Validated HCT-116 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

By Altogen Labs, 11200 Menchaca Road, Suite 203, Austin, TX 78748
Phone: (512) 433-6177 | Email: info@altogenlabs.com

Modeling Drug Resistance in MSI-High Colorectal Cancer

Colorectal cancer is among the most common and deadly malignancies globally, characterized by significant genetic and molecular heterogeneity that complicates treatment and contributes to poor clinical outcomes in advanced stages. While key oncogenic drivers such as APC, KRAS, TP53, and mismatch repair deficiencies have been identified, translating these molecular insights into effective therapies remains hindered by limitations in conventional preclinical models, particularly two-dimensional *in vitro* systems that fail to replicate the tumor microenvironment. Xenograft models, including those derived from established colorectal cancer cell lines and patient tumors, have emerged as essential tools for studying tumor biology *in vivo*, enabling the investigation of tumor-stroma interactions, therapeutic resistance, and biomarker validation in a more physiologically relevant setting. Patient-derived xenografts retain the histological and molecular fidelity of the original tumors, providing a platform for precision oncology and individualized drug screening. The present study leverages the strengths of xenograft modeling to investigate the influence of microenvironmental factors on drug resistance and immune modulation in microsatellite instability-high colorectal cancer, with the broader aim of bridging translational gaps and informing the development of more effective, personalized treatment strategies.

HCT-116 Cell Line

The HCT-116 cell line is a well-characterized human colorectal carcinoma model derived from a male patient and is extensively utilized in cancer research due to its microsatellite instability-high (MSI-H) status and deficiency in the DNA mismatch repair pathway resulting from MLH1 inactivation. It harbors key oncogenic mutations, including KRAS (G13D), and maintains wild-type p53, making it an informative system for studying the molecular underpinnings of colorectal tumorigenesis, chemoresistance, and targeted therapy responses. HCT-116 has been instrumental in advancing understanding of Wnt/β-catenin signaling, cell cycle control, and apoptotic mechanisms, particularly in the context of resistance to chemotherapeutic agents such as 5-fluorouracil, irinotecan, and oxaliplatin. Despite its widespread use, there remains a critical gap in comprehending how the tumor microenvironment, particularly stromal and immune components, modulates drug response and resistance phenotypes in this model. Moreover, although the immunogenic nature of MSI-H tumors suggests susceptibility to immune checkpoint inhibition, the molecular basis of primary or acquired resistance in HCT-116 cells under such treatment conditions remains poorly elucidated, indicating a need for more physiologically relevant models incorporating tumor-stroma and tumor-immune interactions.

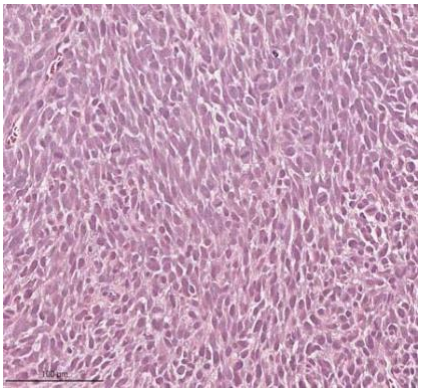

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted HCT-116 tumor (Altogen Labs).

Altogen Labs Validated HCT-116 Xenograft Model

The study design involves subcutaneous transplantation of HCT-116 cells into immunocompromised mice to assess tumor development and therapeutic response. Prior to inoculation, cell cultures are maintained in the exponential growth phase and harvested via trypsinization. Viability is confirmed to exceed 98% using trypan blue exclusion, and cell suspensions are diluted to the required concentration. A total of one million HCT-116 cells suspended in 100 µL of Matrigel are injected subcutaneously into the hind leg of NOD/SCID or athymic BALB/C mice aged 10 to 12 weeks.

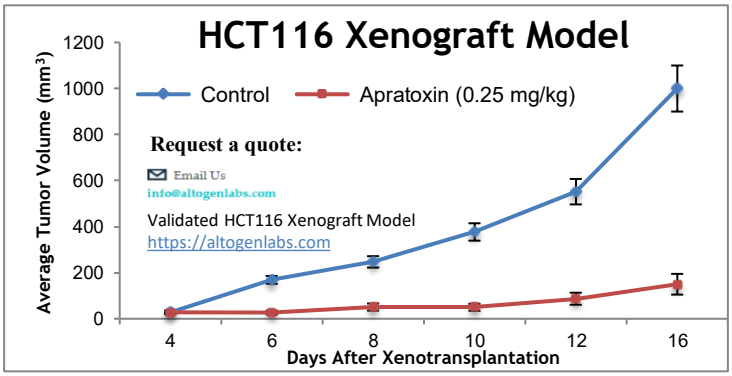

Figure 2. Tumor growth of HCT-116 colon carcinoma xenografts in athymic mice. Data represent mean tumor volume ± standard error of the mean (SEM). Xenograft model and *in vivo* study conducted by Altogen Labs.

Tumor formation is monitored by palpation, and once tumor volumes reach 50–150 mm³, animals are randomized into treatment groups. Compounds of interest are administered according to a predefined schedule, with tumor volumes measured daily using digital calipers and body weights recorded three times per week. Mice are euthanized upon reaching endpoint criteria, and comprehensive necropsy and tissue collection are performed, including excision, weighing, imaging, and preservation of tumors for downstream analyses. Altogen Labs offers a wide range of preclinical oncology services, including over 30 validated Cell Line Derived Xenograft (CDX) models and more than 20 patient-derived xenograft (PDX) models. These *in vivo* systems are suitable for evaluating the functional roles of tumor-associated genes, therapeutic efficacy, and mechanisms of drug resistance. Custom cell line engineering services are available to support studies involving overexpression of oncogenes, tumor suppressors, or gene knockdown via RNA interference, including stable RNAi cell line generation. Quantitative gene expression analysis is offered through RT-PCR platforms, while protein expression can be assessed using capillary-based immunoassays via the ProteinSimple WES system, supporting robust molecular characterization of experimental outcomes.

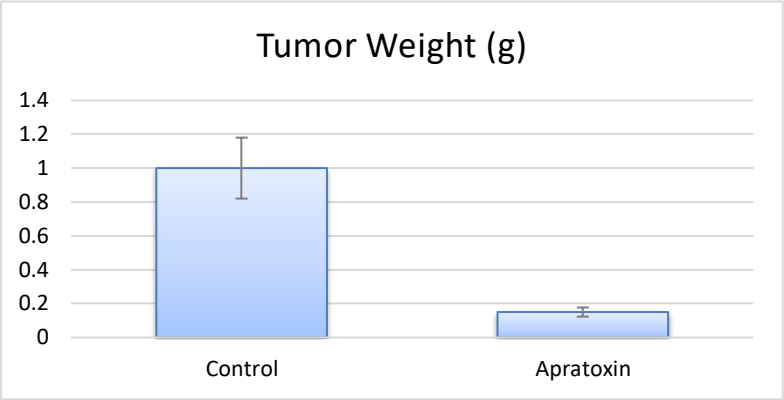

Figure 3. Final tumor weights of HCT-116 xenografts in mice treated daily with Apratoxin (0.25 mg/kg) compared to buffer-only controls. Bars represent mean tumor weight at study endpoint ± standard deviation. Study conducted by Altogen Labs.

Subcutaneous Xenograft Modeling with HCT-116

Subcutaneous xenograft transplantation remains a foundational method in preclinical oncology for evaluating tumor growth kinetics, drug efficacy, and molecular responses *in vivo*. The HCT-116 colorectal carcinoma cell line, characterized by its microsatellite instability-high (MSI-H) status and oncogenic KRAS mutation, has been extensively employed in subcutaneous xenograft models due to its robust tumorigenicity and reproducible growth in immunodeficient mice. Early studies utilizing HCT-116 xenografts helped define dose-response relationships for standard chemotherapeutics such as 5-fluorouracil and irinotecan, while more recent investigations have employed this model to evaluate the activity of targeted agents, including MEK and PARP inhibitors. The subcutaneous implantation of HCT-116 cells into athymic BALB/C or NOD/SCID mice facilitates consistent tumor formation, with palpable masses typically forming within 7–10 days post-inoculation, enabling early intervention and longitudinal therapeutic studies.

Although the subcutaneous model does not fully replicate the complex tumor-stroma interactions of orthotopic sites, it provides several critical advantages, including ease of monitoring tumor progression through caliper-based volume assessments and minimal procedural complexity. It is particularly useful for assessing tumor response in relation to genetic manipulations, such as stable RNAi-mediated knockdown or ectopic gene expression, both of which can be readily introduced into HCT-116 cells. Moreover, the HCT-116 xenograft model has contributed to investigations into immune modulation in MSI-H colorectal cancers, particularly in studies evaluating the impact of PD-L1 expression and immunotherapy resistance mechanisms. Ongoing efforts to enhance the physiological relevance of this model include co-injection with stromal fibroblasts or extracellular matrix components, enabling more accurate simulation of the tumor microenvironment. As such, subcutaneous transplantation of HCT-116 remains a vital and adaptable platform in colorectal cancer research, bridging molecular inquiry with translational application and supporting the development of personalized therapeutic strategies.

HCT-116 Xenografts in Metastatic Cancer Research

Metastatic xenograft transplantation provides a critical platform for modeling the progression of colorectal cancer from primary tumor formation to distant organ colonization. In this context, the HCT-116 cell line serves as a widely adopted model due to its inherent invasive properties and molecular profile characteristic of microsatellite instability-high (MSI-H) colorectal cancer. When introduced into anatomically relevant sites such as the spleen or cecum, HCT-116 cells demonstrate the ability to establish primary tumors and spontaneously disseminate to secondary organs, including the liver and lungs. These transplantation approaches more closely replicate the metastatic cascade observed in human disease, enabling the investigation of tumor cell-intrinsic and microenvironmental factors that drive metastatic competence.

The application of metastatic HCT-116 models supports the evaluation of therapeutic strategies aimed at preventing or eliminating disseminated disease. These models enable detailed examination of the mechanisms underlying metastatic colonization, immune evasion, and drug resistance, offering insight into vulnerabilities that may not be apparent in non-metastatic settings. Additionally, the reproducibility and adaptability of these models make them suitable for genetic manipulation and longitudinal analysis, allowing researchers to track tumor evolution and treatment responses over time. By capturing the biological complexity of metastatic colorectal cancer, HCT-116 xenograft models serve as an indispensable resource for preclinical studies that seek to identify and validate effective therapeutic interventions for advanced-stage disease.

HCT-116 Orthotopic Xenografts in Tumor Progression Studies

Orthotopic xenograft transplantation has become an essential method in colorectal cancer research, offering enhanced biological relevance by recapitulating the native tumor microenvironment and tissue-specific interactions. In contrast to ectopic models, orthotopic transplantation of HCT-116 cells into the cecum or colon of immunodeficient mice enables the study of local tumor invasion, angiogenesis, and spontaneous metastasis in a manner that more closely mirrors clinical progression. The HCT-116 cell line, characterized by microsatellite instability and oncogenic KRAS mutation, reliably forms primary tumors when introduced into the colonic wall, exhibiting invasive phenotypes and the potential to disseminate to distant organs such as the liver and lungs. This localized implantation facilitates investigation into site-specific cues that govern tumor behavior, including interactions with colonic epithelium, extracellular matrix components, and regional vasculature.

The orthotopic HCT-116 model serves as a valuable platform for assessing therapeutic responses within a physiologically relevant setting. By preserving anatomical and microenvironmental context, this approach enhances the predictive validity of preclinical drug studies, particularly those targeting invasion, immune modulation, and metastasis. Furthermore, orthotopic models support integration of advanced imaging technologies and longitudinal monitoring strategies, allowing for dynamic assessment of tumor growth, progression, and response to treatment. The incorporation of genetically modified HCT-116 derivatives, including stable knockdown or overexpression variants, adds further utility by enabling mechanistic dissection of gene function *in vivo*. As such, orthotopic transplantation of HCT-116 cells represents a robust and versatile system that bridges molecular insights with translational research objectives, advancing the understanding of colorectal cancer pathophysiology and informing therapeutic development.

HCT-116 Cell Growth and Migration Inhibited by Triterpenoid Extracts

Ethanol extracts from sporoderm-broken spores of Ganoderma lucidum (BSGLEE) exhibit potent inhibitory effects on HCT-116 colorectal cancer cells by targeting key processes involved in tumor progression. BSGLEE significantly reduces cell viability in a dose- and time-dependent manner, with progressively lower IC50 values observed over 24, 48, and 72 hours. This reduction in proliferation is linked to G0/G1 cell cycle arrest, as shown by flow cytometry, and accompanied by a decrease in the expression of cell cycle regulators including cyclin D1, cyclin E, CDK1, CDK2, and CDK4. At the same time, the tumor suppressor genes p16 and p21 are upregulated, suggesting a shift toward growth suppression. Apoptotic induction is confirmed through nuclear fragmentation visualized with Hoechst staining and increased levels of cleaved caspase-3 and PARP, along with elevated Bax and NAG-1 and reduced Bcl-2 expression. These findings collectively point to a coordinated activation of the intrinsic apoptotic pathway.

In addition to impairing proliferation and inducing apoptosis, BSGLEE disrupts the migratory capacity of HCT-116 cells. Wound healing and Transwell migration assays demonstrate a significant, dose-dependent decline in cell motility. Molecular analysis reveals that BSGLEE downregulates motility-associated genes such as MMP-1, MMP-2, c-Met, and vimentin while enhancing the expression of E-cadherin, a key cell adhesion molecule. These results indicate a suppression of epithelial-mesenchymal transition features. *In vivo*, oral administration of BSGLEE delays tumor formation, reduces tumor volume and weight, and lowers proliferation markers such as Ki-67 in xenograft tumors. Apoptotic markers such as Bax are elevated in tumor sections, supporting the mechanistic observations *in vitro*. No signs of systemic toxicity or significant body weight loss are observed, and liver weights are partially restored in animals treated with higher doses. Together, these findings suggest that BSGLEE may act as a multi-targeted therapeutic agent that interferes with cell cycle progression, promotes apoptosis, and impedes metastatic behavior in colorectal cancer. Further research is warranted to explore its use in combination therapies and to clarify its immunomodulatory effects in immune-competent models.

Case Study: Lagopsis supine Extract Inhibits HCT-116 Tumor Growth

Wei et al., in an article published in *BMC Complementary and Alternative Medicine* journal, demonstrated that *Lagopsis supine* ethanol extract (Ls) exerts significant antitumor effects against HCT-116 colorectal cancer cells through inhibition of cell proliferation, induction of apoptosis, and suppression of JAK2/STAT3 signaling. *In vitro*, Ls reduced HCT-116 cell viability in a dose- and time-dependent manner and promoted apoptosis by increasing the expression of pro-apoptotic markers Bax and caspase-3 while downregulating Bcl-2 and Bak. Western blot and immunohistochemistry confirmed that Ls significantly decreased phosphorylation of JAK2 and STAT3 without affecting total protein levels. *In vivo*, Ls treatment in HCT-116 xenograft-bearing nude mice resulted in dose-dependent tumor growth inhibition, reduced inflammatory infiltration, and no observable systemic toxicity. The study's dual *in vitro* and *in vivo* design strengthens its mechanistic conclusions, although limitations such as the absence of immune system evaluation and pharmacokinetic data highlight the need for further research. These findings suggest Ls as a promising candidate for colorectal cancer therapy, particularly in tumors driven by aberrant JAK/STAT signaling.

Additional Case Study: Tumor Suppressor Role of SLC22A18 in Colorectal Cancer

This comprehensive investigation by Jung et al., published in *Oncotarget* journal, presents compelling evidence for SLC22A18 as a tumor suppressor in colorectal cancer, with HCT-116 cells serving as a key model. The researchers demonstrate that SLC22A18 expression is consistently downregulated in colorectal tumor tissues compared to matched normal tissues, a pattern corroborated by both clinical specimens and TCGA RNA-seq data. Functional assays reveal that ectopic expression of SLC22A18 significantly inhibits colony formation in HCT-116 cells and induces G2/M cell cycle arrest. Flow cytometry shows reduced S-phase entry and an increased G2/M population, accompanied by upregulation of p21 and downregulation of cyclin B1, Cdc25C, and phosphorylated Cdc2, aligning with tumor suppressor behavior. Notably, SLC22A18 expression is suppressed by oncogenic KRAS activity, and KRAS knockdown leads to marked upregulation of SLC22A18 in HCT-116 cells, suggesting a mutually inhibitory interaction. This is further supported by anchorage-independent growth assays in which SLC22A18 expression diminishes KRASG12D-induced transformation of NIH3T3 cells.

The authors strengthen their thesis through a well-integrated experimental design that includes *in vitro* mechanistic studies, *in vivo* xenograft models, and bioinformatic analyses. The xenograft study highlights the translational potential of SLC22A18, as HCT-116 cells overexpressing the gene formed tumors in only 2 out of 15 mice, compared to 100 percent tumor formation in control animals. Kaplan-Meier analyses of TCGA patient data indicate that low SLC22A18 expression correlates with significantly reduced long-term survival, particularly beyond the three-year mark. These findings suggest that SLC22A18 not only functions as a tumor suppressor but may also serve as a prognostic biomarker. Despite the strengths of using matched patient samples, multiple CRC cell lines, and robust validation across platforms, some limitations should be noted. The use of immunodeficient mice precludes examination of immune interactions, and the mechanism by which SLC22A18 mediates tumor suppression, potentially through solute transport, remains unclear. Nevertheless, the data clearly position SLC22A18 as a critical modulator of KRAS-driven oncogenesis in colorectal cancer. Further research should aim to elucidate the molecular substrates of SLC22A18 transport and assess its prognostic utility across larger and more diverse patient cohorts, as well as its therapeutic potential in immune-competent models.

Nrf2 Activation Drives Biphasic Response in HCT-116 Cells

Sulforaphane, a dietary isothiocyanate known for activating the Nrf2 antioxidant pathway, exhibits a biphasic effect on colorectal cancer cells depending on p53 status. In HCT-116 cells with wild-type p53, low concentrations of sulforaphane stimulate proliferation, mitochondrial biogenesis, and antioxidant defense, while higher doses reduce cell viability. This proliferative effect is closely associated with increased nuclear Nrf2 and cytoplasmic HO-1 expression, an elevated Bcl-2 to Bax ratio, enhanced autophagy, and upregulation of mitochondrial biogenesis markers such as PGC1α. These cellular responses are notably absent or reversed in HCT-116 cells lacking p53, which instead display heightened apoptosis, reduced mitochondrial function, and impaired respiration upon sulforaphane treatment. The data suggest that the presence of p53 permits sulforaphane to activate a coordinated cytoprotective and pro-survival response, while its absence shifts the balance toward cell death.

This p53-dependent divergence highlights the critical role of genetic context in modulating cellular responses to phytochemicals. The observed biphasic behavior underscores a risk associated with sulforaphane intake in colorectal cancers retaining wild-type p53, where it may inadvertently support tumor growth. Experimental models further confirm that sulforaphane promotes proliferation in p53-competent xenografts but has minimal effect or promotes apoptosis in p53-deficient tumors. These findings challenge the broad categorization of sulforaphane as universally anticancer and call for more nuanced use of Nrf2-activating compounds.

Altogen Labs offers comprehensive preclinical research services utilizing over 30 standard Cell Line Derived Xenograft (CDX) models and more than 20 Patient-Derived Xenograft (PDX) models. These platforms enable *in vivo* assessment of therapeutic efficacy, tumor growth modulation, and molecular pathway interrogation. For investigators studying gene function in cancer progression, Altogen Labs supports the generation of engineered cell lines with stable overexpression or long-term gene silencing through RNA interference. Quantitative gene expression analysis is conducted via RT-PCR, while protein-level profiling is performed using the WES capillary electrophoresis system (ProteinSimple), allowing for robust molecular characterization. All animal studies are conducted under IACUC-regulated, GLP-compliant conditions at Altogen Labs. Mice are acclimated and sorted by body mass before tumor induction, with daily monitoring for tumor growth and clinical health. Clients receive comprehensive reports including methodology, statistical analyses, raw data, and experimental discussion. For the HCT-116 xenograft model, available services include tumor growth delay (TGD) and inhibition (TGI) studies, flexible dosing regimens, and multiple administration routes including intravenous, intratumoral, and oral gavage. Additional capabilities encompass orthotopic and metastatic engraftment, immunohistochemistry, histopathology, survival analysis, blood chemistry, metabolic and lipid profiling, and advanced imaging modalities such as MRI and fluorescence-based whole-body imaging. A positive control arm using 50 mg/kg cyclophosphamide is also available to benchmark therapeutic efficacy.

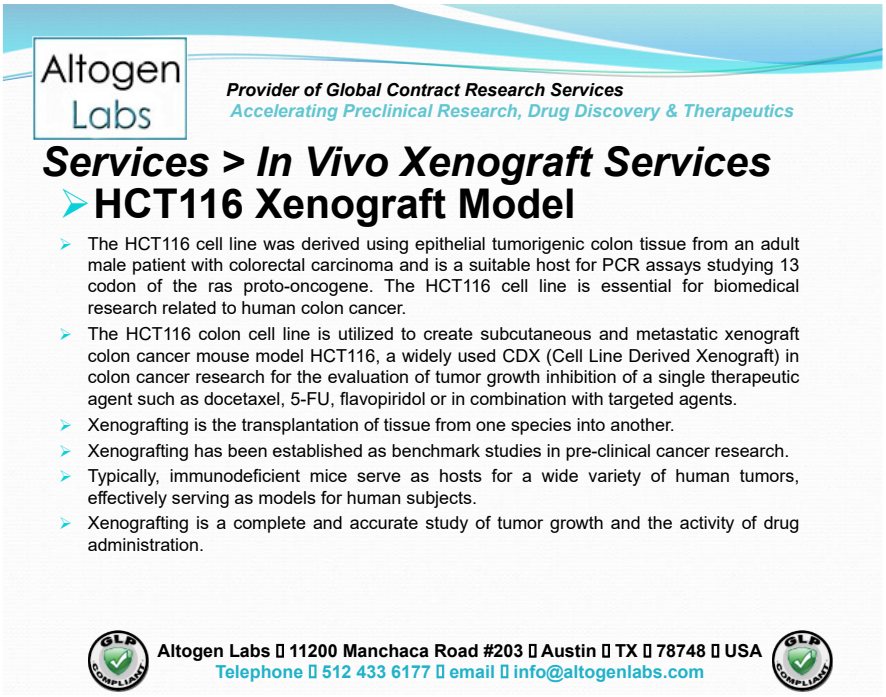

Figure 4. Summary of *in vivo* xenograft services available at Altogen Labs using the HCT-116 colorectal cancer model. This includes the development of subcutaneous and metastatic CDX models for evaluating therapeutic efficacy and tumor growth inhibition in response to single agents or combination regimens.

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