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Study of tumor heterogeneity and subclonality in primary pancreatic and metastatic sites from rapid autopsy patients in PDMR

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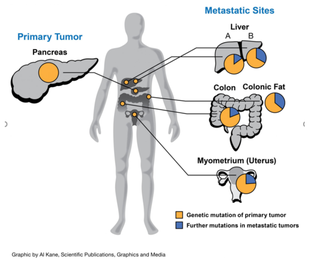
INTRODUCTION

A major set of preclinical models derived from specimens acquired from rapid autopsy patients in the National Cancer Institute Patient-Derived Models Repository[1] (NCI PDMR, https://pdmr.cancer.gov) were from pancreatic adenocarcinoma (PAAD) patients, with metastatic specimens originating from liver, colon, omentum, and lung. Genomic characterization of these preclinical models provides a unique opportunity to study tumor heterogeneity and subclonality associated with the metastatic process and potential treatment resistance.



Pancreatic PDX models from rapid autopsy patients

- To date, 30 rapid autopsy patient-derived xenograft (PDX)/patient-derived cell (PDC)/patient-derived organoid (PDOrg) models derived from pancreatic adenocarcinoma patients (n = 9) have been sequenced using whole-exome sequencing (WES) and RNASeq[2,3]
- Treatment history
- H&E slides
- NGS data: WES and RNAseq

Patient ID	Model ID	Resection Site	Germline?	PDX	PDOrg	PDC	Pt Tumor
521955	521955~158-R2	Liver (A)	Yes	both (6)	both	both	none
521955	521955~158-R3	Liver (B)	Yes	both (6)	none	both	WES
521955	521955~158-R4	Colonic Fat	Yes	both (6)	none	none	both
521955	**521955~158-R5**	**Pancreas**	Yes	**both (5)**	**none**	**none**	**both**
521955	521955~158-R6	Myometrium	Yes	both (6)	both	both	both
521955	521955~158-R7	Colon	Yes	both (2)	both	none	both
521955	521955~158-R8	Diaphragm	Yes	none	none	none	both
217524	217524~143-R1	Pancreas	no	none	none	WES	none
217524	217524~143-R2	Liver	no	none	none	WES	none
217524	**217524~143-R4**	**Pancreas [dd]**	**no**	**both (6)**	**none**	**none**	**none**
217524	217524~143-R5	Liver [A]	no	both (5)	none	WES	none
482815	482815~354-R2	Pancreas head [bb]	no	both (6)	none	none	none
485176	485176~168-R1	Pancreas [cc]	no	none	none	none	WES
485176	485176~168-R4	Lung [mm]	no	both (6)	both	none	none
485368	**485368~065-R1**	**Pancreas (ff)**	**Yes**	**none**	**none**	**none**	**both**
485368	485368~065-R2	Pancreas (gg)	Yes	none	none	none	WES
485368	485368~065-R3	Liver (D)	Yes	both (7)	both	none	WES
485368	485368~065-R4	Liver (E)	Yes	both (6)	none	both	both
485368	485368~065-R8	Liver [E] (cryopreserved)	Yes	both (6)	none	none	none
616732	**616732~234-R1**	**Pancreas**	**no**	**both (4)**	**both**	**none**	**both**
616732	616732~234-R2	Liver	no	both (5)	none	none	both
616732	616732~234-R3	Pleural mass	no	both (5)	none	none	both
777334	**777334~354-R1**	**pancreas head**	**Yes**	**none**	**both**	**both**	**none**
777334	777334~354-R2	pancreas body	Yes	none	none	both	none
777334	777334~354-R3	adrenal [left]	Yes	none	none	none	WES
777334	777334~354-R5	colon	Yes	both (6)	none	both	none
839793	839793~233-R2	**Pancreas-2**	no	none	none	none	WES
839793	839793~233-R4	Liver-2	no	both (7)	none	none	none
454973	454973~116-R2	Pericardium	no	none	both	both	none
454973	454973~116-R3	Omentum	no	none	both	none	none

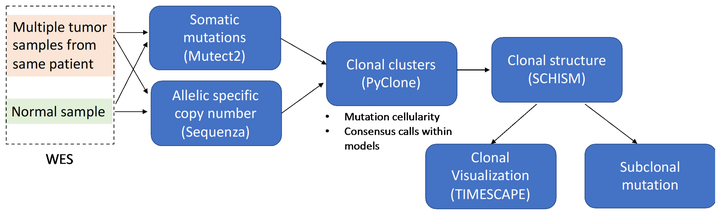
REFERENCES

1. NCI PDMR website: https://pdmr.cancer.gov
2. MoCha NGS pipeline: https://github.com/FNL-MoCha/nextgenseq_pipeline
3. Genomic profiling data, SOPs, data analysis pipeline SOPs available at NCI PDMR website
4. Roth et al., Nature methods, 2014
5. Niknafs et al., PLOS computation biology, 2015

METHODS

Tumor heterogeneity between primary and metastatic sites was studied based on somatic mutation, copy number alteration (CNA) and gene expression data. A bioinformatics workflow was developed to stably infer and visualize the tumor subclonality by integrating the tools of PyClone, SCHISM, and TIMESCAPE, using somatic mutations and site-specific copy number data of multiple samples generated from PDX models in primary and metastatic sites.

Workflow of interfering and visualizing tumor subclonality



❑ PyClone[4]
- A Bayesian clustering method
- Estimating cellular prevalence and accounting for allelic imbalance and normal cell contamination

❑ SCHISM[5]
- Inferring subclonal hierarchy and tumor evolution
- Genetic Algorithm: lineage precedence rule and lineage divergence rule

RESULTS

Among 30 rapid autopsy preclinical models from primary and metastatic sites, liver is the most common metastatic site in PAAD (9/19=47%) compared to other sites. Driver mutations are conserved in all preclinical model specimens derived from a given patient. KRAS p.G12D is present in 28 PDX/PDC/PDOrg models as well as the corresponding patient specimens, and BRAF p.V600E is present in other preclinical models. The fraction of the genome affected by CNA remains stable within a PDX model across passages (n=18, mean=7.63%, sd=5.90%). However, we found that this increased when comparing PDX models derived from metastatic sites versus the primary site (n=16, mean=19.47%, sd=9.69%). This indicates the presence of tumor heterogeneity between metastatic and primary sites. Site-specific subclones were identified in PDX models from two patients (521955 and 485368) and a phylogenetic tree of primary and metastasis sites indicates that one liver metastasis had a unique seeding event compared to the other metastatic sites for both patients.

CONCLUSION

Tumor heterogeneity and subclonality was observed in preclinical models generated from PAAD patients in the NCI PDMR. These models provide a unique resource for preclinical studies in tumor evolution, metastatic spread mediators, and drug resistance.

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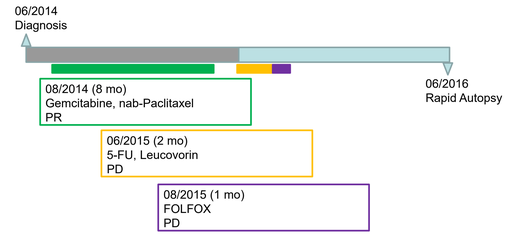
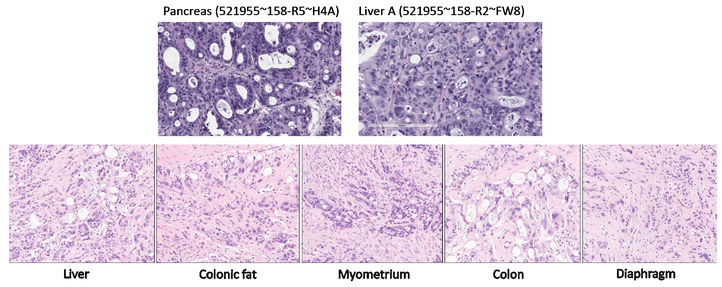
Case study - 521955

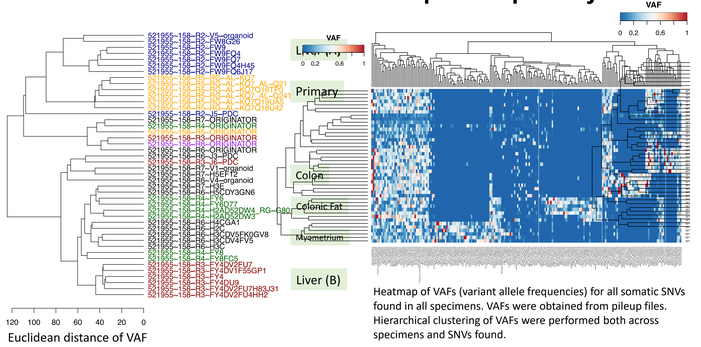
521955, Pancreatic Adenocarcinoma



A. Morphology is consistent between primary and metastatic tumors by H&E

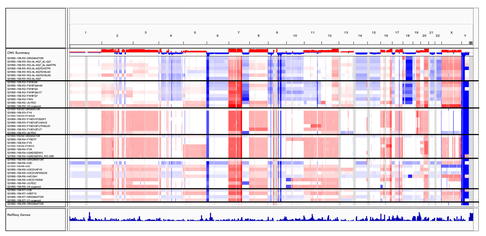


B. Hierarchical clustering on mutation profiles shows different metastatic sites has different relationships with primary



Heatmap of VAFs (variant allele frequencies) for all somatic SNVs found in all specimens. VAFs were obtained from pileup files. Hierarchical clustering of VAFs were performed both across specimens and SNVs found.

C. Copy number profiles indicate tumor heterogeneity between metastatic and primary sites



Site	%CNA changes within model	%CNA changes compare to primary
Primary	4.71%	NA
Liver A	12.30%	15.00%
Liver B	13.31%	32.80%
Colonic Fat	6.35%	35.81%
Myometrium	19.49%	23.83%
Colon	5.82%	19.40%
Diaphragm	NA	21.88%

D. Site-specific subclones were identified in PDX models