The Pro-Apoptotic Effects of Curcumin on HCT116 Colorectal Cancer Cells

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Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, often originating from benign polyps in the colon and rectum that may become malignant if left untreated. While current treatments such as surgery, chemotherapy, and radiation are effective, they often come with severe side effects that impact patient quality of life. This has spurred interest in natural compounds like curcumin, which may offer therapeutic benefits with fewer adverse effects. Curcumin, the main active compound in turmeric, has demonstrated anti-inflammatory, antioxidant, and anticancer properties in various studies, suggesting its potential as an adjunct therapy for CRC. This study investigates the effects of curcumin and 5-Fluorouracil (5-FU), a chemotherapy drug commonly used to treat CRC, on HCT116 colorectal cancer cells. 5-FU works by DNA integration requiring extensive repair, ultimately leading to cell death in cells lacking effective DNA-damage sensing and repair. Cells were treated with varying concentrations of curcumin and 5-FU, followed by viability and apoptotic assays using the eBioscience™ Annexin V Apoptosis Detection Kit, the SYTOX™ viability assay, and by observing morphological changes under the microscope. The primary goal is to determine the LD_{50} concentration of curcumin and observe morphological changes at that concentration. This research aims to provide insights into curcumin's potential as a safe adjunct or alternative to current CRC therapies. By identifying effective concentrations and comparing their effects to those of 5-FU, the study seeks to contribute to more informed treatment strategies for CRC.

Colorectal cancer (CRC) is a form of cancer that affects the colon or rectum, part of the large intestine. It is the third most diagnosed cancer in the United States with 141,902 new cases reported in 2022.[1] Globally, CRC accounted for 16.5 million cases in 2015, and this number is projected to rise to over 2.2 million by 2030.[2] While CRC is most prevalent in Northern and Western Europe and the United States, it is less common in regions such as Africa, Asia, and India.[3] One of the significant challenges in CRC treatment is the lack of clear symptoms at the time of diagnosis (often by routine screening), which leads to delayed detection at advanced stages. Later-stage diagnosis limits treatment effectiveness and significantly reduces survival rates.[3]

The development of colorectal cancer is complex and multifactorial, influenced by factors within the tumor microenvironment. Disruptions in the gut microbial profile, along with a compromised intestinal barrier, can lead to inflammation in the intestines, which plays a key role in the initiation and progression of CRC.[4] Most cases of colorectal cancer begin as neoplastic polyps, benign growth in the mucus-secreting epithelial cells of the colon. While the exact cause of these polyps is unclear, genetic and environmental factors, such as diet and lifestyle, are believed to play a key role in their development.[5]

One critical genetic factor in the pathogenesis of CRC is the mutation of the adenomatous polyposis coli (APC) gene. This tumor suppressor gene plays an essential role in early colorectal tumor development. The mutation and inactivation of this gene represent significant early events specifically linked to the development of colorectal tumors.[6] Mutations that inactivate the APC gene are present in approximately 80% of human colon tumors. Heterozygosity of these mutations leads to autosomal dominant predispositions to colon cancer in humans.[7]

Risk factors associated with the development of CRC include smoking, a diet high in red meats, obesity, alcohol consumption, physical inactivity, and a low intake of dietary fiber.[8] Current treatment options for CRC consist of chemotherapy (regimens such as FOLFOX and FOLFIRI), radiotherapy, and surgical interventions. These treatments are often accompanied by severe side effects, low long-term survival rates, and high rates of cancer recurrence.1 Given these limitations, there is a growing interest in exploring safer modifications to medications and dietary interventions.

Turmeric is a spice known for its diverse health benefits, including antioxidant, antimutagenic, antimicrobial, anticancer, and anti-inflammatory effects.[9] These benefits appear to come from a polyphenolic compound called curcumin. Due to its conjugated double bonds, curcumin acts as an effective electron donor and has been shown to improve systemic markers of oxidative stress by scavenging free radicals, such as reactive oxygen and nitrogen species.[10] This antioxidant activity is critical in preventing DNA damage and lipid peroxidation, which are precursors to oncogenesis.[11]

Studies have observed curcumin's anticancer properties in the context of CRC. In vitro studies on human colon cancer cell lines have shown curcumin's ability to inhibit cell proliferation by inducing cell cycle arrest at the G1 and G2/M phases.[12] Additionally, curcumin promotes apoptosis by targeting multiple molecular pathways in cancer progression.[12] For example, one

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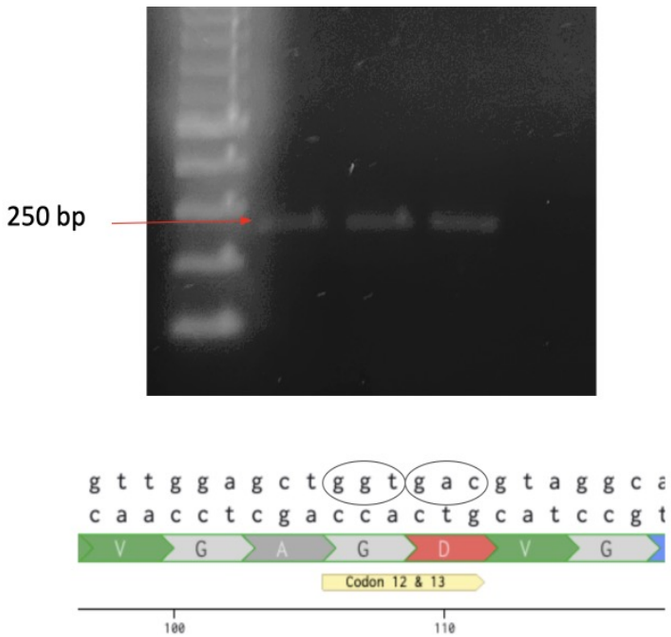

Figure 1 | Gel electrophoresis showing PCR amplification of the KRAS gene from HCT116 colorectal cancer cells. A band at approximately 250 base pairs confirms the successful amplification of the region containing the G13D mutation.

in vitro study on HCT-116 colon cancer cells demonstrates that curcumin induces the production of reactive oxygen species, leading to the downregulation of E2F4, a transcription factor involved in cell growth. This disruption in the cell cycle ultimately leads to apoptosis in HCT-116 cells.[13]

Additionally, an in vivo study was conducted on a mouse model, where the mice were fed diets containing curcumin concentrations of 0.1%, 0.2%, and 0.5% over 15 weeks. The results indicated that the 0.1% concentration did not have significant effects, while the 0.2% and 0.5% concentrations led to a 39% and 40% decrease, respectively, in the number of tumors compared to untreated mice. Additionally, this study suggests that curcumin may be a valuable chemo-preventive agent for human intestinal malignancies associated with APC gene mutations.[14]

Despite curcumin's potential as an anticancer agent, there are challenges related to its solubility and bioavailability in the gastrointestinal tract, limiting its clinical effectiveness.[11] Through the development of nano curcumin, the bioavailability and anticancer activity has been enhanced compared to curcumin, making nano curcumin a more effective option for clinical use.[11]

In addition to its direct anti-cancer properties, curcumin has the potential to alleviate chemotherapy-related side effects. Chemotherapy for CRC is often accompanied by nausea, diarrhea, constipation, neutropenia, and weight loss.[15] Studies have shown that curcumin can protect mitochondria by preventing mitochondrial damage, enhancing the activity of mitochondrial complex enzymes, and reducing oxidative stress. As a result, curcumin can help mitigate some of the side effects of chemotherapy.

Altogether, the potential of curcumin mediating significant anti-cancer properties by inducing apoptosis, reducing tumor growth, and alleviating chemotherapy-related side effects in colorectal cancer make it an attractive drug candidate.

Materials and Methods:

Cell Culture of HCT116 Cells

HCT116 colorectal cancer cells (obtained previous lab member, Allayah Stillwell)[16] were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were maintained at 37°C in a humidified 5% CO_2 incubator. Cells were subcultured every 2–3 days when they reached 70–80% confluence. For passaging, cells were detached using 0.25% trypsin EDTA (1X). The cells were initially at passage #4, and some were cryopreserved at passage #7 in 95% complete media and 5% DMSO, with a final concentration of 1.6×10^6 cells per vial for later use.

Cell Imaging

Cell imaging was performed using an Invitrogen™ EVOS™ XL Core Configured Cell Imager with a mechanical stage. Images were acquired using the appropriate objective lens and analyzed using the built-in software provided by the EVOS™ system.

Cell Counting

Cell counting was performed after trypsinization and prior to cell passage. HCT116 colorectal cancer cells were resuspended in complete growth medium (DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin). A 1:1 dilution was prepared by mixing 100 µL of the cell suspension with 100 µL of 0.4% Trypan Blue solution (Gibco; Lot #2517874) and incubated at room temperature for 3 minutes. The mixture was loaded onto a hemocytometer, and viable (unstained) and non-viable (blue-stained) cells were counted manually in the four corner quadrants under a light microscope using a 10× objective. The total cell concentration was calculated using the following formula:

Cell concentration (cells/mL) = average cell count per quadrant × 10^4 × dilution factor.

% Viability = (number of viable cells / total number of cells) × 100.

These values were used to determine the appropriate seeding density for further experiments.

Reagents For Treatments

Curcumin (Fisher Scientific; Cat. No. NBP2262435G) was initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 10 mM stock solution. The solution was aliquoted and stored at –4°C, protected from light. For cell treatment, the stock solution was diluted in complete culture medium to achieve final concentrations ranging from 0 µM to 100 µM.

5-Fluorouracil (5-FU; Thermo Scientific; Lot: L18E028) was dissolved in DMSO to create a fresh stock solution immediately prior to each treatment. This stock was subsequently diluted in culture media to reach the desired final concentration for each experimental condition.

DMSO (ChemCruz; Lot: G0816) was used as the vehicle control for both curcumin and 5-FU treatments and for preparation of their respective stock solutions. To account for potential cytotoxic effects of

DMSO, the final DMSO concentration was standardized and kept consistent across all treatment groups, including vehicle controls.

MTT Assay for Cell Viability

Cell viability was assessed using the MTT assay (Biosynth; CAS 298-93-1). The MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was prepared as a 5 mg/mL stock solution in sterile phosphate-buffered saline (PBS), protected from light, and stored at 4°C for up to 1–2 weeks before use.

HCT116 cells were seeded into 96-well plates at a density of 10,000 cells per well and incubated for 24 hours at 37°C in an incubator with 5% CO_2 to allow for adherence. After 24 hours, the culture medium was aspirated, and the cells were treated with the desired compounds for 24 hours or the designated treatment duration.

Following treatment, the medium was removed, and 100 µL of MTT working solution (0.5 mg/mL in DMEM, freshly prepared from the stock) was added to each well. Plates were incubated for 4 hours at 37°C to allow viable cells to reduce the MTT reagent to insoluble formazan crystals. After incubation, the MTT solution was aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Plates were gently agitated for 10 minutes to ensure complete solubilization.

Absorbance was measured at 490 nm using a microplate spectrophotometer. Wells containing media without cells were used as negative controls to account for background absorbance. The absorbance values were directly proportional to the number of viable cells, with higher absorbance indicating greater cell viability.

Flow Cytometry for Apoptosis Detection

Apoptosis was evaluated using the Invitrogen™ eBioscience™ Annexin V Apoptosis Detection Kit (Lot #2904486), which contains Annexin V and 7-aminoactinomycin D (7-AAD) for the discrimination of viable, apoptotic, and necrotic cells.

Following treatment, HCT116 cells were harvested by trypsinization and washed twice with phosphate-buffered saline (PBS) to remove residual media. Cells were resuspended in 1X Binding Buffer (provided in the kit) at a density of approximately 1 × 10^6 cells per 100 µL. Staining was performed by adding 5 µL of Annexin V-FITC and 5 µL of 7-AAD to the cell suspension, followed by incubation for 10–15 minutes at room temperature in the dark.

After staining, 200 µL of 1X Binding Buffer was added to each sample to stop the reaction. Samples were immediately ana-

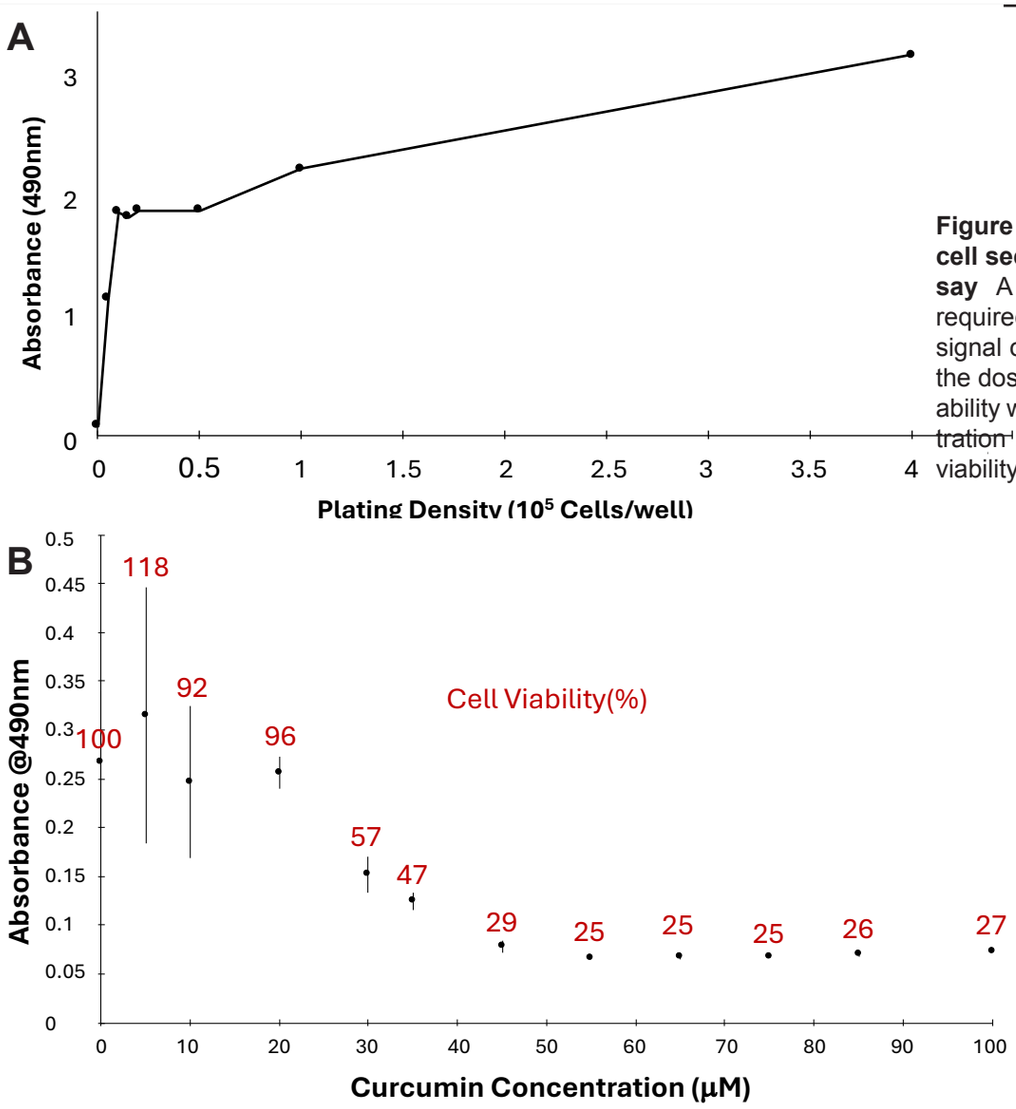

Figure 2 | Optimization of HCT116 cell seeding density and the MTT assay A demonstrates the cell densities required to obtain a strong and reliable signal over the assay time. B illustrates the dose-dependent decrease in cell viability with increasing curcumin concentration (x axis in black) with calculated viability overlaid in red.

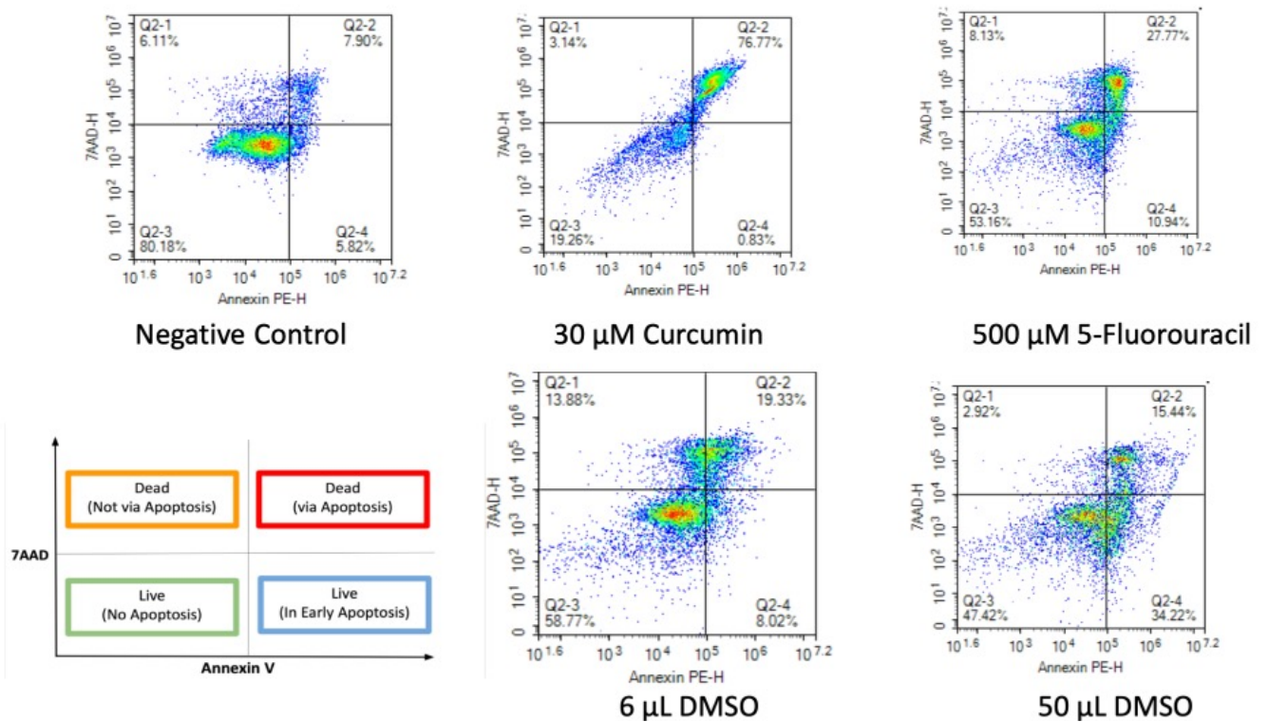

Figure 3 | Detection of apoptosis in HCT116 cells by Annexin V/7-AAD staining following treatment with curcumin and 5-fluorouracil (5-FU). HCT116 cells were treated for 48 hours with either 30 µM curcumin, 500 µM 5-FU, or DMSO controls. Apoptotic and necrotic populations were assessed using flow cytometry, with gates as follows: Q1: dead cells (not via apoptosis), Q2: dead cells via apoptosis, Q3: viable cells, and Q4: early apoptotic cells. Both curcumin and 5-FU treatments increased the proportion of apoptotic cells compared to untreated and DMSO control groups, with curcumin showing a greater extent of apoptosis induction.

lyzed by flow cytometry using a NovoCyte Flow Cytometer (Agilent Technologies). Fluorescence intensities were used to determine the percentage of cells in the following categories:
viable (Annexin V- / 7-AAD-),
early apoptotic (Annexin V+ / 7-AAD-),
late apoptotic (Annexin V+ / 7-AAD+),
and necrotic (Annexin V- / 7-AAD+).

Negative controls (media only) and vehicle controls (DMSO, at volumes equivalent to those used in curcumin and 5-FU treatments) were included to account for background fluorescence and potential solvent effects on apoptosis. Data acquisition and quadrant-based analysis were conducted using NovoExpress software (Agilent Technologies), and results were reported as scatterplots indicating the percentage of cells in each category.

Results

The identity of the HCT116 cell line was confirmed through PCR of a portion of the KRAS gene and electrophoresis on a 2% agarose gel (**Figure 1A**). The resulting single, 250bp band was extracted from the gel and sent for Sanger sequencing, which revealed the characteristic G13D mutation at codon 13, consistent with published HCT116 genomic profiles (**Figure 1B**). This confirmation ensured experimental validity for all subsequent treatments.

Optimization of Viability Assay Conditions

Preliminary optimization of the MTT assay determined that a seeding density of 10,000 cells per well yielded reproducible absorbance readings within the linear range of detection (See **Figure 2A**), avoiding the signal saturation observed at higher densities (≥20,000 cells/well).

Effects of Curcumin on Cell Viability

Treatment of HCT116 cells with increasing concentrations of curcumin (0–100 µM) for 23 hours produced a clear dose-dependent decrease in metabolic activity as measured by the MTT assay See Figure 2B). The calculated LD_{50} was approximately 30 µM. At concentrations ≥50 µM, viability dropped below 20% of the untreated control. DMSO vehicle controls maintained >90% viability at concentrations equivalent to those used for curcumin delivery, although mild cytotoxicity (<10% reduction in viability) was observed at the highest DMSO volume used (**Figure 2C&D**).

Morphological Observations

Phase-contrast imaging supported the cytometric findings. Curcumin-treated cells displayed hallmark apoptotic morphology, including cell rounding, shrinkage, membrane blebbing, and detachment from the substrate. 5-FU-treated cells exhibited apoptotic bodies but retained partial adherence to the plate surface, suggesting slower or less extensive detachment. Untreated negative controls maintained normal epithelial-like morphology, while DMSO controls appeared morphologically comparable to untreated cells, with minor rounding at the highest DMSO levels, but few apoptotic bodies evident (See **Figure 3**).

Comparison of Curcumin and 5-FU Cytotoxicity

Flow cytometry following 48-hour treatments revealed differential patterns of cell death. **Figure 4** illustrates how curcumin at 30 µM induced pronounced apoptosis, with

approximately 45–55% of cells in the late apoptotic (Annexin V+/7-AAD+) quadrant and a smaller fraction (~10–15%) in early apoptosis (Annexin V+/7-AAD-). In contrast, 5-FU at 100 µM produced a moderate apoptotic response, with ~25–30% late apoptotic cells and a similar proportion in early apoptosis. Necrotic cell percentages remained low (<5%) in both treatments, indicating that cell death was primarily apoptotic rather than necrotic.

Overall, curcumin exerted stronger and more rapid pro-apoptotic effects on HCT116 cells than 5-FU under the tested conditions, with both agents acting primarily through apoptotic rather than necrotic pathways.

Discussion

This study provides evidence that curcumin induces significant cytotoxic effects in HCT116 colorectal cancer cells, primarily through apoptosis. Flow cytometric analysis using Annexin V/7-AAD staining revealed that 48-hour curcumin treatment led to a substantial increase in late apoptotic cell populations compared to both the vehicle control and 5-fluorouracil (5-FU), a clinically used chemotherapeutic agent. The pro-apoptotic activity of curcumin was further supported by morphological changes observed under microscopy, including cell rounding, shrinkage, detachment, and the presence of apoptotic bodies—all characteristic of programmed cell death. In contrast, 5-FU treatment resulted in fewer cells undergoing apoptosis at this timepoint, with many remaining adherent and intact, indicating a less robust apoptotic response under the tested conditions.

The dose-response curve from the MTT assay demonstrated that curcumin's cytotoxicity is dose-dependent, with a plateau observed after 50 µM, suggesting saturation of its effect. The calculated LD_{50} of approximately 30 µM is consistent with previously reported values in literature for HCT116 cells, further validating the sensitivity of this cell line to curcumin. Notably, the vehicle control (DMSO) induced early apoptosis at higher concentrations, emphasizing the importance of minimizing solvent concentrations in future experimental designs.

Taken together, these findings reinforce curcumin's potential as an anti-cancer agent capable of inducing apoptosis more effectively than 5-FU in vitro.

However, the differential response also highlights the need for further mechanistic studies to elucidate curcumin's molecular targets and assess its therapeutic synergy or resistance mechanisms when combined with conventional chemotherapy.

Future Direction

There are several directions this project could take in future studies. One important area for investigation is the comparison of curcumin and 5-FU treatments on colorectal cancer cells (HCT116) versus normal colon epithelial cells. By testing these treatments on normal cells, we can determine if they selectively target cancer cells or have similar effects on healthy cells. This would be valuable in understanding whether curcumin and 5-FU could be used in a way that minimizes damage to normal tissue (*i.e.*, results in a favorable therapeutic window), which is a major consideration in cancer therapy.

Another area to explore is the effect of longer treatment periods on cell viability. In this study, curcumin and 5-FU treatments were tested for 24 hours and 48 hours. However, it would be useful to extend the treatment times to 60 or 72 hours to see if a longer exposure period increases the cytotoxic effects on cancer cells. This could help determine the optimal length of treatment for achieving the best results in terms of cell viability and apoptosis.

Finally, a promising area for future research is testing combination treatments. Combining curcumin with 5-FU or other chemotherapy drugs could potentially lead to synergistic effects, where the combination is more effective than either treatment alone. This could improve the overall effectiveness of the treatment and reduce the required doses of each drug, minimizing side effects for patients.

Investigating combination therapies could lead to new strategies for enhancing the efficacy of current cancer treatments.

In conclusion, future research should focus on testing the selectivity of curcumin and 5-FU on normal versus cancer cells, exploring the effects of longer treatment times, and evaluating the potential benefits of combination therapies. These studies could provide important information for improving colorectal cancer treatment.

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Author's Biography

Kuljeet Kaur is a senior in Applied Biological Sciences at KU Edwards. Through her research and clinical experiences, she has gained valuable skills and a deeper understanding of how biology translates to real-world applications. She is eager to apply her knowledge to contribute to healthcare, research, and community-driven initiatives.

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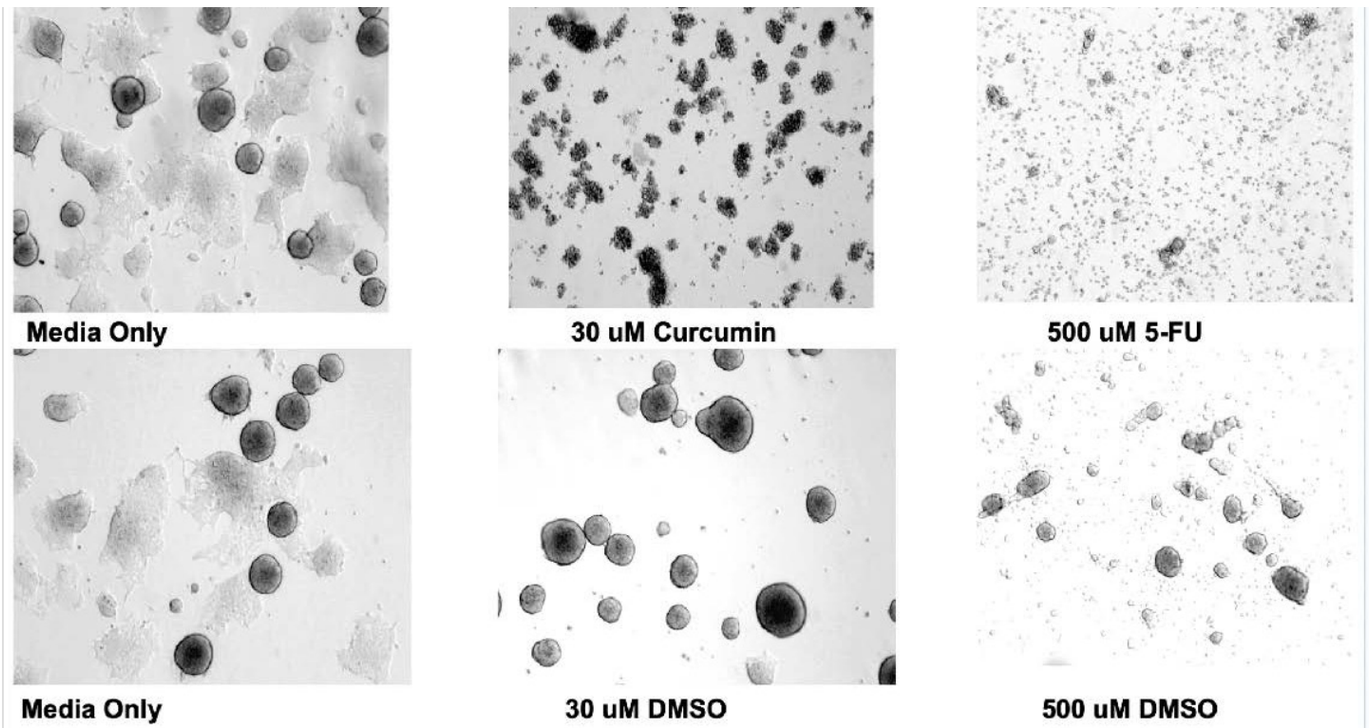

Figure 4 | Morphological changes in HCT116 cells following treatment with curcumin and 5-fluorouracil (5-FU). Cells were treated for 48 hours with 30 μM curcumin or 500 μM 5-FU. DMSO controls (6 μL and 50 μL) were included to match the solvent volumes used in the curcumin and 5-FU treatments, respectively. Curcumin-treated cells appeared smaller and more rounded compared to untreated controls, with frequent aggregation into clusters and the presence of floating cells, indicating detachment. In 5-FU-treated cells, extensive morphological changes were observed, including a pronounced reduction in cell size, fragmentation into apoptotic bodies, and a dramatic loss of confluence, more severe than that seen with curcumin treatment.