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Combination of 5-Fluorouracil and N^1,N^{11}-Diethylnorspermine Markedly Activates Spermidine/Spermine N^1-Acetyltransferase Expression, Depletes Polyamines, and Synergistically Induces Apoptosis in Colon Carcinoma Cells*

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Abstract

The thymidylate synthase inhibitor 5-fluorouracil (5-FU) is used widely for chemotherapy of colorectal carcinoma. Recent studies showed that 5-FU affects polyamine metabolism in colon carcinoma cells. We therefore examined whether combinations of 5-FU with drugs that specifically target polyamine metabolism, *i.e.* N^1,N^{11}-diethylnorspermine (DENSPM) or α-difluoromethyl-ornithine (DFMO), have synergistic effects in killing HCT116 colon carcinoma cells with wild-type or absent p53. Our results showed that simultaneous 5-FU and DENSPM, a spermine analogue, synergistically increased transcript levels of the polyamine catabolism enzyme spermidine/spermine N^1-acetyltransferase, depleted spermine and spermidine, increased acetylated spermidine, and produced synergistic tumor cell apoptosis in both p53 wild-type and p53-null variants. By contrast, simultaneous combination of 5-FU with DFMO, an inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase, depleted putrescine but did not produce synergistic cell killing. Some pre-treatment and post-treatment regimens of DENSPM and DFMO were antagonistic to 5-FU depending on cellular p53 status. Protein and transcriptome expression analysis showed that combined 5-FU and DENSPM treatment activated caspase 9, but not caspase 3, and significantly suppressed NADH dehydrogenases and cytochrome *c* oxidases, consistent with the observed increase in hydrogen peroxide, loss of mitochondrial membrane potential, and release of cytochrome *c*. Our findings demonstrate the importance of the polyamine pathway in 5-FU effects and suggest that the combination of 5-FU with DENSPM has potential for development as therapy for colorectal carcinoma.

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The fluoropyrimidine 5-fluorouracil (5-FU)[1] has been widely used a chemotherapy agent in the treatment of many types of solid tumors (1–3). To improve the efficacy of 5-FU, several other drugs such as leucovorin, irinotecan, or oxaliplatin have been used recently in combination with 5-FU in preclinical and clinical trials that have produced promising results (4 – 6).

5-FU achieves its therapeutic effect by several known mechanisms. It binds to and inhibits thymidylate synthase, which converts 2-deoxyuridine to thymidylate, thus interfering with DNA synthesis. 5-FU incorporates into RNA, leading to disruption of RNA processing (7–9). Our recent functional genomic studies have rediscovered that 5-FU also affects polyamine pathways to reduce the levels of polyamines in treated colon cancer cells (10), an observation that was originally made by Russell *et al.* in 1974 in a liver cancer xenograft mouse model (11). Thus, it appears that 5-FU targets two major cellular processes, *i.e.* polyamine metabolism as well as processing and synthesis of DNA and RNA. These two processes may have intimate inter-relationships because polyamines bind DNA and RNA and affect chromosomal structure, RNA stability, and translation (12).

Spermidine, spermine, and their diamine precursor putrescine are natural polyamines in living organisms and play critical roles in proliferation, differentiation, and homeostasis in both normal and cancer cells. The balance between polyamine biosynthesis and catabolism is tightly regulated to maintain cellular polyamine levels. Mice with disrupted genes important for polyamine biosynthesis, such as ornithine decarboxylase, die early in embryonic development (13). Insufficient levels of polyamines result in retarded cell growth and sometimes cell death. Therefore, cellular polyamine depletion through inhibition of the polyamine biosynthetic pathway has been used for chemotherapeutic intervention (14 –16). DFMO, which is a suicide inhibitor of ornithine decarboxylase, the enzyme that decarboxylates ornithine to produce putrescine, is a well studied inhibitor of the biosynthetic pathway. However, clinical trials using DFMO yielded disappointing results (17–19), perhaps because polyamines are often present at high levels in human diet and intestinal lumen, thus bypassing the suppression of polyamine biosynthesis (20). Nevertheless, because of the low toxicity of DFMO, it has been tested as a chemopreventive agent for colon carcinoma (21).

Interest in polyamine metabolism has shifted to its catabolism, especially because catabolism has been associated with the anti-tumor effects of several polyamine analogues. DENSPM is an analogue of spermine that has been shown to deplete polyamines by inducing expression of the polyamine catabolism enzyme SSAT through Nrf-2 and polyamine-modulated factor-1 interaction on the *SSAT* promoter (22, 23). SSAT acetylates spermine and spermidine, which in turn are substrates for a flavin-dependent polyamine oxidase to produce hydrogen peroxide that is believed to account for the cytotoxicity of DENSPM (24, 25). However, the cytotoxic effect of DENSPM is not observed in all cell types and is often observed only after long exposure, suggesting that the cytotoxicity of DENSPM alone is not very potent.

In this study, we investigated whether combinations of 5-FU with drugs that modulate polyamine metabolism may have synergistic effects in inducing colon cancer cell apoptosis. In addition, to evaluate the role of p53 in the response to combination therapy, we used two

[1]The abbreviations used are: 5-FU, 5-fluorouracil; CI, combination index; CM-H_2DCFDA, 5,6-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; DENSPM, N^1,N^{11}-diethylnorspermine; DFMO, α-difluoromethylornithine; IC_{25}, 25% of inhibitory concentration; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2*H*-tetrazolium; Rho123, rhodamine 123; SSAT, spermidine/spermine N^1-acetyltransferase; wt-p53, wild-type p53; PBS, phosphate-buffered saline.

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isogenic HCT116 colon cancer cell lines that were either wild-type for *p53* (wt-p53) or had the *p53* gene deleted (p53-null) (26, 27).

EXPERIMENTAL PROCEDURES

Reagents

5-FU, DFMO, and antioxidant GSH were purchased from Sigma, and DENSPM was purchased from Tocris (Ellisville, MI). 5-FU was dissolved in Me_2SO, and DFMO and DENSPM were dissolved in water according to the manufacturer's instructions. CM-H_2DCFDA and Rho123 were purchased from Molecular Probes (Eugene, OR) and dissolved in Me_2SO.

Cell Culture

The isogenic HCT116 cell lines with wt-p53 or p53-null background were provided by Dr. Bert Vogelstein (Johns Hopkins University). The cell lines were cultured in Dulbecco's modified Eagle's medium with 10% Nuserum (Collaborative Research Products, Bedford, MA) at 37 °C in a 5% CO_2 incubator. Because fetal bovine serum has abundant thymine and polyamines, we used dialyzed serum to remove these moieties that would interfere with the experimental results. Later experiments showed that fetal bovine serum did not affect the pattern of cellular responses but did shift the dose of drugs required to achieve the same results (data not shown).

MTS Cell Viability Assay

CellTiter 96 Aqueous One Solution Reagent (Promega, G358B, Madison, WI) was used to detect viable cells in cytotoxicity experiments. After 20 μl of MTS solution were added into each well, the cells were incubated at 37 °C with 5% CO_2 for 2 h before absorbance at 490 nm was measured with a Micro-plate reader (MRX, Danatech Laboratory, Houston, TX). All the MTS assays included 3 to 5 replicates for each treatment condition, and the experiments were repeated at least twice.

Drug Treatment Experiments

A total of 3000 cells per well in a volume of 100 μl were plated in each 96-well plate. On the second day, various concentrations of 5-FU were added to the wells, and MTS assays were performed to estimate IC_{25} values (2.5 μM for HCT116 wt-p53 and 15 μM for HCT116 p53-null). Twenty-four hours after the initial cell seeding, the IC_{25} dose of 5-FU alone or combinations with various concentrations of DENSPM (1.25, 2.5, 5, and 10 μM) were added. Three different dosing schedules were evaluated. The post-5-FU schedule was 5-FU for 48 h followed by DENSPM for 48 h; the pre-5-FU schedule was DENSPM for 48 h followed by 5-FU for 48 h; and the simultaneous schedule was 5-FU and DENSPM together for 48 h followed by medium alone for 48 h. After drug exposure, the MTS assay was used to measure cell viability.

Measurement of Cellular Levels of Polyamines

Treated cells were collected and lysed. The polyamine concentrations were determined by high-pressure liquid chromatography as described previously (28).

Cell Cycle Analysis by Flow Cytometry

Cells were seeded in a 10-cm^2 dish (one million cells/dish) in 10 ml of medium supplemented with 10% dialyzed fetal bovine serum. After 24 h at 37 °C, drugs were added for 72 h. After being washed with phosphate-buffered saline, cells were harvested by trypsinization and centrifugation, fixed with 70% of ethanol, and then stained with

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propidium iodide with RNase, followed by analysis on a FACS-Calibur instrument (BD Biosciences, Franklin Lakes, NJ) with CELL Quest software.

Real-time Reverse Transcriptase-PCR Methods for SSAT Expression

SSAT and 18 S rRNA were quantitated using the Applied Bio-system TaqMan method in conjunction with Assays-On-Demand (ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, CA). The PCR mixture consisted of Taqman Master Mix (without uracil-*N*-glycosylase), MultiScribe reverse transcriptase, RNase inhibitors, and 10 ng of total RNA in a final volume of 50 μl. Thermal cycling was initiated with reverse transcription at 48 °C for 30 min and the Taq Gold activation step at 95 °C for 10 min. The thermal profile for the PCR was 95 °C for 15 s and 60 °C for 1 min. Data were obtained during 40 cycles of amplification. PCR were performed in triplicate for SSAT with 18 S as an internal standard. The levels of SSAT in drug-treated samples relative to untreated control were determined.

Microarray Methods for Transcriptome Analysis

Total RNA was isolated, labeled, and hybridized as described (29). After being washed, the slides were scanned with a GeneTAC UC 4 laser scanner (Genomic Solutions, Ann Arbor, MI), and the signal intensities were quantified with ArrayVision™ (Imaging Research Inc., St. Catherine's, Ontario, Canada). The data were analyzed using the in-house program for microarray analysis (30). Each differentially expressed gene was determined from a cutoff value of its *T* value. Generally, a cutoff value of 3 was considered to be statistically significant based on the statistical variations (30). The pathway array includes 2,016 functionally well characterized genes involved in cell death, cell growth, metabolism, cell invasion, cell communication, immune response, DNA repair, and transcription (generated by the M. D. Anderson Cancer Center Genomics Core Laboratory, www3.mdanderson.org/~genomics). GoMiner was used (discover.nci.nih.gov/gominer) for the analysis of functional gene groups.

Western Blotting

Total cellular protein extract was isolated from harvested cells, protein concentration was determined, and Western blotting was carried out as described previously (31). The antibodies used were anti-p53 monoclonal antibody (Ab-6, Oncogene Science, Manhasset, NY), anti-p21^{Waf1} mouse hybridoma (a gift from Dr. Wade Harper at Baylor College of Medicine, Houston, TX), anti-caspase 3 (R & D Systems, Minneapolis, MN), anti-mdm2, anti-cyclin A2, anti-cyclin B1, anti-Fas, anti-β-actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and antibody for cleaved caspase 9 (Cell Signaling, Beverly, MA).

Cytochrome c Release Assay

Drug-treated cells were harvested for separation of mitochondria and cytosol by use of ApoAlert™ cell fractionation kit (BD Biosciences) according to the instructions from the manufacturer. Antibody for cytochrome *c* included in the kit was used in Western blotting analysis.

Cellular Hydrogen Peroxide (H_2O_2) Staining Assay

Cells were seeded in 6-well plates and treated with 5-FU and DENSPM for 48 h. CM-H_2DCFDA was applied to the cells at 1 μM final concentration and incubated for 1 h at 37 °C. The cells were harvested with trypsin and washed twice with ice-cold phosphate-buffered saline. Ten thousand cells were analyzed on the FACS-Calibur instrument (BD Biosciences) with CELL Quest Pro software.

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Mitochondrial Transmembrane Potential ($\Delta\psi_m$) Assay

Cells were treated with Rho123 at 100 nM final concentration to detect the disruption of mitochondrial membrane potential.

Statistical Analysis

The effect of drug combinations was analyzed using median effect algorithms developed by Chou and Talalay (32). Dose-response interactions (antagonism, additivity, synergism) between 5-FU and DENSPM were expressed as a combination index (CI) for every fraction affected. A CI value that is smaller than 1 indicates synergism, a CI value higher than 1 indicates antagonism, and a CI value of 1 indicates additivity. Differences in means were evaluated by two-tailed *t* test assuming unequal variances.

RESULTS

Synergism by Combination of 5-FU with DENSPM

As shown in Fig. 1, treatment of both wt-p53 and p53-null HCT116 cells with combined 5-FU and DENSPM markedly reduced tumor cell viability. For wt-p53 cells, 5-FU and DENSPM had a strong synergistic effect, with a Chou and Talalay CI value in the range of 0.3– 0.6. Moderate synergistic effect ($0.7 < CI < 0.85$) was seen with a post-5-FU schedule of initial 5-FU followed by DENSPM. Interestingly, an antagonistic effect was observed when the wt-p53 cells were pre-treated with DENSPM followed by 5-FU. In p53-null cells, simultaneous drug treatment also had the most synergistic effect, whereas pretreatment with DENSPM before 5-FU had an antagonistic effect.

Flow cytometric analysis (Fig. 2) indicated that simultaneous treatment in both isogenic lines resulted in a marked increase of the apoptotic sub-G_1 population, indicating cytotoxic effect and apoptosis. Absence of *p53* alleles in the null cells appeared to reduce apoptosis in response to either DENSPM alone or the combination treatment. In contrast to the results for 5-FU with DENSPM, the combination of 5-FU with DFMO did not produce synergistic cell killing (Fig. 3).

Depletion of Polyamines and Accumulation of Acetylated Spermidine by Combined 5-FU and DENSPM Treatment

5-FU at the IC_{25} dose did not markedly reduce polyamines. DENSPM decreased the levels of spermidine and spermine as expected, but increased the putrescine level (Fig. 4). However, the combination treatment with 5-FU and DENSPM produced a marked reduction in spermidine and spermine in tumor cells. The levels of acetylated spermidine in the wt-p53 line were markedly increased compared with controls. However, the levels of acetylated spermidine increased only minimally in the p53-null line despite a marked decrease in spermidine in the treated cells.

The cellular polyamine levels in cells treated with DFMO showed a reduction of putrescine and spermidine, which confirmed the effect of DFMO as a biosynthetic inhibitor (Fig. 4). Combination of 5-FU and DFMO also markedly reduced putrescine by half but did not lead to an increase in acetylated polyamines.

Induction of SSAT Gene Expression by 5-FU and DENSPM

To test whether the 5-FU and DENSPM combination had a stronger effect on *SSAT* expression than each individual agent, a real-time PCR analysis was performed to quantify *SSAT* transcript levels in the tumor cells. As shown in Fig. 5, 5-FU alone led to only a minor increase in *SSAT* expression, but DENSPM produced a major increase. The

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combination of the two drugs, however, resulted in a marked induction of *SSAT* expression (24 times higher in wt-p53 cells and 10 times higher in p53-null cells). The extent of *SSAT* mRNA induction appeared to match the levels of acetylated spermidine in wt-p53 cells but not in p53-null cells. Although we did not measure SSAT enzyme activity levels, increased expression of the gene and the marked reduction of spermidine and spermine levels (see above) indicated that an increase in SSAT enzymatic activity occurred.

H_2O_2 Production and Antioxidant Protective Effect

Because SSAT catabolizes polyamines to produce acetylated poly-amines, which serve as substrates for polyamine oxidase that generates hydrogen peroxide (24), we investigated whether combined 5-FU and DENSPM treatment resulted in an increased level of H_2O_2 that would play a role in apoptotic responses as a toxic reactive oxygen species. Measurements of H_2O_2 using CM-H_2DCFDA showed that incubation of cells with simultaneous 5-FU and DENSPM led to increased H_2O_2 production (Fig. 6*A*). Interestingly, 5-FU alone also caused a marked accumulation of H_2O_2, especially in p53-null cells. To determine whether the observed apoptosis involved reactive oxygen species, the cells were incubated with 5-FU and DENSPM in the presence of the antioxidant GSH, and attenuated cytotoxicity was observed, as expected (Fig. 6*B*).

Transcriptome Analysis

From the MTS assay, the combination of 5-FU and DENSPM showed synergistic growth inhibition and cell-killing effects. The gene expression profiles of drug-treated cells at 48 h were investigated to elucidate the molecular pathways of the synergism. Both the wt-p53 and p53-null cell lines showed that a larger number of genes were significantly altered after combination treatment with 5-FU and DENSPM as compared with the single-agent treatment groups (Tables I and II). In the wt-p53 cell line, the numbers of differentially expressed genes among 2,016 cancer pathway-related genes examined in cells treated with 5-FU alone, DENSPM alone, or their combination were 61, 30, and 76, respectively. In the p53-null cell line, 41, 54, and 132 genes were altered. Consistent with the real-time PCR results, marked induction of SSAT mRNA was observed with combined 5-FU and DENSPM treatment in both cell lines (data not shown).

In the wt-p53 HCT116 cell line, the expression of p53 downstream genes (p21^{WAF1}, *mdm2*, *bbc3*, *mic-1*, ferredoxin reductase, *14-3-3-σ*, etc.) was induced, demonstrating that the p53 pathway was activated. These genes were not induced in the p53-null cells. Consistent with the flow cytometry results, the expression of cell cycle arrest genes (p21^{WAF1}, *cyclin D2*, *cyclin A*, *14-3-3-σ*) were also altered. Genes involved in mitochondrial functions were statistically significantly down-regulated (Table I, $p = 0.04$). In the p53-null HCT116 cell line, genes in the categories of oxidative phosphorylation ($p = 0.0007$) and electron transport chain ($p = 0.0001$) were altered. Most of the genes in respiratory chain complex I were suppressed (Table I). Some of the gene expression results were also confirmed at the protein level by Western blot analysis (see Fig. 8).

Loss of Mitochondria Potential and Cytochrome c Release

To test the hypothesis that mitochondria are the potential target for the drug treatment, we measured the disruption of mitochondrial membrane potential ($\Delta\psi_m$) using Rho123. Combined 5-FU and DENSPM treatment resulted in a significant decrease in $\Delta\psi_m$, as shown by a shift in the cell population from high to low fluorescence (Fig. 7). Consistent with the loss of mitochondrial membrane potential, cytochrome *c* was released from mitochondria into the cytoplasm after the combination drug treatment in both cell lines (Fig. 8).

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Caspase 9 Dependence of 5-FU/DENSPM-induced Cell Death

On Western blot using antibodies that specifically recognize activated caspase 3 and cleaved caspase 9, caspase 3 was not activated in 5-FU and DENSPM-treated cells, but activated caspase 9 was detected (Fig. 8). Protein extracts from HL-60 cells with and without Ara-C treatment were used as positive and negative controls for the cleaved and activated forms of caspase 3. This result showed that 5-FU/DENSPM-induced cell death is caspase 9-dependent and caspase 3-independent, which is one of the four types of apoptosis involving different combinations of caspase dependence (33). Western blotting analyses also showed that cyclin A and cyclin B1 were decreased after the combination treatment, confirming our microarray results. Consistent with our previous report (10) that Fas expression was increased in cells treated with 5-FU, 5-FU as well as combined 5-FU and DENSPM also caused Fas up-regulation. Consistent with the results from our transcriptome analysis, 5-FU and DENSPM activated p53 and protein expression of genes of p53 downstream (phosphorylated p53, serine 15, $p21^{Waf1}$, and mdm2) in the HCT116 wt-p53 cell line but not in the p53-null cells (Fig. 8).

DISCUSSION

We reported previously that high-dose 5-FU modulates the polyamine pathway in colon carcinoma cells (10). This observation led us to speculate that 5-FU in combination with poly-amine-targeting drugs may have synergistic effects in cytotoxicity. This present study showed that indeed the combination of 5-FU with the polyamine catabolism-enhancing drug DENSPM synergistically killed colon carcinoma cells independent of their p53 status. Interestingly, antagonistic effects depending on the drug treatment sequence were observed in p53 wild-type and null lines. Most notably, DENSPM pretreatment followed by 5-FU produced a antagonistic effect in both cell lines. This finding indicates that the synergistic effect of simultaneous 5-FU and DENSPM is not because of “priming” of one drug for the other. Rather, the effects of the two drugs may need to augment each other, although we do not know the exact mechanism involved. One possibility is that both 5-FU and DENSPM damage DNA but through different mechanisms (34, 35), which may make DNA repair less effective.

In contrast to 5-FU and DENSPM, combination of 5-FU with the polyamine synthesis inhibitor DFMO did not have synergistic cell killing effects. Furthermore, 5-FU followed by DFMO in the wt-p53 line and DFMO pretreatment followed by 5-FU in the p53-null line had marked antagonistic effects. Although we do not yet understand the mechanisms that account for these different effects, the results underscore the impact of the genetic background of tumor cells on the cellular response to drug treatment and therefore the importance of understanding these relationships.

Modulation of polyamine levels and their cellular responses is a complex process. The synergistic effect of combined DENSPM and 5-FU must be specific at molecular levels. One of the molecular effectors appears to be that SSAT is synergistically induced by the combination of 5-FU and DENSPM but not by 5-FU and DFMO. Previous studies have shown that DENSPM-induced SSAT expression, which is responsible for the depletion of spermine and spermidine via acetylation (36). Consistent with this finding, cellular polyamine levels were markedly reduced by the combination of 5-FU and DENSPM. Acetylated spermidine accumulated in wt-p53 cells but not in p53-null cells. Surprisingly, the levels of putrescine were increased by combined 5-FU and DENSPM in wt-p53 cells but decreased in p53-null cells, indicating that the p53 status has an impact on how polyamines are depleted. p53 could be suppressing the diamine exporter, resulting in enhanced retention of polyamine catabolic products in wt-p53 cells (37).

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Increased putrescine in the absence of spermidine may have another cellular effect because increased putrescine was shown to inhibit hypusine formation and transfer of the butylamine moiety of spermidine to a lysine residue in eukaryotic initiation factor 5A (eIF-5A), resulting in induction of apoptosis (28). This mechanism may involve the ability of deoxyhypusine synthetase to produce homospermidine, as recently shown by Park *et al.* (38).

In the polyamine catabolic pathway, acetylated spermine or spermidine is oxidized by polyamine oxidase. This reaction produces hydrogen peroxide, which at high levels damages mitochondrial membranes resulting in cytochrome *c* release. Release of cytochrome *c* activates members of caspase families and triggers the cascade to apoptosis (25). The anti-mitochondrial effects of acetylated polyamines were reported in previous studies (39, 40). In addition, it was shown that acetylated polyamines cause the loss of mitochondrial DNA (40). Results from our study showed that the combination of 5-FU with DENSPM indeed elevated cellular hydrogen peroxide and damaged mitochondrial membranes.

Consistent with mitochondria being an important target for combined 5-FU and DENSPM, our transcriptome analyses showed that, regardless of the *p53* status, genes involved in mitochondrial functions were preferentially affected. However, mitochondrial alterations are more apparent in p53-null cells where 11 of 136 altered genes are involved in mitochondrial function. Among the 13 genes essential for electron transport chain subunits in the inner mitochondrial membrane (41), 8 genes were suppressed in the p53-null line. Among the mito-chondrial function-related genes that were markedly attenuated in p53-null cells were cytochrome *c* oxidase I and II (*MTCO1* and *MTCO2*), which are subunits of cytochrome *c* oxidase. MTCO2 transfers an electron from cytochrome *c* to the binuclear center of cytochrome a_3-CuB (42) and translocates protons, which are associated with the mitochondrial transmembrane potential (43). NADH dehydrogenase resides in the first complex in the electron transfer chain and catalyzes the transfer of electrons from NADH to coenzyme Q (44). Thus, suppression of MTCO1, MTCO2, and NADH dehydrogenases by the combined drug treatment would likely lead to decreased efficiency of the mitochondrial electron transport chain and subsequently increase the production of superoxides.

In wt-p53 cells, in addition to mitochondria-related genes, many genes involved in the p53 pathway were affected, indicating that the p53-mediated apoptosis pathway most likely played a dominating role in the observed cell death, as expected. One other interesting gene was *dUTPase*, which hydrolyzes dUTP to dUMP and pyrophosphate. Remarkably, increased *dUTPase* levels in colon cancer cell lines have a major role in their resistance to 5-FU and fluorodeoxyuridine (45, 46). Our results showed that the expression of *dUTPase* was suppressed when 5-FU and DENSPM were applied simultaneously in the wt-p53 cell line. This reduction may represent another mechanism that is responsible for the synergistic effects of combined 5-FU and DENSPM in the wt-p53 cell line. Thus, the presence of wt-p53 appears to sensitize cells to 5-FU and DENSPM through multiple processes.

In summary, our study shows that the combination of 5-FU and DENSPM provides more effective cytotoxicity in colon cancer in a cell culture system through effects on the polyamine pathway. The potential of 5-FU and DENSPM as a therapeutic combination for treating colon cancer patients awaits results from preclinical animal model experiments and future clinical trials.

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References

1. Ansfield F, Klotz J, Nealon T, Ramirez G, Minton J, Hill G, Wilson W, Davis H Jr, Cornell G. Cancer. 1997; 39:34– 40. [PubMed: 318916]
2. Moertel CG, Schutt AJ, Reitemeier RJ, Hahn RG. Cancer Res. 1972; 12:2717–2719. [PubMed: 4566290]
3. Fine S, Erlichman C, Kaizer L, Warr D, Gadalla T. Breast Cancer Res Treat. 1994; 30:205–209. [PubMed: 7949219]
4. Calvo E, Cortes J, Rodriguez J, Fernandez-Hidalgo O, Rebollo J, Martin-Algarra S, Garcia-Foncillas J, Martinez-Monge R, de Irala J, Brugarolas A. Clin Colorectal Cancer. 2002; 2:104–110. [PubMed: 12453325]
5. Gil-Delgado MA, Guinet F, Castaing D, Adam R, Coeffic D, Durrani AK, Bismuth H, Khayat D. Am J Clin Oncol. 2001; 24:101–105. [PubMed: 11232943]
6. Goldberg RM, Kaufmann SH, Atherton P, Sloan JA, Adjei AA, Pitot HC, Alberts SR, Rubin J, Miller LL, Erlichman C. Ann Oncol. 2002; 13:1674–1680. [PubMed: 12377659]
7. Parker WB, Cheng YC. Pharmacol Ther. 1990; 48:381–395. [PubMed: 1707544]
8. Peters GJ, Backus HH, Freemantle S, van Triest B, Codacci-Pisanelli G, van der Wilt CL, Smid K, Lunec J, Calvert AH, Marsh S. Biochim Biophys Acta. 2002; 1587:194–205. [PubMed: 12084461]
9. Ghoshal K, Jacob ST. Biochem Pharmacol. 1997; 53:1569–1575. [PubMed: 9264308]
10. Zhang W, Ramdas L, Shen W, Song SW, Hu L, Hamilton SR. Cancer Biol Ther. 2003; 2:572–578. [PubMed: 14614330]
11. Russell DH, Looney WB, Kovacs CJ, Hopkins HA, Marton LJ, LeGendre SM, Morris HP. Cancer Res. 1974; 34:2382–2385. [PubMed: 4367291]
12. Snyder RD. Biochem J. 1989; 260:697–704. [PubMed: 2504149]
13. Pendeville H, Carpino N, Marine JC, Takahashi Y, Muller M, Martial JA, Cleveland JL. Mol Cell Biol. 2001; 21:6549– 6558. [PubMed: 11533243]
14. Elmore E, Stringer DE, Steele VE, Gerner EW, Redpath JL. Anticancer Res. 2001; 21:1163–1165. [PubMed: 11396157]
15. Subhi AL, Diegelman P, Porter CW, Tang B, Lu ZJ, Markham GD, Kruger WD. J Biol Chem. 2003; 278:49868– 49873. [PubMed: 14506228]
16. Belting M, Borsig L, Fuster MM, Brown JR, Persson L, Fransson LA, Esko JD. Proc Natl Acad Sci U S A. 2002; 99:371–376. [PubMed: 11752393]
17. Fabian CJ, Kimler BF, Brady DA, Mayo MS, Chang CH, Ferraro JA, Zalles CM, Stanton AL, Masood S, Grizzle WE, Boyd NF, Arneson DW, Johnson K. Clin Cancer Res. 2002; 8:3105–3117. [PubMed: 12374678]
18. Einspahr JG, Nelson MA, Saboda K, Warneke J, Bowden GT, Alberts DS. Clin Cancer Res. 2002; 8:149–155. [PubMed: 11801552]
19. Levin VA, Uhmk JH, Jaeckle KA, Choucair A, Flynn PJ, Yung WKA, Prados MD, Bruner JM, Chang SM, Kyritsis AP, Gleason MJ, Hess KR. Clin Cancer Res. 2000; 6:3878–3884. [PubMed: 11051233]
20. Sarhan S, Knodgen B, Seiler N. Anticancer Res. 1992; 12:457– 466. [PubMed: 1580563]
21. Meyskens FL Jr, Gerner EW. Clin Cancer Res. 1999; 5:945–951. [PubMed: 10353725]
22. Wang Y, Xiao L, Thiagalingam A, Nelkin BD, Casero RA Jr. J Biol Chem. 1998; 273:34623–34630. [PubMed: 9852135]
23. Wang Y, Devereux W, Stewart TM, Casero RA Jr. J Biol Chem. 1999; 274:22095–22101. [PubMed: 10419538]
24. Ha HC, Woster PM, Yager JD, Casero RA Jr. Proc Natl Acad Sci U S A. 1997; 94:11557–11562. [PubMed: 9326648]
25. Chen Y, Kramer DL, Diegelman P, Vujcic S, Porter CW. Cancer Res. 2001; 61:6437– 6444. [PubMed: 11522638]
26. Waldman T, Kinzler KW, Vogelstein B. Cancer Res. 1995; 55:5187–5190. [PubMed: 7585571]
27. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. Science. 1998; 282:1497–1501. [PubMed: 9822382]

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28. Tome ME, Fiser SM, Payne CM, Gerner EW. Biochem J. 1997; 328:847– 854. [PubMed: 9396730]
29. Hu L, Wang J, Baggerly K, Wang H, Fuller GN, Hamilton SR, Coombes KR, Zhang W. BMC Genomics. 2002; 3:16–23. [PubMed: 12086591]
30. Baggerly KA, Coombes KR, Hess KR, Stivers DN, Abruzzo LV, Zhang W. J Comput Biol. 2001; 8:639– 659. [PubMed: 11747617]
31. Fuller GN, Rhee CH, Hess KR, Caskey LS, Wang R, Bruner JM, Yung WK, Zhang W. Cancer Res. 1999; 59:4228– 4232. [PubMed: 10485462]
32. Chou TC, Talalay P. Adv Enzyme Regul. 1984; 22:27–55. [PubMed: 6382953]
33. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW. Cell. 1998; 94:339–352. [PubMed: 9708736]
34. Basu HS, Schwietert HC, Feuerstein BG, Marton LJ. Biochem J. 1990; 269:329–334. [PubMed: 2386479]
35. Edwards ML, Snyder RD, Stemerick DM. Med Chem. 1991; 34:2414–2420.
36. Fogel-Petrovic M, Kramer DL, Vujcic S, Miller J, McManis JS, Bergeron RJ, Porter CW. Mol Pharmacol. 1997; 52:69–74. [PubMed: 9224814]
37. Xie X, Gillies RJ, Gerner EW. J Biol Chem. 1997; 272:20484–20489. [PubMed: 9252359]
38. Park JH, Wolff EC, Folk JE, Park MH. J Biol Chem. 2003; 278:32683–32691. [PubMed: 12788913]
39. Snyder RD, Beach DC, Loudy DE. Anticancer Res. 1994; 14:347–356. [PubMed: 8017833]
40. Marverti G, Piccinini G, Ghiaroni S, Barbieri D, Quaglino D, Moruzzi MS. Int J Cancer. 1998; 78:33– 40. [PubMed: 9724091]
41. Chinnery PF, Howell N, Andrews RM, Turnbull DM. J Med Genet. 1999; 36:425– 436. [PubMed: 10874629]
42. Hill BC. J Bioenerg Biomembr. 1993; 25:115–120. [PubMed: 8389744]
43. Babcock GT, Wikstrom M. Nature. 1992; 356:301–309. [PubMed: 1312679]
44. Gerhard, M. Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology. John Wiley & Sons Inc; New York: 1998.
45. Canman CE, Radany EH, Parsels LA, Davis MA, Lawrence TS, Maybaum J. Cancer Res. 1994; 54:2296–2298. [PubMed: 8162567]
46. Pugacheva EN, Ivanov AV, Kravchenko JE, Kopnin BP, Levine AJ, Chumakov PM. Oncogene. 2002; 21:4595– 4600. [PubMed: 12096336]

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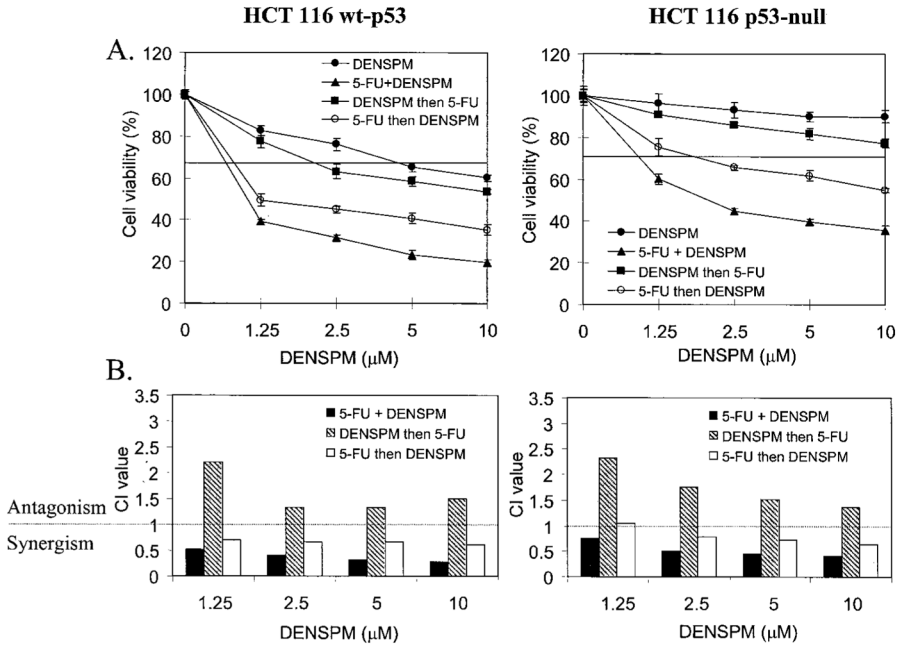

Fig 1. Effects of 5-FU and spermine analogue DENSPM combinations on colon carcinoma cells

HCT116 colon cancer cells were treated with 5-FU at 2.5 μM (IC_{25} for wt-p53 line) or 15 μM (IC_{25} for p53-null line) in combination with increasing doses of DENSPM (simultaneous or one drug following the other). Cell viability at the 96-h end point was measured with MTS assay. *A*, dose-response curves. The straight line at around 70% viability shows the effect by 5-FU alone. The four *curves* represent different regimens: ●, DENSPM alone; ▲, 5-FU + DENSPM (simultaneous treatment for 48 h and drug free medium for 48 h); ■, DENSPM (48 h) then 5-FU (48 h); ○, 5-FU (48 h) then DENSPM (48 h). Simultaneous 5-FU and DENSPM produced the most cytotoxicity followed by the DENSPM post-5-FU regimen. The DENSPM pretreatment regimen had the least effect. *B*, analysis of the combination effect of 5-FU and DENSPM using the Chou and Talalay CI. Dose-response interactions between 5-FU and DENSPM were expressed as a CI value (CI < 1: synergism, CI = 1: additivity, CI > 1: antagonism). Simultaneous treatment with 5-FU and DENSPM resulted in a strong synergistic effect. 5-FU followed by DENSPM had a moderate synergistic effect, but pre-treatment with DENSPM before 5-FU had an antagonistic effect, irrespective of p53 status.

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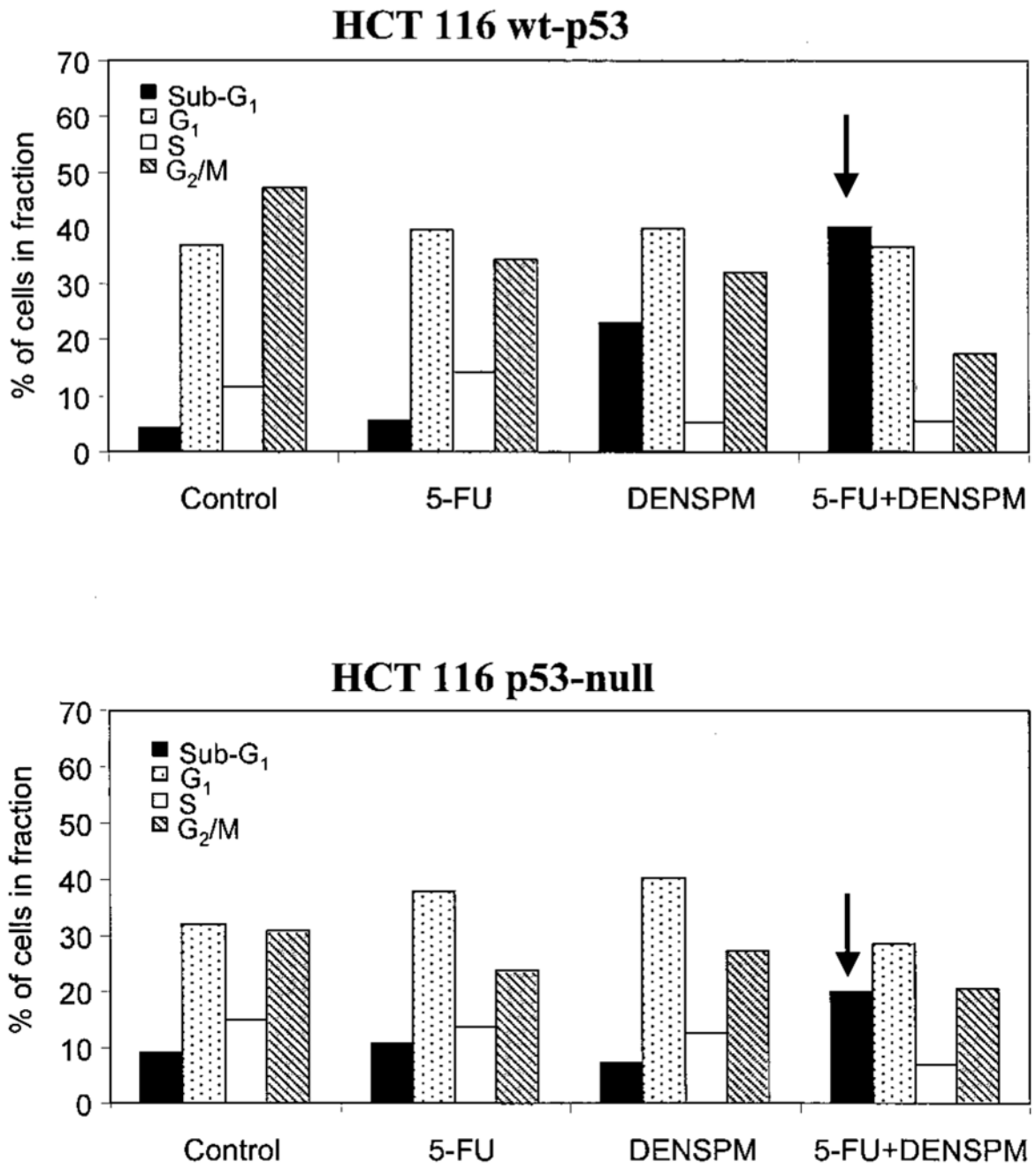

Fig 2. Combined 5-FU and DENSPM treatment causes marked apoptosis in colon carcinoma cells

HCT116 cells with either wt-p53 or p53-null were treated with 5-FU alone, DENSPM alone, or both agents for 72 h and harvested for flow cytometry analysis. Simultaneous treatment produced increases in the apoptotic sub-G_1 population in both isogenic lines (marked by ↓).

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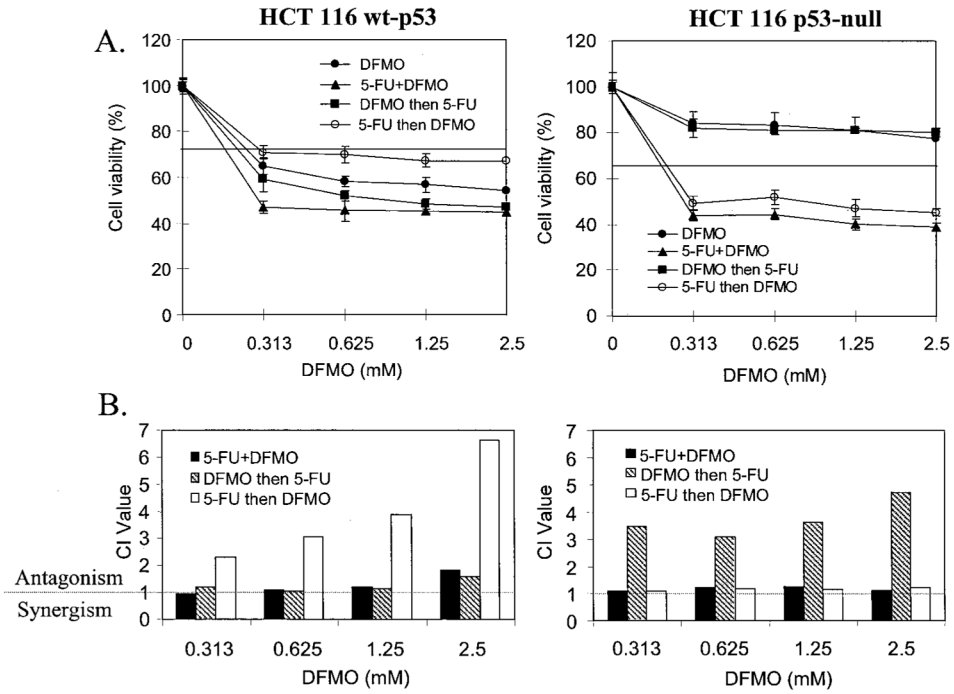

Fig 3. Effects of combined 5-FU and ornithine decarboxylase inhibitor DFMO on colon carcinoma cells

HCT116 colon cancer cells were treated with 5-FU at 2.5 μM (IC_{25} for wt-p53 line) or 15 μM (IC_{25} for p53-null line) in combination with increasing doses of DFMO (simultaneous or one drug following the other). Cell viability at the 96-h end point was measured with MTS assay. *A*, dose-response curves. The *straight line* at around 70% viability shows the effect by 5-FU alone. The four *curves* represent different regimens: ●, DFMO alone; ▲, 5-FU + DFMO (simultaneous treatment for 48 h and drug-free medium for 48 h); ■, DFMO (48 h) then 5-FU (48 h); ○, 5-FU (48 h) then DFMO (48 h). No combination (simultaneous treatment, DFMO post-treatment, or DFMO pre-treatment) produced synergistic cytotoxicity. Some pre-treatment and post-treatment regiments of DFMO are antagonistic to 5-FU depending on the p53 status. DFMO post-treatment and DFMO pre-treatment had the strongest antagonistic effects in wt-p53 and p53-null lines, respectively. *B*, analysis of the combination effect of 5-FU and DFMO using the Chou and Talalay CI. Dose-response interactions between 5-FU and DFMO were expressed as a CI value (CI < 1: synergism, CI = 1: additivity, CI > 1: antagonism). Simultaneous treatment with 5-FU and DFMO only had additive effects in most regimens. 5-FU followed by DFMO had a strong antagonistic effect in the wt-p53 cells and DFMO followed by 5-FU had a strong antagonistic effect in the p53-null cells.

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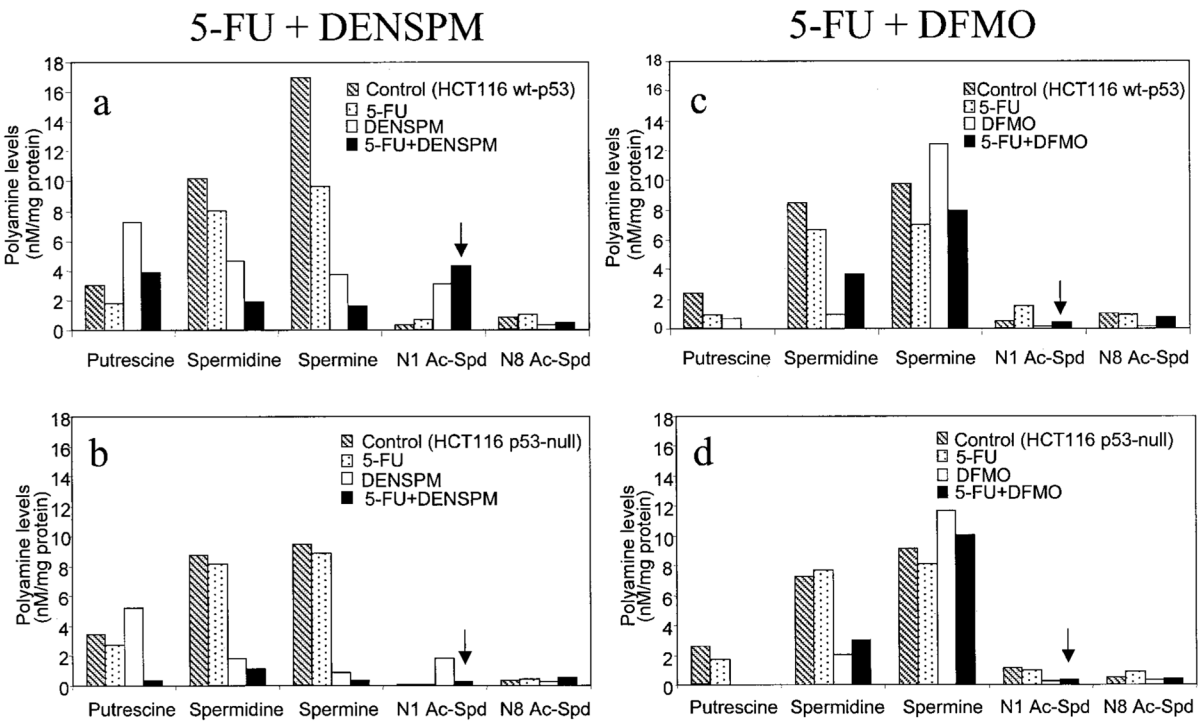

Fig 4. The effect of drug combinations on levels of intracellular polyamines and acetylated spermidine

Cells with wt-p53 (*panels a* and *c*) or p53-null (*panels b* and *d*) were treated with 5-FU alone, DENSPM alone, or their combination (*panels a* and *b*), or with 5-FU alone, DFMO alone, or their combinations (*panels c* and *d*) for 48 h. Surviving cells were collected and the levels of intracellular putrescine, spermidine, spermine, and N1 and N8 spermidine (*Spd*) were measured. Combined 5-FU and DENSPM produced a marked reduction of spermidine and spermine irrespective of the p53 status, but only putrescine was reduced in p53-null cells. Combined 5-FU and DFMO produced a marked reduction of putrescine and spermidine but not spermine. Combined 5-FU and DENSPM produced a marked increase in N1 acetylated spermidine (*arrowheads*) especially in wt-p53 cells, but combined 5-FU and DFMO did not increase acetylated spermidine.

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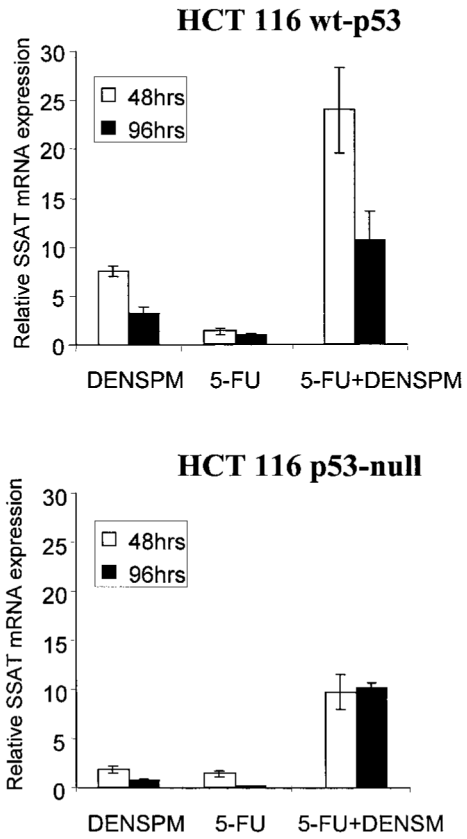

Fig 5. Marked elevation of SSAT mRNA levels after combined 5-FU and DENSPM treatment

The HCT116 cells with either wt-p53 or p53-null were treated with 5-FU alone, DENSPM alone, or their combination for 48 h followed by incubation in drug-free medium for an additional 48 h. Cells were collected at 48 h, total RNA was isolated, and levels of SSAT mRNA were measured by real-time PCR assay. The combination of 5-FU and DENSPM resulted in a marked induction of SSAT mRNA levels in both cell lines.

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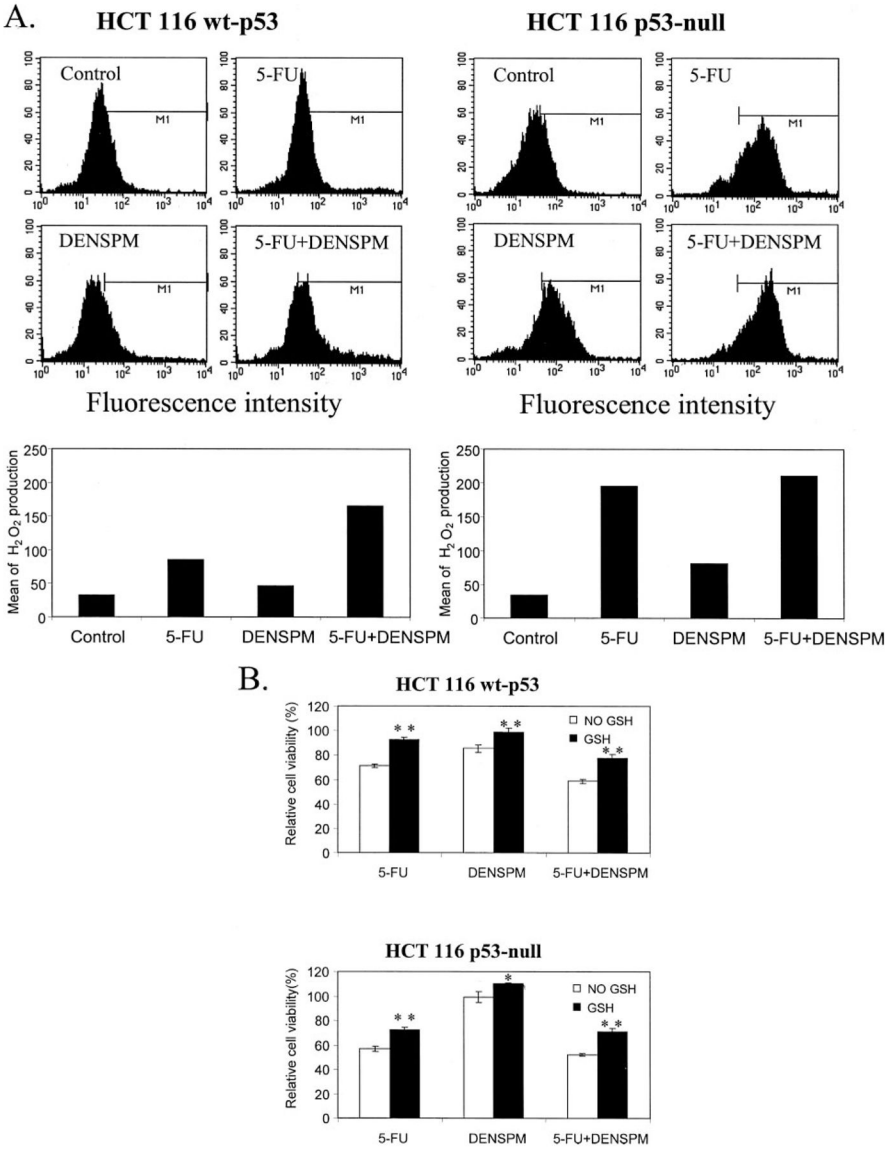

Fig 6. Generation of hydrogen peroxide after drug treatment and free radical scavenger effect of GSH in drug-treated colon carcinoma cell lines

A, the HCT116 cells with either wt-p53 or p53-null background were treated with the indicated regimens for 48 h. Cells were incubated with CM-H_2DCFDA for 1 h at 37 °C and then harvested for analysis by flow cytometry. The mean levels of hydrogen peroxidepositive cells were plotted. Combined 5-FU and DENSPM led to increased hydrogen peroxide levels in the cells. *B*, the cells were incubated with 5-FU (7 μ M for wt-p53 and 25 μM for p53-null), DENSPM (7 μM for wt-p53 and 20 μM for p53-null), or their combination in the presence of the antioxidant GSH (50 μM) for 48 h, then MTS assay was performed to analyze the viability of the cells. GSH attenuated cytotoxicity from 5-FU and DENSPM treatment. The p values of < 0.05 (*) and < 0.01 (**) are indicated.

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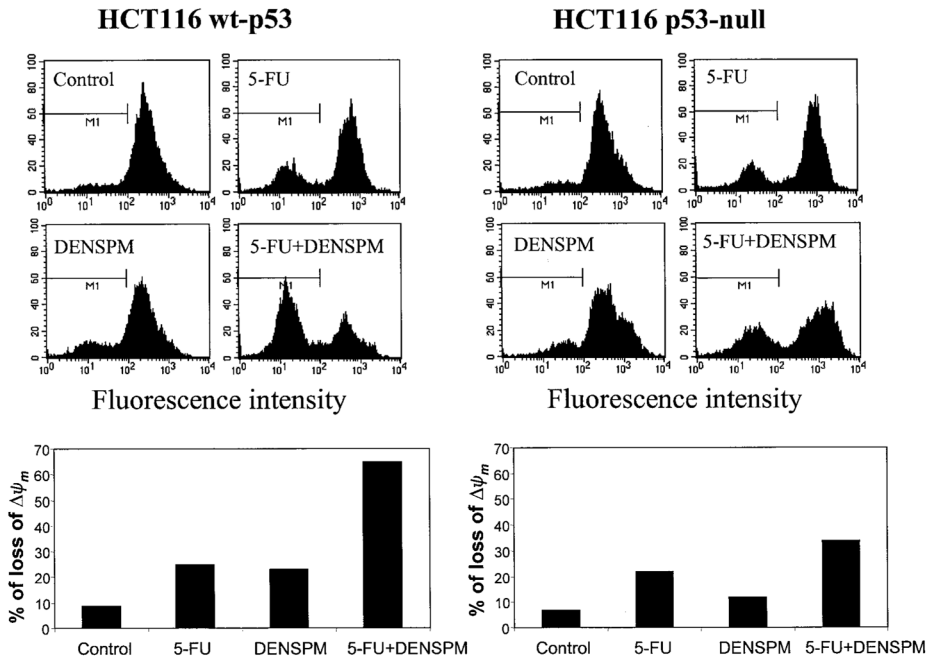

Fig 7. Loss of mitochondrial transmembrane potential in colon carcinoma cells after 5-FU and DENSPM

Mitochondrial membrane potential ($\Delta\psi_m$) was analyzed using Rho123. The HCT116 cells with either wt-p53 or p53-null were treated with 5-FU alone (7 μM for wt-p53 and 25 μM for p53-null), DENSPM alone (7 μM for wt-p53 and 20 μM for p53-null), or their combination for 72 h. Cells were incubated with Rho123 for 1 h and then harvested for analysis by flow cytometry. Combined 5-FU and DENSPM resulted in a significant decrease in $\Delta\psi_m$, as shown by a shift in the cell population from high to low levels of fluorescence intensity.

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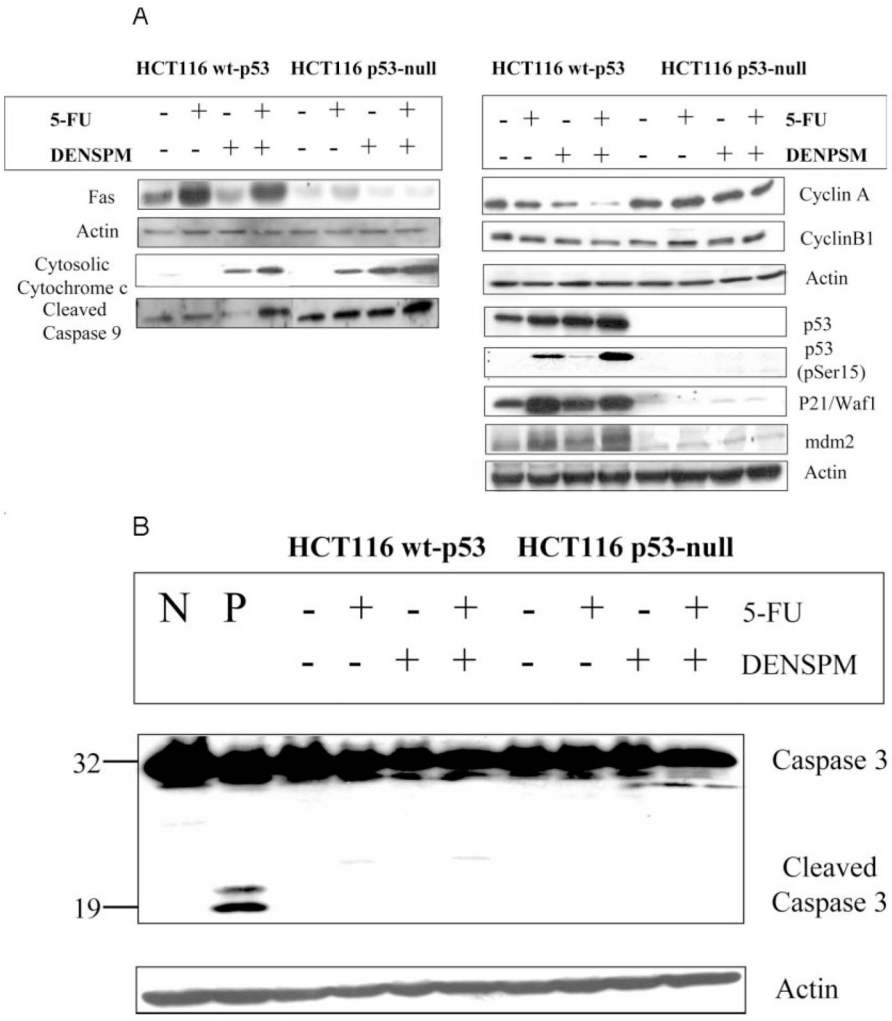

Fig 8. Measurement of protein expression in apoptosis, cell cycle, and p53 pathways in response to drug treatment

A and *B*, HCT116 cells with either wt-p53 or p53-null were treated with 5-FU alone (2.5 μM for wt-p53 and 15 μM for p53-null), DENSPM alone (7 μM for wt-p53 and 20 μM for p53-null), or their combination. Total proteins were extracted (for cytochrome *c*, cytoplasmic fraction without mitochondria was isolated, as described under "Experimental Procedures") for Western blot analyses as indicated on the figure. Three separate blots were used for analyses, and actin was probed on each blot for protein loading control. Cleaved caspase 3 was not detected (*panel B*), but increased levels of cleaved caspase 9 were found after the combined drug treatment. Cytochrome *c* release was most marked with the combined regimen. p53 protein was markedly phosphorylated at Ser-15 in wt-p53 cells after combined drug treatment and after 5-FU alone. The p53 downstream targets, Fas, $p21^{Waf1}$, and mdm2 were induced by 5-FU and by combined 5-FU with DENSPM in wt-p53 cells only. *B: N*, negative control, HL-60 cell lysate. *P*, positive control, HL-60 cells treated with Ara C for 24 h. Cleaved caspase 3 was observed.

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Table I

Altered expression of redox genes encoded by nuclear and mitochondria DNA

The gene functional groups were categorized according to GoMinor (Gene Ontology). All the numbers are the folds of changes relative to the untreated cells. Genes involved in mitochondrial function was significantly enriched in both cell lines (HCT116 wt-p53: $p = 0.04$, HCT116 p53-null: $p = 0.0001$). MT; mitochondria, MTCO1; *Homo sapiens* cytochrome c oxidase I (MTCO1), mRNA; MTCO2; *H. sapiens* cytochrome *c* oxidase II (MTCO2), mRNA; MTATP6; *H. sapiens* ATP synthase 6 (MTATP6), mRNA; MTATP8; *H. sapiens* ATP synthase 8 (MTATP8), mRNA; MTND3, *H. sapiens* NADH dehydrogenase 3 (MTND3), mRNA; MTND4, *H. sapiens* NADH dehydrogenase 4 (MTND4), mRNA; MTND6; *H. sapiens* NADH dehydrogenase 6 (MTND6), mRNA; MTND4L; *H. sapiens* NADH dehydrogenase 4L (MTND4L), mRNA; WEE1; Wee1 + (*S. pombe*) homolog/protein x 013; SDHB, succinate dehydrogenase subunit B; UQCRH, ubiquinol-cytochrome *c* reductase hinge protein; COX8, cytochrome *c* oxidase subunit VIII; SFN, h. sapiens stratifin (SFN), mRNA; FDXR, ferredoxin reductase; DUT, DUTP pyrophosphatase.

Gene description	Accession number	Source	HCT116 wt-p53			HCT116 p53-null		
			5-FU	DENSPM	5-FU + DENSPM	5-FU	DENSPM	5-FU + DENSPM
MTCO1	NM_173704	MT	1.48	1.25	0.78	0.90	0.75	0.27
MTCO2	NM_173705	MT	1.56	0.89	0.66	1.16	0.58	0.14
MTATP6	NM_173702	MT	1.49	0.81	0.66	0.95	0.64	0.23
MTATP8	NM_173703	MT	1.49	0.76	0.64	0.89	0.54	0.21
MTND3	NM_173710	MT	0.87	0.62	0.62	0.68	0.29	0.19
MTND4	NM_173711	MT	1.44	0.82	0.63	0.63	0.47	0.15
MTND6	NM_173714	MT	0.75	0.46	0.65	0.55	0.76	0.54
MTND4L	NM_173712	MT	1.34	0.74	0.63	0.62	0.36	0.14
WEE1	XM_049184	Nuclear	0.49	0.68	0.31	0.68	0.84	0.90
SDHB	NM_003000	Nuclear	1.07	0.75	1.52	1.07	1.24	1.62
UQCRH	NM_006004	Nuclear	0.95	1.16	1.15	1.15	1.33	1.67
COX8	NM_004074	Nuclear	1.25	1.29	1.57	1.12	1.12	1.69
SFN	NM_006142	Nuclear	1.66	1.89	3.03	1.17	0.85	1.20
FDXR	XM_056248	Nuclear	2.00	2.60	2.36	1.18	1.06	1.20
DUT	U31930	Nuclear	0.89	0.76	0.50	0.94	1.48	1.38

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Table II

Altered expression of DNA genes and p53 regulated genes

The gene functional groups were categorized according to GoMinor (Gene Ontology). All the numbers are the folds of changes relative to the untreated cells. DNA replication and chromosome cycle genes in the HCT116 wt-p53 cell line was significantly enriched among altered genes (HCT116 wt-p53: $p = 0.028$). TREX1, *H. sapiens* three prime repair exonuclease 1 (TREX1), transcript variant 3, mRNA; TOP2A, topoisomerase (DNA) II α (170 kD); TOP3, topoisomerase (DNA) III; MSH2, MutS (*E. coli*) homolog 2 (colon cancer, nonpolyposis type 1); WEE1, Wee1 + (*S. pombe*) homolog/protein x 013, MDM2, mouse double minute 2, human homolog of; p53-binding protein; CDKN1A, cyclin-dependent kinase inhibitor 1A (p21, Cip1); BBC3, Bcl-2 binding component 3; FDXR, ferredoxin reductase; MIC-1, macrophage inhibitory cytokine-1 (MIC-1); DDIT1, DNA-damage-inducible transcript 1; SFN, *H. sapiens* stratifin (SFN), mRNA.

Gene description	Accession number	HCT116 wt-p53			HCT116 p53-null		
		5-FU	DENSPM	5-FU + DENSPM	5-FU	DENSPM	5-FU + DENSPM
DNA replication and chromosome cycle							
TREX1	NM_033628	0.66	0.44	0.50	0.73	0.47	0.41
TOP2A	J04088	0.74	0.91	0.35	0.79	1.06	1.13
TOP3	U43431	1.09	0.48	0.52	0.90	1.12	0.73
MSH2	U03911	0.73	0.62	0.38	0.75	0.84	0.65
WEE1	XM_049184	0.49	0.68	0.31	0.68	0.84	0.90
p53 regulated genes							
MDM2	M92424	1.25	0.99	4.63	0.68	1.01	0.75
CDKN1A	L25610	3.50	0.92	5.70	3.24	1.98	1.25
BBC3	NM_014417	2.11	7.84	2.28	1.80	1.66	1.53
FDXR	XM_056248	2.00	2.61	2.36	1.18	1.06	1.20
MIC-1	AF019770	1.99	3.97	3.95	0.71	0.71	0.50
DDIT1	L24498	1.33	0.91	2.53	0.63	1.05	1.26
SFN	NM_006142	1.66	1.89	3.03	1.17	0.85	1.20

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