls.

Gene Set Variation Analysis (gene-set-enrichment-analysis analysis module)

Please refer to the OpenPBTA manuscript for details [4].

Fusion prioritization (fusion_filtering analysis module)

Mutational Signatures (mutational-signatures analysis module)

Tumor Mutation Burden (snv-callers analysis module)

Clinical Data Harmonization

WHO Classification of Disease Types

Molecular Subtyping

Here, we build upon the molecular subtyping performed in OpenPBTA [4].

High-grade gliomas.

A new high-grade glioma entity called infant-type hemispheric gliomas (IHGs), characterized by distinct gene fusions enriched in receptor tyrosine kinase (RTK) genes including *ALK*, *NTRK1/2/3*, *ROS1* or *MET*, was identified in 2021 [doi? 10.1038/s41467-019-12187-5]. To identify IHG tumors, first, tumors which were classified as "IHG" by the DKFZ methylation classifier or diagnosed as "infant type hemispheric glioma" from pathology_free_text_diagnosis were selected [13]. Then, the corresponding tumor RNA-seq data were utilized to seek the evidence for RTK gene fusion. Based on the specific RTK gene fusion present in the samples, IHGs were further classified as "IHG, ALK-altered", "IHG, NTRK-altered", "IHG, ROS1-altered", or "IHG, MET-altered". If no fusion was observed, the samples were identified as "IHG, To be classified".

Atypical teratoid rhabdoid tumors.

Atypical teratoid rhabdoid tumors (ATRT) tumors were categorized into three subtypes: "ATRT, MYC", "ATRT, SHH", and "ATRT, TYR" [14]. In OpenPedCan, the molecular subtyping of ATRT was based solely on the DNA methylation data. Briefly, ATRT samples with a high confidence DKFZ methylation subclass score (>= 0.8) were selected and subtypes were assigned based on the DKFZ methylation subclass [doi10.1038/nature26000?]. Samples with low confidence DKFZ methylation subclass scores (< 0.8) were identified as "ATRT, To be classified".

Neuroblastoma tumors.

Neuroblastoma (NBL) tumors with a pathology diagnosis of neuroblastoma, ganglioneuroblastoma, or ganglioneuroma were subtyped based on their MYCN copy number status as either "NBL, MYCN amplified" or "NBL, MYCN non-amplified". If pathology_free_text_diagnosis was "NBL, MYCN non-amplified" and the genetic data suggested MYCN amplification, the samples were subtyped as "NBL, MYCN amplified". On the other hand, if pathology_free_text_diagnosis was "NBL, MYCN amplified" and the genetic data suggested MYCN non-amplification, the RNA-Seq gene expression level of MYCN was used as a prediction indicator. In those cases, samples with MYCN gene expression above or below the cutoff (TPM >= 140.83 based on visual inspection of MYCN CNV status) were subtyped as "NBL, MYCN amplified" and "NBL, MYCN non-amplified", respectively. MYCN gene expression was also used to subtype samples without DNA sequencing data. If a sample did not fit none of these situations, it was denoted as "NBL, To be classified".

Integration of brain tumor methylation classifications

TP53 Alteration Annotation (tp53_nf1_score analysis module)

Please refer to the OpenPBTA manuscript for details [4].

Prediction of participants' genetic sex

Please refer to the OpenPBTA manuscript for details [4].

Selection of independent samples (independent-samples analysis module)

For analyses that require all input biospecimens to be independent, we use the OpenPedCan-analysis <u>independent-samples</u> module to select only one biospecimen from each input participant. For each input participant of an analysis, the independent biospecimen is selected based on the analysis-specific filters and preferences for the biospecimen metadata, such as experimental strategy, cancer group, and tumor descriptor.

Supplemental Information Titles and Legends

Consortia

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