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Fucoidan Induces Apoptosis through Activation of Caspase-8 on Human Breast Cancer MCF-7 Cells

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Fucoidan is an active component of seaweed that has been shown to inhibit proliferation and induce apoptotic cell death in several tumor cells. However, the detailed mechanisms underlying this process have not yet been elucidated. In the present report, we investigated the effect of fucoidan on the induction of apoptosis in human breast cancer MCF-7 cells. Our data demonstrated that fucoidan reduced the viable cell number of MCF-7 cells in a dose- and time-dependent manner. In contrast, fucoidan did not affect the viable cell number of normal human mammary epithelial cells. Results from the apoptosis assay demonstrated that fucoidan induced internucleosomal DNA fragmentation, chromatin condensation, activation of caspase-7, -8, and -9, and cleavage of poly(ADP ribose) polymerase. Furthermore, expression of Bid was decreased, whereas truncated Bid was increased by fucoidan treatment. There was also a decline in cytosolic Bax and a striking increase of cytosolic cytochrome *c*. Caspase-8-specific inhibitor, z-ITED-fmk, canceled the cytotoxicity of fucoidan, activation of caspase-7, -8, and -9, and a series of changes in Bax, Bid, and cytochrome *c*. However, caspase-9-specific inhibitor exerted a moderate inhibitory effect on the cytotoxicity of fucoidan. These data indicated that fucoidan could induce apoptotic cell death through a caspase-8-dependent pathway in MCF-7 cells.

KEYWORDS: Fucoidan; caspase; apoptosis; breast cancer cell

INTRODUCTION

For a long time, brown seaweeds have been a regular part of the diet in many Asian countries, especially Japan and Korea. Viscous components, such as porphyran, gepsin, and alginic acid oligosaccharide, prevent bacteria from invading these seaweeds. These elements are also known to have various other functions, such as antitumor activity (*1*) and immunomodulating activities (*2*). One of the functional elements, fucoidan, is the collective name for algal sulfated polysaccharides extracted from the brown seaweeds, and the structure of fucoidan consists mainly of polymers formed by branched polysaccharide sulfate esters with a L-fucose building block (*3*). At present, fucoidan is known to have several biological activities, such as antibacterial activity (*4*), antiviral activity (*5*, *6*), antioxidant activity (*7*), and anti-inflammatory bowel disease activity (*8*). In particular, the antitumor activity has recently attracted considerable attention. It was reported that the antitumor effect of "Mekabu" fucoidan appears to be mediated by IFN-γ-activated NK cells, and the antitumor action of fucoidan is due to its anti-angiogenic potency (*9*, *10*). Recently, fucoidan has been reported to induce apoptosis in several cancer cell lines (*11*, *12*), but the mechanism is controversial because it is uncertain which cascade plays a pivotal role in the induction of apoptosis by fucoidan.

Apoptosis is mediated through two major pathways, the death receptor pathway (extrinsic) and the mitochondrial pathway (intrinsic). In the extrinsic pathway, stimulation of death receptors, such as Fas and tumor necrosis factor receptor-1, leads to clustering and formation of a death-inducing signaling complex, which includes the adaptor protein Fas-associated death domain (FADD) and initiator caspases, such as caspase-8. Activated caspase-8 directly activates downstream effector caspases, such as caspase-3 and -7 (*13*). Additionally, caspase-8 can cleave Bid, tBid, and interacts with proapoptotic protein Bax, and the accumulation of Bax in mitochondria promotes cytochrome *c* release to the cytosol (*14*−*16*). In the intrinsic pathway, death receptors transmit death signals to the mitochondria, resulting in the release of several mitochondrial intermembrane space proteins, such as cytochrome *c*, which associate with Apaf-1 and procaspase-9 to form the apoptosome. Activated caspase-9 can cleave and activate effector caspases, such as caspase-3 and -7 (*17*).

In this study, we have demonstrated that fucoidan induces apoptosis in breast cancer MCF-7 cells without affecting the viability of normal epithelial cells. In addition, caspase-8 is considered essential for fucoidan-induced apoptosis.

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MATERIALS AND METHODS

Materials. Fucoidan was presented from the NPO Organization Fucoidan Laboratory. Antibodies to poly(ADP-ribose) polymerase (PARP), Bid, Bax, and cytochrome *c* were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); caspase-7, -8, and -9 were purchased from Cell Signaling Technology, Inc. (Danvers, MA); β-actin was purchased from Sigma (St. Louis, MO); and horseradish peroxidase (HRP)-conjugated anti-mouse IgG and HRP-conjugated anti-rabbit IgG were obtained from Zymed (San Francisco, CA). Fetal bovine serum (FBS) was purchased from Biosource (Camarillo, CA). The caspase-7 inhibitor, z-DEVD-fmk, the caspase-8 inhibitor, z-IETD-fmk, and the caspse-9 inhibitor, z-LEHD-fmk, were purchased from Biovision, Inc. (Mountain View, CA), Kamiya Biomedical Co. (Seattle, WA), and R&D Systems (Lille, France), respectively.

Cells and Cell Culture. Human mammary epithelial cells (HMECs, Cambrex Bio Science Walkersville, Baltimore, MD) and MCF-7 are cell lines derived from normal human mammary epithelial cells and human breast cancer cells, respectively. HMECs were routinely maintained in MEGM (CC-3150) medium (Cambrex Bio Science Walkersville, Baltimore, MD), and MCF-7 cells were maintained in RPMI-1640 medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 5% FBS, at 37 °C in a humid atmosphere containing 5% CO_2. These cells were inoculated at 5.0×10^4 cells/mL, cultured in the respective medium for 24 h, and then cultured in the presence of fucoidan or vehicle [phosphate-buffered saline (PBS)] for appropriate periods. These cells were pretreated with various caspase inhibitors (10 μM) for 1 h before treatment with fucoidan. The number of viable attached cells was counted after trypsinization using 0.2% trypsin−0.025% ethylenediaminetetraacetic acid (EDTA) in PBS using the trypan blue exclusion method.

Flow Cytometry Analysis. Cells were treated with fucoidan or vehicle for appropriate periods. Then, the cells were collected by a trypsinization and washed once with PBS. The cells were fixed with 70% methanol for 30 min at 25 °C and then with 50% ethanol for 24 h. Cells were collected by centrifugation at 300*g* for 5 min and then resuspended in PBS containing 10 μg/mL RNase A (Takara Biochemicals, Tokyo, Japan). After incubation at 37 °C for 20 min, 10 μg/mL propidium iodide was added and the mixture was left on ice for 30 min. Then, the DNA contents of the cells were analyzed using a FACSCalibur (Becton Dickinson, Mountain View, CA). The data were analyzed using the Modi FIT program (Verity Software House, Topsham, ME).

Morphology of Nuclei. Cells were cultured on 18 × 18 mm micro cover glass (Matsunami Glass Industrial, Ltd., Osaka, Japan) in a 35 mm plastic dish (Falcon, Grand Island, NY) in the presence of fucoidan or vehicle for 72 h. Then, cells were washed once with PBS and fixed with 100% methanol for 30 min at 4 °C. After removing methanol, micro cover glasses were dipped into PBS containing 5 μg/mL propidium iodide and 10 μg/mL RNase A and then incubated at 37 °C for 30 min to remove intracellular RNA. The morphology of the nucleus was observed using the Nikon E600 laser scanning confocal microscope (Nikon Instech Co., Ltd., Kanagawa, Japan), and all images were processed using custom software.

Detection of DNA Fragmentation. Cells were treated with fucoidan or vehicle for appropriate periods. The cells were then collected by trypsinization and washed once with PBS. Cell extracts were prepared by suspending cells in a lysis buffer (10 mM Tris-HCl at pH 7.5, 0.5% Triton-X100, and 10 mM EDTA) for 10 min on ice. After the lysates were centrifuged 15000*g* for 5 min, the supernatant was treated with 200 μg/mL RNase A for 1 h at 37 °C and then with 200 μg/mL proteinase K for 30 min at 5 °C. DNA was precipitated with 0.5 M NaCl and 50% isopropanol at −20 °C overnight. After the solution was centrifuged at 13000*g* for 15 min, DNA fragmentation samples were prepared by suspending the pellet in a TE buffer (1 M Tris-HCl at pH 7.4 and 0.5 M EDTA). DNA fragmentation was visualized after electrophoresis using 2% agarose gel containing SYBR Green (Molecular Probes, Eugene, OR) and photographed under UV light.

Immunoblot Analysis. Whole cell extracts were prepared by suspending cells in a lysis buffer containing 50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 1% Triton-X100, 5 mM EDTA, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/mL aprotinin, and 1 mM pervanadate. After standing on ice for 30 min, supernatants were collected by a centrifugation at 15000*g* and 4 °C for 20 min. Meanwhile, extracts of cytosol for detection of Bax and cytochrome *c* were prepared by suspending cells in a cytosol recovery buffer containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.2), 5 mM $MgCl_2$, 10 mM KCl, 250 mM sucrose, 1 mM EDTA, 25 mM NaF, 5 μg/mL PMSF, 8 μg/mL aprotinin, and 1 mM orthovanadate, and then after standing on ice for 5 min, supernatants were collected by a centrifugation at 8500*g* at 4 °C for 5 min. Protein concentrations in the supernatants were determined using the BCA protein assay reagent kit (Pierce). Lysates containing 10 μg of protein were subjected to 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and then transferred to a PROTORAN nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). Samples were mixed with an equal volume of 1× SDS−PAGE buffer containing 2% 2-mercaptoethanol and subjected to electrophoresis. Membrane blocking was performed using 5% bovine serum albumin (BSA) (Roche, Mannheim, Germany) for 1 h for 37 °C. Antibody binding was detected using an enhanced chemiluminescence kit with hyper-ECL (Amersham, Buckinghamshire, U.K.). β-Actin was used as an indicator to establish equality of lane loading.

Statistical Analysis. Data were analyzed by Student's *t* test to evaluate the significance of difference, and $p < 0.05$ was regarded as statistically significant.

RESULTS

Cytotoxic Effect on MCF-7 Cells. Panels **A** and **B** of **Figure 1** show the number of viable attached HMEC and MCF-7 cells after 96 h of cultivation in the presence of 0, 1, 10, 100, 1000 μg/mL fucoidan. As shown in **Figure 1A**, proliferation of HMECs was not affected by the addition of fucoidan. Conversely, fucoidan was shown to reduce the number of MCF-7 cells in a dose-dependent manner. A significant difference was detected between vehicle and fucoidan at a concentration of 10 μg/mL in MCF-7 cells (**Figure 1B**). Next, we examined the cell numbers in cultures of MCF-7 cells treated with 1000 μg/mL fucoidan or vehicle for 24, 48, 72, and 96 h. As shown in **Figure 1C**, cells in the vehicle group showed a continuous increase in cell numbers throughout this culture period. However, the number of fucoidan-treated cells increased more slowly than the vehicle group until 48 h, reached a peak at 48 h, and then began to decrease in the next 48 h. These findings suggest that fucoidan has antiproliferative and cytotoxic effects on MCF-7 cells.

Induction of Apoptosis by Fucoidan. Then, we tried to know whether fucoidan could induce apoptotic cell death. **Figure 2A** shows the sub-G1 population of MCF-7 cells treated with 1000 μg/mL fucoidan or vehicle for appropriate periods. The sub-G1 population of the cells was significantly higher than the control group in the presence of fucoidan after 24 h of cultivation, and its level received an increase in a time-dependent manner. Next, we evaluated the morphology of each nucleus after treatment with fucoidan or vehicle. As shown in **Figure 2B-1**, cells treated with vehicle were normal morphology without any onset of apoptotic cell death, while marked chromatin condensation was detected in the fucoidan-treated cells at 72 h (**Figure 2B-2**). Furthermore, **Figure 2C** shows the effect of fucoidan on DNA internucleosomal fragmentation in MCF-7 cells. When the cells were treated with vehicle for 96 h, DNA fragmentation was not detectable. However, DNA fragmentation was apparent in cells treated with fucoidan at 48, 72, and 96 h. These findings demonstrate that fucoidan induces apoptosis of MCF-7 cells.

Effect of Fucoidan on the Activation of Caspase and Cleavage of PARP. To confirm the involvement of caspases in fucoidan-induced apoptosis, we next tried to evaluate the activation of caspase-7. It is known that caspase-7 is an effector caspase via a caspase-dependent apoptotic pathway in MCF-7 cells (*18*, *19*). **Figure 3** shows the detection of active fragments of caspases and cleavage of PARP on MCF-7 cells treated with 1000 μg/mL fucoidan or vehicle for appropriate periods. The active fragment

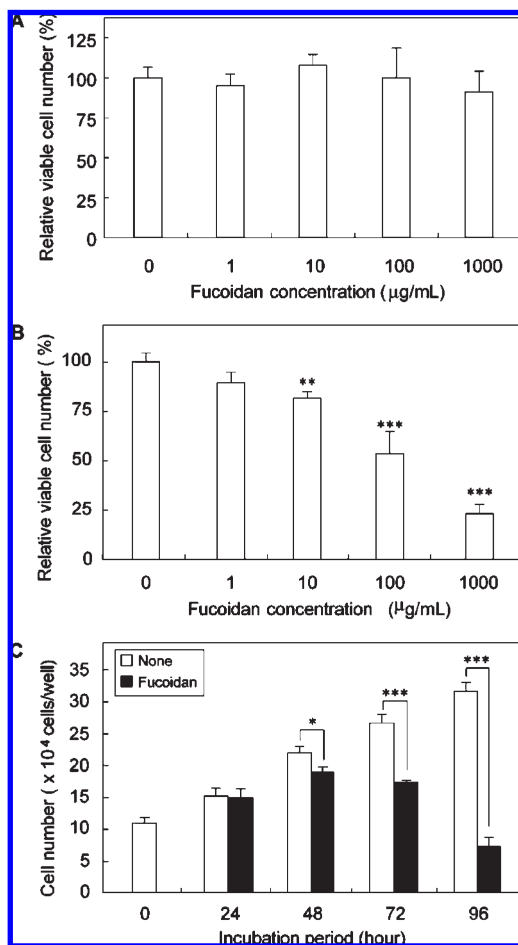

Figure 1. Effect of fucoidan on the proliferation of (A) HMEC and (B and C) MCF-7. (A and B) HMECs (A, 1×10^5 cells/mL) and MCF-7 cells (B, 1×10^5 cells/mL) were treated with 0−1000 μg/mL fucoidan in MEGM medium or RPMI-1640 medium containing 1% FBS for 96 h. (C) Time course of the effect of fucoidan on MCF-7 cells. MCF-7 cells (1×10^5 cells/mL) were treated with 1000 μg/mL fucoidan in RPMI-1640 medium containing 1% FBS for 24, 48, 72, and 96 h. The number of viable attached cells was counted by the trypan blue exclusion method. Data are means ± standard deviation (SD) for three samples. Values with asterisks denote differences from the control group at (∗) $p < 0.05$, (∗∗) $p < 0.01$, and (∗∗∗) $p < 0.001$.

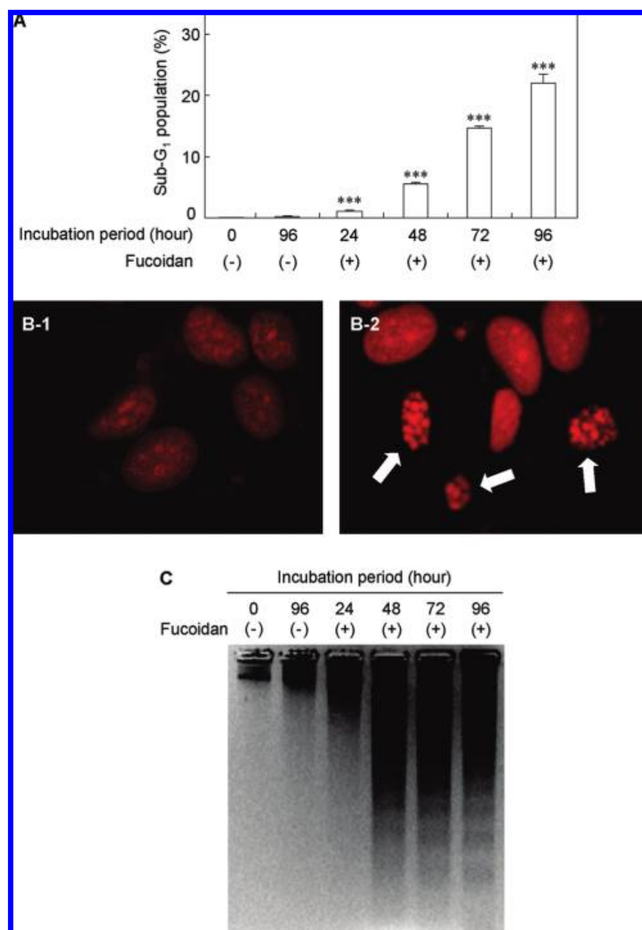

Figure 2. Induction of apoptosis in MCF-7 cells. MCF-7 cells (1×10^5 cells/mL) were treated with 1000 μg/mL fucoidan or vehicle in RPMI-1640 medium containing 1% FBS for 24, 48, 72, and 96 h. (A) Cells were stained with propidium iodide. DNA contents of cells were analyzed by flow cytometry. Each phase was calculated using the Modi FIT program. Data are means ± SD for three samples. Values with asterisks denote differences from the control group at (∗∗∗) $p < 0.001$. (B) Cells were stained with propidium iodide. The morphology of the nucleus was observed using a Nikon E600 laser scanning confocal microscope, and all images were processed using a custom software (1, vehicle; 2, treatment with fucoidan). (C) DNA fragmentation samples from cells were visualized after electrophoresis using 2% agarose gel containing SYBR Green and photographed under UV light.

of caspase-7 was detected at 72 and 96 h in the presence of fucoidan. In addition, the level of cleavage of PARP that is a crucial hallmark of apoptosis via this pathway was increased at 72 and 96 h with an increase in the level of caspase-7 after treatment with fucoidan. Similarly, active fragments of caspase-8 and -9 were detected after treatment with fucoidan. The active fragment of caspase-8 was clearly detected after 24 h; the active fragment of caspase-9 was also detected at 72 and 96 h.

Cleavage of Bid, Translocation of Bax, and Cytochrome *c* Release by Fucoidan. When caspase-8 is activated by apoptotic stimuli, it cleaves Bid to form truncated Bid (tBid), which is fragment of Bid and promotes apoptosis by stimulating the release of mitochondrial cytochrome *c*. It has been shown that a certain kind of apoptotic stimuli triggers a Bax conformational change, translocation to mitochondria, and release of cytochrome *c*. Cytochrome *c* is an important apoptogenic factor in the intrinsic apoptotic pathway, which is released into the cytoplasm and subsequently leads to caspase-9 activation. As shown in **Figure 4A**, expression of Bid was decreased with augmentation of tBid in the presence of fucoidan at 72 and 96 h; however, these expressions were not changed in control MCF-7 cells. Next, we evaluated the expression of Bax. **Figure 4B** shows that the cytosolic Bax level was decreased, although the Bax level in whole cell lysate was increased at 72 and 96 h after treatment with fucoidan. In contrast, it was not changed in the control group. As shown in **Figure 4B**, the cytosolic cytochrome *c* level was increased after 24, 48, 72, and 96 h in the presence of fucoidan in the cytosol; however, the cytochrome *c* level was not changed in the control group.

Suppression of Fucoidan-Induced Apoptosis by Caspase Inhibitors. To determine the involvement of the caspases in fucoidan-induced apoptosis of MCF-7 cells, we used specific inhibitors of caspase-7 (z-DEVD-fmk), caspase-8 (z-IETD-fmk), and caspase-9 (z-LEHD-fmk). As shown in **Figure 5A**, fucoidan was again shown to induce cell death; however, the inhibitors were demonstrated to block the fucoidan-induced apoptotic cell death. In particular, z-IETD-fmk and z-DEVD-fmk almost completely blocked fucoidan-induced cell death, while z-LEHD-fmk partially blocked fucoidan-induced cell death. Next, to verify the

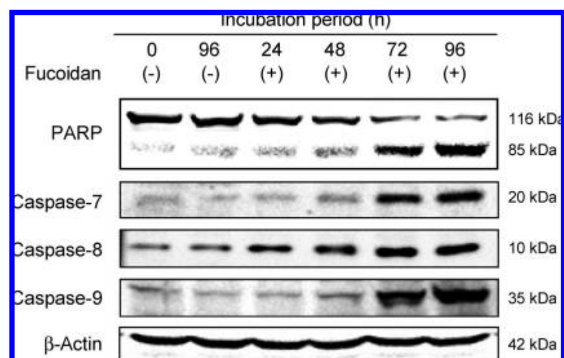

Figure 3. Detection of the active fragment of caspases and cleavage of PARP in MCF-7 cells. Cells (1×10^5 cells/mL) were treated with 1000 μg/mL fucoidan or vehicle in RPMI-1640 medium containing 1% FBS for 24, 48, 72, and 96 h. Cell lysates (10 μg of protein/lane) were fractionated on SDS−polyacrylamide gels and analyzed by Western blotting with antibodies against cleaved proteins (caspase-7, -8, and -9 and PARP).

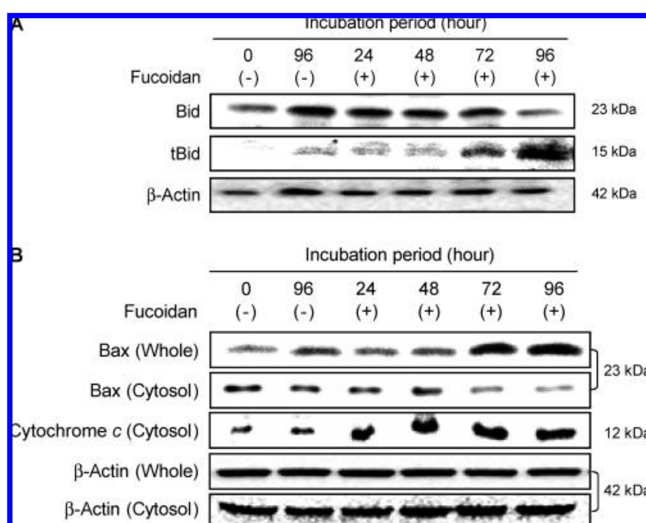

Figure 4. Expression of apoptosis-associated proteins in MCF-7 cells. Cells (1×10^5 cells/mL) were treated with 1000 μg/mL fucoidan or vehicle in RPMI-1640 medium containing 1% FBS for 24, 48, 72, and 96 h. Cell lysates (10 μg of protein/lane) were fractionated on SDS−polyacrylamide gels and analyzed by Western blotting with antibodies against apoptosis-associated proteins (A, Bid and t-Bid; B, Bax and cytochrome *c*).

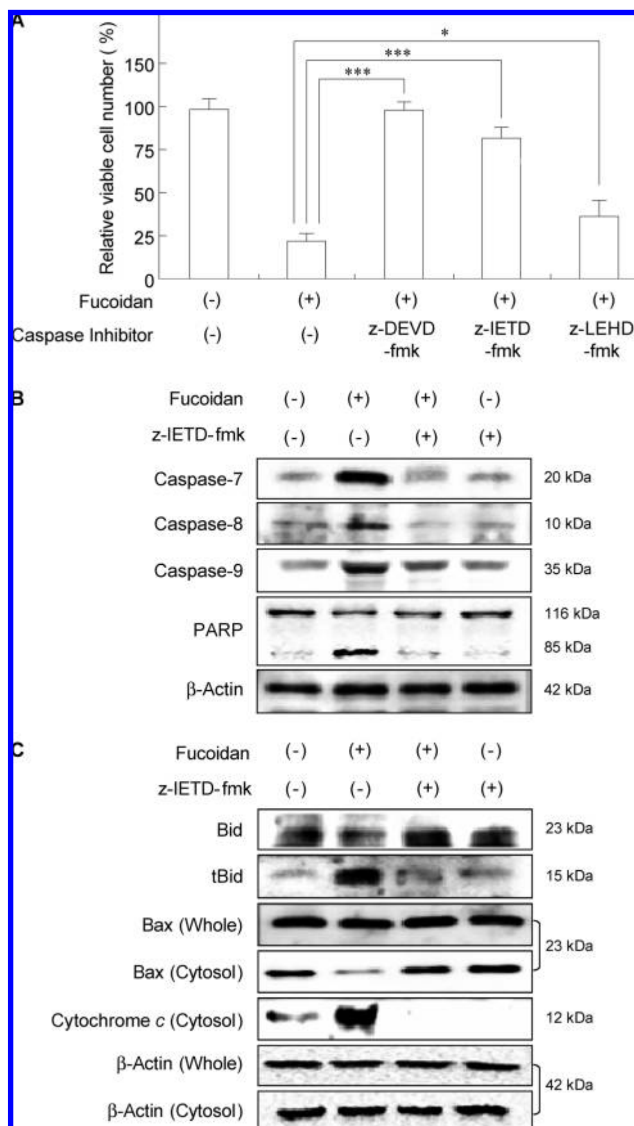

Figure 5. Effect of caspase inhibitors for fucoidan-induced apoptosis in MCF-7. (A) Cells (1×10^5 cells/mL) were pretreated with various caspase inhibitors (10 μM) for 1 h and treated with 1000 μg/mL fucoidan or vehicle in RPMI-1640 medium containing 1% FBS for 96 h. The number of viable attached cells was counted by the trypan blue exclusion method. Data are means ± SD for three samples. Values with asterisks denote differences from the control group at (*) $p < 0.05$ and (***) $p < 0.001$. (B and C) Cells (1×10^5 cells/mL) were pretreated with z-IETD-fmk (10 μM) for 1 h and treated with 1000 μg/mL fucoidan or vehicle in RPMI-1640 medium containing 1% FBS for 72 h. Cell lysates (10 μg of protein/lane) were fractionated on SDS−polyacrylamide gels and analyzed by Western blotting with antibodies against apoptosis-related proteins (B, caspase-7, -8, and -9 and PARP; C, Bid, t-Bid, Bax, and cytochrome *c*).

contribution of caspase-8 to fucoidan-induced apoptosis, we evaluated changes of apoptosis-related proteins after treatment with z-IETD-fmk in the presence of fucoidan. **Figure 5B** shows that after treatment with z-IETD-fmk, treatment with fucoidan did not activate caspase-7, -8, or -9 or cleave PARP. Furthermore, a decrease of Bid with augmentation of tBid, transition of Bax from cytosol, and release of cytochrome *c* to cytosol were canceled after treatment with z-IETD-fmk in the presence of fucoidan (**Figure 5C**).

DISCUSSION

Fucoidan has become a focus of great interest because of its anticancerous activity and is expected to be a new candidate for low toxic cancer therapy. Fucoidan has been consumed as a dietary fiber in many Asian countries for centuries and has been shown to inhibit the growth of a wide variety of tumor cells (*11*, *20*). In this study, we demonstrated that fucoidan, extracted from *Cladosiphon okamuranus*, strongly presented antiproliferative and significant cytotoxic effects on MCF-7 cells in a dose-dependent manner. However, fucoidan did not affect proliferation of human normal epithelial HMECs. It has also been reported that fucoidan inhibited the growth of peripheral blood mononuclear cells (PBMCs) from adult T-cell leukemia patients and human T-cell leukemia virus type 1-infected T-cell lines but not that of normal PBMC (*21*). Therefore, fucoidan may be a good chemopreventive and antitumor candidate without toxic effects on normal cells.

The characteristics of apoptotic cell death are induction of chromatin condensation, fragmentation of nuclei and DNA, and cleavage of specific proteins (*22*, *23*). We found that treatment of fucoidan induced accumulation of sub-G1 population, chromatin

condensation, and internucleosomal fragmentation of DNA. Because these are representative features of apoptosis, fucoidan was shown to induce apoptotic cell death in MCF-7 cells. Effector caspases, such as caspase-3 or -7, activate DNase, resulting in fragmentation of DNA in response to various apoptotic stimuli. MCF-7 cells show a defect in caspase-3 but express caspase-7, which is an executioner caspase capable of cleaving PARP (*18*, *19*). Actually, it is known that activation of caspase-7 and PARP cleavage are hallmarks of apoptosis in MCF-7 cells (*24*). We showed that cleavage of PARP and activation of caspase-7 were induced after treatment with fucoidan in MCF-7 cells and that caspase-7 inhibitor z-DEVD-fmk canceled fucoidan-induced apoptosis. Caspase-3 is known to be activated and plays a pivotal role in fucoidan-induced cell death (*11*, *20*). Taken together, we demonstrated that caspase-7 is required by MCF-7 cells and that activation of caspase-3 is not necessarily a prerequisite for fucoidan-induced apoptosis.

Caspase-8 plays a crucial role in apoptosis triggered by the interaction of ligand with death receptors, such as Fas, tumor necrosis factor receptor (TNFR), and TNF-related apoptosis-inducing ligand receptor (TRAILR) (*13*). Caspase-8 drives its activation through self-cleavage and then amplifies the apoptotic signal by either directly activating downstream effector caspases, such as caspase-3 and -7, or cleaving BH3 domain-only proteins, such as Bid (*25*), leading to caspase-9 activation. In this study, we indicated that caspase-8 was activated by treatment of fuoidan and that fucoidan-induced apoptosis was completely blocked by z-IETD-fmk with suppression of caspase-8 and -7 and PARP cleavage. Here, as far as we know, this is the first time to show that activation of caspase-8 is required for fucoidan-induced apoptosis.

Bid is directly cleaved by caspase-8, whereupon the C-terminal BH3 domain containing a fragment of Bid, tBid, translocates from the cytosol to the outer mitochondrial membrane and triggers cytochrome *c* release, leading to caspase-9 activation (*14*,*26*). In the present study, we detected tBid in fucoidan-treated MCF-7 cells. Furthermore, Bid cleavage was inhibited after treatment with caspase-8 inhibitor z-IETD-fmk. Therefore, it is also suggested that caspase-8 activates the tBid-related apoptotic pathway, leading to caspase-9 activation as well as direct activation of capase-7 in fucoidan-treated cells. Activation of caspase-8 plays a pivotal role in amplifying the apoptotic signal from Bid during apoptosis induced by fucoidan.

Bax is a member of the Bcl-2 family proteins, which can promote apoptosis by forming oligomers on the outer mitochondrial membrane and forming a channel for the release of cytochrome *c* (*15*, *27*). In addition, Bax can be activated by tBid, leading to a change in the conformation of Bax and release of cytochrome *c* from mitochondria (*16*). Cytochrome *c*, which is released from mitochondria, accumulates in the cytosol, where in cooperation with Apaf-1, it participates in ATP-dependent autoactivation of initiator caspase-9 at Asp 315. The activated caspase-9 then proteolytically cleaves and activates executioner caspase-3, -6, and -7 (*28*). In this study, we showed that fucoidan promotes Bax expression and translocation from the cytosol, subsequently inducing the release of cytochrome *c* and activation of caspase-9. However, pretreatment with z-IETD-fmk inhibited the translocation of Bax, release of cytochrome *c*, and cleavage of caspase-9. Moreover, the caspase-9 inhibitor could not fully inhibit fucoidan-induced cell death. Taken together, activation of caspase-9 appears to be a branched pathway of apoptotic cell death, originating from caspase-8 during fucoidan-induced apoptosis.

In summary, our findings demonstrate that fucoidan has cytotoxic effects and induces apoptosis in MCF-7 cells. This apoptotic effect is triggered via a pathway dependent upon caspase-8 activation and subsequent activation of caspase-7 accompanied by activation of caspase-9, which functions as an amplifier caspase via a mitochondrial pathway. On the basis of these findings, it is suggested that fucoidan is a promising candidate for chemoprevention of breast cancer and that the therapeutic value of fucoidan would be an interesting topic for further investigation.

ABBREVIATIONS USED

PARP, poly(ADP-ribose) polymerase; HMEC, human mammary epithelial cell, PMSF, phenylmethylsulfonyl fluoride; FBS, fetal bovine serum; PBS, phosphate-buffered saline; TE buffer, Tris-HCl and ethylenediaminetetraacetic acid (EDTA) buffer; tBid, truncated Bid.

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