Patient-derived tumoroids

Derivation & long-term maintenance of patient-derived tumoroid lines in a defined, serum-free medium

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Key Takeaways

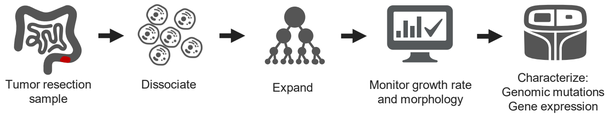
- Optimized serum- and conditioned medium-free system for patient-derived tumoroids
- Consistent growth rates of colorectal and lung tumoroids for 40+ passages
- Demonstrated preservation of tumor phenotype and genotype in culture
- Sustains multiple cancer indications using tissue-specific growth factors
- Scale up to >1 billion cancer organoid cells in easy-to-use suspension culture method

Introduction

Traditional cancer lines do not reflect the complex biology of human cancers. Patient-derived tumoroids (cancer organoids) are an emerging model that enables more representative *in vitro* results; however, current tumoroid culture protocols are limited by laborious formulations and culture formats. We developed a defined, serum- and conditioned-medium free system that improves ease-of-use and supports a scalable suspension workflow. To test this system's ability to maintain the phenotype and genotype of patient-derived tumor cells, we derived and cultured tumoroid lines from a variety of tissue sources for up to a year.

Material and methods

Uncultured primary cancer cells obtained by dissociating fresh tumor resections or from a commercial vendor were expanded in Gibco™ OncoPro™ Tumoroid Culture Medium. Cells were initially cultured embedded in Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix for several passages to generate enough cells to cryopreserve a small bank. Cells were then seeded into suspension culture with dilute (2% volume/volume) Geltrex matrix for long-term culture. Tumoroids were passaged when the average diameter was around 200 µm, typically every 7-10 days. Libraries for next-generation sequencing were prepared on the Ion Chef™ using the Oncomine™ Comprehensive Assay v3 or Ion AmpliSeq™ Transcriptome Human Gene Expression kits and sequenced using the Ion GeneStudio™ S5 System. Gene mutations were called by the Ion Reporter™ software and oncogenic variants were identified using the Oncomine Variants 5.20 filter. Gene ontology analysis was performed with ShinyGO[1].



Results

Tumoroid lines derived from fresh tumor resections or cryopreserved dissociated tumor cells maintain patient-specific morphologies, oncogenic mutations, and gene expression patterns.

Tumoroids were cultured for multiple passages and imaged prior to passaging. Targeted genomic and transcriptomic sequencing were used to compare initial tumor material to the derived tumoroids.

Figure 1. Morphology of patient-derived tumoroids initially and after ten passages. Scale bar = 400 µm.

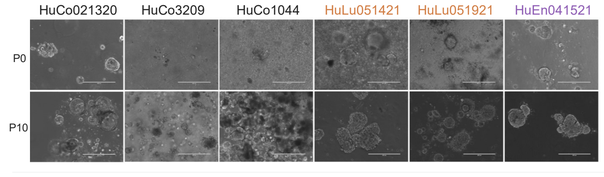


Figure 2. Unique genomic mutations are preserved between primary tumor (P) and tumoroid cultures (T) after 5-10 passages. Oncomine™ Comprehensive Assay v3 was used to identify single base substitutions (bar graphs) and oncogenic mutations (heat map). Each number set denotes a different tumor sample for each cancer indication.

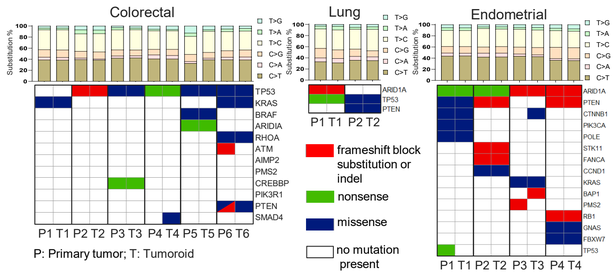


Figure 3. Transcriptomic comparison of primary tumors and early-passage tumoroids in colorectal and lung cancer indicates reduction in immune cell content. Tumor samples contain non-malignant immune, stromal, and endothelial cells that are not supported by the medium long-term. Tumoroid cultures are highly-enriched for epithelial cell adhesion molecule (EpCAM)-positive tumor cells, as demonstrated by immunofluorescence (right) and flow cytometry (data not shown). Differential gene expression analysis revealed differentially expressed genes at fold change >2 and false discovery rate (FDR) <0.05. Gene ontology revealed significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (FDR <0.05), primarily relating to immune cell function.

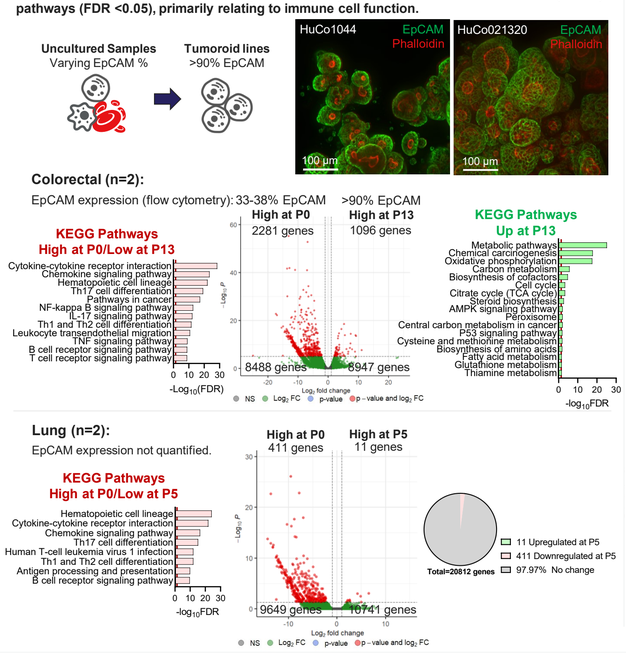


Figure 4. Growth rate and morphology of tumoroid lines during long-term culture. Cumulative population doubling (PD) was measured by cell counts at each passage. Tumoroid lines were recovered from cryopreservation at the beginning of this experiment (day 0), and additional cryopreservation and recovery points are indicated by arrows for each culture.

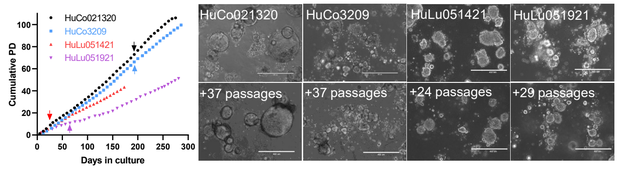


Figure 5. Correlation of single nucleotide variants (SNVs) between early and late passage tumoroid cultures. Each dot represents the variant allelic frequency (VAF) for 1 genetic loci covered by the Oncomine Comprehensive Assay v3. (Below) Single base substitutions (bar graphs) and oncogenic mutations (heat maps) from multiple time points during this study.

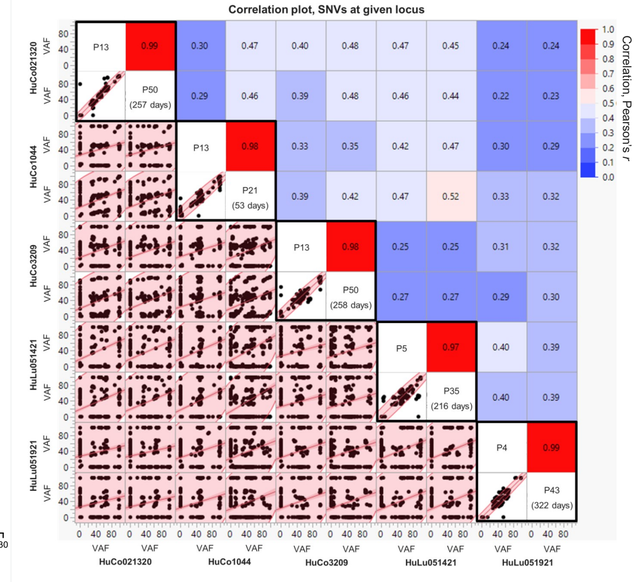


Figure 6. Principal component analysis (PCA) demonstrates that global gene expression patterns are conserved in colorectal and lung tumoroids during long-term culture. Differential gene expression analysis of early versus late passage tumoroids reveals a modest number of differentially expressed genes (DEGs) at fold change >2 and false discovery rate (FDR) <0.05. Gene ontology analysis revealed a few significantly enriched KEGG pathways (FDR <0.05); the top 10 results are shown.

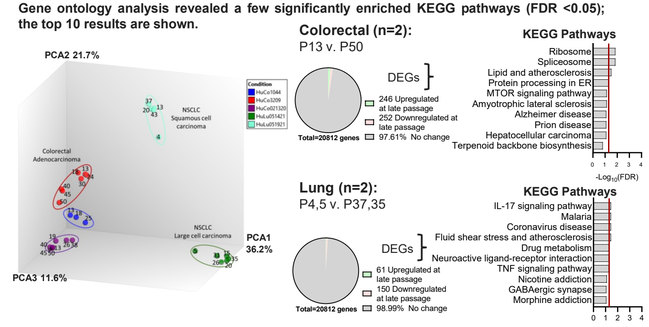
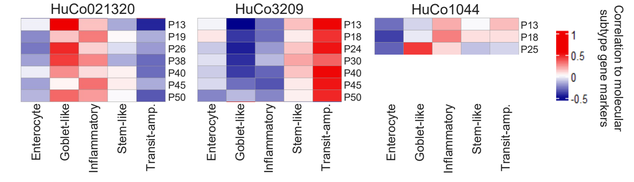


Figure 7. Colorectal patient-derived tumoroids maintain gene expression patterns identifying the consensus molecular subtypes of colorectal cancer[2-3].



References

1. Ge SX, Jung D, and Yao R. (2019) ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinfomatics, Vol.36 (8), pp. 2628-2629.
2. Sadanandam, A.et al. (2013). A colorectal cancer classification system that associates cellular phenotype and responses to therapy. Nat med, Vol.19 (5), pp. 619-625.
3. Sadanandam, A.et al. (2019). Analytical validation of multiplex biomarker assay to stratify colorectal cancer into molecular subtypes. Sci rep, Vol.9 (1).

Acknowledgements

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