From Tumor to Model: Transcriptomic and Therapeutic Insights from Patient-Derived Colorectal Cancer Organoids

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Poster 5457

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Introduction

Colorectal cancer (CRC) is the world's second leading cause of cancer deaths, often diagnosed in advanced stages due to silent onset. To accelerate drug discovery, scientists use both traditional cell lines and patient-derived models. The Human Cancer Models Initiative (HCMI), a collaboration among the National Cancer Institute, Cancer Research UK, Wellcome Sanger Institute, Hubrecht Organoid Technology, and ATCC®, has built a collection of clinically annotated CRC organoids that reflect real tumor biology and genetic diversity.

Comparing organoids with conventional cell lines is essential for improving disease models and developing better therapies. HCMI models show strong concordance with The Cancer Genome Atlas (TCGA), retaining over 80% of oncogenic drivers and preserving key transcriptional and epigenetic features. These models support precision oncology by enabling drug screening, proliferation assays, biomarker discovery, and personalized medicine. Sequencing data and patient clinical information for each model are available through the HCMI portal to enhance their clinical relevance.

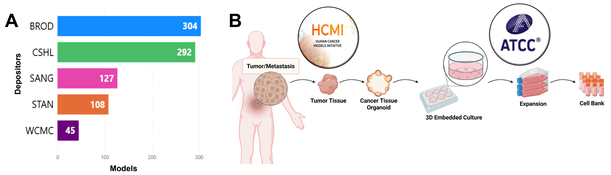


Figure 1: The Human Cancer Models Initiative (HCMI). (A) Institutes involved with depositing HCMI patient-derived cancer models at ATCC®. (B) ATCC® initiates the production pipeline and generates cell banks from this material. Figure created with BioRender.com.

Materials & Methods

Five CRC organoid models from unique donors and four CRC cell lines were expanded and analyzed via RNA sequencing. The organoid models, derived as part of the HCMI collaboration, originated from primary tissues across seven different acquisition sites. Transcriptomic profiles were compared among models and against tumor data from The Cancer Genome Atlas (TCGA). Organoid models (n=5) were screened for drug sensitivity in a medium throughput format using a panel of eight compounds targeting molecular pathways identified by RNA-seq. Drug responses were evaluated using Brightfield microscopy and a luminescent ATP viability assay.

Organoids were sourced from ATCC® and cultured in standard 3-D extracellular matrix embedded conditions using Organoid Growth Kits (ATCC® ACS-7100™ and ATCC® ACS-7103™), Cell Basement Membrane (ATCC® ACS-3035™), and Rock Inhibitor Y27632 (ATCC® ACS-3030™). The organoids were passaged as single cells and cultured for 72 hours to develop into uniform, small organoids prior to seeding and dosing. The organoids were collected and seeded as intact organoids into 96-well plates at the equivalent of $2.5x10^3$ cells/well in 100 µL organoid growth media supplemented with 0.5 mg/mL Cell Basement Membrane. They were tested for sensitivity to a custom panel of 8 chemotherapeutic compounds with varying mechanisms of action and molecular targets reconstituted in either D-PBS (ATCC® 30-2200™) or DMSO (ATCC® 4-X™) and treated with an 8-point, half-log curve in triplicate. After 5 days, the organoids were imaged in Brightfield and measured using a CellTiter-Glo 3-D Cell Viability Assay (Promega, G9681). Responses were normalized to vehicle treatment condition and expressed as percent viability. Figures, non-linear curves, IC50s, and AUCs were generated in GraphPad Prism.

During routine organoid expansion, samples from each model were seeded in 5 replicate wells. Following growth, organoids were harvested individually and pelleted. Cells were lysed using 1% mercaptoethanol in lysis buffer and RNA isolated using a commercially available kit (Thermo Fisher Scientific, 12183020). RNA was stored in RNase-free water and quantified prior to submission for internal sequencing. Automated RNA-seq next-generation sequencing (NGS) library preparation was performed on an Eppendorf epMotion 5075 Liquid Handler using the Illumina Stranded mRNA Prep, Ligation kit. Prepared NGS libraries were assessed using Invitrogen Qubit dsDNA High Sensitivity Assay Kit and an Agilent 4200 TapeStation and D5000 ScreenTape System. Libraries were prepared using an Illumina P3 200-cycle kit and sequenced on the NextSeq 2000 platform.

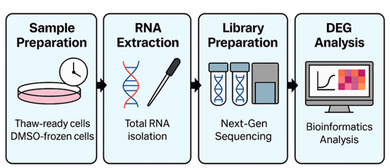


Figure created with BioRender.com.

Results

Table 1: Clinical characteristics of a subset of CRC organoids. CRC organoid models (n=5) were derived from a variety of acquisition sites, genders, races, ages, and clinical stages.

ATCC® No.	Cancer Type	Histological Subtype	Type	Acquisition Site	Gender	Race	Age	Clinical Stage	KRAS Status
PDM-1™	Colorectal	Adenocarcinoma	Primary	Cecum	Male	White	75	Stage I	KRAS-G12A
PDM-45™	Colorectal	Adenocarcinoma	Primary	Transverse colon	Male	--	80	Stage IIIB	KRAS-G12C
PDM-354™	Colorectal	Adenocarcinoma	Primary	Sigmoid colon	Female	--	70	--	No Mutation Detected
PDM-410™	Colorectal	Adenocarcinoma	Metastatic	Liver	Female	Black	56	Stage IVA	No Mutation Detected
PDM-415™	Colorectal	Adenocarcinoma	Metastatic	Peritoneum	Female	Black	48	Stage IIIB	KRAS-G13D

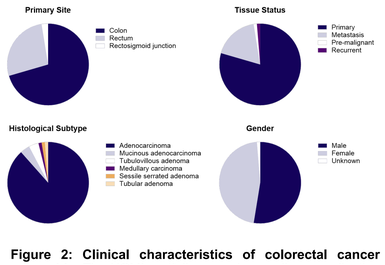


Figure 2: Clinical characteristics of colorectal cancer organoids. The HCMI portfolio comprises a diverse collection of patient-derived colorectal cancer organoids (CCOs). The pie charts illustrate the distribution of organoid models across key clinical attributes—including acquisition site, gender, race, age group, and clinical stage.

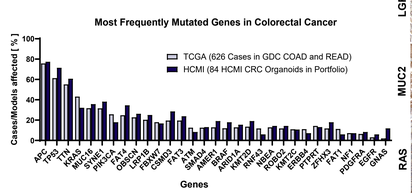


Figure 3: Model concordance in mutation frequencies. Bar graph illustrating the mutation frequency of selected genes, demonstrating strong concordance between HCMI colorectal cancer (CRC) models and clinical tumor profiles.

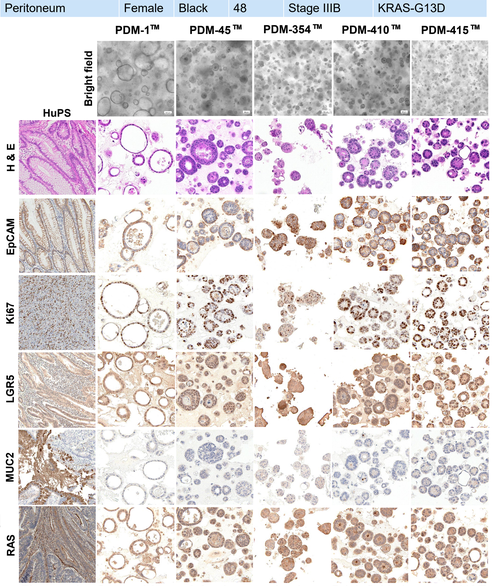


Figure 4: Organoid model characterization. Brightfield images, H&E staining, and IHC panels for five patient-derived colorectal cancer organoids, highlighting morphological diversity and molecular heterogeneity. IHC markers include EpCAM (epithelial identity), Ki-67 (proliferation), LGR5 (stem cell features), MUC2 (goblet cell differentiation), and KRAS (oncogenic pathway activity), collectively illustrating variation in differentiation state, growth dynamics, and signaling across models.

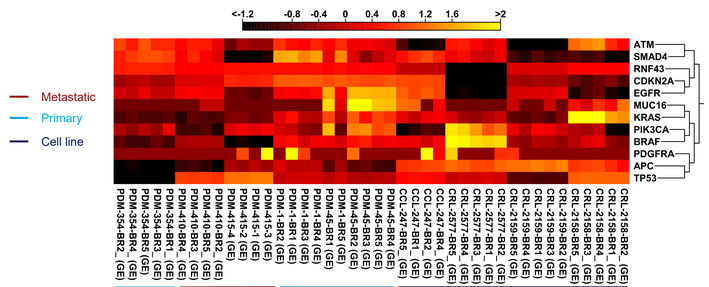
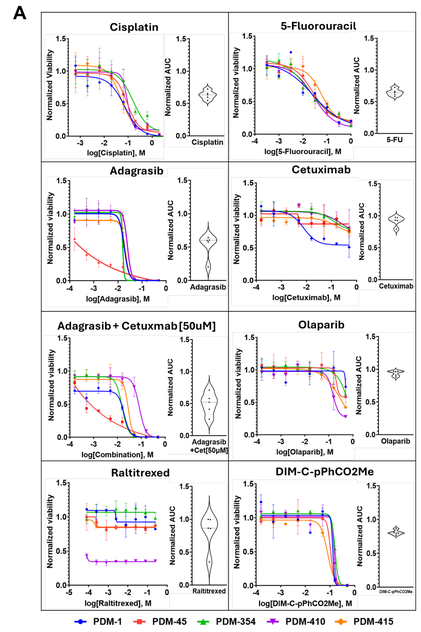


Figure 5: Gene expression heatmap. Heatmap illustrating differential gene expression patterns in primary and metastatic colon cancer cell lines and organoids. Yellow-to-red coloration represents increasing levels of gene upregulation, whereas black indicates downregulation. Higher color intensity corresponds to greater magnitude of expression change.



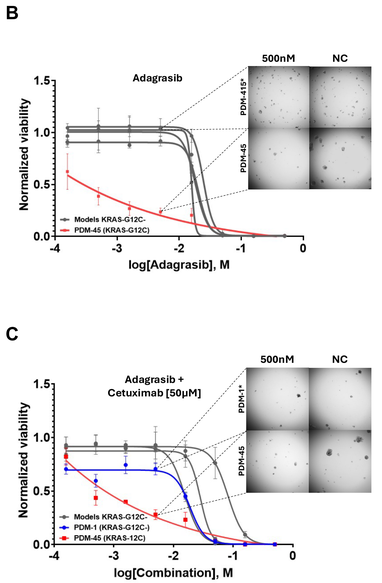


Figure 6: Drug response profiling of colorectal cancer organoids. (A) Dose-response curves showing cell-viability measurements for five colorectal cancer organoids (CCOs) treated with eight chemotherapeutic agents. Three KRAS-G12C$^-$ models show lower sensitivity to the combined drugs, while PDM-1™* displayed increased sensitivity. (B) A KRAS-G12C$^+$ CCO displays increased sensitivity to the FDA-approved KRAS-G12C inhibitor Adagrasib in contrast to four models with no mutation detected in KRAS-G12C. PDM-415™* represents brightfield imaging of KRAS-G12C$^-$ models, n=4. (C) A KRAS-G12C$^+$ CCO displays increased sensitivity to the standard of care, FDA-approved Adagrasib and Cetuximab treatment.

Conclusions

- Patient-derived CRC organoids harbor canonical mutations in key genes, including APC, TP53, and KRAS, and show high genomic concordance with matched tumors.
- Organoids share single-nucleotide variants and exhibit approximately 30–40% overlap in extrachromosomal DNA features with patient tumors.
- Key driver mutations (APC, TP53, KRAS, PIK3CA, SMAD4) were consistently detected and aligned with onco-targets.
- Histopathological analysis confirmed retention of tumor-specific markers.
- Drug screening revealed variable responses across organoid models, with luminescence-based viability assays confirming model-specific sensitivities.
- Differential sensitivity to Adagrasib suggests drug response may be partly driven by genotype.
- Transcriptomic profiling highlighted molecular heterogeneity and distinct subtype-specific expression patterns. Several genes were consistently expressed across models, indicating shared oncogenic pathways.

Explore HCMI Models

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