行政院國家科學委員會專題研究計畫 成果報告

利用蛋白質體與系統生物學解析雌激素與吞噬細胞引發慢性發炎之關係

研究成果報告(完整版)

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中 華 民 國 101 年 10 月 31 日

中 文 摘 要 ： 蝕骨前驅細胞功能如吞噬細胞。蝕骨前驅細胞分化後其蝕骨作用是重要的。雌激素藉由抑制蝕骨細胞分化來提供對骨頭的抗蝕骨作用。脂多醣引起蝕骨前驅細胞遷移和分化。細胞遷移需要肌動蛋白和鈣離子流入。Transient receptor potential (TRP) 通道是讓鈣離子流入非興奮性細胞。因此我們假設脂多醣誘發 TRP 通道增加鈣離子的流入，影響肌動蛋白的聚合作用並導致細胞遷移。雌激素抑制脂多醣誘發的細胞遷移。我們的結果顯示 ionomycin 觸動鈣離子流入倒轉雌激素對脂多醣誘發細胞遷移的阻斷作用。阻斷肌動蛋白聚合作用阻礙了 ionomycin 對抗雌激素抑制在蝕骨前驅細胞中脂多醣誘發的細胞遷移。辣椒素是 TRP vanilloid 1 離子通道的活化劑，其具有和 ionomycin 相同的倒轉雌激素對脂多醣誘發細胞遷移的阻斷作用。我們的發現推測雌激素對脂多醣誘發蝕骨前驅細胞遷移的抑制作用需要肌動蛋白和細胞外鈣離子，而鈣離子的流入來源可能是來自於 TRP 通道。總結來說，雌激素藉由干擾 TRP 通道抑制鈣離子流入，擾亂肌動蛋白聚合作用而因起抑制了脂多醣誘發的細胞遷移而這現象可能經由雌激素受體貝它。

中文關鍵詞： 吞噬細胞, 蝕骨前驅細胞, 細胞遷移, 雌激素, 脂多醣

英 文 摘 要 ： Osteoclast is differentiated monocytes and exhibits a property of mascrophage. Differentiation of osteoclasts from osteoclast precursors is important for osteoclast to provide its function in bone resorption. 17β-estradiol (estrogen, E2) provides an anti-resorptive effect on bone through inhibiting osteoclast differentiation. However, it is still unknown the mechanism of E2 on osteoclast precursor migration. Lipopolysaccharides (LPS) induce osteoclast precursor cell migration and differentiation. Cell migration requires actin polymerization and Ca2+ influx. Transient receptor potential (TRP) channel is a possible pathway for Ca2+ to flow in nonexcitable cells, such as osteoclasts. Therefore, we hypothesized that E2 inhibits LPS-induced migration in osteoclast precursors. According to our data, we suggested that LPS induced TRP channel to increase Ca2+ influx and thus influence actin polymerization and result in cell migration. E2 significantly suppressed LPS-induced cell migration. Triggering Ca2+ influx by

ionomycin reversed the inhibitory effect of E2 on LPS-induced migration. Blocking actin polymerization hindered the counteracting effect of ionomycin on LPS-induced migration which is inhibited by E2 in osteoclast precursors. Capsaicin, a TRP vanilloid 1 ion channel activator, had the same effect as ionomycin to reverse the inhibitory effect of E2 on LPS-induced migration in osteoclast. Our findings indicated that inhibitory effect of activated ERβ on LPS-induced osteoclast precursor cell migration required actin polymerization and extracellular Ca2+ and the influx source of Ca2+ may be from TRP channel. To conclude, we proposed that activated ERβ inhibited Ca2+ influx by interfering TRP, disturbed actin polymerization and thus blocked LPS-induced cell migration.

英文關鍵詞： macrophage, Osteoclast precursor, cell migration, estrogen, LPS

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:利用蛋白質體與系統生物學解析雌激素與吞噬細胞引發慢性發炎之關係

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計畫主持人：蔡美玲
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中　華　民　國　　101 年 10 月 31 日

中文摘要

蝕骨前驅細胞功能如吞噬細胞。蝕骨前驅細胞分化後其蝕骨作用是重要的。雌激素藉由抑制蝕骨細胞分化來提供對骨頭的抗蝕骨作用。脂多醣引起蝕骨前驅細胞遷移和分化。細胞遷移需要肌動蛋白和鈣離子流入。Transient receptor potential (TRP) 通道是讓鈣離子流入非興奮性細胞。因此我們假設脂多醣誘發 TRP 通道增加鈣離子的流入，影響肌動蛋白的聚合作用並導致細胞遷移。雌激素抑制脂多醣誘發的細胞遷移。我們的結果顯示 ionomycin 觸動鈣離子流入倒轉雌激素對脂多醣誘發細胞遷移的阻斷作用。阻斷肌動蛋白聚合作用阻礙了 ionomycin 對抗雌激素抑制在蝕骨前驅細胞中脂多醣誘發的細胞遷移。辣椒素是 TRP vanilloid 1 離子通道的活化劑，其具有和 ionomycin 相同的倒轉雌激素對脂多醣誘發細胞遷移的阻斷作用。我們的發現推測雌激素對脂多醣誘發蝕骨前驅細胞遷移的抑制作用需要肌動蛋白和細胞外鈣離子，而鈣離子的流入來源可能是來自於 TRP 通道。總結來說，雌激素藉由干擾 TRP 通道抑制鈣離子流入，擾亂肌動蛋白聚合作用而因起抑制了脂多醣誘發的細胞遷移而這現象可能經由雌激素受體貝它。

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英文摘要

Osteoclast is differentiated monocytes and exhibits a property of mascrophage. Differentiation of osteoclasts from osteoclast precursors is important for osteoclast to provide its function in bone resorption. 17β-estradiol (estrogen, E2) provides an anti-resorptive effect on bone through inhibiting osteoclast differentiation. However, it is still unknown the mechanism of E2 on osteoclast precursor migration. Lipopolysaccharides (LPS) induce osteoclast precursor cell migration and differentiation. Cell migration requires actin polymerization and Ca^{2+} influx. Transient receptor potential (TRP) channel is a possible pathway for Ca^{2+} to flow in nonexcitable cells, such as osteoclasts. Therefore, we hypothesized that E2 inhibits LPS-induced migration in osteoclast precursors. According to our data, we suggested that LPS induced TRP channel to increase Ca^{2+} influx and thus influence actin polymerization and result in cell migration. E2 significantly suppressed LPS-induced cell migration. Triggering Ca^{2+} influx by ionomycin reversed the inhibitory effect of E2 on LPS-induced migration. Blocking actin polymerization hindered the counteracting effect of ionomycin on LPS-induced migration which is inhibited by E2 in osteoclast precursors. Capsaicin, a TRP vanilloid 1 ion channel activator, had the same effect as ionomycin to reverse the inhibitory effect of E2 on LPS-induced migration in osteoclast. Our findings indicated that inhibitory effect of activated ERβ on LPS-induced osteoclast precursor cell migration required actin polymerization and extracellular Ca^{2+} and the influx source of Ca^{2+} may be from TRP channel. To conclude, we proposed that activated ERβ inhibited Ca^{2+} influx by interfering TRP, disturbed actin polymerization and thus blocked LPS-induced cell migration.

Keywords: macrophage, Osteoclast precursor, cell migration, estrogen, LPS

Introduction

Differentiation of osteoclasts initiates when precursor cells are attracted, recruited and migrated to bone. LPS is able to induce the differentiation of osteoclast precursors [1]. LPS induces macrophage motility and this requires actin polymerization [2]. However, the mechanism of LPS-induced migration is not clear.

Estrogen provides its anti-resorptive effect on bone and its effect is lost after menopause which results in osteoporosis. The bone-preserving effect of estrogen is mainly through regulating the number and development of osteoblasts and osteoclasts from their precursors. Estrogen deficiency induced osteoblast development [3]. Estrogen downregulates osteoblast development [4], while inhibits osteoclast development by suppressing cytokine production by osteoblast or its precursors . Estrogen directly attenuate the development of osteoclast and its resorption activity in osteoclast precursor monocytes in response to M-CSF and RANKL [5].

By reviewing previous studies, the inhibitory effect of estrogen on osteoclast precursor development which results in anti-resorptive effect on bone has long been investigated. Most of the literature reviews demonstrated that estrogen regulates osteoclast precursor proliferation, cytokine production and osteoclastic bone resorption. However, no studies have further investigated the effect of estrogen on the differentiation of osteoclast by inducing osteoclast precursor cell migration.

How estrogen mediates osteoclast precursor cell migration during osteoclast differentiation in order to inhibit bone resorption is not known. The mechanism of LPS on osteoclast precursor cell migration during osteoclast differentiation remains unclear. Based on these unsolved problems, our study is to determine how LPS stimulates osteoclast precursor cell migration and how estrogen provides its anti-resorptive effect on the initiation of osteoclast differentiation through inhibiting precursor cell migration. Thus, the purpose of this study is to 1) characterize Ca^{2+} and actin polymerization in LPS-induced migration and 2) explore the mechanism of estrogen on LPS-induced migration.

Materials and methods

I. Drugs and Reagents

All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Ionomycin and 1, 2- bis (2-aminophenoxy) ethane-N, N, N', N'-tetraacetic acid- acetoxymethyl ester (BAPTA-AM) were purchased from Invitrogen (Taipei, Taiwan). Capsaicin was from Enzo (Plymouth Meeting, PA, USA). Reagent water that was obtained from a Milli-Q Ultrapure Water Purification System (Millipore, Bedford, MA, USA) was used in all experiments.

II. Cell culture

RAW 264.7 cell (a osteoclast precursor cell line), MCF7 (a breast cancer cell line) used in this study were

routinely cultured in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 0.2-0.3 g sodium bicarbonate, 10% fetal bovine serum (Gibco, CA, USA) and 100 units/ mL penicillin-streptomycin (Gibco, CA, USA) at 37°C, 5% CO_2. After reaching 90% confluency, the cells were cultured in serum-free medium for further experiments.

III. Crude cell homogenates

After removal of serum-free medium, the cultured cells were incubated with 100 μL RIPA (150 mM NaCl, 10 mM Tris-base, pH 7.2, 0.1% SDS, 1.0% NP-40, 1% deoxycholate, and 5 mM EDTA) which contained three protease inhibitors including, 1 mM phenylmethansulfonyl flouride, 25 μg/ml leupeptin, and 1 mM sodium orthovanadate. After centrifugation at 14,000 g (4°C for 10 min), the supernatants were collected as crude cell homogenates. The total proteins measured by Lowry assay. Bovine serum albumin was used as the standard.

IV. Analysis of cell viability

1. Tryptan blue exclusion assay

The number of living cells was determined by tryptan blue exclusion assay. In each assay, 100 μl of tryptan blue (0.4%) was added to 100 μl of cell suspensions. 10 μl of the cell suspension was then applied to a hemocytometer, and the number of cells was counted for each data point in a total of eight microscopic fields.

2. Cell viability assay

Cell viability assay was measured by MTT (3-4, 5-dimethylthiazol-2-yl)- 2,5- diphenyl tetrazolium bromide) assay and was to determine cell viability. Five thousand RAW 264.7 cells were seeded onto a 96-well plate. Subsequently, cells were cultured in serum-free medium for 12 hours before treatment of drugs for an additional 24 hours or indicated times. 200 μl of MTT (5μg/μl) was then added to each well. By reacting with mitochondrial succinate dehydrogenase of living cells, the yellow colored MTT converted to formazan. 200 μl of dimethyl sulfoxide was added to dissolve the formazan crystals at 37°C for 10 min. The absorption value at 490 nm was detected by an ELISA reader (Sunrise, Tecan, Switzerland).

V. Western blot analysis

Equal amounts of cell homogenate (60 μg) were loaded to each lane on a 10% sodium dodecyl sulfate-polyacrylamide gel. Molecular weight markers were loaded simultaneously. After electrophoresis at 4°C for 2-4 hrs, separated proteins in the gel were transferred onto a polyvinyldifluoride membrane. The membrane containing transferred-proteins was treated with 5 % non-fat milk in Phosphate Buffer Saline & Tween buffer to block nonspecific binding. After incubation of the membrane with primary antibodies including anti-ERα (Santa Cruz, CA; SC-543), anti-ERβ (Upstate, NY; 05-824) and anti-β actin (Chemicon, CA; MAB1501) and then with secondary antibodies (Table 1.), the membrane was treated with chemiluminescence reagents to enhance signals. Finally, exposure of the membrane to the light-imagine film (Kodak, MA, USA) visualized the bands. The protein abundance of β actin was detected as an internal control.

VI. Migration assay

Chemotactic migration ability of osteoclast precursors was analyzed by Boyden chamber (AP48, Neuro probe Inc, MD, USA), which is a 48 well chemotaxis chamber. RAW 264.7 cells were cultured in serum-free medium for 12 hours. Later on, 1x 10^5 cells/ well suspended in serum-free DMEM with indicated drugs and placed into the upper chamber of Boyden chamber with a polycarbonate filter (8 μm pore size, GE osmonics labstore, MN, USA). The lower chamber was filled with medium containing 10% fetal bovine serum as a chemoattractant and indicated drugs. The indicated drugs loaded into both the upper and lower chambers are LPS, E2, cytochalasin B, EGTA (Ethylene glycol- bis (2-aminoethylether)- N,N,N′,N′-tetraacetic acid), BAPTA-AM, ionomycin, DPN (diarylpropionitrile), PHTPP, or Ruthenium Red. After incubated for indicated times, cells in the upper chamber were removed with a cotton swab. The cells present on the lower surface of the filters were fixed with methanol for 10 mins. Filters were thereafter stained with hematoxylin (Vector Laboratories Inc, CA, USA) for 30 mins, and then washed out with MQ water. Subsequently, the nuclei stained in brown color cells in three different fields of each well were imaged at 200x, counted and averaged as a representative number of migrated cells per field.

VII. Data analysis and statistical evaluation

Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by a pair-wise comparison in the means model of SPSS (SYSTAT 11.0, SPSS Science, CA, USA), with $p < 0.05$ considered significant. All experiments were carried out at least three independent times. Data represent as the means ± SEM (standard errors).

Results:

Objective I: Effect of actin and calcium influx on LPS-induced migration

Migration of osteoclast precursor cells is a critical step to initiate osteoclast differentiation. Cell migration requires actin polymerization and the influx of Ca^{2+}. Therefore, our first objective is to explore the role of actin and Ca^{2+} on LPS-induced cell migration. We used cytochalasin B to test whether actin is involved in LPS-induced osteoclast precursor cell migration. Compared with the vehicle, cytochalasin B at a concentration of 10^{-6} M and treatment for 6 hrs increased migrated cell numbers but attenuated the increase of migrated cells by LPS (Fig. 1). These data suggest that LPS-induced migration of osteoclast precursors is due to actin polymerization.

Our next study was to investigate whether Ca^{2+} influx involved in LPS-induced migration. To test this hypothesis, intracellular or extracellular Ca^{2+} chelators were used to influence Ca^{2+} concentrations. Addition of EGTA, a chelator of extracellular Ca^{2+}, decreased the LPS-induced migration in a dose-dependent manner to the minimum concentration (5x 10^{-3} M) which has no effect on cell migration as compared with vehicle (Fig. 2). BAPTA-AM, which can easily pass through the cell

membrane and then convert to BAPTA by intracellular esterase, was further used as an intracellular Ca^{2+} chelator. However, addition of BAPTA-AM at a concentration of 10^{-6} M was not found to alter the cell migration in cells treated with or without LPS (Fig. 3). Ionomycin, a Ca^{2+} ionophore, introduces a channel on the membrane and permits Ca^{2+} influx into the cell [6]. According to Supplementary Fig. 4 and 5, ionomycin at the concentration of 10^{-8} M did not affect cell viability and migration ability. Thus, ionomycin (10^{-8} M) was used in the following experiments. Ionomycin significantly increased cell migration induced by LPS at the concentration of 0.1 μg/mL, but not at 0.5μg/mL (Fig. 4). The results indicate that extracellular Ca^{2+} is required for LPS-induced migration. Thus, we further investigated the role of actin on Ca^{2+} dependent LPS-induced migration. The LPS-induced cell migration triggered by ionomycin was reduced by cytochalasin B (Fig. 5).

To explore whether the source of Ca^{2+} is from during LPS-induced migration, capsaicin, a TRP vanilloid 1 ion channel activator, was used. Capsaicin enhanced the ability of LPS to induce migration and such an effect was even greater as compared with the vehicle (Fig. 6). Collectively, these data demonstrate that LPS-induced migration on osteoclast precursor cells is actin polymerization and Ca^{2+} dependent. The Ca^{2+} influx may be through TRP channel.

Objective II: Effect of 17β-estradiol on LPS-induced migration

Our second objective was to investigate the effect of E2 on LPS-induced migration. First, the effect of E2 on the viability of RAW 264.7 cells was examined. Based on data from Supplementary Fig. 2, E2 at a concentration of 10^{-8} M alone did not affect the viability of RAW 264.7 cells. Hence, E2 at 10^{-8} M was used for later experiments.

The number of migrated cells was amplified by LPS in exposure period for 6 hrs. After 6-hr treatment with LPS, E2 was able to reduce the number of migrated cells (Fig. 7). To further explore whether E2 lowers the number of migrated cells via its estrogen receptors, we first measured the expression of ERα and ERβ in RAW 264.7 cell. In our Supplementary Fig. 3 showed that RAW 264.7 cells expressed ERβ mainly. Therefore, our next experiment was to explore whether inhibitory effect of E2 on LPS-induced migration is ERβ dependent. DPN which is an ERβ agonist and antagonist was used in our study. Further treatment of DPN (10^{-8} M) for 6 hrs suppressed LPS-induced migration (Supplementary Fig. 7). Taken together, these data suggest that LPS is required for the ability of estrogen to inhibit LPS-induced migration in osteoclast precursor cells under our experimental conditions and this mechanism of estrogen action is likely through ERβ.

Thereafter, we studied whether the inhibitory effect of E2 on LPS-induced migration is actin- and Ca^{2+}- dependent. We first examined the effect of ionomycin on E2 mediated LPS-induced migration. Ionomycin at 10^{-8} M reversed the decrease of LPS-induced migration by E2 and this effect was also superior to vehicle (Fig. 8). We clarified whether the inhibition of cell migration by E2 in LPS-induced migration is mediated by actin. Cytochalasin B blocked the effect of ionomycin on E2 mediated cell migration induced by LPS (Fig. 9). Also, the blockage effect of cytochalasin B on LPS-induced migration ability even turned down migrated cell numbers to the level similar to vehicle. This data suggest that the inhibitory effect of estrogen on Ca^{2+} -dependent migration induced by LPS was altered by an interference with actin polymerization.

We further determined to verify the source of Ca^{2+} is from during estrogen mediated migration induced by LPS. TRP channels are a family of ion channels permeable for Ca^{2+}, and some also for Mg^{2+} [55]. To test the hypothesis that Ca^{2+} influx was through TRPV1 channel, capsaicin, a TRP vanilloid 1 ion channel activator, and ruthenium red, a non-selective TRP channel blocker, were used to determine the role of TRP channels in the inhibition of LPS-induced migration by estrogen. The migrated cell numbers in capsaicin- and ruthenium red- mediated LPS-induced migration inhibited by E2 had similar effect with vehicle. Capsaicin reversed the inhibitory effect of estrogen on LPS-induced migration, while ruthenium red inhibited LPS-induced migration (Fig. 10). Taken together, TRP channel may be the possible channel regulated by estrogen to exert its inhibitory effect on cell migration in response to LPS.

Discussion

Summary of this study

We hypothesized that Ca^{2+} and actin polymerization were involved in LPS-induced migration and that estrogen inhibited LPS-induced migration. Our data clearly showed that actin polymerization disrupting drug and Ca^{2+} chelator lowered LPS-induced migration ability. TRP channel was the possible pathway for Ca^{2+} to influx during LPS-induced migration. A 6-hr treatment with E2 declined the LPS-induced migration. E2 inhibited LPS-induced migration is actin- and Ca^{2+}-dependent. Above all, we proposed a pathway of the inhibitory effect of estrogen on LPS-induced migration in osteoclast precursors (Fig. 11). The inhibitory effect of E2 on LPS-induced migration is suggested to be mediated by influencing TRP channel, subsequently reduced influx of Ca^{2+} and actin polymerization.

LPS-induced migration

Migration of osteoclast precursors (the family of monocyte/macrophage lineage cells) into bone is a critical process to initiate osteoclast differentiation.

In Supplementary Fig. 1, we first characterize that mononuclear osteoclast precursors are able to differentiate into multinucleated osteoclasts in response to LPS (0.5 μg/ml) as shown in previous study by Islam et al. [6].

Cytoskeleton and extracellular Ca^{2+} are considered as the two important components involved in cell migration [7-10]. LPS, a component of Gram positive bacteria cell wall, not only induces immune response and activates macrophages in the host but also triggers the differentiation of osteoclast precursors. RAW 264.7 as an osteoclast precursor cell can differentiate into macrophage-like osteoclasts in response to LPS [6]. Thus, we used LPS to trigger osteoclast differentiation and examine its function on osteoclast precursor cell migration. The importance of our finding is to demonstrate that actin polymerization and Ca^{2+} influx play a critical role in LPS-induced migration.

Effect of Ca^{2+} on LPS-induced migration

LPS increased the amount of Ca^{2+} inside the cytosol of macrophage. During migration, the influx of Ca^{2+} may come from extracellular compartment or intracellular compartment. Our data demonstrated that extracellular Ca^{2+} is more important in LPS-induced migration. Based on Fig.4 and Supplementary Fig. 6, the effect of LPS on inducing cell migration reached maximum at the concentration of 0.5 μg/mL, and thus ionomycin could not increase more cells to migrate. However, the induction of migration mediated by ionomycin was shown when LPS-induced migration was inhibited by cytochalasin B. Such the case, LPS-induced migration is Ca^{2+}-dependent. Here, we showed that extracellular Ca^{2+} entered cells during LPS-induced migration and this result was consistent with previous studies.

Role of TRP channel on Ca^{2+}-dependent LPS-induced migration

The source of Ca^{2+} in cells is considered to be from voltage-dependent or voltage-independent ion channels. TRP channel is recognized as the channel permeable for Ca^{2+} in nonexcitable cells, such as osteoclast precursors. Among the subfamilies of TRP channel, Idris et al. [13] demonstrated that mouse osteoclasts expressed TRP vanilloid 1 (TRPV1) mRNA and protein and TRPV1 antagonist repressed osteoclast formation and bone-resorptive activity. TRPV2 mRNA was found in LPS-induced RAW 264 macrophages and TRPV2 involved in the increase of intracellular Ca^{2+} in LPS-induced RAW 264 [14]. However, the role of TRP channel in LPS-induced cell migration remains uncertain. Thus, we used TRP channel activator and TRP channel non-selective blocker to examine the role of TRP channel in LPS-induced migration. In Supplementary Fig. 8, capsaicin increased LPS-induced migration. The upregulation of LPS-induced migration by capsaicin is inhibited by EGTA. Our novel finding is that TRP channel (more specifically TRPV1) is involved in the Ca^{2+}-dependent LPS-induced migration

Effect of actin on LPS-induced migration

Actin is one of the components of cytoskeleton that are most recognized to play a role in cell migration. LPS increased actin reorganization in macrophage. We showed that actin polymerization is important for LPS-induced migration. Blocking actin polymerization by cytochalasin B disrupted the Ca^{2+}-dependent cell migration triggered by LPS.

Effect of E2 on LPS-induced migration

Estrogen inhibits bone remodeling by regulating osteoblastic bone formation and osteoclastic bone resorption. Estrogen inhibits bone resorption through suppressing osteoclast development. It is well known that E2 restrains cytokine- mediated osteoclast precursor cell number and bone resorption activity regulated by osteoblast. However, how estrogen mediates osteoclast differentiation at the first step through modulating precursor cell migration remains unknown.

In our study, we hypothesized that estrogen inhibited osteoclast precursor cell migration induced by LPS. To test our hypothesis, we used RAW 264.7 cells, which are the precursor of osteoclasts, were chosen as our cell model in an attempt to identify the mechanism of estrogen to influence LPS-induced migration in these cells. Our report showed that Estrogen reduced LPS-induced migration in an exposure period of 6 hrs.

The osteoprotective effect of E2 on bone is mediated through ERα. Though both ERα and ERβ are both found in human osteoclast, the role of activated ERβ on osteoclast precursors is rarely discussed. Our Supplementary Fig. 3 showed that RAW 264.7 are ERβ dominant cells. According to our Supplementary Fig. 7, we suggested that activated ERβ by E2 is likely to regulate LPS-induced migration.

Significance of this study

The significance of our study is to demonstrate that LPS-induced migration required Ca^{2+} influx through TRP channel and actin polymerization. This is the first study to demonstrate that the inhibitory effect of estrogen on LPS-induced migration is actin- and Ca^{2+}-dependent. In response to LPS, Ca^{2+} influx through TRP channel for cells to migrate during osteoclast differentiation. TRP channel is the downstream target of the genomic pathway of E2 to inhibit LPS-induced osteoclast differentiation. However, whether TRP channel can be regulated by estrogen receptors requires further investigation.

References

1. Tajima T, Murata T, Aritake K, Urade Y, Hirai H, Nakamura M, Ozaki H, Hori M: Lipopolysaccharide induces macrophage migration via prostaglandin D(2) and prostaglandin E(2). J Pharmacol Exp Ther 2008, 326:493-501.
2. Kleveta G, Borzecka K, Zdioruk M, Czerkies M, Kuberczyk H, Sybirna N, Sobota A, Kwiatkowska K: LPS induces phosphorylation of actin-regulatory proteins leading to actin reassembly and macrophage motility. J Cell Biochem 2012, 113:80-92.
3. Jilka RL, Takahashi K, Munshi M, Williams DC, Roberson PK, Manolagas SC: **Loss of estrogen upregulates osteoblastogenesis in the murine bone marrow. Evidence for autonomy from factors released during bone resorption.** *J Clin Invest* 1998, **101:**1942-1950.
4. Robinson JA, Harris SA, Riggs BL, Spelsberg TC: **Estrogen regulation of human osteoblastic cell proliferation and differentiation.** *Endocrinology* 1997, **138:**2919-2927.
5. Sorensen MG, Henriksen K, Dziegiel MH, Tanko LB, Karsdal MA: **Estrogen directly attenuates human osteoclastogenesis, but has no effect on resorption by mature osteoclasts.** *DNA Cell Biol* 2006, **25:**475-483.
6. Le Clainche C, Carlier MF: **Regulation of actin assembly associated with protrusion and adhesion in cell migration.** *Physiol Rev* 2008, **88:**489-513.
7. Naccache PH, Showell HJ, Becker EL, Sha'afi RI: **Transport of sodium, potassium, and calcium across rabbit polymorphonuclear leukocyte membranes. Effect of chemotactic factor.** *J Cell Biol* 1977, **73:**428-444.
8. Sanchez-Madrid F, del Pozo MA: **Leukocyte polarization in cell migration and immune interactions.** *EMBO J* 1999, **18:**501-511.
9. Schiffmann E: **Leukocyte chemotaxis.** *Annu Rev Physiol* 1982, **44:**553-568.
10. Zigmond SH: **Chemotaxis by polymorphonuclear leukocytes.** *J Cell Biol* 1978, **77:**269-287.
11. Mormann M, Thederan M, Nackchbandi I, Giese T, Wagner C, Hansch GM: **Lipopolysaccharides (LPS) induce the differentiation of human monocytes to osteoclasts in a tumour necrosis factor (TNF) alpha-dependent manner: a link between infection and pathological bone resorption.** *Mol Immunol* 2008, **45:**3330-3337.
12. Zhuang L, Jung JY, Wang EW, Houlihan P, Ramos L, Pashia M, Chole RA: **Pseudomonas aeruginosa lipopolysaccharide induces osteoclastogenesis through a toll-like receptor 4 mediated pathway in vitro and in vivo.** *Laryngoscope* 2007, **117:**841-847.
13. Idris AI, Landao-Bassonga E, Ralston SH: **The TRPV1 ion channel antagonist capsazepine inhibits osteoclast and osteoblast differentiation in vitro and ovariectomy induced bone loss in vivo.** *Bone* 2010, **46:**1089-1099.
14. Yamashiro K, Sasano T, Tojo K, Namekata I, Kurokawa J, Sawada N, Suganami T, Kamei Y, Tanaka H, Tajima N, et al: **Role of transient receptor potential vanilloid 2 in LPS-induced cytokine production in macrophages.** *Biochem Biophys Res Commun* 2010, **398:**284-289.

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: 利用蛋白質體與系統生物學解析雌激素與吞噬細胞引發慢性發炎之關係
	計畫主持人: 蔡美玲
	計畫編號: 100-2629-B-006-003- 學門領域: 性別主流科技計畫

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：蔡美玲	計畫編號：100-2629-B-006-003-
計畫名稱：利用蛋白質體與系統生物學解析雌激素與吞噬細胞引發慢性發炎之關係	

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	3	3	100%		1. Pei-Yu Wu, Mei-Ling Tsai. Involvement of activated estrogen receptor beta in phenotypic shift of LPS-activated macrophages. The 26th Joint Annual Conference of Biomedical Science (2011). 2. Yi-Jing Huang, Ying-Yi Liu, Mei-Ling Tsai. Correlation analysis of angiogenesis and tumor size in

							microtubule dynamics in 4T1-bearing mice. The 7th Congress of FAOPS in Taipei (2011).
		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	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%		
國外	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	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。)		no					

	成果項目	量化	名稱或內容性質簡述
科教	測驗工具(含質性與量性)	0	
	課程/模組	0	

處計畫加填項目	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

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)

Our findings indicated that inhibitory effect of activated ERβ on LPS-induced osteoclast precursor cell migration required actin polymerization and extracellular Ca2+ and the influx source of Ca2+ may be from TRP channel. To conclude, we proposed that activated ERβ inhibited Ca2+ influx by interfering TRP, disturbed actin polymerization and thus blocked LPS-induced cell migration.