Patient-derived tumoroids

Expansion of established patient-derived tumoroids in a novel serum-free, Wnt agonist-free media system

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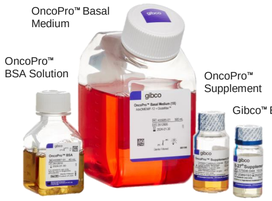
Key Takeaways

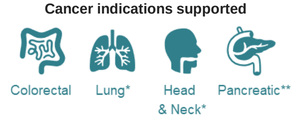
- Established tumoroids can be readily expanded in serum- and conditioned medium-free Gibco™ OncoPro™ Tumoroid Culture Medium
- Mutational status and gene expression levels of the original tumoroid cultures are maintained in OncoPro™ over multiple passages in both embedded and suspension formats
- Similar signaling pathways are active across culture conditions, and Wnt-related signaling pathways are not differentially activated following culture in Wnt agonist-free OncoPro™ Tumoroid Culture Medium

Introduction

Tumoroid technology enables culture of patient tissue-derived cancer cells in 3D, with retention of key characteristics (genotype, phenotype) from the original patient tumor. However, current tumoroid culture relies on labor-intensive media formulations and culture workflows that limit their utility. We have developed and tested OncoPro™ Tumoroid Culture Medium, a novel serum-free, conditioned medium-free cell culture media system specifically designed for tumoroids derived from multiple cancer indications. To test the compatibility of the media system with pre-existing tumoroid (cancer organoid) lines, we expanded and characterized tumoroid models established by and available from the U.S. National Cancer Institute (NCI) Patient-Derived Models Repository (PDMR).



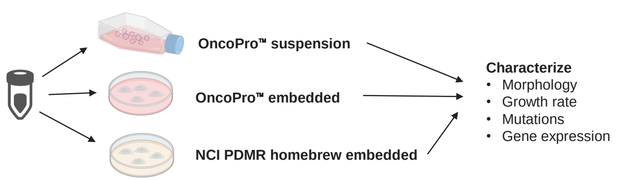
Cancer indications supported



* requires FGF10 ** requires FGF10 and gastrin

Materials and methods

Cryopreserved tumoroids were received from the NCI PDMR and cultured in one of three study arms: (1) embedded culture (Geltrex™ domes) using NCI PDMR prescribed homebrew media; (2) embedded culture using OncoPro™ Tumoroid Culture Medium; and (3) suspension culture using OncoPro™ Tumoroid Culture Medium. Cell counts at each passage were used to monitor cumulative population doublings. Following expansion, the mutational status of tumoroids from each condition was characterized and compared to the initial cells received from the NCI PDMR using the targeted NGS Oncomine™ Comprehensive Assay v3C. Similarly, gene expression levels were quantified using the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit.



Results

Expansion of NCI PDMR tumoroid lines in OncoPro™ Tumoroid Culture Media

Tumoroid lines were thawed and expanded in parallel in multiple media and culture conditions. Tumoroids were imaged prior to subculturing. Dissociated cell counts at each passage were used to track cumulative population doublings over time.

Figure 1. Morphology of NCI PDMR tumoroid lines expanded in NCI PDMR homebrew media (embedded culture), or in OncoPro™ embedded or suspension culture. Scale bar = 400 µm.

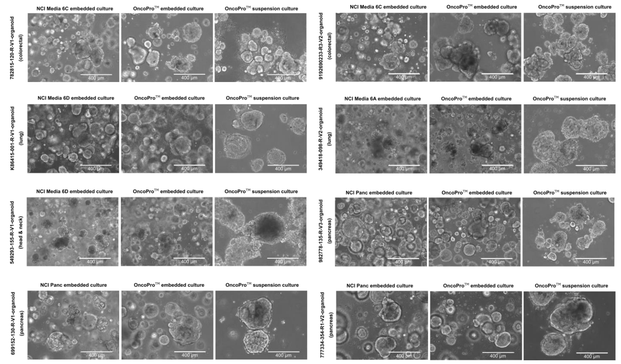
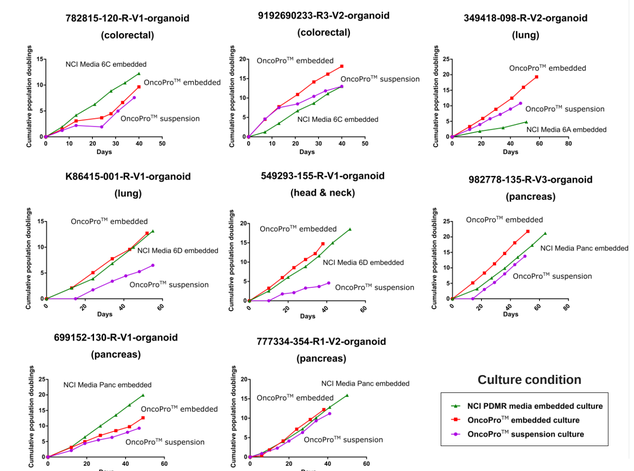


Figure 2. Cumulative population doublings of cells expanded in OncoPro™ suspension culture (purple), OncoPro™ embedded culture (red), or NCI PDMR media embedded culture (green). In general, tumoroids had comparable growth rates in each culture condition.



NCI PDMR tumoroid lines are genetically stable in OncoPro™ Media

Samples for DNA isolation and analysis were collected after tumoroid lines were expanded in parallel for 3-7 passages in NCI PDMR homebrew media, or in OncoPro™ Tumoroid Culture Medium in embedded or suspension formats. Both SNV variant allele frequency (VAF) and substitutions (below) and ploidy values (not shown) were conserved from initial starting material sampled at thaw.

Figure 3. Heatmap illustrating correlation coefficients between initial tumoroid banks received from NCI PDMR and tumoroids expanded in multiple culture conditions. Color indicates value of Pearson correlation coefficient between allele frequency of single nucleotide variants (SNVs) detected for indicated samples. Inset provides further detail on multivariate correlation comparison. SNVs were identified using the Oncomine™ Comprehensive Assay v3C.

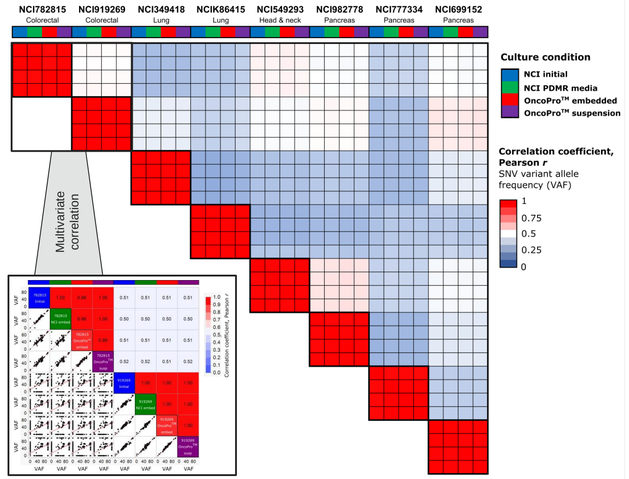
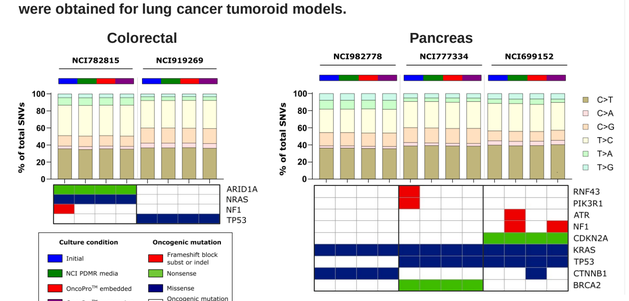


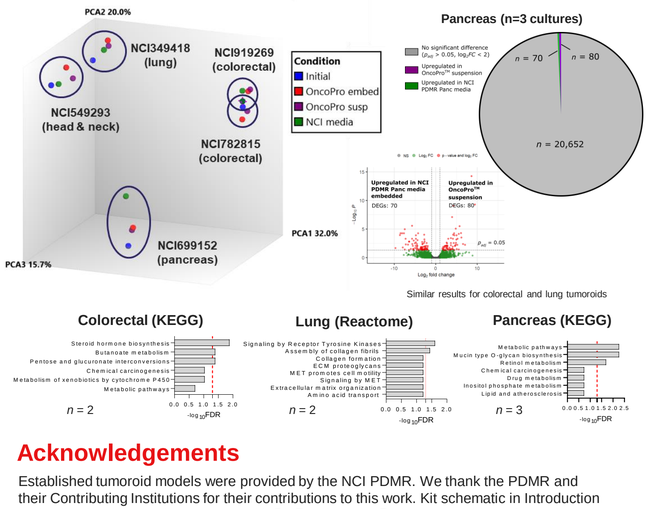
Figure 4. Single base substitution plots and oncogenic variants in colorectal and pancreatic tumoroids expanded in NCI PDMR media or OncoPro™ media and compared to initial starting material (cryopreserved tumoroids from NCI PDMR). Oncogenic variants were called using Oncomine™ variant 5.20 filter. Similar results were obtained for lung cancer tumoroid models.



NCI PDMR tumoroid lines are transcriptionally stable in OncoPro™ Media

Tumoroid lines were expanded in parallel in multiple media and culture conditions for 3-7 passages post-thaw prior to quantification of expression levels across over >20,000 human RefSeq genes using the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit. Analysis revealed that donor-specific characteristics were maintained, with few differentially expressed genes or changes in signaling pathways.

Figure 5. Principal component analysis (PCA) plot (top left) comparing gene expression levels across donors and culture conditions. Differential gene expression (top right) and pathway analysis (KEGG or Reactome; bottom) revealed few differences between tumoroids expanded in OncoPro suspension culture and in embedded culture in NCI PDMR homebrew media. In particular, changes in Wnt-related pathways were not observed in Wnt agonist-free OncoPro™ media during tumoroid culture across multiple cancer indications.



Acknowledgements

Established tumoroid models were provided by the NCI PDMR. We thank the PDMR and their Contributing Institutions for their contributions to this work. Kit schematic in Introduction and cell culture illustrations created with BioRender.com. Pathway analysis performed using ShinyGO (DOI: https://doi.org/10.1093/bioinformatics/btz931).

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