GENOMIC LANDSCAPE OF ACQUIRED UNIPARENTAL DISOMY IN NCI PDMR PATIENT-DERIVED XENOGRAFT MODELS

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Introduction

Uniparental disomy (UPD), also known as copy-neutral loss of heterozygosity (LoH), refers to the duplication of one homologous chromosome or chromosomal arm accompanied by loss of the other homolog. Acquired UPD (aUPD) is common in cancer and may result from mitotic nondisjunction or anaphase lag during mitosis. Functionally, aUPD confers a selective advantage during tumor evolution through loss of function of one or more tumor suppressor genes and/or a gain in oncogene expression[1,2]. Many cancer histologies are known to possess recurrent chromosomal arm-level alterations which may be prognostic of outcome. Although arm and chromosome-level aneuploidy has been previously investigated in the TCGA and other data sets[3], the characterization of aUPD specifically and its stability in pre-clinical models has not been broadly explored.

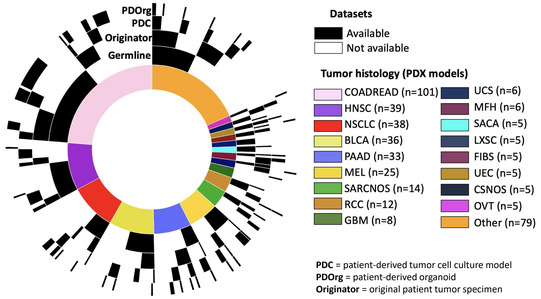
This study aims to characterize arm-level aUPD in the National Cancer Institute's Patient-Derived Models Repository (PDMR; https://pdmr.cancer.gov). The PDMR includes patient-derived xenograft (PDX), organoid and cell culture models established from multiple tumor histologies representing different passages and lineages. The associated clinical annotation and genomic data make it possible to assess the prevalence of aUPD in the PDMR cohort and stability of aUPD between passages and lineages within individual PDX models.

Methods

High tumor purity in the PDX specimens (after removal of mouse reads representing the stroma) enabled highly accurate assessment of LoH. As a first step in the identification of arm-level aUPD events, variants called by GATK HaplotypeCaller from whole exome sequencing (WES) data were used to identify segments of homozygosity using BCFtools/RoH (run of homozygosity)[4]. After excluding acrocentric arms (i.e. 13p, 14p, 15p, 21p and 22p), runs of homozygosity could be called in 39 chromosomal arms. Copy number segment data were obtained using CNVkit where the segment mean value indicated the log2 ratio of copy number compared to a pool of normal samples. Copy neutral RoH segments were obtained by intersecting the RoH segments with the copy neutral segments (defined as absolute segment mean less than 0.3); and then copy neutral %LoH at the arm level was calculated as the percentage of total length of copy neutral RoH segments in each chromosome arm. If the mean copy neutral %LoH of multiple PDX samples from the same model on a chromosome arm was >80% we considered the model to have aUPD at the arm level. Microsatellite Instability (MSI) status was estimated using mSINGS and evaluated for associations with aUPD. Fisher's exact test was used to test for associations between aUPD and MSI.

Results

NCI Patient-Derived Models Repository Cohort (n = 427 PDX models)



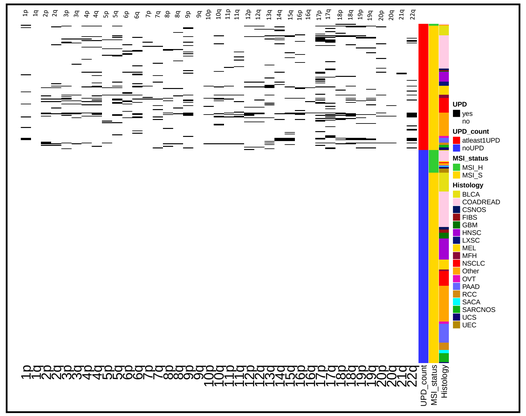
Datasets
- Available
- Not available

Tumor histology (PDX models)
- COADREAD (n=101)
- HNSC (n=39)
- NSCLC (n=38)
- BLCA (n=36)
- PAAD (n=33)
- MEL (n=25)
- SARCNOS (n=14)
- RCC (n=12)
- GBM (n=8)
- UCS (n=6)
- MFH (n=6)
- SACA (n=5)
- LXSC (n=5)
- FIBS (n=5)
- UEC (n=5)
- CSNOS (n=5)
- OVT (n=5)
- Other (n=79)

PDC = patient-derived tumor cell culture model
PDOrg = patient-derived organoid
Originator = original patient tumor specimen

OncoTree code	Cancer Type	OncoTree code	Cancer Type
COADREAD	Colorectal Adenocarcinoma	UCS	Uterine Carcinosarcoma
HNSC	Head and Neck Squamous Cell Carcinoma	MFH	Undifferentiated Pleomorphic Sarcoma/Malignant Fibrous Histiocytoma/High-Grade Spindle Cell Sarcoma
NSCLC	Non-Small Cell Lung Cancer	SACA	Salivary Carcinoma
BLCA	Bladder Urothelial Carcinoma	LXSC	Larynx Squamous Cell Carcinoma
PAAD	Pancreatic Adenocarcinoma	FIBS	Fibrosarcoma
MEL	Melanoma	UEC	Uterine Endometrioid Carcinoma
SARCNOS	Sarcoma, NOS	CSNOS	Breast Invasive Carcinosarcoma, NOS
RCC	Renal Cell Carcinoma	OVT	Ovarian Epithelial Tumor
GBM	Glioblastoma Multiforme		

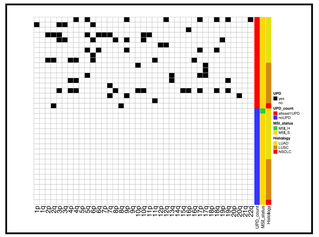
Overview of aUPD in the PDMR



- 37% of all PDMR models had at least 1 chromosomal arm with aUPD
- aUPD was observed most frequently at arms 17p (~7%) and 17q (~7%)

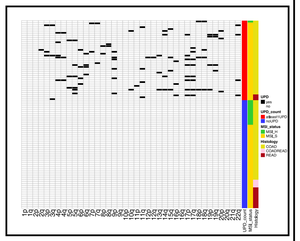
Originator and Passage Zero (P0) specimens determined to have human stromal contamination were removed from this analysis, leaving 417 evaluable PDX models. A chromosomal arm was required to have >80% LoH to be designated as having aUPD.

aUPD in NSCLC Models



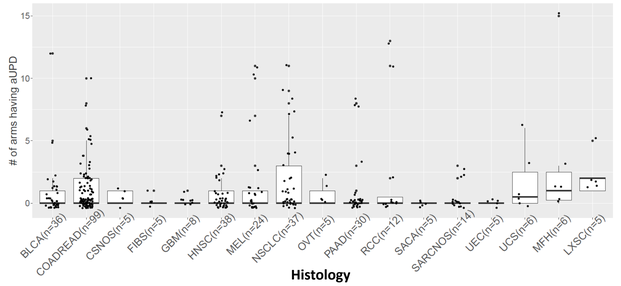
- 37 evaluable NSCLC PDX models included 19 LUAD, 16 LUSC and 2 NSCLC NOS
- 49% of NSCLC models had at least 1 arm with aUPD
- 3p LoH (copy neutral and copy loss) was observed in 90% of LUSC[3] but 3p aUPD (i.e. copy-neutral LoH) was seen in <10% of LUSC cases in the PDMR

aUPD in COADREAD Models



- 90 evaluable COADREAD PDX models included 81 COAD, 14 READ and 4 COADREAD
- 42% of COADREAD models had at least 1 arm with aUPD
- In COADREAD cases where aUPD was seen in either 17p or 17q, co-occurrence of aUPD in both arms was observed at a frequency of 60% (6/10).

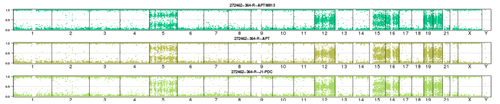
Prevalence of aUPD by Cancer Subtype



- Only histologies represented by ≥ 5 models were included in this analysis

Some PDMR Models Exhibited Extensive aUPD

60 year-old male with RCC and aUPD observed in 11 of 39 evaluable chromosomal arms*

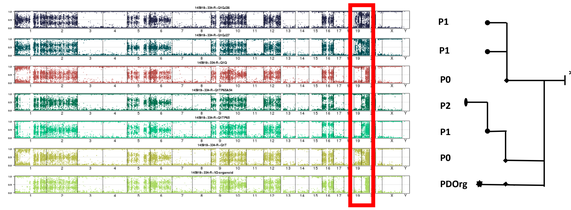


P0 = PDX Passage 0, P1 = PDX Passage 1, PDC = Patient-derived tumor cell culture model
***copy-neutral LoH (aUPD) was observed in 11/39 arms; copy-loss LoH was observed in the remaining arms**

- 11 of 417 evaluable PDMR models had extensive aUPD (>25% of chromosomal arms)

Uniformity of aUPD status between lineages, passages and model types within a model

A COADREAD model with aUPD differences between PDM lineages and passages for chromosomal arm 19p



- 500 chromosomal arms from 155 aUPD models (835 samples) were assessed for mean and standard deviation (SD) of copy neutral %LoH across different samples from the same model
  - 460 aUPD chromosomal arms (92%) from 143 PDMR models had uniform aUPD status across all lineages, passages and model types within a model (SD of %LoH between all samples was ≤20)
  - 40 aUPD chromosomal arms (8%) from 21 PDMR models had differences in aUPD status between lineages, passages and model types within a model (SD of %LoH was >20)
- Differences in aUPD status between different samples within a model may be due to tumor heterogeneity or to clonal selection with passaging

* The originator specimen was not available for analysis in this model

MSI-H Status Was Strongly Associated with the Absence of aUPD

# of models	Without aUPD	With aUPD	Total
MSI-H	28	2	30
MSI-S	234	153	387
Total	262	155	417

P = 0.0001, Fisher's exact test

Summary

- We have performed an in-depth analysis of acquired UPD, a subtype of arm-level aneuploidy, in NCI's PDMR cohort
- 37% of all PDMR models had at least 1 chromosomal arm with aUPD
- The level of aUPD varied between histologies and within a given histology; 17p (~7%) and 17q (~7%) had the highest prevalence of aUPD
- aUPD status was consistent across different passages and lineages within a model, with some differences observed likely due to tumor heterogeneity or clonal evolution
- MSI-H status was strongly associated with the absence of aUPD

Future Directions

- Preclinical drug studies using NCI PDMR models may suggest appropriate therapeutic options for cancers with aUPD
- Investigation of additional subtypes of aneuploidy in the PDMR such a copy-loss LoH and arm-level copy gain are ongoing

References

1. Tuna et al., Uniparental Disomy in Cancer, Trends Mol Med (2009), 15, 120-128
2. Makishima et al., Pathogenesis and Consequences of Uniparental Disomy in Cancer (2011), 10.1158/1078-0432.CCR-10-2900
3. Taylor et al., Genomic and Functional Approaches to Understanding Cancer Aneuploidy, Cancer Cell (2018), 33, 676-689
4. Narasimhan et al., BCFtools/RoH: A Hidden Markov Model Approach for Detecting Autozygosity from Next-Generation Sequencing Data, Bioinformatics (2016), 32, 1749-1751

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