ISSN : 1225-9918
Journal of Life Science **2010** Vol. 20. No. 12. 1784~1791 DOI : 10.5352/JLS.2010.20.12.1784

Inhibition of Cell Invasion by Ethyl Alcohol Extracts of *Hizikia fusiforme* in AGS Human Gastric Adenocarcinoma Cells

Yung Hyun Choi*

Department of Biochemistry, College of Oriental Medicine, Research Institute of Oriental Medicine, Blue-Bio Industry Regional Innovation Center and Department of Biomaterial Control (BK21 program), Graduate School, Dongeui University, Busan 614-052, Korea

Received October 27, 2010 / Accepted November 2, 2010

In this study, we investigated the effects of ethyl alcohol extracts of *Hizikia fusiforme* (EHF) on the correlation between tightening of tight junctions (TJs) and anti-invasive activity in human gastric adenocarcinoma AGS cells. Inhibitory effects of EHF on cell proliferation, motility, and invasiveness were found to be associated with increased tightness of the TJs, which was demonstrated by an increase in transepithelial electrical resistance. Activities of matrix metalloprotease (MMP)-2 and -9 in AGS cells were dose-dependently inhibited by treatment with EHF, and this was also correlated with a decrease in expression of their mRNA and proteins; however, tissue inhibitor of metalloproteinase (TIMP)-1 and -2 mRNA levels were increased. Additionally, immunoblotting results indicated that EHF repressed the levels of claudin proteins (claudin-1, -3, and -4), major components of TJs that play key roles in control and selectivity of paracellular transport. Furthermore, EHF decreased expression of insulin such as growth factor-1 receptor proteins, while concurrently increasing that of thrombospondin-1 and E-cadherin. In conclusion, these results suggest that EHF treatment may inhibit tumor cell motility and invasion, and therefore act as a dietary source to decrease the risk of cancer metastasis.

Key words : *Hizikia fusiforme*, invasion, tight junction, MMPs

Introduction

Epidemiological studies have indicated that ubiquitous consumption of seaweeds, which are important dietary components in Asian communities, is a protective factor against some types of cancer [4,26]. Previous results showed that extracts of various edible seaweeds exhibited suppressive effects against chemically induced tumorigenesis through suppression at the initiation and promotion phases [10,22,28,29]. Of these, *Hizikia fusiforme*, a kind of brown seaweed, which mainly grows in the temperate seaside areas of the northwest Pacific, including Korea, Japan, and China, has been used widely as a health food for hundreds of years [7,26]. However, the biochemical mechanisms underlying *H. fusiforme*-mediated anti-cancer activity, including anti-metastatic effect, have not yet been clarified.

Tumors are characterized by a high rate of proliferation, turnover, and polarization, with a structurally distinct apical side. Two parameters, movement and invasiveness of cancer cells, are important for their ability to spread or metastasize to other sites [21]. During metastasis, cancer cells undergo disruption of the cell-cell junction and also dysregulation of junctional complex proteins [9,14]. Gastric adenocarcinoma is one of the most common malignancies and the leading cause of cancer deaths worldwide [11]. Despite recent efforts in the search for molecular mechanisms responsible for development of this cancer, the pathways that are important for initiation and development of stomach malignant transformation have remained elusive. In addition, gastric adenocarcinoma cells characteristically lack tight junctions (TJs) [13,23], which is consistent with the idea that loss of cell–cell adhesion is essential for tumor invasion. However, only a relatively small number of papers investigating the function of TJs in human gastric cancer cells have been published.

As part of an ongoing study to isolate chemopreventive or therapeutic compounds from seaweeds, we isolated several extracts of *H. fusiforme* that caused cell growth inhibition in various human cancer cell lines. In this study, [16] L. Zhang, J. Shi, J. Feng, H. Klocker, C. Lee and J. Zhang, Type IV collagenase (matrix metalloproteinase-2 and -9) in prostate cancer, *Prostate Cancer Prostatic Dis.* **7** (2004), pp. 327–332. View Record in Scopus Cited By in Scopus (18) we ex-

*Corresponding author
Tel : +82-51-850-7413, Fax : +82-51-853-4036
E-mail : choiyh@deu.ac.kr

amined the effects of ethyl alcohol extracts of *H. fusiforme* (EHF) on invasive parameters, including cell motility, matrigel membrane invasion, zymography for matrix metalloproteases (MMP) activities, and gene expression for TJ- and metastasis-associated proteins, in human gastric adenocarcinoma AGS cells.

Materials and Methods

Preparation of ethyl alcohol extracts of *H. fusiforme*, cell culture, and cell viability

Ethyl alcohol extract of *H. fusiforme* was prepared as previously described [12]. Briefly, dried *H. fusiforme* were extracted in 80% ethyl alcohol at a ratio of 10 ml/g for 24 hr in a refrigerator at 4°C. Extracts (ethyl alcohol extracts of *H. fusiforme*, EHF) were vacuum-filtered through a Whatman #2 filter and lyophilized, yielding a dried residue, and then kept at -80°C. The powder was dissolved in dimethyl sulfoxide (DMSO) as a stock solution at 100 μg/ml and stored in aliquots at −20°C. The human gastric adenocarcinoma AGS cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in DMEM medium (Invitrogen Corp., Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO-BRL, Gaithersburg, MD), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO-BRL) at 37°C in a 5% CO_2 incubator. For the cell viability study, AGS cells were grown to 70% confluence and treated with EHF. Control cells were supplemented with complete media containing 0.1% DMSO (vehicle control). Following treatment, cell viability was determined by MTT [3-(4,5-Dimethyl-2 thiazolyl)-2,5-diphnyl-2H-tetrazolium bromide, MTT, Sigma, St Louis, MO] assay, which is based on conversion of MTT to MTT-formazan by mitochondria.

Cell motility assay

AGS cells were grown to confluency on 30-mm cell culture dishes coated with rat tail collagen (20 μg/ml, BD Biosciences, Bedford, MA). Confluent cells were wounded by scraping with a pipette tip. After wounding, cultures were washed twice with PBS and control cells were exposed to medium alone. Cells were incubated with 1% FBS-containing medium supplemented with 300 μg/ml of EHF for 48 hr. Wound closure of cells was observed and photographed under the microscope at 40X magnification. Culture treatments were repeated twice and each sample was assayed in triplicate.

Measurement of transepithelial electrical resistance

Transepithelial electrical resistance (TER) was measured using an EVOM Epithelial Tissue Voltohmmeter (World Precision Instruments, FL) equipped with a pair of STX-2 chopstick electrodes. Briefly, AGS cells were seeded into the 8.0 μm pore size insert (upper chamber) of a Transwell® (Corning Costar Corp., NY) and allowed to reach full confluence, after which fresh medium was replaced for further experiments. Inserts without cells, inserts with cells in medium, and inserts with cells with EHF were treated for 48 hr. Electrodes were placed at the upper and lower chambers, and resistance was measured with the voltohmmeter.

In vitro invasiveness assay

Matrigel invasion assays were used to assess the ability of AGS cells to penetrate the extracellular matrix in the presence or absence of EHF, as previously described [5]. Briefly, cells were exposed to EHF for 6 h, and treated cells (50,000) were then plated onto the apical side of Matrigel-coated filters in serum-free medium containing either DMSO or EHF. Medium containing 20% FBS was placed in the basolateral chamber to function as a chemoattractant. After 48 hr, cells on the apical side were wiped off with a Q-tip. Cells on the bottom of the filter were stained with hematoxylin (Sigma) and counted (three fields of each triplicate filter) with an inverted microscope.

RNA extraction and reverse transcription-PCR

Total RNA was prepared using an RNeasy kit (Qiagen, La Jolla, CA) and primed with random hexamers for synthesis of complementary DNA using AMV reverse transcriptase (Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed in a Mastercycler (Eppendorf, Hamburg, Germany) with the primers indicated in Table 1. Amplification products obtained by PCR were separated on a 1% agarose gel and visualized by ethidium bromide (EtBr) staining.

Protein extraction and Western blot analysis

Total cell lysates were prepared in an extraction buffer [25 mM Tris-Cl (pH 7.5), 250 mM NaCl, 5 mM ethylendiaminetetra acetic acid, 1% nonidet P-40, 0.1 mM sodium

Table 1. Sequences of the primer pairs employed in the RT-PCR reactions

Name		Sequence of primers
TIMP-1	sense	5′-TGG-GGA-CAC-CAG-AAG-TCA-AC-3′
	antisence	5′-TTT-TCA-GAG-CCT-TGG-AGG-AG-3′
TIMP-2	sense	5′-GTC-AGT-GAG-AAG-GAA-GTG-GAC-TCT-3′
	antisence	5′-ATG-TTC-TTC-TCT-GTG-ACC-CAG-TC-3′
MMP-2	sense	5′-GGC-CCT-GTC-ACT-CCT-GAG-AT-3′
	antisence	5′-GGC-ATC-CAG-GTT-ATC-GGG-GA-3′
MMP-9	sense	5′-CGG-AGC-ACG-GAG-ACG-GGT-AT-3′
	antisence	5′-TGA-AGG-GGA-AGA-CGC-ACA-GC-3′
GAPDH	sence	5′-CGG-AGT-CAA-CGG-ATT-TGG-TCG-TAT-3′
	antisence	5′-AGC-CTT-CTC-CAT-GGT-GGT-GAA-GAC-3′

orthovanadate, 2 μg/ml leupeptin, and 100 μg/ml phenylmethylsulfonyl fluoride]. Protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad, Laboratories, Hercules, CA). For Western blot analysis, proteins were separated by 8~13% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then electrotransferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH). Membranes were blocked with 5% skim milk for 1 hr, and membranes then were subjected to immunoblot analysis with the desired antibodies. Proteins were then visualized by the enhanced chemiluminescence (ECL) method, according to the recommended procedure (Amersham Co.). Primary antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and Calbiochem (Cambridge, MA). Peroxidase-labeled donkey anti-rabbit immunoglobulin and peroxidase-labeled sheep anti-mouse immunoglobulin were purchased from Amersham Co.

Gelatin zymographic analysis of secreted MMPs

Following incubation with EHF, cell culture supernatants were collected and centrifuged at 400x *g* for 5 min. Cell-free supernatant was mixed with 2X sample buffer (Invitrogen) and zymography was performed using precast gels (10% polyacrylamide and 0.1% gelatin). Following electrophoresis, gels were washed twice at room temperature for 30 min in 2.5% Triton X-100, and subsequently washed in buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, and 0.02% NaN_3 at pH 7.5, and incubated in this buffer at 37°C for 24 hr. Thereafter, gels were stained with 0.5% (w/v) Coomassie brilliant blue G-250 (Bio-Rad) for 1 hr, then lightly destained in methanol:acetic acid:water (3:1:6). Clear bands appear on the Coomassie stained blue background in the areas of gelatinolytic activity. Gels were scanned and images were processed for extraction of the blue channel signal, converting it to black and white, and inverting it for quantification of gelatinolytic activities from the integrated optical density.

Statistical analysis

All data are presented as mean±SD. Significant differences among the groups were determined using the unpaired Student's t-test. A value of $*p<0.05$ was accepted as an indication of statistical significance. All of the figures shown in this article were obtained from at least three independent experiments.

Results

Inhibition of cell growth and motility by EHF in AGS cells

To investigate the effects of EHF on cell viability, AGS cells were treated with various concentrations of EHF and subjected to MTT assay. We found that EHF had strong inhibitory effects on cell proliferation in a dose-dependent manner (Fig. 1A). When compared with the controls, treatment with 300 μg/ml and 500 μg/ml of HEF resulted in approximately 27% and 40% inhibition of cell growth, respectively. To determine whether or not EHF inhibits motility of AGS cells, wound healing experiments were performed. Results demonstrated that 300 μg/ml of EHF delayed the motility of AGS cells compared to that of control cells (Fig. 1B).

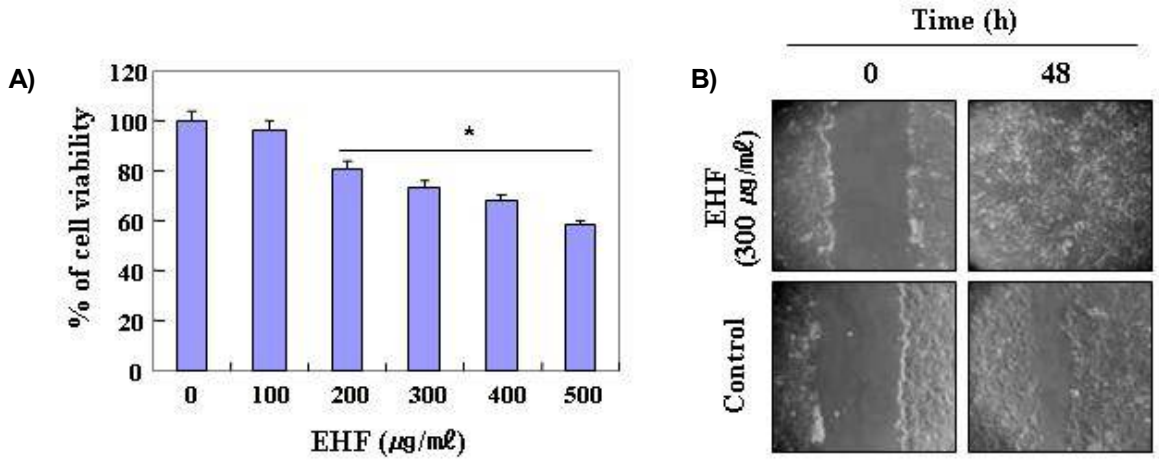

Fig. 1. Effects of EHF on cell viability and motility of AGS cells. (A) AGS cells were plated at a concentration of $1x10^5$ cells per 60-mm plate and then incubated for 24 hr, after which they were treated with various concentrations of EHF for 48 hr; cell viability was estimated by MTT assay. Each point represents the mean±SD of three independent experiments. Significance was determined using a Student's *t*-test (*p<0.05 versus untreated control). (B) Cells were grown to confluency on 30-mm cell culture dishes; a scratch was then made though the cell layer using a pipette tip. After washing with PBS, serum free media (to prevent cell proliferation) containing either vehicle or 300 μg/ml of EHF was added for 48 hr. Photographs of the wounded area were taken immediately after the scratch was made for evaluation of cell movement into the wounded area.

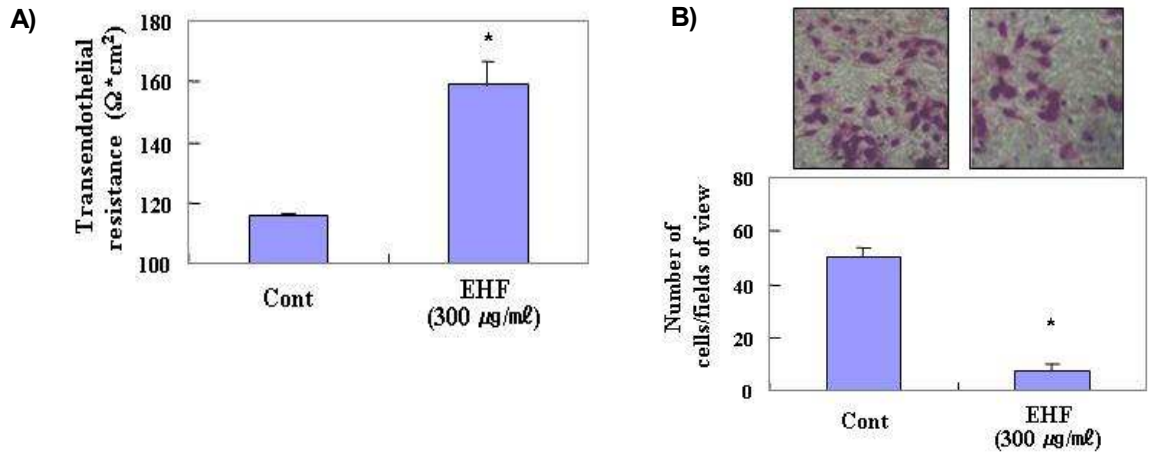

Fig. 2. Increased TER values and decreased invasion by EHF in AGS cells. (A) AGS cells were treated with or without 300 μg/ml of EHF for 48 hr and TER values were measured as described in the materials and methods section. Results are shown as the mean±SD of three independent experiments. Significance was determined using a Student's *t*-test (*, p<0.05 versus untreated control). (B) Cells pretreated with or without 300 μg/ml of EHF for 6 hr were plated onto the apical side of matrigel coated filters in serum-free medium containing either vehicle or EHF. Medium containing 20% FBS was placed in the basolateral chamber to act as a chemoattractant. After 48 hr, cells on the apical side were wiped off using a Q-tip. Next, cells on the bottom of the filter were stained using hematoxylin and Eosin Y, and then counted. Data are shown as the mean of triplicate samples and represent invasive cell numbers compared with those of control cells. Significance was determined using a Student's t-test (*, p<0.05 versus untreated control).

Increased TER values and decreased cell invasion by EHF in AGS cells

TER (a measure of tight junction formation) [21] values were measured for examination of the relationship between tightening of TJs and invasive activity of AGS cells treated with EHF. As shown in Fig. 2A, incubation of AGS cells with EHF increased their TER levels, suggesting that EHF increased TJ function in AGS cells (approximately 1.5-fold

by 300 μg/ml of EHF). Using a Boyden chamber invasion assay, we next examined the question of whether or not EHF decreases the activity of cell invasion. As shown in Fig. 2B, treatment of cells with 300 μg/ml of EHF reduced cell invasion through the Matrigel chamber (approximately 0.16-fold by 300 μg/ml of EHF). These results indicated that up-regulation of TER by EHF was associated with inhibition of cell invasion in AGS cells.

Modulation of expression levels of TJ- and metastasis-related proteins by EHF in AGS cells

To elucidate the question of whether or not EHF reduces levels of TJ components and TJ regulators, AGS cells were treated with EHF and levels of their key proteins, such as claudins, E-cadeherin, IGF-1R, and TSP-1, were examined using Western blot analyses. As shown in Fig. 3, EHF treatment time-dependently decreased levels of claudin proteins (claudin-1, -3, and -4), the most important components of the TJ [9,20], suggesting that this modulation contributed to TJ tightening. EHF treatment also decreased protein levels of IGF-1R, a promoter of tumor invasion and metastasis in various human cancers [3,17], but increased levels of E-cadherin, an adherent junction protein that regulates TJ formation [2]. In addition, EHF treatment time-dependently increased expression of TSP-1, which limits tumor growth [6,31].

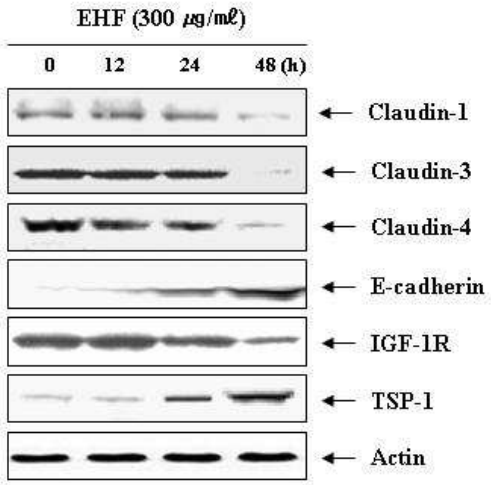

Fig. 3. Effects of EHF on expression of claudins, E-cadherin, IGF-1R, and TSP-1 proteins in AGS cells. Cells were treated with 300 μg/ml of HEF for the indicated times. Cells were lysed and equal amounts of cell lysate were separated by electrophoresis on SDS - polyacrylamide gels and transferred to nitrocellulose membranes. Next, the membranes were probed with the indicated antibodies, and the proteins were visualized using an ECL detection system. Actin was used as an internal control.

Inhibition of activities and expression of MMP-2 and -9 by EHF in AGS cells

Because cell migration plays an important role in the metastasis process and since invasion of the basement membrane is primarily mediated by gelatinase MMPs [8,25], we tested the effects of EHF on TIMPs and MMPs mRNA levels

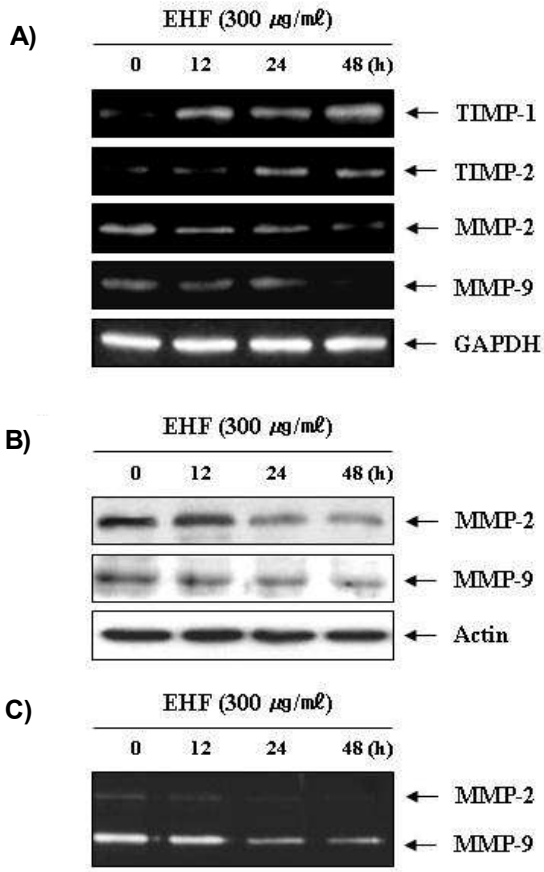

Fig. 4. Effects of EHF on expression and activity of TIMPs and MMPs in AGS cells. (A) AGS cells were grown under the same conditions, as shown in Fig. 3, and total RNAs were isolated and reverse-transcribed. Resulting cDNAs were then subjected to PCR and the reaction products were subjected to electrophoresis in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as an internal control. (B) Cells were lysed and proteins were separated by electrophoresis on SDS-polyacrylamide gels. Western blotting was then performed using anti-MMP-2 and anti-MMP-9 antibodies, and an ECL detection system. Actin was used as an internal control. (B) Following incubation with 300 μg/ml of EHF for the indicated times, the medium was collected, and the activities of MMP-2 and -9 were measured by zymography.

by RT-PCR. As shown in Fig. 4A, EHF treatment increased TIMP-1 and -2 mRNA levels, but decreased MMP-2 and -9 levels in a time-dependent manner. We next investigated the effects of EHF on protein levels and activities of MMPs using Western blot and gelatin zymography. Data indicated that activities of MMP-2 and -9 in AGS cells were significantly decreased by EHF treatment in a dose-dependent manner, which was connected with a concurrent down-regulation of their mRNA and protein levels (Fig. 4). These results suggest that the anti-invasive effect of EHF is associated with increased TIMP-1 and -2 levels, as well as inhibition of MMP-2 and -9 mRNA, protein expression, and activity in AGS cells.

Discussion

Cell migration and invasion are critical steps in metastasis. Therefore, inhibition of tumor cell migration and invasion are important mechanisms in the anti-metastatic effects of anti-cancer drugs. In the present study, we investigated the effects of ethyl alcohol extract of *H. fusiforme* (EHF) on cell migration and invasion of gastric cancer cells using an AGS cell line. We found that EHF markedly inhibited cell motility and invasive activity by tightening TJs and decreasing MMP activity.

Changes in permeability properties and loss of cell polarity are hallmarks of epithelial cell tumorigenesis. Modulation of TJs, which are structures critical for maintenance of these functions in epithelial cells, in a number of epithelial cancers, including gastric adenocarcinoma, has been demonstrated [1,13,20,21,23]. Thus, TJ disruption and dysregulation of its composite proteins play critical roles in cancer progression, invasion, and metastasis, particularly epithelial cancers [6,27]. For example, Soler *et al.* [24] first demonstrated that TER of colon carcinoma tissue was significantly lower than that of normal colon tissues but showed higher transepithelial paracellular permeability, which confirmed the loss of TJs. In this study, EHF treatment increased the TER of AGS cells in a concentration-dependent manner, which was associated with lower cell motility and invasiveness (Fig. 1 and 2). Since TJ leakiness is associated with cancer progression, TJ tightening may have anti-cancer activity [20,21]. Thus, our results indicate that the anti-invasive activity of HEF may be due, in part, to its ability to enhance TJ activity.

Many components of TJs have recently been identified. Among these, the members of claudin family, which are transmembrane proteins with extracellular domains, interact with other claudins associated with adjacent cells for regulation of paracellular permeability [9,14]. Emerging evidence indicates that TJ disruption and dysregulation of TJ protein are early events in cancer cell invasion and metastasis [1,6,27,31]. These observations also indicate that claudins are dysregulated in many types of cancers and that the nature of the dysregulation is highly type-specific for given cancers. In addition, these data suggest that claudin proteins may prove to be useful biomarkers for detection and diagnosis of certain cancers. In the present study, EHF treatment markedly down-regulated claudin levels (-1, -3, and -4), indicating that down-regulation of claudin expression by EHF relates to increased TJ tightening.

In addition, we measured the effects of EHF on metastasis and TJ proteins such as E-cadherin, IGF-1R, and TSP-1. E-cadherin is an adherent junction protein and type I transmembrane glycoprotein, and low expression is associated with cancer invasion and metastasis [2]. Expression of TSP-1 is decreased in tumor cells, resulting in suppression of tumor growth, and high TSP-1 expression is inversely correlated with invasiveness and lymph node metastasis [15,30]. However, IGF-1R is highly expressed in many types of human cancer and promotes tumor invasion and metastasis *in vivo* [3,17]. In the present study, EHF markedly increased levels of E-cadherin and TSP-1, but decreased IGF-1R in a time-dependent manner (Fig. 3), indicating a potential mechanism for change of TER values and TJ permeability.

MMPs are important proteolytic enzymes during organ development and tissue regeneration; however, they also play important roles in cancer invasion and metastasis. In particular, MMP-2 and -9 play important roles in tumor invasion and angiogenesis; therefore, tumor metastasis can be inhibited by blocking MMPs synthesis and activity. MMP-2 and -9 in particular play important roles in tumor invasion and angiogenesis, and tumor metastasis can be inhibited by blocking MMPs synthesis and activity [8,25]. MMPs activity is tightly controlled by transcriptional activation, by a complex proteolytic activation cascade, and by an endogenous system of TIMPs. TIMPs inhibit MMPs by formation of 1:1 stoichiometric complexes for regulation of matrix turnover [18,19]. As shown in Fig. 4, EHF markedly inhibited the levels of MMP-2 and -9 mRNA, as well as protein levels and expression of TIMP1- and -2 mRNA. Data suggest that the anti-invasive activity of HEF in AGS cells was associated with inhibition of MMP-2 and -9 activities through elevation

of TIMPs expression, and increased TIMPs/MMPs ratio is a key factor in regulation of the anti-metastatic process.

Although this study will require validation, we tentatively suggest that EHF inhibits cell motility and invasion in AGS cells through an influence on expression of several important tumor invasiveness and metastasis-related genes, and MMPs activity. Taken together, the data indicate that *H. fusiforme* may be a promising new dietary source for decreasing the risk of cancer cell metastasis.

Acknowledgment

This research was a part of the project funded by the Ministry of Land, Transport and Maritime Affairs, Korea.

References

1. Anderson, J. M. 2006. Molecular structure of tight junctions and their role in epithelial transport. *News Physiol. Sci.* **16**, 126-130.
2. Battle, E., E. Sancho, C. Franci, D. Dominguez, M. Monfar, J. Braulidia, and A. G. Herreros. 2001. The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumor cells. *Nat. Cell Biol.* **2**,84-89.
3. Brodt, P., L. Fallavollita, A. M. Khatib, A. A. Samini, and D. Zhang. 2001. Cooperative regulation of the invasive and metastatic phenotypes by different domains of the type I insulin-like growth factor receptor β subunit. *J. Biol. Chem.* **276**, 33608-33615.
4. Carper, J. 1989. Seaweed, or Kelp. In The Food Pharmacy. pp. 264-268, Bantan Books, New York.
5. Choi, W. Y., C. Y. Jin, M. H. Han, G. Y. Kim, N. D. Kim, W. H. Lee, S. K. Kim, and Y. H. Choi. 2009. Sanguinarine sensitizes human gastric adenocarcinoma AGS cells to TRAIL-mediated apoptosis via down-regulation of AKT and activation of caspase-3. *Anticancer Res.* **29**, 4457-4465.
6. de Oliveira, S. S., I. M. de Oliveira, W. de Souza, and J. A. Morgado-Diaz. 2005. Claudins upregulation in human colorectal cancer. *FEBS Lett.* **579**, 6179-6185.
7. Dobashi, K., T. Nishino, M. Fujihara, and T. Nagumo. 1989. Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed *Hizikia fusiforme*. *Carbohydr. Res.* **194**, 315-320.
8. Egeblad, M. and Z. Werb. 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* **2**, 161-174.
9. Hewitt, K. J., R. Agarwal, and P. J. Morin. 2006. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer* **6**, 186-193.
10. Hiqashi-Okaj, K., S. Otani, and Y. Okai. 1999. Potent suppressive effect of a Japanese edible seaweed, *Enteromorpha prolifera* (Sujiao-nori) on initiation and promotion phases of chemically induced mouse skin tumorigenesis. *Cancer Lett.* **140**, 21-25.
11. Hyatt, B. J., P. E. Paull, and W. Wassef. 2009. Gastric oncology: an update. *Curr. Opin. Gastroenterol.* **25**, 570-578.
12. Kim, T. Y., C. Y. Jin, G. Y. Kim, I. W. Choi Y. K. Jeong, T. J. Nam, S. K. Kim, and Y. H. Choi. 2009. Ethyl alcohol extracts of *Hizikia fusiforme* sensitize AGS human gastric adenocarcinoma cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis. *J. Med. Food* **12**, 782-787.
13. Kimura, Y., H. Shiozaki, M. Hirao, Y. Maeno, Y. Doki, M. Inoue, T. Monden, Y. Ando-Akatsuka, M. Furuse, S. Tsukita, and E. Monden. 1997. Expression of occludin, tight-junction-associated protein, in human digestive tract. *Am. J. Pathol.* **151**, 45-54.
14. Kominsky, S. L., P. Argani, D. Korz, E. Evron, V. Raman, E. Garrett, A. Rein, G. Sauter, O. P. Kallioniemi, and S. Sukumar. 2003. Loss of the tight junction protein Claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast. *Oncogene* **22**, 2021-2033.
15. Lawler, J. 2002. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *J. Cell Mol. Med.* **6**, 1-12.
16. Li, B., X. J. Wei, Y. L. Sun, and S. Y. Xu. 2006. Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed, *Hizikia fusiforme*. *Carbohydr. Res.* **341**, 1135-1146.
17. Long, L., R. Rubin, and P. Brodt. 1998. Enhanced invasion and liver colonization by lung carcinoma cells overexpressing the type 1 insulin-like growth factor receptor. *Exp. Cell Res.* **238**, 116-121.
18. Malemud, C. J. 2006. Matrix metalloproteinases (MMPs) in health and disease: an overview. *Front Biosci.* **11**, 1696-1701.
19. Mannello, F., G. Tonti, and S. Papa. 2005. Matrix metalloproteinase inhibitors as anticancer therapeutics. *Curr. Cancer Drug Targets* **5**, 285-298.
20. Morin, P. 2005. Claudin proteins in human cancer: promising new targets for diagnosis and therapy. *Cancer Res.* **65**, 9603-9606.
21. Mullin, J. M., N. Agostino, E. Rendon-Huerta, and J. J. Thornton. 2005. Keynote review: epithelial and endothelial barriers in human disease. *Drug Discov. Today* **10**, 395-408.
22. Okai, Y., K. Higashi-Okai, S. Nakamura, Y. Yano, and S. Otani. 1994. Suppressive effects of the extracts of Japanese edible seaweeds on mutagen-induced *umu* C gene expression in *Salmonella typhhimurium* (TA 1535/pSK 1002) and tumor promotor-dependent ornithine decarboxylase induction in BALB/c 3T3 fibroblast cells. *Cancer Lett.* **87**, 8725-8732.
23. Ouban, A. and A. A. Ahmed. 2010. Claudins in human cancer: a review. *Histol. Histopathol.* **25**, 83-90.
24. Soler, A. P., K. V. Laughlin, and J. M. Mullin. 1993. Effects of epidermal growth factor versus phorbol ester on kidney epithelial (LLC-PK1) tight junction permeability and cell

division. *Exp. Cell Res.* **207**, 398-406.
25. Sternlicht, M. D. and Z. Werb. 2001. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.* **17**, 463-516.
26. Tokudome, S., K. Kuriki, and M. A. Moore. 2001. Seaweed and cancerprevention. *Jpn. J. Cancer Res.* **92**, 1008-1009.
27. van Itallie, C. M. and J. M. Anderson. 2006. Claudins and epithelial paracellular transport. *Annu. Rev. Physiol* **68**, 403-429.
28. Yamamoto, I., H. Maruyama, and M. Moriguchi. 1987. The effect of dietary seaweeds on 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis in rats. *Cancer Lett.* **35**, 109-118.
29. Yamamoto, I. and H. Maruyama. 1985. Effect of dietary seaweed preparations on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. *Cancer Lett.* **26**, 241-251.
30. Yao, L., Y. L. Zhao, S. Itoh, S. Wada, L. Yue, and I. Furuta. 2000. Thrombospondin-1 expression in oral squamous cell carcinomas: correlations with tumor vascularity, clinicopathological features and survival. *Oral Oncol.* **36**, 539-544.
31. Yokoyama, K., N. Kamata, E. Hayashi, T. Hoteiya, N. Ueda, R. Fujimoto, and M. Ngayama. 2001. Reverse correlation of E-cadherin and Snail expression in oral squamous cell carcinoma cells *in vitro*. *Oral Oncol.* **37**, 65-71.

초록 : AGS 인체 위암세포에서 톳 에탄올 추출물에 의한 침윤성 저해

최영현*
(동의대학교 한의과대학 생화학교실, 한의학연구소, 블루바이오 소재개발센터 및 대학원 바이오물질제어학과)

본 연구에서는 AGS 인체 위암세포에서 톳 에탄올 추출물(EHF)의 항침윤성과 tight junctions (TJs)의 tightening과의 관계를 조사하였다. EHF에 의한 AGS 위암세포의 증식억제와 연관된 세포이동성 및 침윤성의 감소는 transepithelial electrical resistance의 증가와 연계된 Js의 tightness 증가와 연관성이 있었다. EHF는 matrix metalloprotease (MMP)-2 및 -9의 활성을 억제하였으며, 이는 MMPs의 mRNA 및 단백질 발현 감소에 의한 것이었으나 tissue inhibitor of metalloproteinase (TIMP)-1 및 -2의 mRNA 발현은 증가시켰다. 또한 EHF는 TJs의 주요 조절인자인 claudin family 단백질들(claudin-1, -3 및 -4)의 발현을 감소시켰으며, insulin like growth factor-1 receptor 단백질은 감소된 반면 thrombospondin-1 및 E-cadherin의 발현은 증가되었다. 본 연구의 결과는 톳 추출물이 암의 전이를 효과적으로 억제하는 효능이 있음을 보여주는 결과이다.