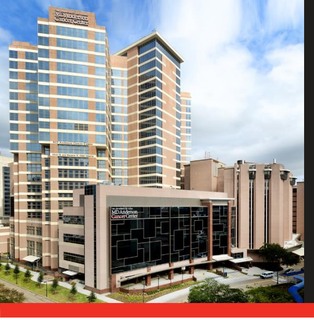

Appendiceal Adenocarcinoma PDX Models Have Improved Tumor Growth in an Orthotopic Tumor Environment

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Background

Appendiceal Adenocarcinoma (AA) is a rare cancer that most commonly metastasizes to the peritoneal cavity. Very few preclinical models of AA exist. Patient-derived xenograft (PDX) models have the advantage of maintaining molecular and histologic features of human tumors as well as inherent intratumoral heterogeneity.

Most PDX models involve engraftment of tumors in the subcutis of immunodeficient mice. However, heterotopic tumor implantation may alter tumor growth and behavior. We hypothesized that orthotopic engraftment in the peritoneal cavity would more faithfully recapitulate the tumor microenvironment in metastatic AA. Improved preclinical modeling is critical for studying tumor biology of human cancers and accurately predicting patient responses to novel therapies.

Methods

- Three AA PDX tumor models were developed to compare the peritoneal cavity and subcutis.

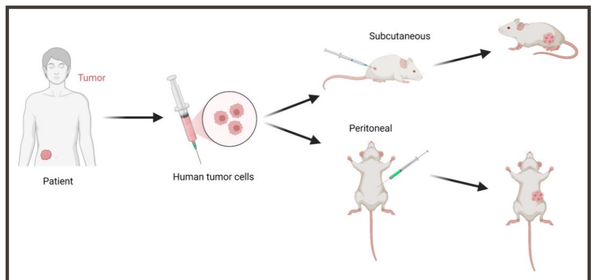


Fig. 1 Experimental Design. A diagram explaining the process of inserting a PDX subcutaneously and into the peritoneal cavity of NSG mice.

- Tumor size was measured over time to calculate and normalize tumor growth.
- Tumors were removed and RNA sequencing was performed.
- H&E and Immunohistochemical (IHC) staining was performed. Ki-67 staining was used to evaluate cell proliferation. Serial sections were stained with human marker Ku80, GI epithelial marker CDX2, and mesenchymal marker vimentin.
- Slides were scanned with an Aperio AT2 whole slide digital scanner. Images were deconvoluted and merged using HALO v3.6.

Antibody	Vendor	Cat. No.	Host and Clonality	Species Reactivity	Dilution
CDX2	Abcam	76541	Rabbit IgG mAb	M, R, Hu	1:8000
KU80	Cell Signaling	2180	Rabbit IgG mAb	Hu, NHP	1:100
Vimentin	Cell Signaling	5741	Rabbit IgG mAb	H, M, R, Mk	1:200

Fig. 2 Information on the antibodies used.

Results

1.) Increased Appendiceal Adenocarcinoma Tumor Growth Rate In Peritoneal Microenvironment

A

Model	Histopathology	Mucinous	Grade	Source
TM00351	Mucinous Adenocarcinoma	Yes	G3	Jackson Laboratory
PMCA-3	Pseudomyxoma Peritonei	Yes	G3	Flatmark Lab
AAP16	Invasive, non-mucinous adenocarcinoma	No	G2.5	MDACC

B

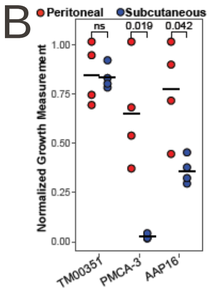
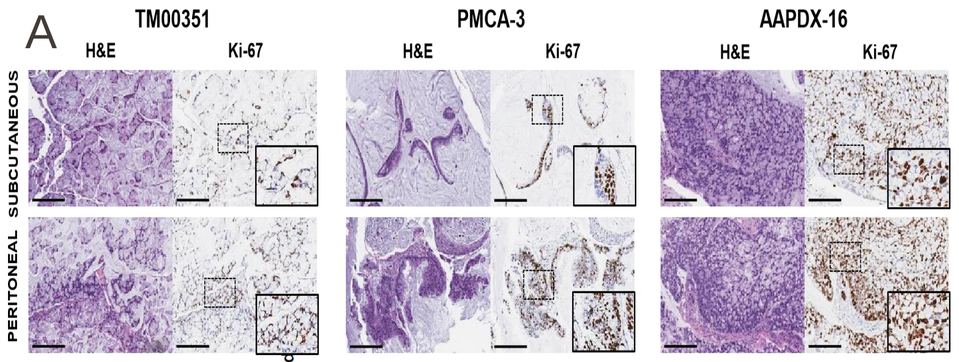


Fig. 3 Appendiceal Adenocarcinoma PDX Models. **A.** Information on the tumors used to generate the PDX. **B**. Normalized growth measures for peritoneal and subcutaneous PDX. 2/3 of the tumor models evaluated showed a faster growth rate in the peritoneal cavity.

2.) Increased Appendiceal Adenocarcinoma Tumor Cell Proliferation In Peritoneal Microenvironment



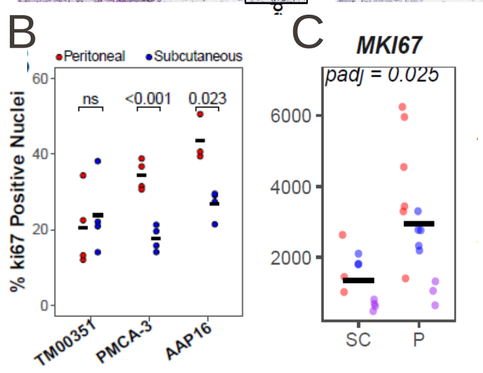


Fig. 4 Appendiceal Adenocarcinoma PDX Model Tumor Proliferation. A. H&E and immunohistochemical staining of peritoneal and subcutaneous implanted tumors showing increased cellularity and increased Ki67 staining. **B**. Percent of Ki-67 positive nuclei Is increased in peritoneal engraftment. **C.** Ki-67 was recorded with higher amounts in the peritoneal PDX compared to the subcutaneous PDX via RNA seq.

3.) Human Appendiceal Adenocarcinoma Cells Persist in PDX Murine Models

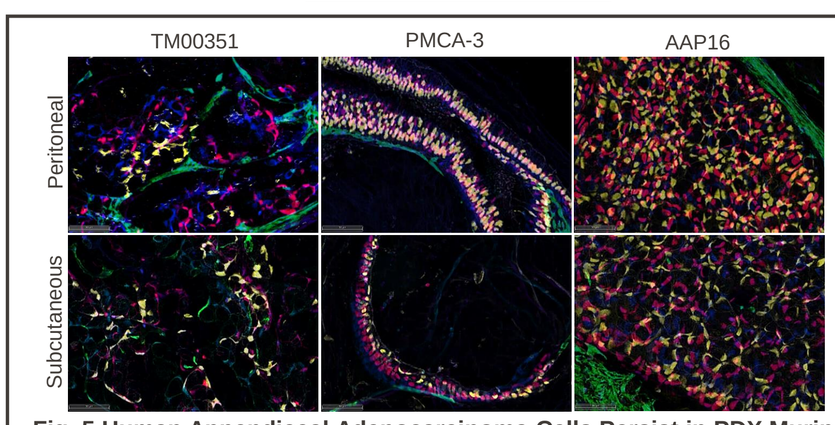


Fig. 5 Human Appendiceal Adenocarcinoma Cells Persist in PDX Murine Models,. Differential staining at a 40x magnification. The CDX2 is marked with gold, the KU80 is marked with red, and the Vimentin is marked with green. From this image, we can see a distinct overlap between the CDX2 and KU80 creating the orange coloring.

Results cont'd

4.) Murine Stroma Replaces Human Stroma in Appendiceal Adenocarcinoma PDX Tumors

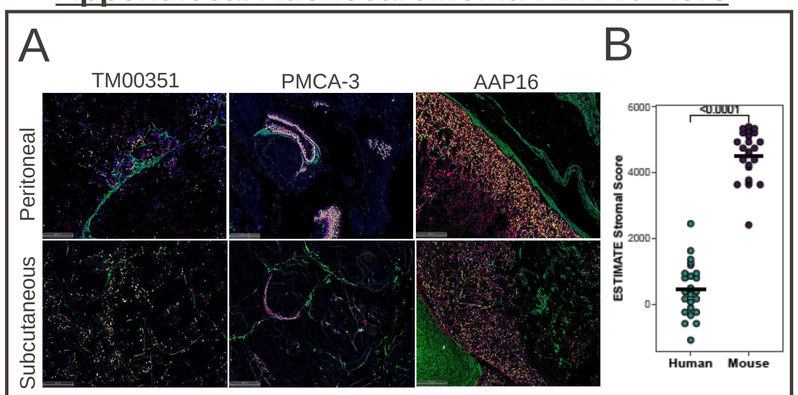


Fig. 6 Stroma is Replaced by Murine Cells. A. Differential staining at a 10x magnification. The CDX2 is marked with gold, the KU80 is marked with red, and the Vimentin is marked with green. The Vimentin marks the stroma. **B**. Estimate stromal score.

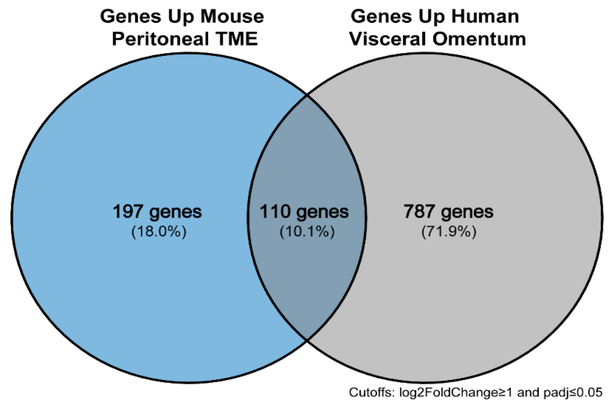


Fig 7. Comparison of Murine and Human Peritoneal Stroma. Out of the 307 genes unique to the mouse peritoneal tumor environment and the 897 unique genes to the human visceral omentum there is a 10% overlap.

Conclusions

1.) Orthotopic engraftment in the peritoneal cavity is superior to subcutaneous engraftment in preclinical PDX models due to improved tumor cell persistence and proliferation

2.) Murine stroma derived from the peritoneal cavity more faithfully recapitulates tumor microenvironment derived stroma in human patients and plays a role in supporting tumor growth.

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