Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the world.\(^1\) CRC treatment strategies are highly dependent on tumor stage. Metastatic CRC (stage IV) is treated with combined chemotherapy, including 5-fluorouracil (5-FU) plus either irinotecan (CPT-11) or oxaliplatin (L-OHP). Recently, it has been reported that combined chemotherapy plus biological agents (anti-angiogenic or anti-epidermal growth factor receptor molecules) increased stage IV patients’ median survival period.\(^2\) Despite many therapeutic options, an efficient drug combination remains to be found for CRC patients. In addition, tailored therapy for each CRC patient is still a challenge.

It is well-established that epigenetic alterations such as DNA methylation and histone modifications play a crucial role in the initiation and progression of cancer, including CRC. Unlike genetic alterations, epigenetic alterations are reversible and can be targeted by epigenetic modifiers.\(^3\) Epigenetic modifiers such as DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors have been administered for several years and have been evaluated as a treatment approach for a variety of cancers in the United States. DNMT inhibitors, such as decitabine (5-aza-2’-deoxycytidine; DAC) and 5-azacytidine (AC), are being used for the treatment of myelodysplastic syndromes (MDS), but the efficacy is variable.\(^4,5\) In addition, DAC is used usually for patients with acute myeloid leukemia who are not candidates for standard remission, including chemotherapy.\(^6\) On the other hand, HDAC inhibitors, such as vorinostat and romidepsin, have been approved for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma, respectively.\(^7\)

\textit{In vitro} experiments and animal models have shown that DNMT and HDAC inhibitors have anti-tumor activities in CRC.\(^3\) However, minor efficacy of epigenetic modifiers has been reported in solid tumors, including CRC in humans.\(^1,8\) On the other hand, epigenetic mechanisms have been reported to trigger resistance to conventional anticancer drugs in CRC.\(^3\) Thus, combinatorial chemotherapy with epigenetic modifiers may pave the way to the reversal of chemoresistance. DNMT inhibitors and HDAC inhibitors have undergone major preclinical investigation and clinical development to overcome chemoresistance in solid tumors, including CRC.\(^1\)

For example, it has been reported that DNMT inhibitors, such as DAC and AC, showed synergic effects on the cytotoxicity induced by anticancer drugs except for SN-38 plus Zeb, while HDAC inhibitors, trichostatin A, suberoylanilide hydroxamic acid and valproic acid, showed antagonistic effects. DAC showed the most potent synergic effects among the epigenetic modifiers studied. Thus, we examined whether the synergic effect of DAC is observed in other different CRC cell lines, HT29, SW48 and HCT116 cells. In all 4 CRC cell lines, the cytotoxicity of L-OHP was enhanced in a synergic manner by cotreatment with DAC. However, synergic effects of DAC with 5-FU or CPT-11 (SN-38) were not observed in 4 CRC cell lines.

**Key words** DNA methyltransferase inhibitor; synergism; colorectal cancer; isobologram; histone deacetylase inhibitor

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DAC, AC and zebularine (Zeb) as DNMT inhibitors and TSA, SAHA and valproic acid (VPA) as HDAC inhibitors. The results obtained from this study about the effects of epigenetic modification on the cytotoxicity induced by anticancer drugs will provide information to help understand how to incorporate epigenetic modifiers into the current anticancer armamentarium.

MATERIALS AND METHODS

**Materials**  Three human colon adenocarcinoma cell lines (HT29, SW480 and SW48) and human colon carcinoma cell line HCT116 were purchased from DBPharmBiomedical (Osaka, Japan). McCoy's 5A medium, Leibovitz L-15 medium (L-15), penicillin–streptomycin and fetal bovine serum (FBS) were purchased from Life Technologies Corp. (Carlsbad, CA, U.S.A.). L-OHP, DAC, AC, Zeb, TSA and SAHA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 5-FU was purchased from Nacalai Tesque Inc. (Kyoto, Japan). CPT-11 hydrochloride, 7-ethyl-10-hydroxycamptothecin (SN-38) and VPA sodium salt were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.). cis-Diamineplatinum(II) dichloride (CDDP) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

**Cells and Cell Culture**  HT29 and HCT116 were grown in McCoy's 5A supplemented with 10% FBS, 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37°C in 5% CO$_2$–95% air while SW480 and SW48 were grown in L-15 supplemented with 10% FBS, 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37°C in 100% air.[19]

**Drug Treatment**  Cells were seeded at a density of 2×10$^3$ (HT29, HCT116) or 3×10$^5$ (SW480, SW48) in 96-well plates. After culturing for 24 h, cells were treated with the combination of an anticancer drug with an epigenetic modifier and cultured for 72 h. In the combination experiments, epigenetic modifiers were used at approximately IC$_{50}$ or lower concentration; anticancer drugs were used at below, around or above the IC$_{50}$ value.

**Cytotoxicity Assay**  A cytotoxicity assay was performed using a Cell Counting-8 kit (Dojindo Laboratories, Kumamoto, Japan). After drug treatment for 72 h, cells were washed with L-15 medium and incubated with 100 µL L-15 medium (due to the high background absorbance of McCoy's 5A medium) and 10 µL of WST-8 solution for 1–4 h at 37°C. Conversion of WST-8 to its formazan by living cells (active mitochondrial dehydrogenase) was determined using a microplate reader with a wavelength of 450 nm for the indicator color and 655 nm for the background using a microplate reader. For all assays, reactions containing no cells were used to determine blank values, which were subtracted from values obtained from the assays with cells. The IC$_{50}$ values were determined by fitting the following equations to the obtained data using a non-linear least-squares regression analysis program, Microsoft Excel Solver: $V_{\text{drug}}=V_{\text{control}}\times\text{IC}_{50}^C/(C^\gamma+\text{IC}_{50}^C)$, where $V_{\text{drug}}$ and $V_{\text{control}}$ are cell viability in the presence or absence of drugs, respectively. $C$ and $\gamma$ represent the concentration of drugs and sigmoid factor, respectively.

**Analysis of Combination Effects**  In isobologram analysis, we used the method of Steel and Peckham because it can cope with any agents with unclear cytotoxic mechanisms and a variety of dose–response curves of anticancer drugs.[20–22]

![Fig. 1](image-url)  (Fig. 1). Dose–response interactions between anticancer drugs and epigenetic modifiers were evaluated at the IC$_{50}$ level by the isobologram method. The concentrations which produced 50% growth inhibition are expressed as 1.0 on the ordinate and abscissa of isobolograms. Three isoeffect curves, mode I and mode II, were constructed based on the dose–response curves of each anticancer and each epigenetic modifier drug. Mode I line: if the two agents acted additively by independent mechanisms, the combined data points would lie near the mode I line (heteroadddition). Mode II lines: if the agents act additively by similar mechanisms, the combined data points would lie near the mode II lines (isoadddition). When both agents have the same dose–response curves, the mode II lines converge to make a straight line connecting the 1.0 points on the ordinate and abscissa. When both agents have a linear dose–response curve, the mode I and mode II lines converge to make a straight line connecting the 1.0 points on the ordinate and abscissa. Thus, when the data points of the drug combination fell within the area surrounded by mode I and/or mode II lines (i.e., within the envelope of additivity), the combination was described as additive. A combination that gives data points to the left of the envelope of additivity (i.e., the combined effect is caused by lower doses of the two agents than is predicted) can confidently be described as supraadditive (synergistic). A combination that gives data points to the right of the envelope of additivity, but within the square or on the line of the square can be described as subadditive (i.e., the combination is superior or equal to a single agent but is less than additive). A combination that gives data points outside the square can be described as protective (i.e., the combination is inferior to cytotoxic action to a single agent). A combination with both subadditive and/or protective interactions can confidently be described as antagonistic. The isobologram method of Steel and Peckham has an area of uncertainty (envelope of additivity), while many other approaches have an additive line. Therefore, the isobologram of Steel and Peckham is generally stricter regarding synergism and antagonism than other methods.[20–21]

The combination effects were also evaluated using the combination index (CI) method of Chou and Talay.[23] In combination index analysis, the combination effect was evalu-
The CI was defined as follows: CI calculated using CalcuSyn software (Biosoft, Cambridge, U.K.).

The sigmoid factor, and a is the fraction affected. The dose f

HCT116 and HT29), SW480 cells showed the largest IC 50

Cell Lines

Among the 4 CRC cell lines (SW48, SW480, HCT116, and HT29), SW480 cells showed the largest IC 50

The DRI value was defined as follows: CI calculated based on the refined and expanded method of Chou and Talay.23 CI values indicate the following:

Table 1. IC 50 Values of DNA Methyltransferase and Histone Deacetylase Inhibitors in SW480 Cells

Table 2. IC 50 Values of 5-FU, CPT-11, SN-38 and L-OHP in 4 CRC Cell Lines

Table 2. IC 50 Values of DNA Methyltransferase and Histone Deacetylase Inhibitors in SW480 Cells

Table 3. IC 50 Values of 5-FU, CPT-11 or SN-38 and L-OHP in 4 CRC Cell Lines

Cells were treated with various concentrations of drugs for 72 h, and cell viability was measured by the WST-8 assay. Each value represents the mean±S.D. of three independent experiments. The IC 50 value was calculated as described in Materials and Methods. 5-FU: 5-fluorouracil, CPT-11: irinotecan, SN-38: 7-ethyl-10-hydroxy camptothecin, L-OHP: oxaliplatin.

RESULTS

Cytotoxicity Induced by Anticancer Drugs in 4 CRC Cell Lines The results for combinations of anticancer drugs and epigenetic modifiers were analyzed with the help of both isobologram20 and CI23 values. Isobologram analysis provides a graphical presentation of the nature of the interaction of two drugs, and actual combined data points (C1/C1,1 and C2/C2,2) were plotted. Based on the dose–response curves of an anticancer drug alone and epigenetic modifier alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC50 levels were generated based on these dose–response curves for the combinations. Combined data points in the isobologram method by Steel and Peckham may be additive although CI

Fig. 2. Effects of Anticancer Drugs on the Growth of SW480 Cells

SW480 cells were treated with various concentrations of drugs for 72 h, and cell viability was measured by the WST-8 assay. Each point represents the mean±S.D. of three independent experiments. 5-FU: 5-fluorouracil, CPT-11: irinotecan, SN-38: 7-ethyl-10-hydroxy camptothecin, L-OHP: oxaliplatin.

Table 1. IC 50 Values of 5-FU, CPT-11, SN-38 and L-OHP in 4 CRC Cell Lines

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RESULTS

Cytotoxicity Induced by Anticancer Drugs in 4 CRC Cell Lines Among the 4 CRC cell lines (SW48, SW480, HCT116 and HT29), SW480 cells showed the largest IC50 values of 3 anticancer drugs (5-FU, CPT-11 or SN-38 and L-OHP) (Fig. 2, Table 1). Therefore, SW480 cells were used for experiments on the combinatorial effects of the anticancer drugs and epigenetic modifiers below.

Cytotoxicity Induced by Epigenetic Modifiers in SW480 Cells The IC50 values of DNMT inhibitors and HDAC inhibitors in SW480 cells are shown in Table 2. The cytotoxicity induced by DAC showed a slower sigmoid curve than that induced by other epigenetic modifiers examined (Fig. 3).

Cytotoxicity Induced by DAC in 4 CRC Cell Lines The IC50 values of DAC were HT29>SW480>SW48>HCT116. The difference in IC50 values between HT29 and HCT116 cells was about 824-fold (Table 3).

Combinatorial Effects of Anticancer Drugs and Epigenetic Modifiers in SW480 Cells The results for combinations of anticancer drugs and epigenetic modifiers were analyzed with the help of both isobologram20 and CI23 values. Isobologram analysis provides a graphical presentation of the nature of the interaction of two drugs, and actual combined data points (C1/C1,1 and C2/C2,2) were plotted. Based on the dose–response curves of an anticancer drug alone and epigenetic modifier alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC50 levels were generated based on these dose–response curves for the combinations. Combined data points in the isobologram method by Steel and Peckham may be additive although CI
values are synergistic or antagonistic, since their isobologram is generally stricter regarding synergism and antagonism than other methods. Therefore, in this study, the CI values in each Table, which were regarded as synergistic or antagonistic in isobologram analysis, are shown. In addition, different combination concentrations of 2 drugs were used in this study, since synergism and antagonism can be different at dose or effect levels.23) In the combination study, anticancer drugs and epigenetic modifiers were used at below, around or above the IC50 value, and at approximately IC50 or at a lower concentration, respectively.

Supplementary Figure 1 shows the isobologram of SW480 cells treated with both anticancer drugs and DNMT inhibitors. Regarding DAC, all or some combined data points fell in the synergistic area regardless of the types of anticancer drugs (Supplementary Fig. 1A). However, in AC or Zeb treatment, most of combined data points fell in the antagonistic area and within the envelope of additivity in combination with 5-FU, CPT-11 or SN-38 (Supplementary Figs. 1B, C). In addition, as shown in CI value analysis of Table 4, CI values were lowest with DAC treatment than AC or Zeb treatment, indicating that DAC showed more synergistic effects in the cytotoxicity induced by 5-FU, CPT-11 (SN-38) and L-OHP. Notably, only with AC treatment, the combination effect at a lower concentration (0.5 μM) tended to be more potent than that at a higher concentration (2.0 μM) (Supplementary Fig. 1B). On the other hand, when using HDAC inhibitors, almost all data points fell in the antagonistic area and within the envelope of additivity (Supplementary Figure 2). CI values were >1, the criteria of additivity and antagonism, in the treatment with all 3 HDAC inhibitors except for VPA plus CPT-11 (Table 4).

Synergism between drugs allows for the reduction of doses used to minimize unwanted adverse effects while maintaining therapeutic efficacy. Thus we calculated the DRI. A DRI >1 correlates with a synergistic interaction. Combinations of anticancer drugs with DAC allowed for maximum dose-reduction of 31-fold with DAC and 26-fold with 5-FU, 71-fold with DAC and 6.6-fold with CPT-11, 726-fold with DAC and 14-fold with SN-38, 235-fold with DAC and 26-fold with L-OHP (Table 4). However, DRI values in combinations with AC or Zeb were less than those in combinations with DAC (Table 4). Most of the DRI values in combinations with HDAC inhibitors included 1 (Table 4).

Comparison of Combinatorial Effects of Anticancer Drugs and DAC among 4 CRC Cell Lines

Combinatorial effects of anticancer drugs and DAC were examined in HT29, SW48 and HCT116 cells in addition to SW480 cells. Furthermore, another platinum anticancer drug, CDDP, was also examined for the combinatorial effect of co-treatment with DAC in 4 CRC cell lines. Supplementary Figure 3 shows the isobologram of HT29, SW48 and HCT116 cells treated with both anticancer drugs and DAC. In addition, as shown in Table 4, CI values were lowest with DAC treatment than AC or Zeb treatment, indicating that DAC showed more synergistic effects in the cytotoxicity induced by 5-FU, CPT-11 (SN-38) and L-OHP. Notably, only with AC treatment, the combination effect at a lower concentration (0.5 μM) tended to be more potent than that at a higher concentration (2.0 μM) (Supplementary Fig. 1B). On the other hand, when using HDAC inhibitors, almost all data points fell in the antagonistic area and within the envelope of additivity (Supplementary Figure 2). CI values were >1, the criteria of additivity and antagonism, in the treatment with all 3 HDAC inhibitors except for VPA plus CPT-11 (Table 4).

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strong to very strong synergism (Table 5). However, with 5-FU or CPT-11 (SN-38), similar results were not observed in HT29, SW48 and HCT116 cells. CDDP also showed a synergistic effect by co-treatment with DAC (Supplementary Fig. 4, Supplementary Table 1). The DRI values in combinations, which were regarded as synergistic in isobologram analysis, were >1 in both DAC and each anticancer drug (Table 5, Supplementary Table 1).

**DISCUSSION**

Recent improvements in combination chemotherapy have extended the survival duration in patients suffering from CRC. However, the effects of epigenetic modifiers on combination therapy remain to be elucidated. In addition, the effects could be influenced by the types of drugs or cancer cells. Thus, using 4 CRC cell lines, including MSI-present/absent cells, we examined the effects of typical epigenetic modifiers, DNMT or HDAC inhibitors, on the cytotoxicity induced by several anticancer drugs.

First, we examined the combinatorial effects of two types of epigenetic modifiers, DNMT or HDAC inhibitors, with 5-FU, CPT-11: irinotecan, SN-38: 7-ethyl-10-hydroxycamptothecin, L-OHP: oxaliplatin, DAC: decitabine, AC: 5-azacytidine, Zeb: zebularine, TSA: trichostatin A, SAHA: suberoylanilide hydroxamic acid, VPA: valproic acid.

In Table 4, CI values, graded symbols and DRI values of combination study in SW480 cells are shown. CI and DRI values represent minimum–maximum value and symbols represent graded symbols defined in Table 4. CI values were calculated as described in Materials and Methods. CI and DRI values are shown which were regarded as synergistic or antagonistic in isobologram analysis. Combination data, which were regarded as additive in isobologram analysis, are shown as ±: nearly additive, +: slight antagonism, ++: moderate antagonism, +++: strong antagonism, +++++: very strong antagonism.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>5-FU</th>
<th>CPT-11</th>
<th>SN-38</th>
<th>L-OHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29</td>
<td>1.2–3.3</td>
<td>0.58–8.1</td>
<td>0.45–8.8</td>
<td>1.3</td>
</tr>
<tr>
<td>SW48</td>
<td>0.30–0.32</td>
<td>9.46–3.6×10^5</td>
<td>3.1–3.3</td>
<td>1.9</td>
</tr>
<tr>
<td>HCT116</td>
<td>1.5–3.8</td>
<td>0.34–0.76</td>
<td>1.3–7.2</td>
<td>0.43</td>
</tr>
</tbody>
</table>

In Table 5, CI values, graded symbols and DRI values of combination of DAC with anticancer drugs in HT29, SW48 and HCT116 cells are shown. CI and DRI values represent minimum–maximum value and symbols represent graded symbols defined below. CI values were calculated as described in Materials and Methods. CI values were regarded as synergistic or antagonistic in isobologram analysis. Combination data, which were regarded as additive in isobologram analysis, are shown as ±: nearly additive, +: slight antagonism, ++: moderate antagonism, +++: strong antagonism, +++++: very strong antagonism.
genetic modifiers as chemo-sensitizers in combination with chemotherapeutic drugs. In order to decide the concentration of epigenetic modifiers in the combination study, IC\textsubscript{50} values of epigenetic modifiers were examined in SW480 cells. The cytotoxicity induced by DAC showed a slower sigmoid curve than that induced by other epigenetic modifiers examined. In the combination study, anticancer drugs and epigenetic modifiers were used below, around or above the IC\textsubscript{50} value, and at approximately IC\textsubscript{50} or a lower concentration, respectively.

In the combination study using SW480 cells, contrasting results were observed. On the whole, DNMT inhibitors had synergic effects on the cytotoxicity induced by the anticancer drugs examined, except for SN-38 plus Zeb, while HDAC inhibitors had antagonistic effects. In addition, the synergic effect of DAC was stronger than that of AC or Zeb. The allowed dose-reduction in combinations of anticancer drugs with DAC was greater than that with AC or Zeb. Notably, only in AC treatment, the combination effect at a lower concentration (0.5\textmu M) tended to be more potent than that at a higher concentration (2.0\textmu M). As previously demonstrated, low-dose AC, as well as DAC, has been associated with different therapeutic responses compared with high-dose treatment with the same drug, including not only many different leukemia cell lines but also solid cancer cell lines.\textsuperscript{24} These drugs act as DNMT inhibitors at a low dose, but at a high dose, cytidine analogue such as AC exert their effects by incorporation into not only DNA but also RNA. Indeed, AC was developed as a toxic nucleoside analogue well before the field of epigenetics had truly emerged. Furthermore, AC and DAC have been reported to have different mechanisms of action.\textsuperscript{25}

Next, we further examined whether the synergic effect of DAC is observed in 4 different CRC cell lines. Two of the 4 CRC cell lines were MSI (SW48 and HCT116 cells) and 2 were MSS (HT29 and SW480 cells).\textsuperscript{19} First, the IC\textsubscript{50} value of DAC was compared among 4 CRC cell lines. A marked difference in IC\textsubscript{50} values, 824-fold between HT29 and HCT116 cells, was observed. This difference was substantially larger than that observed in IC\textsubscript{50} values of anticancer drugs among the 4 CRC cell lines examined. The synergic effect of DAC by co-treatment with SN-38 or Zeb (SN-38) was not necessarily observed in other CRC cell lines, HT29, SW48 or HCT116 cells. On the other hand, co-treatment with DAC and L-OHP showed a synergic effect in the 4 CRC cell lines examined. Even in HT29 cells, with the highest IC\textsubscript{50} value, DAC showed a synergic effect against SN-38 or L-OHP. Inversely, HCT116 cells, which were the most sensitive to DAC, showed an antagonistic effect with DAC plus 5-FU or SN-38. These results suggest that the intensity of cytotoxicity by DAC is not associated with that of synergic effects. Also, L-OHP may enhance the sensitivity of DAC, although L-OHP itself did not show an inhibitory effect on DNA methylation (data not shown).

In addition, we examined whether another platinum anticancer drug, CDDP, has a synergic effect on the cytotoxicity induced by anticancer drugs. CDDP showed a similar synergic effect on the cytotoxicity induced by anticancer drugs. This implies that the synergic effect observed by co-treatment with DAC is not specific to L-OHP, and could be a common phenomenon when using other platinum anticancer drugs. Therefore, L-OHP plus DAC was suggested to be a suitable combination for CRC cells.

Regarding DAC and AC, in in vitro studies using CRC cell lines, synergic effects such as enhanced cytotoxicity or reversal of multidrug resistance induced by anticancer drugs have been reported.\textsuperscript{3,11} An in vivo xenograft study showed that low-dose DAC has a synergistic effect in combined therapy with CPT-11.\textsuperscript{23} In addition, Morita et al. reported the synergistic effect of DAC and 5-FU on SW48 colon tumor xenografts, which corresponds to the results in this study.\textsuperscript{26} However, there is little information on whether these synergic effects could apply to different cells. In contrast to this study, HDAC inhibitors showed synergic effects in combination with anticancer drugs in vitro studies using CRC cell lines. Pretreatment with HDAC inhibitors such as TSA or SAHA increased the killing efficacy of anticancer drugs in several types of cancer cell lines, but treating the cells in reverse order had no greater cytotoxic effect, indicating that the treatment schedule is dependent on the combinatorial effect.\textsuperscript{12} Previous research suggested that combinatorial effects by HDAC inhibitors may differ among the types of anticancer drugs as well as cancer cells.\textsuperscript{13,14,27} L-OHP has been reported to show a stronger synergic effect than 5-FU by co-treatment with HDAC inhibitors.\textsuperscript{27,28} Furthermore, the results in this study should be confirmed in vivo, such as by using tumor xenograft models since the results of an in vitro study may not correspond to an in vivo study.\textsuperscript{29} Nevertheless, this in vitro study is indispensable in order to decide the type of cancer cells for the tumor xenograft model experiment. We should also evaluate the combination efficacy of more than two agents because the standard chemotherapy for colorectal cancer combines 5-FU with CPT-11 (SN-38) or L-OHP. Further studies to clarify the detailed mechanisms of the combinatorial effects and for clinical application are also needed, and currently we are examining these issues.

This study suggests that DNMT inhibitors have a synergic effect on the cytotoxicity induced by anticancer drugs, while HDAC inhibitors have an antagonistic effect on most resistant SW480 cells among the 4 CRC cell lines. Among the DNMT inhibitors examined, DAC showed the strongest synergic effect on the cytotoxicity induced by anticancer drugs, 5-FU, CPT-11 (SN-38) and L-OHP, in SW480 cells. Furthermore, in all 4 CRC cell lines, the cytotoxicity of L-OHP was enhanced in a synergic manner by co-treatment with DAC, the cytotoxicity of which showed a marked difference among the 4 CRC cell lines. These findings provide insight into comprehensive understanding of the appropriate combination of epigenetic modifiers and anticancer drugs for CRC cells. However, we should be aware that the synergic effect by the combination of DAC and 5-FU or CPT-11 (SN-38) depends on the type of CRC cells.

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Vol. 37, No. 1


