行政院國家科學委員會專題研究計畫　成果報告

巨噬細胞中調控油酸所誘導的脂肪滴形成之訊息路線研究

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摘 要

我們之前的結果顯示，PMA 可以使經由油酸處理後的 RAW 264.7 巨噬細胞堆積更多的脂肪滴。在此，我想進一步探討的是 PMA 這種效應是否乃經由活化 mitogen-activated protein kinase (MAPK) 而達成。以 0.1 μM PMA 處理巨噬細胞可使 ERKs (p44 及 p42 MAPK) 在 10 分鐘內迅速磷酸化，但隨即在大約 3 小時時回到原來的程度。免疫轉漬的結果顯示以 PMA 處理 4 小時可使 adipophilin 的表現量增加，但以 100 μM PD 98059 這種 MEK 的抑制劑共同處理卻無法抑制 PMA 所引起的 adipophilin 增加之效果。除了不會影響脂質生成外，PD 98059 亦不會促進脂質分解。綜合以上的結果，我假設 ERK 這種 MAPK 的活性跟巨噬細胞內油酸所引起的脂質代謝作用無關。

關鍵詞

巨噬細胞；油酸；adipophilin；mitogen-activated protein kinase

ABSTRACT

Our previously result showed that PMA increased lipid droplet accumulation induced by oleic acid in RAW 264.7 macrophages. In this report, I further studied whether this effect is mediated via activation of mitogen-activated protein kinase (MAPK). Treatment of macrophages with 0.1 μM PMA induced rapid phosphorylation of ERKs (p44 and p42 MAPK) within 10 min, followed by returning to basal level at about 3 h. Results from immunoblot showed that PMA increased adipophilin expression at 4 h; however, cotreatment with 100 μM PD 98059, a MEK inhibitor, did not inhibit adipophilin protein level enhanced by PMA. In addition to have no effect on lipogenesis, PD 98059 did not stimulate lipolysis. Taken together, these results suggest that activity of ERK is not involved in lipid metabolism induced by oleic acid in RAW 264.7 macrophages

Keywords
macrophage; oleic acid; adipophilin; mitogen-activated protein kinase

INTRODUCTION

Adipophilin (also known as adipose differentiation-related protein, ADRP) is ubiquitously expressed in lipid droplet-containing cells in addition to adipocytes and steroidogenic cells. It can also be induced by oleic acid in RAW macrophages (Chen et al., 2001). Moreover, the expression of adipophilin protein level is enhanced by treatment with PMA (Chen et al., 2002). Nevertheless, the exact function of adipophilin in lipid metabolism and through what mechanism is it regulated remain uncertain. Moreover, nothing is known about its function during lipolysis. It should be clarified that adipophilin is necessary for lipid droplet formation or it simply acts by stabilization of lipid droplets.

Activation of mitogen-activated protein kinase (MAPK) in response to mitogen, cytokine, or various stresses plays a key role in transducing extracellular stimulus into intracellular signals which regulate a wide variety of cellular function. MAPK is composed of 3 distinct modules in vertebrates, i.e., extracellular-signal regulated kinase (ERK), c-Jun amino terminal kinase (JNK) and p38 (Hwang and Rhee, 1999). Recent studies indicate that oxidized LDL activate MAPK pathway via a PKC-dependent manner in smooth muscle cells and macrophages (Kusuhara et al., 1997; Yang et al., 2000), the two cell types involved in atherogenesis. Similar stimulation of MAPK by fatty acids has also been shown in rat liver epithelial WB cells (Hii et al., 1995) and U-937 cells (Barry et al., 1999). In adipocytes, activation of ERK has been shown to stimulate lipolysis via phosphorylation of hormone-sensitive lipase (Greenberg et al., 2001). However, these studies did not examine the correlation between MAPK activation and lipid droplet formation. Combined with our previous results (Chen et al., 2001) which demonstrated that lipid droplet accumulation and adipophilin expression are both up-regulated by PKC, it is thus necessary to investigate the putative involvement of PKC/MAPK in lipogenesis and adipophilin induction.

PURPOSES

Though adipophilin has been demonstrated to be located around lipid droplets, little is known about its functions and regulation in lipid metabolism. We utilize in vitro cultured RAW 264.7 macrophages to study the biochemical properties during lipogenesis and lipolysis. Since lipid-laden macrophage foam cells is one of the early characteristic in atherosclerosis, investigating the regulatory mechanisms of lipid metabolism in these cells may be helpful in understanding the process of atheroscerosis.

MATERIALS AND METHODS

Cell culture and drug treatment

RAW 264.7 macrophages (ATCC TIB-71) were purchased from the American Type Culture Collection (Rockville, MD) and cultured on coverglasses in DMEM (GibcoBRL, Long Island, NY) supplemented with 10% CPSR-1 (a low-lipid serum replacement) (Sigma), 100 units/ml of penicillin, and 100 μg/ml of streptomycin. To study lipid droplet accumulation, macrophages were loaded with 100 μg/ml of oleic acid (Sigma) (Jepson et al., 1996) in the presence of 0.1 μM PMA (Sigma) which activates ERK, or 100 μM PD 98059 (Calbiochem) which inhibits ERK.

Immunoblotting

RAW 264.7 macrophages were scraped off from the culture dishes, homogenized by sonication and determined for protein concentration (protein assay kit, BioRad). Cell lysates (50 μg/lane) were electrophoresed on 10 % SDS polyacrylamide gel (Fritz et al., 1989). Proteins were then transferred onto nitrocellulose membrane. After blocking with 5% non-fat milk for 30 min, the membranes were reacted with rabbit anti-adiophilin, mouse anti-phosphorylated ERK, followed by incubating with peroxidase-conjugated anti-rabbit and anti-mouse IgG. The blots were developed with the enhanced chemiluminence (ECL) system and exposed to Hyperfilm-ECL film (Amersham Pharmacia biotech, England).

Measurement of fatty acid release

After incubated with various agents, lipolysis was determined by measuring the extracellular free fatty acid using a colorimetric assay (Roche, Indianapolis; USA).

RESULTS

Fig. 1 shows that treatment of cells with oleic acid does not enhance obviously the phosphorylation of ERK 1 (p44 MAPK), though a slightly increase in ERK 2 (p42 MAPK) phosphorylation is observed. Incubation with 0.1 μM PMA significantly elevates phosphorylation levels of both p44 and p42 at 10 min in the presence or absence of oleic acid, followed by a decline to nearly basal level at about 3 h. As PMA potentiates lipid droplet accumulation & adipophilin expression, it is interesting to investigate whether PMA stimulates lipogenesis through activation of ERKs. Immunoblot results (Fig. 2)

shows that the level of adipophilin in PMA-treated cells is significantly elevated as compared with that in the absence of PMA. Unfortunately, PD 98059, a MEK inhibitor, at 100 μM does not inhibit the effect of PMA, nor does it affect adipophilin level induced by oleic acid alone. These results show that the PMA-enhanced adipophilin expression and lipid droplet accumulation is not mediated through activation of ERKs.

Though ERK is not involved in lipogenesis, it is worth to examine the effect of ERK on lipolysis. Measurement of extracellular fatty acid reveals that PD 98059 does not stimulate lipolysis in oleic acid-induced lipid-laden macrophages. All these findings indicate that ERK activity is irrelevant to lipid metabolism in RAW 264.7 macrophages incubated with oleic acid.

DISCUSSION

In this report, I present the data which demonstrate that both PMA-potentiated lipid droplet formation and adipophilin expression are not mediated via activation of ERK in RAW 264.7 macrophages incubated with oleic acid; moreover, inhibition of ERK activity does not stimulate lipolysis.

Previous results showed that PMA enhanced the oleic acid-induced PKC isozyme translocation in RAW 264.7 macrophages (Chen et al., 2002). It is possible that this increased translocation of PKC isozymes may explain the PMA-induced elevation of adipophilin expression and lipid droplet formation. Nevertheless, the downstream signaling mechanisms involved in this pathway remain unknown. Recent findings indicate that oxidized LDL-induced lipid-laden foam cell formation is mediated through activation of MAPK pathway via a PKC-dependent manner in smooth muscle cells and macrophages (Yang et al., 2000). Moreover, lipolysis in adipocytes is stimulated by activation of ERK (Greenberg et al., 2001). It is therefore my interest to explore the possibility that MAPK is also involved in lipid metabolism in macrophages incubated with oleic acid. My results show that, unlike adipocytes, neither lipogenesis nor lipolysis is regulated by activation or inhibition of ERK activity. These results indicate that lipolysis is regulated via different mechanisms between adipocytes and lipid-loaded RAW 264.7 macrophages, which may be due to the fact that adipocytes are “endowed” with lipid, whereas RAW 264.7 macrophages are “forced” to include exogenous lipid.

Though ERK does not participate in lipid metabolism in RAW 264.7 macrophages, the plausibility that this process is regulated by another modules of MAPK (JNK and p38) (Hwang and Rhee, 1999) can not be excluded out. Further studies are required to investigate the possible candidates involved in oleic acid- and PMA-induced lipid metabolism in RAW 264.7 macrophages.

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計劃結果自評

本計劃的結果初步排除了 MAP kinase 在 oleic acid 形成脂肪滴的過程所扮演的角色。比較此結果與先前其他作者在脂肪細胞所得到的結果顯示，並非所有含有脂肪滴的細胞其脂質代謝的訊息機制皆相同。至於巨噬細胞透過何種途徑將所攝入的 oleic acid 轉變成脂肪滴堆積在細胞質中則有待進一步的研究。