

# Sulforaphane enhances apoptosis induced by *Lactobacillus pentosus* strain S-PT84 via the TNF $\alpha$ pathway in human colon cancer cells

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**Abstract.** Sulforaphane and *Lactobacilli* induce apoptosis in several cancer cells. Sulforaphane, a dietary isothiocyanate, is an attractive agent due to its potent anticancer effects. Sulforaphane suppresses the proliferation of various cancer cells *in vitro* and *in vivo*. The present study investigated the effect of sulforaphane and a co-culture with *Lactobacillus*-treated peripheral blood mononuclear cells (PBMCs) in human colon cancer cells. The combination markedly induced apoptosis in human colon cancer HCT116 and SW480 cells. A pan-caspase inhibitor markedly inhibited apoptosis, and a tumor necrosis factor (TNF) receptor/Fc chimera partially inhibited apoptosis in both cells. The amount of TNF $\alpha$  secretion in the culture supernatant was significantly increased by co-culture with *Lactobacillus*-treated normal human PBMCs. On the other hand, the expression of cellular inhibitor of apoptosis-2 (cIAP-2), an anti-apoptotic protein, was increased by co-culture with *Lactobacillus*-treated PBMCs in colon cancer cells, but sulforaphane treatment significantly suppressed the induction of cIAP-2. The present results revealed that sulforaphane enhances apoptosis in human colon cancer cells under co-culture with *Lactobacillus*-treated PBMCs via the TNF $\alpha$  signaling pathway.

## Introduction

Malignant tumors are a serious disease and since 1981 have the highest risk of death in Japan. In particular, colon cancer

is the second most common type of cancer death (1). The association between fruit and vegetable intake and colorectal cancer risk has been investigated by many epidemiologic studies (2-4). Fruit and vegetables contain cancer-preventive agents, and dietary chemoprevention strategies have gained significant interest (5-7).

Sulforaphane is an isothiocyanate that is abundant in cruciferous vegetables, including broccoli sprouts, cabbage, and cauliflower (8,9). Several reports showed that sulforaphane, a well-known phytochemical, induces cell cycle arrest and apoptosis in cancer cells (10-14). Sulforaphane is a promising compound for cancer prevention and therapy (15-18). Sulforaphane downregulates the expression of Bcl-2, inhibitor of apoptosis (cIAP)-1/2, and XIAP in prostate cancer (14) and the expression of Bcl-2 family proteins in human hepatoma cells (19).

There are several reports that suggest that *Lactobacilli* have antitumor activity. Takeda *et al* (20) found that drinking fermented products containing *Lactobacilli* have immunological effects on the immune system of healthy individuals, and *Lactobacilli* have anticancer effects *in vitro* and *in vivo* (21-23). Ishikawa *et al* (24) found in randomized clinical studies that a *Lactobacillus* strain prevents colorectal tumors. *Lactobacillus* strains induce tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), tumor necrosis factor (TNF)  $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-10 from normal human peripheral blood mononuclear cells (PBMCs) (25-27).

Izumo *et al* (28,29) and Hayama *et al* (30) reported that the *Lactobacillus* (*L.*) *pentosus* strain S-PT84, isolated from Kyoto pickles, has inhibitory activity against influenza virus, *Salmonella Typhimurium*, and *Candida albicans* infection in mice, and S-PT84 enhances IFN- $\alpha$  production from plasmacytoid dendritic cells by virus stimulation (31).

Previously, we proposed 'combination-oriented molecular-targeting prevention' of cancer (32). A combination of two agents can synergistically enhance their preventive effects, even if the effect of each single agent is weak. A combination of sulforaphane and curcumin has a synergistic anti-inflammatory effect (33), and a combination of sulforaphane and epigallocatechin-gallate has a synergistic growth-inhibitory effect in human colon carcinoma cells (34). Additionally, sulforaphane enhances TRAIL-induced

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**Abbreviations:** PBMCs, peripheral blood mononuclear cells; TNF, tumor necrosis factor

**Key words:** sulforaphane, *Lactobacillus*, tumor necrosis factor  $\alpha$ , apoptosis, colon cancer

apoptosis in human cancer cells (35,36). TRAIL is a member of the TNF family of cytokines, which is crucial for cancer prevention.

Therefore, we hypothesized that the combination of sulforaphane and a TRAIL inducer, such as *Lactobacillus*, may have a beneficial effect for cancer prevention. In this study, we evaluated the effect of a combination of sulforaphane and co-culture with *L. pentosus* S-PT84-treated PBMCs in human colon cancer cells.

## Materials and methods

**Reagents and antibodies.** Sulforaphane was purchased from LKT Laboratories (St. Paul, MN, USA) and dissolved in dimethyl sulfoxide (DMSO). *Lactobacillus pentosus* strain S-PT84 was kindly gifted from the Suntory Global Innovation Center (Kyoto, Japan) (37). Heat-killed *L. pentosus* S-PT84 was freeze-dried and used for the following experiments: Propidium iodide was purchased from Sigma (St. Louis, MO, USA). 5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was purchased from FUJIFILM Wako pure chemical corporation (Osaka, Japan). Annexin V-FITC apoptosis detection kit was purchased from Nacalai Tesque (Kyoto, Japan). Antibodies against cIAP-1, cIAP-2, Bcl-xL, acetylated-Histone H4, and Histone H4 were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibody against TNFR1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against Bcl-2 and Bim were purchased from Abcam (Cambridge, MA, USA). Antibody against Bax was purchased from BD Biosciences (San Jose, CA, USA). Antibody against GAPDH was purchased from HyTest (Turku, Finland). Antibodies against XIAP, human recombinant TNF $\alpha$ /Fc chimera, human recombinant DR5/Fc chimera, human recombinant Fas/Fc chimera, and zVAD-fmk were obtained from R&D Systems (Minneapolis, MN, USA). Granzyme B inhibitor I was obtained from Calbiochem (San Diego, CA, USA). HRP-conjugated anti-mouse IgG and HRP-conjugated anti-rabbit IgG were purchased from GE Healthcare (Piscataway, NJ, USA).

**Cell culture.** Human colon cancer HCT116 and SW480 cells and normal human colon epithelial CCD 841 CoN cells were obtained from the American Type Culture Collection (Rockville, MD, USA). HCT116 and SW480 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, 4 mmol/l glutamine, 50 U/ml of penicillin G, and 100  $\mu$ g/ml of streptomycin. CCD 841 CoN cells were maintained in Eagle's minimum essential medium supplemented with 10% (v/v) fetal bovine serum, 4 mmol/l glutamine, 50 U/ml of penicillin G, and 100  $\mu$ g/ml of streptomycin. Normal human PBMCs were isolated as previously described (25). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

For co-culture, HCT116 or SW480 cells were seeded onto 12-well plates (BD Falcon, no. 353503). The next day, PBMCs were seeded on cell culture inserts (0.4  $\mu$ m pores; BD Falcon no. 353494) in a 12-well plate.

**Cell proliferation assay.** The number of viable cells was determined using the Cell Counting Kit-8 according to the

manufacturer's instructions (Dojindo Molecular Technology, Kumamoto, Japan).

**Detection of sub-G1 population.** Cells were stained with 10  $\mu$ g/ml of propidium iodide. Flow cytometry was performed with FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA) and CellQuest software (BD Biosciences).

**Detection of apoptosis (JC-1).** Cells were stained with 2  $\mu$ mol/l JC-1 and incubated for 15 min. Cells were washed twice and resuspended with PBS. Flow cytometry was performed with FACSCalibur and CellQuest software.

**Detection of apoptosis (Annexin V).** Annexin V-FITC apoptosis detection kit was used according to the manufacturer's protocol. Briefly, cells were resuspended with Annexin V binding buffer and incubated with Annexin V and propidium iodide for 15 min. Flow cytometry was performed with FACSCalibur and CellQuest software.

**Enzyme-linked immunosorbent assay (ELISA).** Cells were centrifuged and the supernatant was collected. A human TNF $\alpha$  ELISA kit (Abcam, Cambridge, UK) was used according to the manufacturer's instructions.

**Western blotting.** Cells were lysed in buffer containing 50 mmol/l Tris-HCl (pH 7.5), 1% SDS, 2  $\mu$ g/ml leupeptin, 2  $\mu$ g/ml aprotinin, and 1 mmol/l dithiothreitol (DTT). The lysate was sonicated and centrifuged at 14,000 g for 20 min at 4°C, and the supernatant was collected. Equal amounts of lysate were analyzed by SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The blots were blocked in blocking buffer for 1 h at room temperature and incubated with the appropriate primary antibody in blocking buffer for 1 h at room temperature. The blots were then washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody for 1 h, and signals were detected using a Chemilumi-one chemiluminescent kit (Nacalai Tesque, Kyoto, Japan) or Immobilon Western Chemiluminescent HRP Substrate (Millipore). The band intensities were quantified by ImageJ software (NIH).

**Statistical analysis.** Data are the means  $\pm$  standard deviation of three experiments. For simple group means analysis, data were analyzed using a Student's t-test and differences were considered significant at  $P < 0.05$ . For between-group comparisons, one-way ANOVA and Tukey's post hoc test were performed using Microsoft Excel 2007 (Microsoft Corporation).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Sulforaphane enhances apoptosis induced by co-culture with *Lactobacillus*-treated PBMCs in human colon cancer cells.** Sulforaphane, a well-known phytochemical, induces apoptosis in cancer cells (10-14). We investigated the effect of sulforaphane on cell growth in human colon cancer HCT116 and SW480 cells and found that sulforaphane suppressed the growth of both cells in a dose-dependent manner (Fig. 1A).

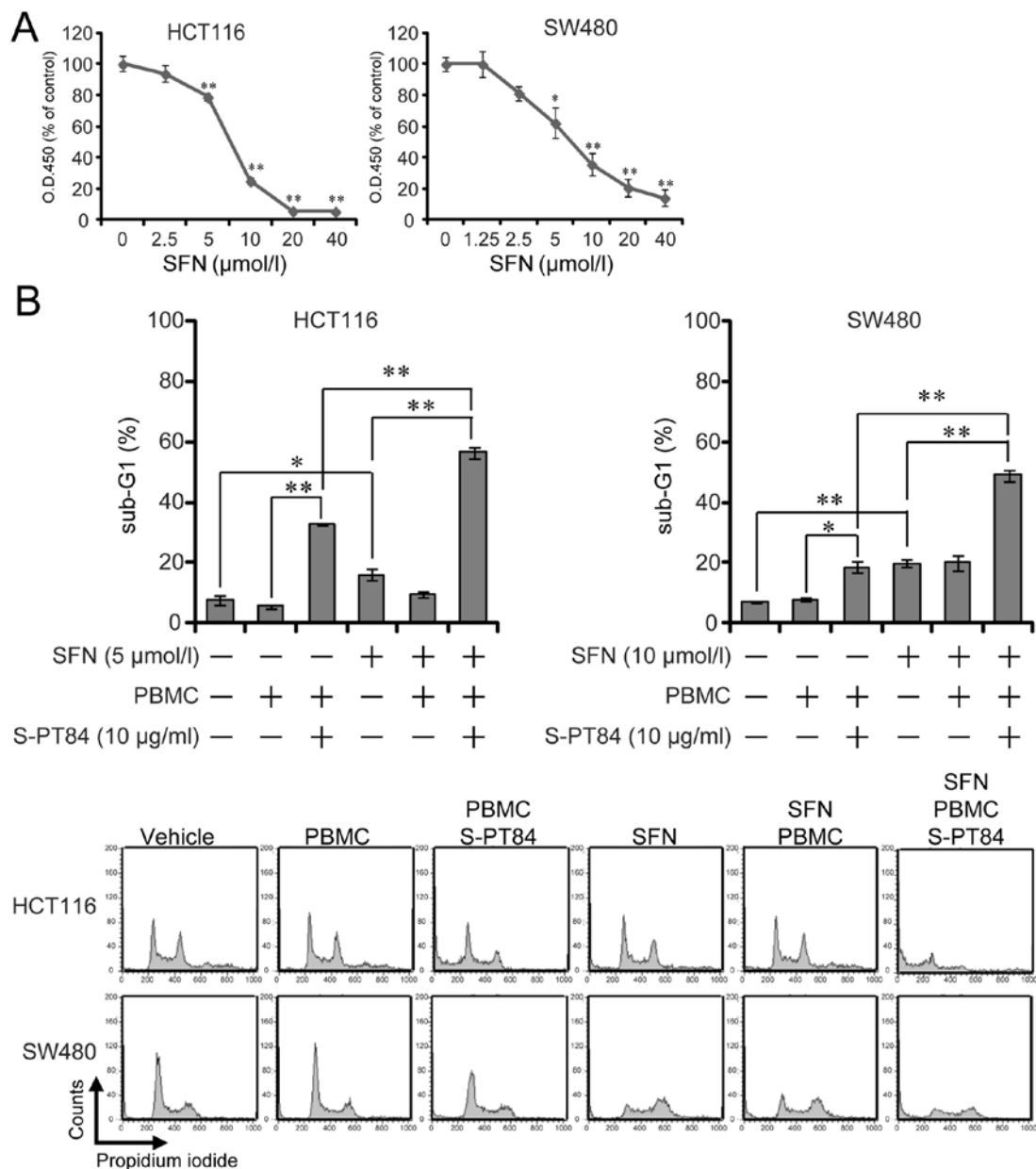


Figure 1. SFN enhances apoptosis induced by *Lactobacillus* in human colon cancer cells. (A) Cell proliferation assay. HCT116 and SW480 cells were treated with SFN at various concentrations for 72 h, and cell growth was determined using Cell Counting Kit-8. \* $P < 0.05$  and \*\* $P < 0.01$  vs. 0  $\mu\text{mol/l}$  SFN. (B) Detection of sub-G1 population cells. PBMCs were pre-incubated with 10  $\mu\text{g/ml}$  *L. pentosus* S-PT84 for 24 h. HCT116 or SW480 cells were co-incubated with PBMCs (E/T ratio=100:1), incubated with or without SFN for 48 h, and then harvested. After fixation and staining with propidium iodide, HCT116 and SW480 cells were subjected to cell cycle analysis using FACSCalibur. Representative histogram patterns from HCT116 and SW480 cells. Data are presented as the mean  $\pm$  standard deviation of three experiments. \* $P < 0.05$  and \*\* $P < 0.01$ , as indicated. SFN, Sulforaphane; PBMCs, peripheral blood mononuclear cells.

Previously, we showed that *Lactobacillus* strains facilitated natural killer activity against human cancer cells *in vitro* (25). Here, we evaluated the effect of *L. pentosus* S-PT84 on cellular immunity by inducing apoptosis in human colon cancer HCT116 and SW480 cells in co-culture with PBMCs treated by *L. pentosus* S-PT84. First, isolated PBMCs from a healthy volunteer were pre-treated by *L. pentosus* S-PT84 for 24 h. Second, PBMCs were co-cultured with human colon cancer cells. After 48 h, the sub-G1 population was examined by flow cytometry. Although non-treated PBMCs showed basal levels of cytotoxicity, *Lactobacillus*-treated PBMCs induced apoptosis against HCT116 and SW480 cells (Fig. 1). Similarly, the sub-G1 population was significantly increased

by sulforaphane in both human colon cancer cell lines tested (Fig. 1B).

We evaluated the effect of the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs in human colon cancer cells. As shown in Fig. 1B, the addition of sulforaphane increased the sub-G1 population from 33 to 57% in HCT116 cells and from 19 to 49% in SW480 cells under co-culture with *Lactobacillus*-treated PBMCs. Next, Annexin V/propidium iodide staining was used to analyze apoptosis by treatment in human colon cancer HCT116 and SW480 cells or in normal human colon CCD 841 CoN cells. Sulforaphane markedly enhanced apoptosis induced by co-culture with *Lactobacillus*-treated PBMCs in human

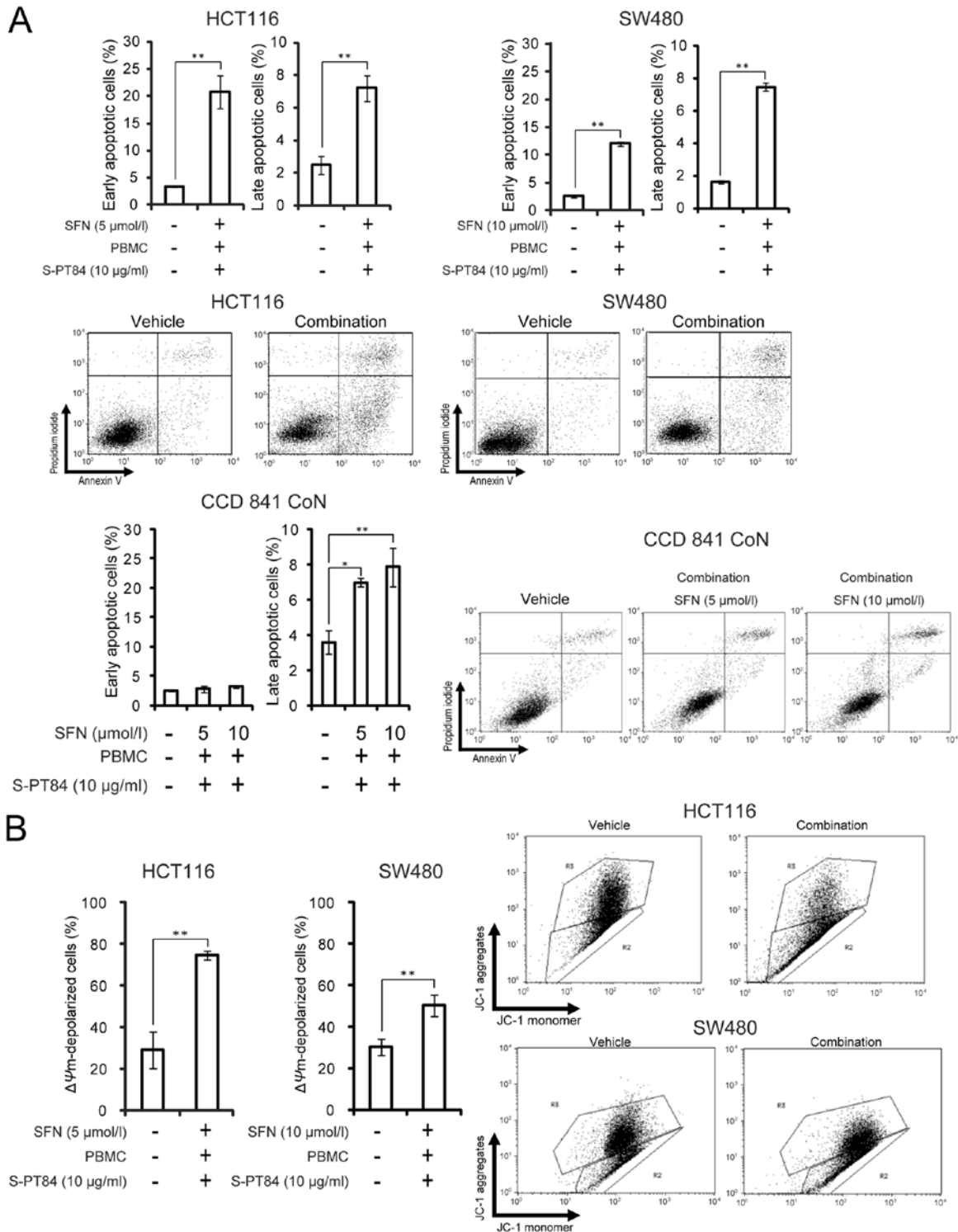


Figure 2. SFN enhances apoptosis induced by *Lactobacillus* in human colon cancer cells. (A) Annexin V and propidium iodide staining. PBMCs were pre-incubated with 10 μg/ml *L. pentosus* S-PT84 for 24 h. HCT116, SW480, or CCD 841 CoN cells were co-incubated with PBMCs (E/T ratio=100:1), incubated with SFN for 48 h, and then harvested. After staining with Annexin V and propidium iodide, the cells were subjected to apoptosis analysis using FACSCalibur. The bottom right quadrant indicates early apoptotic cells, whereas the top right quadrant indicates late apoptotic cells. (B) JC-1 staining to measure mitochondrial depolarization. HCT116 or SW480 cells were co-incubated with PBMCs (E/T ratio=100:1), incubated with SFN for 48 h, and then harvested. PBMCs were pre-incubated with 10 μg/ml *L. pentosus* S-PT84 for 24 h. After staining with JC-1, the cells were subjected to apoptosis analysis using FACSCalibur. The top gate indicates normal mitochondria (healthy cells, red fluorescence), whereas the bottom gate indicates depolarized mitochondria (apoptotic cells, green fluorescence). Data are presented as the mean ± standard deviation of three experiments. \*P<0.05 and \*\*P<0.01, as indicated. SFN, Sulforaphane; PBMCs, peripheral blood mononuclear cells.

colon cancer cells more than in normal human colon cells (Fig. 2A). In addition, we used PBMCs from another volunteer to assess the combination of sulforaphane and *L. pentosus*

S-PT84-treated PBMCs (Fig. S1). As a result, the combination also induced apoptosis in HCT116 cells markedly. Furthermore, we assessed the mitochondrial transmembrane

potential in human colon cancer HCT116 and SW480 cells by JC-1 staining (Fig. 2B). To investigate the mitochondria membrane potential ( $\Delta\Psi_m$ ) involved in apoptotic induction, we analyzed apoptosis using a mitochondria-specific dye JC-1. As shown in Fig. 2B, the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs markedly decreased  $\Delta\Psi_m$  in human colon cancer HCT116 and SW480 cells, which indicates an increase in apoptosis.

*Sulforaphane enhances apoptosis induced by Lactobacillus in human colon cancer cells via the TNF $\alpha$  pathway.* Three death factors (TNF $\alpha$ , FasL, and TRAIL) and their receptors have been identified (38). Furthermore, cytotoxic lymphocytes induce apoptosis in target cells by these death factors and/or perforin and granzyme B (39,40). To investigate the apoptosis pathway induced by sulforaphane and co-culture with *Lactobacillus*-treated PBMCs, we used a TNFR/Fc chimera, DR5/Fc chimera, Fas/Fc chimera, Granzyme B inhibitor I, and pan-caspase inhibitor. Apoptosis was markedly blocked by the pan-caspase inhibitor zVAD-fmk, which indicates that apoptosis was caspase-dependent in HCT116 and SW480 cells (Fig. 3). Furthermore, the TNFR/Fc chimera partially inhibited apoptosis in both cells (Fig. 3). On the other hand, the DR5/Fc chimera, Fas/Fc chimera, and Granzyme B inhibitor I did not affect apoptosis. These results suggest that the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs induced caspase-dependent apoptosis by the TNF $\alpha$ -TNFR pathway.

*Secretion of TNF $\alpha$  is increased by addition of Lactobacillus.* We found that TNF $\alpha$  was involved in apoptosis by a combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs. Microbial stimuli increases the secretion of various cytokines, including TNF $\alpha$ , from PBMCs (26). Therefore, we evaluated the amount of TNF $\alpha$  secretion from the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs using culture supernatant by ELISA. As shown in Fig. 4, TNF $\alpha$  concentration markedly increased by co-culture with *Lactobacillus*-treated PBMCs, regardless of sulforaphane treatment, in both cells.

*Effects of sulforaphane and/or co-culture with Lactobacillus-treated PBMCs on the expression of various intracellular regulators of apoptosis on human colon cancer cells.* To investigate the extrinsic and intrinsic pathways of apoptosis induced by the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs, we examined the expression of apoptosis-related proteins using western blotting (Fig. 5), and normalized histograms were shown in Fig. S2. As shown in these data, cIAP-1 and cIAP-2, anti-apoptotic IAP family proteins, were significantly upregulated by co-culture with *Lactobacillus*-treated PBMCs in both cancer cells. However, sulforaphane treatment suppressed the induction of cIAP-1 and cIAP-2 (Figs. 5A, B and S2). Co-culture with *Lactobacillus*-treated PBMCs slightly induced the expression of TNFR1, a TNF $\alpha$  receptor, in HCT116 cells (Figs. 5A and S2). Additionally, sulforaphane further increased the induction of TNFR1 in HCT116 cells (Figs. 5A, B and S2). XIAP was markedly downregulated by sulforaphane in SW480 cells with or without co-culture with *Lactobacillus*-treated

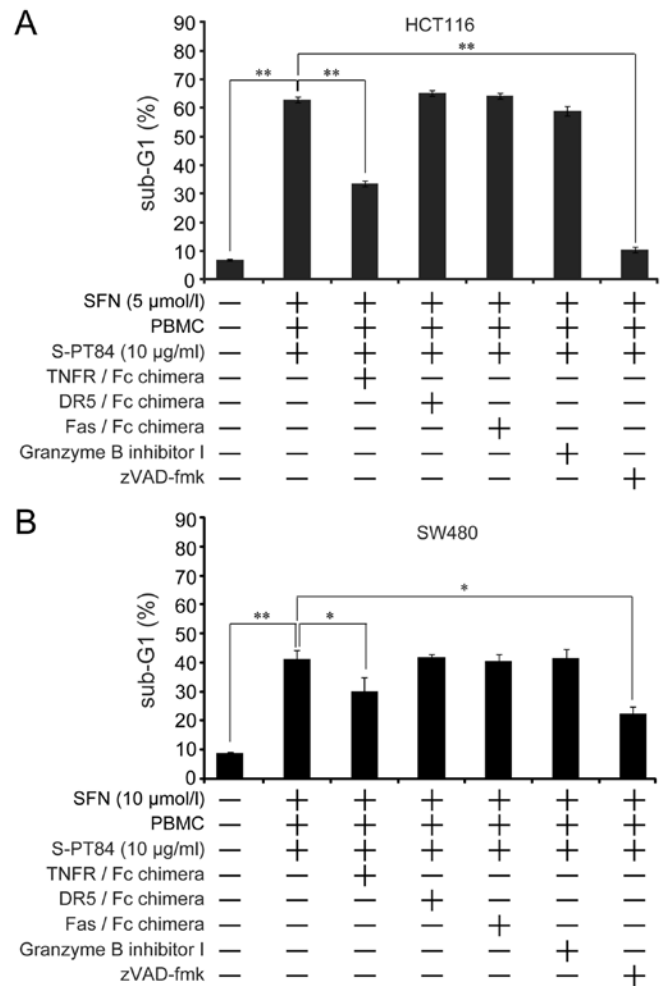


Figure 3. SFN enhances apoptosis induced by *Lactobacillus* in human colon cancer cells via the TNF $\alpha$  pathway. PBMC ( $2 \times 10^6$ /ml) were pre-incubated with 10  $\mu$ g/ml *L. pentosus* S-PT84 for 24 h. HCT116 and SW480 cells were co-incubated with PBMCs under treatment of SFN with or without 1  $\mu$ g/ml TNFR/Fc chimera, 1  $\mu$ g/ml DR5/Fc chimera, 1  $\mu$ g/ml Fas/Fc chimera, 20  $\mu$ mol/l Granzyme B inhibitor I, or 20  $\mu$ mol/l zVAD-fmk for 48 h, and then harvested. After fixation and staining with propidium iodide, (A) HCT116 or (B) SW480 cells were subjected to cell cycle analysis using FACSCalibur. Data are presented as the mean  $\pm$  standard deviation of three experiments. \* $P < 0.05$  and \*\* $P < 0.01$ , as indicated. SFN, Sulforaphane; PBMC, peripheral blood mononuclear cells; TNFR, tumor necrosis factor receptor.

PBMCs (Figs. 5B and S2). The expression of Bcl-2 was slightly induced by sulforaphane with *Lactobacillus*-treated PBMCs in HCT116 cells (Figs. 5A and S2). Co-culture with *Lactobacillus*-treated PBMCs increased Bax in SW480 cells with or without sulforaphane treatment (Figs. 5B and S2). The expression of Bim, Bcl-xL and Bcl-2 did not significantly change in SW480 cells (Figs. 5B and S2). These results raise the possibility that TNFR1 in the extrinsic pathway and cIAP-1, cIAP-2 and Bax in the intrinsic pathway were involved in apoptosis by the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs in human colon cancer cells.

## Discussion

Combinations of different chemopreventive agents may synergistically enhance their preventive effects, and 'combination-oriented molecular-targeting prevention' of cancer

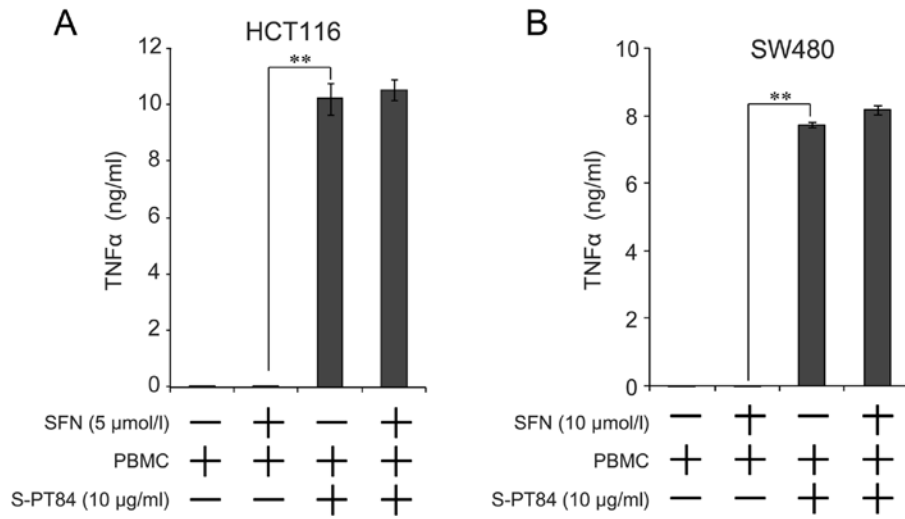


Figure 4. Secretion of TNFα is increased in PBMCs stimulated with *Lactobacillus*. PBMCs ( $2 \times 10^6$ /ml) were pre-incubated with  $10 \mu\text{g/ml}$  *L. pentosus* S-PT84 for 24 h. (A) HCT116 or (B) SW480 cells were co-incubated with PBMCs under SFN treatment for 48 h, and the culture supernatant was collected. Concentrations of TNFα in the culture supernatant were measured by ELISA. Data are presented as the mean  $\pm$  standard deviation of three experiments. \*\* $P < 0.01$ , as indicated. SFN, Sulforaphane; PBMCs, peripheral blood mononuclear cells; TNFα, tumor necrosis factor.

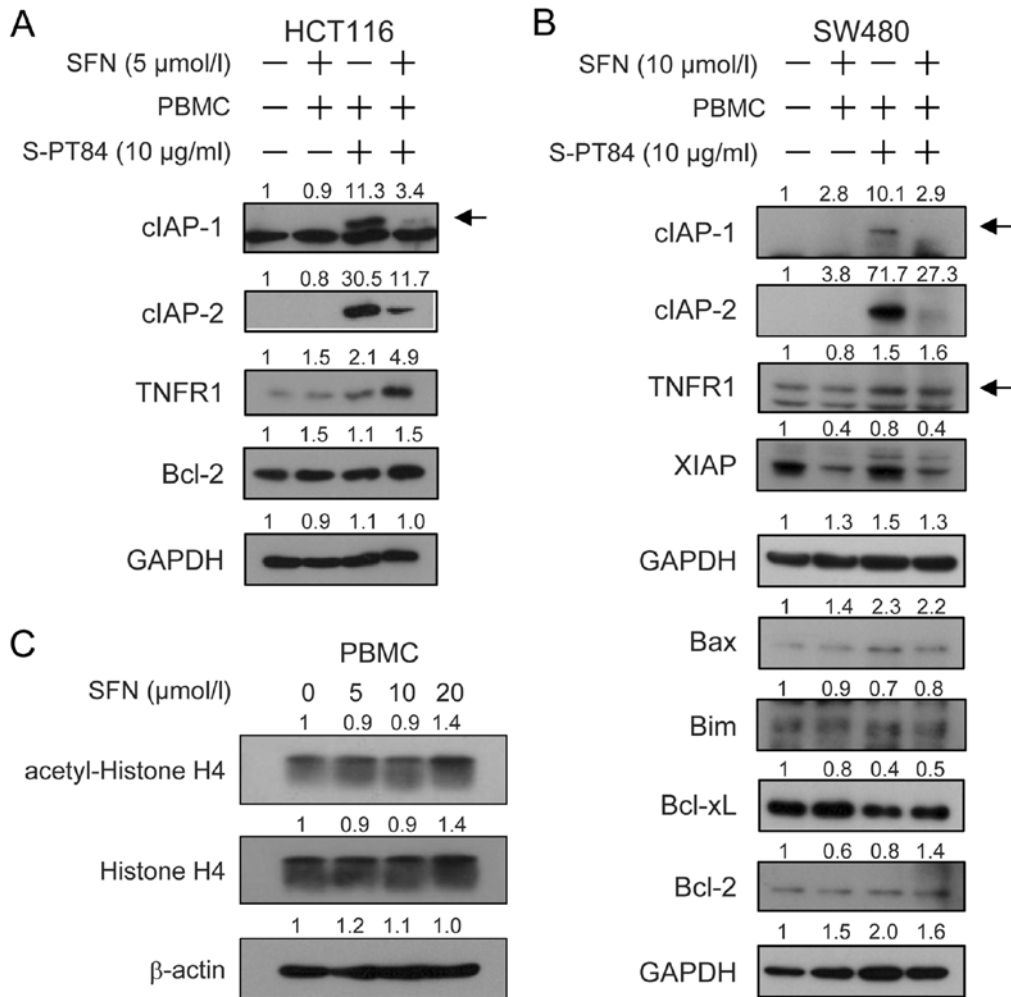


Figure 5. Effects of SFN and/or PBMCs stimulated with *Lactobacillus* on the expression of various intracellular regulators of apoptosis on human colon cancer cells. PBMCs ( $2 \times 10^6$ /ml) were pre-incubated with  $10 \mu\text{g/ml}$  *L. pentosus* S-PT84 for 24 h. HCT116 or SW480 cells were co-incubated with PBMCs under SFN treatment for 48 h. Cell extracts from (A) HCT116 or (B) SW480 cells were prepared for western blotting as indicated. GAPDH was used as a loading control. (C) Effect of SFN on acetylation of Histone H4 in PBMCs. PBMCs were cultured with or without  $5\text{--}20 \mu\text{mol/l}$  SFN for 48 h. Cell extracts from PBMCs were prepared for western blotting as indicated.  $\beta$ -actin was used as a loading control. The band intensities were quantified with ImageJ software. Band intensities were normalized by (A and B) GAPDH or (C)  $\beta$ -actin. Fold changes relative to the control (vehicle) lane were calculated. Arrows indicate the cIAP-1 or TNFR1 bands. SFN, Sulforaphane; PBMCs, peripheral blood mononuclear cells; TNFR, tumor necrosis factor receptor; cIAP, cellular inhibitor of apoptosis.

is a practical approach for the chemoprevention of human malignant tumors (32).

Sulforaphane induces apoptosis in several types of cancer cells (9-13), and *Lactobacilli* also induce apoptosis in cancer cells by activating immune cells (25,41). We found that sulforaphane enhanced apoptosis in human colon carcinoma cells by co-culture with *Lactobacillus*-treated PBMCs.

*Lactobacillus* strains upregulate TRAIL production in human immune cells (25), and sulforaphane induces the expression of DR5, a TRAIL receptor, in human cancer cells (35,36). Therefore, apoptosis induced by a combination of sulforaphane and the *Lactobacillus* strain *L. pentosus* S-PT84 may depend on the TRAIL pathway. However, we found that apoptosis was TRAIL-independent because exogenous addition of a soluble human recombinant DR5/Fc chimera, which binds soluble TRAIL and prevents TRAIL binding to its receptor, failed to prevent apoptosis. Furthermore, the concentration of TRAIL in the culture supernatant was low from the combined treatment of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs (data not shown). Instead, apoptosis was likely caused by TNF $\alpha$  because the TNFR/Fc chimera significantly inhibited apoptosis. Moreover, soluble human recombinant Fas/Fc chimera and a granzyme B inhibitor did not attenuate apoptosis by the combination of sulforaphane and co-culture with *Lactobacillus*-treated PBMCs in HCT116 and SW480 cells. On the other hand, apoptosis was markedly inhibited by zVAD-fmk, a pan-caspase inhibitor, and induced mitochondria membrane depolarization. Sulforaphane induces apoptosis in various tumor cells via a mitochondria-dependent pathway by inducing reactive oxygen species (ROS) (42-44). These results suggest that this combination was dependent on both the extrinsic and intrinsic pathways of apoptosis. Sulforaphane suppresses LPS-induced cell signaling, including TNF $\alpha$  (45), but we found that sulforaphane did not inhibit TNF $\alpha$  production induced by *L. pentosus* S-PT84 in both cell types. Sulforaphane acts as a histone deacetylase (HDAC) inhibitor (46,47), and several HDAC inhibitors suppress the activities of immune cells (48-50). Therefore, sulforaphane may potentially suppress immune activities by inhibiting HDAC. We investigated the effect of sulforaphane on histone acetylation by western blotting, but acetylation of histone H4 was not changed by 5 and 10  $\mu$ M sulforaphane in PBMCs.

The expression of cIAP-1 and cIAP-2 was also markedly induced by co-culture with *Lactobacillus*-treated PBMCs in both cells. The induction of cIAP-1 and cIAP-2 may contribute to the prevention of apoptosis in colon cancer cells in co-culture with *Lactobacillus*-treated PBMCs despite upregulation of TNF $\alpha$ . Interestingly, sulforaphane suppressed the expression of cIAP-1 and cIAP-2 induced by co-culture with *Lactobacillus*-treated PBMCs. Sulforaphane downregulates cIAP-1 and cIAP-2 in human prostate cancer cells (14). Proteins in the IAP family are widely and highly expressed in many cancers and are an important target for anticancer therapy (51).

We found that sulforaphane enhanced apoptosis in human colon cancer cells under co-culture with *Lactobacillus*-treated PBMCs. Production of TNF $\alpha$  from PBMCs was induced by co-culture with *Lactobacillus*, and sulforaphane suppressed the expression of cIAP-1 and cIAP-2 in colon cancer cells. Furthermore, to verify the preventive effect of the combination for colon carcinogenesis, the verification by long-term

experiments with exposure to an exogenous chemical carcinogen are needed.

Sulforaphane and *Lactobacillus* are contained in a wide variety of foods; therefore, consumption of sulforaphane and *Lactobacillus* may have a protective effect against colon cancer.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

SY, MH and TS conceived and designed the study, and wrote the manuscript. SY and MH conducted the experiments, and performed the data analysis. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

PBMCs were acquired from healthy volunteers after obtaining written informed consent. The present study was approved by the Kyoto Prefectural University of Medicine Research Ethics Committee (permission. no. RBMR-C-919).

### Patient consent for publication

Not applicable.

### Competing interests

Toshiyuki Sakai received funding from Suntory Co. (Japan) who also supplied the *Lactobacillus pentosus* strain S-PT84 used in the study.

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