**Fucoidan Promotes Apoptosis and Inhibits EMT of Breast Cancer Cells**

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Fucoidan is an active component of seaweed, and could inhibit proliferation and induce apoptotic cell death in several tumor cells. However, the function of fucoidan in breast cancer is largely unknown. In the present study, we evaluated the anti-cancer potential of fucoidan in human breast cancer MCF-7 cells. Adult Sprague–Dawley rats were randomized to receive fucoidan (200 or 400 mg/kg body weight per day) or normal saline via gastric gavage for 3 consecutive days. Serum samples were prepared from these rats, and used for subsequent experiments to examine the potential effects in MCF-7 cells. Cell viability was determined using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Apoptosis was examined with Hoechst33258 staining and flow cytometry. Cell migration and invasion were measured by wound scratch assay and Transwell assay, respectively. Western blot and enzyme-linked immunosorbent assay (ELISA) were used to examine the expression of secretory E-cadherin and matrix metalloproteinase-9 (MMP-9). Conditioned serum from fucoidan-treated rats significantly suppressed cell proliferation and enhanced apoptosis. Cell migration and invasion were also significantly decreased. Observed effects of conditioned serum were associated with upregulation of E-cadherin and downregulation of MMP-9. Conditioned serum of rats treated with fucoidan could inhibit the proliferation and promote apoptosis of MCF-7 cells. Cell invasion and migration were inhibited, possibly via decreased epithelial–mesenchymal transition (EMT) process. Fucoidan may be a promising therapeutic agent for human breast cancers.

**Key words** breast cancer cell; fucoidan; drug serum; apoptosis; epithelial–mesenchymal transition

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**INTRODUCTION**

Fucoidan is composed of l-fucose, sulfate groups and other biologically active components such as D-xylose, D-mannose, D-galactose, D-rhamnose, arabinose, glucose, D-glucuronic acid and acetyl groups. It is primarily extracted from brown seaweeds and has been intensively studied due to its diverse biological properties, including antithrombotic, anticoagulant, antiviral and antitumor activities.

Breast cancer is a heterogeneous disease with several defined molecular subgroups, more than one-and-half million new breast cancer cases are reported worldwide each year. Unfortunately, most chemotherapeutic agents and hormonally directed drugs for breast cancer are designed to broadly target common deregulated mechanisms within breast cancer cells. Many healthy tissues are also affected and patients often experience clinically significant toxic effects. Subsequent studies demonstrated that fucoidan could induce cell cycle arrest in a chemoresistant non-small-cell bronchopulmonary carcinoma line. Accumulating evidence has indicated that fucoidan could significantly suppress cell viability and promote apoptosis of cancer cells. Fucoidan also has beneficial effects as it can resist toxicity associated with chemoradiotherapy. Previous studies have demonstrated that fucoidan induced apoptosis of human breast cancer MCF-7 cells via activating caspase-8. In addition, brown algae containing fucoidan is widely spread in resources, and in recent years has drawn intense research interests.

However, as a polysaccharide molecule, it is difficult to purify the fucoidan monomer, and the effect of the crude extract is not very precise when tested in vitro. The effects of fucoidan on the onset and progression of human breast cancer are still unelucidated. Therefore, in the present study, serum samples were obtained from female Sprague–Dawley (SD) rats, which were fed by fucoidan or an equivalent volume of normal saline, respectively. Serum from each group was incubated in vitro with MCF-7 cells to study the effect of fucoidan on the proliferation, migration and apoptosis of breast cancer cells.

**MATERIALS AND METHODS**

**Cells** Breast cancer MCF-7 cells (Shanghai Life Science of Chinese Academy of Sciences, Shanghai, China) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) high glucose (0.45 g/L n-glucose; Hyclone Biotechnology, Beijing, China) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Waltham, MA, U.S.A.) and 100 U/mL penicillin/0.1 mg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

**Animals** Twenty adult female SD rats (2 months of age, weighing 120–160 g each; Shandong Lukang Pharmaceutical Co., Qingdao, China) were housed in a standard animal facility with free access to drinking water and natural light. The
rats were randomized to receive normal saline once daily (the control group), or fucoidan from *Fucus vesiculosus* (fucoandal sulfate, C18H27O21S3…, 675.6 KD, F8190-500MG, 95% purity; Sigma, St. Louis, MO, U.S.A.) at 200 or 400 mg/kg body weight (BW) per day *via* gastric gavage for 3 consecutive days. All rats were sacrificed 1 h after the last drug treatment. Blood was collected and clarified by centrifugation, and serum samples were stored at −80°C.

The study protocol was approved by the by Institutional Ethical Committee for Care and Use of Laboratory Animals of Shandong University Medical College and all animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the U.S. National Institute of Health.

3-[(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT) Assays The MTT assays were performed as previously described.11) In brief, the cells were seeded in 96-well plates at a density of 2.5 × 10^4/mL. After the cells became adherent, serum from the control group and the fucoidan treatment groups was added at a concentration of 0.2% (v/v), respectively. After 48-h incubation, the cells were treated with 20 μL MTT (M2128-250MG, 98% purity; Sigma). After removal of the supernatant, 150 μL dimethyl sulfoxide was added in each well, and the absorbance (optical density (OD) value) was measured at 490 nm. The experiment was performed in triplicate at least three times independently, and the mean value was calculated to reflect the proliferative activity of the cells. The inhibition ratio was calculated as follows:

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\text{Inhibition ratio} = \left(1 - \frac{\text{OD of a treatment group}}{\text{OD of the control group}} \right) \times 100\%
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**Apoptosis Assays** MCF-7 cells were seeded in 6-well plates and incubated with serum from rats treated with high or low doses of fucoidan or controls. After 48 h, cells were harvested, washed twice with phosphate buffered saline (PBS), and then resuspended in 400 μL 1× binding buffer at a concentration of approximately 1 × 10^6 cells/mL. Five microliters of fluorescein isothiocyanate (FITC)-annexin V (Chinese Beyotime Biotechnology Research Institute, Shanghai, China) was added and gently mixed for 15 min at 4°C in the dark. Finally, 10 mL propidium iodide was added and mixed for 5 min at 4°C in the dark. Then, fluorescence-activated cell sorting analysis was performed by the flow cytometer and Cell Quest software (Becton Dickinson, Franklin Lakes, NJ, U.S.A.).

**Fluorescence Microscopy** MCF-7 cells were seeded in a 6-well plate at a concentration of 1 × 10^4 cells/mL. After the cells became adherent, they were treated with serum containing 0.2% (v/v) low-dose, high-dose fucoidan or blank control for 48 h. Cells were fixed by 4% polyformaldehyde for 1 h at 4°C, and then washed with PBS three times. The cells were stained with 0.5 mL Hoechst 33258 (10 mg/mL; Chinese Beyotime Biotechnology Research Institute) at 37°C for 10 min in the dark and observed under a fluorescence microscope (excitation wavelength 365 nm). Each experiment was performed at least 3 times.

**Wound Scratch Assays** Migration was evaluated by wound scratch assays. MCF-7 cells were seeded in 6-well plates and incubated with serum. After 24 h, scratch wounds were created in the confluent monolayer using a sterile 200 μL pipette tip. After removal of floating cells, cells were cultured with serum-free medium. Cell migration into the wound space was estimated at 0 and 48 h after wounding using a microscope (Carl Zeiss Inc., Thornwood, NY, U.S.A.) equipped with a digital camera, and analyzed using the NIH ImageJ software. Wound closure was determined as the difference between wound width at 0 and 48 h.

**Transwell Assays** MCF-7 cells were incubated with serum for 24 h, and then harvested and washed with PBS. Then, the cells were resuspended in fresh serum-free medium to a concentration of 5 × 10^5/mL, and 200 μL cell suspension was added to the Boyden chamber (EMD Millipore), which was coated with 50 μL Matrigel (in 1 mg/mL final concentration). The lower chamber was added with 10% serum-containing medium. After 24 h at 37°C, the cells on the upper chamber were wiped off to remove the non-invaded cells. The invaded cells were detected by staining with crystal violet and counted by light microscopy under the high-power field (×200). Five fields were counted in each of three different experiments and the average of migrated cells was reported.

**Enzyme-Linked Immunosorbent Assay (ELISA)** MCF-7 cells were seeded in 6-well plates and incubated with serum. After 24 h, the levels of E-cadherin and matrix metalloproteinase-9 (MMP-9) were measured by ELISA assays (Chinese Beyotime Biotechnology Research Institute) according to ELISA kit instructions. The OD value was measured using a microplate reader (PerkinElmer, Inc., U.S.A.) at 490 nm.

**Western Blotting Assays** Cellular lysates of MCF-7 cells were prepared as previously described. Immunoblotting assays were performed as depicted earlier and antibodies against the following proteins were used E-cadherin (1:2000), MMP-9 (1:1000), and β-actin (1:1000) (all from Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). Protein bands were visualized using the enhanced chemiluminescence method (Amerham Pharmacia Biotech, Buckinghamshire, U.K.).

**Statistical Analysis** Data were expressed as mean ± standard deviation (S.D.). All data comparison was performed using one-way ANOVA, followed by Dunnett’s *post-hoc* test. All statistical analyses were done with SPSS 17.0 software (SPSS Inc., Chicago, IL, U.S.A.), p values <0.05 were considered statistically significant.

**RESULTS**

**Serum from Fucoidan-Treated Rats Inhibits Proliferation and Induces Apoptosis of MCF-7 Cells** We examined the effect of serum derived from fucoidan-treated rats on the viability of MCF-7 cells using MTT assays. The OD at 48 h of MCF-7 cells treated with serum from rats receiving high and low doses of fucoidan was 0.424 ± 0.063 and 0.516 ± 0.049, respectively, and was significantly lower than that of the control group (0.793 ± 0.048) (*p* < 0.05). Furthermore, the high fucoidan dose group had a significantly higher inhibition ratio (44.1%) *versus* the low fucoidan dose group (32.0%) (*p* < 0.05).

Then, we examined the effect of serum derived from fucoidan-treated rats on the apoptosis of MCF-7 cells. We found that the ratio of apoptotic cells in the control group was 5.1%, which was significantly lower than that of the high and low fucoidan dose group (12.3 and 32.9%, respectively) at 48 h. Noticeably, the ratio of early apoptotic cells in the high fucoidan dose group increased 13.1 fold compared with the control group (28.2 versus 2%, *p* < 0.05) while the ratio of late...
apoptotic cells increased only 51.6% versus the controls (4.7 versus 3.1%, \( p < 0.05 \)) (Fig. 1). Furthermore, fluorescence microscopy revealed increased chromatin condensation, margination, nuclear fragmentation and formation of apoptotic bodies in MCF-7 cells treated with serum from fucoidan-treated rats (Fig. 2).

The above findings indicated that serum from fucoidan-treated rats significantly suppressed the growth of MCF-7 cells in vitro and prominently induced early apoptosis of MCF-7 cells.

Serum from Fucoidan-Treated Rats Inhibits the Migration of MCF-7 Cells

We further assessed the effect of serum derived from fucoidan-treated rats on the migration of MCF-7 cells using scratch wound assays. We found that serum derived from rats treated with low and high doses of fucoidan significantly inhibited the relative migration of MCF-7 cells (low fucoidan, 63.94 ± 27.38; high fucoidan, 42.57 ± 11.32) at 48 h after wounding versus the control (138.25 ± 36.17) (\( p < 0.01 \)) (Fig. 3A). Transwell assays further showed that serum derived from fucoidan-treated rats significantly reduced the number of migrated cells compared to the controls (Fig. 3B).

DISCUSSION

Breast cancer is one of the most devastating diseases and the major cause of cancer mortality among women globally.\(^{12,13}\) Endocrine therapy and targeted therapies for women whose tumor overexpresses human epidermal growth factor receptor 2 (EGFR2) have improved the survival of breast cancer patients.\(^{14}\) However, these receptors are lacking in breast cancer tissues of some patients, such as those with triple negative breast cancer, which renders breast cancer

**Fig. 1. Fucoidan-Derived Serum Markedly Induces Apoptosis of MCF-7 Cells**

MCF-7 cells were treated with serum from rats treated with high or low doses of fucoidan as detailed in Materials and Methods. The cells were stained with annexin V-FITC (the vertical axis) and propidium iodide (the horizontal axis) and apoptotic cells were detected by flow cytometry. The left, mid and right panels are the control, high and low fucoidan dose group. Representative graphs of at least three independent experiments are shown. 1: control cells; 2: cells treated with drug serum from low-dose fucoidan (200 mg/kg·BW) treated rats; 3: cells treated with drug serum from high-dose fucoidan (400 mg/kg·BW) treated rats.

**Fig. 2. MCF-7 Cells Exhibit Typical Morphological Features of Apoptosis 48h after Treatment with Serum from Rats Treated with Fucoidan**

An apoptotic cell has special morphologic features such as cell shrinkage, chromatin condensation and margination as well as forming apoptotic bodies. In order to determine whether the drug serum was attributable to the induction of apoptosis, we stained the MCF-7 cells were stained with Hoechst 33258 and cellular morphology was examined by fluorescence microscopy. DNA fragmentation and chromatin condensation are observed in the treated cells. The left, mid and right panels are the control, high and low fucoidan dose group. Magnification, ×400. 1: control cells; 2: cells treated with drug serum from low-dose fucoidan (200 mg/kg·BW) treated rats; 3: cells treated with drug serum from high-dose fucoidan (400 mg/kg·BW) treated rats.

**Fig. 3. Serum from Fucoidan-Treated Rats Modulates the Expression of E-Cadherin and MMP-9 in MCF-7 Cells**

ELISA showed that serum from fucoidan-treated rats significantly upregulated the expression of E-cadherin and downregulated MMP-9 expression (Fig. 4A). Meanwhile, Western blotting assays further showed that the expression of E-cadherin increased while the expression of MMP-9 decreased in MCF-7 cells treated with serum from fucoidan-treated rats at 48 h, which are consistent with the ELISA results (Fig. 4B).
treatment rather daunting because of the highly aggressive nature of these cancer types. Moreover, these treatments are associated with complications and long-term side effects. Accordingly, intensive research efforts have been undertaken to improve existing therapies in terms of increased efficacy and diminished toxicities and to discover novel treatment modalities, including those that offer cures for the disease. For many years, investigators have focused on plants and microorganisms, mainly because these organisms are readily available and folk traditions have described beneficial effects. Fucoidan extracted from brown seaweed has various biological functions including anticancer effect. Some studies revealed that fucoidan inhibited proliferation and induced apoptosis of human lymphoma HS-Sultan cell lines and non-small-cell lung cancer cells. There is little knowledge about the effect of fucoidan on the onset and progression of human breast cancer. Because of readily available and anticancer effect, we sought to delineate the significant role of fucoidan in invasion, metastasis and angiogenesis of breast cancer cells.

Our study primarily focused on the effects of serum from fucoidan-treated rats on the growth of MCF-7 cells; the MTT assays demonstrated that serum from fucoidan-treated rats could effectively inhibit proliferation of MCF-7 cells. Apoptosis assays further showed that serum obtained in such a manner significantly induced the apoptosis of MCF-7 cells, which showed typical morphological features of apoptosis such as cell shrinkage, chromatin condensation and margination. Interestingly, serum from fucoidan-treated rats also suppressed the migration of MCF-7 cells.

Reports in the literature have suggested that E-cadherin and MMP-9 play a key role in tumor metastasis. E-Cadherin has been implicated as a tumor invasion suppressor gene. E-Cadherin is a specific biological characteristic, and cancer cell invasion and metastasis are closely related to the distribution of epithelial cell adhesion molecules. E-Cadherin is thought...
to be a protein intimately implicated in the invasion and metastasis of human malignant tumor cells, and its inactivation plays an important role in tumor development.\textsuperscript{25} E-Cadherin expression and functional abnormality can be detected in a variety of human tumors.\textsuperscript{26} MMP-9, which is the largest molecular weight MMP-family of MMPs, plays an important role in promoting tumor invasion and metastasis, especially after activation. The degradation of gelatin and collagen IV were close to tumor cells, so that the tumor cells along the absence of infiltration and metastasis. MMP-9 in breast cancer invasion and metastasis is also very close.\textsuperscript{19} In epithelial–mesenchymal transition (EMT), when epithelial cells become fibroblast-like, intercellular adhesion between cells will decrease and motility will increase, which further promote tumor progression.\textsuperscript{27} Western blotting assays and ELISA showed that serum from fucoidan-treated rats upregulated the expression of E-cadherin while downregulating MMP-9 expression. These results indicated that fucoidan could contribute significantly to EMT progression. Hsu et al.\textsuperscript{28} found similar results in 4T1 and MDA-MB-231 cells. Fucoidan effectively reverses transforming growth factor receptor (TGF\textbeta)-induced EMT morphological changes, upregulates epithelial markers, downregulates mesenchymal markers. In addition, it recently reported the protective effects of fucoidan on EMT of retinal pigment epithelial cells.\textsuperscript{29}

In conclusion, this study demonstrated that serum from fucoidan-treated rats could effectively inhibit the proliferation of breast cancer MCF-7 cells and induce tumor cell apoptosis, and suppress tumor cell migration by modulating E-cadherin and MMP-9 expression. It may be a potential promising therapeutic agent for breast cancer. However, the mechanism underlying the tumor inhibitory effects of fucoidan remains to be elucidated.

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Conflict of Interest The authors declare no conflict of interest.

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REFERENCES

18. Tran KN, Tran PH, Vo TV, Tran TT. Design of fucoidan functionalized iron oxide nanoparticles for biomedical applications. Curr.


