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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Komal S. Rathi	Formal analysis, Investigation, Methodology, Writing – Original draft
Yuankun Zhu	Data curation, Formal analysis, Investigation, Methodology, Supervision
Bailey K. Farrow	Data curation, Software
Daniel P. Miller	Formal analysis
Mariarita Santi	Investigation, Validation, Writing - Review and editing
Adam A. Kraya	Methodology
Xiaoyan Huang	Formal analysis
Bo Zhang	Data curation, Formal analysis
Brian M. Ennis	Data curation, Formal analysis
Ryan J. Corbett	Formal analysis
Sharon J. Diskin	Investigation, Supervision, Validation, Funding acquisition, Writing - Review and editing
Nicholas Van Kuren	Data curation, Software
Noel Coleman	Data curation
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 CAVATICA 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 OpenPedCan methylation-preprocessing module.

Methylation beta-values summaries

We comprehensively summarized gene-level and isoform-level metrics for the methylation `beta-values` 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 dkfz's brain classifier version 12.5, a comprehensive DNA methylation-based classification of CNS tumors across all entities and age groups [13]. Unprocessed IDAT-files from the Children's Brain Tumor Network (CBTN) `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 focal-cn-file-preparation 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..

Atypical teratoid rhabdoid tumors..

Neuroblastoma tumors...

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 independent-samples 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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