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Docosahexaenoic acid induces proteasome-dependent degradation of estrogen receptor α and inhibits the downstream signaling target in MCF-7 breast cancer cells☆

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Abstract

About two thirds of breast cancers in women are hormone-dependent and require estrogen for growth, its effects being mainly mediated through estrogen receptor α (ERα). Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) have opposite effects on carcinogenesis, with DHA suppressing and AA promoting tumor growth both in vitro and in vivo. However, the mechanism is not clear. Here, we examined whether the effect is mediated through changes in ERα distribution. MCF-7 cells, an ERα-positive human breast cancer cell line, was cultured in estrogen-free medium containing 0, 10 or 60 μM DHA or AA, then were stimulated with estradiol. DHA supplementation resulted in down-regulation of ERα expression (particularly in the extranuclear fraction), a reduction in phosphorylated MAPK, a decrease in cyclin D1 levels and an inhibition in cell viability. In contrast, AA had no such effects. The DHA-induced decrease in ERα expression resulted from proteasome-dependent degradation and not from decreased ERα mRNA expression. We propose that breast cancer cell proliferation is inhibited by DHA through proteasome-dependent degradation of ERα, reduced cyclin D1 expression and inhibition of MAPK signaling.

Keywords: Breast cancer; Docosahexaenoic acid; Arachidonic acid; Estrogen receptor α; MAPK; Cyclin D1; Estradiol; Proteasome activity

1. Introduction

Breast cancer is the most common cancer in women worldwide [1], and about two thirds of cases have hormone-dependent cancers that contain estrogen receptors (ERs) and require estrogen for tumor growth [2]. The actions of estrogen are mainly mediated through two ERs, ERα and ERβ, which are members of the steroid/nuclear hormone receptor superfamily [3–5]. ERα and ERβ play different roles in mediating the actions of estrogen, with ERα, but not ERβ, being essential for female mammary gland development [6]. In addition, ERα promotes, while ERβ inhibits, estrogen-stimulated breast cancer cell proliferation [7–10].

ERα is a hormone-activated transcription factor for genomic signaling and is mainly located in the nucleus in target cells and human breast tumors [5,11,12] but is also detected in the cytosol in human breast tumors [13]. In MCF-7 human breast cancer cells and in ERα-transfected COS-7 cells, it has been demonstrated that 17-β-estradiol (E_2), the major form of estrogen, stimulates the translocation of cytosolic ERα to lipid rafts [14], where membrane-associated ERα activates growth receptors and/or E_2-triggered nongenomic signaling, especially the mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) pathway [15–17]. In addition, drug resistance can result from long-term treatment with the antiestrogen tamoxifen, which increases the translocation of ERα out of the nucleus and enhances E_2 stimulation of the MAPK pathway in MCF-7 cells [18]. These results indicate that extranuclear ERα plays an important role in the E_2-mediated triggering of MAPK signaling, causing breast cancer proliferation and drug resistance. We hypothesized that reducing ERα expression, particularly in the extranuclear compartment, might inhibit the proliferation of estrogen-responsive breast cancers.

Polyunsaturated fatty acids (PUFAs) are classified into n-3 and n-6 PUFAs and are primarily found in phospholipids in the plasma membrane [19,20]. Most animal or cell culture studies have shown that n-3 PUFAs reduce, and n-6 PUFAs increase, the risk of breast cancer [20–22]. However, the mechanism is not clear. We hypothesized that breast cancer growth may be inhibited by docosahexaenoic acid (DHA, 22:6n-3) through changes in ERα distribution and related downstream signaling, while arachidonic acid (AA, 22:4n-6) would have no such effects.

Abbreviations: AA, arachidonic acid (20:4n-6); BSA, bovine serum albumin; CD-FBS, charcoal/dextran-stripped fetal bovine serum; DHA, docosahexaenoic acid (22:6n-3); DMSO, dimethylsulfoxide; EPA, eicosapentaenoic acid (20:5n-3); ER, estrogen receptor; MAPK, mitogen-activated protein kinase; PUFA, polyunsaturated fatty acids.

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2. Materials and methods

2.1. Cell line and culture conditions

Culture media were purchased from Gibco Invitrogen (Grand Island, NY, USA), and unless otherwise specified, all chemicals were from Sigma (St. Louis, MO, USA). MCF-7 cells were obtained from the Bioresource Collection and Research Center (Hsing-Jue, Taiwan) and routinely cultured in Dulbecco's modified Eagle medium (DMEM) with L-glutamine and 110 mg/L of sodium pyruvate containing 5% fetal bovine serum (FBS), 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37°C in a 5% CO_2 incubator. For experiments, the cells (5×10^4 cells/ml) in DMEM containing 5% FBS were seeded in dishes or plates for 48 h, then the medium was replaced with phenol red-free DMEM with 1% charcoal/dextran-stripped FBS (CD-FBS) supplemented with vehicle (bovine serum albumin, or BSA) or BSA-bound DHA or AA (see below) and/or MG132 and the cells incubated for 24–48 h before stimulation with 10 nM E_2 for the indicated time. E_2 (Steraloids Inc. Wilton, NH, USA) was dissolved in ethanol, while MG132 and fluorogenic proteasome substrate III (Calbiochem, San Diego, CA, USA) was dissolved in dimethylsulfoxide (DMSO). The final concentrations of BSA, ethanol and DMSO were 1%, 0.1% and 0.1%, respectively.

2.2. Preparation of albumin-bound DHA or AA

All steps were at room temperature. DHA or AA was solubilized by preparing a complex of the sodium salt with fatty acid-free BSA at a molar ratio of 3:1. In brief, pure DHA (18.25 μl) or AA (16.51 μl) (Nu-Chek Prep, Elysian, MN, USA) was mixed with 0.25 ml of 0.2 M NaOH (equimolar amounts), then 1.089 g of fatty acid-free BSA (0.33 mM) and 50 ml of 25 mM HEPES buffer, pH 7.0, were added and the mixture shaken for 5 h. The BSA-bound DHA or AA was filtered through a 0.22-μm filter and stored as aliquots in a −20°C freezer.

2.3. Preparation of charcoal/dextran-stripped FBS

All steps were at room temperature. The FBS was stripped twice with charcoal and dextran to remove steroid hormones. Five grams of charcoal was washed twice with 500 ml of distilled water, then 0.5 g of dextran (Amersham Bioscience, Buckinghamshire, England) and 500 ml of distilled water were added and the mixture stirred for 10 min, followed by centrifugation at 6000×*g* for 10 min. The supernatant was discarded and the rinsed charcoal/dextran stirred with 500 ml of FBS for 30 min, then the mixture was centrifuged at 6000×*g* for 30 min. The supernatant was collected and stirred with a fresh preparation of rinsed charcoal/dextran for 30 min, then the mixture was centrifuged as above and the charcoal/dextran-stripped FBS (CD-FBS) filtered through a 0.22-μm filter and stored as aliquots in a −80°C freezer.

2.4. Preparation of whole cell lysates and extranuclear and nuclear protein extracts

To prepare whole cell lysates, the cells were washed with ice-cold PBS, then scraped off and sonicated in lysis buffer [20 mM HEPES, pH 7.3, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5 mM dithiothreitol and a protease inhibitor cocktail mix (Complete, Roche, Basel, Switzerland)]. Extranuclear and nuclear fractions were prepared by extraction with hypotonic and hypertonic buffers as described previously [23], with some modifications. In brief, after washing, the cells were incubated for 15 min in hypotonic buffer (10 mM KCl, 10 mM HEPES, pH 7.9, 1 mM EDTA, 0.6% Triton X-100, 1 mM PMSF, 0.5 mM dithiothreitol and protease inhibitor cocktail), then were gently scraped off and collected in an Eppendorf tube, passed through a pipette 50 times every 5 min for 30 min and centrifuged at 18,000×*g* at 4°C for 15 min. The supernatant was collected as the extranuclear fraction. The pellet was resuspended in PBS and centrifuged again at 18,000×*g* at 4°C for 15 min to remove extranuclear protein, then the pellet was resuspended in hypertonic buffer (400 mM KCl, 20 mM HEPES, pH 7.9, 1 mM EDTA, 20% glycerol, 1 mM PMSF, 0.5 mM dithiothreitol and protease inhibitor cocktail), and the suspension vortexed well, incubated on ice for 15 min and centrifuged at 18,000×*g* at 4°C for 15 min. The final supernatant was collected as the nuclear fraction. The lysates were stored at a −80°C freezer until analysis.

2.5. Western blot analysis of ERα, cyclin D1 and MAPK expression

The protein concentration of the lysates was measured using a Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), then 25 μg of protein was electrophoresed on SDS gels and subjected to Western blotting using rabbit polyclonal antibodies (1:1000) against human ERα (Santa Cruz Biotech.) or human phospho-p44/42 MAPK or rat p44/42 MAPK (both from Cell Signaling) or mouse monoclonal antibodies against human cyclin D1 (1:2000) or rabbit GAPDH (1:2500) (both from Cell Signaling) as described previously [24].

2.6. Proteasome activity assay

The proteasome activity in the extranuclear fraction was measured using a fluorogenic proteasome substrate as described previously with some modifications [25]. The extranuclear fraction was prepared as described above but using hypotonic buffer without the PMSF, dithiothreitol or protease inhibitor cocktail. Each sample was assayed in triplicate in the absence (DMSO vehicle) or presence of 10 μM MG132 in 96-well plates. A sample of extranuclear protein (15 μg; 2–5 μl, made to 5 μl with the above hypotonic buffer) was added to 150 μl of 20 mM Tris–HCl, pH 7.8, 0.5 mM EDTA and 0.035% SDS, then the reaction was initiated by adding 1.5 μl of fluorogenic proteasome substrate III (Suc-Leu-Leu-Val-Tyr-AMC, Calbiochem) (final concentration, 100 μM) and the sample incubated for 15 min at room temperature. The increase in fluorescence was measured every 30 min for 2 h using a fluorometric plate reader with excitation at 360

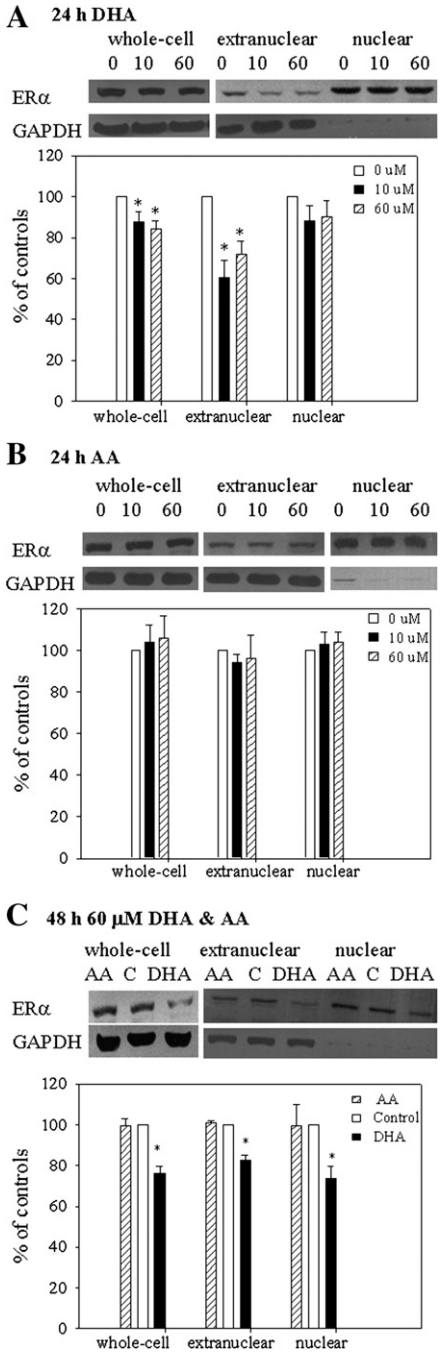

Fig. 1. Effect of DHA and AA supplementation on ERα expression in whole cell lysates and extranuclear and nuclear fractions of MCF-7 cells by Western blotting. Cells were pretreated with 0, 10 or 60 μM DHA (A) or AA (B) for 24 h or with 60 μM DHA or AA for 48 h (C), then were stimulated with 10 nM E_2 for 4 h. Western blot analysis was performed on whole cell lysates and the extranuclear and nuclear fractions using anti-ERα antibody. GAPDH was used as the loading control for comparison of whole cell lysate and extranuclear fraction proteins. Because GAPDH was barely detectable in the nuclear fraction, nuclear ERα expression was expressed as a percentage of the value in the 0 μM DHA or AA control. Asterisk indicates a significant difference compared to controls. The data are the mean±S.E.M. for four independent experiments for all treatments, except for the 48 h 60 μM DHA- or AA-treated whole cell lysate samples (n=3).

nm and emission at 460 nm. The difference in fluorescence between the samples with and without MG-132 was taken as an estimate of proteasome activity and was expressed as a percentage of that in the controls.

2.7. Reverse transcription polymerase chain reaction for ERα mRNA expression

Total RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer's instructions. The RNA (2 μg) was reverse transcribed with oligo(dT) using ImProm-II Reverse Transcriptase (Promega) in a 20-μl reaction volume. The reverse transcription (RT) product corresponding to 0.1 μg of initial RNA was subjected to 25 cycles of polymerase chain reaction (PCR) amplification. The amplification conditions were denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 60 s. The specific primer pairs were ERα (forward, 5′-AGCACCCAGTGAAGCTACTG-3′; reverse, 5′-GAGGCACACAAACTCCTCTC-3′; product 154 bp) and GAPDH (forward, 5′-GATGACATCAAGAAGGTGGTG-3′; reverse, 5′-GCTGTAGCCAAATTCGTTGTC-3′; product 197 bp). The PCR products were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and photographed under UV light using a digital image capture system.

2.8. Quantification of cell viability

Cell viability was quantified by measuring mitochondrial dehydrogenase activity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Cells in 24-well plates were treated with 0, 10 or 60 μM DHA or AA for 24 h with or without subsequent treatment with 10 nM E_2 for 24 h, then MTT (0.5 mg/ml) was added and incubation continued at 37°C for 4 h. The formazan crystals were solubilized in DMSO and the absorbance at 570 nm was read on a spectrophotometer.

2.9. Statistical analysis

The data are presented as mean±S.E.M. Statistical differences between the results of treatments were determined by one-way ANOVA followed by Tukey's test using the SAS program (version 9.1.3, SAS Institute, Cary, NC, USA). A two-sided P value of $\leq .05$ was considered statistically significant.

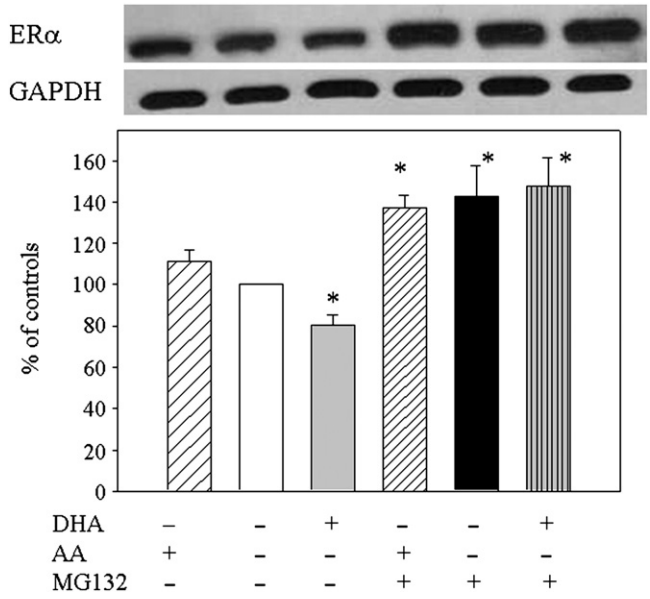

Fig. 3. Western blots showing the effect of a proteasome inhibitor on the DHA-induced decrease in ERα expression in MCF-7 cells. Cells were pretreated with 0 or 60 μM AA or DHA in the absence or presence of 10 μM MG132 for 48 h, then were stimulated with 10 nM E_2 for 4 h. Western blot analysis was performed on whole cell lysates using anti-ERα antibody. GAPDH was used as the loading control. The levels are expressed as a percentage of the control value. The data are the mean±S.E.M. for three independent experiments for all treatments. Asterisk indicates a significant difference compared to the untreated controls.

3. Results

3.1. Effect of DHA and AA supplementation on ERα expression in whole cell lysates and the extranuclear and nuclear fractions

ERα expression in the whole cell lysate and extranuclear fraction, but not the nuclear fraction, was significantly reduced after 24 h supplementation with 10 or 60 μM DHA compared to no DHA supplementation (Fig. 1A). In contrast, 24-h supplementation with 10 or 60 μM AA had no effect on ERα levels in any of the samples (Fig. 1B).

We also examined ERα expression in cells treated for 48 h with 0 or 60 μM DHA or AA and found that ERα levels in the DHA-treated cells were significantly decreased in the whole cell lysate and extranuclear and nuclear fractions (Fig. 1C). In contrast, the AA-treated cells showed no change in ERα levels in any of the samples.

3.2. Lack of effect of DHA and AA on ERα mRNA expression

In order to determine whether the DHA-mediated decrease in ERα levels resulted from altered ERα mRNA expression, ERα mRNA

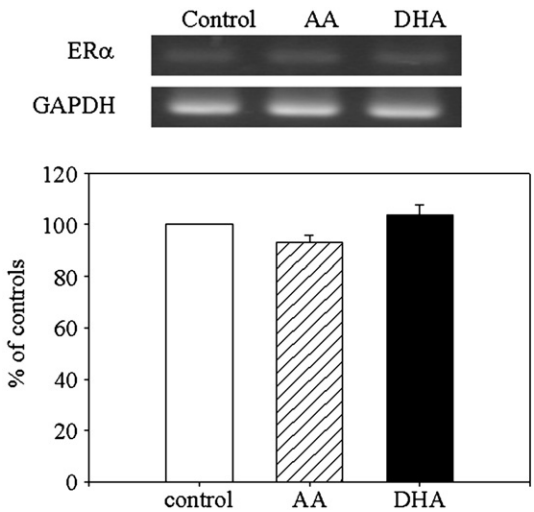

Fig. 2. Lack of effect of DHA and AA supplementation on ERα mRNA levels in MCF-7 cells as shown by RT-PCR. Cells were pretreated with 0 or 60 μM AA or DHA for 48 h, then stimulated with 10 nM E_2 for 4 h, and ERα mRNA levels were determined. GAPDH mRNA was used as the loading control. The levels are expressed as a percentage of the control value. The data are the mean±S.E.M. for four independent experiments for all treatments.

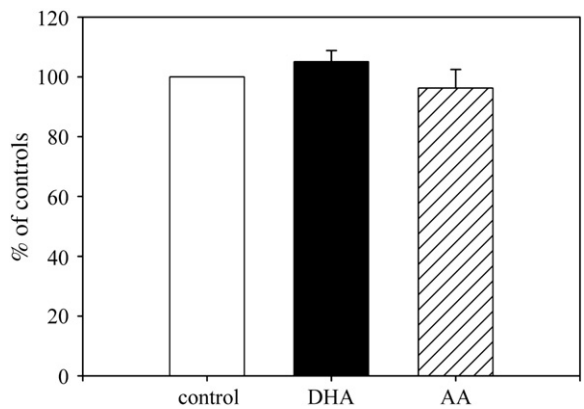

Fig. 4. Lack of effect of DHA and AA supplementation on proteasome activity in the extranuclear fraction of MCF-7 cells. Cells were pretreated with 0 or 60 μM DHA or AA for 48 h, then stimulated with 10 nM E_2 for 4 h. Proteasome activity in the extranuclear fraction was determined using fluorogenic proteasome substrate III and is expressed as a percentage of that of the controls without DHA or AA supplementation. The data are the mean±S.E.M. for four independent experiments for all treatments.

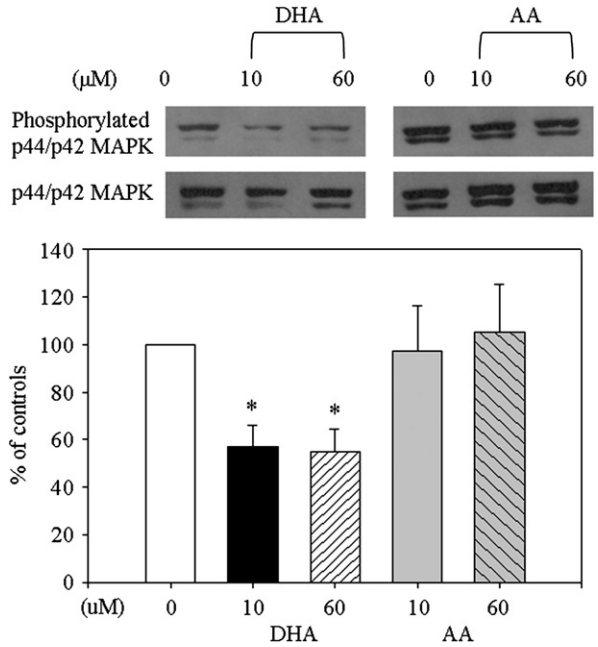

Fig. 5. Effect of DHA and AA supplementation on phosphorylated and total p44/p42 MAPK levels in MCF-7 cells. Cells were pretreated with 0, 10 or 60 μM DHA or AA for 24 h, then were incubated with 10 nM E_2 for 15 min. Western blot analysis was performed on whole cell lysates using the indicated antibodies. Total p44/p42 MAPK was used as the loading control. The levels are expressed as a percentage of the levels in the 0 μM DHA or AA controls. Asterisk indicates a significant difference compared to controls. The data are the mean±S.E.M. for four independent experiments for all treatments.

expression was measured in cells treated for 48 h with 0 or 60 μM DHA or AA and was found to be unaffected by DHA or AA (Fig. 2).

3.3. Effect of a proteasome inhibitor on the DHA-induced decrease in ERα expression

We then used the proteasome inhibitor MG132 to examine whether the DHA-induced decrease in ERα expression resulted from proteasome-dependent degradation. As shown in Fig. 3, MG132 blocked ERα degradation in DHA-treated cells. There was no significant difference in ERα expression in MG132-treated cells in the presence or absence of DHA or AA, and the values were significantly higher than in those in non-MG132-treated controls.

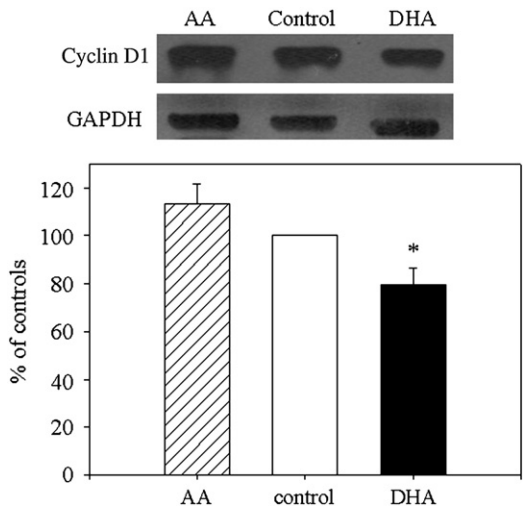

Fig. 6. Effect of DHA and AA supplementation on cyclin D1 expression in MCF-7 cells. Cells were pretreated with 0 or 60 μM DHA or AA for 48 h, then were stimulated with 10 nM E_2 for 4 h. Western blot analysis was performed on whole cell lysates using the indicated antibodies. GAPDH was used as the loading control. The levels are expressed as a percentage of the levels in the 0 μM DHA or AA controls. Asterisk indicates a significant difference compared to controls. The data are the mean±S.E.M. for three independent experiments for all treatments.

3.4. Lack of effect of DHA or AA on proteasome activity

To determine whether DHA induced ERα degradation by increased proteasome activity, we measured proteasome activity in the extra-nuclear fraction of cells treated for 48 h with 0 or 60 μM DHA or AA and found that it was unchanged by DHA or AA (Fig. 4).

3.5. Effect of DHA and AA on the MAPK signaling pathway

We then examined the effect of DHA on the MAPK signaling pathway. Phosphorylated MAPK induced by E_2 stimulation for 15 min were significantly reduced after 24 h supplementation with 10 or 60 μM DHA compared to controls (0 μM) (Fig. 5). In contrast, when AA supplementation was used, the difference in phosphorylated MAPK levels in 0, 10 and 60 μM AA-treated cells was nonsignificant.

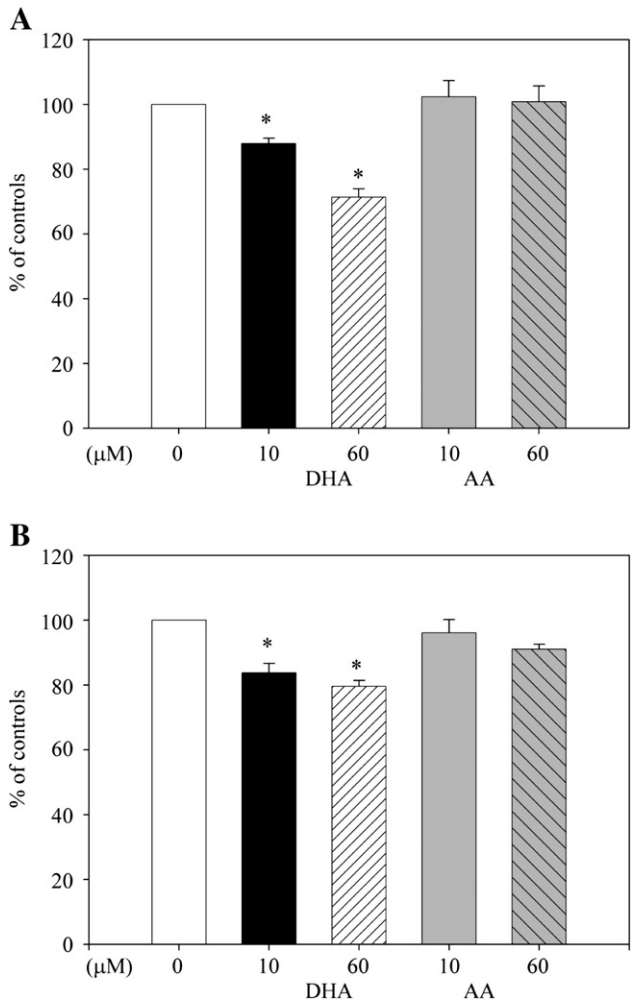

Fig. 7. Effect of DHA and AA supplementation on the viability of MCF-7 cells. Cells were pretreated with 0, 10, or 60 μM DHA or AA for 24 h alone (A) or followed by addition of 10 nM E_2 for 24 h (B), then were incubated with MTT for 4 h. Cells without DHA or AA supplementation (0 μM) were used as the controls. The data are the mean±S.E.M. for five independent experiments for all treatments. * indicates a significant difference compared to controls.

3.6. Effect of DHA and AA on cyclin D1 expression

As cyclin D1 is a known downstream target of ERα [26], we examined whether the DHA-induced decrease in ERα expression had any effect on cyclin D1 levels. Expression of cyclin D1 was significantly reduced after 48 h supplementation with 60 μM DHA compared to controls (0 μM) (Fig. 6). In contrast, using AA supplementation, cyclin D1 expression tended to increase, but the difference was not significant.

3.7. Effect of DHA and AA on cell viability

We next examined whether the reduced ERα expression, cyclin D1 levels and MAPK signaling seen with DHA supplementation inhibited MCF-7 cell proliferation. As shown in Fig. 7A, viability was significantly decreased in cells supplemented for 24 h with 10 or 60 μM DHA compared to controls but was not affected in 10 or 60 μM AA-treated cells. The viability of cells treated for 24 h with DHA or AA, followed by E_2 stimulation for 24 h, was similar to the above (Fig. 7B).

4. Discussion

To our knowledge, this is the first study demonstrating that DHA supplementation reduces levels of ERα, phosphorylated MAPK and cyclin D1 in a human breast cancer cell line. In contrast, AA had no such effects. The DHA-induced decrease in ERα expression resulted from proteasome-dependent degradation and not from decreased ERα mRNA expression, and neither DHA nor AA had effect on proteasome activity. We conclude that DHA inhibited breast cancer cell proliferation by proteasome-dependent degradation of ERα and reduced cyclin D1 expression and MAPK signaling, while AA had no such effects. We propose that the n-3 PUFA DHA has potential as both a natural antibreast cancer supplement that can inhibit cancer growth and as an alternative adjunctive endocrine therapy to attenuate the resistance and hypersensitivity of estrogen-responsive breast cancers.

It is interesting to know that, like the antiestrogen compound ICI [27], DHA decreased ERα expression in MCF-7 cells without affecting ERα mRNA expression. It has been reported that E_2 increases ERα degradation and that the proteasome inhibitor MG132 blocks this effect, suggesting that E_2-induced ERα degradation occurs through the proteasome-dependent pathway [28,29]. We found that E_2-induced ERα degradation was enhanced by DHA and that this effect was blocked by MG132, indicating DHA increased ERα degradation through the proteasome-mediated pathway. This result is supported by the finding that DHA stimulates the proteasome-dependent pathway to increase the degradation of β-catenin in HCT116 and SW480 human colorectal cancer cells [30] and of nuclear sterol regulatory element binding protein-1 in primary rat hepatocytes [31]. In addition, peroxisomal proliferator-activated receptor γ agonists also stimulate the proteasome-dependent pathway to enhance ERα and cyclin D1 degradation in MCF-7 cells [32]. Since it has been suggested that DHA metabolites are ligands of peroxisomal proliferator-activated receptor γ [33], it would be interesting to know whether the DHA-induced decrease in ERα expression involves peroxisomal proliferator-activated receptor γ signaling. We found that DHA had no effect on proteasome activity; however, in an animal study, DHA increased muscle proteasome activity in rats fed an n-3 PUFA-rich diet [34].

Proteasomes are large multi-subunit complexes including the 20S and 26S proteasome and have multiple proteolytic activities, including chymotrypsin-like, trypsin-like and peptidylglutamyl peptide hydrolyzing-like activities [35,36]. In this study, we measured 20S chymotrypsin-like activity (so-called 20S proteasome activity) and found it was unaffected by DHA. Whether DHA-induced ERα degradation is due to 26S proteasome activity or other peptidase activities or involves signals in ERα for ubiquitinylation and degradation requires further study.

Tamoxifen therapy is the most widely used and most effective treatment for hormone-dependent breast cancers [37]. However, initially responsive tumors eventually acquire tamoxifen resistance, resulting in more severe tumor recurrence [38]. In addition, adaptive hypersensitivity to estrogen in breast cancer develops in patients with estrogen deprivation caused by removal of the ovaries or administration of aromatase inhibitors [39]. Studies of MCF-7 cells treated long-term with tamoxifen or by estrogen deprivation have shown increased ERα levels in the cytosol as well as enhanced E_2-stimulated MAPK signaling and growth of cancer cells [18,40], indicating that extranuclear ERα levels and the associated nongenomic signaling may be important mediators of the resistance and adaptive hypersensitive of estrogen-responsive breast cancers. In addition, cyclin D1, a downstream target of ERα and MAPK signaling, also plays an important role in causing drug resistance of breast cancers [26,41]. In the present study, we found that extranuclear ERα levels, MAPK signaling, cyclin D1 expression and cell viability were reduced in DHA-treated MCF-7 cells. It is important to determine whether these findings can be confirmed in breast cancer cell models of tamoxifen resistance or long-term estrogen deprivation to determine whether DHA has potential as an alternative adjunctive endocrine therapy against the resistance and adaptive hypersensitivity of estrogen-responsive breast cancers by reducing ERα and cyclin D1 levels.

E_2 can trigger rapid nongenomic signaling by the MAPK and Akt pathways. MAPK signaling in human breast cancer cells is mediated by ERα [16,17,42,43] and MAPK signaling plays a critical role in the control of cell growth, proliferation, differentiation and many other functions [44]. In the present study, we found that ERα and phosphorylated MAPK expression were reduced by DHA in MCF-7 cells. This finding is supported by the observation that DHA decreases phosphorylated MAPK levels in HT-29 cells, a human colon cancer cell line [45], and in FM3A cells, a mouse mammary cancer cell line [46]. Whether the Akt pathway is affected by DHA requires further studies.

Evidence is accumulating that supplementation with the n-3 PUFAs DHA and eicosapentaenoic acid (EPA, 20:5n-3), but not with other fatty acids, inhibits the growth of breast cancers. At 30 μM, DHA or EPA activates peroxisome proliferator-activated receptor γ to up-regulate syndecan-1 and promote apoptosis in MCF-7 cells [47]. Supplementation with 100 μM DHA or DHA+EPA, but not linoleic acid (18:2n-6), reduces the viability of MDA-MB-213 cells, an ERα-negative breast cancer cell line, by inhibiting proliferation and increasing caspase activity to induce apoptosis [48], and changes raft lipid composition and reduces epidermal growth factor receptor levels [49]. Supplementation with 20–100 μM DHA or EPA, but not oleic acid (18:1n-9) or linoleic acid, inhibits the growth of MDA-MB-231 cells by activating neutral sphingomyelinase to induce apoptosis [50]. At a concentration of 10 μM, DHA, but not AA, inhibits the proliferation of FM3A cells, a mouse mammary cancer cell line, by inhibiting MAPK signaling, up-regulating $P27^{kip1}$ levels and arresting cell cycle progression [46]. In addition, DHA or EPA, but not AA or oleic acid, increases BRCA1 and BRCA2 mRNA levels in MCF-7 and MDA-MB-231 cells [51]. In this study, we found that supplementation with 10 or 60 μM DHA, but not AA, decreased ERα levels, phosphorylated MAPK, cyclin D1 expression and MCF-7 viability. All the above evidences indicate a specific effect of n-3 PUFAs in inhibiting the growth of breast cancers.

Our findings provide a potential mechanism for the decrease in viability of MCF-7 cells caused by DHA, involving a reduction in ERα, phosphorylated MAPK and cyclin D1 expression. The DHA-induced decrease in ERα expression resulted from proteasome-dependent degradation. In contrast, AA had no such effects. We propose that DHA may have potential both as a natural antibreast cancer supplement

that can inhibit cancer growth and as an adjunctive endocrine therapy to attenuate the resistance or adaptive hypersensitivity of estrogen-responsive breast cancers.

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