Establishing a platform for the generation of organoids from diverse tumor types as part of the NCI patient-derived models (PDM) initiative.

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Introduction

Cancer Organoids are discrete multicellular structures that recapitulate tumor microanatomy. These reagents can be generated by extended culture of partially or fully dissociated tumor samples in three-dimensional matrices. By maintaining tumor cells in an appropriate context, they provide a biosimilar platform for studying disease pathogenesis and cellular pharmacology. Similarly, cancer organoid culture is useful for propagating slow growing tumors or those requiring heterotypic cell-cell interactions. The NCI patient-derived models (PDM) initiative aims to develop a national repository of patient-derived cancer models (PDMs) consisting of clinically annotated patient-derived xenografts (PDXs) and patient-derived tumor cell cultures (PDCs) prepared from primary and metastatic tumors. In this study, the feasibility and utility of integrating organoid generation into current workflows was explored for diverse tumor types. All animal studies were conducted in AAALACi accredited facilities under an approved IACUC protocol.

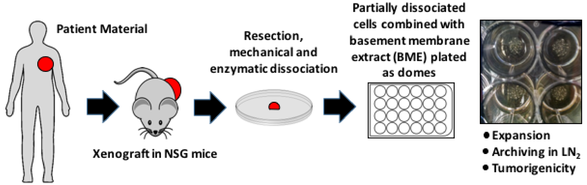

Figure 1: Procedure for organoid generation

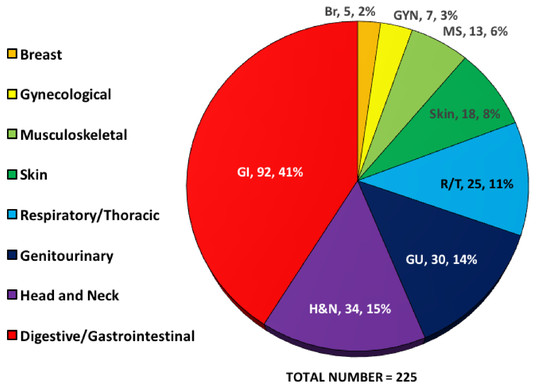

Figure 2: Total number of unique samples attempted - subdivided by tumor type

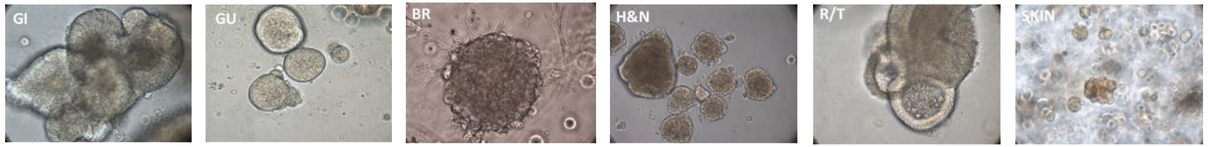

Figure 3: Representative organoid morphologies for different tumor types.

Results and Discussion

In this study, fragments from 225 early-passage tumor xenografts, currently being grown as part of the PDM initiative, were evaluated for the ability to form organoids. An SOP was developed wherein mouse xenograft resections were subjected to mechanical dissociation followed by enzymatic dissociation with collagenase/Dispase. Cells were pelleted, Basement Membrane Extract (BME) added and 35µL domes of the cell/BME mixture plated per well of a 24-well plate. The plate was then inverted, incubated at 37° to promote gel formation, reverted and the appropriate media added. The total number of xenograft samples attempted subdivided by tumor type is shown in Figure 2. Cultures were evaluated weekly for ability to form organoids, fed and expanded as needed. Representative organoid morphologies for tumor categories are shown in Figure 3. Following expansion, dispase was added to all BME-organoid wells to dissolve the BME, a small aliquot of organoids was dissociated into single cells using TrypLE and cell number per mL determined. Organoids were frozen in LN_2 as intact organoids at 10^6 cells/vial. To test for tumorigenicity intact organoids (approximately 10^6 cells) were implanted subcutaneously into NSG mice (see Figures 5 and 6). Results show that organoid cultures in BME were extremely effective at generating xenografts and that the resultant tumor had the same histopathological features as the patient tissue xenograft. To optimize media culture conditions for the different cancer types, whole tumors were enzymatically dissociated with collagenase/Dispase, BME/cell domes plated and 10-12 medias with different additives added to 5 wells each (Table 1). Cultures were examined weekly for organoid formation and viability. Data demonstrated that, although tumor-specific media formulations were effective in general, there were some instances where many tumors appear to have unique growth requirements within categories. Lastly, all organoid cultures were used to generate 2D cell lines. In some instances, organoid cultures were successful in the generation of 2D lines after previous attempts using standard culture methods had failed (see example, Figure 4).

*Media Type/Component**	6B/Colon	6A	6C/Colon	6D	6E	6F	6G	6H	6I	6J	BREAST	PANC	SCLC
L-WRN CM ATCC CRL-3276 50%		+	+		+		+		+	+	+	+	+
hEGF , 5,10,or 50ng/mL			+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+
hFGF10, 10 or 100 ng/mL					+	+	+	+	+	+	+	++	+
hFGF2, 1ng/mL					+	+	+	+	+	+	+		+
PGE2, 1µM					+	+	+	+	+	+			+
NSC756649, 500 nM							+	+		+		+	+
SB202190, 10µM							+	+		+			
Hydrocortisone, 0.3µg/mL									+	+	+		
Insulin, 1µg/mL									+	+	+		
Gastrin, 10nM												+	
β-estradiol, 0.5nM											+		
DHT, 1nM													
Breast											2/2		
Digestive/Gastrointestinal	33/45	12/15	22/36	4/9	4/9	3/9	4/9	3/9	3/9	3/9		3/5	
Genitourinary	3/9	7/13	2/7	2/7	5/11	2/7	3/7	2/7	2/6	3/7			
Head & Neck	0/2	1/3	0/2	2/3	0/2	0/2	0/2	0/2	0/2	0/2			
Respiratory/Thoracic	1/4	6/8	0/2	1/4	2/4	1/2	1/2	1/2	1/2	1/2			2/3
Skin	2/4	5/7	1/4	3/7	2/4	1/4	1/4	1/4	2/4	1/4			

Table 1: Media matrix testing results. Media formulations with final concentrations are shown along with the proportion of samples tested that formed organoids. *All media formulations based on - DMEM F12, HEPES, Glutamax, Pen/Strep, N-acetylcysteine, 1.25mM, Nicotinamide 10mM, B-27 Supplement 1X, N2 Supplement 1X, Y-27632 compound 10µM.

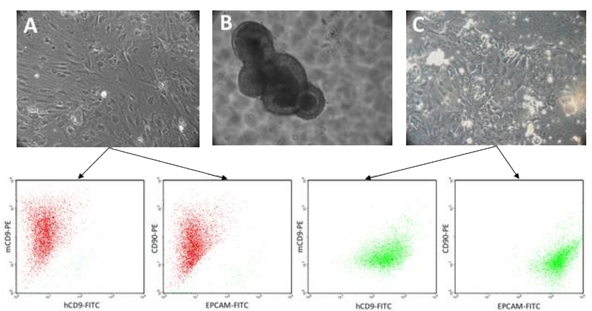

Figure 4: For certain samples intermediary organoid culture was essential for cell line generation. For the above CRC sample, direct plating of cells in 2D culture resulted in tumor cell death and outgrowth of murine fibroblasts (A). However, growth as organoids (B) generated EPCAM+ cells that were amenable to 2D growth (C).

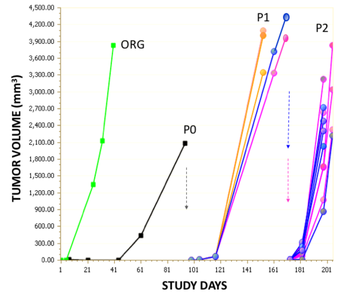

Figure 5 – Organoid growth *in vivo*. Patient material was implanted into 1 NSG mouse (#Z66) on day 1 (passage 0). Tumor was harvested 94 days post-implant and passed into 5 mice (VU4, VU5, VU6, VU7, VU8 – passage 1). Resulting tumors were harvested 49-74 days post-implant on experimental days 153-169 (range 3339-4092 mg). Tumors harvested from VU5 and VU8 (day 169 - 74 days post-implant) were each passaged into 5 new mice (U63-U67, U68-U72 respectively). All 10 passage 2 tumors (U63 – U72) reached 2036 - 3835 mg by 30 - 37 days post implant (experimental days 198 – 205) . An organoid culture prepared from the xenograft growing in NSG mice was harvested and implanted into 1 NSG mouse (FYF) on day 1. The resulting tumor was >1000 mg within 25 days of implant and achieved a final mass of 3832 mg on day 40 post-implant.

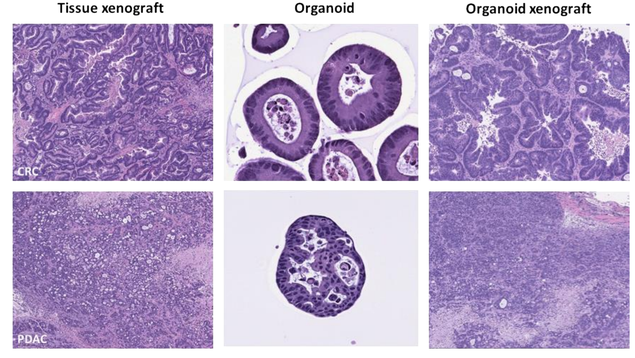

Figure 6: H&E staining of tissue xenografts versus *in vitro* cultured organoids and subsequent organoid-derived xenografts.

Conclusions

In this study, culture techniques and media formulations were developed that allowed xenograft-derived organoids to be generated from colon, pancreatic, breast, melanoma, head & neck and lung cancers. These organoids were scalable, could be archived in LN2 and were amenable to analysis by FACS and Immunohistochemistry. For some samples, intermediary organoid culture was shown to be essential for the development of 2D cell lines. Interestingly, organoids were also found to be efficient at generating xenografts which were histomorphologically similar to initial tissue xenografts from direct tumor implantation. Although considerable additional effort is required to further optimize growth conditions, progress made thus far suggests that organoid generation from early-passage xenografts is feasible.