PDX models generated from a patient with metastatic colon adenocarcinoma display both spatial and temporal tumor heterogeneity

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Abstract:

Background: Patient-derived Xenograft (PDX) models are being widely used in preclinical studies to identify biomarkers of drug response and to enhance our understanding of cancer biology. Since patients with metastatic cancer have both intra-tumor and inter-site heterogeneity, PDX models generated from different tumor sites may provide a way to study tumor heterogeneity. Characterization of the genomic landscape in these models may also provide better insights into treatment response or resistance. It is rare to have multiple PDX models generated from a single patient over multiple time points during a treatment trajectory. Here, we report the genomic profiles of PDX models generated from 4 distinct tissue specimens over a 7-month period from a patient with metastatic colon adenocarcinoma. The first 2 PDX models were generated from circulating tumor cells (CTCs) and a liver biopsy prior to treatment with a combination pan-AKT + MEK inhibitor regimen. A third PDX model was generated from a liver biopsy while on-treatment and a fourth from an adrenal gland resection at progression. Clinically, all reported metastatic sites, except the adrenal gland, responded to the combination therapy.

Results: Genomic characterization of the specimens obtained from these 4 PDX models led to the following observations: 1) PIK3CA E545K and KRAS G12D are present in all the specimens tested for all 4 models and are likely truncal driver mutations; 2) exclusive inter-model SNVs (single nucleotide variants) were identified, and may be model-specific variants representing inter-site heterogeneity in the patient; 3) variants involved in known resistance mechanisms to MEK inhibition were not present in any specimens, although LOH was observed in chr15 where MAP2K1 gene (MEK) is located; 4) overexpression of AKT3 has been reported as a resistance mechanism to a pan-AKT inhibitor and higher levels of AKT3 expression was observed in the adrenal tissue from the patient but not in any other PDX models derived from this patient; 5) intra-model and inter-model heterogeneity in whole genome CNV (copy number variant) profiles was observed between individual PDXs obtained from the pre-treatment CTC-derived model and the on-treatment liver biopsy model. Interestingly, one of the PDXs from the CTC-derived model presented a sub-clonal tumor fraction closely related to the on-treatment liver biopsy model. The multiple inter-model CNV profiles in the liver biopsy derived PDX models represent temporal heterogeneity within a tissue.

Conclusions: We observed genomic heterogeneity in PDXs generated from specimens from a patient with metastatic colon adenocarcinoma. Both truncal and sub-clonal variants were identified representing various tumor fractions in these models. This case study illustrates how genomic profiling of multiple tumor sites at different times during course of treatment can provide insight into the complexity of tumor heterogeneity and tumor evolution in patients with metastatic disease.

Introduction:

- PDX models can capture the tissue complexity present in corresponding patient tumors, including the tumor heterogeneity
- Tumor heterogeneity in a patient can arise in:
  - lesions from different sites (spatial, intertumoral)
  - distinct cell populations within the same lesion (spatial, intratumoral)
  - lesions from different time points during therapy (temporal)
- PDX models can be used as surrogates to investigate molecular profiles and conduct pre-clinical studies on multiple lesions sampled at different times from the same patient
- We present genomic characterization of tumor heterogeneity observed in 4 PDX models derived from a single colon adenocarcinoma patient

Clinical history of Patient 172845:

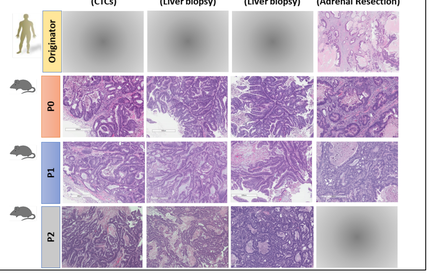
43 year-old female with colon adenocarcinoma and known metastatic sites in the liver, lung, adrenal gland, and bone

Date of diagnosis: March, 2010

Therapy during tissue collection: MK-2206 (AKTi) and Selumetinib (MEKi) were initiated after collection of blood and biopsy in May 2013. Adrenal mass was unresponsive to study agents while all other sites of disease (lung and liver) initially responded and then progressed.

Date Regimen Started	Standardized Regimen	Best Response	Duration (Months)
May, 2010	Bevacizumab, FOLFOX	Partial Response	3
Oct, 2010	Oxaliplatin	No data available	5
Oct, 2010	5-Fluorouracil, Bevacizumab, Leucovorin	No data available	15
March, 2012	FOLFIRI	No data available	4
August, 2012	Onalespib	Non-evaluable	1
November, 2012	Indimitecan	Disease Progression	1

Patient 172845 tumors passaged in NSG mice show histopathology similar to the originator specimen

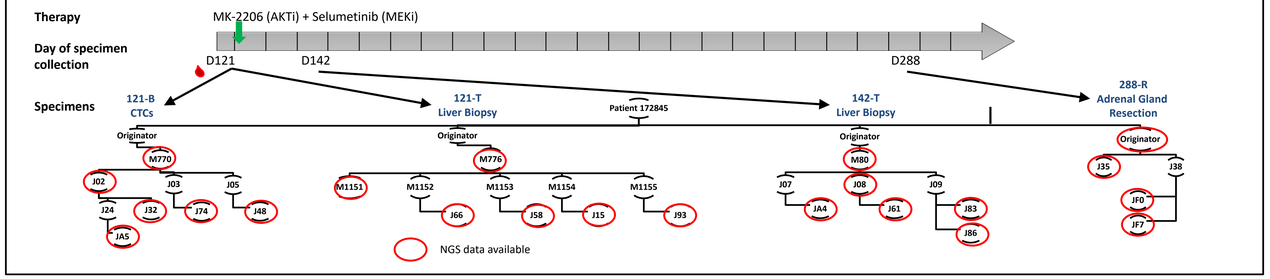


H&E staining of originator tissue from patient and tissues obtained from PDX models from different passages

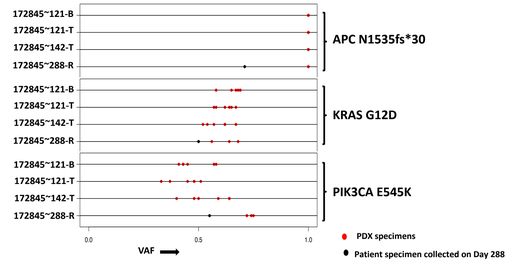
No residual Patient Material (Originator) **available** from 121-B, 121-T, or 142-T collection time points

288-R: Resection material from adrenal gland metastasis - Moderate to well differentiated colon adenocarcinoma

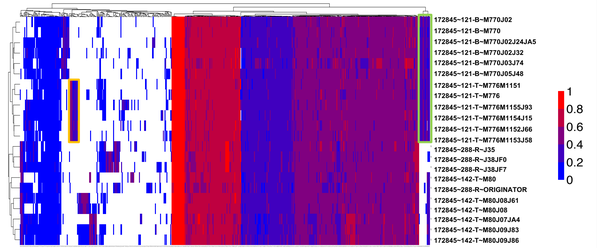
Timeline and lineage of PDX models derived from patient 172845



Common driver mutations present in all models at similar variant allele frequencies



Exclusive model-specific SNVs are identified using variant heatmap

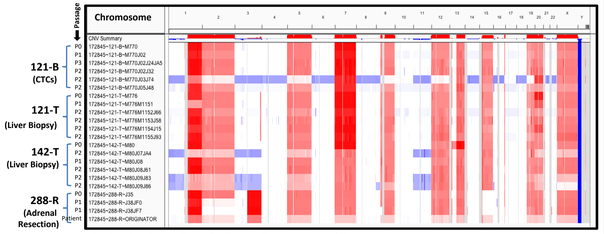


Heatmap of VAFs (variant allele frequencies) for all putative somatic SNVs found in all specimens. VAFs were obtained from pileup files. Hierarchical clustering of VAFs were performed both across specimens and SNVs found

Exclusive SNVs in 172845~121-T model

Exclusive SNVs found in only models derived from tissue obtained on day 121

CNV profiles show distinct patterns of genomic heterogeneity - unique CNV alterations are found in 2 out of 4 PDX models generated from the same patient

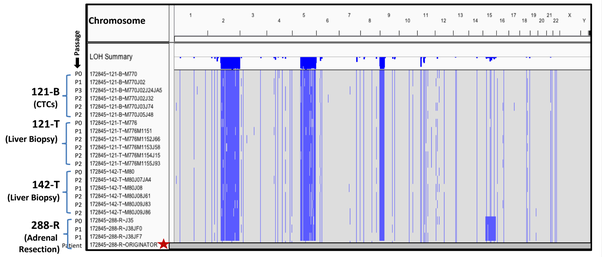


Molecular IDs confirm all mice are from same model

Copy gain region

Copy loss region

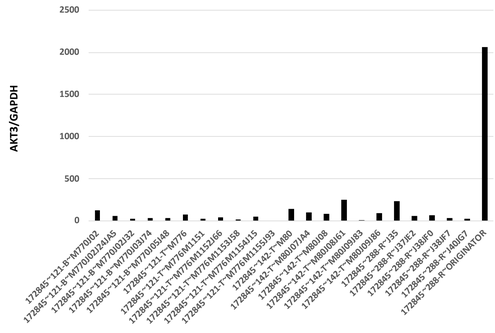
Loss of heterozygosity (LOH) profiles show a distinct pattern of possible copy neutral LOH in chr15 that is unique to PDXs generated from the treatment-resistant adrenal gland resection



LOH region (>15Mb)

★ 172845~288-R-Originator specimen had ~30% stromal component and can't be used for high fidelity LOH calls.

AKT3 over-expression is observed in the patient specimen from the adrenal gland resected material but is not present in other specimens from the same model



GAPDH normalized AKT3 TPM values are plotted as histograms for all specimens

Overexpression of AKT3 has been reported as a resistance mechanism to a pan-AKT inhibitor (Stottrup et al, 2016, Mol Can Ther).

Summary:

- **We observed genomic heterogeneity in PDXs generated from specimens from a patient with metastatic colon adenocarcinoma**
- **We have observed both spatial and temporal heterogeneity in genomic profiles**
- **Both truncal and sub-clonal variants were identified representing various tumor fractions in these models**
- **This case study illustrates how genomic profiling of multiple tumor sites at different times during course of treatment can provide insight into the complexity of tumor heterogeneity and tumor evolution in patients with metastatic disease**

Resources and References:

1. NCI PDMR website: https://pdmr.cancer.gov
2. Genomic profiling data, SOPs, data analysis pipeline SOPs available at NCI PDMR website
3. Stottrup C, Tsang T, Chin YR (2016), Upregulation of AKT3 Confers Resistance to the AKT Inhibitor MK2206 in Breast Cancer, 15(8):1964-74. doi: 10.1158/1535-7163.MCT-15-0748

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