ISSN : 1225-9918
Journal of Life Science 2011 Vol. 21. No. 11. 1501~1510 DOI : http://dx.doi.org/10.5352/JLS.2011.21.11.1501

Effects of Seaweeds on Matrix Metalloproteinases Derived from Normal Human Dermal Fibroblasts and Human Fibrosarcoma Cells

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Received August 18, 2011 / Revised November 14, 2011 / Accepted November 17, 2011

In recent years novel potential pharmocological candidates have been looked for in animal, seaweed, sponge, fungi and marine bacteria resources. In this study, matrix metalloproteinases (MMPs) that play an important role in metastasis, arthritis, chronic inflammation and wrinkle formation were used as target enzymes to screen therapeutic agents. The inhibitory effects of several marine algae including green algae (5 species), red algae (18 species) and brown algae (4 species) methanolic extracts on MMPs were investigated in human dermal fibroblasts and human fibrosarcoma cell line (HT1080 cells) using gelatin zymography. In human dermal fibroblasts, the inhibition of MMP-2 was observed in *Laurencia okamurae, Polysiphonia japonica, Grateloupia lanceolate and Sinkoraena lancifolia* of red algae. In contrast, MMP-2 activation was enhanced in *Enteromorpha compressa and E. linza* of green algae, and *Peltaronia bighamiae and Sargassum thunbergii* of brown algae. In human fibrosarcoma cells, MMP-9 activation was decreased in the presence of *S. thunbergii* of brown algae, *Polysiphonia japonica* in red algae and *E. compressa and E. linza* of green algae. The interesting finding is that *E. compressa and E. linza* of green algae, and *S. thunbergii* of brown algae exhibited a positive effect on MMP-2 in normal cells, but a negative effect on MMP-9 in cancer cell lines. These results suggest that *E. compressa and E. linza* of green algae, and *S. thunbergii* of brown algae contain potential therapeutic ingredients for cancer treatment.

Key words : Matrix metalloproteinase, seaweeds, gelatin zymography; HT1080 cells, human dermal fibroblasts

Introduction

MMPs have been reported to be involved in the degradation of extracellular matrix in process of several diseases such as chronic inflammation, periodontal disease, chronic obstructive pulmonary disease, arthritis, and cancer metastasis, and also in the aging process, such as skin wrinkling [4,6,20,22,25]. MMPs are a family of secreted or transmembrane zinc-endopeptidases that are capable of digesting extracellular matrix (ECM), such as fibrillar and non-fibrillar collagens, fibronectin, laminin, elastin and basement membrane glycoproteins under physiological conditions [3]. The MMPs in tumor growth and invasion have been identified revealed by studies with either MMP-2 knockout mice having reduced melanoma tumor progression and angiogenesis [13]. In recent years it was reported that ultraviolet B-induced enhancement of gelatinase activity in the skin contributes to wrinkle formation through the destruction of basement membrane structure and dermal collagen and thus topical application of inhibitors of MMPs may be an effective way to overcome this problem [12]. Therefore, inhibition of MMP activity in the extracellular space has been extensively studied as an approach to inhibit metastasis and wrinkle formation. At present, several MMP inhibitors are under clinical trials and most of these MMP inhibitors are synthetic peptides, chemically modified tetracyclines, bisphosphonates or compounds isolated from natural sources. However, most of these drugs are reported to exert side effects such as, musculoskeletal pain in tendons and joints [18]. Until now, MMP inhibitors that are capable of applying to clinical trials have not been reported from marine resources. Therefore, we took an effort to screen marine algal species possessing MMP inhibitory compounds. Based on our screening studies, the extracts of 3 marine algae present in the subtidal regions of Jeju Island in Korea had an excellent efficacy to inhibit MMP

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activities. *Enteromorpha,* an edible green alga, produces a dizzying array of bioactive compounds that have proven to be useful in the treatment of certain bacterial & viral infections, cancer and inflammation [10,19]. *Enteromorpha compressa,* a marine green algal species, grows extensively in North coastal Andhra Pradesh. Besides its nutritional importance it has also been identified as source of anti-anaphylactic compounds [25]. Green algae of the genus Ulva (syn. Enteromorpha) are common, green macroalgae found throughout the world in the upper intertidal zone of seashores and as a fouling organism on a variety of man-made structures including ships' hulls [2]. *Sargassum fulvellum* known as Hejo is used as a food additive , and recorded many uses in treatment of lump, dropsy, swollen and painful scrotum, and urination problems with no side effects. [5,14].

Therefore, in the present work we carried out a detailed study to investigate the inhibitory effects of several algae on MMPs secreted from normal human cells and human fibrosarcoma cells.

Materials and Methods

Chemicals

Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, penicillin/streptomycin/ amphotericin (10,000 U/ml, 10,000 μg/ml, and 2,500 μg/ml, respectively) and fetal bovine serum (FBS) were obtained from Gibco BRL (Grand island, NY, USA). HT1080 cells were obtained from American Type of Culture Collection (Manassas, VA, USA). Human dermal fibroblasts (HDFs) were kindly donated by LG HG & CM Research Institute (Daejeon, Korea). MTT reagent, gelatin and PMA (phorbol 12-myristate 13-acetate) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other materials required for culturing of cells were purchased from Gibco BRL.

Preparation of seaweeds extract used in this study

The seaweeds used in this study are as follows: *Pterocladiella capillacea, Scinaia okamurae, Laurencia okamurae, Grateloupia lanceolate, Chondrophycus undulates, Gracilaria textorii, Polysiphonia japonica, Halymenia dilatata, Gloiopeltis furcata, Glacilaria verrucosa, Chondria cssicaulis, Chondrus crispus, Prionitis cornea, Ecklonia cava, Martensia denticulate, Lomantaria catenata, Acrosorium flabellatum, Sinkoraena lancifolia, Geldium amansii, Shizymenia dubyi, G. elliptica, Capopeltis affinis, G. filicina, Pterocladiella capillacea, Scinaia okamurae, G. lanceolate* of red algae, *Codium contractum, Hizikia fusiform, Enteromorpha compressa, Ulva conglobata , U. pertusa, Monostroma nitidum* of green algae, *Myagropsis myagroides, Sargassum horneri, Ishige sinicola, Pachydictyon coriaceum, Idhige okamurai, S. siliquastrum, S. coreanum, Peltaronia bighamiae, S. thunbergii, S. thunbergii, Padina arborescen, S. fulvellum, Myagropsis myagroides* of brown algae. They were collected along Jeju Island coast of Korea. Fresh seaweeds were washed three times with tap water to remove salt, epiphytes and sand attached to the surface of the samples and stored at -20℃. The frozen samples were lyophilized and homogenized using a grinder before extraction. The dried seaweeds powder (1 kg) was extracted three times with MeOH (1:10 w:v) at room temperature for 24 hr while stirring, filtered using Whatman No. 41 and evaporated in vacuum. For the bioassay, the fractions were dissolved in dimethylsulfoxide (DMSO) and distilled water according their solubility, respectively.

Cell culture

Cell lines were separately grown as monolayers in T-75 tissue culture flasks (Nunc, Denmark) at 5% CO_2 and 37°C humidified atmosphere using appropriate media supplemented with 10% fetal bovine serum, 2 mM glutamine and 100 μg/ml penicillin-streptomycin. DMEM was used as the culture medium for human fibrosarcoma cells (HT1080 cells) and human dermal fibroblasts (HDFs) cultured primarily from human fetal skin. Cells were passaged 3 times a week by treating with trypsin-EDTA and used for experiments after 5 passages.

MTT assay

Cytotoxic levels of EC extract on HT1080 cells, and HDFs were measured using MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide). The cells were grown in 96-well plates at a density of $5×10^3$ cells/well. After 24 hr, cells were washed with fresh medium and were treated with different concentrations of seaweeds extract. After 48 h of incubation, cells were rewashed and 20 μl of MTT (5 mg/ml) was added and incubated for 4 hr. Finally, DMSO (150 μl) was added to solubilize the formazan salt formed and amount of formazan salt was determined by measuring the OD at 540 nm using an GENios® microplate reader (Tecan Austria GmbH, Austria). Relative cell viability was determined by the

amount of MTT converted into formazan salt. Viability of cells was quantified as a percentage compared to the control (OD of treated cells − OD of blank / OD of control − OD of blank×100) and dose response curves were developed. The data were expressed as mean from at least three independent experiments and $p<0.05$ was considered significant.

Gelatin zymography

Activities of MMP-2 and MMP-9 were determined by zymography as described previously [11] in the presence or absence of seaweeds extract. After cells were exposed to various concentrations of seaweeds extract for 1 hr prior to treatment of 10 ng/ml and 100 ng/ml PMA for HDFs and HT1080 cells respectively, incubation was continued in FBS-free medium for 3 days. Conditioned medium containing 50 μg of total protein (or reaction products of various concentrations of EC extract with active MMP-2) was resuspended in a sample buffer (125 mM Tris-HCl, pH 6.8, 3% SDS, 40% glycerol, 0.02% bromophenol blue) without boiling and electrophoresed under non-reducing conditions on 10% polyacrylamide gels containing 1.5 mg/ml gelatin. After electrophoresis, the gels were washed twice with 50 mM Tris-HCl (pH 7.5) containing 2.5% Triton X-100 and incubated overnight at 37°C in a developing buffer containing 10 mM $CaCl_2$, 50 mM Tris-HCl, and 150 mM NaCl. The gels were stained with 0.25% Coomassie Blue R-250 in 30% methanol and 10% acetic acid, and de-stained in the same solution without the Coomassie Blue dye. Gelatinolytic bands were observed as clear zones against the blue background and the intensity of the bands was estimated using Image Master Software, Amersham Pharmacia Biotech (Sweeden).

Statistical analysis

Comparisons of all data were performed using two-tailed, unpaired Student's t-test. A P value less than 0.05 was considered statistically significant. Data are expressed as means ±SE.

Results

Effect of seaweeds methanol extract on cell growth

The aim of this study was to investigate the inhibitory effect of seaweeds methanolic extracts on MMPs activity in normal human dermal cells and human fibrosarcoma cells. First of all, the cytotoxic effects of seaweeds methanolic extracts were examined by MTT assay. As shown in Fig. 1, even though all data were not shown here, all seaweeds methanolic extracts did not exert any cytotoxic effect on HDFs at the 1 μg/ml. Therefore, all seaweeds methanolic extracts with no cytotoxity could be focused for screening of therapeutic candidates in this study.

Inhibitory effect of seaweeds methanol extracts on MMPs activity in human dermal fibroblasts

In order to investigate whether seaweeds methanolic extracts inhibits MMPs secreted from HDFs cultured primarily from human fetal skin, conditioned medium of HDFs treated with seaweeds methanolic extracts at 1 μg/ml for 6 day after treated with PMA to induce MMPs expression was subjected to gelatin zymography. As shown in Fig. 2A, HDFs secreted mainly proMMP-2 and a little amount of proMMP-9. It also observed that MMP-2 was activated by treatment with doxycycline at 1 μg/ml, a positive control. In this experiment the *M. myagroides* methanolic extract greatly increased MMP-2 activity. In contrast, *G. lanceolate* methanolic extract inhibited MMP-2 activity. It showed higher inhibition of MMP-2 activity compared to that of doxycycline at same concentration. As shown in Fig. 2B, in this study the *E. linza* methanolic extract remarkably activated pro-MMP-2 compared with PMA treatment group. On the other hand, *P.*

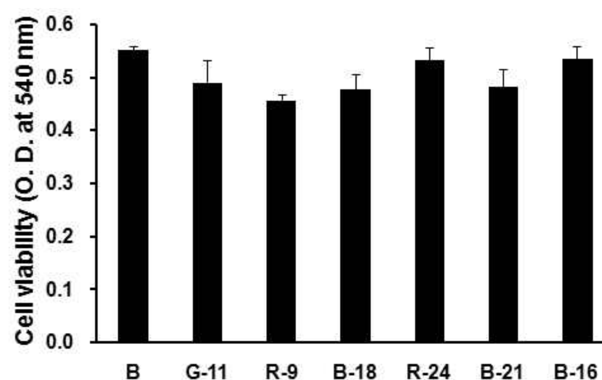

Fig. 1. Effect of seaweeds methanolic extracts on viability of human dermal fibroblasts. The cells were treated with 1 μg/ml of seaweeds methanolic extracts and cell viability was determined by MTT assay after 24 hr. Data are given as means of values±SD from three independent experiments. The seaweeds used in this experiment were as follows: G11 (*M. nitidum*), R9 (*L. okamurae*), B18 (*M. myagroides*), R24 (*P. japonica*), B21 (*P. coriaceum*) and B16 (*S. coreanum*).

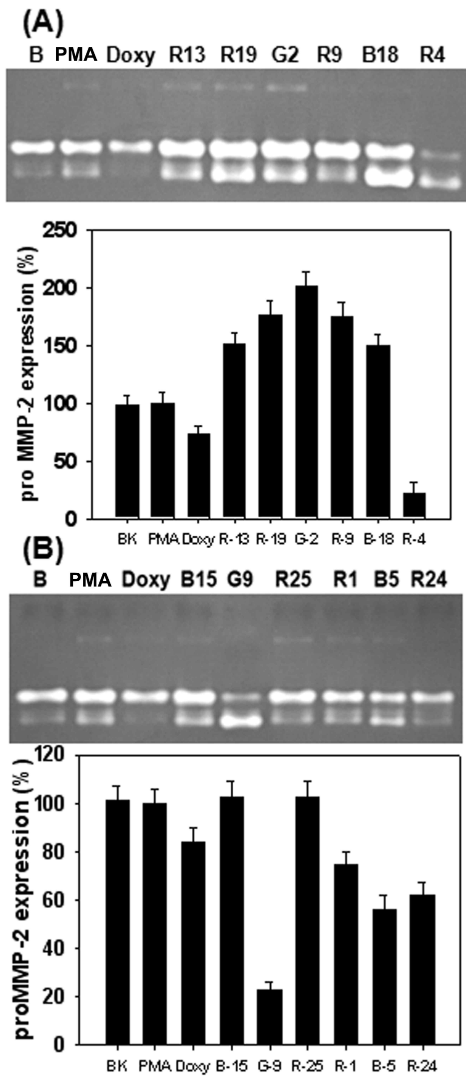

Fig. 2. Effects of seaweeds methanolic extracts on activities of MMP-2 and MMP-9 from HDFs. The cells were treated with 10 ng/ml of PMA under serum-free conditions to induce MMP expressions. After 6 days of incubation, conditioned media were reacted with various concentrations of seaweeds methanolic extracts for 1 hr, and then gelatin zymography was performed. Doxycycline (Doxy) was used as the positive control. (A) The seaweeds used in this experiement were as follows: R13 (*P. capillacea*), R19 (*S. okamurae*), G2 (*C. contractum*), R9 (*L. okamurae*), B18 (*M. myagroides*) and R4 (*G. lanceolate*). (B) The seaweeds used in this experiement were as follows: B15 (*S. horneri*), G9 (*S. okamurae*), R25 (*C. undulates*), R1 (*G. textorii*), B5 (*I. sinicola*) and R24 (*P. japonica*). Lower panel represents respective relative enzyme activities as percent of blank group.

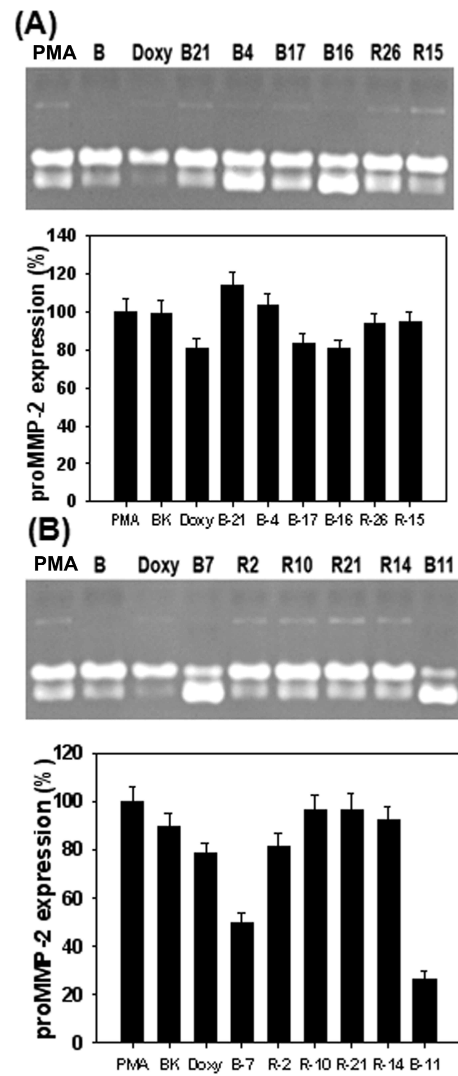

Fig. 3. Effects of seaweeds methanolic extracts on activities of MMP-2 and MMP-9 from HDFs. The cells were treated with 10 ng/ml of PMA under serum-free conditions to induce MMP expressions. After 6 days of incubation, conditioned media were reacted with various concentrations of seaweeds methanolic extracts for 1 hr, and then gelatin zymography was performed. Doxycycline (Doxy) was used as the positive control. (A) The seaweeds used in this experiement were as follows: B21 (*P. coriaceum*), B4 (*I. okamurai*), B17 (*S. siliquastrum*), B16 (*S. coreanum*), R26 (*H. dilatata*) and R15 (*G. furcata*). (B) The seaweeds used in this experiement were as follows: B7 (*P. bighamiae*), R2 (*G. verrucosa*), R10 (*C. cssicaulis*), R21 (*C. crispus*), R14 (*P. cornea*) and B11 (*S. thunbergii*). Lower panel represents respective relative enzyme activities as percent of blank group.

japonica methanolic extract inhibited MMP-2 activation by more 30 % than doxycycline treatment group. In next experiment, both *I. okamurai* and *S. coreanum* methanolic extracts showed MMP-2 inhibition by 50% compared with Blank group. However, it was found that *G. furcata* methanolic extract inhibited MMP-2 activation compared with PMA treat-

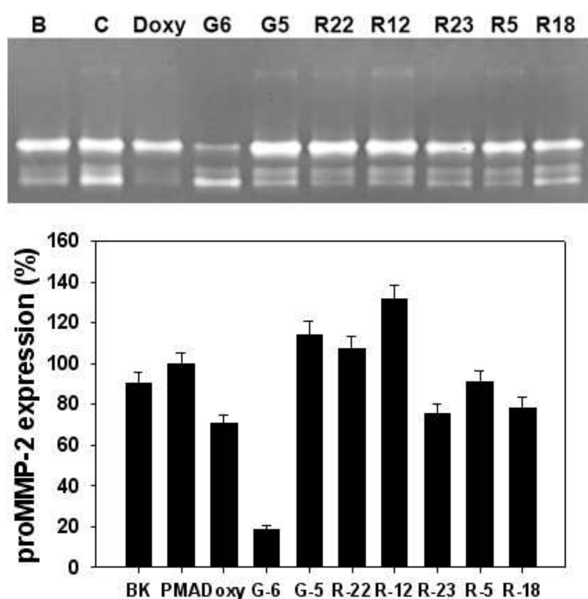

Fig. 4. Effects of seaweeds methanolic extract on activities of MMP-2 and MMP-9 from HDFs. The cells were treated with 10 ng/ml of PMA under serum-free conditions to induce MMP expressions. After 6 days of incubation, conditioned media were reacted with various concentrations of seaweeds methanolic extracts for 1 hr, and then gelatin zymography was performed. Doxycycline (Doxy) was used as the positive control. The seaweeds used in this experiement were as follows: G6 (*E. compressa*), G5 (*U. pertusa*), R22 (*M. denticulate*), R12 (*L. catenata*), R23 (*A. flabellatum*), R5 (*S. lancifolia*) and R18 (*G.* amansii). Lower panel represents respective relative enzyme activities as percent of blank group.

ment group, as shown in Fig. 3A. In contrast, both *P. bighamiae and S. thunbergii* methanolic extracts greatly enhanced MMP-2 activity compared to PMA treatment group, as shown in Fig. 3B. They exhibited 3 times more inhibition of MMP-2 activity than PMA treatment group. The excellent inhibitory effect of *E. compressa* methanolic extract on MMP-2 activity was observed in Fig. 4. Furthermore, 50% more inhibition of MMP-2 activity was observed at 1 µg/ml compared with doxycycline treatment group.

Inhibitory effect of seaweeds methanol extracts on MMPs activity in human fibrosarcoma cells

In this experiment, the effect of seaweeds methanolic extracts on MMPs was examined in human fibrosarcoma cells, HT1080 cell line that has widely used to study metastasis. In a similar method to normal human dermal fibroblasts, the conditioned medium of HT1080 cells treated with seaweeds methanolic extracts at 1 µg/ml for 3 d after treated

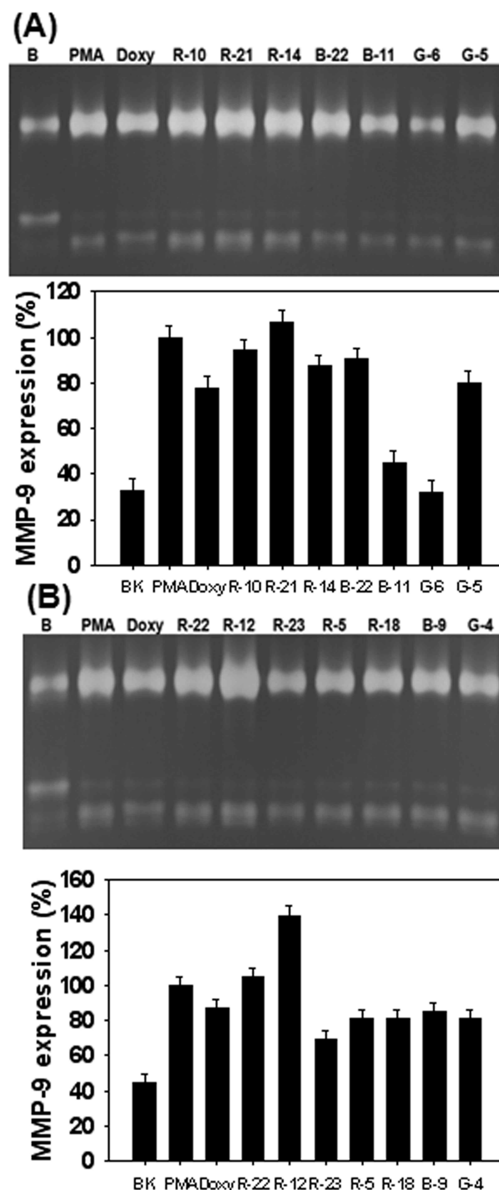

Fig. 5. Effects of seaweeds methanolic extracts on expression and activity of MMP-2 and MMP-9 in HT1080 cells. The cells stimulated with 10 ng/ml of PMA to induce MMP expression were treated with various concentrations of seaweeds methanolic extracts under serum-free conditions for 3 days. MMP activities in conditioned media were determined by zymography as described in the text. Doxycycline (Doxy) was used as the positive control. (A) The seaweeds used in this experiement were as follows: R10 (*C. cssicaulis*), R21 (*C. crispu*), R14 (*P. cornea*), B22 (*E. cava*), B11 (*S. thunbergii*), G6 (*E. compressa*) and G5 (*U. pertusa*). (B) The seaweeds used in this experiement were as follows: R22 (*M. denticulate*), R12 (*L. catenata*), R23 (*A. flabellatum*), R5 (*S. lancifolia*), R18 (*G. amansii*), B9 (*H. fusiform*) and G4 (*U. conglobata*). Lower panel represents respective relative enzyme activities as percent of blank group.

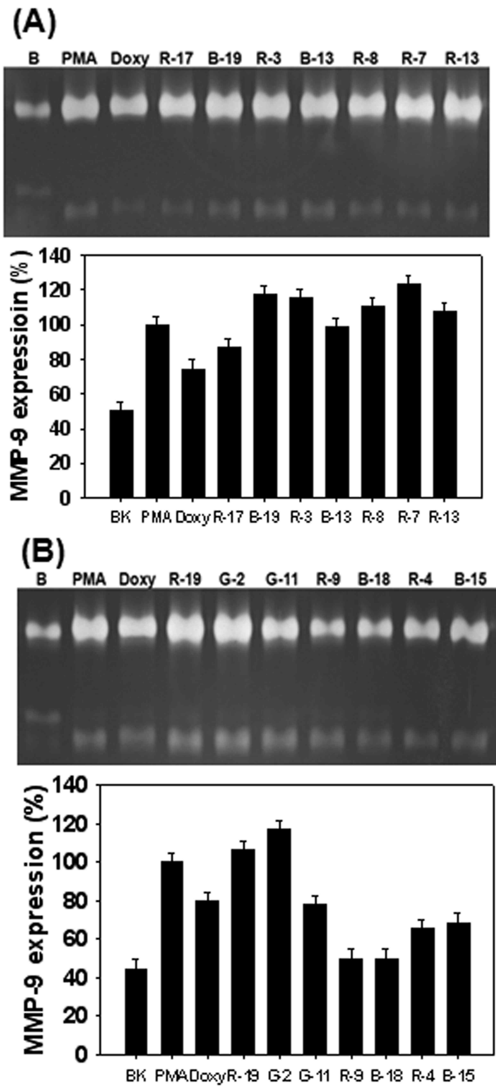

Fig. 6. Effects of seaweeds methanolic extracts on expression and activity of MMP-2 and MMP-9 in HT1080 cells. The cells stimulated with 10 ng/ml of PMA to induce MMP expression were treated with various concentrations of seaweeds methanolic extracts under serum-free conditions for 3 days. MMP activities in conditioned media were determined by zymography as described in the text. Doxycycline (Doxy) was used as the positive control. (A) The seaweeds used in this experiement were as follows: R17 (*S. dubyi*), B19 (*P. arborescens*), R3 (*G. elliptica*), B13 (*S. fulvellum*), R8 (*C. affinis*), R7 (*G. filicina*) and R13 (*P. capillacea*). (B) The seaweeds used in this experiement were as follows: R19 (*S. okamurae*), G2 (*C. contractum*), G11 (*M. nitidum)*, R9 (*L. okamurae*), B18 (*M. myagroides*), R4 (*G. lanceolate*) and B15 (*S. horneri*). Lower panel represents respective relative enzyme activities as percent of blank group.

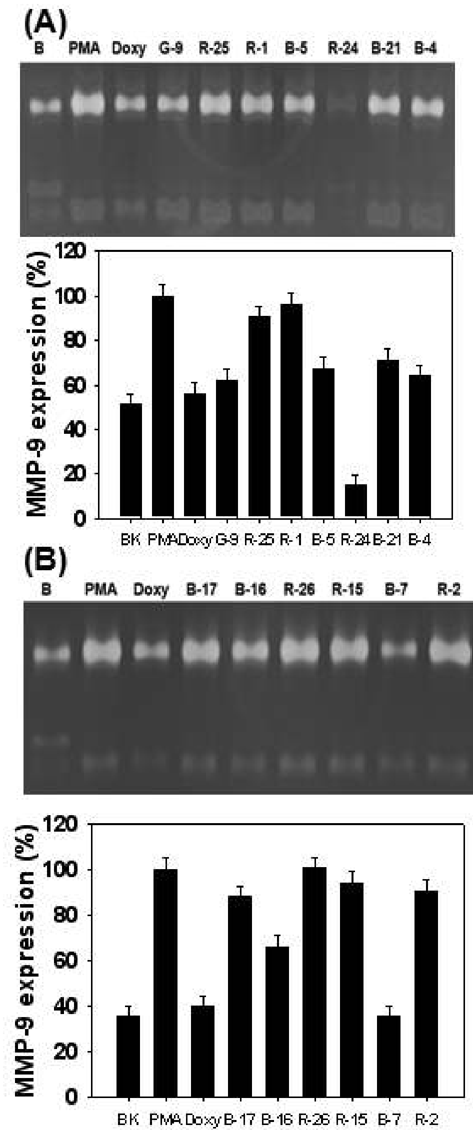

Fig. 7. Effects of seaweeds methanolic extracts on expression and activity of MMP-2 and MMP-9 in HT1080 cells. The cells stimulated with 10 ng/ml of PMA to induce MMP expression were treated with various concentrations of seaweeds methanolic extracts under serum-free conditions for 3 days. MMP activities in conditioned media were determined by zymography as described in the text. Doxycycline (Doxy) was used as the positive control. (A) The seaweeds used in this experiement were as follows: G9 (*E. linza*), R25 (*C. undulates*), R1 (*G. textorii)*, R24 (*P. japonica*), B21 (*P. coriaceum*) and B4 (*I. okamurai*). (B) The seaweeds used in this experiement were as follows: B17 (*S. siliquastrum*), B16 (*S. coreanum*), R26 (*H. dilatata)*, R15 (*G. furcata*), B7 (*P. bighamiae*) and R2 (*G. verrucosa*). Lower panel represents respective relative enzyme activities as percent of blank group.

with PMA was analyzed to analyzed using gelatin zymography. As shown in Fig. 5A, the gelatinolytic activity at 92 kDa, which corresponds to the molecular mass of pro-MMP-9, was more clearly detected in the conditioned

medium from PMA-stimulated HT1080 cells than HDFs. The inhibitory effect of doxycycline at 1 μg/ml on MMP-9 activity was clearly observed in zymogram. Among seaweeds methanolic extracts in this experiment, *S. thunbergii* and *E. compressa* methanolic extracts greatly reduced MMP-9 activity as well as MMP-activity. However, there was no significant difference in inhibitory effect on MMP-9 activity between other seaweeds and PMA treated group. Fig. 5B illustrated that *L. catenata* methanolic extract greatly enhanced MMP-9 activation compared to PMA treated group. In contrast, *A. flabellatum* showed the highest inhibitory effect on MMP-9 activity in other seaweeds. It was not observed that there was any effective algae with inhibition or activation on MMP-2 and MMP-9 in Fig. 6A. In the next experiment, as shown in Fig. 6B, several seaweeds exhibited an effective inhibition on MMP-2 and MMP-9 activities. In particular, both *L. okamurae* and *G. amansii* methanolic extracts exhibited a significant inhibitory effect on MMP-9 activity. As shown in Fig. 7A. two seaweeds were found to be effective in inhibiting MMP-2 and MMP-9 activities. *E. linza* methanolic extract showed a similar inhibitory effect to doxycycline treatment group. The other seaweed, *P. japonica* methanolic extract completely inhibited MMP-2 and MMP-9 activities. In results of Fig. 7B, it was found that *Peltaronia bighamiae* methanolic extract has a inhibitory effect on MMP-9 activity.

Inhibitory effect of 4 seaweeds methanol extracts in a dose dependent manner on MMP-2 and -9 activies in human fibrosarcoma cells

In order further confirm whether the seaweeds selected from above screening assay exert an inhibitory effect on MMP-2 or MMP-9 activity, 4 kinds of seaweeds methanolic extracts with increasing concentration were treated to HT1080 cells. As shown in Fig. 8, *S. thunbergii* methanolic extract did not show any clear inhibitory effect on MMP-9 activity, wheras it at 10 μg/ml exhibited an inhibitory effect

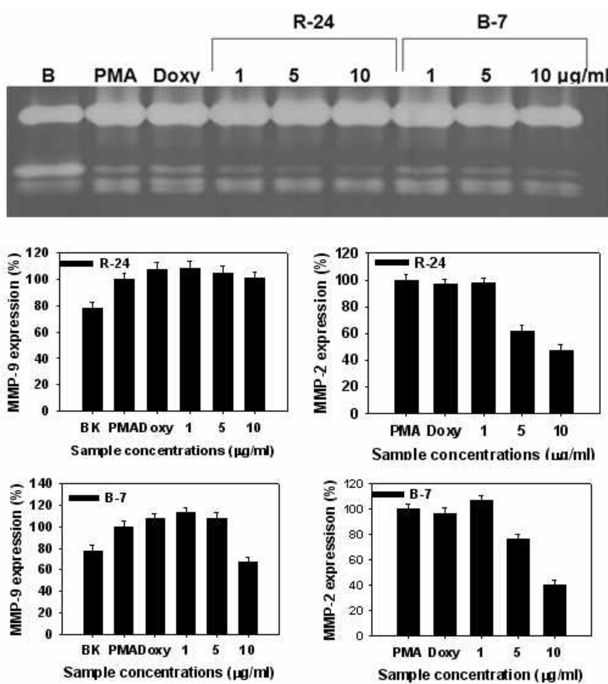

Fig. 8. Effects of seaweeds methanolic extracts on expression and activity of MMP-2 and MMP-9 in HT1080 cells. The cells stimulated with 10 ng/ml of PMA to induce MMP expression were treated with various concentrations of *S. thunbergii* and *E. linza* methanolic extracts under serum-free conditions for 3 days. MMP activities in conditioned media were determined by zymography as described in the text. Doxycycline (Doxy) was used as the positive control. Lower panel represents respective relative enzyme activities as percent of blank group.

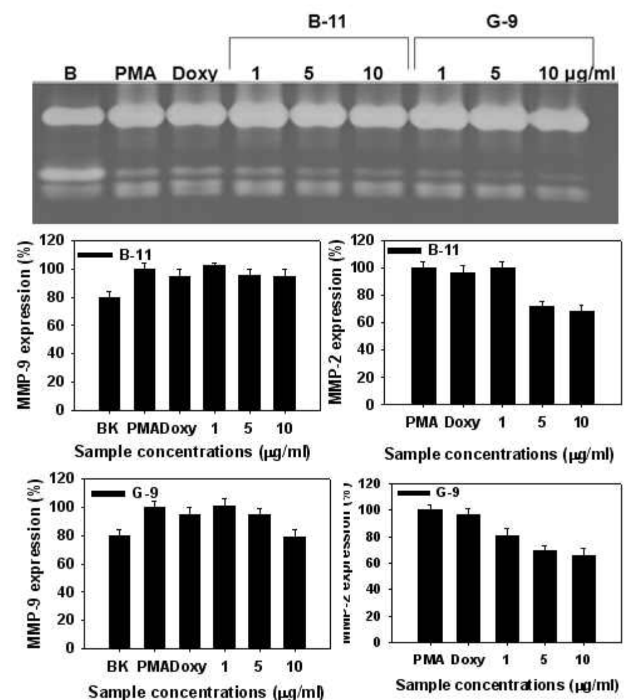

Fig. 9. Effects of seaweeds methanolic extracts on expression and activity of MMP-2 and MMP-9 in HT1080 cells. The cells stimulated with 10 ng/ml of PMA to induce MMP expression were treated with various concentrations of R24 (*P. japonica*) and B7 (*P. bighamiae*) methanolic extracts under serum-free conditions for 3 days. MMP activities in conditioned media were determined by zymography as described in the text. Doxycycline (Doxy) was used as the positive control. Lower panel represents respective relative enzyme activities as percent of blank group.

on MMP-2 activity. In contrast, *E. linza* methanolic extract not only revealed an inhibitory effect on MMP-9 activity at a concentration of 10 µg/ml but also exerted a dose dependent inhibitory effect on MMP-2 activity. Other two seaweeds, *P. japonica* and *P. bighamiae*, were investigated for the effect on MMPs activity. As shown in Fig. 9, it was observed that while *P. japonica* methanolic extract did not exhibit any inhibitory effect on MMP-9 activity, it showed a clear inhibitory effect on MMP-9 activity at a concentration of 10 µg/ml. However, *P. bighamiae* methanolic extract exerted an inhibitory effect on both MMP-9 and MMP-2 activities in a dose dependent manner, indicating that it may be a potential candidate for chemoprevention of metastasis

Discussion

In our study several marine seaweeds including green algae, brown algae and red algae collected from the southern coast of Korea was used to screen novel MMP inhibitors from marine natural resources. Methanol was applied to the seaweeds to extract as much ingredients as possible. ethanol. In order to study the inhibitory effects of these extracts on MMPs activity, gelatin zymography was utilized as an assay because of its accuracy and reproducibility even though there are many ways for study of MMPs. Using this assay, we investigated inhibitory effect of the seaweeds methanolic extracts on MMPs released from the cultured cells corresponding to normal human fibroblasts and human fibrosarcoma cells. For reliability of this experiment, doxycycline was used as a positive control to compare their effects. Doxycycline is a synthetic tetracycline derivative that can inhibit MMP activity and potentially an effective therapeutic agent [1]. Longitudinal double-blind studies on humans with adult periodontitis have demonstrated that a sub-antimicrobial dose of doxycycline, previously reported to suppress collagenase activity in the periodontal pocket, is safe and effective and has recently been approved by the FDA as an adjunct to scaling and root planning [7]. Furthermore, Periostat® (CollaGenex Pharmaceuticals Inc.), a tetracycline analog containing doxycycline has been reported to inhibit collagenase activity but failed to act as an antibiotic [8]. We wondered whether a pothetial candidate could be screen from seaweeds and what is difference in effect on MMPs activity between normal cells and cancer cells. Our results demonstrate that same seaweeds are able to exert different effects on MMPs activity. These results suggest a possibility that a therapeutic agent can act an efficacy in normal individual and patient with cancer diffentially. In our study especially *P. bighamiae* , a brown algae, and *E. linza,* a green algae, revealed any clear inhibitory effect on both MMP-9 and MMP-2 activities in human fibrosarcoma cells, indicating that they can possibly be a candidate to further study a concrete mechanism and single compound from them. Previous study on *Peltaronia bighamiae* have reported that high content of active compounds such as polysaccharides >30 kDa phlorotannins is responsible for anticoagulant activitiy [26]. *Enteromorpha linza* was reported to have an anti-inflammatory activity [16]. Furthermore, its inhibitory effects on MMP-2 and MMP-9 activity at the same concentration were more effective and that of doxycycline. In addition, our results demonstrated that both *P. japonica*, a red algae, and *P. bighamiae*, a brown alga, methanolic extracts revealed an excellent inhibitory effect on MMP-2 activity. Our findings are consistent with previous study in detail on *P. japonica* that it attenuates Wnt/beta-catenin signaling via activation of NF-kappaB and can potentially be used as a chemopreventive agent against colon cancer [9]. On the other hand, recent study has shown that a water-soluble extract of *P. binghamiae* inhibits the expression of adipogenic regulators in 3T3-L1 preadipocytes and reduces adiposity and weight gain in rats fed a high-fat diet [15]. Moreover, the inhibitory effects on MMP-2 were observed in *L. okamurae, P japonica, G.. lanceolate and S. lancifolia* of only red algae. In contrast, the positive effects on MMP-2 activity were found in *E. compressa and E. linza* of green algae, and *P. bighamiae and S. thunbergii* of brown algae. In human fibrosarcoma cells, the inhibitory effects on MMP-9 were observed in *S. thunbergii* of brown algae, *P. japonica* in red algae and *E. compressa and E. linza* of green algae. The positive effects on MMP-9 activity were not observed in any algae used in this study. HT1080 cells mainly release proMMP-9 and a little amount of proMMP-2. In contrast, proMMP-2 is mainly expressed in HDFs. The interesting finding is that *E. compressa and E. linza* of green algae, and *S. thunbergii* of brown algae exhibited a positive effect on MMP-2 in normal cells, but a negative effect on MMP-9 in cancer cell line. Previously it has been reported that fibrosarcoma HT1080 cells secrete type IV collagenase, MMP-2 and MMP-9, and that these enzymes play a major role in cancer metastasis [27]. Furthermore, MMP-2 and MMP-9 can degrade type IV collagen of base membranes and known to play a crucial role in cancer invasion such as ovarian carcinoma [22].

Moreover, above results confirmed that the innibitory effect on MMP activity exert depending on cell types. Therefore, it can be presumed that *E. compressa and E. linza* of green algae, and *S. thunbergii* of brown algae contain potential therapeutic ingredients for cancer treatment. In particular, *S. thunbergii* that inhibits MMP-9 in this study was reported to contain not only (2*S*)-1-*O*-(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadeca trienoyl)-3-*O*-*β*-d-galactopyranosyl-*sn*-glycerol and (2*S*)-1-*O*-(9Z,12Z,15Z-octadecatri enoyl)-2-*O*-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-3-*O*-β-d-galactopyranosyl-*sn*-glycerol [17] but also 9-(3,4-dihydro-2,8-dimethyl-6-hydroxy-2*H*-1-benzopyran-2-yl)-6-methyl-2-(4-methyl-3-pent enyl)-(2*E*,6*E*)-nonadienoic (hunbergols A) acid, 10-(2,3-dihydro-5-hydroxy-7-methyl-1-benzofuran-2-yl)-10-hydroxy-6-methyl-2-(4-methyl-3-pentenyl)-(2*E*,6*E*)-undecadienoic acid (hunbergols B) and tetraprenyltoluquinols [23]. Therefore, these results suggest that the activity which *S. thunbergii* exerts MMP-9 inhibition as well as ROS scavenging effect may be caused by hunbergols and tetraprenyltoluquinols.

In conclusion, although our results show in vitro effects of the seaweeds methanolic extracts on MMP-2 and MMP-9 activities, it provides the first experimental evidence that some seaweeds can be a potential candidate to develop active compounds capable of inhibiting MMP activity for chemoprevention of metastasis.

Acknowledgement

This work was supported by Dong-Eui University (2011AA100).

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초록 : 사람피부섬유아세포 및 섬유아육종세포로부터 유래된 기질금속단백질효소에 대한 해조류의 효능

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최근에 해양자원에 있는 동물, 해조류 곰팡이 세균에서 신규 잠재적인 후보약효제가 조사되어 왔다. 본 연구에서는 치료제를 탐색하기 위하여 암전이, 관절염, 만성염증 및 주름형성에 주요한 역할을 하는 기질금속단백질분해효소(s) (MMPs)를 목적효소로 이용하였다. 5종의 녹조류, 18종의 홍조류, 4종의 갈조류를 포함한 다양한 해조류가 사람피부섬유아세포 및 섬유아육종세포로부터 유래된 기질금속단백질효소에 미치는 영향을 gelatin zymography를 이용하여 조사하였다. 사람피부섬유아세포에서는 홍조류중에서 *Laurencia okamurae, Polysiphonia japonica, Grateloupi*a lanceolate 및 *Sinkoraena lancifolia*에서 MMP-2 억제효과가 관찰되었다. 반면에 녹조류의 *Enteromorpha compressa*와 *Enteromorpha linza,* 갈조류의 *Peltaronia bighamiae and Sargassum thunbergii*에서는 MMP-2 활성화가 관찰되었다. 사람섬유아육종세포에서는 MMP-9 활성화가 갈조류인 *Sargassum thunbergii,* 홍조류의 *Polysiphonia japonica*, 녹조류의 *Enteromorpha compressa*와 *Enteromorpha linza*의 존재 하에서는 감소되었다. 본 연구에서 흥미로운 발견은 녹조류의 *E. compressa*와 *E. linza* 및 갈조류의 *S. thunbergii*는 정상세포에서는 MMP-2에 대하여 활성화 효과를 나타내었으나, 암세포에서는 MMP-9응 억제하는 효과를 나타낸 것이다. 이러한 결과는 녹조류의 *E. compressa*와 *E. linza* 및 갈조류의 *S. thunbe*rgii는 항암 효능을 발휘할 수 있는 성분을 함유하고 있다는 것을 암시하고 있다.