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Synergistic effects of 5-fluorouracil in combination with diosmetin in colorectal cancer cells

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Abstract

Colorectal cancer (CRC) is among the most common occurring cancer. The management of CRC includes laparoscopic surgery, radiotherapy, chemotherapies and neoadjuvant. However, the conventional chemotherapies have poor impact on combating CRC and are associated with severe toxic effects and high relapse. Therefore, searching for a new combination regimen is a favorable consideration. The aim of this study was to elucidate the synergistic effect of 5-fluorouracil (5-FU) and diosmetin in an *in vitro* model on colorectal cancer cells. Findings showed that the dose reduction index (DRI) of 5-FU was three-fold lower in the combination with a combination index (CI) value less than one, which indicates a synergistic effect. AO/PI microscopic results revealed signs of apoptosis and dead cells after 72 h of treatment. Flow cytometry analysis confirmed the apoptotic effect of combination was more prominent as compared to 5-FU alone. The findings of this study offered a potential strategy to reduce the cytotoxicity and enhance the efficacy of 5-FU on colorectal cancer cells through a synergistic study model.

Keywords: Colorectal cancer; Synergism; 5-Fluorouracil; Diosmetin; CI & DRI

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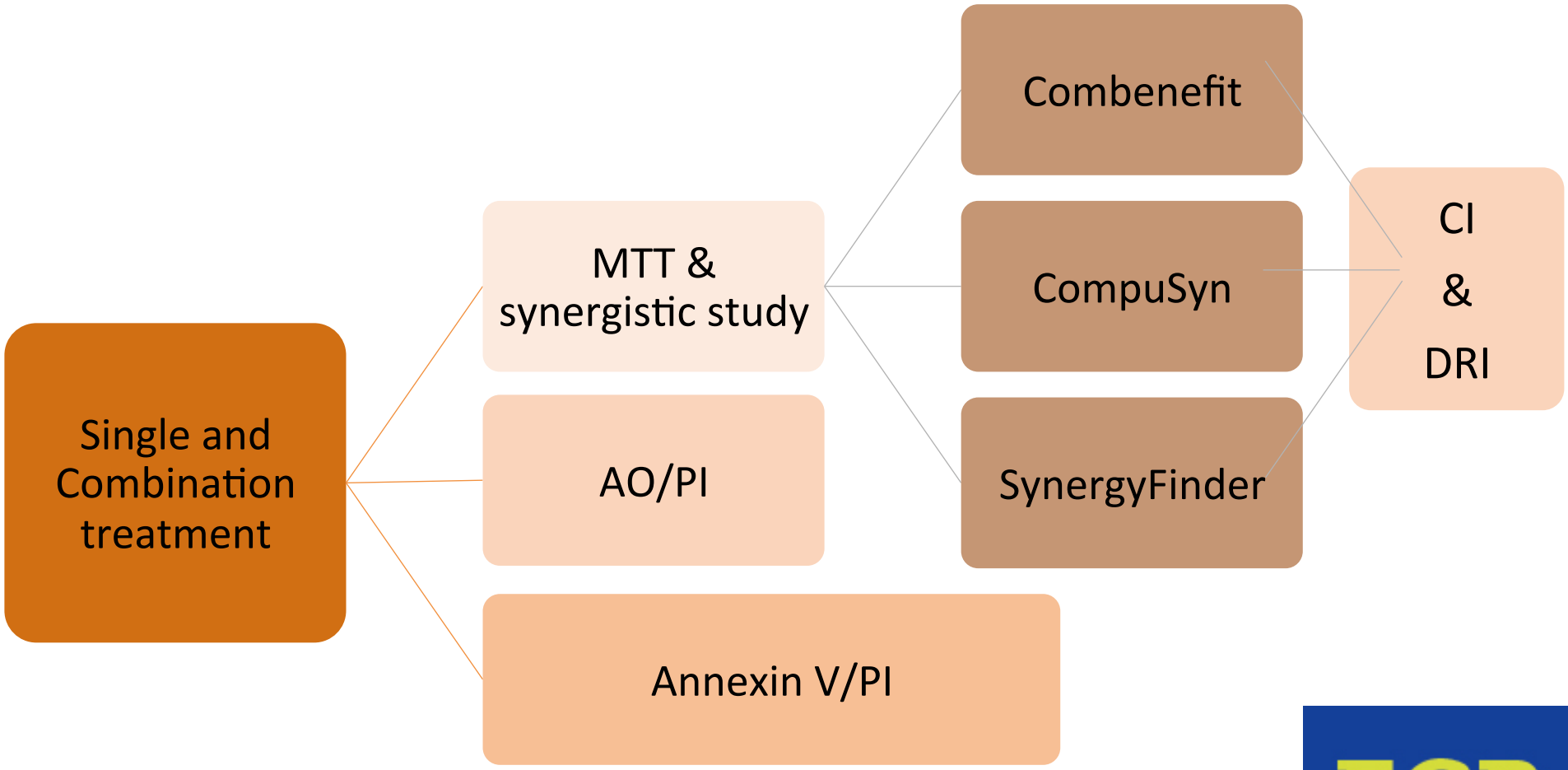
Experimental design



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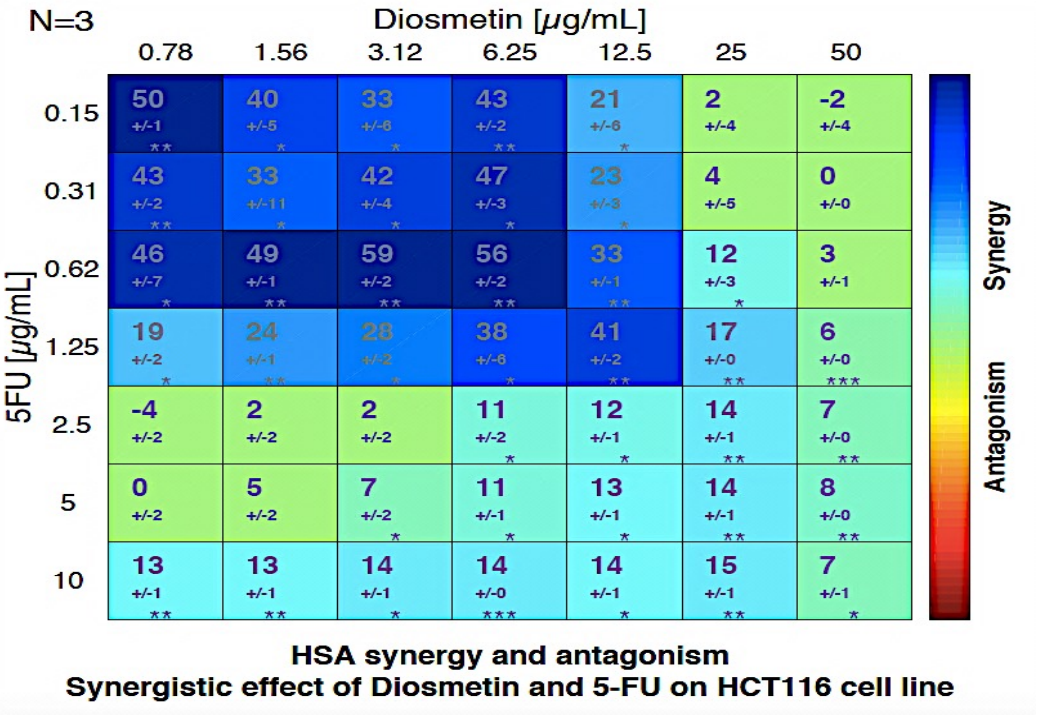
Results & Discussion

MTT

- Individual treatment: Diosmetin (IC50): 4.16±1.3 μg/mL; 5-FU (IC50): 0.83±0.0 μg/mL
- Combination treatment: Diosmetin (IC50): 1.38±0.8 μg/mL; 5-FU (IC50): 0.27±1.1 μg/mL
- CI: 0.66 (<1); DRI of 5-FU: 3.0 (Three-fold reduction)
- Combenefit: Synergistic doses: Diosmetin: 5-0.78 to 6.25 μg/mL; 5-FU: 0.15 to 0.62 μg/mL)
- Synergy score: 17.051 ± 1.67 (>10 is synergistic) 17.051% response



HSA synergy and antagonism
Synergistic effect of Diosmetin and 5-FU on HCT116 cell line

Figure 1. Combenefit analysis of diosmetin and 5-FU combination. Data were obtained from three individual experiments.

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Results & Discussion

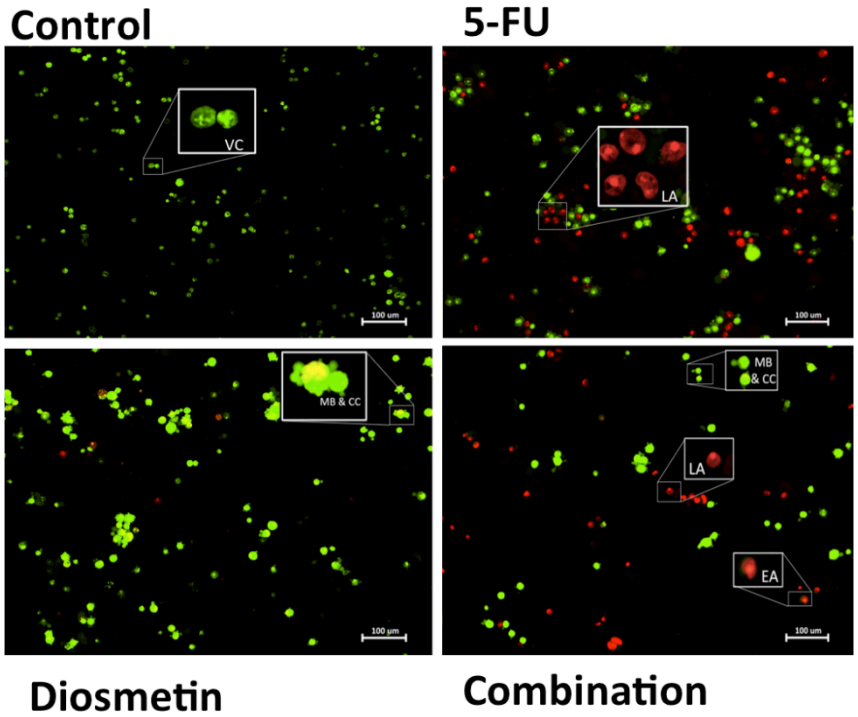

Figure 2. AO/PI staining of HCT-116 cells after 72h treatment with the IC_{50} of 5-FU, diosmetin and combination as compared to untreated control cells. VC: viable cells, LA: late apoptosis, MB: membrane blebbing, CC: chromatin condensation, EA: early apoptosis and N: necrosis. 10x magnification.

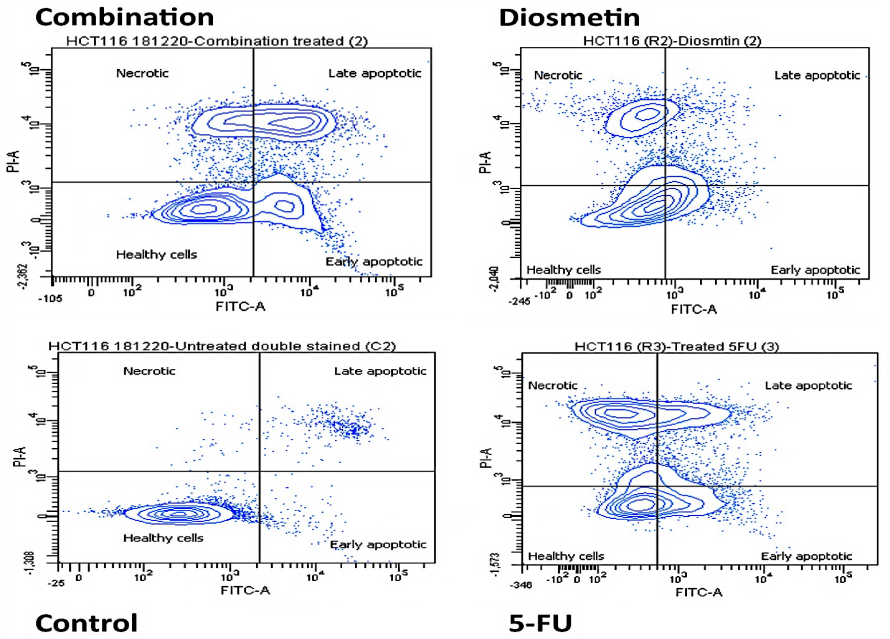

Figure 3. Flowcytometry graph of annexin V-FITC analysis in HCT116 cells. Cells were treated with the IC_{50} doses of the individual drugs and in combination.

- AO/PI: combination therapy induced apoptosis (membrane blebbing & chromatin condensation), with less necrosis compare to 5-FU.
- Annexin V: combination therapy induced apoptosis with 41.9% apoptosis compared to 37.3% of 5-FU treatment. This confirms Phosphatidylserine (PS) translocation

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Conclusions

Overall, this study has provided evidence that 5-FU and diosmetin exert a synergistic effect against HCT-116 cells via apoptosis induction. However, further assessments are required to detect the molecular mechanism of the combination therapy.

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