Patient-derived tumoroids

How low can you go: Maintenance of tumoroid phenotype with a highly scalable and automation-compatible reduced-ECM suspension culture method

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KEY TAKEAWAYS

- Traditional embedded tumoroid culture is highly manual, costly, and incompatible with high-throughput workflows.
- Our Gibco™ OncoPro™ Tumoroid Culture Medium pairs with a suspension culture method which is:
  - Compatible with existing tumoroid lines
  - Demonstrates preservation of patient-specific phenotype and genotype in culture comparable to embedded method

Introduction

- **Tumoroids**, also known as **cancer organoids**, have been shown to be more physiologically relevant by maintaining patient-specific mutational and gene expression profiles.
- Despite the physiological relevance of tumoroid models, they have yet to supplant traditional cancer cell lines. One reason for this is the relative difficulty of embedded culture.
- We have developed a novel tumoroid culture medium and method which maintains patient-specific characteristics comparably to embedded culture while leveraging the benefits of a suspension culture.

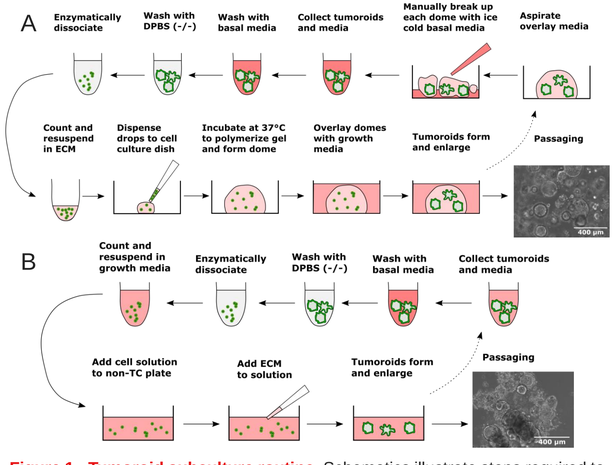

Figure 1. Tumoroid subculture routine. Schematics illustrate steps required to passage tumoroids in (A) ECM domes (embedded) and (B) suspension culture

Benefits of reduced-ECM suspension culture method

Scale (for one passage)	OncoPro Suspension Culture	Homebrew Embedded Culture
10 million	1 X T75	2 X 10 cm dishes (50 domes)
50 million	5 X T75	10 X 10 cm dishes (250 domes)
100 million	10 X T75	20 X 10 cm dishes (500 domes)

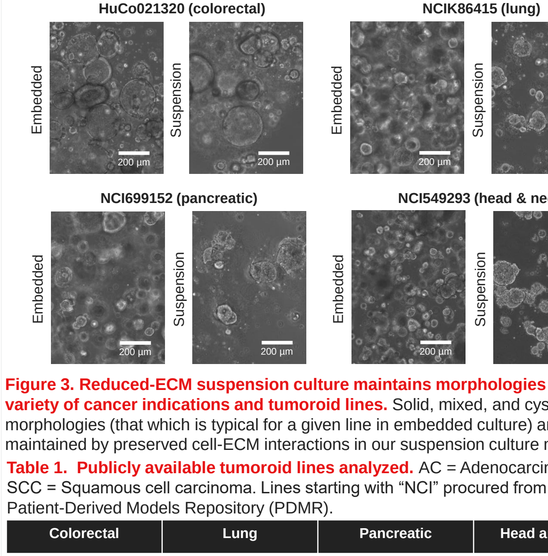

Figure 2. Suspension culture workflow has lower resource requirements. The number of domes to get 100 million cells in one passage would be difficult for one user to achieve, as opposed to using suspension (top). Amount of ECM required to harvest 100 million cells for 9 tumoroid lines (bottom). On average, suspension culture required less ECM than embedded culture (Mean ± SEM, not significant).

Suspension maintains embedded patient-specific morphologies

Figure 3. Reduced-ECM suspension culture maintains morphologies for a variety of cancer indications and tumoroid lines. Solid, mixed, and cystic morphologies (that which is typical for a given line in embedded culture) are maintained by preserved cell-ECM interactions in our suspension culture method.

Table 1. Publicly available tumoroid lines analyzed. AC = Adenocarcinoma, SCC = Squamous cell carcinoma. Lines starting with "NCI" procured from NCI Patient-Derived Models Repository (PDMR).

Colorectal	Lung	Pancreatic	Head and Neck
[NCI] 782815-120-R-V1-organoid Colon AC	[NCI] 349418-098-R-V2-organoid Lung AC	[NCI] 699152-130-R-V1-organoid Pancreas AC	[NCI] 549293-155-R-V1-organoid Lip/oral cavity SCC
[NCI] 919269-233-R3-V2-organoid Rectosigmoid AC	[NCI] K86415-001-R-V1-organoid Lung AC	[NCI] 982778-135-R-V3-organoid Pancreas AC	[NCI] 832693-133-R-V1-organoid Lip/oral cavity SCC
Tumoroid line Z Colorectal cancer – caecum	--	--	--

Suspension maintains mutational profiles

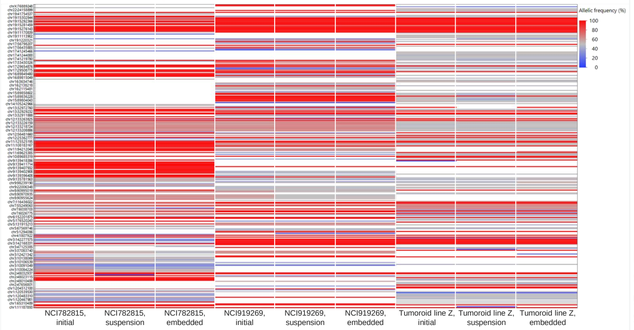

Figure 4. Patient-specific mutational profiles maintained in suspension culture. Heat map of allelic frequency for single nucleotide variants. Cultures in OncoPro Tumoroid Culture Medium were >90% correlated with the initial material.

Suspension maintains gene expression profiles

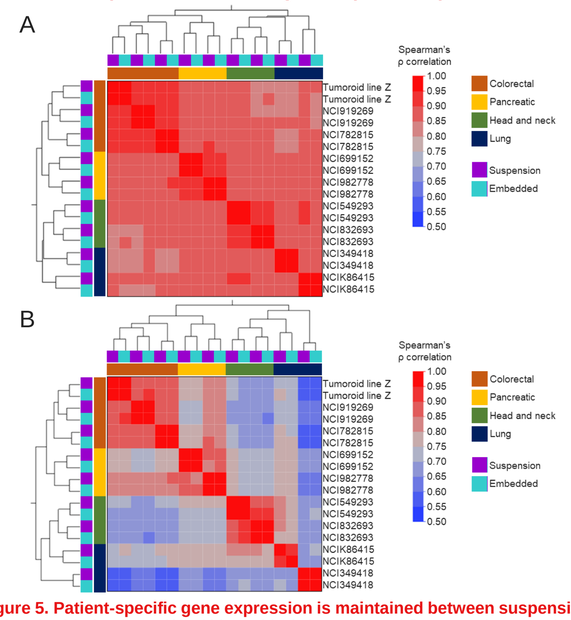

Figure 5. Patient-specific gene expression is maintained between suspension and embedded culture. Ward hierarchical clustering and Spearman's ρ correlations of gene expression for (A) the full transcriptome (20802 genes) and (B) a panel of 1423 cancer-related genes for all samples tested (Table 1).

Differentially expressed genes (DEGs) between culture methods

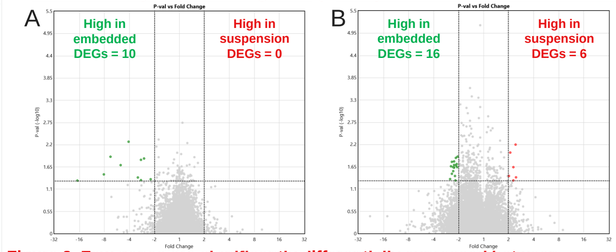

Figure 6. Few genes are significantly differentially expressed between suspension and embedded. Out of 20802 genes, less than one percent of the genes are significantly differentially expressed ($p < 0.05$, log2FC = 1) for (A) all samples tested combined (Table 1) or (B) only colorectal cancer. TAC 4.0 software.

Gene ontology (GO) analysis of differentially expressed genes

A

Gene ID	Embedded Avg (log2)	Suspension Avg (log2)	Fold Change	p-value
CA9	6.63	2.56	16.85	0.0487
EGLN3	8.45	5.43	8.13	0.034
ANKRD37	6.4	3.65	6.72	0.0126
ANGPTL4	4.65	2.31	5.07	0.02
NDRG1	8.55	6.52	4.09	0.0053
ENO2	5.75	4.09	3.16	0.0409
VLDLR	3.43	1.89	2.92	0.0474
PPFIA4	2.49	0.96	2.89	0.0148
MIR210HG	2.36	0.94	2.68	0.0139
BNIP3L	7.51	6.36	2.22	0.0454

B

GO biological process (GOBP)	No. of mapped IDs, Homo sapiens reference	No. of mapped IDs from genes analyzed	Raw p-value	FDR
response to hypoxia	277	5	5.59E-08	0.000877
response to decreased oxygen levels	290	5	7E-08	0.000549
response to oxygen levels	319	5	1.12E-07	0.000584

Figure 7. Only one significant 'Complete GO' hierarchy called for DEGs. (A) DEGs between embedded and suspension for Fig. 6A. Red outline denotes genes mapped to significant 'GOBP complete' hierarchy called in Fig. 7B. List of DEGs for Fig. 6B did not return any overrepresented terms in 'Complete GO' annotation data-sets. (B) Significantly overrepresented GO terms (FDR < 0.05) in 'GOBP complete' analysis; table adapted from software output. PANTHER Overrepresentation Test (released 20221013); DOI 10.5281/zenodo.6799722 released 2022-07-01. GO references (DOI): 10.1038/75556; 10.1093/nar/gkaa1113; 10.1093/nar/gky1038.

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