PATIENT-DERIVED ORGANOID AND CELL CULTURE MODELS FROM THE NCI PATIENT-DERIVED MODELS REPOSITORY (NCI PDMR) PRESERVE GENOMIC STABILITY AND HETEROGENEITY OF PATIENT TUMOR SPECIMENS

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ABSTRACT

Background: The National Cancer Institute (NCI) has developed a Patient-Derived Models Repository (PDMR; https://pdmr.cancer.gov) of preclinical models including patient-derived xenografts (PDX), cell cultures (PDC) and organoids (PDOrg). Extensive clinical annotation and genomic datasets are available for these preclinical models. However, it is unclear if the molecular profiles of the corresponding patient tumors are stably propagated in these models. We have previously demonstrated that PDX models from the NCI PDMR faithfully represent the patient tumors both in terms of genomic stability and tumor heterogeneity. Here, we conduct an in-depth investigation of genomic representation of patient tumors in the PDOrgs and PDCs.

Methods: PDOrgs (n=79) and PDCs (n=96) were established from tumor fragments (i.e., initiator specimens) obtained either from patient specimens or from PDX specimens of early in vivo passaged tumor. For some models (n=23), both PDOrgs and PDCs were generated from the same tumor tissue; in fewer cases (n=4), PDCs were established from organoids derived from patient specimens. Whole Exome Sequencing and RNA-Seq were performed on all PDCs and PDOrgs, and data were compared with patient specimens (Originator specimens).

Results: A majority of the PDOrgs and PDCs have stably inherited the genome of the corresponding patient specimens based on the following observations: (1) >72% of PDOrgs and PDCs maintained similar copy number alteration profiles compared with the patient specimens (originator) of the preclinical model; (2) the variant allele frequency (VAF) of clinically relevant mutations remained consistent between the PDOrgs, PDCs, and the originator specimens, with none of the PDCs or PDOrgs deviating by >=17% VAF; and (3) clinically relevant biomarkers (e.g., MSI, LOH, mutational signatures etc.) are concordant amongst the PDOrgs, PDCs, and the originator specimens. We observed that the majority of SNVs and indels present in the originator specimens were also found in the PDOrgs and PDCs, suggesting almost all the tumor heterogeneity was preserved in these preclinical models.

Conclusions: This large and histologically diverse set of PDOrgs and PDCs from the NCI PDMR exhibited genomic stability and faithfully represented the tumor heterogeneity observed in corresponding patient specimens. These preclinical models thus represent a valuable resource for researchers interested in pre-clinical drug or other studies.

RESULTS

NCI PDMR DATASETS FOR ORGANOID AND CELL CULTURE MODELS

	PDC	PDOrg
Total number of models	**96**	**79**
Total number of models derived from Patient Originator Specimen	30	0
Total number of models derived from patient PDX/Organoid Specimen	66	79
Total number of models with germline	46	38
Total number of models with Patient Originator Specimen	31	22
Total number of models with germline and Patient Originator Specimen	18	11

Tumor histology	OncoTree	PDC	PDOrg
Bladder	BLCA	6	3
Colon Adenocarcinoma	COAD	16	36
Non-small Cell Lung Carcinoma	NSCLC	4	5
Head and Neck Squamous Cell Carcinoma	HNSC	14	2
Melanoma	MEL	9	2
Pancreatic Adenocarcinoma	PAAD	11	6
Sarcoma	SARCNOS	3	
Other histologies		33	25

Clinical and Histo-Pathological datasets:

- Clinical annotation of donors
- Limited clinical history including previous treatment regimens
- Donor demographics including self-declared ethnicity, smoking history
- STR profile
- Histo-pathological annotations and images of individual specimens

Genomic Datasets:

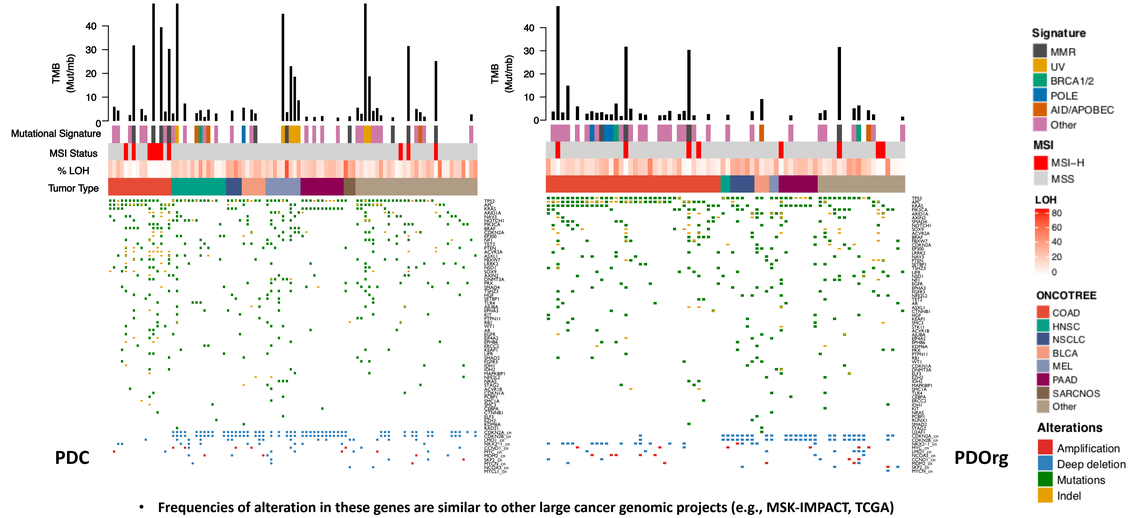
Whole Exome:

- Raw FASTQ files
- VCF files (from our GATK pipeline)
- OncoKB annotated variant calls
- Inferred ancestry determination from WES data using SNPweights

Whole transcriptome:

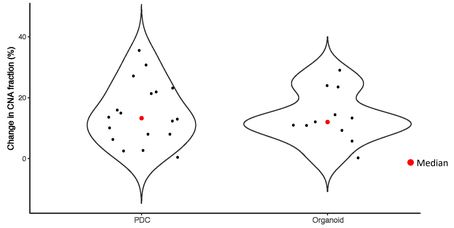
- Raw FASTQ files
- Gene expression data

INTEGRATED VIEW OF GENOMIC BIOMARKERS OF CLINICAL AND BIOLOGICAL RELEVANCE PRESENT IN NCI PDMR ORGANOID AND CELL CULTURE MODELS



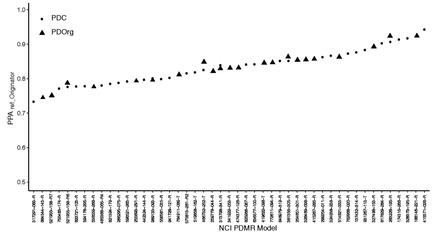
- Frequencies of alteration in these genes are similar to other large cancer genomic projects (e.g., MSK-IMPACT, TCGA)

STABILITY OF COPY NUMBER ALTERATIONS IN ORGANOID AND CELL CULTURE MODELS



- Only specimens with both originator and germline available were considered for this analysis
- Stability in Copy Number Alterations (CNA) were determined by measuring the fraction of genome changed (represented by 280,000 bins). Significantly changed CNA segment in a specimen compared to originator when $\log_2(CN_R/CN_O)$ =<-0.4 or >=0.4 (CN_R/CN_O = ratio of copy number of the segment of PDX specimen and Originator). Corrections for tumor cellularity were applied in originator specimens
- Copy number alteration (CNA) profiles of PDOrgs and PDCs models are stable when compared to the originator patient specimen
- In majority of the PDCs and PDOrgs, fraction of genome changed is less than 20% compared to their respective Originator specimens

MAJORITY OF VARIANTS OBSERVED IN PATIENT ORIGINATOR SPECIMENS WERE PRESERVED CELL CULTURE AND ORGANOID MODELS



- We tested the whether variants (SNV and Indels) observed in Originator specimens were also represented faithfully in PDCs and PDOrg models.
- This was measured from observed Positive Percent Agreement (PPA) for SNV and Indel variants between individual specimens and originator specimen (as reference) ($PPA_{ref\_originator}$) (In this case, the $PPA_{ref\_originator}$ for a specimen represent the percentage of detected variants also detected in originator specimen)
- We observed the minimum $PPA_{ref\_originator}$ >73% and median $PPA_{ref\_originator}$ > 82% suggesting majority of SNV and indels observed in the patient originator specimens are also present in the PDOrg and PDC specimens

CLINICALLY RELEVANT BIOMARKERS ARE CONCORDANT AMONGST THE PDOrgs, PDCs, AND THE ORIGINATOR SPECIMENS

- **Variant allele frequency (VAF) of driver mutations remained consistent between the PDCs, PDOrgs and the originator specimens**

Model	Tumor Histology	Mut_ID	VAF (PDC)	VAF (PDOrg)	VAF obs (Originator)	Estimated Tumor fraction (from Sequenza)	Estimated Stromal Corrected VAF (Originator)
466732-252-T	Adenocarcinoma of small intestine	BRAF:D594N	0.66	0.57	0.33	0.46	0.72
328469-098-R	Adenocarcinoma - colon	KRAS:G12R		0.47	0.17	0.43	0.40
519858-162-T	Adenocarcinoma - colon	KRAS:G12V	0.59		0.44	0.68	0.65
931267-113-T	Colorectal cancer, NOS	KRAS:G12D	0.36		0.17	0.43	0.40
328469-098-R	Adenocarcinoma - colon	PIK3CA:H1047R		0.50	0.18	0.43	0.42
931267-113-T	Colorectal cancer, NOS	PIK3CA:E545K	0.37		0.17	0.43	0.40
328373-195-R	H & N squamous cell car.	TP53:R342*	1.00		0.56	0.41	1.37*
466732-252-T	Adenocarcinoma of small intestine	TP53:R280T	0.98	1.00	0.38	0.46	0.83
762968-020-R	Adenocarcinoma - colon	TP53:R248Q	0.95		0.88	0.88	1.00

Only PDC and PDOrg specimens with both originator and germline available were considered for this analysis
* Sequenza algorithm reported lower confidence in estimation of tumor content in this originator compared to other specimens

- **Microsatellite instability status is 100% concordant between PDC and PDOrg and their respective originator specimens**
  - MSI status for 54 PDC and PDOrg specimens from 46 models (40 patients) were evaluated (MSI calls available for both originator and PDC or PDOrg specimens)
  - In 5 PDC and PDOrg specimens, MSI status were high and were concordant with their respective Originator specimen
  - For 49 PDC and PDOrg specimens, MSI status were stable and were also concordant with their respective Originator specimen

SUMMARY

- This large diverse set of preclinical models of patient derived cell line cultures and organoids provide researchers an excellent resource for drug discovery efforts.
- These preclinical models are associated with clinical history, inferred ancestry, genomic datasets (mutations, copy number alterations, MSI status)
- Both patient derived cell line cultures and organoid preclinical models exhibited genomic stability (both for driver mutations and copy number alterations)
- Variants observed in patient tumor specimens were preserved in these preclinical models
- Consistency of MSI status and driver mutation allele frequencies were observed in preclinical models compared to the patient specimens

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