Chapter 9
Antitumor and Antimetastatic Effects of Marine Algal Polyphenols

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Abstract Recently, bioactive substances from marine organisms have gained notable attention from various fields throughout the globe. Among marine organisms, algae have been studied widely for the isolation and characterization of biologically active components and polyphenols are one of the most abundant among them. Vast majority of algal polyphenols are consisted of phlorotannins which are derived mainly from brown algae and shown to possess numerous bioactivities such as antioxidant, anti-inflammatory, antidiabetic, antihypertensive, anti-allergic and so on. Moreover, marine polyphenols are reported to act against tumor growth and show anti-cancer properties. Results indicate that these substances demonstrate varying mechanisms of action and significant activities towards cancer and tumor-related complications. Herein, some recent findings towards the anticancer and antimetastatic characteristics of marine algal polyphenols are reviewed. Their efficiency, source and molecular mechanisms are presented.

Keywords Algal polyphenols · Anti-cancer · Antitumor · Antimetastatic · Marine algae · Phlorotannins

9.1 Background

Among all life-threatening diseases of modern world, cancer has stirred enormous difficulties for the fields of medicine and immunology. Discovery and development of novel and efficient compounds from natural sources have been the key aspect

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of concern for researches, especially in the pharmaceutical field. Nature contains a broad variety of organisms including microbial inhabitants and lithosphere, atmosphere and hydrosphere altogether present an excellent source of distinctive chemical compounds with great potential to be used as therapeutic agents. Present trends credit drugs derived from natural sources to have notable impact on the antitumor agent discovery approach [1]. Natural products are considered to possess this giant potential due to their bioavailability, specific and strong binding to drug targets, ability to bind proteins with minimal entropy loss. In addition, it has been reported that compounds of natural origin are known to be adoptive to diverse conformations in aqueous and lipophilic environments [2].

The utilization of organisms present in folk medicine is being widely investigated worldwide. Among all traditional medicinal organisms, plant materials occupy a large part of natural products which are being recognized for their renowned bioactivities such as anticancer, antiviral, antioxidant and antibacterial. Starting from ancient times, folk medicines use marina algae intensely, and in this regard several species, especially brown algaehave emerged as abundant source of nutrition, hence consumed as a kind of seasoned vegetable in various coastal areas worldwide [3–5]. Moreover, it has been treated as a source of natural marine product due to its biological activity in a broad range. Marine algae already reported to contain various phlorotannin derivates, which have been regarded as potential pharmacological polyphenols [6–7]. Phlorotannins are oligomeric form of phloroglucinol and have been revealed to have antioxidant, antibacterial, anti-inflammation, anti-allergy, anti-matrix metalloproteinase (MMP), apoptosis-induction, and so on [8–10].

9.2 Phlorotannins

Phlorotannins are natural compounds which are formed by the polymerization of 1,3,5-trihydroxybenzene (phloroglucinol) monomer units and are known to be biosynthesized through acetate–malonate pathway. Highly hydrophilic phlorotannins are found to be between 126 Da and 650 kDa molecular weight [11]. Phlorotannins of different molecular weights are mainly accumulated in marine brown algae which contains a wide range of phloroglucinol-based polyphenols. There four main types of phlorotannins based on linkage of monomers, namely fuhalols (phlorotannins with an ether linkage), fucols (with a phenyl linkage), fucophloroethols (with an ether and phenyl linkage) and eckols (with a dibenzodioxin linkage). Reports indicate several isolated and elucidated phlorotannins from marine sources such as phloroglucinol, eckol, fucodiphloroethol G, phlorofucofuroeckol A, 7-phloroeckol, dieckol, and 6,6′-bieckol [12]. In addition, triphloroethol A, 8,8’-bieckol, and 8,4’”-dieckol have been isolated. Marine brown alga *Ecklonia cava* is extensively studied and promoted to be a rich and dependable source of phenolic compounds in comparison to other brown algae [13]. Stressful conditions of marine environments and herbivore danger are considered to be battled by phlorotannins in case of brown algae. Owing to the health beneficial various biological activities of marine brown

algae, phlorotannins are reported to be important compounds for future development and discovery of therapeutic agents.

The bioavailability of plant polyphenols have already been studied and discussed in vivo [14–16]. According to these reports it could be said that around 70% out of consumed polyphenolics amount has been shown potent bioavailability. However, these reports mostly direct the issues through mouse models systems which present a need for further researches that investigates phlorotannin bioavailability in human subjects.

9.3 Anti-cancer Effect of Marine Algal Polyphenols

Harada and Kamei [17] showed that phlorotannins exhibit anticarcinogenic effects. Study presented that brown alga *Laminaria japonica* fractionated phlorotannin extract (PE) has shown considerable anti-proliferative activity in the hepatocellular carcinoma cells (BEL-7402) and also on leukemic cell lines (P388) with the IC_{50} values of 120 and >200 μg/ml, respectively. Microscopic observations have revealed that the morphologic features of tumor cells treated with PE and 5-fluorouracil (a commercial chemotherapy drug) are markedly different from the normal control group suggesting the anti-proliferative effect of PE [18]. Moreover, dioxinodehydroeckol which was isolated from *E. cava* has shown to possess a notable anti-proliferative effect on human breast cancer cells (MCF-7). Dioxinodehydroeckol inhibited the proliferation of MCF-7 cells with rates of approximately 25, 40, 53, 56 and 64% at concentrations of 1, 5, 10, 50 and 100 μM, respectively, compared to the control group. Study credited the potential anti-proliferative activity of dioxinodehydroeckol to its ability to induce of apoptosis through nuclear factor kappa-light-chain-enhanced activated B cells (NF-κB) family and NF-κB dependent pathway [19]. In another research, *E. cava* has been subjected to enzymatic extraction along crude polyphenolic and polysaccharide fractions. All aforementioned fractions of *E. cava* have been evidently shown to possess antiproliferative and antiradical activities. Especially the CphF at an IC_{50} of 5.1 μg/ml has inhibited cell proliferation in murine colon cancer cell line (CT-26) significantly. A nuclear cell staining assay suggested that this anti-proliferative effect of CphF is associated with apoptotic cell demise in CT-26 [20]. The direct correlation between the anti-proliferative effect of the algae and their polyphenolic content is evidently documented. In this context, the anti-proliferative effects of red alga, *Palmaria palmate* and three kelp Laminaria setchellii, Macrocystis integrifolia, Nereocystis leutkeana extracts has been studied on human cervical adenocarcinoma cell line (HeLa cells). HeLa cell proliferation was inhibited between 0 and 78% by *P. palmate*; 0 and 55% by *L. setchellii* and 0 and 69% by *M. integrifolia* and *N. leutkeana* at 0.5–5 μg/ml algal extract concentration range. This investigation suggests the effectiveness of polyphenolic compounds in controlling tumor growth and brings front a fact that marine algae could serve beneficial for anticancer properties [21]. In addition, in vivo tests also presented valuable data regarding antitumor

effects of polyphenols. Dietary inclusion of brown algal polyphenols in pre-tumor bearing mouse feeding at the rates of 0.1 and 0.5% has notably reduced tumor proliferation by 45 and 56% and tumor mass by 54 and 65%, respectively, for each application rate. Moreover, the topical application of polyphenols at 3 and 6 mg has significantly decreased tumor proliferation by 60 and 46% and tumor mass by 66 and 57%, respectively. In case of action mechanism, it is believed that brown algal polyphenols inhibit the cyclooxygenase-2 activity and cell proliferation hence preventing the tumor progression [22].

9.4 Marine Algal Polyphenols as MMP Inhibitors

Matrix metalloproteinase enzymes (MMPs) play a significant role in the digestion of extra cellular matrix components, hence directly associated with chronic inflammation, wrinkle formation, arthritis, osteoporosis, periodontal diseases, tumor invasion, and metastasis in pathological conditions. During current decade, a detailed presentation of MMP inhibitory effects of phlorotannins derived from *E. cava* has been documented for the first time [23]. In this report, a novel gelatin digestion assay was able to visualize complete inhibition of bacterial collagenase-1 activity with introduction of 20 μg/ml of *E. cava* extract during preliminary screening assays. In addition, a sensitive fluorometric assay has been carried out and it showed that phlorotannin content of E. cava can specifically inhibit both MMP-2 and MMP-9 activities ($p < 0.001$) at 10 μg/ml. Also, artificially induced activities of MMP-2 and MMP-9 in human dermal fibroblast and HT 1080 cells have been successfully suppressed by E. cava extract in a comparable manner to that of positive control doxycycline. More interestingly, EC extract did not exert any cytotoxic effect even at 100 μg/ml, anticipating, its potential use as a safe MMP inhibitor. Therefore, it can easily be suggested that phlorotannins would be a potent natural source for the development of therapeutic agents against MMP and cancer.

In another research, 3-(3, 4-dihydroxy-phenyl)-acrylic acid phenethyl ester (caffeic acid phenethyl ester, CAPE) has also been isolated and characterized as biologically active compound with health benefits from methanol extracts prepared from roots of *Rhodiola sacra* and quadrifida [24, 25]. In this regard, Lee et al. evidently proposed that these active compounds can down regulate artificially enhanced MMP-9 activities, indicating a notable antitumor effect [26].

Comparison of 29 seaweed extracts in regard to their inhibitory efficiencies on transcriptional activities of MMP-1 expression has been performed by Joe et al. [27]. Research has concluded that the eckol and dieckol from Ecklonia species have showed strong inhibition of both NF-κB and AP-1 reporter activity, which were well related with their abilities to inhibit MMP-1 expression. In addition, MMP-1 expression was dramatically attenuated by treatment with the eckol or dieckol.

It has been also known that matrix metalloproteinases (MMPs) are crucial components in photoaging of the skin, especially due to high and long exposure to ultraviolet A. Reports indicate that enhanced activity of MMPs and increased

photoaging appear to be stimulated by UV-irradiation-associated generation of reactive oxygen species (ROS). Ryu et al. demonstrates that the alga Corallina pilulifera methanol extract which has been shown a high phenolic content, reduced the expression of UV-induced MMP-2 and -9 of human dermal fibroblasts in a dose dependent fashion, and has also exhibited strong antioxidant activity by scavenging free radicals [28].

In a murine asthma model, it has been documented that MMP-9 expression was significantly reduced by the administration of *E. cava* extracts. And it has been presented that *E. cava* extracts were able to notable suppress the cytokine signaling-3 (SOCS-3) expression and reduce the increased eosinophil peroxidase (EPO) activities [29].

Also aforementioned phlorotannins, namely eckol, dieckol, 6,6′-bieckol and 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6″-trihydroxy-phenoxy)-2,4,9-trihydroxydibenzo-1,4,-dioxin were also extracted from brown alga, *E. cava*, and in regard it has been reported that these compounds were able to inhibit the expression of MMP-1, -3 and -13 induced by proinflammatory cytokines [30].

In short, polyphenols, especially from marine sources, have excellent MMP inhibitory activities; however potent cytotoxicity of polyphenols come front as a major drawback. Therefore, the pharmaceutical applications of these MMP inhibitors are usually limited.

In this regard, future researches should turn their attention to reduce their toxicity levels by altering the chemical structure in a way to preserves bioactivity while converting the compound to be biologically safe. Following these improvements, MMP inhibitor polyphenols will gain a huge potential to be used in clinical applications.

9.5 Conclusions

In conclusion, due to the abundant presence and distinctive chemical distribution of polyphenols among marine sources, especially algae, future focus should be directed to biological and pharmacological of novel polyphenols from marine sources with higher efficiency against cancer and less cytotoxicity to non-cancer cells. It is recommended to screen phlorotannins from other marine macro algae and evaluate their anticancer activities as a comparative study. With the latest advancements in the fields of molecular biology and biochemistry, a sophisticated approach to study the interactions of polyphenols with human cellular systems could prove beneficial in understanding and altering parameters like bioavailability and cytotoxicity of these compounds. Also, detailed studies on molecular interactions of phenolic compounds involved in the management of various human diseases would pave the way for development of novel therapeutic agents with superior efficiency. On the other hand, broadening the ways and studies that are developed to screen more biologically efficient polyphenols will definitely provide promising drug candidates for pharmaceutical purposes.

References

1. da Rocha AB, Lopes RM, Schwartsmann G (2001) Natural products in anticancer therapy. Curr Opin Pharmacol 1(4):364–369
2. Koehn FE, Carter GT (2005) The evolving role of natural products in drug discovery. Nat Rev Drug Discov 4(3):206–220
3. Ali MS, Jahangir M, Saleem M, Pervez MK, Hameed S, Ahmad VU (2000) Metabolites of marine algae collected from Karachi-coasts of Arabian sea. Nat Prod Sci 6(2):61–65
4. Kim S-K, Kong C-S (2010) Anti-adipogenic effect of dioxinodehydroeckol via AMPK activation in 3T3-L1 adipocytes. Chem Biol Interact 186(1):24–29
5. Maegawa M, Yokohama Y, Aruga Y (1987) Critical light conditions for young Ecklonia cava and Eisenia bicyclis with reference to photosynthesis. In: Ragan M, Bird C (eds) Twelfth international seaweed symposium, developments in hydrobiology, vol 41. Springer, The Netherlands, pp 447–455
6. Shibata T, Kawaguchi S, Hama Y, Inagaki M, Yamaguchi K, Nakamura T (2004) Local and chemical distribution of phlorotannins in brown algae. J Appl Phycol 16(4):291–296
7. Kang C, Jin YB, Lee H, Cha M, Sohn ET, Moon J et al (2010) Brown alga Ecklonia cava attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. Food Chem Toxicol 48(2):509–516
8. Kang HS, Chung HY, Kim JY, Son BW, Jung HA, Choi JS (2004) Inhibitory phlorotannins from the edible brown alga Ecklonia stolonifera on total reactive oxygen species (ROS) generation. Arch Pharm Res 27(2):194–198
9. Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002) Bactericidal activity of phlorotannins from the brown alga Ecklonia kurome. J Antimicrob Chemother 50(6):889–893
10. Yang YI, Shin HC, Kim SH, Park WY, Lee KT, Choi JH (2012) 6,6′-Bieckol, isolated from marine alga Ecklonia cava, suppressed LPS-induced nitric oxide and PGE(2) production and inflammatory cytokine expression in macrophages: the inhibition of NFkappaB. Int Immunopharmacol 12(3):510–517
11. Ahn G-N, Kim K-N, Cha S-H, Song C-B, Lee J, Heo M-S et al (2007) Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Eur Food Res Technol 226(1–2):71–79
12. Eom S-H, Kim Y-M, Kim S-K (2012) Antimicrobial effect of phlorotannins from marine brown algae. Food Chem Toxicol 50(9):3251–3255
13. Heo S-J, Park E-J, Lee K-W, Jeon Y-J (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 96(14):1613–1623
14. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130(8):2073S–2085S
15. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Amer J Clin Nutr 79(5):727–747
16. Yang CS, Sang S, Lambert JD, Lee M-J (2008) Bioavailability issues in studying the health effects of plant polyphenolic compounds. Mol Nutr Food Res 52(S1):S139–S151
17. Harada H, Kamei Y (1997) Selective cytotoxicity of marine algae extracts to several human leukemic cell lines. Cytotechnology 25(1–3):213–219
18. Yang H, Zeng M, Dong S, Liu Z, Li R (2010) Anti-proliferative activity of phlorotannin extracts from brown algae *Laminaria japonica* Aresch. Chin J Oceanol Limnol 28(1):122–130
19. Kong C-S, Kim J-A, Yoon N-Y, Kim S-K (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. Food Chem Toxicol 47(7):1653–1658
20. Athukorala Y, Kim K-N, Jeon Y-J (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food Chem Toxicol 44(7):1065–1074

21. Yuan YV, Walsh NA (2006) Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food Chem Toxicol 44(7):1144–1150
22. Hwang H, Chen T, Nines RG, Shin HC, Stoner GD (2006) Photochemoprevention of UVB-induced skin carcinogenesis in SKH-1 mice by brown algae polyphenols. Int J Cancer 119(12):2742–2749
23. Kim M-M, Ta QV, Mendis E, Rajapakse N, Jung W-K, Byun H-G et al (2006) Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. Life Sci 79(15):1436–1443
24. Mook-Jung I, Kim H, Fan W, Tezuka Y, Kadota S, Nishijo H et al (2002) Neuroprotective effects of constituents of the oriental crude drugs, Rhodiola sacra, R. sachalinensis and Tokaku-joki-to, against beta-amyloid toxicity, oxidative stress and apoptosis. Biol Pharm Bull 25(8):1101–1104
25. Yoshikawa M, Shimada H, Shimoda H, Murakami N, Yamahara J, Matsuda H (1996) Bioactive constituents of Chinese natural medicines. II. Rhodiolae radix. (1). Chemical structures and antiallergic activity of rhodiocyanosides A and B from the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey. (Crassulaceae). Chem Pharm Bull (Tokyo) 44(11):2086–2091
26. Lee KW, Kang NJ, Kim JH, Lee KM, Lee DE, Hur HJ et al (2008) Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B. Genes Nutr 2(4):319–322
27. Joe MJ, Kim SN, Choi HY, Shin WS, Park GM, Kang DW et al (2006) The inhibitory effects of eckol and dieckol from *Ecklonia stolonifera* on the expression of matrix metalloproteinase-1 in human dermal fibroblasts. Biol Pharm Bull 29(8):1735–1739
28. Ryu B, Qian Z-J, Kim M-M, Nam KW, Kim S-K (2009) Anti-photoaging activity and inhibition of matrix metalloproteinase (MMP) by marine red alga, *Corallina pilulifera* methanol extract. Radiat Phys Chem 78(2):98–105
29. Kim SK, Lee DY, Jung WK, Kim JH, Choi I, Park SG et al (2008) Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: role of suppressor of cytokine signaling. Biomed Pharmacother 62(5):289–296
30. Ryu B, Li Y, Qian ZJ, Kim MM, Kim SK (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: implication for chronic articular disease. Chem Biol Interact 179(2–3):192–201