S-10-6 Marine Natural Products against Tumor Development

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I. INTRODUCTION

Marine natural products provide unique information for cancer research. Lyngbyatoxin A, isolated from a Hawaiian blue-green alga, was shown to be the first tumor promoter derived from a marine organism in a two-stage carcinogenesis experiment on mouse skin. Subsequently, various kinds of marine natural products were reported to be tumor promoters, such as debromoaplysiatoxin, aplysiatoxin and palytoxin. Lyngbyatoxin A and aplysiatoxins, like 12-O-tetradecanoylphorbol-13-acetate (TPA), are potent activators of protein kinase C and thus are called the TPA-type tumor promoters [1] (TABLE 1). Recently, we have demonstrated that okadaic acid, dinophysistoxin-1 (35-methylokadaic acid) and calyculin A are tumor promoters as strong as the TPA-types on mouse skin, and act on the cells differently than the TPA-types. Okadaic acid, dinophysistoxin-1 and calyculin A bind to the same receptors, protein phosphatases 1 and 2A, and inhibit their activities, we called these the okadaic acid class of the non-TPA type tumor promoters [1]. The first part of this paper reviews tumor promotion of the okadaic acid class compounds and their mechanisms of action.

TABLE 1
 Tumor promoters derived from marine organisms

TPA-type	non-TPA type
Teleocidin class	Okadaic acid class
Lyngbyatoxin A (Teleocidin A-1)	Okadaic acid
Lyngbyatoxins B and C	Dinophysistoxin-1 (35-methylokadaic acid)
Aplysiatoxin class	Calyculins A, B, C, D, E, F, G and H
Debromoaplysiatoxin	Microcystins-LR ^{**} , YR and RR
Aplysiatoxin	
Bromoaplysiatoxin	
Oscillatoxin A	Palytoxin class
Anhydrodebromoaplysiatoxin	Palytoxin

^{**} Microcystins belong to the okadaic acid class. But the tumor promoting activity of microcystin LR was shown in the liver.

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Fig. 1. Structures of some tumor promoters of the okadaic acid class.

The second part of this paper reports that some marine natural products inhibit tumor promotion of okadaic acid on mouse skin. We have used a test on inhibition of tumor promotion for screening for anticarcinogenic agents, in particular, cancer chemopreventive agents. The aim of cancer chemoprevention is to inhibit the process of tumor development. Additionally, many of these compounds can be expected to inhibit regrowth after tumor resection surgery in humans. This paper first reports the results of discodermin A, and reviews the anticarcinogenic effects of sarcophytol A as well.

II. SIGNIFICANCE OF OKADAIC ACID CLASS COMPOUNDS IN TUMOR PROMOTION

Okadaic acid and dinophysistoxin-1 were isolated from the black sponge, *Halichondria okadai*, and calyculin A was isolated from the marine sponge, *Discodermia calyx* (Fig. 1). These three compounds bound to a catalytic subunit of protein phosphatases 1 and 2A, which catalyze dephosphorylation of phosphoserine and phosphothreonine, and strongly inhibited their activities. For example, the IC_{50} for the catalytic subunit of protein phosphatase 2A, isolated from human erythrocytes, was 0.07 nM for okadaic acid and 0.13 nM for calyculin A, respectively. The inhibitory activity of dinophysistoxin-1 was comparable to that of okadaic acid [1].

Potent tumor promoting activities of the three okadaic acid class compounds were shown in two-stage carcinogenesis experiments. Namely, initiation was conducted by a single application of 7,12-dimethylbenz(a)-anthracene (DMBA) to the skin of the backs of mice. From one week after initiation, 1 μ g of each compound was applied to the same area on the skin, twice a week, until week 30. The groups treated with DMBA plus okadaic acid, DMBA plus dinophysistoxin-1 and DMBA plus calyculin A all resulted in high percentages of mice with tumors, namely, maximal percentages of tumor-bearing mice were 86.7%, 100% and 93.3%, respectively. DNA isolated from tumors of the three groups of mice contained a mutation in the second nucleotide of codon 61 of the mouse c-H-ras gene. Thus, CAA changed to CTA, and glutamine changed to leucine. Since the same mutation had been found in DNA isolated from tumors of the group treated with DMBA plus TPA, we thought that tumor promoters of the okadaic acid class, as well as the TPA-types, induced clonal expansion, which is a specific growth of the initiated cells containing the mutation, through two different pathways; the okadaic acid pathway, through inhibition of protein phosphatase 1 and 2A activities, and the TPA-pathway, through activation of protein kinase C.

Fig. 2. Structures of discodermin A and sarcophytol A.

III. OKADAIC ACID PATHWAY AS A GENERAL MECHANISM OF TUMOR PROMOTION

Inhibition of dephosphorylation of proteins by okadaic acid resulted in an increase of phosphoproteins in the cells. These phosphoproteins seem to play a significant role in tumor promotion of various organs. We have recently demonstrated that okadaic acid in drinking water promoted carcinogenesis in the rat glandular stomach initiated with N-methyl-N'-nitro-N-nitrosoguanidine. Microcystin-LR, which is isolated from blue-green algae found in fresh and brackish water (Fig. 1) and belongs to the okadaic acid class (TABLE 1), promoted induction of glutathione S-transferase placental form foci, a biochemical marker of preneoplastic nodules, in rat liver initiated with diethylnitrosamine. Thus, tumor promotion in various organs can now be discussed as a common biochemical process [1].

Next, the okadaic acid pathway should be discussed in relation to human cancer development. There are endogenous proteinous inhibitors of protein phosphatase 1, inhibitors 1 and 2, in the cells. Inhibitory activity of inhibitor 2 was as strong as okadaic acid, based on molar ratios. Although a potent inhibitor of protein phosphatase 2A has not yet been identified, we think the endogenous inhibitors toward protein phosphatases 1 and 2A might act as tumor promoters in humans, when these inhibitors remain constitutively active, due to a disturbance in the normal regulation. Thus, we assume that the inhibitors take the okadaic acid pathway in the process of tumor promotion in human cancer development.

IV. DISCODERMIN A, A NEW INHIBITOR OF TUMOR PROMOTION

Discodermin A was isolated from the marine sponge, *Discodermia kiiensis* (Fig. 2). The compound was first found to be a peptide with antimicrobial activity against gram-positive bacteria and to have inhibitory activity on phospholipase A₂ [2]. Figure 3 shows inhibition of tumor promotion by discodermin A. A two-stage carcinogenesis experiment on mouse skin was initiated with a single application of 100 µg of DMBA, followed by repeated applications of 1 µg okadaic acid, twice a week. 15 min before each treatment with okadaic acid, 500 µg of discodermin A, the amount of which was 300 times more than that of okadaic acid, was applied on the same area of mouse skin. Discodermin A treatment reduced the percentages of tumor-bearing mice from 86.7% to 46.7% and the average numbers of tumors per mouse from 4.7 to 1.1 in week 20. The inhibitory activity on phospholipase A₂ might be partly involved in inhibition of tumor promotion.

V. SARCOPHYTOL A, AS A POSSIBLE CANCER CHEMOPREVENTIVE AGENT

Sarcophytol A, isolated from the soft coral, *Sarcophyton glaucum*, is one of the main compounds of the cembrane-type diterpenes. In 1989, we first reported that sarcophytol A inhibited tumor promotion of teleocidin on mouse skin. Later, we further showed that sarcophytol A inhibited tumor promotion of TPA, aplysiatoxin and okadaic acid. In addition, sarcophytol A inhibited chemical and viral carcinogenesis in other organs than the

Fig. 3. Inhibition of tumor promotion by discodermin A. The groups treated with DMBA and okadaic acid (○) and DMBA and okadaic acid plus discodermin A (●).

skin, which will be reviewed briefly. Treatment with a diet containing 0.01% sarcophytol A significantly inhibited development of large bowel cancer induced by N-methyl-N-nitrosourea in female CD-Fischer rats. A diet containing 0.05% sarcophytol A prolonged 50% mortality due to spontaneous thymic lymphoma in female AKR mice by 5 weeks. Treatment with a diet containing sarcophytol A inhibited spontaneous mammary tumors in female SHN mice and spontaneous liver tumors in male C3H/HeN mice [3]. As for the mechanisms of action of sarcophytol A, we think that sarcophytol A inhibits multifunctional processes in the cells. Antioxidant activity is one of the important effects of sarcophytol A. The study with ^3H -sarcophytol A revealed that its bioavailability seems to be very wide in various organs. Sarcophytol A has great potential to be developed as a non-toxic agent for the purpose of cancer prevention in humans.

Summary

This review article dealt with significant effects of marine natural products in carcinogenesis, namely as chemical probes to understand the process of carcinogenesis and as possible cancer preventive agents in humans.

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