行政院國家科學委員會專題研究計畫 成果報告

Syndecan 在多元不飽和脂肪酸對調控肝癌及乳癌細胞轉移作用之影響

研究成果報告(精簡版)

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中 華 民 國 100 年 11 月 01 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。

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. However, the effects of docosahexaenoic acid (DHA) and linoleic acid (LA) on metastasis of breast cancer cells have not been fully clarified. The model of TPA-induced MCF-7 breast cancer cell metastasis was used in this study. The results showed that TPA-induced MMP-9 gene expression and enzyme activity in a dose-dependent manner, and 200 μM DHA and LA significantly decreased the TPA-induced MMP-9 expression, cell migration and invasion. Treatment with JNK, ERK1/2, PI3K, and PKC inhibitors caused a marked decrease in TPA-induced MMP-9 expression； however, only TPA-induced activation of ERK1/2 and Akt was attenuated by DHA and LA. The result of EMSA showed that DHA and LA decreased TPA-induced NF-κB and AP-1 DNA binding activity. Moreover, DHA, but not LA, dramatically increased HO-1 expression in a dose- and time-dependent manner. HO-1 siRNA

alleviated the DHA inhibition of MMP-9 protein and enzyme activity in the presence of TPA in MCF-7 cells. Taken together, these results suggest that DHA and LA inhibit TPA-induced cell migration and invasion by reducing MMP-9 activation, mainly via ERK1/2 and PI3K/Akt pathways and subsequently NF-κB and AP-1 trans-activation. Furthermore, the inhibition of TPA-induced MMP-9 activation by DHA is at least in part through induction of HO-1 expression in 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

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計畫主持人：李健群
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計畫參與人員：林俐伶、呂佳陽

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　100　年　10　月　31　日

中文摘要

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關鍵字: 二十二碳六烯酸、亞麻油酸、基質金屬蛋白酶-9、轉移、MCF-7

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. However, the effects of docosahexaenoic acid (DHA) and linoleic acid (LA) on metastasis of breast cancer cells have not been fully clarified. The model of TPA-induced MCF-7 breast cancer cell metastasis was used in this study. The results showed that TPA-induced MMP-9 gene expression and enzyme activity in a dose-dependent manner, and 200 μM DHA and LA significantly decreased the TPA-induced MMP-9 expression, cell migration and invasion. Treatment with JNK, ERK1/2, PI3K, and PKC inhibitors caused a marked decrease in TPA-induced MMP-9 expression; however, only TPA-induced activation of ERK1/2 and Akt was attenuated by DHA and LA. The result of EMSA showed that DHA and LA decreased TPA-induced NF-κB and AP-1 DNA binding activity. Moreover, DHA, but not LA, dramatically increased HO-1 expression in a dose- and time-dependent manner. HO-1 siRNA alleviated the DHA inhibition of MMP-9 protein and enzyme activity in the presence of TPA in MCF-7 cells. Taken together, these results suggest that DHA and LA inhibit TPA-induced cell migration and invasion by reducing MMP-9 activation, mainly via ERK1/2 and PI3K/Akt pathways and subsequently NF-κB and AP-1 trans-activation. Furthermore, the inhibition of TPA-induced MMP-9 activation by DHA is at least in part through induction of HO-1 expression in MCF-7 cells.

Keywords: Docosahexaenoic acid; Linoleic acid; MMP-9; Migration; MCF-7

Introduction

Breast cancer is the most common female cancer and is the second leading cause of cancer deaths in Western women. About 30% to 40% of women with this form of cancer will develop metastases and eventually die of this disease. According to the statistical data of Department of Health of Taiwan, the incidence of breast cancer has increased 4.5 fold in the past twenty years, and is the fourth leading cause of cancer death in Taiwanese women.

Metastatic spread of cancer cells is the main cause of death of 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 including collagenases, gelatinases, stromelysin, and elastases. Activation of MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) is intensely correlated with the tumor invasion and metastasis in different types of cancer cell, including human breast, hepatoma, prostate and lung cancer cells. In general, MMP-2 is constitutively expressed in highly metastatic tumors, whereas MMP-9 can be stimulated by the growth factor, 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 modulating the activation of transcription factors such as NF-κB and AP-1 through PKC, PI3K and mitogen-activated protein kinase (MAPK) signaling pathways. Recent studies showed that the dietary factors such as α-lipoic acid, capsaicin, and conjugated linoleic acid (CLA) are protective against cancer migration, invasion and angiogenesis by suppressing MMP-9 expression or enzyme activity. In our previous study, phenobarbital-induced JNK1/2 and ERK2 activation was down-regulated by DHA which suggests DHA may possess the ability to suppress the MMP-2 or MMP-9 activation. In other words, DHA can be the potential candidate for antitumor.

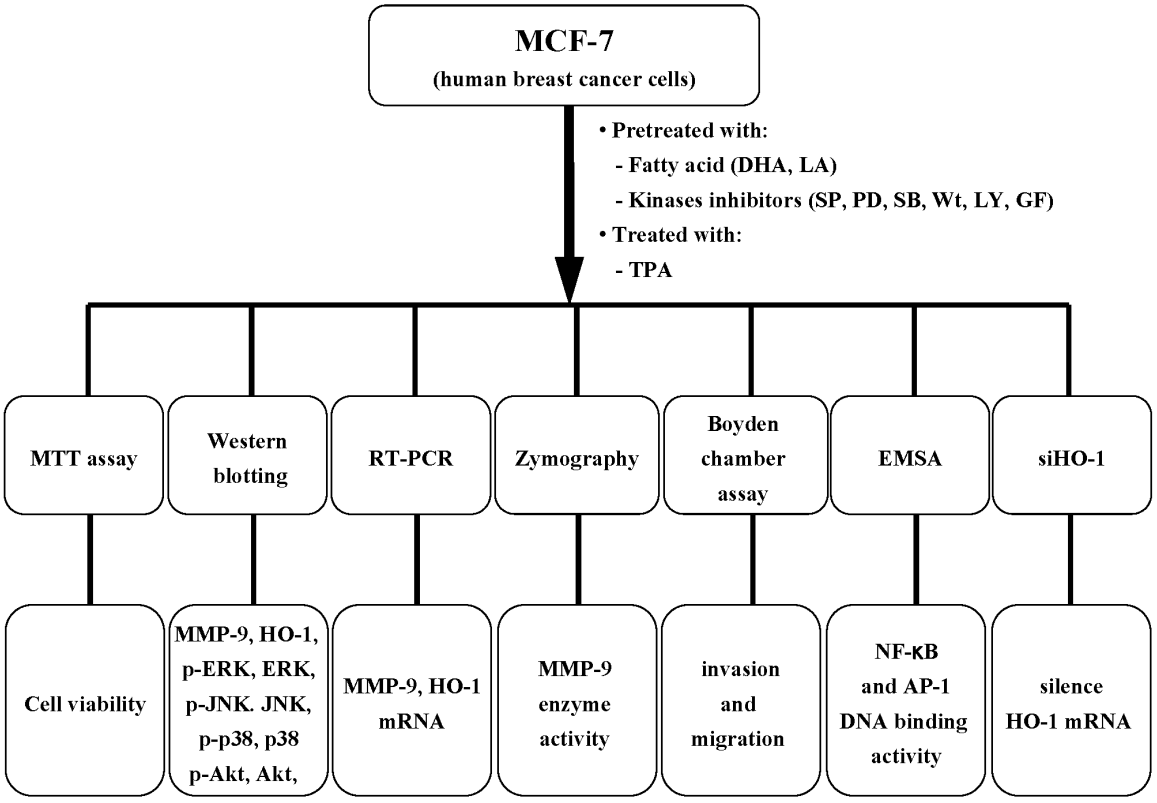
Dietary lipids are important to human beings because of their role in energy and essential fatty acids supplies. Linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) are essential fatty acids that must be obtained from diets. These polyunsaturated fatty acids (PUFAs) and their metabolic products play critical roles 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 n-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fatty fish is associated with a reduced risk for breast cancer. Experimental animal and cell culture studies provided evidences that dietary n-3 and n-6 PUFAs inhibit the promotion and progression stages 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 expressed 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. In addition to anti-oxidant and anti-inflammatory activities of HO-1, HO-1 has also been shown to possess anti-tumorigenic action in breast cancer cells. It is also shown that HO-1 is induced by a wide array of phytochemicals through Nrf2. In addition to the above mentioned stimuli, induction of HO-1 by DHA in BV-2 microglia and mouse peritoneal macrophages was reported. However, the effect of n-3 and n-6 PUFAs on HO-1 induction in human cancer cells lacks.

Because of the HO-1 induction capability of DHA, it is possible that DHA can exert antitumor activity. According to previous studies describing the antitumor activity of n-3 and n-6 PUFAs, we investigated the metastasis and invasion inhibition effects of n-3 and n-6 PUFAs in TPA-induced MCF-7 human breast cancer cell and the possible mechanism involved.

Materials and Methods

Dulbecco's Modified Eagle Medium (DMEM), OPTI-MEM, 25% trypsin-EDTA, and penicillin-streptomycin solution were from GIBCO-BRL (Grand Island, NY); fetal bovine serum (FBS) was from HyClone (Logan, UT); 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), albumin, bovine serum essentially fatty acid free (BSA), sodium bicarbonate, calcium chloride, Triton X-100, 12-O-tetradecanoylphorbol 13-acetate (TPA), GF109203X (PKC kinase inhibitor), wortmannin, and LY294002 (PI3K kinase inhibitor) were from Sigma-Aldrich, Inc. (St. Louis, MO); SP600125 (JNK inhibitor), PD98059 (ERK inhibitor), SB203580 (p38 inhibitor) were from TOCRIS (Ellisville, MO); docosahexaenoic acid and linoleic acid were from Cayman Chemical (Ann Arbor, MI); collagen was from Collaborative Biomedical Products (Bedford, MA); TRIzol reagent was from Molecular Research Center, Inc (Cincinnati, OH); antibodies against Akt, phospho-Akt (T308 and S473), ERK1/2, phospho-ERK1/2, p38, and phospho-p38were from Cell Signaling Technology (Danvers, MA); antibodies against JNK1 and phospho-JNK1/2 were from Santa Cruz Biotechnology (Santa Cruz, CA); antibody against HO-1was from Calbiochem (Darmstadt, Germany); and DharmaFECT 1 Transfection Reagent was from Dharmacon (Lafayette, CO).



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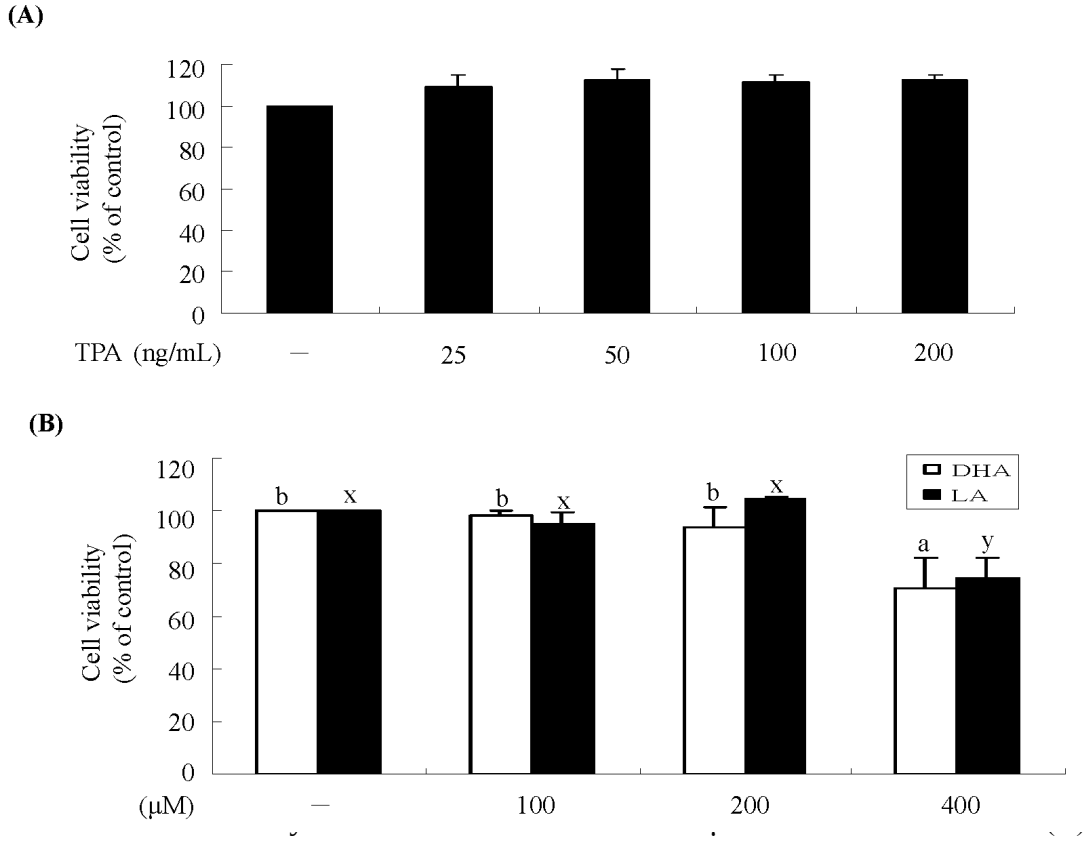
Result



h. (B) Effects of DHA Effects of LA or DHA with TPA on cell viability. Cells were pretreated with 0-200 μM LA or DHA for 24 h followed by incubation with 100 ng/mL of TPA for another 24 h. Cell viability was measured by using the MTT assay. Values are means ± SD of three independent experiments. Bars not sharing the same letters or symbols are significantly different (p<0.05).

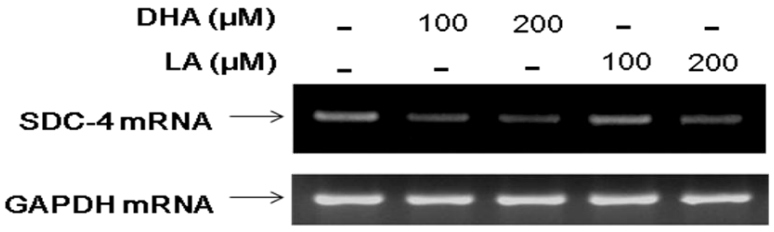


Figure 2. Effect of DHA or LA on SDC-4 mRNA expression. DHA or LA inhibits SDC-4 mRNA expression migration in MCF-7 cells. MCF-7 cells were treated with or without 100, 200 μM LA or DHA for 20 h. Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different (p<0.05).

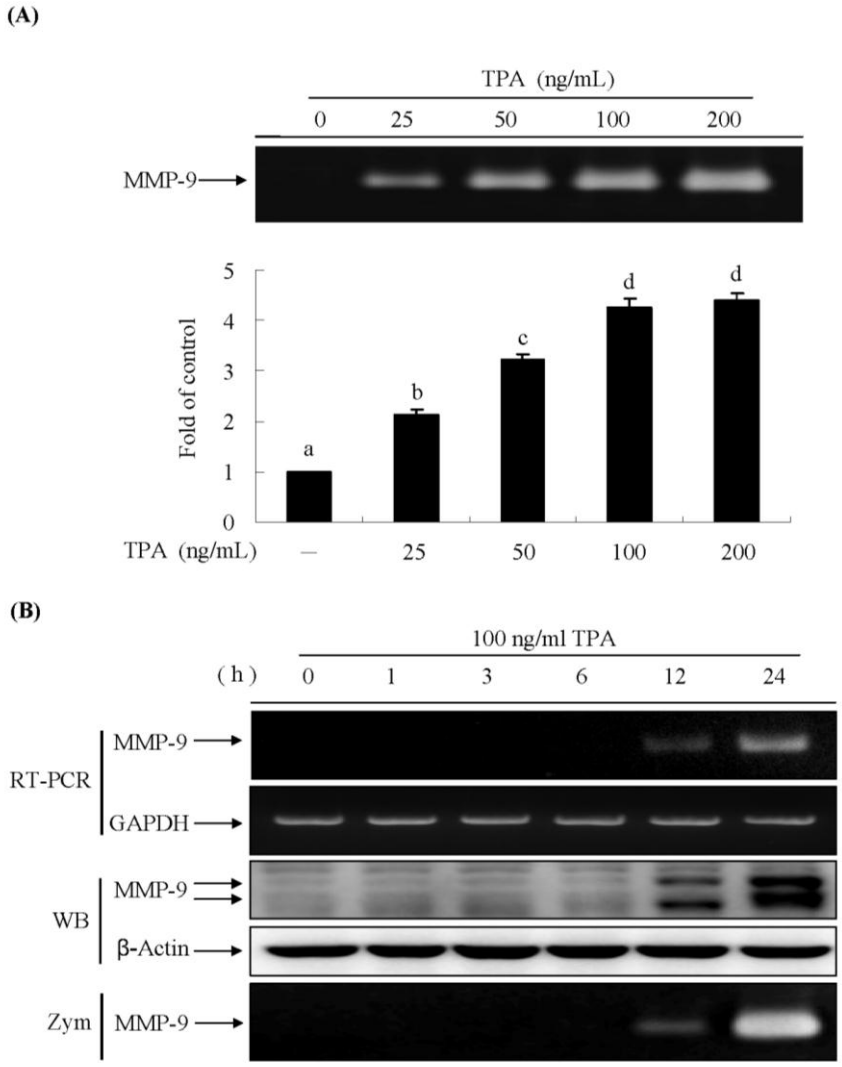


Figure 3. Effect of TPA on MMP-9 gene expression and enzyme activity in MCF-7 cells. (A) TPA induces MMP-9 mRNA expression in a dose-dependent manner. MCF-7 cells were treated with various doses of TPA for 24 h. Total RNA (0.1 μg/μL) were used to detect the MMP-9 mRNA expression, which was measured by RT-PCR. (B) TPA induces MMP-9 mRNA and protein expression and enzyme activity in a time-dependent manner. MCF-7 cells were treated with 100 ng/mL of TPA for 0-24 h. MMP-9 protein expression was measured by Western blotting (WB) and MMP-9 enzyme activity was measured by gelatin zymography assay (Zym). Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different (p<0.05).

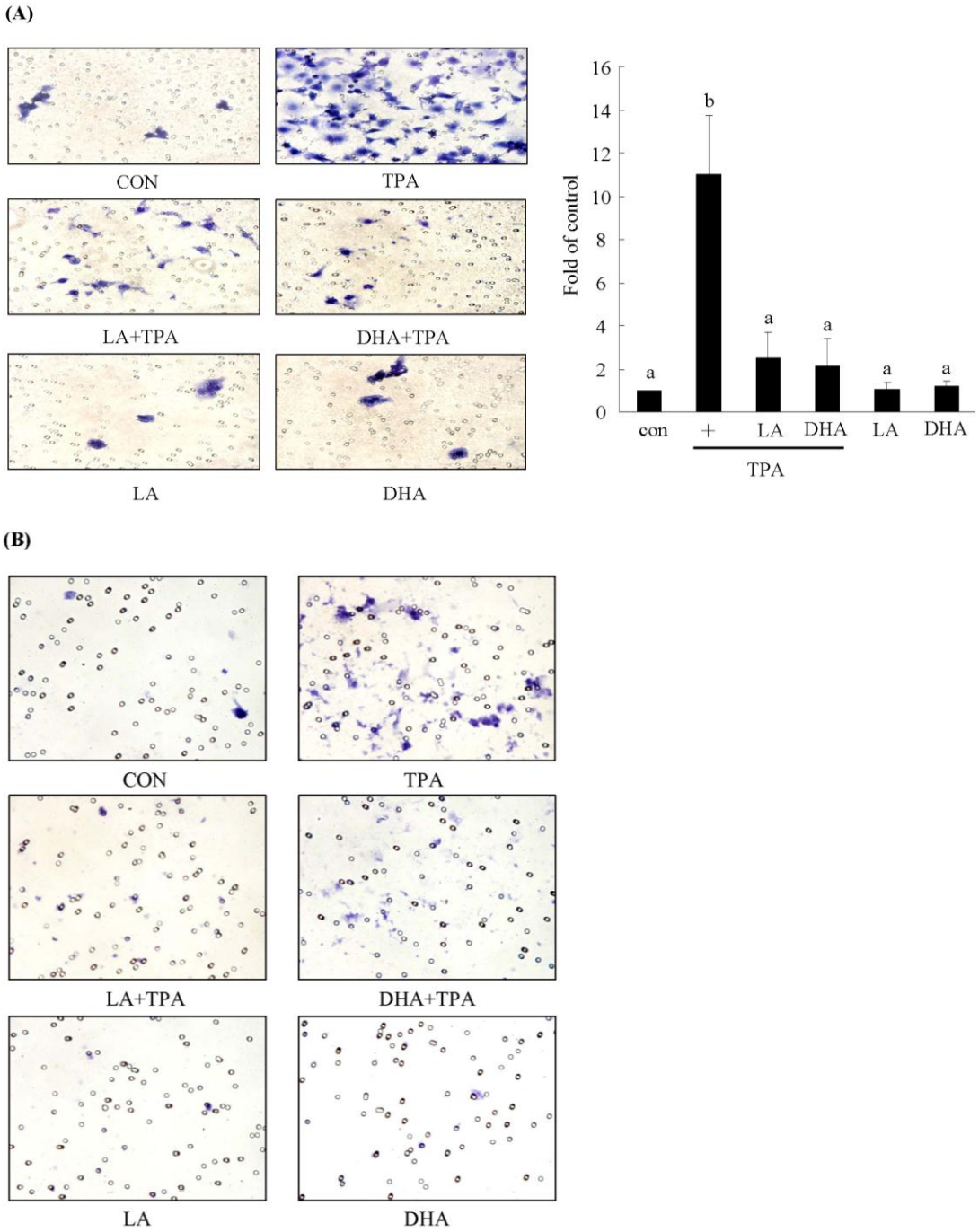


Figure 4. Effect of DHA or LA on TPA-induced migration and invasion in MCF-7 cells. (A) LA or DHA inhibits TPA-induced cell migration in MCF-7 cells. (B) LA or DHA inhibits TPA-induced cell invasion in MCF-7 cells. MCF-7 cells were pretreated with or without 200 μM LA or DHA for 20 h followed by incubation with or without 100 ng/mL of TPA for an addition 24 h. Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different (p<0.05).

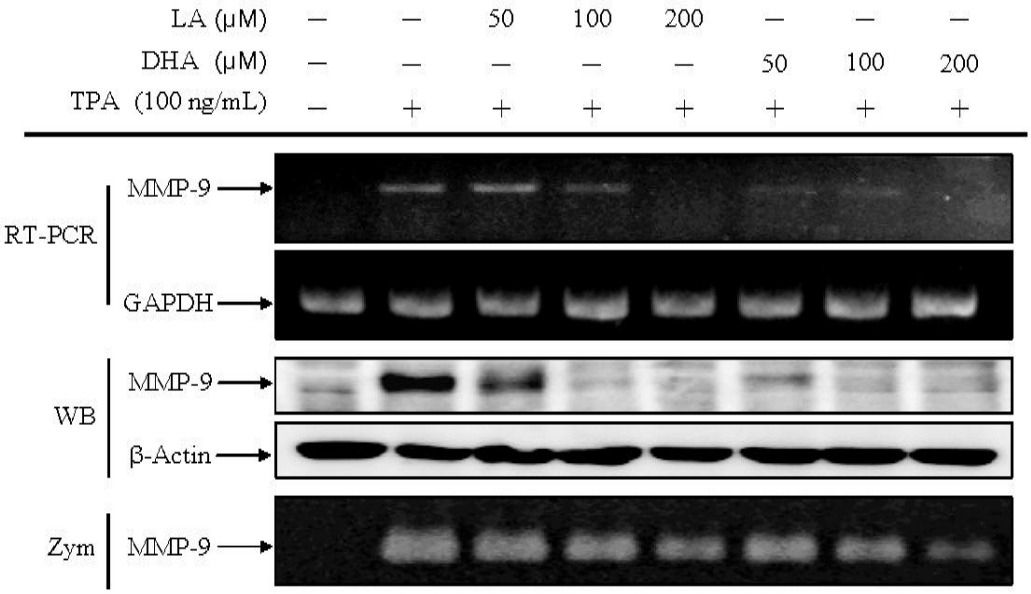


Figure 5. Effect of LA or DHA on TPA-induced MMP-9 expression in MCF-7 cells. Effect of LA or DHA on TPA-induced MMP-9 mRNA and protein expression, and MMP-9 enzyme activity. MCF-7 cells were treated with 100 ng/mL of TPA for **0**-24 h. MMP-9 mRNA expression was measured by RT-PCR. MMP-9 protein expression was measured by Western blot (WB) and MMP-9 enzyme activity was measured by gelatin zymography assay (Zym). Total RNA (0.1 μg/μL) were used for RT-PCR. Aliquot of cell lysates (20 μg) were used for Western blot assay. One representative experiment out of three independent experiments is shown. Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different (p<0.05).

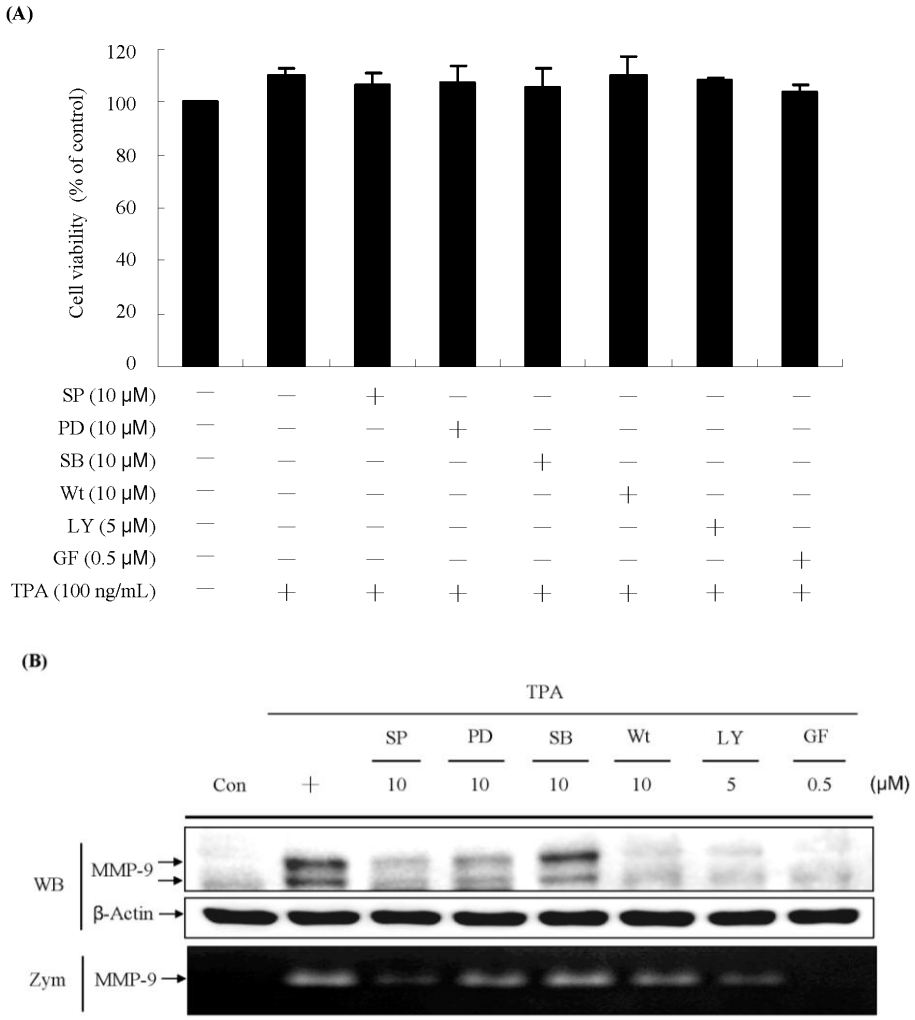


Figure 6. Effect of protein kinase inhibitors on TPA-induced MMP-9 expression in MCF-7 cells. (A) Effects of protein kinase inhibitors on MCF-7 cell viability. Cells were pretreated with pharmacological inhibitors of MAPKs, PI3K and PKC including SP600125 (JNK inhibitor, SP), PD98059 (ERK inhibitor, PD), SB203580 (p38 inhibitor, SB), wortmannin and LY294002 (PI3K inhibitors, Wt/LY), and GF109203X (non-selective PKC inhibitor, GF) for 24 h followed by incubation with 100 ng/mL of TPA for another 24 h. Cell viability was measured by using the MTT assay. (B) Effects of protein kinase inhibitors on TPA-induced MMP-9 protein expression and enzyme activity. MCF-7 cells were treated with 100 ng/mL of TPA for 0-24 h. MMP-9 protein expression was measured by Western blot (WB) and MMP-9 enzyme activity was measured by gelatin zymography assay (Zym). Aliquot of cell lysates (20 μg) were used for Western blot assay. One representative experiment out of three independent experiments is shown. Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different ($p<0.05$).

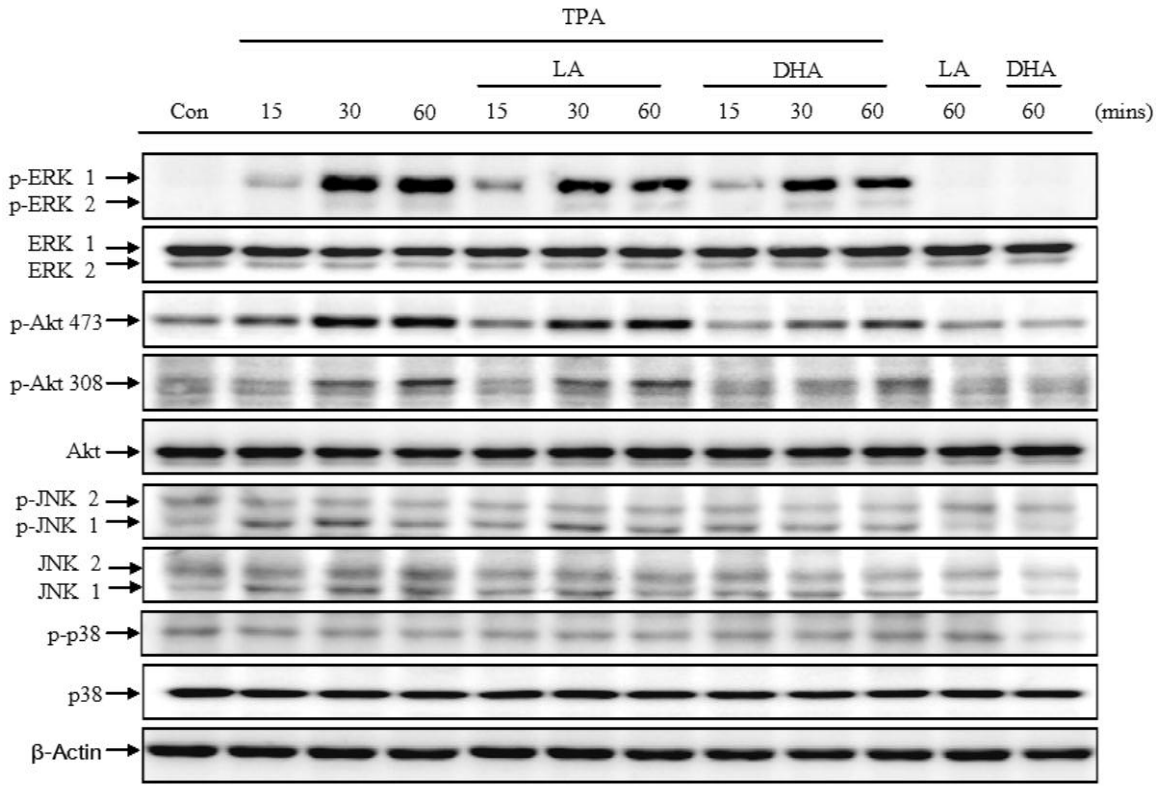


Figure 7. Effect of LA or DHA on TPA-induced MAPKs and Akt activation. Cells were treated with or without 200 μM LA or DHA for 24 h followed by incubation with or without 100 ng/mL of TPA for indicated time periods. The phosphorylation of protein kinases was measured by Western blot. Aliquots of cell lysates (20 μg) were used. One representative experiment out of three independent experiments is shown.

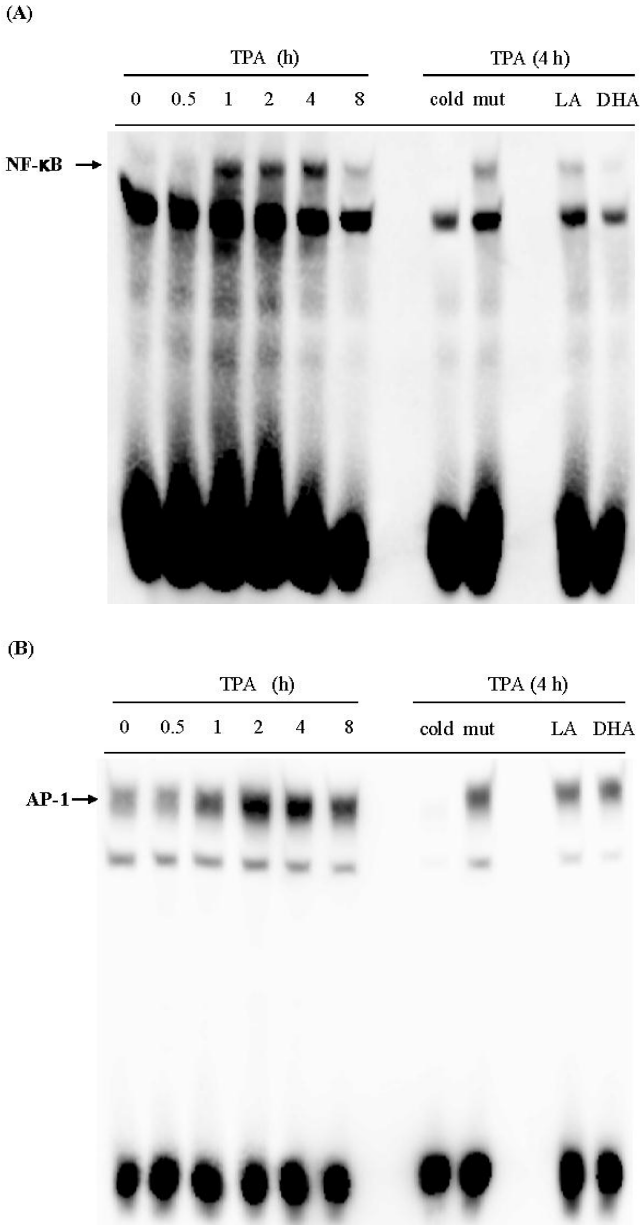


Figure 8. Effects of LA or DHA on TPA-induced AP-1and NF-κB DNA-binding activity. (A) Effects of LA or DHA on TPA-induced NF-κB DNA-binding activity. (B) Effects of LA or DHA on TPA-induced AP-1 DNA-binding activity. MCF-7 cells were treated with 100 ng/mL of TPA for indicated time periods, and cells pretreated with 200 μM LA or DHA for 24 h followed by incubation with 100 ng/mL of TPA for 4 h. Aliquots of nuclear extracts (10 μg) were used for EMSA. To confirm the specificity of the nucleotide, 25-fold of cold probe (biotin-unlabeled AP-1 or NF-κB binding site, cold) and biotin-labeled double-stranded mutant AP-1 or NF-κB oligonucleotide (mut, 4 μg) were included in the EMSA. One representative experiment out of three independent experiments is shown.

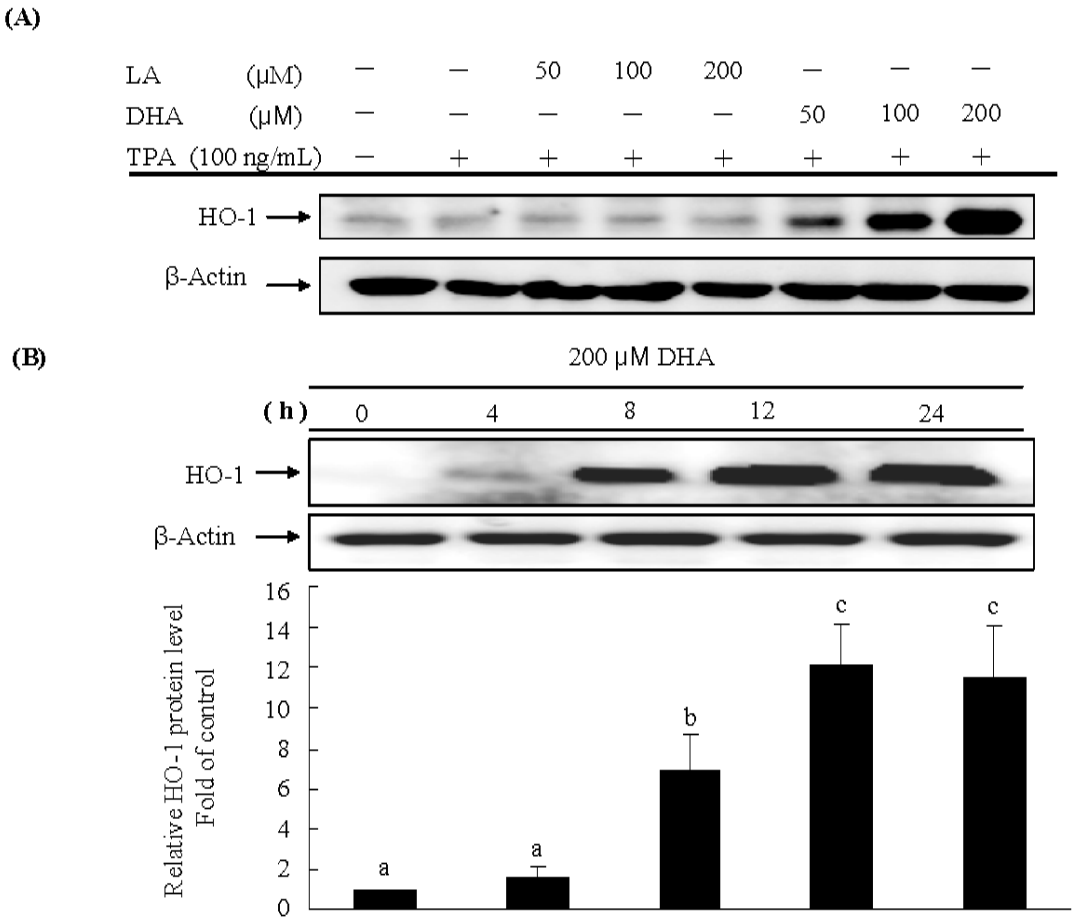


Figure 9. Effect of DHA on HO-1 Expression of MCF-7 Cells in the Presence of TPA. (A) DHA induced HO-1 protein expression in a dose-dependent manner. MCF-7 cells were pretreated with various doses of LA or DHA for 20 h, followed by treatment with 100 ng/mL of TPA for another 24 h. (B) DHA induced HO-1 protein expression in a time-dependent manner. MCF-7 cells were treated with 200 μM DHA for indicated time periods. Aliquots of cell lysates (20 μg) were used for Western blot assay. One representative experiment out of three independent experiments is shown. Values are means ± SD of three independent experiments. Values not sharing the same letter are significantly different (p<0.05).

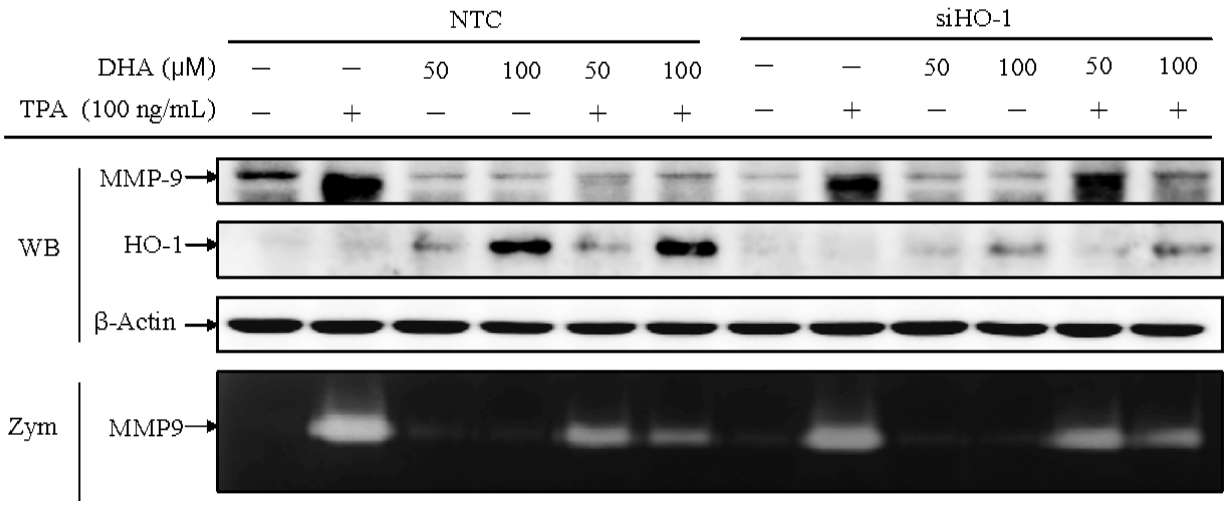


Figure 10. Effect of siHO-1 on the inhibition of MMP-9 expression by DHA.
An HO-1 siRNA system was used to silence HO-1 mRNA in cells and to create a siRNA knockdown MCF-7 cell model. Aliquots of cell lysates (20 μg) were used for Western blot (WB). MMP-9 enzyme activity was measured by gelatin zymography assay (Zym). One representative experiment out of three independent experiments is shown.

Conclusion

In the present study, we demonstrate that DHA and LA inhibits TPA-induced cell migration and invasion by reducing MMP-9 activation, mainly via ERK1/2 and PI3K/Akt pathways and sequentially NF-κB and AP-1 trans-activation. Furthermore, the inhibition of TPA-induced MMP-9 activation by DHA is at least in part through induction of HO-1 expression in MCF-7 breast cancer cells.

國科會補助專題研究計畫成果報告自評表

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：（以 100 字為限）

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）

乳癌好發於女性，也是美國女性癌症死亡的第二大原因，流行病學研究調查指出，在所有因癌症死亡的美國女性中，高達 15%是死於乳癌。乳癌的高致死率主要來自於乳癌細胞的高度轉移能力，使癌細胞易擴散並侵犯鄰近組織、器官所致。然而，對於如何提升乳癌細胞已發生轉移的乳癌患者之存活率，至今仍無顯著的改善。因此，在施以手術或化療、放射療等其它治療方式後，如何進一步有效抑制癌細胞複發及轉移，即成為防患乳癌死亡率增加的重要手段。本實驗室致力於研究乳癌細胞轉移之分子作用機轉，期望能找出降低癌細胞因發生轉移所導致高死亡率的治療方式。本研究結果發現二十二碳六烯酸(Docosahexaenoic acid, DHA)和亞麻油酸(Linoleic acid, LA) 可顯著減少 TPA 所誘發 NF-κB 及 AP-1 與 DNA 的結合能力進而降低與癌細胞轉移高度相關的基質金屬蛋白酶-9 (Matrix metalloproteinase-9, MMP-9)的表達，其中 DHA 抑制 TPA 誘發 MMP-9 活化過程中，少部分可能是透過抑制 SDC-4 並活化 HO-1 表現所影響。由此結果可知，飲食因子如魚油抑制乳癌細胞轉移之作用機轉可能與其活化 SDC-4 或 HO-1 有關，進一步研究可以觀察是否其他飲食因子或化合物也可透過調控此一訊號路徑減少乳癌細胞的轉移，開發出更具潛力的相關保健食品與藥物。

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/19

國科會補助計畫	計畫名稱: Syndecan在多元不飽和脂肪酸對調控肝癌及乳癌細胞轉移作用之影響
	計畫主持人: 李健群
	計畫編號: 99-2313-B-040-007- 學門領域: 食品及農化

無研發成果推廣資料

99 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：99-2313-B-040-007-
計畫名稱：Syndecan 在多元不飽和脂肪酸對調控肝癌及乳癌細胞轉移作用之影響	

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	90%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	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	0	100%	人次	
		博士生	1	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	0	2	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	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。)	無

	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)

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