Validated SW480 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

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Modeling Tumor-Stroma Interactions in Colorectal Cancer Xenografts

Colorectal cancer (CRC) is a major global health burden, with high mortality rates driven by tumor heterogeneity, treatment resistance, and limited efficacy of current therapeutic strategies. Although advances in molecular profiling and targeted therapies have improved disease management, significant gaps remain in understanding the mechanisms underlying tumor progression and drug resistance. Xenograft models have become essential in preclinical cancer research by providing a biologically relevant system to study tumor behavior and therapeutic responses *in vivo*. These models allow for the evaluation of oncogenic pathways, drug efficacy, and tumor-microenvironment interactions within a controlled setting. While patient-derived xenografts (PDXs) capture greater histological and genetic complexity, cell line-derived xenografts (CDXs) such as those developed from SW480 cells offer reproducibility and are widely used for mechanistic studies. This research utilizes xenograft models to investigate how stromal interactions influence Wnt/β-catenin signaling and chemoresistance in CRC, aiming to address current limitations in model systems and to contribute to the development of more effective and durable treatment strategies.

SW480 Cell Line

The SW480 cell line, derived from a primary adenocarcinoma of the colon, is a widely utilized *in vitro* model in colorectal cancer research due to its well-characterized molecular profile and clinical relevance to early-stage disease. These epithelial cells harbor pathogenic mutations in KRAS (G12V), APC, and TP53, rendering them a representative model of canonical colorectal tumorigenesis involving dysregulated Wnt/β-catenin signaling and impaired apoptotic pathways. SW480 cells have been instrumental in delineating mechanisms of therapeutic resistance, particularly to 5-fluorouracil and oxaliplatin, and serve as a platform for evaluating the roles of non-coding RNAs, including HOTAIR and miR-21, in promoting tumor cell proliferation, invasion, and survival. Despite extensive characterization in monoculture systems, key gaps remain regarding the influence of the tumor microenvironment, including cancer-associated fibroblasts and immune interactions, on SW480 behavior and drug responsiveness.

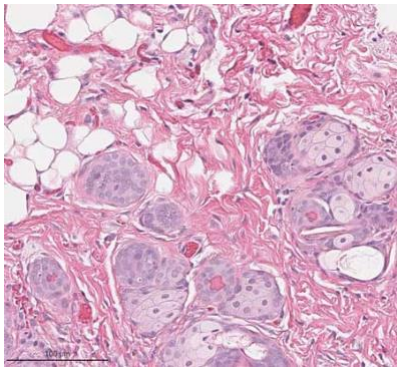

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted SW480 tumor (Altogen Labs).

Altogen Labs Validated SW480 Xenograft Model

The SW480 xenograft model is derived from a human colon adenocarcinoma cell line established from a primary tumor in a 50-year-old male patient. This model is commonly employed in preclinical research due to its well-defined genetic background, including elevated p53 protein levels and the expression of several oncogenes such as c-myc, K-ras, H-ras, and fos. SW480 cells produce carcinoembryonic antigen (CEA) and keratin and exhibit an epithelioid morphology, forming gland-like structures in xenografts. Although tumorigenic, SW480 cells are considered less metastatic and less resistant to apoptosis than their metastatic counterpart, SW620. Their limited metastatic potential makes them suitable for evaluating early-stage colorectal cancer therapies.

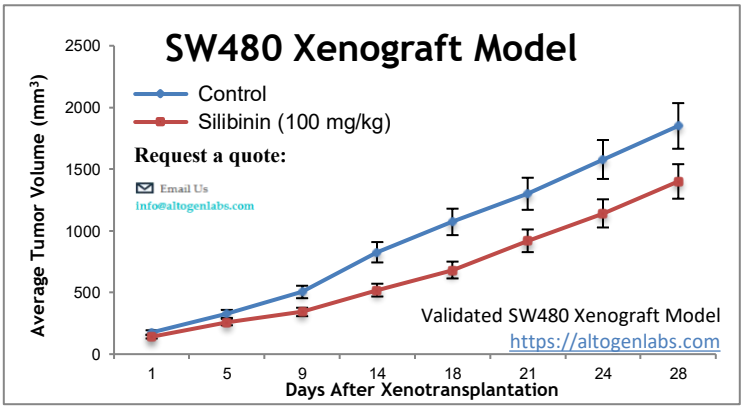

Figure 2. Tumor growth kinetics and chemotherapeutic evaluation of the Altogen Labs in-house validated SW480 xenograft model of colorectal cancer. Immunodeficient mice bearing subcutaneous SW480 tumors were randomized to receive treatment with silibinin (100 mg/kg) or vehicle control (buffer only). Data are presented as mean tumor volumes ± standard error of the mean (SEM).

The SW480 xenograft model has been utilized in various preclinical investigations, including studies targeting the β-catenin pathway, EGFR inhibition with cetuximab, and visualizing hypoxia-related pathways using GFP-linked HIF-1α expression systems. This model continues to serve as a platform for studying therapeutic agents that modulate cell proliferation, apoptosis, and signaling networks relevant to colorectal cancer progression. In the typical study design, SW480 cells are assessed for viability using trypan blue exclusion before being suspended in Matrigel and injected subcutaneously into the flank of immunocompromised mice at a concentration of 10,000 cells/μL in a 100-200 μL volume. Tumor growth is monitored using digital calipers, and mice are randomized into treatment groups once tumors reach a size of 75-125 mm³. Experimental compounds are administered according to a defined dosing regimen, with tumor size measured daily and body weight recorded three times weekly. Upon reaching the pre-specified tumor endpoint, animals are humanely euthanized, and tumors are harvested, weighed, and imaged. Tissues may be preserved in RNA-Later, snap frozen, or fixed in formalin for downstream analysis such as histology, RNA or protein isolation, and gene expression profiling. Conducted in a GLP-compliant, IACUC-regulated facility, Altogen Labs supports over 90 CDX and 30 PDX xenograft models, offering comprehensive preclinical services including toxicity testing, ELISA assay development, liposome encapsulation of nucleic acids, and generation of stable or inducible gene knockdown cell lines.

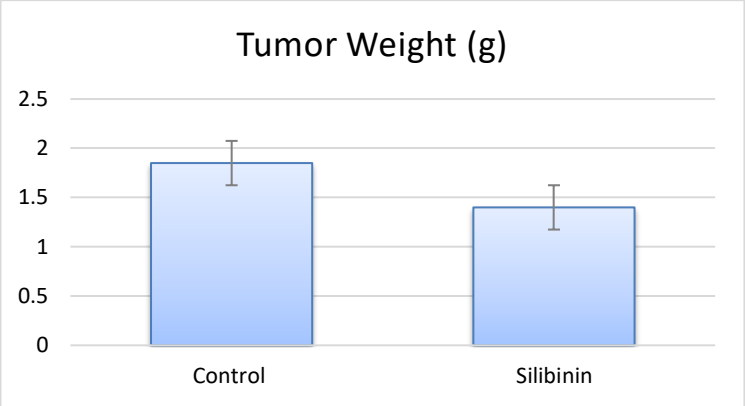

Figure 3. Tumor weights of SW480 xenografts harvested from mice treated with the silibinin (100 mg/kg) or vehicle control (buffer only). Tumor weights were recorded on Day 28 of the study and are presented as mean ± SEM. The study performed using Altogen Labs in-house validated SW480 xenograft model.

SW480 Subcutaneous Xenografts in Colorectal Cancer Research

Subcutaneous xenograft transplantation is a widely adopted preclinical approach for studying colorectal cancer, providing a reproducible and accessible platform for *in vivo* evaluation of tumor growth and therapeutic responses. The SW480 cell line, derived from a primary colon adenocarcinoma, is frequently used in these models due to its well-characterized mutations in APC, TP53, and KRAS, and its consistent tumor-forming capacity in immunodeficient mice. SW480-derived xenografts are particularly valuable for assessing drug resistance mechanisms, especially to chemotherapeutic agents such as 5-fluorouracil and oxaliplatin, reflecting clinically relevant treatment challenges. These tumors typically display histopathological features of epithelial adenocarcinomas and exhibit moderate growth kinetics, making them suitable for long-term studies involving tumor volume tracking, pharmacological intervention, and histological analysis.

Recent research has expanded the utility of SW480 xenografts by incorporating elements of the tumor microenvironment, including co-injection with cancer-associated fibroblasts or extracellular matrix components, to study stromal influence on signaling pathways and drug sensitivity. Additionally, these models have enabled the investigation of targeted therapies directed at Wnt, MAPK, and PI3K pathways, revealing connections between oncogenic mutations and therapeutic vulnerability. While the lack of immune components and anatomical relevance limits their translational capacity, subcutaneous SW480 xenografts remain foundational to preclinical oncology due to their consistency, scalability, and capacity for mechanistic exploration. As xenograft models evolve to better reflect tumor complexity, the SW480 subcutaneous system continues to play a central role in advancing our understanding of colorectal cancer biology and informing the development of more effective treatment strategies.

Orthotopic SW480 Xenografts for Colorectal Cancer Modeling

Orthotopic xenograft transplantation represents a critical advancement in preclinical modeling of colorectal cancer by allowing tumor cells to be implanted into their site of origin within the host animal. In contrast to subcutaneous models, orthotopic transplantation more accurately recapitulates the tumor microenvironment, including local tissue architecture, stromal interactions, and organ-specific factors that influence tumor behavior. When applied to the SW480 cell line, orthotopic models offer an opportunity to study early-stage colorectal cancer progression in a physiologically relevant setting. The orthotopic model using SW480 cells is particularly valuable for examining tumor-host interactions that regulate growth kinetics, angiogenesis, and immune modulation. Placement of tumor cells into the colonic or rectal wall facilitates access to native extracellular matrix components and local vasculature, which can significantly influence gene expression profiles and treatment response. This model also permits longitudinal monitoring of disease progression and

evaluation of therapeutic interventions under conditions that closely mimic human pathology. While orthotopic transplantation requires technical precision and often involves longer tumor latency periods compared to ectopic models, the increased biological fidelity justifies its use in mechanistic studies and translational research. Incorporating SW480 into orthotopic frameworks enhances the relevance of preclinical findings and strengthens the predictive value of experimental outcomes for informing clinical strategies in colorectal cancer.

Modeling Metastasis with SW480 Xenograft Transplants

Metastatic xenograft transplantation provides a critical platform for investigating the biological mechanisms that drive colorectal cancer dissemination and for evaluating novel anti-metastatic therapies. Although the SW480 cell line originates from a primary colorectal adenocarcinoma and is traditionally considered less aggressive in standard subcutaneous models, it can be adapted to study metastatic behavior when introduced into organ-specific environments or modified to mimic advanced disease states. These models are essential for replicating the multi-step cascade of metastasis, including local invasion, intravasation, circulation, extravasation, and colonization of distant organs. When used in appropriate transplantation settings, SW480 xenografts allow researchers to assess tumor cell plasticity, microenvironmental interactions, and the molecular transitions that underlie metastatic progression.

The application of SW480 cells in metastatic xenograft models enables the exploration of dynamic processes such as epithelial-to-mesenchymal transition, immune modulation, and extracellular matrix remodeling in a physiologically relevant context. By introducing SW480 cells into vascularized sites such as the spleen or tail vein, researchers can monitor their capacity to survive in circulation and establish secondary lesions in target organs, particularly the liver and lungs. These models are particularly useful for evaluating the efficacy of therapeutics aimed at interrupting metastatic spread and for identifying gene expression changes associated with the transition from localized to systemic disease. Although challenges remain in achieving consistent metastatic colonization, these models continue to advance our understanding of colorectal cancer metastasis and contribute to the development of strategies for therapeutic intervention and disease management.

Case Study: ZG16 Suppresses PD-L1 and Enhances Immune Activation in Colorectal Cancer

In a study published by *Translational Oncology* journal, Meng et al. investigate the immunomodulatory function of Zymogen Granule Protein 16 (ZG16) in colorectal cancer, focusing on its relationship with PD-L1 expression and innate immune activation. A central component of the experimental design is the use of SW480 cells, a well-characterized colorectal cancer line, to explore how ZG16 influences tumor-immune interactions. The authors demonstrate that ZG16 expression is inversely correlated with PD-L1 levels in colorectal tumor tissues and that overexpression of ZG16 in SW480 cells suppresses PD-L1 protein expression without altering its mRNA transcript levels. This post-transcriptional regulation suggests ZG16 may target glycosylated PD-L1 for degradation, potentially via its lectin-binding domain. *In vitro* assays further show that SW480 cells engineered to overexpress ZG16 (SW480-ZG16) promote the survival and proliferation of co-cultured natural killer (NK) cells and CD8+ T cells, primarily through upregulation of the NKG2D receptor. *In vivo*, ZG16 overexpression in SW480 xenografts resulted in reduced tumor volume and increased NKG2D expression within the tumor microenvironment, confirming its role in immune-mediated tumor suppression.

Patterns in the data consistently indicate that ZG16 functions as a negative regulator of immune checkpoint signaling and a promoter of innate immune activation. The inverse correlation between ZG16 and PD-L1 was substantiated through both IHC in patient samples and Western blotting in SW480 and HCT116 cells. Notably, the fact that ZG16 did not alter PD-L1 transcript levels but reduced protein expression implicates a post-translational mechanism, an insight that challenges the conventional assumption of transcriptional regulation in PD-L1 expression. The use of both SW480 and HCT116 strengthens the generalizability of findings, though the SW480 model was pivotal in demonstrating immune activation via NK cell and CD8+ T cell assays. Methodologically, the study is sound, employing authenticated cell lines, reproducible biological replicates, and both *in vitro* and *in vivo* models. However, limitations include a modest sample size in the animal study and the need for mechanistic validation of ZG16's interaction with PD-L1 glycoforms. Overall, this work positions SW480 as a valuable model for dissecting immune regulation in colorectal cancer and underscores ZG16 as a candidate biomarker for immunotherapy stratification.

Additional Case Study: TRIM67 Suppresses Notch Signaling and EMT in SW480 Cells

In their study published in *BMC Cancer* journal, Bond CE, et al. examine the tumor-suppressive function of PRDM5 in colorectal cancer, focusing on its ability to inhibit proliferation, invasion, and chemoresistance in SW480 cells. The authors demonstrate that PRDM5 expression is frequently downregulated in colorectal tumors and that its restoration in SW480 cells significantly reduces cell viability, colony formation, and invasive capacity. Apoptosis is notably increased, accompanied by the activation of caspase-3 and downregulation of the anti-apoptotic protein Bcl-2. Importantly, PRDM5 overexpression enhances the sensitivity of SW480 cells to 5-fluorouracil (5-FU), a frontline chemotherapeutic agent for colorectal cancer. *In vivo*, xenograft tumors formed from PRDM5-overexpressing SW480 cells display reduced size and elevated apoptotic activity, further supporting its tumor-inhibitory role.

The findings support the hypothesis that PRDM5 serves as a suppressor of colorectal tumor progression and a modulator of chemotherapy response. The study employs a combination of molecular assays, functional tests, and xenograft modeling, providing robust evidence for the impact of PRDM5 on SW480 cellular behavior. While the data are compelling, the investigation is limited by its use of a single cell line and lacks mechanistic depth regarding PRDM5's downstream signaling networks. Nonetheless, the results suggest that PRDM5 could function as both a predictive biomarker and therapeutic target in colorectal cancer, particularly in patients exhibiting poor response to 5-FU. Further research should explore PRDM5-mediated regulatory pathways and validate these findings across diverse cellular and clinical models.

Regulation of Smad2 and EMT by miR-145 in SW480 Cells

SW480 colorectal cancer cells display well-characterized changes in behavior and molecular phenotype in response to modulation of epithelial-mesenchymal transition (EMT) regulators. Upregulation of miR-145 in these cells leads to a significant reduction in proliferation and migratory capacity, which is accompanied by decreased expression of mesenchymal markers such as vimentin and N-cadherin. In parallel, the epithelial marker E-cadherin is upregulated, indicating a shift toward an epithelial phenotype and suppression of EMT. These changes reflect a reprogramming of cellular identity that aligns with reduced invasiveness and metastatic potential. At the molecular level, miR-145 directly downregulates Smad2 expression, a key mediator of the TGF-β signaling pathway, implicating this microRNA in the control of transcriptional networks that drive tumor progression.

The relationship between miR-145 and tumor-suppressive activity in SW480 cells highlights the importance of microRNA-mediated regulation in colorectal cancer biology. By restoring miR-145 levels, it is possible to reverse the mesenchymal phenotype and impair cellular mechanisms associated with metastasis. These findings are supported by consistent alterations in gene and protein expression, as well as reduced performance in functional assays measuring cell migration and invasiveness. Techniques such as qPCR, western blotting, and transwell migration assays provide the necessary resolution to confirm these phenotypic effects. Although this insight is primarily drawn from *in vitro* models, the data suggest that miR-145 has significant therapeutic potential. Future research may focus on evaluating miR-145 analogs or delivery systems to assess their ability to inhibit EMT in more complex models of colorectal cancer. The SW480 cell line continues to serve as a valuable platform for dissecting these molecular interactions and advancing translational applications in cancer therapy.

CRMP-4 Knockdown Suppresses SW480 Cell Proliferation and Tumor Growth

CRMP-4 is a cytoplasmic protein that contributes to the regulation of cell proliferation and tumor progression in colorectal cancer. In SW480 colorectal cancer cells, elevated expression of CRMP-4 has been linked to enhanced cell growth and tumorigenicity. When CRMP-4 expression is silenced using RNA interference techniques, there is a notable reduction in both mRNA and protein levels, which correlates with decreased cellular proliferation. Functional assays such as MTT and BrdU incorporation consistently show impaired growth in SW480 cells following CRMP-4 knockdown. *In vivo*, SW480 cells with suppressed CRMP-4 expression form smaller tumors in xenograft models, confirming its role in promoting tumor expansion. These findings suggest that CRMP-4 functions as a positive regulator of colorectal cancer cell proliferation, likely through intracellular pathways that influence cell cycle progression and survival.

The consistency of these data across multiple experimental systems highlights CRMP-4 as a potential molecular target for therapeutic intervention in colorectal cancer. The SW480 cell line, with its well-defined genetic background, serves as a suitable model to investigate such regulatory mechanisms. While the reduction in tumor growth following CRMP-4 inhibition is well established, additional research is needed to identify the downstream signaling pathways and molecular interactions governed by this protein.

SW480 as a Model for Oncogenic Pathway Analysis

SW480 colorectal cancer cells provide a valuable system for examining the regulatory roles of specific oncogenes in tumor progression. KRAS, a proto-oncogene frequently mutated in colorectal cancer, exhibits constitutive activation in SW480 cells due to a missense mutation. This results in persistent signaling through downstream pathways such as MAPK and PI3K, contributing to increased proliferation, resistance to apoptosis, and enhanced invasive potential. Experimental silencing of KRAS in SW480 cells significantly reduces their growth rate, migration ability, and colony-forming efficiency, highlighting its essential role in maintaining the malignant phenotype. The expression of epithelial-mesenchymal transition (EMT) markers is also altered following KRAS knockdown, with reduced vimentin and increased E-cadherin, suggesting that KRAS contributes directly to the mesenchymal, motile phenotype characteristic of invasive colorectal tumors.

In addition to KRAS, the expression of other oncogenes such as survivin and c-Myc is elevated in SW480 cells. Survivin, a member of the inhibitor of apoptosis (IAP) family, is linked to resistance against apoptotic stimuli and promotes survival in response to stress or chemotherapeutic agents. c-Myc functions as a transcriptional regulator of genes involved in cell cycle progression and metabolism and is consistently overexpressed in these cells. The combined effect of these oncogenes creates a signaling environment that favors unchecked proliferation, escape from programmed cell death, and increased adaptability. The methodologies used to obtain these findings include gene silencing through siRNA, immunoblotting for protein detection, and functional assays for migration, invasion, and growth.

Altogen Labs offers a comprehensive suite of preclinical laboratory services employing over 90 validated cell line-derived xenograft (CDX) models and more than 30 patient-derived xenograft (PDX) models to support oncology drug development. In staged xenograft studies, dosing of the experimental compound is initiated when tumors reach a predefined average volume, typically between 75 and 125 mm^3, allowing for consistent baseline measurements across treatment groups. In unstaged studies, dosing begins 4 to 5 days following xenotransplantation, enabling evaluation of early drug effects. All animal procedures are performed in an IACUC-regulated and GLP-compliant facility to ensure ethical and scientific rigor. Clients are provided with a comprehensive report that includes detailed methodologies, statistical analyses, raw data, and interpretive discussion of results. Altogen Labs also offers a variety of molecular and cellular analysis services, such as tissue harvesting, histology, total protein and RNA isolation, and gene expression profiling to support downstream biomarker discovery and mechanistic studies.

Figure 4. Overview of *in vivo* xenograft services featuring the SW480 colorectal cancer model, highlighting its use in preclinical drug efficacy and toxicity studies (Altogen Labs).

For researchers utilizing the SW480 xenograft model, Altogen Labs provides a broad range of experimental endpoints and procedural customizations tailored to specific therapeutic objectives. Study options include tumor growth delay (TGD) and tumor growth inhibition (TGI) analyses to quantify treatment efficacy, with adjustable dosing schedules and administration routes such as intravenous, intraperitoneal, oral gavage, subcutaneous, intratumoral, and intramuscular injections. Advanced engraftment techniques are also available, including orthotopic implantation and metastatic modeling via tail vein, left ventricular, or mammary fat pad injection. Additional capabilities include immunohistochemical staining of SW480 tumor tissue, comprehensive blood chemistry assessments, gross necropsy, and detailed histopathological examination. Toxicological evaluations and survival analysis can be integrated to assess systemic effects and treatment tolerability. Optional positive control groups employing cyclophosphamide at 50 mg/kg administered intramuscularly allow for benchmarking of investigational compounds. High-resolution imaging services, including fluorescence-based whole-body imaging, are available to monitor tumor burden and biodistribution in real time.

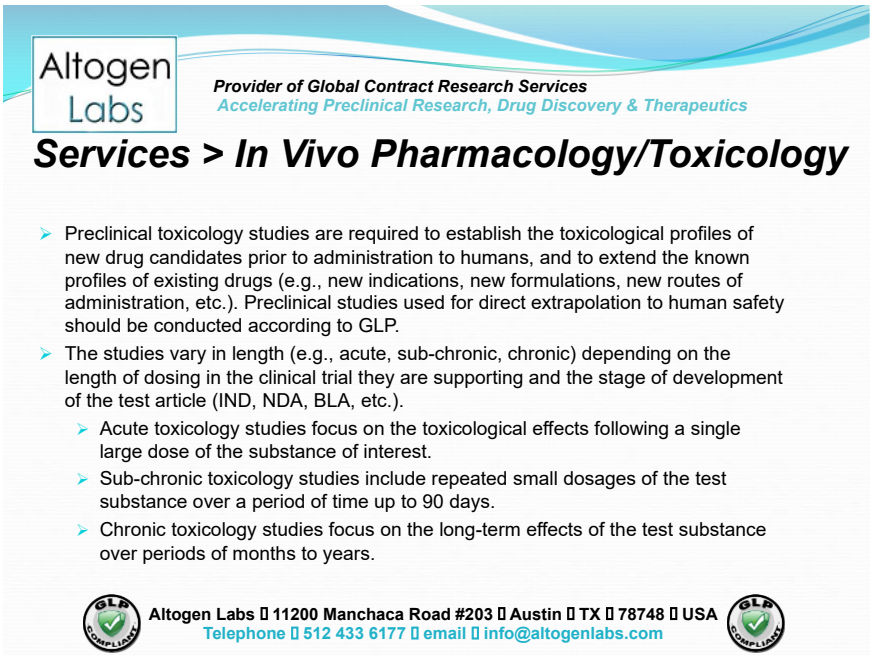

Figure 5. Summary of *in vivo* pharmacology and toxicology services, including acute, sub-chronic, and chronic study designs used to evaluate the safety profiles of test substances under GLP-compliant conditions (Altogen Labs).

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Keywords: SW480, colon, colorectal, xenograft, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, colon cancer, PDX, CDX, metastatic, orthotopic