Research Article

The Extracts of Some Marine Invertebrates and Algae Collected off the Coast Waters of Vietnam Induce the Inhibitory Effects on the Activator Protein-1 Transcriptional Activity in JB6 Cl41 Cells

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It has previously been shown that inhibition of the transcriptional activity of the oncogenic nuclear factor AP-1 can result in cancer prevention. Marine invertebrates and alga are a rich source of natural compounds that possess various biological activities. The inhibitory effects of the extracts of Vietnamese marine organisms in relation to the AP-1 transcriptional activity were examined by the luciferase method using JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by AP-1 DNA binding sequence. As was found, 71 species of marine sponges out of 148 species studied contain inhibitors of the AP-1 transcriptional activity. Therefore, marine organisms as a source of biologically active compounds have a great potential for isolation of the new cancer preventive compounds that inhibit the oncogenic AP-1 nuclear factor.

1. Introduction

The activator protein-1 (AP-1) transcription factor is a heterodimeric complex that contains members of the JUN, FOS, ATF, and MAF protein families. AP-1 activity is induced by a plethora of physiological stimuli and regulates a wide range of cellular processes, including cell migration, proliferation, differentiation, inflammation, apoptosis and survival, transformation, and tumor promotion [1–5]. The upregulation of such AP-1 proteins as c-FOS, FOSB, and c-JUN was found to correlate with a positive effect on cell transformation [6–10]. The AP-1 has increased expression in different cancers including breast, ovarian, cervical, colorectal, lung, bladder, and many others [11–15]. Thus, AP-1 has been considered primarily to be an oncogene. Later, some of the AP-1 proteins, such as Jun-B and c-Fos, were shown to have tumor-suppressor activity both *in vitro* and *in vivo* [16, 17]. Activation of another AP-1 protein, c-Jun, is required for the induction of Fas L-mediated apoptosis in PC12 and human leukemia HL-60 cells [18, 19]. Activation

of both AP-1 and NF- κ B nuclear factors is necessary for DNA damaging agents- and ceramide-induced apoptosis in T lymphocytes and Jurkat T cells [20, 21]. The balance between AP-1 family members, c-Jun, and ATF-2 governs the choice between differentiation and apoptosis in PC12 cells [22]. The ultimate fate of the cells relies on the relative abundance of AP-1 complex, the composition of the AP-1 dimers, cell type, and cellular environment [23].

AP-1 transcription factor plays a role in regulatory processes essential for the specific function of cells in the immune, endocrine, nervous, cardiovascular, and other physiological systems [24, 25], and therefore it participates not only in cancer but also in such diseases as cerebral ischaemia, stroke, seizure [26, 27], psoriasis [28], and mastitis [29].

Taken together, this suggests that AP-1 is a promising target for prevention and therapy in cancer and many other diseases [30].

Many works from our laboratory and other laboratoring clearly suggested that some cancer preventive and therapeutic

compounds, including those of marine origin, can inhibit AP-1 activity, whereas other such compounds can induce it. For example, cancer preventive compounds resveratrol from grape skin, actinoporin RTX-A from sea anemones, steroidal glycoside LSG from starfishes are among agents that inhibit AP-1 activity [31–33]. On the other, hand anticancer drug vinblastine, cancer preventive flavonoids kaempferol and genistein, marine natural product 3-demethylubiquinone Q2 from ascidian *Aplidium glabrum*, and its synthetic analogs induce AP-1 activity [34–39].

In this study, the *in vitro* effects of ethanol extracts of 148 marine invertebrate species (mainly sponges) and algae on the basal AP-1-dependent transcriptional activity in mouse epidermal JB6 Cl41 cells were evaluated.

2. Experimental

2.1. Marine Invertebrates and Alga. The specimens of marine invertebrates and algae were collected off the coast of Vietnam at a depth of 2–440 m by scuba diving and trawling during the 34th scientific expedition onboard the research vessel “Academic Oparin” in May–June 2007 and were immediately extracted with ethanol. The animals and algae were identified by Dr. V. B. Krasokhin. The sponges having the same names in Table 1 belong to one and the same genus, but they may have different specific names, undetermined yet, and were collected at the different places of the coast of Vietnam. The voucher specimens are kept in the collection of G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia.

2.2. Cell Culture. The mouse epidermal JB6 Cl41 AP-1 cells were cultured in monolayers at 37°C and 5% CO₂ in MEM containing 5% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin [40].

2.3. MTS Cytotoxicity Assay. Each ethanol extract of a marine invertebrate or alga was evaporated to dryness. Then, the solutions of the dry extract residue in 5% FBS/MEM with the residue concentrations of 1.0; 0.9; 0.8; 0.7; 0.6; 0.5; 0.4; 0.25; 0.125; and 0.0625 mg/mL were prepared. The effect of these solutions on JB6 Cl41 AP-1 cells viability was evaluated using MTS reduction into its formazan product [41] as described in [42]. In brief, the JB6 Cl41 AP-1 cells were cultured for 12 h in 96-well plates (6,000 per well in 0.1 mL of 5% FBS/MEM). The media was then replaced with fresh 5% FBS/MEM containing the studied substances at various concentrations in a total volume of 0.1 mL and the cells were incubated with obtained solutions for 22 h. Then, 20 μ L of the MTS reagent was added into each well and MTS reduction was measured 2 h later spectrophotometrically at 492 and 690 nm as background using the Multiskan MS microplate reader (Labsystems, Finland). The solution with the first nontoxic concentration (more than 80% of living cells in the corresponding experiment) of an extract residue (see Table 1) was used in the luciferase assay (see Section 2.4).

TABLE 1: The effects of the extracts of the marine invertebrates and alga on AP-1-dependent transcriptional activity in JB6 Cl41 cells.

Num.*	Name ^x	Conc. ^ε mg/mL	AP-1 [¥]
O14-34	<i>Callyspongia</i> sp.	0.6	103
O15-34	<i>Oscarella</i> sp.	0.5	94
O16-34	<i>Dysidea</i> sp.	0.5	100
O17-34	<i>Mycale</i> sp.	0.5	134
O18-34	<i>Chondrilla</i> sp.	0.6	110
O19-34	<i>Monanchora</i> sp.	0.125 (0.24)**	40
O20-34	<i>Haliclona</i> sp.	0.6	96
O21-34	<i>Spirastrella</i> sp.	0.4	111
O22-34	Ascidian unidentified	0.8	80
O23-34	<i>Petrosia</i> sp.	0.8	64
O24-34	Demospongiae, unidentified	0.6	88
O25-34	<i>Spirastrella</i> sp.	0.6	41
O26-34	<i>Callyspongia</i> sp.	0.5	71
O27-34	<i>Spongia</i> sp.	0.5	98
O28-34	<i>Mycale</i> sp.	0.8	106
O29-34	<i>Callyspongia</i> sp.	0.6	101
O30-34	<i>Chondrilla</i> sp.	0.6	89
O31-34	Alga unidentified	0.5	98
O33-34	<i>Callyspongia</i> sp.	0.5	89
O34-34	<i>Callyspongia</i> sp.	0.5	70
O35-34	<i>Xestospongia</i> sp.	0.6	29
O36-34	<i>Spongia</i> sp.	0.5	35
O37-34	<i>Spirastrella</i> sp.	0.5	122
O38-34	Demospongiae, Haplosclerida, fam. unidentified	0.5	90
O41-34	<i>Phakellia</i> sp.	0.7	59
O42-34	<i>Halichondria</i> sp.	0.5	74
O43-34	<i>Halichondria</i> sp.	0.4	125
O44-34	<i>Haliclona</i> sp.	0.6	105
O45-34	<i>Halichondria</i> sp.	0.6	75
O46-34	<i>Haliclona</i> sp.	0.6	93
O47-34	Ascidian unidentified	0.6	95
O48a-34	Ascidian unidentified	0.8	79
O48b-34	Ascidian unidentified	0.8	96
O49-34	Echinoidea unidentified	0.8	105
O50-34	Pennatulacea unidentified	0.5	91
O52-34	Alga unidentified	1.0	95
O53-34	Alga unidentified	0.6	76
O54-34	Alga unidentified	0.6	89
O55-34	Alga unidentified	0.7	93
O56-34	Alga unidentified	0.5	169
O57-34	<i>Petrosia</i> sp.	0.5	105
O58-34	Demospongiae unidentified	0.125 (0.27)**	19
O59-34	<i>Haliclona</i> sp.	0.8	74
O60-34	<i>Pachastrella</i> sp.	0.0625 (0.17)**	47

TABLE 1: Continued.

Num.*	Name ^x	Conc. [£] mg/mL	AP-1 [¥]
O61-34	<i>Spirastrella</i> sp.	0.5	73
O62-34	<i>Spongia</i> sp.	0.6	71
O63-34	<i>Spongia</i> sp.	0.5	102
O64-34	<i>Stelletta</i> sp.	0.5	62
O65-34	<i>Spirastrella</i> sp.	0.25 (0.35)**	52
O66-34	<i>Dysidea</i> sp.	0.125	77
O67-34	<i>Spongia</i> sp.	0.6	84
O68-34	Algae unidentified	0.5	67
O69-34	Opisthobranchia unidentified	0.25	88
O70-34	Opisthobranchia unidentified	0.125 (0.22)**	121
O71-34	Opisthobranchia unidentified	0.8	96
O72-34	Opisthobranchia unidentified	0.5	149
O73-34	<i>Spongia</i> sp.	0.0625 (0.1)**	137
O74-34	<i>Myxilla</i> sp.	0.125 (0.23)**	14
O75-34	<i>Spirastrella</i> sp.	0.4	108
O76-34	<i>Xestospongia</i> sp.	0.5	117
O77-34	<i>Stelletta</i> sp.	0.0625	91
O78-34	<i>Spongia</i> sp.	0.25 (0.54)**	38
O79-34	<i>Callyspongia</i> sp.	0.5	108
O81-34	Gorgonian gen unidentified	0.5	35
O82-34	Gorgonian gen unidentified	0.25	104
O83-34	Ascidia	0.6	91
O84-34	<i>Haliclona</i> sp.	0.6	74
O85-34	<i>Spirastrella</i> sp.	0.8	60
O86-34	<i>Halichondria</i> sp.	0.8	63
O87-34	<i>Spongia</i> sp.	0.8	60
O88-34	<i>Xestospongia</i> sp.	0.6	109
O89-34	<i>Clathria</i> sp.	0.8	105
O90-34	Ascidian unidentified	1.0	105
O92-34	<i>Spongia</i> sp.	0.6	93
O93-34	<i>Spirastrella</i> sp.	0.5	124
O94-34	<i>Spongia</i> sp.	0.6	77
O95-34	<i>Spongia</i> sp.	0.6	105
O98-34	Crinoidea	0.6	71
O99-34	Demospongiae, unidentified	0.8	93
O100-34	Demospongiae, unidentified	0.8	87
O101-34	Demospongiae, unidentified	0.25 (0.4)**	127
O102-34	<i>Spirastrella</i> sp.	0.5	84
O103-34	<i>Petrosia</i> sp.	0.5	54
O104-34	<i>Halichondria</i> sp.	0.5	104
O105-34	Alga unidentified	0.25	99
O106-34	Alga unidentified	0.0625 (0.11)**	169
O107-34	Alga unidentified	0.6	144
O115-34	Crinoidea	0.25 (0.42)**	73
O117-34	Nudibranchia unidentified	0.25 (0.37)**	77
O119-34	<i>Spirastrella</i> sp.	0.6	118

TABLE 1: Continued.

Num.*	Name ^x	Conc. [£] mg/mL	AP-1 [¥]
O120-34	<i>Haliclona</i> sp.	0.6	88
O121-34	Demospongiae unidentified	0.6	111
O122-34	<i>Spongia</i> sp.	0.5	38
O123-34	<i>Callyspongia</i> sp.	0.0625	101
O124-34	<i>Axinella</i> sp.	0.6	52
O125-34	<i>Aplysina</i> sp.	0.6	60
O126-34	<i>Spongia</i> sp.	0.6	57
O127-34	<i>Spongia</i> sp.	0.0625 (0.16)**	46
O129-34	Crinoidea unidentified	0.6	84
O130-34	Crinoidea unidentified	0.5	90
O131-34	Crinoidea unidentified	0.125	83
O132-34	Nudibranchia unidentified	0.0625	84
O133-34	Ascidian unidentified	0.25 (0.37)**	73
O134-34	<i>Haliclona</i> sp.	0.0625	110
O135-34	<i>Callyspongia</i> sp.	0.5	135
O136-34	<i>Spongia</i> sp.	0.5	79
O137-34	Demospongiae unidentified	0.25	84
O140-34	<i>Spongia</i> sp.	0.8	41
O141-34	<i>Clathria</i> sp.	0.8	63
O145-34	<i>Callyspongia</i> sp.	0.8	43
O146-34	<i>Axinella</i> sp.	0.6	33
O149-34	<i>Suberites</i> sp.	0.8	83
O152-34	Lithistida unidentified	0.8	67
O153-34	<i>Halichondria</i> sp.	0.8	102
O154-34	<i>Penares</i> sp.	0.25 (0.34)**	37
O155-34	<i>Petrosia</i> sp.	0.8	67
O156-34	<i>Pheronema raphanus</i>	0.0625	90
O157-34	<i>Pachastrella</i> sp.	0.8	49
O158-34	Lithistida unidentified	0.8	75
O159-34	<i>Geodia</i> sp.	0.125	98
O160-34	Demospongiae unidentified	0.8	55
O162-34	<i>Halichondria</i> sp.	0.8	60
O163-34	<i>Spirastrella</i> sp.	0.5	101
O165-34	<i>Petrosia</i> sp.	0.8	53
O167-34	<i>Rhabdastrella globostellata</i>	0.5	37
O168-34	<i>Petrosia</i> sp.	0.125	81
O169-34	<i>Halichondria</i> sp.	0.25 (0.48)**	36
O170-34	<i>Acanthodendrilla</i> sp.	0.0625 (0.1)**	53
O171-34	<i>Callyspongia</i> sp.	0.8	30
O172-34	<i>Carteriospongia</i> sp.	0.0625 (0.11)**	19
O173-34	<i>Carteriospongia</i> sp.	0.25 (0.32)**	76
O174-34	<i>Axinella</i> sp.	0.25 (0.41)**	33
O175-34	<i>Halichondria</i> sp.	0.25 (0.33)**	66
O176-34	<i>Petrosia</i> sp.	0.125	110
O177-34	<i>Spongia</i> sp.	0.125 (0.19)**	54

TABLE 1: Continued.

Num.*	Name ^x	Conc. [£] mg/mL	AP-1 [¥]
O178-34	<i>Petrosia</i> sp.	0.8	56
O179-34	<i>Hyalonema</i> sp.	0.25	80
O181-34	Porifera, Lithistida unidentified	0.5	43
O186-34	Porifera, Calcarea, unidentified	0.6	55
O187-34	<i>Callyspongia</i> sp.	0.0625	101
O188-34	<i>Agelas</i> sp.	0.8	50
O189-34	<i>Axinella</i> sp.	0.0625 (0.16)**	67
O192-34	<i>Agelas</i> sp.	0.8	47
O193-34	<i>Petrosia</i> sp.	0.5	42
O194-34	<i>Amphimedon</i> sp.	0.25 (0.39)**	42
O196-34	<i>Spongia</i> sp.	0.125 (0.23)**	60
O197-34	<i>Axinella</i> sp.	0.5	60
O198-34	<i>Plakina</i> sp.	0.5	41

*The number of the voucher specimen.

^xSystematical name of the specimen.

[£]Concentration of the extract dry residue in the MEM.

[¥]AP-1-dependent transcriptional activity, percentage of the control.

**IC₅₀.

2.4. Luciferase Assay for AP-1-Dependent Transcriptional Activity. The effect of the studied substances on AP-1 transcriptional activation was investigated in JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by an AP-1 DNA binding sequence as described earlier [43]. In brief, viable JB6 Cl41 AP-1 cells (8×10^3) suspended in 100 μ L of 5% FBS/MEM were added into each well of a 96-well plate. Plates were incubated overnight and the media was then replaced with fresh 5% FBS/MEM containing the extract residue at subtoxic concentration (see Section 2.3) in a total volume of 0.1 mL and the cells were incubated with the extract residue for 24 h. Then, the cells were disrupted for 1 h at room temperature with lysis buffer (0.1 M potassium phosphate buffer at pH 7.8, 1% Triton X-100, 1 mM DTT, 2 mM EDTA) and 30 μ L of lysate from each well were transferred into a plate for luminescent analysis and luciferase activity was measured. Results are expressed in Table 1 as an AP-1-dependent transcriptional activity in percentage relative to untreated control cells (100%).

3. Results and Discussion

In continuation of our search for cancer preventive compounds from marine organisms [32, 33, 38, 43, 44], we studied the influence of the extracts from 148 species of marine invertebrates and algae on the AP-1-dependent transcriptional activity in mouse epidermal JB6 Cl41 cells. The JB6 Cl41 cells undergo the malignant transformation under the treatment of EGF as a promoter. The transformation involves the activation of AP-1 nuclear factor which regulates the transcription of various genes related to inflammation, proliferation and metastasis. Thus, it can be said that the extracts which show the inhibition of the AP-1-dependent transcriptional activity in JB6 Cl41 cells may contain some

cancer preventive compounds. To study the effects of the ethanol soluble substances from the studied species on the AP-1 transcriptional activity, we used the luciferase assay and JB6 Cl41 AP-1 cells stably expressing a luciferase reporter gene controlled by an AP-1 DNA binding sequence. The results of the study are presented in Table 1.

We studied 148 species of marine invertebrates and algae, and there are 116 sponges, 7 ascidians, 10 species of algae, 1 species of Echinoidea, 4 species of Opisthobranchia, 2 species of Nudibranchia, 2 species of Gorgonacea, 5 species of Crinoidea, 1 species of Pennatulacea. The extracts of 71 species studied showed inhibitory effects (less than 80% of activity in comparison to control) on AP-1-dependent transcriptional activity at noncytotoxic concentrations, whereas extracts of 12 species demonstrated activation (more than 120% of activity in comparison to control) of the AP-1 nuclear factor in JB6 Cl41 cells. As shown in Table 1, sponges, belonging to genera *Spongia*, *Petrosia*, *Halichondria*, *Callyspongia*, *Spirastrella*, and *Axinella* are very promising sources of the AP-1 nuclear factor inhibitors, whereas 2 species of Opisthobranchia and 3 species of Algae contain activators of the AP-1 activity. We hope that our study will facilitate the further search and isolation of the AP-1 nuclear factor inhibitors and activators from marine sources.

4. Conclusion

In conclusion, the extracts of 71 species of marine organisms out of 148 species studied showed inhibitory effects on the AP-1 transcriptional activity in JB6 Cl41 AP-1 cells, and only 12 extracts demonstrated activation of the AP-1 nuclear factor. The search for natural compounds that inhibit or induce AP-1 activity may lead to the development of the new promising preventive or therapeutic drugs against various diseases including cancer.

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