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Inhibitory effects of fucoidan on activation of epidermal growth factor receptor and cell transformation in JB6 Cl41 cells

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Abstract

Algal fucoidan is a marine sulfated polysaccharide with a wide variety of biological activities including anti-thrombotic, anti-inflammatory, and anti-tumor activities. In this study, we tested the hypothesis that fucoidan may suppress neoplastic cell transformation by inhibiting the phosphorylation of epidermal growth factor receptor (EGFR) in mouse epidermal JB6 Cl41 cells. Our results provided the first evidence that fucoidan from *Laminaria guryanovae* exerted a potent inhibitory effect on EGF-induced phosphorylation of EGFR. Consistent with its inhibitory action on phosphorylation of EGFR, fucoidan clearly suppressed the phosphorylation of extracellular signal-regulated kinase or *c-jun* N-terminal kinases induced by EGF. Moreover, EGF-induced the *c-fos* and *c-jun* transcriptional activities were inhibited by fucoidan, resulting to suppressing of activator protein-1 (AP-1) activity and cell transformation induced by EGF. Taken together, these results indicate that fucoidan might exert chemopreventive effects through the inhibition of phosphorylation of the EGFR.
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Keywords: Fucoidan; AP-1; Cell transformation; EGFR; c-fos; c-jun

1. Introduction

Brown seaweeds are known to produce different polysaccharides, namely alginates, laminarans and fucoidans (Painter, 1983; Percival, 1967). The latter polysaccharides usually contain large proportions of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, mannose and glucuronic acid (Duarte et al., 2001; Percival, 1967). Several biological activities have been attributed to the fucoidans: anti-coagulant (Chevolot et al., 2001), anti-thrombotic (Mourao, 2004), anti-inflammatory (Cumashi et al., 2007), anti-tumoral (Itoh et al., 1993; Maruyama et al., 2006) (Teas et al., 1984), and anti-viral (Thompson and Dragar, 2004). Also, it was reported that fucoidan increases the level of nitric oxide (NO) production in quiescent macrophages, which was related with p38 kinase-dependent NF-kB activation (Nakamura et al., 2006). Although several studies on the biological activities of fucoidans have been performed, with particular focus on its anti-tumorigenic activity, it is unclear if fucoidan inhibits the neoplastic cell transformation and AP-1 transactivation activity induced by tumor promoter, such as EGF.

The epidermal growth factor receptor (EGFR), one of the receptor tyrosine kinases, plays a pivotal role in regulating cell proliferation, differentiation, and transformation (Chen et al., 1987). The EGFR is an important target for cancer therapy (Yarden and Sliwkowski, 2001). Many carcinomas are promoted by EGFR activation, which can result from mutation of the receptor (Humphrey et al., 1990), its overexpression (Gorgoulis et al., 1992), or from EGFR stimulation through autocrine loops (Sizeland and Burgess, 1992).

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To elucidate the mechanism of the anti-tumorigenic effects of fucoidan, we studied the effects of fucoidan extracted from *Laminaria guryanovae* on the phosphorylation of EGFR and neoplastic cell transformation induced by EGF in mouse epidermal JB6 cells. Because the activation of EGFR has an important role in tumorigenesis, the results of this investigation may provide new insights in the mechanism of fucoidan in tumor suppression and the possibility for its application in tumor prevention and treatment.

2. Materials and methods

2.1. Reagents and antibodies

Eagle's minimal essential medium (MEM), L-glutamine, gentamicin, and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). EGF was purchased from Calbiochem–Novabiochem (San Diego, CA). Polyvinylidene difluoride (PVDF) membrane was from Millipore (Bedford, MA). The Dual-luciferase reporter assay system and CellTiter 96 non-radioactive cell proliferation assay kit were purchased from Promega (Madison, WI). Antibodies against phospho-MEK1/2, -ERK1/2, -p90RSK, -Elk1, -JNK, -*c-jun*, -EGFR, and total GAPDH were purchased from Cell Signaling Tech. Inc. (Danvers, MA).

2.2. Cell culture and transfection

JB6 Cl41 mouse epidermal cells were cultured in MEM supplemented with 5% FBS. Cells were transfected by a cationic liposome transfection method, Lipofectamine (Invirogen Co., Carlsbad, CA).

2.3. Polysaccharide extraction

L. guryanovae samples were collected in Troitsa Bay, Sea of Japan. The isolation and separation of water-soluble polysaccharides were carried out by the modified methods (Zvyagintseva et al., 1999). The fresh or deep-frozen seaweeds (3 kg) were initially treated with ethanol, acetone, and chloroform successively. A defatted seaweed *L. guryanovae* was stirred for 5 h in 0.1 M HCl for 5 h at room temperature, and the extracts were combined. The remaining seaweed was then extracted with water for 5 h at 60 °C. The extracts were concentrated to 1/5 of volume by ultrafiltration with the use of Millipore 3 kDa membrane, and polysaccharides were precipitated with four volumes of 96% ethanol. The precipitates were washed with 96% ethanol and acetone and air-dried.

2.4. Anion exchange chromatography

A solution of polysaccharide in 0.1 M NaCl (2.3 g in 50 ml) was applied onto a DEAE-cellulose column (Cl^- form, 3 × 21 cm) equilibrated with 0.1 M NaCl. The column was then successively eluted with 0.1, 0.5, 1, and 2 M NaCl solutions, each time until the disappearance in elute of positive reaction for carbohydrates by the phenol–sulfuric acid method (Dubois et al., 1956). The correspondent polysaccharides fractions were concentrated by ultrafiltration (1 kDa cutoff), dialyzed, and lyophilized. Fucoidan was dissolved in sterile water to test the biological activity in JB6 Cl41 cells.

2.5. Analytical procedures

Neutral carbohydrates were quantified by the phenol–sulfuric acid method (Dubois et al., 1956); reducing carbohydrates were determined according to Nelson method (Nelson, 1944). Monosaccharide composition was determined by HPLC with a LC-5001 carbohydrate analyzer (a Durrum DA-X8-11 column (385 × 3.2 mm) (Biotronic), bicinhoniate assay, and a C-R2 AX integrating system (Shimadzu) after hydrolysis by 2 M TFA (6 h, 100 °C).

2.6. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

To estimate cell cytotoxicity, JB6 Cl41 cells were seeded (1×10^4) in 96-well plates in 100 μl of 5% FBS-MEM at 37 °C in a 5% CO_2 incubator. After culturing for 24 h, the various concentrations of fucoidan were treated and incubated for additional 24 h at 37 °C in a 5% CO_2 incubator. After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (15 μl) were added to each well, and cells were then incubated for 4 h at 37 °C in a 5% CO_2. Absorbance was measured at 570 nm.

2.7. Anchorage-independent cell transformation assay (soft agar assay)

EGF-induced cell transformation was investigated in JB6 Cl41 cells. In brief, cells (8×10^3/ml) were exposed to EGF (1 ng/ml) with or without fucoidan in 1 ml of 0.3% basal medium Eagle (BME) agar containing 10% FBS, 2 mM L-glutamine, and 25 μg/ml gentamicin. The cultures were maintained at 37 °C, in a 5% CO_2 incubator for 10 days, and the cell colonies were scored using a microscope and the Image-Pro PLUS computer software program (Media Cybernetics, Silver Spring, MD) as described by Colburn et al. (1981). The effects of the fucoidan on cell transformation of JB6 C141 cells are presented as an inhibition of cell transformation compared with EGF-stimulated cells in soft agar.

2.8. Reporter gene assays

The reporter gene assay for firefly luciferase activity was performed using lysates from transfected cells. In addition, the reporter gene vector phRL-SV40 (Promega) was co-transfected into each cell line and the *Renilla* luciferase activity generated by this vector was used to normalize the results for transfection efficiency. Cell lysates were prepared by first washing the transfected JB6 Cl41 cells once in phosphate buffered saline (PBS) at 37 °C. After removing the PBS completely, passive lysis buffer (PLB, Promega) were added, and then cells were incubated for 1 h with gentle shaking. The supernatant fraction was used for the measurement of firefly and *Renilla* luciferase activities. Cell lysates (20 μl each) were mixed with 100 μl of luciferase assay II reagent (Promega) and firefly luciferase light emission was measured by TriStar LB 941 (Berthold Tech. GmbH and Co. KG, Germany). Subsequently, 100 μl of *Renilla* luciferase substrate (Promega) was added in order to normalize the firefly luciferase data. *c-Fos* promoter luciferase (pGL3-Fos) and *c-jun* promoter luciferase (JC6GL3) constructs were kindly provided by Dr. Ron Prywes (Columbia University, NY). The AP-1 luciferase reporter plasmid (−73/+63 collagenase–luciferase) was kindly provided by Dr. Dong Zigang (Hormel Institute, University of Minnesota, MN).

2.9. Immunoblotting

The proteins were resolved by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto PVDF membranes. The membranes were blocked, hybridized with the appropriate primary antibody overnight at 4 °C. Protein bands were visualized by the chemiluminescence detection kit (ECL of Amersham Biosciences Corp., Piscataway, NJ) after hybridization with the horseradish peroxidase (HRP)-conjugated secondary antibody from rabbit or mouse.

3. Results

3.1. Purification and cell viability of fucoidan from L. gurjanovae

The brown seaweed *L. gurjanovae* was collected in the coast of island Big Shantar (Okhotsk sea). Polysaccharides were isolated from seaweed by a combination of methods

according material and methods (Kusaykin et al., 2006). Recently, we reported the characteristics of polysaccharide fractions obtained by a successive extraction of seaweed *L. gurjanovae* (Shevchenko et al., 2007b). According to the results, the seaweed *L. gurjanovae* contained laminaran consisting of glucose, and fucoidan built up of galactose and fucose. An analysis of all the obtained information shows that fucoidan from *L. gurjanovae* is a partially acetylated galactofucan of a block structure, in which both monosaccharide (fucose and galactose) residues are sulfated and a partially acetylated (Fig. 1A). We used fucoidan from *L. gurjanovae* which was eluted with 2 M NaCl and was 85–90% at purity (Shevchenko et al., 2007b), to elucidate the molecular mechanism of the anti-tumorigenic effects of fucoidan.

To test the effect of various concentrations of fucoidan from *L. gurjanovae* (0, 1, 10, 30, 50, 100, 200 μg/ml) on the cell proliferation in JB6 Cl41 cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to examine. The treatment of fucoidan did not show any decrease of the rate of proliferation in JB6 Cl41 cells (Fig. 1B).

3.2. Fucoidan inhibits the EGFR signaling pathway induced by EGF

In this study, we investigated the effects of fucoidan on the EGF-induced phosphorylation of EGFR in mouse skin epidermal JB6 Cl41 cells. JB6 Cl41 cells were treated with 1 ng/ml EGF for 15 min, in the absence or presence of various concentrations of fucoidan (1, 10, 50, 100 μg/ml) and assayed by immunoblotting with the anti-phospho-EGFR and anti-EGFR (1005) antibodies, respectively. Results indicated that treatment of fucoidan significantly decreased the phosphorylation of EGFR, but not the EGFR total protein level (Fig. 2A).

One of the most important protein kinase cascades activated by tumor promoters, such as epidermal growth factor (EGF), are the mitogen-activated protein kinases (MAPKs) (Bode and Dong, 2003; Cowley et al., 1994), following from the activation of EGFR. Next, we examined the inhibitory effect of fucoidans on the MAP kinases, such as MEK1/2, ERK1/2, p90RSK, or Elk-1 of JB6 Cl41 cells

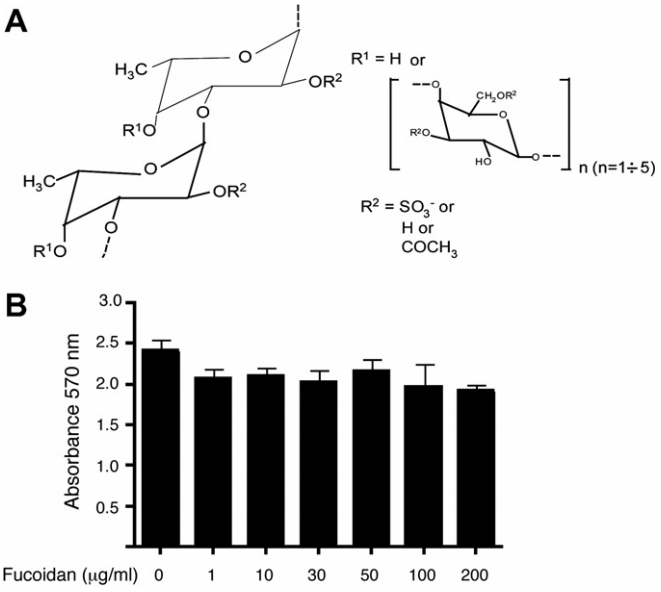

Fig. 1. The structure and cell viability of fucoidan. (A) Structure of the sulfated fucan isolated from the brown seaweed *L. guryanovae.* (B) JB6 Cl41 cells were seeded (1×10^4/well) in 96-well plates in 100 μL of 5% FBS-MEM, and then treated with a range of concentrations of fucoidan (1–200 μg/ml) for 72 h. The cell proliferation was estimated using the CellTiter 96 non-radioactive cell proliferation assay kit by absorbance at 570 nm. Each bar indicates the mean ± SD of values obtained from triplicate experiments.

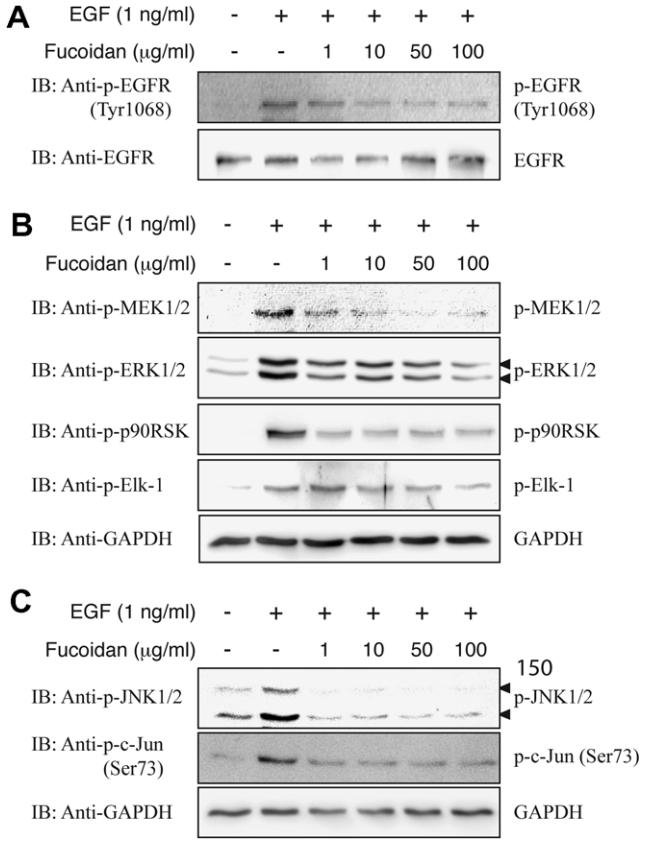

Fig. 2. Inhibitory effect of fucoidan on the EGFR signaling pathway. (A) Fucoidan inhibits EGFR phosphorylation induced by EGF. JB6 Cl41 cells were treated with different concentration of fucoidan for 12 h, and then exposed to EGF (1 ng/ml) for 15 min. Proteins in whole cell lysates were separated by SDS–PAGE and immunoblotted with antibodies to detect phosphorylation of EGFR (1005), as indicated. (B) Fucoidan inhibits ERK1/2 phosphorylation induced by EGF. JB6 Cl41 cells were treated with different concentration of fucoidan for 12 h, and then exposed to EGF (1 ng/ml) for 15 min. Proteins in whole cell lysates were separated by SDS–PAGE and immunoblotted with antibodies to detect phosphorylation of MEK1/2, ERK1/2, p90RSK, Elk-1 and GAPDH, as indicated. (C) Fucoidan inhibits JNK1/2 phosphorylation induced by EGF. JB6 Cl41 cells were treated with different concentration of fucoidan for 12 h, and then exposed to EGF (1 ng/ml) for 15 min. Proteins in whole cell lysates were separated by SDS–PAGE and immunoblotted with antibodies to detect phosphorylation of JNK1/2, phosphorylation of *c-jun* (Ser63 or Ser73) and GAPDH, as indicated.

cultured with fucoidan. JB6 Cl41 cells were treated with 1 ng/ml EGF for 15 min, in the absence or presence of various concentrations of fucoidan (1, 10, 50, 100 μg/ml) and assayed by immunoblotting with anti-phospho-MEK1/2, anti-phospho-ERK1/2, anti-phospho-p90RSK, or anti-phospho-Elk-1 antibodies, respectively. It shows that fucoidan significantly inhibit EGF-induced phosphorylation of MEK1/2, ERK1/2, p90RSK, or Elk-1, respectively (Fig. 2B), suggesting that MAPK signaling cascade is the targets of fucoidan.

EGF-induced MEKK1 phosphorylation can activate ERK as well as JNK, resulting to the activation of *c-jun* promoter (Gupta and Prywes, 2002). EGF-induced *c-jun* transcriptional activation is closely connected with cell proliferation, differentiation, and neoplastic cell transformation (Shaulian and Karin, 2002). Therefore, we first examined whether fucoidan can inhibit the EGF-induced phosphorylation of JNKs as well as *c-jun*. Our results showed that pretreatment with fucoidan almost completely inhibited the EGF-induced phosphorylation of JNKs and *c-jun* (Ser63 and Ser73) in JB6 Cl41 cells (Fig. 2C) that may lead to downregulation of *c-jun* transcriptional activity.

3.3. Fucoidan inhibits EGF-induced c-fos or c-jun transcriptional activity

c-Jun and c-Fos are nuclear proto-oncoproteins whose expression is stimulated by a variety of growth-promoting agents and activated oncogenes (Herschman, 1991). To investigate whether fucoidan suppress the EGF-induced *c-fos* or *c-jun* transcriptional activity, we took advantage of the availability of the reporter plasmid carrying the *luc* gene under the control of the murine *c-jun* or *c-fos* promoter (Gupta and Prywes, 2002; Zhu et al., 1997). Twenty-four hours after transfection with these reporters in JB6 Cl41 cells, cells were starved for another 24 h by incubating in serum-deprived MEM at 37 °C in a 5% CO_2 atmosphere. At 12 h of starvation, cells were pretreated or not treated with fucoidan (1, 10, 50, and 100 μg/ml), and then treated or not treated with EGF (1 ng/ml) for 12 h. As shown in Fig. 3A and B, EGF-promoted *c-fos* or *c-jun* transcriptional activity was significantly suppressed by fucoidan. These data support our notion that the suppression of the *c-jun* or *c-fos* promoter by EGF is one of the mechanisms explaining the antitumorigenetic effect of fucoidan.

3.4. Fucoidan suppressed AP-1 transactivation activity and neoplastic cell transformation

The AP-1 transcription factor is a dimeric complex that comprises members of the Jun, Fos, activating transcription factor, and musculoaponeurotic fibrosarcoma protein families (Eferl and Wagner, 2003). The regulation of cell proliferation by AP-1 might be of crucial importance for the multistage development of tumors (Liu et al., 2002).

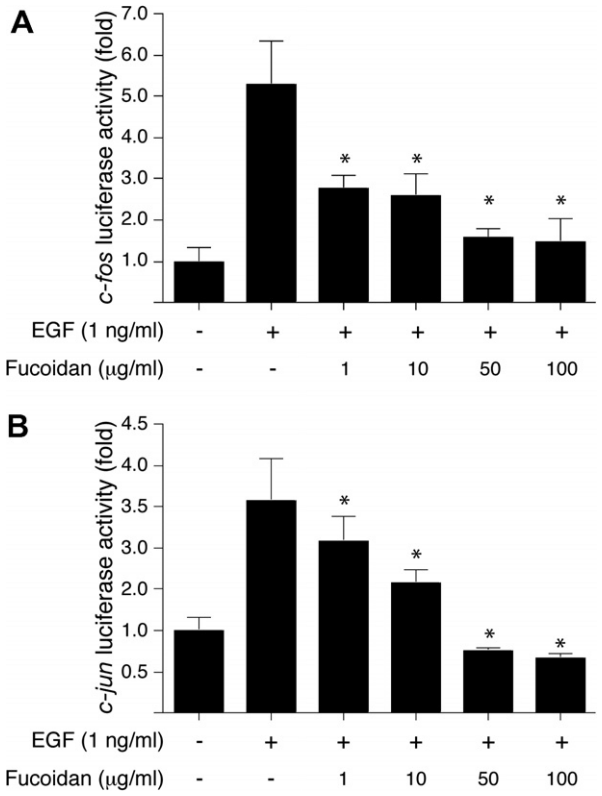

Fig. 3. Inhibitory effect of fucoidan on the *c-fos* and *c-jun* promoter activity induced by EGF. (A and B) JB6 Cl41 cells was transfected with a plasmid mixture containing *c-fos* luciferase reporter gene (0.5 μg; A) or the *c-jun* luciferase reporter gene (0.5 μg; B), each with the *phRL-SV40* gene (0.5 ng). At 24 h after transfection, cells were starved for 24 h by incubating in serum-deprived MEM at 37 °C in a 5% CO_2 atmosphere. At 12 h of starvation, the different concentration of fucoidan was treated, and then incubated in the presence of 1 ng/ml EGF for additional 12 h. The *firefly* luciferase activity was determined in cell lysates and normalized against *renilla* luciferase activity. All experiments were done at least twice. *Columns*, mean of triplicate samples; *bars*, SE. Data were recorded as relative luciferase activity (fold) using a TriStar LB 941. Significant differences were evaluated using the Student's *t*-test (*, $P < 0.05$), significant decrease in EGF-induced *c-fos* or *c-jun* activity in fucoidan-treated JB6 Cl41 cells compared with positive control cells.

AP-1 is induced by several external stimuli, such as EGF, which increase MAPK activity (Tyagi et al., 2003). To determine whether the anti-tumorigenetic effect of fucoidan is responsible for the inhibition of AP-1 activation response to EGF, we next cotransfected the AP-1 luciferase reporter plasmid and the phRL-SV40 gene into JB6 Cl41 cells. At 24 h after transfection, cells were starved for an additional 24 h by incubating in serum-deprived MEM at 37 °C in a 5% CO_2 atmosphere. At 12 h of starvation, we did pretreatment of fucoidan (1, 10, 50, and 100 μg/ml). They were then treated or not treated with EGF (1 ng/ml) for 12 h. The EGF-induced AP-1 activation response in JB6 Cl41 cells was significantly inhibited by fucoidan similar to that observed for *c-jun* or *c-fos* activity (Fig. 4A).

Next, we studied the effect of fucoidan on EGF-promoted cell transformation using our previously developed methods (Dong and Cmarik, 2002). JB6 Cl41 cells were

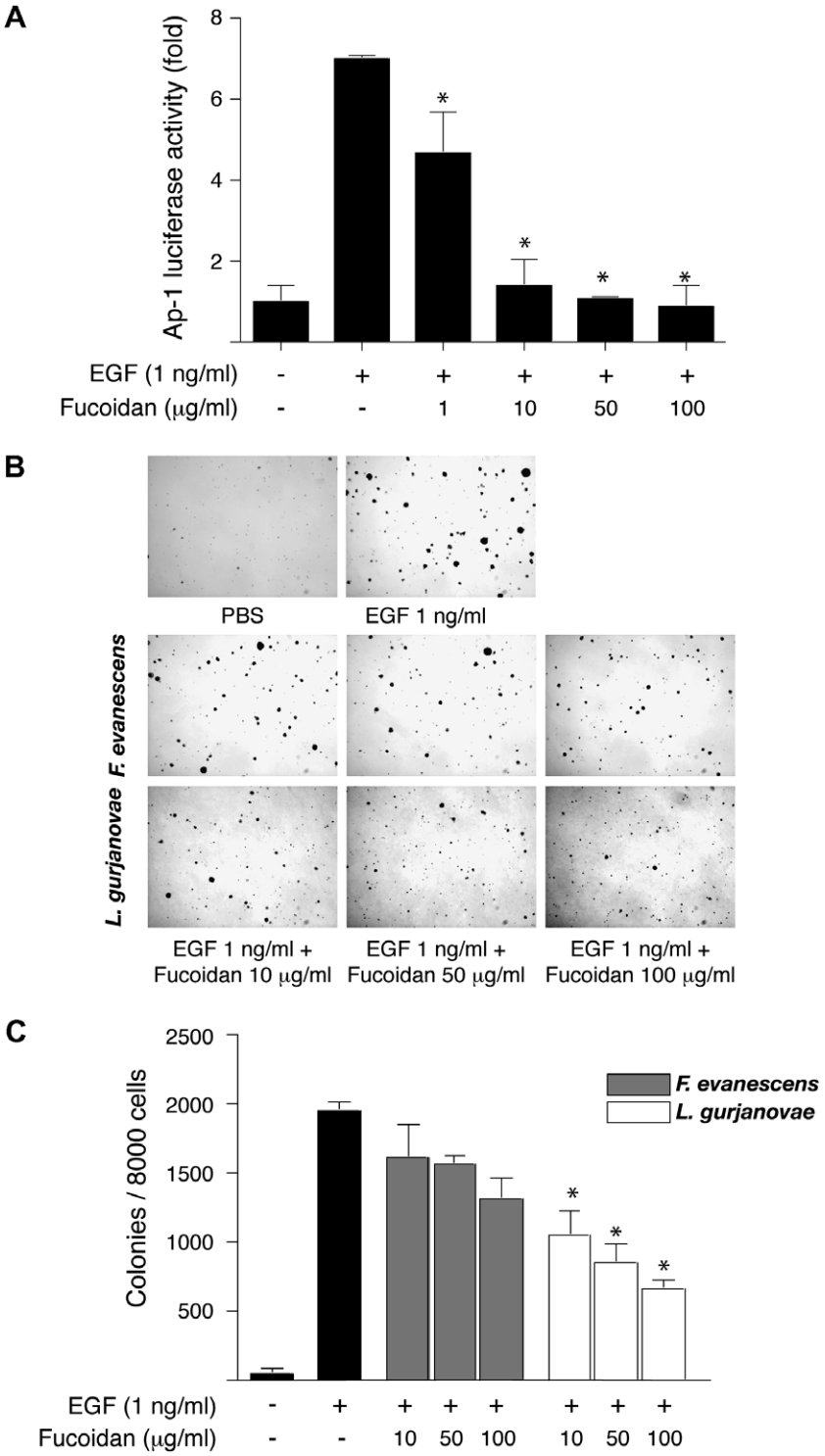

Fig. 4. Inhibitory effects of fucoidan on the *AP-1* activity and cell transformation induced by EGF. (A) JB6 Cl41 cells were cotransfected with a plasmid mixture containing the *AP-1* luciferase reporter gene (1 μg) with the *phRL-SV40* gene (1 ng). At 24 h after transfection, cells were starved for 24 h by incubating in serum-deprived MEM at 37 °C in a 5% CO_2 atmosphere. At 12 h of starvation, the different concentration of fucoidan was treated, and then incubated in the presence of 1 ng/ml EGF for additional 12 h. The *firefly* luciferase activity was determined in cell lysates and normalized against *renilla* luciferase activity. All experiments were done at least twice. *Columns*, mean of triplicate samples; *bars*, SE. Data were recorded as relative luciferase activity (fold) using a TriStar LB 941. Significant differences were evaluated using the Student's *t*-test (*, $P < 0.05$), significant decrease in EGF-induced *AP-1* activity in fucoidan-treated JB6 Cl41 cells compared with positive control cells. (B and C) JB6 Cl41 cells were used to assess cell transformation in a soft agar assay. Cells (8×10^3/ml) treated with/without fucoidans, from *L. gurjanovae* or *F. evanescens,* were exposed to EGF (0 or 1 ng/ml) in 1 ml of 0.3% basal medium Eagle's agar containing 10% FBS. The culture was maintained at 37 °C in a 5% CO_2 atmosphere for 10 days; then, colonies were counted automatically. The average colony number was calculated and photographed from three separate experiments. Significant differences were evaluated using the Student's *t*-test (*, $P < 0.05$), significant decrease in EGF-induced cell transformation in fucoidan-treated cells compared with positive control cells.

treated separately with EGF (1.0 ng/ml) in the absence or presence of various concentrations of fucoidan (10, 50, or 100 μg/ml) in a soft agar matrix and incubated at 37 °C in a 5% CO_2 incubator for 10 days. Also, we compared the effects of the fucoidans, from *L. gurjanovae*, sulfated 36.2% (Shevchenko et al., 2007b), and from *Fucus evanescens*, sulfated at 36.3% (Cumashi et al., 2007), on the EGF-induced cell transformation, to exam whether the anti-tumor activity of fucoidan results from the its sulfated group. Our results showed that fucoidan, from *L. gurjanovae*, significantly inhibited the formation of EGF-promoted colonies (Fig. 4B and C). The inhibition was evident not only in colony number but also in colony size. The fucoidan from *F. evanescens* had a very weak activity to inhibit cell transformation compared with it from *L. gurjanovae* (Fig. 4B and C), suggesting that anti-tumor activity of fucoidan may result from not only the sulfate groups but also a distinctive feature of structure, such as a monosaccharide composition, position of branches, or rate of branches on the fucoidan. Taken together, these data strongly support the idea that fucoidan plays an inhibitory role of neoplastic cell transformation in epidermal mouse skin cells stimulated with EGF, resulting from inhibiting of AP-1 activity.

4. Discussion and conclusion

Fucoidans represent the family sulphated homo- and hetero-polysaccharides mainly build up of α-L-fucose with sulphate and acetate units. Fucoidan from *L. gurjanovae* differs from similar polysaccharides, isolated from other *Laminaria* species, which are sulfated poly-(1 → 3)-β-*L*-fucans (Chizhov et al., 1999); although, an increased content of galactose was described for fucoidans from *L. japonica* (Honya et al., 1994) and *Ecklonia kurome* (Nishino and Nagumo, 1992). Most studies have used a commercial available fucoidan from *Fucus vesiculosus* (Sigma, St. Louis, MO), widely used in research presents a heterogeneous composition of more than 15 different fucans with varying proportions of individual monosaccharide (Nishino et al., 1994). In the present work, we have purified fucoidans from *L. gurjanovae* to determine anti-tumor activity dependent of structure.

Here, we investigated the effects of fucoidan from *L. gurjanovae* on AP-1 transactivation and subsequent cell transformation in the mouse epidermal JB6 Cl41 cells. The standard oncogenic transformation assay for cell lines is usually performed in soft agar because of the higher transformation efficiency of cells in soft agar (Dong and Cmarik, 2002). The JB6 Cl41 cell line is a well-established system used extensively as an in vitro model for the study of tumor promotion and anti-tumor promotion (Amstad et al., 1997). Recently, Low molecular weight fucoidan (LMWF), a sulfated polysaccharide from brown seaweeds that mimic some biological activities, has been shown to bind to vascular endothelial growth factor 165 ($VEFF_{165}$) and its receptors, resulting to promotes the binding of ($VEGF_{165}$) to VEGF receptor-2 and Neuropilin-1 (NRP1) (Lake et al., 2006). However, natural and oversulfated fucoidans significantly suppressed the mitogenic and chemotactic actions of $VEGF_{165}$ on HUVEC by preventing the binding of $VEGF_{165}$ to its cell surface receptor (Koyanagi et al., 2003). Therefore, we hypothesized that fucoidan may bind with EGF, resulting to inhibit EGF-induced cell transformation. Our results indicated that fucoidan from *L. gurjanovae*, blocked EGF-induced phosphorylation of EGFR, suggesting that fucoidan may bind with EGF and prevent the binding of EGF to EGFR. Fucoidan also blocked EGF-induced ERK and JNK activity, which most likely explains their inhibitory effect on AP-1 transactivation and the subsequent reduction in cell transformation induced by EGF.

As a sequence-specific transcriptional activator, AP-1 mediates a broad range of external stimuli that lead to gene transcription. Many stimuli, including TPA, growth factor, and UV radiation that induce AP-1, are associated with tumorigenesis (Angel and Karin, 1991). Neoplastic cell transformation is often associated with a dramatic increase in AP-1 activity (Vallone et al., 1997), and this transient induction of AP-1 has been shown to be involved in the promotion of epidermal tumors (Domann et al., 1994). Constitutive AP-1 activity has been associated with the malignant conversion of papillomas to carcinomas as well (Yuspa, 1998). Chemopreventive agents or modification of AP-1 proteins that inhibit AP-1 activation are effective in preventing cell transformation or tumorigenesis (Dong et al., 1997a). EGF is the tumor promoter, which can stimulate malignant transformation. In our experiments, fucoidan from *L. gurjanovae* suppressed EGF-stimulated JB6 Cl41 cells transformation on soft agar. A corresponding inhibition of EGF-induced AP-1 activity was also found, suggesting that the inhibition of tumorigenesis by this polysaccharide is through the inhibition of AP-1 activity.

Many mechanisms are involved in the up- and down-regulation of AP-1 activity (Angel and Karin, 1991). MAPKs are the most common pathways known to mediate AP-1 function (Karin, 1995), and in the current experiment, EGF activated members of the MAPK family: JNK, ERKs. Fucoidan from *L. gurjanovae* were able to block EGF-stimulated ERKs and JNKs activities. Although both ERKs and JNKs of the MAPK family have been reported to be able to induce AP-1 activity, each of the kinases may activate different AP-1 components resulting in the transcription of different genes (Karin, 1995). Many reports indicated that JNKs are critical in mediating AP-1 transactivation and malignant transformation (Dong et al., 1997b). For instant, it was reported that TNF-α induced cell transformation requires activation of JNKs (Huang et al., 1999). It was also reported that TPA-induced skin trumorigenesis was strikingly suppressed in JNK-2-deficient mice (Chen et al., 2001). In our experiments, the inhibition of EGF-induced JNK activity by fucoidan agreed well with the inhibitory effects of it on EGF-induced AP-1 activity and cell transformation.

The fucoidan from *L. gurjanovae* were effective in inhibiting cell transformation induced by EGF in the mouse epidermal JB6 Cl41 cells. The inhibition was found to be associated with the inhibitory effects of this compound on AP-1 activity. Fucoidan also blocked the phosphorylation of EGFR, a receptor of EGF, suggesting that EGFR may be a critical target for fucoidan in inhibiting AP-1 activity and cell transformation.

Conflict of interest statement

I, Hong Seok Choi, declare that I have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, *Inhibitory Effects of Fucoidan on Activation of Epidermal Growth Factor Receptor and Cell Transformation in JB6 Cl41 Cells*.

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