Life Sciences xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Effects of Antarctic krill docosahexaenoic acid on MCF-7 cell migration and invasion induced by the interaction of CD95 with caveolin-1

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ARTICLE INFO

Keywords:
Antarctic krill
DHA
CD95
Caveolin-1
Migration
Invasion

ABSTRACT

Tumor metastasis leads to a poor prognosis in breast cancer, yet the mechanisms remain unclear. Docosahexaenoic acid (DHA) extracted from Antarctic krill is an optical isomer of common DHA and has a much stronger anti-neoplastic effect. In this work, the migration and invasion abilities of MCF-7 cells treated with low concentrations of Antarctic krill DHA were evaluated. Low concentrations of Antarctic krill DHA significantly reduced the numbers of migrating and invasive MCF-7 cells, whereas the cell numbers decreased slowly in the CD95-silenced MCF-7 cells, which implies that CD95 might be involved in cell migration and invasion. Additionally, co-immunoprecipitation and Western blotting demonstrated that Antarctic krill DHA induced the accumulation of CD95 and caveolin-1 interaction, resulting in the down-regulation of MMP2 expression through the FAK/SRC/PI3K/AKT signaling pathway. In conclusion, Antarctic krill DHA enhanced the interaction between CD95 and caveolin-1, which may led to an inhibitory effect on cell migration and invasion *via* the FAK/SRC/PI3K/AKT signaling pathway. Our study indicates that Antarctic krill DHA has great potential for tumor therapy and has revealed a new metastatic mechanism mediated by the interaction of CD95 with caveolin-1.

1. Introduction

As one of the most common cancers, breast cancer has the highest cancer incidence in women and is one of the leading causes of cancer-associated morbidity and mortality [1,2]. There is an estimated annual mortality of 465,000, and > 1.38 million new patients are diagnosed with breast cancer every year worldwide [2]. Breast cancer development is a multi-step process [3,4]. Once breast cancer develops into the later stages, metastatic lesions develop in other organs and/or tissues, which are more difficult to treat, leading to more suffering by the patients and higher mortality. A series of interrelated processes leads to the occurrence of metastasis, including local invasion, cell transport, cell arrest, adherence, extravasation, and proliferation [5,6]. Although many studies have previously explained this complex process, the detailed mechanisms of metastasis have not been elucidated.

CD95 (also known as Fas) is a death receptor expressed on all cell surfaces that receives the signal to induce cell apoptosis [7]. CD95 is also related to cell migration and invasion [8,9]. Caveolin-1 is the main protein in caveolae on the cell membrane, which play important roles in vesicle trafficking, signal transduction, calcium signaling and membrane recycling [10,11]. Similar to caveolae, caveolin-1 plays an important role in endocytosis, cholesterol homeostasis, vesicle trafficking, lipid transport and storage, the regulation of protein turnover, the control of cell signaling, and cell death [12–14]. Caveolin-1 is also closely related to cell migration and invasion [10]. The interaction of CD95 with caveolin-1 has been shown to be related to cell apoptosis [15,16]. However, the effect of the interaction between CD95 and caveolin-1 on breast cancer metastasis is unclear and requires further study.

Docosahexaenoic acid (DHA) is an omega-3 poly-unsaturated fatty acid that is mainly obtained from fish oil. As an essential nutrient, DHA plays key roles in the neurogenesis [17], anti-viral [18], and anti-aging [19] processes. In the 1970s, DHA was found to exhibit an anti-tumor effect [20,21]. Previous studies showed that DHA induced tumor cells to undergo apoptosis through certain cell signaling pathways [22–24]. Previous work by our group isolated an *E*-configuration DHA from Antarctic krill oil, which exhibited a more effective growth inhibition effect on several tumor cell lines than normal DHAs [25]. However, the anti-neoplastic mechanism needs further study.

A previous study indicated that DHA induced CD95 translocation to lipid rafts [26]. Caveolae are one type of lipid raft with multiple biological functions. Furthermore, the expression level of caveolin-1, the main protein in caveolae, has been reported to be up-regulated in MCF-7 cells, leading to an increase in metastasis [27,28]. Therefore, CD95

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https://doi.org/10.1016/j.lfs.2017.11.011
Received 24 July 2017; Received in revised form 9 October 2017; Accepted 7 November 2017

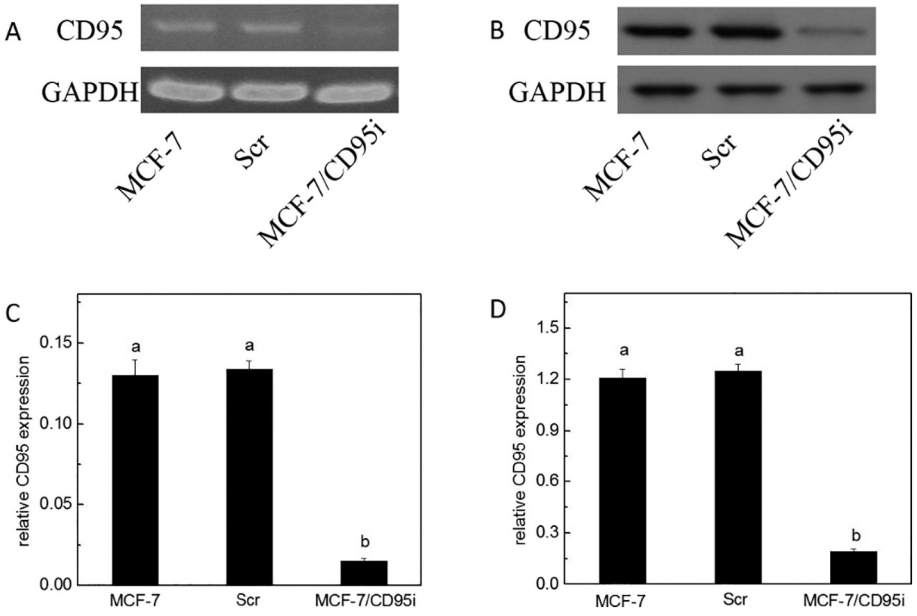

Fig. 1. CD95 interference efficiency detected by RT-PCR (A) and Western blotting (B). The relative transcription (C) and expression (D) levels of CD95 were normalized to the transcription and expression levels of GAPDH. Values sharing a common (a–b) in each dot did not show any statistically significant differences. $P < 0.05$.

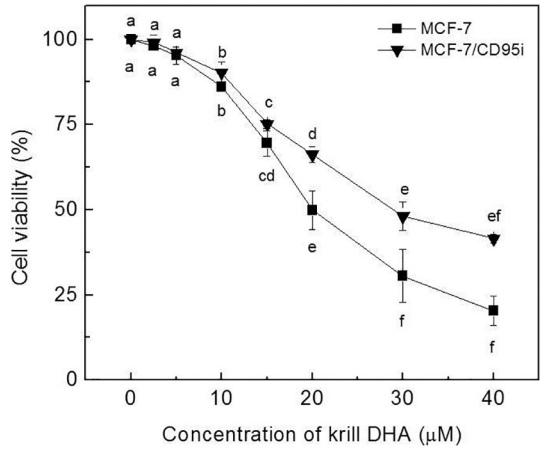

Fig. 2. The inhibitory effect of Antarctic krill DHA on the growth of MCF-7 and MCF-7/CD95i cells. Antarctic krill DHA was dissolved in DMSO to concentrations of 20 μM and 40 μM, and 0.5% DMSO was used as a control. Values sharing a common (a–f) in each dot did not show any statistically significant differences. $P < 0.05$.

may interact with caveolin-1 following DHA exposure. Our study aims to investigate the inhibitory effect of Antarctic krill DHA, which has a stronger anti-neoplastic effect, on migration and invasion and to elucidate the underlying mechanism.

2. Materials and methods

2.1. Materials

MCF-7 cells were purchased from Cell Bank, Chinese Academy of Sciences, Shanghai, China. Antarctic krill DHA was obtained from Antarctic krill oil. The medium (DMEM), fetal bovine serum (FBS), and antibiotics were purchased from HyClone, Logan, USA. The CCK-8 kit was purchased from KeyGen Biotech, Nanjing, China. DMSO was purchased from Coolaber, Beijing, China.

2.2. Cell culture and cell viability

The MCF-7 cell line was purchased from Cell Bank, Chinese Academy of Sciences (Shanghai, China), and the cells were cultured in DMEM supplemented with 10% FBS (HyClone, USA) and 1% antibiotics (the final concentrations of penicillin and streptomycin were 100 U/mL and 100 μg/mL, respectively). The cells were grown in a 25-cm^2 tissue culture flask at 37 °C in 5% humidified CO_2. After harvesting by trypsinization, three thousand cells per well were seeded into a 96-well plate and cultured for 24 h to allow adherence. Then, the cells were exposed to each sample and incubated for 16–18 h. DHA was dissolved in DMSO, and 0.5% DMSO was used as a negative control. After treatment, 10 μL of the CCK-8 reagent (KeyGen Biotech, China) was added to the wells and incubated for an additional 2 h. The absorbance (A) of each well was measured using a multi-well reader (Thermo Fisher Scientific, USA) at 450 nm and 630 nm. Cell viability was calculated as follows: Cell viability (%) = $(A_{450} - A_{630})/(A_{450} - A_{630})*100$.

2.3. Lentiviral infection

Short interfering oligonucleotides specific for CD95 were designed on the Open Biosystems website (http://rnaidesigner.thermofisher.com/rnaiexpress/). The oligonucleotides were annealed and then ligated into the pHBLV-U6-ZsGreen-Puro plasmid (Hanbio Biotechnology), which was digested with *Bam*HI and *Xho*I (Takara, Japan). The recombinant plasmid was co-transfected into human embryonic kidney 293T cells with two packaging plasmids (pVSV-G and psPAX2) using the Liposomal Transfection Reagent (HieffTrans, China) according to the manufacturer's protocol. After transfection, the supernatant containing retrovirus was collected at 24 h, 48 h and 72 h. Then, the supernatant was centrifuged at 4000 r/min for 30 min in a 100-kDa ultrafiltration centrifuge tube. MCF-7 cells were infected with the viral supernatant in the presence of 5 μg/mL of polybrene (Sigma, USA). Down-regulation of CD95 in the MCF-7 cell line was confirmed, and the cells were labeled MCF-7/CD95i cells.

2.4. RT-PCR analysis

Total RNA was prepared from the MCF-7 cells using TRIzol (Takara, Japan). Then, 5 μg of total RNA was transcribed into cDNA with oligo dT using the AMV reverse transcriptase (Takara). The rTaq DNA polymerase (Takara) was used to amplify the cDNA with the following primers: CD95-Forward: 5′-TACGAGTGACTTGGCTGGAG-3′ and CD95-Reverse: 5′-CGGGACTAAGACGGGGTAAG-3′; and GAPDH-Forward: 5′-TGACGATGCGGACGAGAT-3′ and GAPDH-Reverse: 5′-GCAGGAGGC AGGGAGATAG-3′. Agarose gel electrophoresis (1%) was used to analyze the PCR products, and the band intensities were measured with the