行政院國家科學委員會補助專題研究計畫成果報告

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※ Regulation of insulin signaling by extracellular matrix ※
※ in mammary epithelial cells ※
※ 細胞外間質調節乳腺上皮細胞對胰島素刺激所引發 ※
※ 訊息傳遞的機轉 ※

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計畫主持人：李宜儒

共同主持人：

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執行單位：中山醫學大學免疫學研究所

中　華　民　國　90 年　10 月　31 日

行政院國家科學委員會專題研究計畫成果報告

細胞外間質調節乳腺上皮細胞對胰島素刺激所引發訊息傳遞的機轉

Regulation of Insulin Signaling by Extracellular Matrix in Mammary Epithelial Cells

計畫編號：NSC 89-2320-B-040-064
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主持人：李宜儒　中山醫學大學免疫學研究所
計畫參與人員：林義宗　中山醫學大學醫學研究所

一、中文摘要

細胞外間質可經由影響生長因子的訊息傳遞而改變細胞的生理反應。以前的結果顯示若將乳腺上皮細胞培養在類似基底膜的間質上，胰島素的訊息傳遞則較強，尤其是這些在胰島素受體以下的訊號。經由動力學分析，我們更進一步證明細胞貼附於基底膜的時間愈長，胰島素所刺激的 IRS-1 胳氨酸磷酸化的程度就愈高。此外，胰島素可與 IGF-I 的受體結合，而且細胞與基底膜的接觸可促進 IRS-1 轉移到 IGF-I 受體上。In-gel PTP assay 的結果顯示細胞外間質會影響去酸磷酶的表現。細胞培養在基底膜或培養皿，其去酸磷酶的表現則不同，這可能對調節 IRS-1 胳氨酸磷酸化有關。因此，我們認為對乳腺上皮細胞而言基底膜提高胰島素訊息傳遞的機轉在於促進 IRS-1 移附到受體上，以利 IRS-胳氨酸磷酸化及其下游的訊息傳遞。

關鍵詞：基底膜、胰島素、IRS-1

Abstract

Extracellular matrix (ECM) influences cellular responses by affecting growth factor-activated signaling. Our previous work has shown that mammary cell adhesion to basement membrane (BM) confers better signal propagation downstream of insulin receptor. In this study, we further demonstrated that persisted adhesion to BM was optimal for tyrosine phosphorylation of IRS-1. Beside, insulin could signal through IGF-I receptor, and the recruitment of IRS-1 to IGF-I receptor in response to insulin was BM-dependent. By the in-gel PTP assay, we found that cells cultured on BM exhibited a different pattern of PTP expression compared to those cultured on plastic, which might have a role in the regulation of IRS-1 tyrosine phosphorylation. Thus, the requirement of BM for insulin signaling in mammary epithelial cells is controlled by the permissiveness of signal relay between the receptor complex and IRS-1.

Keywords: basement membrane, insulin, IRS-1

二、緣由與目的

Extracellular matrix (ECM) not only provides physical supports for tissues but also transmits biochemical signals into cells through its cell surface receptors. It often acts in conjunction with growth factor to affect various cellular responses such as proliferation, differentiation, migration and survival (1). Mounting evidence has demonstrated a cross-talk between ECM- and growth factor-triggered signaling pathways (1).

Mammary epithelial cells reside on basement membrane (BM) *in vivo*. Expression of the milk protein β-casein, the hallmark of mammary cell differentiation, requires the presence of lactogenic hormones (prolactin, insulin and hydrocortisone) and BM (2, 3). Moreover, both insulin and BM confers mammary cell survival (4). One

explanation for duel requirement of growth factor and BM for mammary functions is that cell adhesion to BM renders these cells full responsiveness to growth factors (5).

Insulin initiates cascades of signaling by promoting tyrosine phosphorylation of the insulin receptor (IR). The active receptor then recruits and phosphorylates the insulin receptor substrate (IRS) and Shc. IRS protein contains multiple tyrosine residues, which serves as docking sites for a number of signaling molecules including PI3K, Grb2, SHP-2 and Nck (6). It has been shown that ECM regulates insulin signaling in several cell types (7-10). In mammary epithelium, insulin signaling propagates better in cells cultured on BM than in those cultured on collagen I or tissue culture plastic (11). Greater extents of tyrosine phosphorylation of IRS-1 and its association with PI3K as well as PKB activation take place in cells cultured on BM; however, tyrosine phosphorylation of IR is indiscernible in cells on both substrata (4, 11). Thus, it is conceivable that ECM modulates insulin signaling at the level of tyrosine phosphorylation of IRS-1 in mammary epithelial cells. Here we further investigated the possibilities that signal relay between IR and IRS-1 is disrupted, leading to substantial levels of IR activation but a defective in IRS-1 tyrosine phosphorylation, and that the involvement of PTP in downregulation of IRS-1 phosphorylation.

三、結果與討論

Prolonged adhesion to BM is required for optimal tyrosine phosphorylation of IRS-1 in response to insulin. All of our previous work was performed in long-term cultures where mammary cells were in contact with the substrata for 3-4 days. In this study, we were interested in whether short-period adhesion to substrata would give rise to the same results as the long-term cultures. Moreover, we wished to unravel the nature of the "inhibitory" or "stimulatory" effect conferred by collagen and BM, respectively, on tyrosine phosphorylation of IRS-1. A time course experiment was performed for this purpose whereby trypsinized cells were plated on either collagen I or BM for 1h, 6h, 24h and 48h, stimulated with insulin for 15 min, and IRS-1 tyrosine phosphorylation was monitered.

Lots of cells adhered to collagen I 1 h after plating, and some of them started to spread. After 6 h, all adhered cells spread out. Virtually all cells adhered on BM after 1h, but these cells remained round-shaped. Cell clusters formed after 6 h, with very few single cells left. Regarding IRS-1 tyrosine phosphorylation, there was no difference between cells cultured on collagen I and BM 1h after plating (Fig. 1). The extent of phosphorylation increased as cell adhesion to BM persisted, and reached the maximum after 2 days (Fig. 1). IRS-1 phosphorylation in cells cultured on collagen I also elevated slightly as adhesion continued, but it was much lower than those in cells cultured on BM. Thus, optimal IRS tyrosine phosphorylation requires prolonged adhesion to BM.

Recruitment of IRS-1 to IGF-I receptor in response to insulin is BM-dependent. We have found that insulin at the physiological concentration signaled through the IGF-I receptor in mammary cells cultured on BM, and IRS-1 is co-precipitated with IGF-I receptor after insulin treatment. To examine whether this also occurred in mammary cells cultured on plastic, similar experiments were performed in cells cultured on plastic and BM. IR was co-precipitated with IGFIR in cells cultured on both substrata, but greater extents of IRS-1 were associated with IGFIR in cells cultured on BM in response to insulin (Fig. 2). Thus, formation of the IR/IGFIR complex is irrespective of the substrata that cells reside, whereas insulin signal relay between the receptor complex and IRS-1 requires cell anchorage to BM.

Examination of PTP expression in mammary cells cultured on BM and plastic. Our previous work has revealed that vanadate can somehow improve the tyrosine phosphorylation of IRS-1 in cells cultured on collagen I or plastic (11), suggesting PTP may be, at least in part, involved in the ECM-mediated modulation of IRS-1

phosphorylation. In search for the possible PTPs associated with IRS-1, in-gel PTP assay was performed (12). Phosphorylated poly(glutamate-tyrosine) was incorporated into acrylamide gels, and samples were separated by SDS-PAGE. Following electrophoresis, gels were subjected to denaturation and renaturation treatment, and PTP activity was detected by autoradiography. The clear bands in a black background represented where PTPs localized.

Whole cell PTP expression in mammary cells cultured on plastic and BM were obtained by using total cell lysates for in-gel PTP assay. Similar profile but different expression levels of PTP were observed in these cells (Fig. 3). PTP with the molecular weight of ~125 kDa and ~65 kDa were predominantly expressed in cells cultured on plastic, while those at the size of ~35 kDa and ~40 kDa were detected mainly in cells cultured on BM. Insulin stimulation did not alter PTP expression. This result suggests that cell-ECM interactions affect PTP expression in mammary epithelial cells.

Higher extent of SHP-2 is associated with IRS-1 upon insulin stimulation in cells cultured on BM. We then examined the possibility that cell-ECM interactions influence the association of PTPs with IRS-1. Thus, IRS-1 immunoprecipitates were subjected to in-gel PTP assay. Insulin induced the association of an ~70 KDa PTP with IRS-1 in cells cultured on both substrata, but the extents was greater in cells cultured on BM (Fig. 4). This PTP is likely to be SHP-2 since it migrated at about the same size as the major band from SHP-2 immunoprecipitates (data not shown).

The extent of association between SHP-2 and IRS-1 was further examined by co-immunoprecipitation analysis. In agreement with the results in figure 4, insulin stimulated the association of SHP-2 with IRS-1, and the extent of association was higher in cells cultured on BM (Fig. 5). These results suggest that SHP-2 is not responsible for the downregulation of IRS-1 tyrosine phosphorylation in cells cultured on plastic. This was not too surprising since SHP-2 has been shown to play a positive role in insulin signaling (13). It is likely that other PTPs are involved in the inhibition of IRS-1 tyrosine phosphorylation but they could not be detected by the in-gel PTP assay.

四、計畫成果自評

We have accomplished almost 80% of the experiments proposed in this project. The rest of them are undergoing at the moment. We shall have a manuscript ready for submission in the near future.

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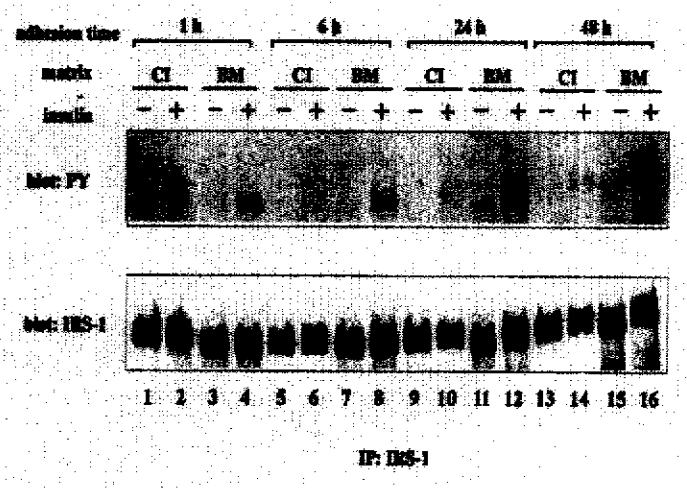

Figure 1. Kinetic analysis of the effect of cell adhesion to ECM on IRS-1 tyrosine phosphorylation. Primary mammary cells were trypsinized and kept in suspension for 1h or plated on collagen I (CI) and BM for 1, 6, 24 and 48 h. Cells were then incubated in the absence or presence of insulin for 15 min. Total cell lysates were immunoprecipitated with IRS-1 antibody followed by immunoblotting with anti-phosphotyrosine antibody (PY). The blot were stripped and re-probed with IRS-1 antibody.

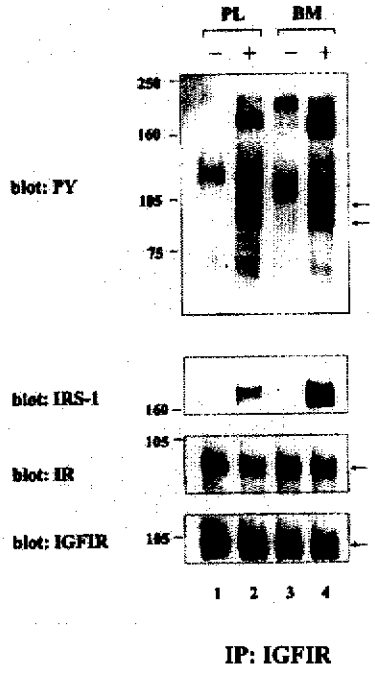

Figure 2. Recruitment of IRS-1 to IGFIR in response to insulin is BM-dependent. Cells cultured on plastic (PL) or BM were incubated in the absence or presence of 100 nM insulin for 15 min. Cell lysates were immunoprecipitated with anti-IGFIR antibody, followed by immunoblotting with anti-phosphotyrosine antibody (PY). The blot was stripped and re-probed with antibodies to IRS-1, IR and IGFIR.

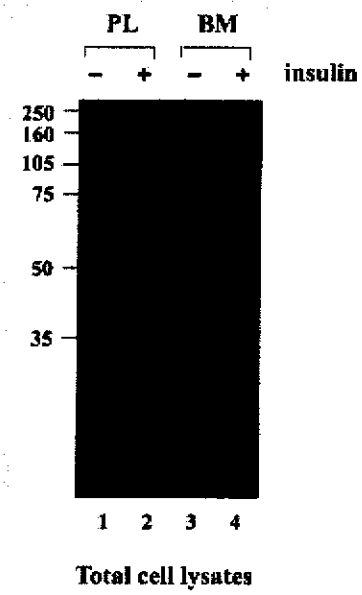

Figure 3. PTP expression profile in mammary cells cultured on plastic and BM. Mammary cells cultured on plastic (PL) and BM were incubated in the absence and presence of 100 nM insulin for 15 min. Total cell lysates were subjected to in gel PTP assay.

Figure 4. PTP association with IRS-1. Cells cultured on plastic (PL) and BM were incubated in the absence or presence of 100 nM insulin for 15 min. Total cell lysates were immunoprecipitated with antibodies to IRS-1, followed by in gel PTP assay.

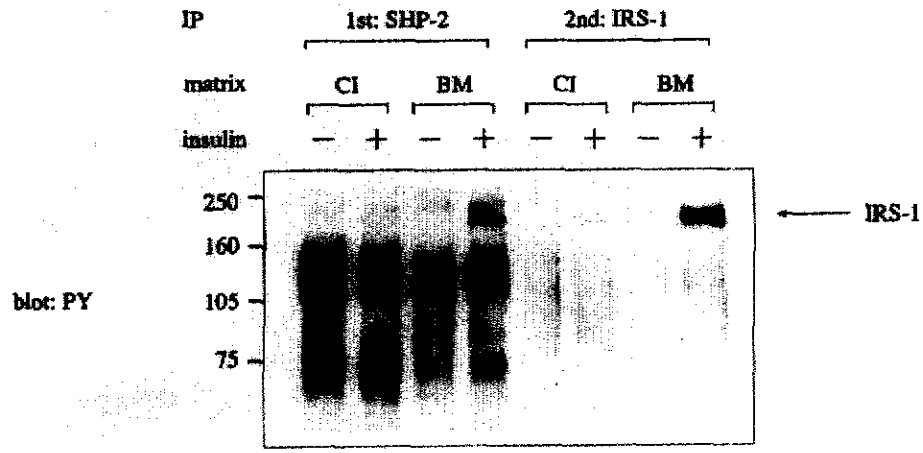

Figure 5. Insulin stimulated higher extent of association between SHP-2 and IRS-1 in mammary cells cultured on BM. Cells cultured on collagen I (CI) or BM were incubated in the absence or presence of insulin for 15 min. Cell lysates were firstly immunoprecipitated with anti-SHP-2 antibody, and the supernatant was subjected to another round of immunoprecipitation by IRS-1 antibody. These immunoprecipitates were then loaded on SDS-PAGE, transferred to membrane, and blotted with anti-phosphotyrosine antibody (PY).