行政院國家科學委員會專題研究計畫 成果報告

Syndecan 和 heme oxygenase-1 對多元不飽和脂肪酸調控乳癌細胞轉移機制之探討

研究成果報告(精簡版)

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計 畫 主 持 人 ：李健群

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中 華 民 國 101 年 10 月 31 日

中文摘要： 基質金屬蛋白酶-9 (Matrix metalloproteinase-9, MMP-9)的表達在癌細胞轉移過程中扮演關鍵角色。不少研究顯示多不飽和脂肪酸對多種人類癌細胞具有抗癌功效。但對於二十二碳六烯酸 (Docosahexaenoic acid, DHA)和亞麻油酸(Linoleic acid, LA)是否影響乳癌細胞轉移及其相關機制仍尚未清楚。本研究以 12-O-tetradecanoylphorbol-13-acetate (TPA)誘發 MCF-7 人類乳癌細胞轉移為實驗模式，探討 DHA 和 LA 對 TPA 所誘發的乳癌細胞移行(migration)及侵襲(invasion)之影響。實驗結果顯示，TPA 誘發 MMP-9 酵素活性呈現劑量依賴關係，給予 200 μM DHA 和 LA 則顯著抑制 TPA 所誘發 MMP-9 表現和癌細胞的移行及侵襲。分別給予 JNK、ERK1/2、PI3K 和 PKC 抑制劑可減少 TPA 所誘發 MMP-9 表現及其酵素活性。然而，預處理細胞 DHA 和 LA 只可抑制 TPA 所誘發 ERK1/2 和 AKT 磷酸化作用。先前研究結果顯示 NF-κB 及 AP-1 轉錄因子在啟動 MMP-9 基因轉錄活化過程中扮演重要角色，本研究利用 electrophoretic mobility shift assay (EMSA)證實 DHA 和 LA 亦可顯著減少 TPA 所誘發 NF-κB 及 AP-1 與 DNA 的結合能力。此外，本研究首度發現 DHA 可誘發 MCF-7 乳癌細胞血基質氧化酶(Heme oxygenase-1； HO-1)基因表現且呈現劑量和時間依賴性。利用 siRNA 將 HO-1 基因 knockdown 後，原本受 DHA 所抑制的 MMP-9 酵素蛋白質表現及活性均可回復。綜合上述結果，DHA 及 LA 均可能透過抑制 ERK1/2 和 PI3K/Akt 訊號傳遞路徑的活化，減少 NF-κB 及 AP-1 對 MMP-9 基因的轉錄活化作用，最終抑制 TPA 誘發 MCF-7 乳癌細胞的移行和侵襲。此外，在 DHA 抑制 TPA 誘發 MMP-9 活化過程中，少部分可能是透過活化 HO-1 表現所影響。本研究結果也可說明多元不飽和脂肪酸可減少乳癌細胞轉移，具有降低乳癌進程的潛力。

中文關鍵詞： 二十二碳六烯酸、血基質氧化酶-1、亞麻油酸、基質金屬蛋白酶-9、MCF-7 乳癌細胞

英文摘要： Matrix metalloproteinase-9 (MMP-9) plays a crucial role in the tumor metastasis. Previous studies showed that polyunsaturated fatty acids exhibited anti-cancer effect in various human carcinoma cells. The effect of both polyunsaturated fatty acids, docosahexaenoic acid (DHA) and linoleic acid (LA), on metastasis of breast cancer cells was not fully clarified. We used TPA-induced MCF-7 breast cancer cells to study the anti-metastasis potential of DHA and LA. The results showed that TPA induced MMP-9

enzyme activity in both dose- and time-dependent manners, and 200 μM DHA and LA significantly inhibited the TPA-induced MMP-9 mRNA and protein expression, enzyme activity, cell migration, and invasion. Treatment with ERK1/2, PI3K, and PKC inhibitors resulted in a marked decrease in TPA-induced MMP-9 protein expression and enzyme activity. In addition, TPA-induced activation of ERK1/2 and Akt was attenuated by DHA and LA treatment. PKC inhibitor, GF, suppressed TPA-induced ERK1/2 activation. EMSA results showed that DHA, LA, and PD98059 as well as wortmannin decreased TPA-induced NF-κB and AP-1 DNA binding activity. Furthermore, DHA rather than LA dramatically increased HO-1 expression in both dose- and time-dependent manners. HO-1 siRNA alleviated the DHA inhibition of TPA-induced MMP-9 protein expression and enzyme activity in MCF-7 cells. HO-1 shRNA reversed the DHA inhibition of TPA-induced cell migration in MCF-7 cells. Taken together, these results suggest that DHA and LA share the same signaling pathways, ERK1/2 and PI3K/Akt, to inhibit TPA-induced MMP-9 activation. However, an induction of HO-1 is exclusively involved in the inhibition of TPA-induced MMP-9 activation and subsequent cell migration by DHA rather than by LA in MCF-7 cells. On the basis of these findings, DHA and LA share both similar and divergent signaling pathways in the suppression of TPA-induced MCF-7 metastasis.

英文關鍵詞： Docosahexaenoic acid / Heme oxygenase 1 / Linoleic acid / matrix metalloproteinase-9/ MCF-7 cells

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

（計畫名稱）

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執行機構及系所：

計畫主持人：李健群
共同主持人：
計畫參與人員：林俐伶

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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中　華　民　國 101 年 10 月 31 日

中文摘要

基質金屬蛋白酶-9 (Matrix metalloproteinase-9, MMP-9)的表達在癌細胞轉移過程中扮演關鍵角色。不少研究顯示多不飽和脂肪酸對多種人類癌細胞具有抗癌功效。但對於二十二碳六烯酸(Docosahexaenoic acid, DHA)和亞麻油酸(Linoleic acid, LA)是否影響乳癌細胞轉移及其相關機制仍尚未清楚。本研究以 12-O-tetradecanoylphorbol-13-acetate (TPA)誘發 MCF-7 人類乳癌細胞轉移為實驗模式，探討 DHA 和 LA 對 TPA 所誘發的乳癌細胞移行(migration)及侵襲(invasion)之影響。實驗結果顯示，TPA 誘發 MMP-9 酵素活性呈現劑量依賴關係，給予 200 μM DHA 和 LA 則顯著抑制 TPA 所誘發 MMP-9 表現和癌細胞的移行及侵襲。分別給予 JNK、ERK1/2、PI3K 和 PKC 抑制劑可減少 TPA 所誘發 MMP-9 表現及其酵素活性。然而，預處理細胞 DHA 和 LA 只可抑制 TPA 所誘發 ERK1/2 和 AKT 磷酸化作用。先前研究結果顯示 NF-κB 及 AP-1 轉錄因子在啟動 MMP-9 基因轉錄活化過程中扮演重要角色，本研究利用 electrophoretic mobility shift assay (EMSA)證實 DHA 和 LA 亦可顯著減少 TPA 所誘發 NF-κB 及 AP-1 與 DNA 的結合能力。此外，本研究首度發現 DHA 可誘發 MCF-7 乳癌細胞血基質氧化酶(Heme oxygenase-1; HO-1)基因表現且呈現劑量和時間依賴性。利用 siRNA 將 HO-1 基因 knockdown 後，原本受 DHA 所抑制的 MMP-9 酵素蛋白質表現及活性均可回復。綜合上述結果，DHA 及 LA 均可能透過抑制 ERK1/2 和 PI3K/Akt 訊號傳遞路徑的活化，減少 NF-κB 及 AP-1 對 MMP-9 基因的轉錄活化作用，最終抑制 TPA 誘發 MCF-7 乳癌細胞的移行和侵襲。此外，在 DHA 抑制 TPA 誘發 MMP-9 活化過程中，少部分可能是透過活化 HO-1 表現所影響。本研究結果也可說明多元不飽和脂肪酸可減少乳癌細胞轉移，具有降低乳癌進程的潛力。

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Abstract

Matrix metalloproteinase-9 (MMP-9) plays a crucial role in the tumor metastasis. Previous studies showed that polyunsaturated fatty acids exhibited anti-cancer effect in various human carcinoma cells. The effect of both polyunsaturated fatty acids, docosahexaenoic acid (DHA) and linoleic acid (LA), on metastasis of breast cancer cells was not fully clarified. We used TPA-induced MCF-7 breast cancer cells to study the anti-metastasis potential of DHA and LA. The results showed that TPA induced MMP-9 enzyme activity in both dose- and time-dependent manners, and 200 μM DHA and LA significantly inhibited the TPA-induced MMP-9 mRNA and protein expression, enzyme activity, cell migration, and invasion. Treatment with ERK1/2, PI3K, and PKC inhibitors resulted in a marked decrease in TPA-induced MMP-9 protein expression and enzyme activity. In addition, TPA-induced activation of ERK1/2 and Akt was attenuated by DHA and LA treatment. PKC inhibitor, GF, suppressed TPA-induced ERK1/2 activation. EMSA results showed that DHA, LA, and PD98059 as well as wortmannin decreased TPA-induced NF-κB and AP-1 DNA binding activity. Furthermore, DHA rather than LA dramatically increased HO-1 expression in both dose- and time-dependent manners. HO-1 siRNA alleviated the DHA inhibition of TPA-induced MMP-9 protein expression and enzyme activity in MCF-7 cells. HO-1 shRNA reversed the DHA inhibition of TPA-induced cell migration in MCF-7 cells. Taken together, these results suggest that DHA and LA share the same signaling pathways, ERK1/2 and PI3K/Akt, to inhibit TPA-induced MMP-9 activation. However, an induction of HO-1 is exclusively involved in the inhibition of TPA-induced MMP-9 activation and subsequent cell migration by DHA rather than by LA in MCF-7 cells. On the basis of these findings, DHA and LA share both similar and divergent signaling pathways in the suppression of TPA-induced MCF-7 metastasis.

Keywords: Docosahexaenoic acid / Heme oxygenase 1 / Linoleic acid / matrix metalloproteinase-9/ MCF-7 breast cancer cells

Introduction

Metastatic spread of cancer cells is the main cause of death for breast cancer patients. Breakdown of the extracellular matrix (ECM) by proteinases is an essential step in cancer metastasis. Matrix metalloproteinases (MMPs), a family of ECM degrading proteinases, are divided into four subclasses based on the substrate specificity including collagenases, gelatinases, stromelysin, and elastases. Activation of MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) is closely associated with the tumor invasion and metastasis in different types of cancer cells, including human breast, hepatoma, prostate and lung cancer cells. In general, MMP-2 is constitutively expressed in highly metastatic tumors, whereas MMP-9 is stimulated by the growth factors, such as epidermal growth factor and transforming growth factor beta (TGF-β), the inflammatory cytokine such as tumor necrosis factor-α (TNF-α), ultraviolet radiation, or phorbol ester.

The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter, stimulates renal tumor cell proliferation through activation of protein kinase C (PKC). TPA-induced MMPs activation was mediated by transcription factors such as NF-κB and AP-1 through activation of PKC, phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinases (MAPKs) signaling pathways. Recent studies showed that dietary factors including α-lipoic acid, capsaicin, and conjugated linoleic acid are protective against cancer cell migration, invasion and angiogenesis by suppressing MMP-9 expression or enzyme activity. Because of the roles of PKC, PI3K, and MAPKs in TPA-induced MMPs activation and cancer cell metastasis, substances which inhibit these signaling pathways may confer antitumor potential. In our previous study, docosahexaenoic acid (DHA) was shown to suppress phenobarbital-induced JNK1/2 and ERK2 activation which suggests DHA may be a candidate for anti-carcinogenesis.

Due to their roles in energy and essential fatty acids supplies, dietary lipids are important to human beings. Linoleic acid (LA) is an n-6 essential fatty acid and DHA is an n-3 essential fatty acid. These two polyunsaturated fatty acids (PUFAs) and their metabolic products are known to be involved in a variety of physiological processes, such as regulation of inflammation, insulin resistance, blood pressure, and lipid metabolism. Epidemiologic studies showed that high consumption of fatty fish rich in n-3 PUFAs such as eicosapentaenoic acid (EPA) and DHA is associated with a reduced risk for breast cancer. In addition, both animal and cell studies support the notion that dietary n-6 and n-3 PUFAs inhibit promotion and progression of carcinogenesis.

Heme oxygenase 1 (HO-1) is one of the members of HO system. HO-1 is also known as HSP32 (heat shock protein of 32 kDa), and it is an inducible enzyme and expression is relatively low in most tissues under basal conditions. HO-1 is induced by a wide variety of stimuli such as ultraviolet A radiation, endotoxin, and cytokines. Additionally, HO-1 is induced by numerous phytochemicals as well as DHA. HO-1 was shown to have anti-oxidant and anti-inflammatory activities in HaCaT cells, and it has recently been reported to play an anti-tumorigenic role in a variety of carcinoma cells including PC3 prostate cancer cells, hepatocellular carcinoma cells, and MCF-7 breast cancer cells. Because of the HO-1 induction potential of DHA, it is inferred that DHA may possess antitumor activity.

Aim of the study

According to the antitumor activity of n-3 and n-6 PUFAs demonstrated in previous studies, we investigated the metastasis and invasion inhibition of n-3 and n-6 PUFAs in TPA-induced MCF-7 human breast cancer cell and the possible mechanism(s) involved.

Methods

1. Cell culture-The human breast cancer cell line MCF-7 was cultured in DMEM (pH 7.2) supplemented with 1.5 g/l $NaHCO_3$, 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37℃ in a 5% CO_2 humidified incubator.
2. Cell viability was assessed by the MTT assay.
3. Western blot analysis
4. Nuclear extract preparation
5. PKC translocation
6. RNA interference by small interfering RNA of HO-1
7. Migration and invasion assays

Results

Fig.1 DHA or LA suppresses TPA-induced migration and invasion of MCF-7 cells

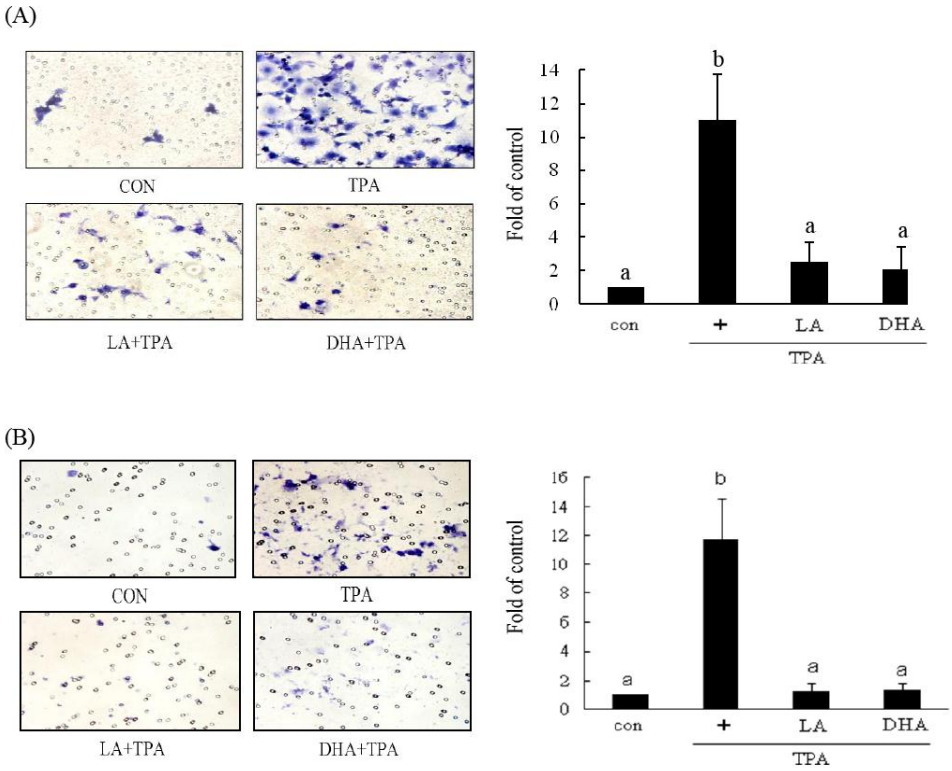


Fig. 2 DHA and LA inhibited TPA-induced MMP-9 gene expression and enzyme activity in MCF-7 cells

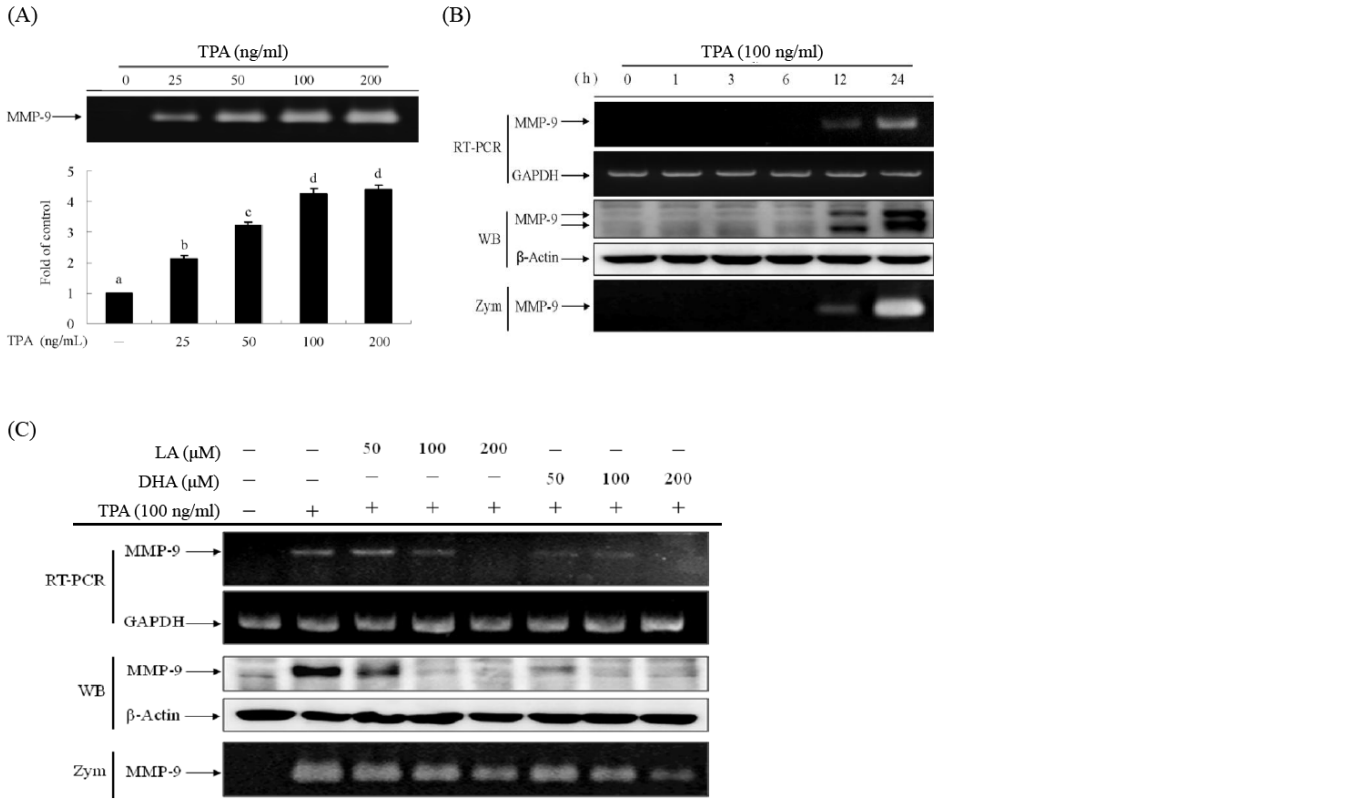


Fig. 3 DHA down-regulates TPA-induced MMP-9 expression via ERK, PI3K/Akt, and PKC signaling pathways and LA via ERK signaling pathway

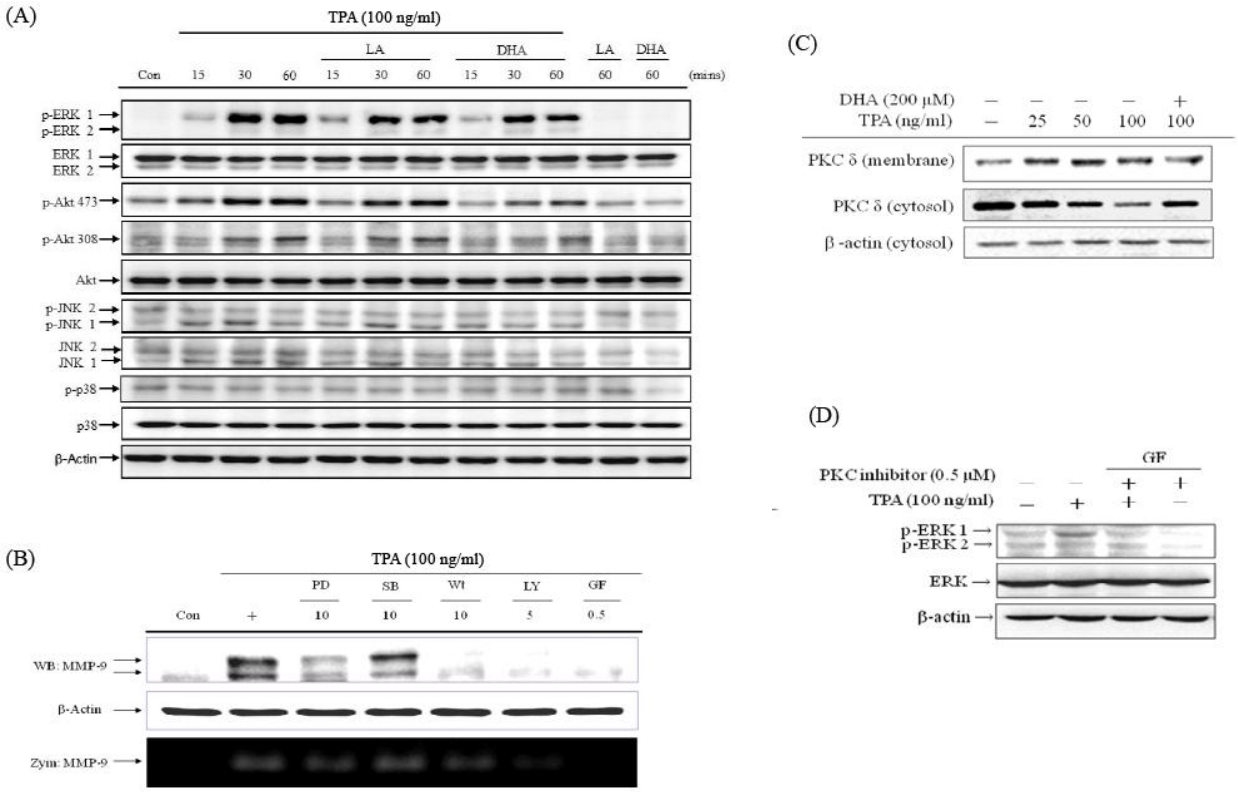


Fig. 4 DHA and LA inhibit TPA-induced DNA binding activities of NF-κB and AP-1

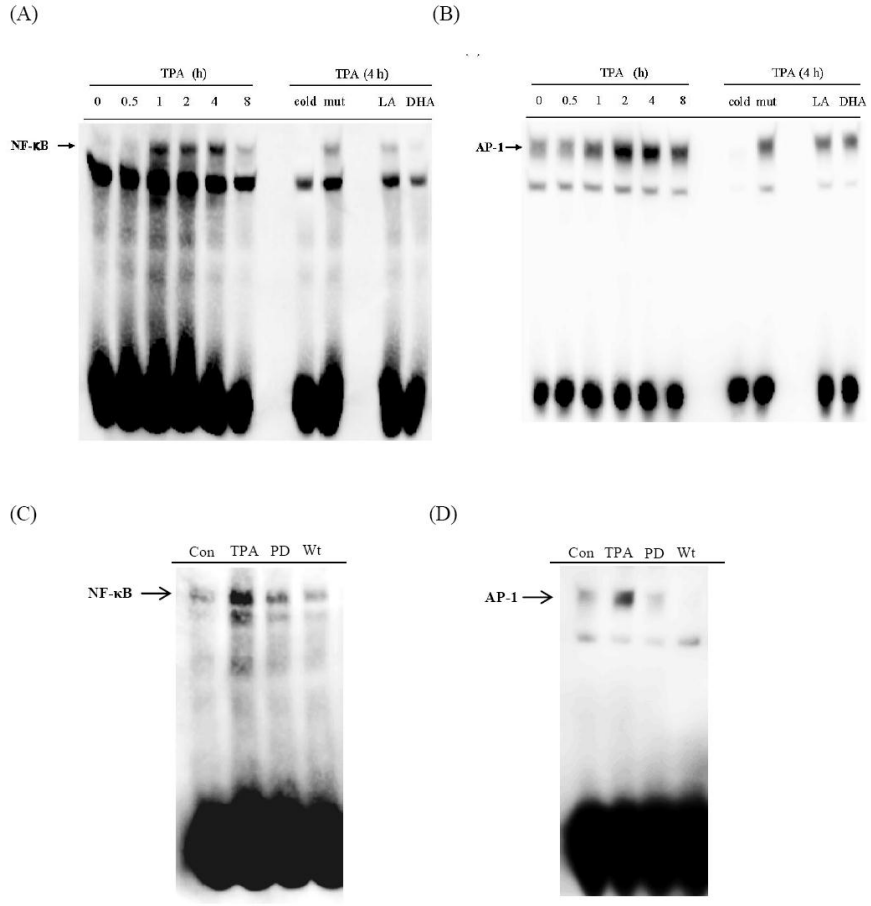
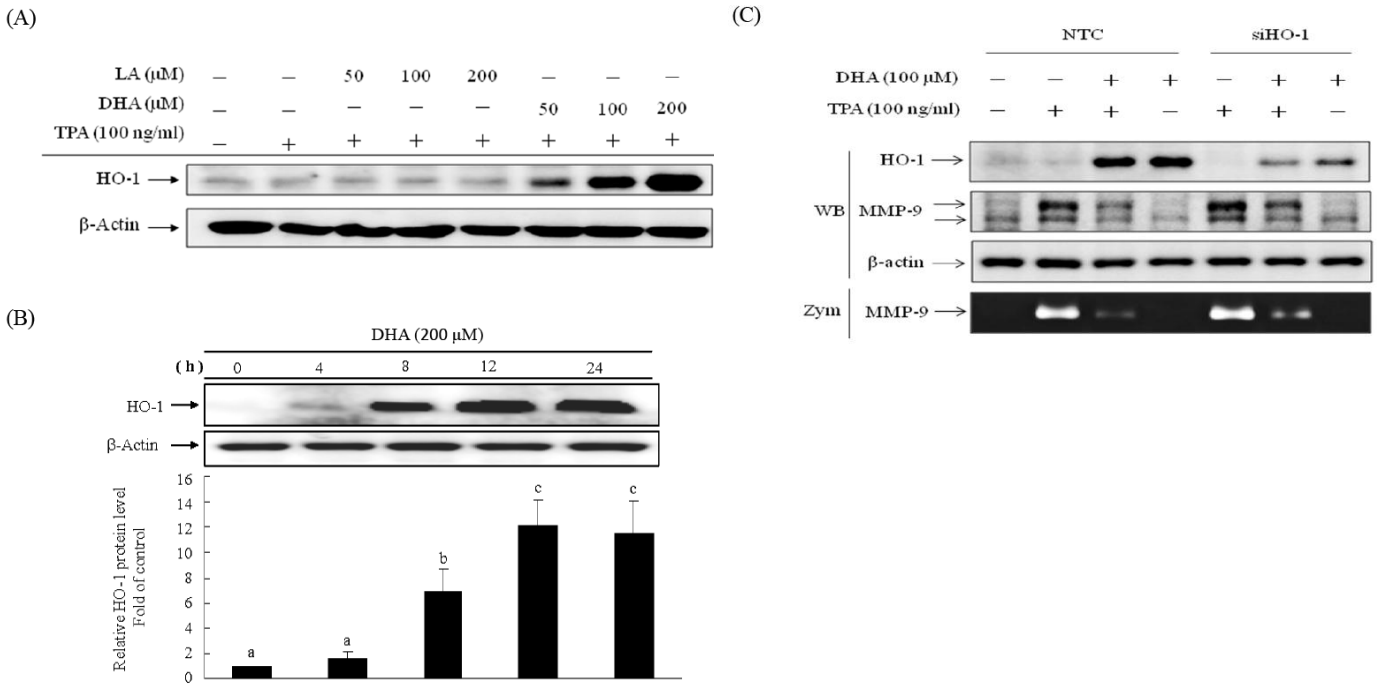


Fig. 5 DHA induces HO-1 gene expression and HO-1 siRNA alleviates DHA inhibition of TPA-induced MMP-9 protein expression and enzyme activity



Discussion

In this study, we demonstrated that both DHA and LA effectively inhibited TPA-induced MMP-9 expression and MCF-7 cell migration and invasion and that this inhibition was associated with an up-regulation of HO-1 expression by DHA but not by LA. Both DHA and LA suppressed TPA-induced ERK1 activation, and DHA showed an additional inhibition on Akt activation.

Experimental evidences indicate that n-3 PUFAs may have an anti-tumor activity, whereas total fat, saturated, and n-6 PUFAs may stimulate various mammary tumor growth and metastasis. This study was designed to examine the effect of n-3 and n-6 PUFAs, such as DHA and LA, on MCF-7 breast cancer cell migration and invasion and to clarify the possible molecular mechanisms involved.

MCF-7 breast cancer cells are recognized to have weak invasiveness, however, the invasive potential of MCF-7 cells could be significantly increased by TPA. In the present study, the MMP-9 activity which is associated with cancer cell invasion and migration was induced by TPA in a dose-dependent manner (Figure 2A) and both mRNA and protein levels were dramatically increased after treatment with 100 ng/ml of TPA for 24 h in MCF-7 breast cancer cells (Figure 2B), which is in line with the results of a previous study.

Previous studies showed that the low concentrations of DHA or EPA (25 μM) alone showed minimal inhibitory effect on cell migration against serum, but the relatively high concentration of 100 μM or 152 μM (about 50 ng/ml) DHA significantly decreased cell migration and invasion in MDA-MB-231 breast cancer cells. Although 100 μM PUFAs have been shown to cause rat primary hepatocyte damage and 200 μM DHA treatment for 72 h predominantly inhibited MCF-7 cell viability and induced apoptosis, treatment of MCF-7 cells with 200 μM DHA or LA for 24 h showed no cytotoxic effect. The cytotoxic effect of PUFAs might be dependent on the cell type and exposure period. Both 200 μM DHA and LA showed a significant suppressive effect on TPA-induced cell migration and invasion (Figures 1A and B). Pharmacokinetic study showed that the human plasma concentration of DHA is able to achieve 120 mg/l (about 315 μM) after daily administration of 3 g fish oil supplement for 2 weeks. The mean concentration of plasma phospholipid-esterified LA is about 824 μM. These results supported the used dosage of DHA and LA in the present study is physiologically achievable.

It is well established that tumor cell migration and invasion depend on MMP-2 and MMP-9 expression and enzyme activities. Current studies found that medicinal herb such as kalopanaxsaponin A and flavonoid quercetin inhibit TPA-induced cell invasion by reducing MMP-9 expression in MCF-7 cells. A previous study indicated that dietary DHA and CLA are capable of reducing MMP-2 and MMP-9 production in reproductive tissues of pregnant rats. In the present study, we provide the evidence that TPA-induced MMP-9 expression

and activity were down-regulated by DHA and LA in MCF-7 cells (Figure 2C). Beneficial effect of DHA and LA on breast tumor metastasis has been demonstrated in another study. An early study showed that high intakes of n-6 PUFA and saturated fat are more likely to increase risk of breast cancer; however, 300 μM LA was shown to suppress colorectal cancer cell growth by inducing oxidant stress and mitochondrial dysfunction. In the present study, MMP-9 expression and enzyme activity were significantly suppressed by 200 μM LA (Figure 2C), which suggests the suppression of MMP-9 expression contributes at least in part to the inhibition of TPA-induced migration and invasion of MCF-7 cells by LA.

TPA increases the migration and invasion of various cancer cells by activating MMP-9 via PKCs, MAPKs, and PI3K/Akt signaling pathways and related transcription factors. In the present study, TPA-induced MMP-9 expression and activity were significantly inhibited by ERK inhibitor (PD98059, PD), PI3K inhibitors such as wortmannin (Wt) and LY294002 (LY), and non-selective PKC inhibitor (GF109203X, GF) (Figure 3B). Several studies indicated the PKCs including α, β, δ, and ε isoforms played crucial roles in TPA-induced MMP-9 production in different cell types. A recent study showed TPA-induced MMP-9 activation, mainly via PKCδ/ERK pathways in MCF-7 cells. DHA significantly inhibited TPA-induced translocation of two PKC isoforms, PKCα and PKCε, from cytosol to the plasma membrane, but failed to inhibit the TPA-induced translocation of PKCδ isoform in NIH/3T3 cells. In the present study, DHA decreased the TPA-induced translocation of PKCδ from cytosol to the plasma membrane (Figure 3C), which suggested PKCδ and its downstream pathway may be associated with DHA down-regulation of TPA-induced MMP-9 expression. On the basis of DHA inhibition of TPA-induced ERK1, PI3K/Akt, and PKCδ signaling pathways and LA suppression of TPA-induced ERK1 signaling pathway, both PUFAs may have anti-tumor potential via similar and divergent signaling pathways.

TPA-induced MMP-9 expression was mediated by activating transcription factors such as NF-κB and AP-1 through PKCs, PI3K and MAPKs signaling pathways. A recent study reported that Kalopanaxsaponin A (KPS-A) inhibits TPA-induced invasion by reducing MMP-9 activation, mainly via PI3K/Akt/NF-κB and PKCδ/ERK/AP-1 pathways in MCF-7 cells. Dihydroartemisnin inhibits TPA-induced MMP-9 activation through suppression of PKCα/Raf/ERK and JNK signaling pathways and subsequent NF-κB and AP-1 trans-activation in HT-1080 cells. Meanwhile, dihydroartemisnin directly suppressed IκBα degradation and decreased p65 nuclear translocation. These results supported the notion that modulation of IκBα/p65 may be involved in TPA-induced MMP-9 activation. EMSA results showed the DNA binding activities of NF-κB and AP-1 began 1 h after TPA treatment and sustained until 4 h (Figures 4A and B). Both DHA and LA treatments attenuated the TPA-induced DNA binding activities of NF-κB and AP-1 (Figures 4A and B), and these results were consistent with the observation proposed in a recent study. Inhibition of TPA-induced DNA binding activities of NF-κB and AP-1 by Wt and PD (Figures 4C and D), which suggests TPA-induced MMP-9 expression and activity may be via PI3K/Akt/NF-κB, PKC/ERK/NF-κB, PI3K/Akt/AP-1, and PKC/ERK/AP-1 pathways. Cordycepin inhibited MMP-9 expression by suppressing AP-1 but not NF-κB activation in MCF-7 cells. Hypericin-mediated photodynamic therapy (PDT) down-regulated MMP-9 expression via inhibition of GM-CSF production, and subsequently suppressed both NF-κB and AP-1 transcriptional activities in human nasopharyngeal cancer cells. Signaling pathways involved in the inhibition of MMP-9 expression and subsequent cell migration and invasion in different cell types may depend on specific treatment employed. In the present study, DHA and LA inhibit TPA-induced migration and invasion by reducing MMP-9 expression and activity may be via the four signaling pathways mentioned above.

The induction of HO-1 by butein and phloretin is mediated through the ERK/Nrf2 pathway and exerts anti-inflammatory and anti-oxidant activities. In addition to anti-oxidant and anti-inflammatory activities of HO-1, HO-1 has also been shown to suppress breast cancer migration and invasion in recent studies. The activation of PKCα/ERK/AP-1 signaling with induction of reactive oxygen species (ROS) production was involved in TPA-induced invasion of MCF-7 cells, and TPA-induced ROS production and MMP-9 activation was abolished by overexpression of HO-1 protein in MCF-7 cells.In the present study, DHA but not LA time- and dose-dependently induced HO-1 expression (Figures 5A,5B). HO-1 silence attenuated DHA inhibition of TPA-induced MMP-9 protein expression, enzyme activity (Figure 5C). These results indicate the importance of HO-1 in the inhibition of TPA-induced MMP-9 expression and cell migration by DHA. In BV-2 microglia, HO-1 expression was significantly induced after 6 h of DHA exposure via PI3K/Akt and ERK signaling pathways. It supported the possible of DHA is capable of inducing HO-1 expression in short times and subsequently suppressing TPA-induced cell migration and invasion in breast cancer cell.

Taken together, these results suggest that DHA and LA down-regulate TPA-induced MMP-9 gene expression and MCF-7 breast cancer cell migration and invasion are at least in part through inhibition of PKC/ERK1 and PI3K/Akt signaling pathways and reduction of NF-κB and AP-1 transcriptional activation. In addition to the signaling pathways mentioned above, DHA inhibition of TPA-induced MMP-9 activation in MCF-7 cells is via its HO-1 induction activity.

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請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □ 未達成目標（請說明，以 100 字為限） □ 實驗失敗 □ 因故實驗中斷 □ 其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：□已發表 ■未發表之文稿 □撰寫中 □無 專利：□已獲得 □申請中 □無 技轉：□已技轉 □洽談中 □無 其他：目前投稿中

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500 字為限）

基質金屬蛋白酶-9 (Matrix metalloproteinase-9, MMP-9)的表達在癌細胞轉移過程中扮演關鍵角色。不少研究顯示多不飽和脂肪酸對多種人類癌細胞具有抗癌功效。但對於二十二碳六烯酸 (Docosahexaenoic acid, DHA)和亞麻油酸(Linoleic acid, LA)是否影響乳癌細胞轉移及其相關機制仍尚未清楚。本研究以 12-O-tetradecanoylphorbol-13-acetate (TPA)誘發 MCF-7 人類乳癌細胞轉移為實驗模式，探討 DHA 和 LA 對 TPA 所誘發的乳癌細胞移行(migration)及侵襲(invasion)之影響。實驗結果顯示，給予 200 μM DHA 和 LA 則顯著抑制 TPA 所誘發 MMP-9 表現和癌細胞的移行及侵襲。分別給予 JNK、ERK1/2、PI3K 和 PKC 抑制劑可減少 TPA 所誘發 MMP-9 表現及其酵素活性。本研究亦利用 EMSA 證實 DHA 和 LA 可顯著抑制 TPA 所誘發 NF-κB 及 AP-1 與 DNA 的結合能力。本研究首度發現 DHA 可誘發 MCF-7 乳癌細胞血基質氧化酶(Heme oxygenase-1; HO-1)基因表現且呈現劑量和時間依賴性。利用 siRNA 將 HO-1 基因 knockdown 後，原本受 DHA 所抑制的 MMP-9 酵素蛋白質表現及活性均可回復。綜合上述結果，DHA 及 LA 均可能透過抑制 ERK1/2 和 PI3K/Akt 訊號傳遞路徑的活化，減少 NF-κB 及 AP-1 對 MMP-9 基因的轉錄活化作用，最終抑制 TPA 誘發 MCF-7 乳癌細胞的移行和侵襲。此外，在 DHA 抑制 TPA 誘發 MMP-9 活化過程中，少部分可能是透過活化 HO-1 表現所影響。本研究結果也可說明多元不飽和脂肪酸可減少乳癌細胞轉移，具有降低乳癌進程的潛力。

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/31

國科會補助計畫	計畫名稱: Syndecan 和 heme oxygenase-1 對多元不飽和脂肪酸調控乳癌細胞轉移機制之探討
	計畫主持人: 李健群
	計畫編號: 100-2313-B-040-006- 學門領域: 食品及農化

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：李健群	計畫編號：100-2313-B-040-006-
計畫名稱：Syndecan 和 heme oxygenase-1 對多元不飽和脂肪酸調控乳癌細胞轉移機制之探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	本計畫結果證實魚油中的DHA可透過增加HO-1表現抑制TPA所誘發的MCF-7乳癌細胞轉移，此結果可說明魚油對於乳癌細胞的轉移具有顯著抑制效果，未來可望在臨床乳癌治療過程，給予適度魚油攝取建議。此結果已彙整相關研究結果並撰寫成期刊論文，進行投稿。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

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■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

研究成果已完成論文撰寫並投稿發表。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

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