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Title	Studies on antitumor and anti-cachectic activities of eicosapentaenoic acid-enriched phospholipids derived from marine echinoderms [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(水産科学) 甲第12213号
Issue Date	2016-03-24
Doc URL	http://hdl.handle.net/2115/61623
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Type	theses (doctoral - abstract and summary of review)
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File Information	Lei_Du_abstract.pdf (論文内容の要旨)

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学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）　　氏名：杜　　磊

学 位 論 文 題 目

Studies on antitumor and anti-cachectic activities of eicosapentaenoic acid-enriched phospholipids derived from marine echinoderms

（棘皮動物に豊富な EPA 結合型リン脂質の抗腫瘍ならびに抗悪液質作用に関する研究）

During the past 30 years, n-3 PUFAs, especially EPA, has received much attention because of its beneficial effects on human health, including its use in the prevention and treatment of cancer and cancer-associated cachexia (CAC). However, most of these clinical studies have been carried out using EPA bound to triacylglycerol (TAG) or ethyl esters (EE). Little information has been known on the effects of EPA bound to phospholipids (PLs). With their increasing bioavailability, new evidence has emerged on their biological activities and differences to EPA bound to TAG or EE. It has been reported that the marine echinoderms such as starfish *Asterias amurensis* and sea cucumber *Cucumaria frondosa* are good natural sources of EPA-enriched phospholipids (EPA-PL). Thus, the starfish *A. amurensis* phospholipids (SFP) and the sea cucumber *C. frondosa* phospholipids (SCP) were extracted and then the antitumor and anti-cachectic activities of SFP and SCP were investigated both *in vitro* and *in vivo*.

It is well known that the major portion of PLs have been considered to be hydrolyzed in small intestine. In addition, since commercial PLs produced from plant sources are usually processed from soybean, the soy-PL liposome was also prepared and used it as control. Therefore, the transport and uptake effects of liposomes consisted of SFP, SCP or soy-PL in small intestinal epithelial cell models were investigated in chapter 1. The results showed that SFP and SCP contained 42 % and 47.9 % EPA, respectively. The liposomes composed of SFP or SCP exhibited higher transport and uptake effects in both the Caco-2 and M cell monolayer models than soy-PL liposomes and it may be due to the proper size of the liposomes and increase in unsaturation of intestinal epithelial cell membrane and affection on tight junctions of Caco-2 and M cell monolayers.

In chapter 2, the results manifested that SFP and SCP liposomes exhibited potent antitumor effects on several kinds of cancer cells and in S180 ascitic tumor-bearing mice. SFP and SCP liposomes showed a dose-dependent inhibitory effects on the viability of S180, P388, HepG2 and HeLa cells. In S180 ascitic tumor-bearing mice, a rapid increase in ascitic fluid with tumor cells growth and high early mortality rate were observed. Dietary SFP and SCP liposomes (100 mg/kg/day) for totally 14 consecutive days prolonged the life span of tumor-bearing mice. In addition, it was also found that oral treatment of SFP and SCP liposomes decreased the ascitic fluid volume and the tumor cells viability in tumor-bearing mice. It may be concluded that SFP and SCP increase the life span of tumor-bearing mice via decreasing the ascitic fluid volume and killing the tumor cells. The present data also revealed that oral administration of SFP and SCP liposomes significantly restored GSH

levels as well as SOD and CAT activities in the liver of tumor-bearing mice, suggesting that the antitumor property of SFP and SCP may also be partly attributed to their potent antioxidant activities. Moreover, the observation of acridine orange (AO) and ethidium bromide (EB) staining indicated that SFP and SCP killed the S180 ascitic tumor cells via induction of apoptosis. The data in this chapter also implied that the mechanism of SFP and SCP in inducing apoptosis of tumor cells must involve activation of mitochondrial apoptotic pathways.

During the experimental period in chapter 2, it was found that the body weight of the S180 ascitic tumor-bearing mice were consecutively lost but oral administration of SFP and SCP liposomes could suppress the decline significantly. Therefore, the anti-cachectic activity of SFP and SCP in S180 ascitic tumor-bearing mice (CAC mice) were next investigated in chapter 3. The results demonstrated that oral administration of SFP and SCP liposomes (100 mg/kg/day) for totally 14 consecutive days could suppress the cachectic body weight loss in tumor-bearing mice. Excessive loss of white adipose tissue (WAT) and significant increase of serum non-esterified fatty acids (NEFA) level in CAC mice were also observed in this study, but these changes were suppressed by SFP and SCP administration, suggesting the involvement of lipolysis in tumor-induced weight loss and the amelioration of SFP and SCP for weight loss by inhibiting lipolysis in CAC mice. However, dietary SFP and SCP could not ameliorate the anorexia of CAC mice. Thus, SFP and SCP prevented cachectic weight loss in CAC mice, most likely by preservation of adipose tissue through inhibiting lipolysis and the mechanisms of anti-cachectic actions of SFP and SCP must be due to their anti-inflammatory properties, inhibiting the expression of lipid mobilizing factor and key rate-limiting lipases, preventing the WAT browning and partially recovering the function of lipogenesis in CAC mice.

Although the data in chapter 3 revealed that SFP and SCP were effective in preventing adipose tissue loss in CAC mice, it is currently unclear whether SFP and SCP can suppress the cytokine-induced lipolysis in adipocytes. Therefore, to better understand the antilipolytic effects of SFP and SCP and further elucidate the possible mechanisms by which SFP and SCP inhibit over lipolysis, pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) were employed and then used to stimulate the over lipolysis of 3T3-L1 mature adipocytes in chapter 4. The results showed that treatment with SFP and SCP could inhibit the proinflammatory cytokine-induced lipolysis in 3T3-L1 adipocytes significantly. These findings suggest that the antilipolytic actions of SFP and SCP in 3T3-L1 adipocytes are responsible for activation of AMP-activated protein kinase (AMPK) and upregulating the expression of regulators of key rate-limiting lipases such as G0/G1 switch gene 2 (G0S2) and perilipin as well as ameliorating the function of fatty acids uptake into adipocytes. Moreover, the data in this chapter also revealed that SFP and SCP could increase the glucose consumption and uptake in 3T3-L1 adipocytes in the absence or presence of TNF-α via activation of phosphoinositide-3-kinase (PI3K) and upregulating the mRNA expression of glucose transporter type 4 (GLUT4). It indicates that EPA-PL may attenuate the TNF-α-induced insulin resistance in adipocytes.

In conclusion, based on all data in this study, it is suggested that the EPA-PL derived from starfish *A. amurensis* and sea cucumber *C. frondosa* exert potent antitumor and anti-cachectic activities both *in vitro* and *in vivo*. Although further study on human intervention trial is needed and further investigations are also warranted to confirm the structure-activity relationship of PLs types and the variety of n-3 PUFAs, these encouraging findings may provide the basis for marine PLs supplementation in cancer and cancer-associated cachexia therapy.