Biochemical and Biophysical Research Communications 458 (2015) 727–732

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Anti-metastatic effects of the sulfated polysaccharide ascophyllan isolated from *Ascophyllum nodosum* on B16 melanoma

Ryogo Abu [a], Zedong Jiang [b], Mikinori Ueno [a], Shogo Isaka [a], Satoru Nakazono [a], Takasi Okimura [c], Kichul Cho [d, e], Kenichi Yamaguchi [a], Daekyung Kim [d, e, *], Tatsuya Oda [a, **]

[a] *Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan*
[b] *College of Food and Biological Engineering, Jimei University, Xiamen, Fujian Province 361021, China*
[c] *Research and Development Division, Hayashikane Sangyo Co., Ltd., Shimonoseki, Yamaguchi 750-8608, Japan*
[d] *Jeju Center, Korea Basic Science Institute (KBSI), Jeju 690-756, Republic of Korea*
[e] *Korea University of Science & Technology, Daejeon 305-350, Republic of Korea*

ARTICLE INFO

Article history:
Received 23 December 2014
Available online 23 January 2015

Keywords:
Ascophyllan
Sulfated polysaccharide
Ascophyllum nodosum
B16 melanoma
Melanoma metastasis

ABSTRACT

We previously found that ascophyllan, a sulfated polysaccharide isolated from brown seaweed *Ascophyllum nodosum*, exhibited antitumor activity in sarcoma-180 tumor-bearing mice. In this study, we found that ascophyllan inhibited the migration and adhesion of B16 melanoma cells by reducing the expression of N-cadherin and enhancing the expression of E-cadherin in a concentration-dependent manner. Transwell invasion assay revealed that ascophyllan suppressed the invasion ability of B16 cells. It also inhibited the expression of matrix metalloprotease-9 (MMP-9) mRNA and the secretion of MMP-9 protein in B16 cells, a process that may involve the extracellular signal-regulated kinase (ERK) signaling pathway. Furthermore, ascophyllan administered intraperitoneally at 25 mg/kg showed anti-metastatic activity in a mouse model of metastasis induced by intravenous injection of B16 cells, and the number of lung surface metastatic nodules in ascophyllan-treated mice was significantly reduced compared to that in the untreated control mice. Since splenic natural killer cell activity enhanced in the mice injected with ascophyllan intraperitoneally, we suggest that ascophyllan may exhibit *in vivo* anti-metastatic activity on B16 melanoma cells through activation of the host immune system in addition to a direct action on cancer cells.

1. Introduction

Metastasis of cancer cells is the major cause of cancer-related mortality. The development of cancer metastasis consists of multiple steps, in which cancer cells migrate from the primary tumor site, invade surrounding tissues, move through the blood or lymphatic system to distant tissues, extravasate from the vasculature, and eventually proliferate to form secondary tumors at new sites. Hence, inhibition of any of these processes could be an effective anticancer approach [1]. It is well documented that natural products are one of the most important sources of potential anticancer agents [2]. Naturally occurring polysaccharides show potent immunotherapeutic properties in terms of cancer prevention and treatment [3,4]. Some of these polysaccharides act as biological response modifiers. Of special interest is fucoidan, a sulfated fucan found in brown seaweeds, which is known to show antitumor activity [5].

Ascophyllan (xylofucoglycuronan) is a sulfated polysaccharide structurally similar to, but distinguishable from, fucoidan [6]. We previously found that ascophyllan has various bioactivities such as a growth-promoting effect on MDCK cells [7] and induction of cytokine secretion from mouse macrophage RAW264.7 cells [8]. Furthermore, we recently found that intraperitoneally administered ascophyllan had significant antitumor activity in a sarcoma-180 solid tumor model [9]. In this study, we investigated ascophyllan's effects on B16 melanoma metastasis by using *in vitro* and *in vivo* approaches.

* Corresponding author. Fax: +82 64 805 7800.
** Corresponding author. Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan. Fax: +81 95 819 2831.
E-mail addresses: dkim@kbsi.re.kr (D. Kim), t-oda@nagasaki-u.ac.jp (T. Oda).

http://dx.doi.org/10.1016/j.bbrc.2015.01.061
0006-291X/

2. Materials and methods

2.1. Materials

Matrigel® was obtained from Becton & Dickinson Biosciences (Franklin Lakes, NJ, USA). Antibodies for nonphosphorylated and phosphorylated p38, JNK, and ERK MAP kinases were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Specific inhibitors for extracellular-regulated kinase (ERK) (PD98059), p38 (SB202190), and c-jun NH_2-terminal kinase (JNK) (SP600125) mitogen-activated protein (MAP) kinases were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Anti-E-cadherin and anti-N-cadherin monoclonal antibodies were obtained from Sigma (St. Louis, MO, USA).

2.2. Preparation of ascophyllan and desulfated ascophyllan

Ascophyllan was prepared from *Ascophyllum nodosum* as described previously [6,7], and desulfated ascophyllan was prepared from ascophyllan using a previously described method [10].

2.3. Cell culture

Murine B16 melanoma and lymphoma YAC-1 cells were obtained from the Institute of Development, Aging, and Cancer of Tohoku University, and cultured at 37 °C in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml). RAW264.7 (mouse macrophage) cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured as described previously [8].

2.4. Animals

C57BL/6 mice (8 weeks old, 20.0 ± 2.0 g) were obtained from Texam Corp. (Nagasaki, Japan). All mice were treated according to the Guidelines of the Japanese Association for Laboratory Animal Science and the Guidelines for Animal Experiments of Nagasaki University, Japan as described previously [9].

2.5. Cytotoxicity assay

Adherent B16 cells in 96-well plates (1×10^4 cells/well) were cultured with varying concentrations of ascophyllan (0–1000 μg/ml) in the growth medium for 24 h at 37 °C, and then, cell viability was determined using MTT assay as described previously [11].

2.6. Wound healing assay

B16 melanoma cells were seeded into a well of 6-well plates (1×10^6 cells/well). After 24 h at 37 °C, a wound in the cell monolayer was formed by scraping with a 200-μl tip. After the cells were washed thrice with the growth medium, varying concentrations of ascophyllan (0, 10, 100, or 1000 μg/ml) were added to the wells and incubated for 0, 6, 12, and 24 h at 37 °C. Then, migrated cells in the scraped area were photographed under a phase-contrast inverted microscope.

2.7. Adhesion assay

Adhesion assay was performed as described previously [12] with slight modifications. Briefly, 100 μl of type I rat skin collagen (0.1 mg/ml) in 5 mM acetic acid was added into each well of 48-well plates and incubated at room temperature for 1 h, after which the supernatants were removed. The wells were dried at room temperature for 2 h and then washed twice with phosphate-buffered saline (PBS). The suspensions of B16 melanoma cells (3×10^5 cells/well) were added to each well in the presence or absence of ascophyllan (10, 100 or 1000 μg/ml). After 1 h incubation at 37 °C, the wells were washed twice with PBS. To determine the viable adherent cells on each well, MTT assay was conducted as described above.

2.8. Nitrite assay for the estimation of NO

NO-inducing activities of ascophyllan and desulfated ascophyllan were measured by Griess assay as described previously [8].

2.9. Transwell invasion assay

Cell invasion ability was determined using a transwell invasion assay as described previously [13]. To analyze invasive ability, transwell chambers (BD, Franklin Lakes, NJ, USA) were set up with 8-μm pore-size filters. The filter's lower surface was coated with 2 μg/50 μl fibronectin and the upper surface with 10 μg/50 μl Matrigel. Coated filters were washed with PBS and dried immediately before use. B16 melanoma cell suspensions (3×10^5 cells/ml, 300 μl) with or without ascophyllan were added to the chamber's upper compartment and incubated at 37 °C. After 24 h incubation, the non-invaded cells on the upper surface were wiped with a cotton swab. The invaded cells on the filter's lower surface were fixed with 30% methanol and stained with 0.5% crystal violet in 30% methanol. The stained cells were counted under a microscope.

2.10. Western blot analysis

Adherent B16 melanoma cells in 6-well plates (5×10^5 cells/well) were treated with varying concentrations of ascophyllan in a growth medium at 37 °C. After 1.5 h incubation for the analysis of MAP kinases or 24 h incubation for cadherins, the cells were washed thrice with ice-cold PBS and harvested in lysis buffer, and the proteins were extracted as described previously [8]. Equal amounts of proteins (30 μg) were separated by 10% SDS-PAGE, transferred onto polyvinylidene difluoride membranes, and probed with specific primary antibodies against E-cadherin, N-cadherin, and nonphosphorylated and phosphorylated p38, JNK, and ERK MAP kinases, and subsequently with HRP-conjugated secondary antibodies. The blots were detected using an enhanced chemiluminescence kit following manufacturer instructions.

2.11. RNA isolation and RT-PCR

Adherent B16 melanoma cells in 6-well plates (3×10^5 cells/well) were incubated with varying concentrations of ascophyllan (0–20 μg/ml) in the growth medium for 24 h at 37 °C; then, the cells were subjected to PCR as described previously [14]. Extracted total RNA was reverse-transcribed into single-strand cDNA using the PrimeScript® 1st strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan). cDNA was amplified by PCR with the following primers: MMP-9, 5′-AGTCCGGCAGACAATCCTTGCA-3′ (sense) and 5′-ATCCACGCGAATGACGCTCTGG-3′ (antisense); MMP-2, 5′-ATCGCTCAGATCCGTGGTG-3′ (sense), 5′-TGTCACGTGGTGTCACTGTCC-3′ (antisense); GADPH, 5′-GGAGCCAAAAGGGTCATCAT-3′ (sense), 5′-GTGATGGCATGGACTGTGGT-3′ (antisense). Amplified products were analyzed in 2% agarose gels containing 0.1 μg/ml ethidium bromide under UV light, and images were captured with Light capture (ATTO Co., Tokyo, Japan).

2.12. Gelatin zymography

Matrix metalloprotease-9 (MMP-9) activity was determined using gelatin zymography as described previously [13]. B16 cells

(1×10^7 cells/ml) were incubated in a serum-free medium with the indicated concentrations of ascophyllan at 37 °C for 24 h. Supernatant aliquots (10 μl) were removed from the total culture medium (200 μl), mixed with 5 μl of sample buffer (62.5 mM Tris–HCl, pH 6.8, containing 10% glycerol, 0.00125% bromophenol blue, 12% SDS) without reducing regent, and subjected to SDS-PAGE at 4 °C in 10% polyacrylamide gels that were copolymerized with 1 mg/ml gelatin. After electrophoresis, the gels were washed twice with rinsing buffer (2.5% Triton X-100, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% NaN_3) for 30 min at room temperature and incubated at 37 °C in the incubation buffer (50 mM Tris–HCl, pH 7.5, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% NaN_3). After 24 h incubation, the gels were stained with 0.5% (w/v) Coomassie brilliant blue R-250 solubilized in 30% methanol and 10% acetic acid for 30 min, and then incubated in a destaining solution (30% methanol and 10% acetic acid). The stained gels were photographed.

2.13. In vivo experimental lung metastasis

The experimental metastasis assay was carried out by the method described previously [15]. B16 cells were harvested, washed with PBS, and resuspended in PBS. The cell suspensions (3×10^5 cells/100 μl) were injected into mice via tail vein. An hour after the injection, mice were randomly divided into two groups (n = 8 for each group). The control group received intraperitoneal injection of PBS, and the treatment group received intraperitoneal injection of ascophyllan (25 mg/kg) in PBS. The treatment was continued daily for 10 days, and then the mice were observed for a further 10 days. The mice were sacrificed on day 21 after the injection of B16 cells, and the number of metastatic B16 colonies on the lung surface was counted under microscopic observation.

2.14. NK cell activity of splenic cells of C57BL/6 mice

Ascophyllan (25 mg/kg/day) was intraperitoneally injected into mice for 3 consecutive days. The control group received PBS alone. Three mice were used for each treatment group. After treatment, spleen cells were prepared from the mice, and their NK cell activity was measured by calcein-release assay using YAC-1 cells as target cells as described previously [11].

2.15. Statistical analysis

All experiments were repeated at least three times. Data were expressed as mean ± standard deviation (S.D.), and data were analyzed by paired Student's *t*-test to evaluate significant differences. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Cytotoxic effect of ascophyllan on B16 melanoma cells

As shown in Fig. 1A, ascophyllan showed no significant cytotoxic effect on B16 melanoma cells up to 1000 μg/ml.

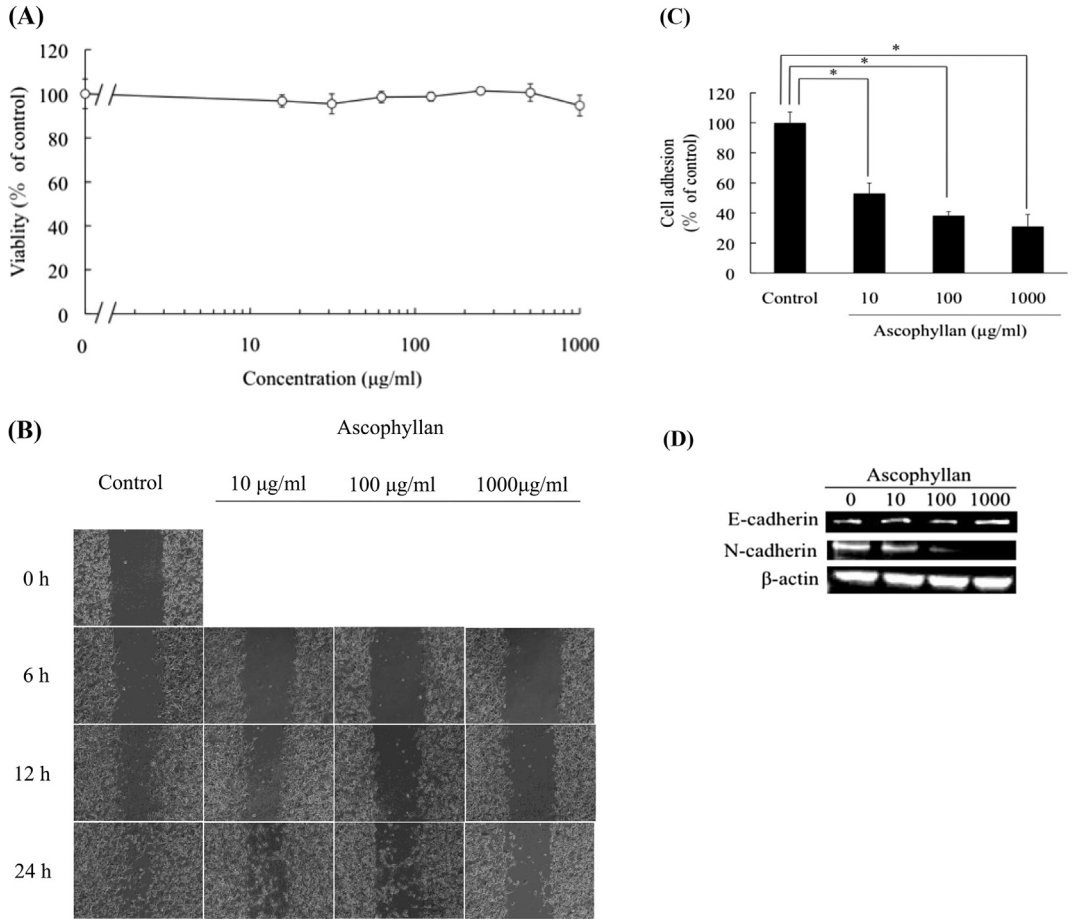

Fig. 1. The effects of ascophyllan on viability (A), migration (B), adhesion (C), and expression levels of E-cadherin and N-cadherin (D) of B16 melanoma cells. (A) Cytotoxicity of ascophyllan was measured by MTT assay. (B) The cells migrated into open area were photographed during incubation in the presence of ascophyllan (0–1000 μg/ml). (C) Extent of cell adhesion on collagen-coated plates is expressed as % of the control. Each value represents the mean ± S.D. (*($p < 0.05$) of triplicate measurements. (D) The expression levels of E-cadherin and N-cadherin were determined by Western blot analysis.

3.2. Effects of ascophyllan on migration of B16 melanoma cells

Effects of ascophyllan on cell migration were investigated by wound healing assay. It was observed that B16 melanoma cells moved gradually from the edge of the wound to open area during 24 h incubation, and ascophyllan inhibited the motility of B16 melanoma cells in a concentration-dependent manner (Fig. 1B).

3.3. Effects of ascophyllan on adhesion of B16 melanoma cells

Ascophyllan inhibited the adhesion of B16 melanoma cells to type I collagen, and the inhibitory effects of ascophyllan were approximately 47%, 62%, and 69% at the concentrations of 10, 100, and 1000 μg/ml, respectively (Fig. 1C).

3.4. Effects of ascophyllan on the expression of E-cadherin and N-cadherin in B16 melanoma cells

As shown in Fig. 1D, ascophyllan reduced N-cadherin levels significantly and slightly increased E-cadherin levels.

3.5. Effects of ascophyllan on invasion of B16 melanoma cells

To investigate whether ascophyllan affects the invasion ability of B16 melanoma cells, an *in vitro* transwell invasion assay system was used. As shown in Fig. 2A, ascophyllan at 5, 10, and 20 μg/ml strongly reduced the number of invaded B16 cells through Matrigel by 57%, 67%, and 78%, respectively.

3.6. Effects of ascophyllan on the activity of MMP-9 in B16 melanoma cells

As shown in Fig. 2B, ascophyllan suppressed expression levels of MMP-9 mRNA in B16 melanoma cells in a concentration-dependent manner, and a significant inhibitory effect of ascophyllan on the secretion of MMP-9 was observed at 20 μg/ml (Fig. 2C). Expression of MMP-2 in B16 melanoma cells was at undetectable levels under our experimental conditions (data not shown).

3.7. Effects of ascophyllan on MAP kinase signaling pathways in B16 melanoma cells

As shown in Fig. 2D, the level of phosphorylated ERK selectively decreased with ascophyllan administration, whereas the levels of phosphorylated p38 and phosphorylated JNK were not significantly changed. Furthermore, the ERK-specific inhibitor significantly reduced the expression level of MMP-9, while inhibitors specific for p38 and JNK showed no inhibitory effect on MMP-9 expression; rather, they slightly enhanced expression (Fig. 2E). These results suggest that ascophyllan down-regulates the expression of MMP-9 through inactivation of ERK signaling pathways.

3.8. Effects of ascophyllan on experimental lung metastasis with B16 melanoma cells

Intraperinoneal administration of ascophyllan (25 mg/kg/day) from day 0 to day 10 after tumor injection resulted in significant decrease in the number of metastatic nodules on the lung surface compared to untreated control group (Fig. 3A and B). Body weights of mice were slightly reduced during ascophyllan-treatment, but they recovered to normal levels at the end of the experiment (data not shown).

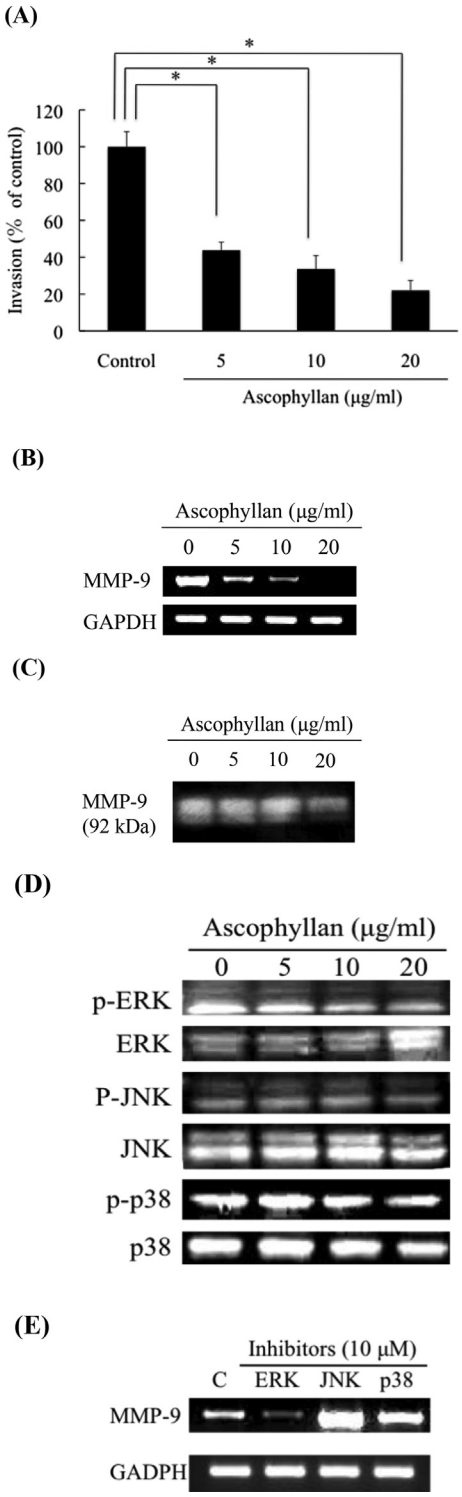

Fig. 2. Analyses of the invasion behavior (A), expression of MMP-9 (B, C), and MAP kinase systems (D, E) in ascophyllan-treated B16 melanoma cells. (A) Data obtained by invasion assay are shown as mean ± S.D. (*$p < 0.05$) of triplicate measurements. (B) The levels of MMP-9 mRNA were determined by PCR. (C) MMP-9 activity in the culture supernatant of the cells treated as described in (B) was analyzed by gelatin zymography. (D) The levels of nonphosphorylated and phosphorylated p38, JNK, and ERK MAP kinases in cells treated for 1.5 h were determined by Western blot analysis. (E) The effects of specific MAP kinase inhibitors on the expression of MMP-9. Cells were incubated with 10 μM of each inhibitor for 24 h and then subjected to PCR analysis.

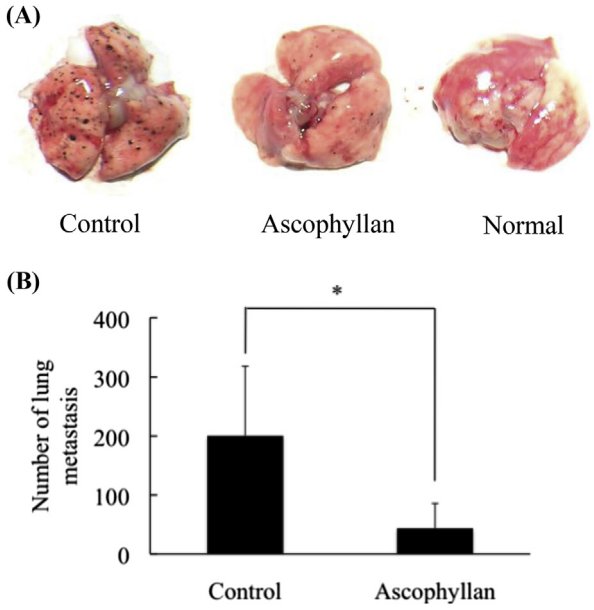

Fig. 3. The effect of ascophyllan on experimental lung metastasis with B16 melanoma cells. (A) B16 cells were injected into the tail vein of C57BL/6 mice on day 0. Starting on day 1, ascophyllan was administered i.p. at 25 mg/kg/day for 13 consecutive days PBS was administered to control mice with the same schedule. At 21 days after injection of B16 cells, lungs were excised, and metastatic nodules on the lung surface were photographed (A) and counted (B). Each value represents the mean ± S.D. ($^*p < 0.05$) of triplicate measurements.

3.9. *Effects of intraperitoneally administered ascophyllan on splenic NK cell activity of C57BL/6 mice*

The spleen weights of mice intraperitoneally administered with ascophyllan at 25 mg/kg for 3 consecutive days were significantly greater than those of control mice injected with PBS (data not shown), and the NK cell activities of splenic cells prepared from the mice injected with ascophyllan were evidently enhanced compared to those of control mice (Fig. 4).

3.10. *Bioactivities of desulfated ascophyllan*

Consistent with previous results [10], NO-inducing activity of desulfated ascophyllan toward RAW264.7 cells was reduced by 75% relative to native ascophyllan (Table 1). Cell-adhesion inhibitory effect of desulfated ascophyllan on B16 cells was also reduced to a similar extent (Table 1).

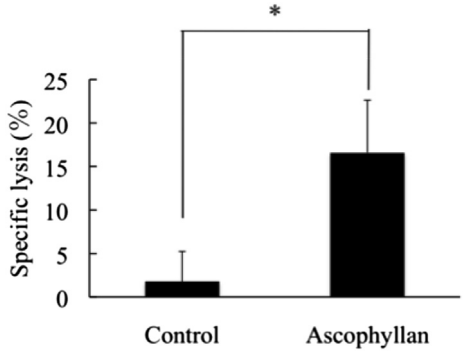

Fig. 4. The effect of intraperitoneal administration of ascophyllan on splenic NK cell activity. After 3 consecutive days of i.p. administration of ascophyllan at 25 mg/kg/day, splenic NK cell activities of the treated mice were measured. PBS was administered to control mice with the same schedule. Data represent mean ± S.D. ($n = 3$; $^*p < 0.05$).

Table 1
Bioactivities of desulfated ascophyllan.

	Sulfate level[a] (%)	Cell adhesion inhibitory activity (%)[b]			NO inducing activity[c] (%)
		Sample conc. (μg/ml)			Sample conc. (μg/ml)
		10	100	1000	100
Desulfated ascophyllan	79.1 ± 0.6	86.0 ± 2.3	88.7 ± 6.0	82.9 ± 4.5	74.8 ± 5.2

[a] Determined by turbidity assay as described previously [10], and expressed % relative to native ascophyllan.
[b] Determined as described in the text and expressed % relative to native ascophyllan.
[c] Determined by Griess assay as described previously [8] and expressed % relative to native ascophyllan.

4. Discussion

Our previous study demonstrated that intraperitoneally administered ascophyllan showed antitumor effects in sarcoma-180 solid tumor-bearing mice [9]. Since increases in splenic NK cell activity and serum levels of TNF-α, IL-12, and IFN-γ were observed in the ascophyllan-treated mice, whereas ascophyllan showed no direct cytotoxic effect on sarcoma-180 cells, it was suggested that ascophyllan exhibited antitumor activity through activation of the host immune system [9].

In the present study, we investigated the effects of ascophyllan on tumor metastasis using B16 melanoma cells. Our *in vitro* analysis revealed that ascophyllan inhibited migration and adhesion of B16 cells in a concentration-dependent manner (Fig. 1B and C). Since no significant cytotoxic effect of ascophyllan on B16 cells was observed up to 1000 μg/ml (Fig. 1A), it may be ruled out that the anti-migration and anti-adhesion properties of ascophyllan are due to cytotoxicity.

Epithelial-mesenchymal transition (EMT) is a critical process in cancer development and metastasis [16,17], and invasion of cancer cells is enabled by EMT, which is often associated with reduction of E-cadherin (epithelial marker) expression and increase of N-cadherin (mesenchymal marker) expression [18]. To investigate whether or not the inhibitory effects of ascophyllan on adhesion and migration of B16 cells is due to the inhibition of EMT, we investigated the expression levels of E-cadherin and N-cadherin in ascophyllan-treated B16 cells. As expected, significant decrease in N-cadherin expression concomitant with partial increase in E-cadherin expression was observed in ascophyllan-treated cells (Fig. 2D).

Tumor cell invasion to the ECM and basement membrane is the most important step in the tumor metastasis processes [19]. The results obtained by transwell invasion assay showed that ascophyllan significantly inhibited B16 cell invasion at a lower concentration range than the concentrations used in migration and adhesion assays, and ascophyllan even at 5 μg/ml suppressed more than 50% of B16 cell invasion (Fig. 2A).

It is well known that MMPs play a pivotal role during tumor metastasis by degrading proteins in the ECM and basement membranes. Among them, MMP-2 and MMP-9 are expressed abundantly in various cancer cells [20,21] and degrade type IV collagen, which is a major component of the basement membrane [22]. Hence, we investigated the effects of ascophyllan on the expression and secretion of MMP-9 in B16 cells. The results obtained by PCR and zymography showed that ascophyllan reduced the expression levels of MMP-9 mRNA and the secretion levels of MMP-9 protein. Expression levels of MMP-2 in B16 cells were undetectable by PCR (data not shown). These results suggest that

ascophyllan is capable of suppressing multiple steps of metastasis through inhibition of tumor cell migration, adhesion, and MMP-9-mediated invasion.

It has been reported that expression and secretion of MMPs are up-regulated by activation of ERK signaling pathways in some cell types [23]. Furthermore, a recent study on amsacrine, a drug with anti-leukemia activity, demonstrated that this drug induced down-regulation of MMP-2 and MMP-9 in U937 cells as well as inactivation of ERK and activation of p38 and JNK MAP kinases [24]. Consistent with these findings, the level of phosphorylated ERK was selectively decreased in ascophyllan-treated B16 cells, while no significant changes in phosphorylated p38 and JNK were observed (Fig. 2D), suggesting that ascophyllan suppressed MMP-9 expression through the inhibition of ERK activation. Involvement of ERK in MMP-9 expression in B16 cells was also confirmed by the fact that a specific ERK inhibitor significantly reduced the expression of MMP-9 (Fig. 2E).

Regarding the structure–activity relationship of ascophyllan, the sulfate level seems to be an important structural element for its bioactivities. Since macrophage-stimulating activity as judged by NO-induction on RAW264.7 cells and the cell-adhesion inhibitory activity of desulfated ascophyllan on B16 cells were reduced to a similar extent as compared to native ascophyllan (Table 1), a common mechanism of action through sulfate groups might be responsible for both activities. Further studies are necessary to clarify how sulfate groups are involved in these bioactivities.

Based on these *in vitro* data, we further evaluated anti-metastatic activity of ascophyllan by *in vivo* experimental lung metastasis assay, in which B16 melanoma cells were injected into tail veins of C57BL/6 mice. Intraperitoneal administration of ascophyllan resulted in significant reduction of metastatic nodules of the lung surface compared to the untreated control group (Fig. 3). Although the exact mechanism of action of ascophyllan in the *in vivo* experimental metastasis model is still unclear, host immune system activation by ascophyllan may be partly responsible for the suppression of B16 melanoma metastasis. NK cells play a critical role in host defense against tumors [25] and are involved in prevention of tumor metastasis [26]. In agreement with our previous studies [9,11], intraperitoneal administration of ascophyllan resulted in significant increase in the splenic NK cell activity in recipient C57BL/6 mice (Fig. 4). Further studies are required to clarify how ascophyllan inhibited B16 metastasis in *in vivo* metastasis model.

In conclusion, we have found for the first time that ascophyllan exhibits anti-metastatic activity through inhibiting tumor cell migration, adhesion, and invasion at a non-cytotoxic concentration range. Ascophyllan may also demonstrate anti-metastatic activity via stimulation of the host immune system.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a Project fund (C34290) to D. Kim from Jeju Center at the Korea Basic Science Institute of Korea.

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2015.01.061.

References

[1] G.L. Nicloson, Organ specific tumor metastasis: role of preferential adhesion, invasion and growth of malignant cell at specific sites, Cancer Metastasis Rev. 7 (1988) 143–188.
[2] M. Gordaliza, Natural products as leads to anticancer drugs, Clin. Transl. Oncol. 9 (2007) 767–776.
[3] K.O.A.L. Lins, D.P. Bezerra, A.P.N.N. Alves, N.M.N. Alencar, M.W. Lima, V.M. Torres, W.R.L. Farias, C. Pessoa, M.O. De Moraes, L.V. Costa-Lotufo, Anti-tumor properties of a sulfated polysaccharide from the red seaweed *Champia feldmannii* (Diaz-Pifferer), J. Appl. Toxicol. 29 (2009) 20–26.
[4] H. Wang, L.C.M. Chiu, V.E.C. Ooi, P.O. Ang Jr., A potent antitumor polysaccharide from the edible brown seaweed *Hydroclathrus clathratus*, Bot. Marin. 53 (2010) 265–274.
[5] H. Itoh, H. Noda, H. Amano, C. Zhuaug, T. Mizuno, H. Ito, Antitumor activity and immunological properties of marine algal polysaccharides, especially fucoidan, prepared from *Sargassum thunbergii* of Phaeophyceae, Anticancer Res. 13 (1993) 2045–2052.
[6] S. Nakayasu, R. Soegima, K. Yamaguchi, T. Oda, Biological activities of fucose-containing polysaccharide ascophyllan isolated from the brown alga *Ascophyllum nodosum*, Biosci. Biotechnol. Biochem. 73 (2009) 961–964.
[7] Z. Jiang, T. Okimura, T. Yokose, Y. Yamasaki, K. Yamaguchi, T. Oda, Effects of sulfated fucan, ascophyllan, from the brown Alga *Ascophyllum nodosum* on various cell lines: a comparative study on ascophyllan and fucoidan, J. Biosci. Bioeng. 110 (2010) 113–117.
[8] Z. Jiang, T. Okimura, K. Yamaguchi, T. Oda, The potent activity of sulfated polysaccharide, ascophyllan, isolated from *Ascophyllum nodosum* to induce nitric oxide and cytokine production from mouse macrophage RAW264.7 cells: comparison between ascophyllan and fucoidan, Nitric Oxide 25 (2011) 407–415.
[9] Z. Jiang, R. Abu, S. Isaka, S. Nakazono, M. Ueno, T. Okimura, K. Yamaguchi, T. Oda, Inhibitory effect of orally-administered sulfated polysaccharide ascophyllan isolated from *Ascophyllum nodosum* on the growth of sarcoma-180 solid tumor in mice, Anticancer Res. 34 (2014) 1663–1672.
[10] Z. Jiang, M. Ueno, T. Nishiguchi, R. Abu, S. Isaka, T. Okimura, K. Yamaguchi, T. Oda, Importance of sulfate groups for the macrophage-stimulating activities of ascophyllan isolated from the brown alga *Ascophyllum nodosum*, Carbohydr. Res. 380 (2013) 124–129.
[11] K. Nakano, D. Kim, Z. Jiang, M. Ueno, T. Okimura, K. Yamaguchi, T. Oda, Immunostimulatory activities of the sulfated polysaccharide ascophyllan from *Ascophyllum nodosum* in *in vivo* and *in vitro* systems, Biosci. Biotechnol. Biochem.. 76 (2012) 1573–1576.
[12] M. Hatai, H. Kato, Y. Yaoi, Inhibition of cell adhesion by proteolytic fragments of type IV collagen, Cell. Struct. Funct. 18 (1993) 53–60.
[13] X.M. Zhang, S.P. Huang, Q. Xu, Quercetin inhibits the invasion of murine melanoma B16-BL6 cells by decreasing pro-MMP-9 *via* the PKC pathway, Cancer Chemother. Pharmacol. 53 (2004) 82–88.
[14] T.W. Chung, H.J. Choi, J.Y. Lee, H.S. Jeong, C.H. Kim, M. Joo, J.Y. Choi, C.W. Han, S.Y. Kim, J.S. Choi, K.T. Ha, Marine algal fucoxanthin inhibits the metastatic potential of cancer cells, Biochem. Biophys. Res. Commun. 439 (2013) 580–585.
[15] D.R. Welch, Technical considerations for studying cancer metastasis *in vivo*, Clin. Exp. Metastasis 15 (1997) 272–306.
[16] C.K. Saito, H. Shirako, T. Takeuchi, Y. Kawakami, Cancer metastasis is accelerated through immunosuppression during snail-induced EMT of cancer cells, Cancer Cell 15 (2009) 195–206.
[17] T.W. Chung, S.K. Moon, Y.C. Chang, J.H. Ko, Y.C. Lee, G. Cho, S.H. Kim, J.G. Kim, C.H. Kim, Novel and therapeutic effect of caffeic acid and caffeic acid phenylester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism, FASEB J. 18 (2004) 1670–1681.
[18] H. Peinado, D. Olmeda, A. Cano, Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype, Nat. Rev. Cancer 7 (2007) 415–428.
[19] S.J. Lee, H. Sakuraia, K. Oshimac, S.H. Kim, I. Saiki, Anti-metastatic and anti-angiogenic activities of a new matrix metalloproteinase inhibitor, TN-6b, Eur. J. Cancer 39 (2003) 1632–1641.
[20] C. Simon, H. Goepfert, D. Boyd, Inhibition of the p38 mitogen-activated protein kinase by SB 203580 blocks PMA-induced Mr 92,000 type IV collagenase secretion and in vitro invasion, Cancer Res. 58 (1998) 1135–1139.
[21] N. Johansson, R. Ala-aho, V. Uitto, R. Grénman, Expression of collagenase-3 (MMP-13) and collagenase-1 (MMP-1) by transformed keratinocytes is dependent on the activity of p38 mitogen-activated protein kinase, J. Cell. Sci. 113 (2000) 227–235.
[22] L.L. Johnson, R. Dyer, D.J. Hupe, Matrix metalloproteinases, Curr. Opin. Chem. Biol. 2 (1998) 466–471.
[23] J.H. Park, H.J. Han, Caveolin-1 plays important role in EGF-induced migration and proliferation of mouse embryonic stem cells: involvement of PI3K/Akt and ERK, Am. J. Physiol. Cell. Physiol. 297 (2009) C935–C944.
[24] W.H. Liu, Y.J. Chen, J.H. Chien, L.S. Chang, Amsacrine suppresses matrix metalloproteinase-2 (MMP-2)/MMP-9 expression in human leukemia cells, J. Cell. Physiol. 229 (2014) 588–598.
[25] J. Wu, L.L. Lanier, Natural killer cells and cancer, Adv. Cancer Res. 90 (2003) 127–156.
[26] Z.K. Ballas, C.M. Buchta, T.R. Rosean, J.W. Heusel, M.R. Shey, Role of NK cell subsets in organ-specific murine melanoma metastasis, PLoS One 8 (2013) e65599.