An Open Pediatric Brain Tumor Atlas

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Abstract

Introduction

Introduction will go here.

Materials and Methods

Biospecimen collection

The Pediatric Brain Tumor Atlas specimens are comprised of samples from Children's Brain Tumor Tissue Consortium (CBTTC) and the Pediatric Pacific Neuro-oncology Consortium (PNOC).

Children's Brain Tumor Tissue Consortium (CBTTC)

The CBTTC [1] is a collaborative, multi-institutional (16 institutions worldwide) research program dedicated to the study of childhood brain tumors. All CBTTC data can be download from the Gabriella Miller Kids First Data Resource Center (KF-DRC), [2]. The deidentified patient's blood and tumor tissue were prospectively collected by the consortium from patients enrolled within the CBTTC.

The cell lines were generated by the CBTTC from either fresh tumor tissue obtained firectly from surgery performed at Children's Hospital of Philadelphia (CHOP) or from prospectively collected tumor specimens stored in Recover Cell Culture Freezing media (cat# 12648010, Gibco). The tissue was dissociated using enzymatic method with papain as described [3]. Briefly, tissue was washed with HBSS (cat# 14175095, Gibco), minced and icubated with activated papin solution (cat# LS003124, SciQuest) for up to 45 minutes. The papain was inactivated using ovomucoid solution (cat# 542000, SciQuest), tissue was briefly treated with DNase (cat# 10104159001, Sigma) and passed through the 100µm cell strainer (cat# 542000, Greiner Bio-One). Two cell culture conditions were initiated based on the number of cells avialble. For cultures utilizing the fetal bovine serum (FBS), a minimum density of 3×10^5 cells/ml were plated in DMEM/F-12 medium (cat# D8062, Sigma) supplemented with 20% FBS (cat# SH30910.03, Hyclone), 1% GlutaMAX (cat# 35050061, Gibco), Penicillin/Streptomycin-Amphotericin B Mixture (cat# 17-745E, Lonza) and 0.2% Normocin (cat# ant-nr-2, Invivogen). For the serum-free media conditions cells were plated at minimum density of 1×10^6 cells/ml in DMEM/F12 media supplemented with 1% GlutaMAX, 1x B-27 supplement minus vitamin A (cat# 12587-010, Gibco), 1x N-2 supplement (cat# 17502001, Gibco), 20 ng/ml epidermal growth factor (cat# PHG0311L, Gibco), 20 ng/ml basic fibroblast growth factor (cat# 100-18B, PeproTech), 2.5µg/ml heparin (cat# H3149, Sigma), Penicillin/Streptomycin-Amphotericin B Mixture and 0.2% Normocin.

Pacific Pediatric Neuro Onclology Consortium (PNOC)

The Pacific Pediatric Neuro-Oncology Consortium (PNOC) is an international consortium dedicated to bringing new therapies to children and young adults with brain tumors. PNOC collected blood and tumor biospecimens from newly-diagnosed DIPG patients as part of the clinical trial PNOC003/NCT02274987 [4].

Nucleic acids extraction and library preparation

PNOC samples

The Translational Genomic Research Institute (TGEN; Phoenix, AZ) performed DNA and RNA extractions on tumor biopsies using a DNA/RNA AllPrep Kit (Qiagen, #80204). All RNA used for library prep had a minimum RIN of 7 but no QC thresholds were implemented for the DNA. For library

preparation, 500ng of nucleic acids were used as input for RNA-Seq, WXS, and targeted DNA panel (panel). The RNA prep was performed using the TruSeq RNA Sample Prep Kit (Illumina, #FC-122-1001) and the exome prep was performed using KAPA Library Preparation Kit (Kapa Biosystems, #KK8201) using Agilent's SureSelect Human All Exon V5 backbone with custom probes. The targeted DNA panel developed by Ashion (formerly known as the GEM Cancer panel) consisted of exonic probes against 541 cancer genes. Both panel and WXS assays contained 44,000 probes across evenly spaced genomic loci used for genome-wide copy number analysis. For the panel, additional probes tiled across intronic regions of 22 known tumor suppressor genes and 22 genes involved in common cancer translocations for structural analysis. All extractions and library preparations were performed according to manufacturer's instructions.

CBTTC samples

Blood, tissue, and cell line DNA/RNA extractions were performed at Biorepository Core (BioRC) at CHOP. Briefly, 10-20 mg frozen tissue, 0.4-1ml of blood or 2×10^6 cells pellet was used for extractions. Tissues were lysed using a Qiagen TissueLyser II (Qiagen) with 2×30 sec at 18Hz settings using 5 mm steel beads (cat# 69989, Qiagen). Both tissue and cell pellets processes included a $CHCl_3$ extraction and were run on the QiaCube automated platform (Qiagen) using the AllPrep DNA/RNA/miRNA Universal kit (cat# 80224, Qiagen). Blood was thawed and threated with RNase A (cat#, 19101, Qiagen); 0.4-1ml was processed using the Qiagen QIAsymphony automated platform (Qiagen) using the QIAsymphony DSP DNA Midi Kit (cat# 937255, Qiagen). DNA and RNA quantity and quality was assessed by PerkinElmer DropletQuant UV-VIS spectrophotometer (PerkinElmer) and an Agilent 4200 TapeStation (Agilent, USA) for RINe and DINe (RNA Integrity Number equivalent and DNA Integrity Number equivalent respectively). Library preparation and sequencing was performed by the NantHealth sequencing center. Briefly, DNA sequencing libraries were prepared for tumor and matched-normal DNA using the KAPA Hyper prep kit (cat# KK8541, Roche); tumor RNA-Seq libraries were prepared using KAPA Stranded RNA-Seq with RiboErase kit (cat# KK8484, Roche). Whole genome sequencing (WGS) was performed at an average depth of coverage of 60X for tumor samples and 30X for germline. The panel tumor sample was sequenced to 470X and the normal panel sample was sequenced to 308X. RNA samples were sequenced to an average of 200M reads. All samples were sequenced on the Illumina HiSeq platform (X/400) (Illumina) with 2 × 150bp read length.

Data generation

NantHealth Sequencing Center (Culver City, CA) performed whole genome sequencing (WGS) on all paired tumor (~60X) and constitutive (~30X) DNA samples. WGS libraries were 2x150 bp and sequenced on an Illumina X/400. NantHealth Sequencing Center performed ribosomal-depleted whole transcriptome stranded RNA-Seq to an average depth of 100M reads for CBTTC tumor samples. The Translational Genomic Research Institute (TGEN; Phoenix, AZ) performed paired tumor (~200X) and constitutive whole exome sequencing (WXS) or targeted DNA panel (panel) and poly-A selected RNA-Seq (~200M reads) for PNOC tumor samples. PNOC WXS and RNA-Seq libraries 2x100 bp and sequenced on an Illumina HiSeq 2500.

DNA WGS Alignment

We used BWA-MEM [5] v0.7.17 for alignment of paired-end DNA-seq reads. The alignment reference that we used was Homo Sapiens Human Genome (hg) version 38, patch release 12, fasta file obtained from UCSC [6]. Alignments were futher processed using following the Broad Institute's Best Practices [7] for processing BAMs in preparation for variant discovery. Duplicates were marked using Samblaster[8] v0.1.24, BAMs merged and sorted using Sambamba [9] v0.6.3. Lastly, resultant BAMs were processing using Broad's Genome Analysis Tool Kit (GATK) [10] v4.0.3.0, BaseRecalibrator submodule.

Quality Control of Sequencing Data

NGSCheckmate [doi:10.1093/nar/gkx193] was peformed on matched tumor/normal CRAMs to confirm sample matches and remove mis-matched samples from the dataset. CRAM inputs were preprocessed using bcftools to filter and call 20k common SNPs using default parameters[11] and the resulting VCFs were used to run NGSCheckmate using this workflow in the D3b GitHub repository. Per author guidelines, <= 0.61 was used as a correlation coefficient cutoff at sequencing depths >10 to predict mismatched samples. For RNA-Seq, read strandedness was determined by running the `infer_experiment.py` script on the first 200k mapped reads. If calculated strandedness did not match strandedness information received from the sequencing center, samples were removed from analysis. We required at least 60% of RNA-Seq reads mapped to the human reference or samples were removed from analysis.

Somatic Single Nucleotide Variant Calling

SNV and INDEL calling

We used four variant callers to call SNVs and INDELS from targeted DNA panel, WXS, and WGS data: Strelka2, Mutect2, Lancet, and VarDict. The same input interval BED files were used for both panel and WXS data and intervals were padded by 100 bp on each side for all variant calling algorithm runs. The BED files for WGS were not padded for Mutect2 and Strelka2 runs, were padded by 300 bp on each side for Lancet, and by 100 bp on each side for VarDict. Strelka2 [12] v2.9.3 was run using default parameters on human genome reference hg38, canonical chromosomes only (chr1-22, X,Y,M), as recommended by the authors. The final Strelka2 VCF was filtered for PASS variants. Mutect2 from GATK v4.1.1.0 was run following Broad best practices outlined from their Workflow Description Language (WDL) [13]. The final Mutect2 VCF was filtered for PASS variants. Lancet [14] v1.0.7 [15] was run using default parameters, unless noted below. For input intervals to Lancet, a reference BED was created by using only the UTR, exome, and start/stop codon features of the GENCODE 31 reference. Per recommendations by the New York Genome Center, the Lancet input intervals were augmented with PASS variant calls from Strelka2 and Mutect2 as validation. VarDictJava [16] v1.58 [17] was run using the hg38 fasta reference with the same BED intervals used for Mutect2.
Parameters and filtering followed BCBIO standards except that variants with a variant allele frequency (VAF) >= 0.05 (instead of >= 0.10) were retained. The 0.05 VAF increased the true positive rate for INDELs and decreased the false positive rate for SNVs when using VarDict in consensus calling. The final VCF was filtered for PASS variants with TYPE=StronglySomatic.

VCF annotation and MAF creation

We filtered outputs from both callers on the “PASS” filter, and annotated using The ENSEMBL Variant Effect Predictor [18], reference release 93, and created MAFs using MSKCC’s vcf2maf [19] v1.6.17.

Somatic Copy Number Variant Calling

We used Control-FREEC [20,21] v8.7 and CNVkit [22] v0.9.3 for copy number variant calls. CNVkit was run using default parameters on human genome reference hg38 and using the batch command for tumor-normal pairs rather than a panel of normals.

Somatic Structural Variant Calling

We used Manta SV [23] v1.4.0 for structural variant (SV) calls. Manta SV calling was also limited to regions used in Strelka2. We also ran LUMPY SV [24] v0.2.13 in express mode using default parameters. The hg38 reference used was also limited to canonical chromosome regions. The somatic

DNA workflow for SNV, INDEL, copy number, and SV calling can be found in the KidsFirst Github repository.

Gene Expression Abundance Estimation

We used STAR [25] v2.6.1d to align paired-end RNA-seq reads. This output was used for all subsequent RNA analysis. The reference we used was that of ENSEMBL's GENCODE 27 [26], "Comprehensive gene annotation." We used RSEM [27] v1.3.1 for transcript- and gene-level quantification. We also added a second method of quantification using kallisto [28] v0.43.1. This method differs in that it uses pseudoaligments using fastq reads directly to the aforementioned GENCODE 27 reference.

RNA Fusion Calling and Prioritization

Gene fusion detection

We set up Arriba v1.1.0 and STAR-Fusion 1.5.0 [29] fusion detection tools using CWL on CAVATICA. For both these tools we used aligned BAM and chimeric SAM files from STAR as inputs and GRCh38_gencode_v27 GTF for gene annotation. We ran STAR-Fusion with default parameters and annotated all fusion calls with GRCh38_v27_CTAT_lib_Feb092018.plug-n-play.tar.gz provided in the STAR-fusion release. For Arriba, we used a blacklist file (blacklist_hg38_GRCh38_2018-11-04.tsv.gz) from the Arriba release tarballs to remove recurrent fusion artifacts and transcripts present in healthy tissue. We also provided Arriba with strandedness information or set it to auto-detection for polyA samples. The RNA expression and fusion workflows can be found in the KidsFirst GitHub repository.

Fusion prioritization

We built a fusion prioritization pipeline to filter and annotate fusions. We considered all inframe and frameshift fusion calls with 1 or more junction reads and fused genes expressed with TPM greater than one to be true calls. If a fusion call had large number of spanning fragment reads compared to junction reads (spanning fragment minus junction read greater than ten) or if either 5 or 3 genes fused to more than five different genes we removed these calls as a potential false positive. We also removed fusions if the 5 or 3 ends were the same gene, and these were tagged as non-canonical splicing or duplication. We used a list of curated fusion calls for each histology to capture each occurrence of the fusion as a putative driver fusion. We prioritized a union of fusion calls as true calls if the fused genes were detected by both callers, the same fusion was recurrent in histology (>2 samples) or the fusion was specific to the broad histology. We annotated putative driver fusions and prioritized fusions lists with kinases, oncogenic, tumor suppressor, transcription factor, fused genes and known TCGA fusions from curated datasheets. We also added chimerDB [30] annotations to both driver and prioritized fusion list.

Clinical Data Harmonization

WHO Classification of Disease Types

The `disease_type_old` field in the `pbta-histologies.tsv` file contains the diagnosis denoted from the patient's pathology report. The `disease_type_new` field in the `pbta-histologies.tsv` file includes updates to `disease_type_old` and these changes are documented in the `Notes`. For instance, any diagnosis denoted as "Other" in `disease_type_old` was modified to capture the pathology report diagnosis in `disease_type_new`. Additionally, `disease_type_old` was modified to `disease_type_new` if the presence of specific molecular alterations defined a biospecimen as having an alternate diagnosis. The `broad_histology` denotes the broad 2016 WHO classification

[doi:10.1007/s00401-016-1545-1] for each tumor. The `short_histology` is an abbreviated version of the `broad_histology`.

Molecular Subtyping

The `molecular_subtype` column in the `pbta-histologies.tsv` file contains molecular subtype information derived as described below. Medulloblastoma subtypes SHH, MYC, Group 3, and Group 4 were predicted using an RNA expression classifier on the RSEM FPKM data.

Survival

Overall survival was calculated as days since initial diagnosis.

Prediction of participants' genetic sex

The clinical metadata provided included a reported gender. We used DNA data, in concert with the reported gender, to predict participant genetic sex so that we could identify sexually dimorphic outcomes. This analysis could also reveal samples that may have been contaminated in certain circumstances. We used the idxstats utility from SAMTOOLS [31] to calculate read lengths, the number of mapped reads, and the corresponding chromosomal location for reads to the X and Y chromosomes. We used the fraction of total normalized X and Y chromosome reads that were attributed to the Y chromosome as a summary statistic. We reviewed this statistic in the context of reported gender and determined that a threshold of less than 0.2 clearly delineated female samples. Fractions greater than 0.4 were predicted to be males. Samples with values in the range [0.2, 0.4] were marked as unknown. We ran this analysis through CWL on Cavatica. Resulting calls were added to the clinical metadata as `germline_sex_estimate`.

Results

Results section stub.

Conclusions

Stub in conclusions section

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