行政院國家科學委員會專題研究計畫 期末報告

免疫相關基因對基質調控乳腺結構和功能所扮演的角色

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 101-2320-B-040-005-
執 行 期 間 ：101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ：中山醫學大學微生物免疫研究所

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中 華 民 國 102 年 10 月 30 日

中文摘要：微環境中的細胞外基質對組織的構形及功能具極大的影響。在乳腺組織中，上皮細胞貼附於基底膜，形成具有空腔的腺體結構。在體外，若將細胞培養於由基底膜基質所構成的 3D 環境則能重建此種結構；再加入泌乳荷爾蒙（泌乳激素、胰島素和氫皮質酮）還能進一步刺激乳腺細胞的分化。相對於此，細胞貼附在一般的塑膠盤或是第一型膠原蛋白薄層所構成的 2D 培養環境時，僅能形成單一且扁平的細胞層，並且喪失對泌乳荷爾蒙的反應以及分化的能力。基於微環境的重要性，我們比較以 2D 及 3D 方式培養時細胞的基因表現，希望能藉此找出調控乳腺形態及功能的基因。微矩陣分析的結果顯示，有兩類基因在 3D 的培養條件下呈現增加的情形；一類是與清除凋亡細胞有關的基因，另一類則具有先天免疫功能。由於培養在基底膜上的乳腺細胞會形成空腔，而空腔的形成又是因為腺泡內部的細胞進行凋亡而後再被清除所致，因此我們推測這兩類基因可能參與了乳腺內腔的清空。在本篇研究，我們確認先前微矩陣分析所得之結果，發現培養在基底膜的乳腺細胞 CD14、lipopolysaccharide-binding protein、complement 3、growth arrest specific 6 及 milk-fat globule epidermal growth factor 8 的表現較培養在塑膠盤的乳腺細胞高。此現象發生在腺泡空腔形成之前，顯示這些基因可能參與了乳腺內腔的清空。我們並發現細胞外基質的類型對誘發免疫相關基因扮演了重要的角色，因為當把細胞重新貼附在第一型膠原蛋白薄層或改變基質所刺激的 RhoA/Rac 活性時，免疫相關基因的表現也隨之被影響。由於一些免疫相關基因為急性期基因（acute phase gene），而 Stat3 對誘發急性期基因十分重要，所以我們也檢測了 Stat3 在乳腺細胞的表現量及磷酸化的程度。結果顯示，二者在培養於基底膜的乳腺細胞中皆較高；因此，Stat3 可能為調控免疫相關基因表現的上游訊息分子。總結而言，內腔的形成與細胞分化在乳腺發育過程中扮演了重要的角色。當調控失當時可能會產生內腔充填（luminal filling）和不必要的發炎反應以及分化能力喪失的情形；這些反而有利於腫瘤的進展。我們希望這些研究能使我們對乳腺的正常發育有更深入的了解，並進而找出治療乳癌的策略。

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of mammary cells display this morphology when they are grown on 3D BM matrix. Furthermore, these cells acquire functional differentiation in the presence of lactogenic hormone. By contrast, cells cultured on tissue culture plastic or thin-layer of collagen I, a 2D environment, form monolayers. They are unresponsive to the stimulation of lactogenic hormone. Owing to the importance of the microenvironment, we compared gene expression in cells cultured on 2D plastic and 3D BM to find out genes that control breast architecture and function. Two groups of gene are especially upregulated in cells cultured on BM. One contributes to the clearance of apoptotic cells; the other has innate immune function. As cells cultured on BM exhibit 3D acinar morphology with a lumen, and lumen formation requires apoptosis and the subsequent removal of apoptotic cells, we speculate that these genes are involved in luminal clearance of mammary gland. Here we confirm our microarray data and show that the expression levels of CD14, LBP, C3, GAS6 and MFGE8 are augmented in cells cultured on BM. This precedes lumen formation, suggesting these genes might have a role in luminal clearance of the mammary gland. The type of ECM is critical for induction of the immune-related genes as re-adhesion of cells onto 2D collagen I or altering ECM-elicited RhoA/Rac activity affects their expression. Some genes identified by microarray analysis are acute phase genes. Given that Stat3 is a key factor to induce acute-phase genes, we have also examined the expression and phosphorylation levels of Stat3 in mammary cells. Our results showed that both of them are higher in cells cultured on BM, implying that activation of Stat3 acts upstream of gene induction. Lumen formation and differentiation are key events in development. When they are dysregulated, adverse consequences such as luminal filling and undesirable inflammation take place, which facilitate tumor progression. We hope our work helps decipher the control of normal development of mammary gland and furthermore, devise strategies for cancer therapy.

英文關鍵詞： mammary gland, matrix, morphogenesis, differentiation, immune response

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

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共同主持人：
計畫參與人員：杜軍毅、黃俊浩、趙育麟

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中　華　民　國 102 年 10 月 30 日

目錄

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英文摘要及關鍵詞

Within the context of 3D microenvironment, extracellular matrix (ECM) greatly influences tissue architecture and function. In mammary glands, epithelial cells contact basement membrane (BM) to form glandular structures with a central lumen. In vitro cultures of mammary cells can display this morphology as long as they are grown in a 3D environment made up with malleable BM matrix. Furthermore, these cells acquire functional differentiation in the presence of lactogenic hormone (prolactin, insulin and hydrocortisone). By contrast, cells cultured on tissue culture plastic or thin-layer of collagen I, a 2D environment, form monolayers. They are unresponsive to the stimulation of lactogenic hormone, and lose the ability to differentiate. Owing to the importance of the microenvironment, we compared gene expression in cells cultured on 2D plastic and 3D BM, the conditions with least and most resemblance to the in vivo state, respectively, in order to find out genes that control breast architecture and function. Two groups of gene are especially upregulated in cells cultured on BM. One contributes to the clearance of apoptotic cells; the other has innate immune function. As cells cultured on BM exhibit 3D acinar morphology with a lumen, and lumen formation requires apoptosis and the subsequent removal of apoptotic cells, we speculate that these genes are involved in luminal clearance of mammary gland. Here we confirm our microarray data and show that the expression levels of CD14, LPS-binding protein, complement 3, growth arrest-specific 6 and milk fat globule-EGF factor 8 are augmented in cells cultured on BM. This precedes lumen formation, suggesting these genes might have a role in luminal clearance of the mammary gland. The type of ECM is critical for induction of the immune-related genes as re-adhesion of cells onto 2D collagen I or altering ECM-elicited RhoA/Rac activity affects their expression. Some genes identified by microarray analysis are acute phase genes. Given that Stat3 is a key factor to induce acute-phase genes, we have also examined the expression and phosphorylation levels of Stat3 in mammary cells. Our results showed that both of them are higher in cells cultured on BM, implying that activation of Stat3 acts upstream of gene induction. Lumen formation and differentiation are key events in development. When they are dysregulated, adverse consequences such as luminal filling, undesirable inflammation and loss of differentiation take place, which facilitate tumor progression. We hope our work helps decipher the control of normal development of mammary gland and furthermore, devise strategies for cancer therapy.

Key words: mammary gland, matrix, morphogenesis, differentiation, immune response

報告內容

一、前言

Normal tissue architecture is essential for tissues to function properly, and also provides restraint to prevent tumor progression. Within the context of 3D microenvironment, the extracellular matrix (ECM) is the key factor to mold tissue morphology. Both the composition and rigidity of the ECM affect this process by activating biochemical and mechanical signals. Mammary gland displays glandular structure with a central lumen, and its constituent epithelial cells contact the basement membrane (BM) *in vivo*. This feature can be reestablished *in vitro* by culturing mammary cells on pliable 3D BM matrix. Furthermore, these cells acquire functional differentiation in the presence of lactogenic hormones (prolactin, insulin and hydrocortisone). By contrast, cells cultured on 2D substratum, such as on the tissue culture plastic or thin-layer of collagen I (the dominant stromal matrix in mammary glands), form monolayers, and they are unable to differentiate even with the stimulation of lactogenic hormones. Thus, ECM greatly affects mammary morphology and function.

二、研究目的

In light of the importance of tissue architecture, gene expression profiling was exploited to study the correlation between morphology and gene expression, with the desire to find out signature genes that link to specific functions, or even predict outcome for breast cancer. We thus compared gene expression in primary mouse mammary epithelial cells cultured on 2D plastic and 3D BM, the conditions with least and most resemblance to the in vivo state, respectively.

三、文獻探討

ECM in the microenvironment provides the positioning signal to establish apico-basal polarity, and instructs morphogenesis. Maintaining tissue structure is essential for normal functions. Taking the mammary gland as an example, apical secretion of milk into the lumen of alveoli and the transport of milk through ducts require correct tissue structure and polarity. Moreover, normal tissue architecture poses a barrier to block tumor progression, and losing tissue architecture facilitates the advance of malignancy. The classical example for it is the effect of Rous sarcoma virus on tumor formation. Injection of the virus into chicken embryos did not generate tumors. But when the embryos were dissociated and cultured in a Petri dish, transformed phenotype quickly appeared (Bissell and Hines, 2011). Another study shows that mutant p53 disrupts mammary architecture via the mevalonate pathway. Depletion of the mutant p53 or inhibition of the pathway by statin or geranylgeranyl transferase inhibitor reverts breast cancer cells to a more normal morphology (Freed-Pastor et al., 2012). Therefore, identification of the factors jeopardizing tissue architecture might shed new light on cancer therapy. Owing to the importance of tissue architecture, gene expression profiling is used to study the correlation between morphology and gene expression. A large-scale study using 25 breast cell lines show that these cells cultured on 3D BM adopt different types of morphology, referred to as: Round class, Mass class, Grape-like class and Stellate class. Interestingly, cell lines of similar morphologies share certain pattern of gene expression (Kenny et al., 2007). Another study is to compare gene expression profiles in 2D and 3D cultures of 2 breast cell lines, which have relatively normal phenotype. The genes downregulated in 3D condition can be used as prognosis markers for breast cancer with high accuracy (Fournier et al., 2006).

Lumen formation is a key event of morphogenesis for many organs. Different mechanisms have been

elucidated (Bryant and Mostov, 2008). For mammary glands and salivary glands, lumen develops by cavitation. Initially, epithelial cells aggregate to form cell clusters. As cells inside the cluster are devoid of contact with BM, they undergo anoikis (Schafer et al., 2009). p38α plays a role in anoikis during mammary morphogenesis, and inhibition of this pathway leads to luminal filling reminiscent of that observed in ductal carcinoma in situ (DCIS)(Wen et al., 2011). Cells inside the cluster also undergo autophagy because of hypoxia and nutrient deprivation (Mills et al., 2004). Apart from these types of cell death, lysosomal-mediated cell death and entosis have been detected (Kreuzaler et al., 2011; Overholtzer et al., 2007). Dead cells are then removed by neighboring epithelial cells or incoming professional phagocytes, resulting in the formation of lumen (Monks et al., 2005). As for kidney and vascular development, lumen is formed by hollowing mechanism. This does not require cell apoptosis, and is mediated by membrane separation of epithelial cells (Bryant and Mostov, 2008).

The clearance of apoptotic cells, also termed efferocytosis, requires a crosstalk between the dying cells and phagocytes. Apoptotic cells release “find-me” signals such as lysophosphatidylcholine, CX3CL1, ATP/UTP, S19 ribosomal dimers, endothelial monocyte-activating polypeptide II, and TGF-β to attract phagocytes. They also display “eat-me” signals. A major “eat-me” signal is phosphatidylserine (PS) which can be bound by a panel of soluble molecules, such as β2-glycoprotein I (β2-GPI), growth arrest specific 6 (GAS6), annexin I, milk-fat globule epidermal growth factor 8 (MFGE8), transglutaminase 2 (TG2), thrombospondin-1 (TSP-1) and serum-derived protein S. These molecules are then recognized by receptors on phagocytes, including β2-GPI receptor, Mer, PS receptor (PSR), αvβ3 integrin and CD36. Another type of the “eat-me” signal is oxidized low-density lipoprotein, which interacts with an array of scavenger receptors on phagocytes. The innate immunity also plays a part in efferocytosis. Complements and pentraxins can bridge apoptotic cells and phagocytes by interacting with apoptotic-cell-associated molecular pattern (ACAMP) on dying cells and CD14/calreticulin/CD91complex on phagocytes. Engagement and clustering of these receptors on phagocytes stimulate the engulfment and the subsequent immunosuppression (Chao et al., 2012; Ravichandran and Lorenz, 2007).

Communication between immune cells and mammary cells contributes to the normal development of the mammary gland. During puberty, macrophages, eosinophils and mast cells are distributed around the terminal end buds, and helps ductal outgrow and branching morphogenesis. Macrophages and eosinophils also play a role during pregnancy and lactation as the blockade of their recruitment to mammary glands leads to a decrease in milk production. Lymphocytes are found the lactating mammary glands as well. Their secreted products (ex: IgA) are transported to milk, providing passive immunity for the newborn. After weaning, mammary glands undergo involution with massive apoptosis taking place. More macrophages come in to hasten the removal of apoptotic cells (Atabai et al., 2007; Reed and Schwertfeger, 2010). It has been shown that lymphocytes and plasma cells are also infiltrated to the mammary gland at the later stage of involution (Stein et al., 2004).

Mammary epithelia itself possesses immune functions. Many constituents in milk such as lysozyme and xanthine oxidoreductase that are synthesized by mammary cells are known to have antimicrobial function. This can protect the newborn from infection, and also protect the mother from mastitis. In fact, the mammary gland is considered to be evolved from the innate immune system (Vorbach et al., 2006). Furthermore, in vitro experiments reveal that mammary cells synthesize Th2 cytokines (IL-4 and IL-13) in the course of differentiation. This is accompanied by a decrease in the production of Th1 cytokines (IL-12 and TNF-α). In agreement with these results, levels of Stat6 phosphorylation and GATA3 expression are elevated during

pregnancy. Stat6 is the downstream signaling molecule of IL-4/IL-13, and GATA3 is the target gene of Stat6. Interestingly, Stat6-deficinet and IL-4/IL-13-doubly deficient mice display similar phenotype in mammary glands, with a delayed development of lubuloalveolar structures (Khaled et al., 2007; Watson et al., 2011).

Gene expression profiling of mammary gland development reveals that a large number of genes involved in immune function are induced during involution. These include cytokines, acute phase proteins, soluble defense factors and immunoglobulins. Stat3 and CCAAT/enhancer binding protein δ (C/EBPδ), two key transcription factors for induction of acute phase genes, are also upregulated (Clarkson et al., 2004; Stein et al., 2004). Conditional deletion of Stat3 in mammary epithelium impairs the acute phase response, and polarizes macrophages towards the M1 phenotype (Hughes et al., 2012). Even so, the role for induction of these immune-related genes during involution is not completely clear. As dramatic tissue remodeling in mammary glands occurs after weaning, it has been suggested that these genes are involved in a wound healing-like process. The increased expression of cytokines and immune mediators at this stage is thus implicated in the incidence and poor prognosis of pregnancy-associated breast cancer (Asztalos et al., 2010; Schedin, 2006). On the other hand, clearance of apoptotic cells leads to immunosuppression by producing TGF-β and IL-10. Some acute phase proteins such as pentraxin 3, serum amyloids A, ceruloplasmin, uterocalin and secretory leukocyte protease inhibitor, also exhibit anti-inflammatory functions. This can counteract the inflammatory responses to avoid overt inflammation (Atabai et al., 2007; Clarkson et al., 2004).

四、研究方法

Substrata and Cell Cultures

Collagen I thin gel-coated dishes are prepared by incubating plates overnight at 4 °C with collagen I at 8 μg/cm^2. The plates are washed extensively with PBS before use. For cells embedding in 3D collagen gel, cells suspension is mixed with the neutralized collagen I and then plated in 24-well plates. After the gel is solidified, medium supplemented with 10% FCS and 50 mg/ml of ascorbic acid are added to each well. Relaxed collagen gels are prepared by gently dislodging the anchored collagen gels from the underlying plates by a spatula. Reconstituted basement membrane matrix (Matrigel) is coated onto dishes at 14 mg/ml. To fix matrigel, 2% paraformaldehyde is added and incubated with gel at room temperature for 30 min. The reaction is stopped by aspiration of the fixative, and the gel is washed with 0.1 M glycine in PBS for 6-8 times. Primary epithelial cultures are prepared from mid-pregnant ICR mice and plated on different substrata in nutrient mixture F-12 containing 10% fetal bovine serum, 1 mg/ml fetuin, 5 ng/ml EGF, 5 μg/ml insulin and 1 μg/ml hydrocortisone. After 72 h, medium is changed to Dulbecco's modified Eagle's medium DMEM)/nutrient mixture F-12 containing hydrocortisone, insulin and prolactin (3 μg/ml). Second passage cells are obtained by trypsinization of cells cultured on collagen I or BM and replated on different substrata. Mouse mammary gland epithelial cells, NMuMG, are grown in DMEM supplemented with 10% fetal bovine serum and 10 μg/ml insulin.

RNA Extraction and RT-PCR

Cells are lysed by TRIzol reagent, and total RNA is extracted. Reverse transcription is performed on 1 μg of total RNA using reverse transcriptase and oligo(dT) primers. The reverse transcription products are then used as templates for PCR amplification using gene-specific primers. PCR products are separated on 1.5% agarose gel and analyzed by ethidium bromide incorporation.

Immunoprecipitation and Western Blot Analysis

Cells are lysed in lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1 mM Na_3VO_4, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1% Triton-100. Cell lysates containing equal amounts of protein are incubated with 1-2 μg of antibody and 20-50 μl of protein A-Sepharose beads for 2-4 h at 4°C. Immunoprecipitates or whole cell lysates are subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with antibody. Proteins are visualized using an ECL kit.

Immunofluorescence Microscopy

Cells are fixed in 4% paraformaldehyde in PBS, quenched with 0.1 M glycine, and then permeabilized with 0.2% Triton X-100 in PBS for 20 min. The samples are blocked for 1 h with the blocking solution containing 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 0.05% NaN_3, 10% goat serum, 0.2% Triton X-100 and 0.05% Tween 20 and incubated with primary antibody for 3-4 h at room temperature followed by 6 washes with PBS. The samples are then incubated with secondary antibody for 2 h at room temperature followed by 6 wishes with PBS. After mounting the samples on glass slides, slides are observed under a confocal microscope.

五、結果與討論

Many genes upregulated in primary mouse mammary epithelial cells cultured on BM have functions for clearance of apoptotic cells and innate immunity.

In searching of signature genes for mammary cells cultured on 2D and 3D environment, primary mouse mammary epithelial cells were cultured on plastic or BM for 4 d, and total RNA was collected for microarray analysis. Unexpectedly, two groups of gene were upregulated in cells cultured on 3D BM. One is involved in the clearance of apoptotic cells; the other has innate immune functions. As primary cultures contain heterogeneous populations of cell despite that the majority is epithelial cells, we wondered if the immune-related genes were expressed by leukocytes. To clarify it, a normal mouse mammary gland epithelial cell line NMuMG was used. Cells were cultured on 2D collagen I and 3D BM for 1 d, and total RNA was subjected to microarray analysis. To our surprise, many immune-related genes were upregulated in cells cultured on BM. Some genes were detected in both primary cells and the cell line. These results suggest that mammary cells themselves are capable to express genes involved in immune regulation.

To confirm microarray data, we examined the expression of CD14, lipopolysaccharide-binding protein (LBP), complement 3 (C3), GAS6 and MFGE8 by RT-PCR. The first three genes confer immune function, and the latter two gene products participate in clearance of apoptotic cells. For comparison, the experiment set-up here was the exactly the same as that for microarray experiments. Thus, primary mammary epithelial cells were cultured on plastic or BM for 4 d, NMuMG cells were cultured on collagen I or BM for 1 d, and total RNA was subjected to RT-PCR analysis. Except for MFGE8, all genes were upregulated in both cells cultured on 3D BM (Fig. 1). This is consistent with the microarray data. To keep it simple, the following experiments were conducted mainly in primary cells.

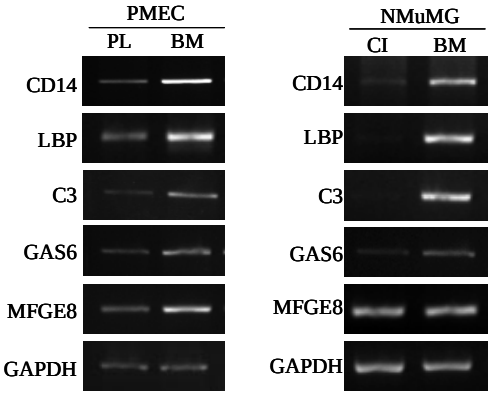

Figure 1. Upregulation of clearance- and immune-related genes in mammary cells cultured on 3D BM.

Kinetic analysis of clearance- and immune-related gene expression and lumen formation in primary mouse mammary epithelial cells.

Given that cells cultured on BM exhibit acinar morphology with a central lumen, and lumen formation requires apoptosis and the subsequent removal of apoptotic cells, we wonder if the clearance- and immune-related genes are involved in luminal clearance of mammary glands. We started with the examination of a temporal link between gene expression and luminal clearance. If this does exist, there is a good chance that these genes are involved in lumen formation of mammary glands. Primary mammary cells were cultured on plastic or BM for 1, 2 or 4 d, total RNA and cell lysates were collected for RT-PCR and immunoblotting analysis, respectively. Upregulation of CD14, LBP, C3, GAS6 and MFGE8 was observed in cells cultured on BM for 1, 2 and 4 d. But the great differences in gene expression between these two culture conditions occurred on day 2 (Fig. 2). To examine lumen formation, cells were cultured on BM for 1, 2 and 4 d, stained with Hoescht 33258 and rhodamin-phalloidin, and visualized under confocal microscope. The strong staining of phalloidin dictates the intensive localization of F-actin, particularly on the apical surface of an acinus. The lack of nuclear stain in the middle of the acinus indicates where the lumen forms. Proper lumen formation was observed in cells cultured on BM for 4 d (Fig. 3). Thus, the expression of clearance- and immune-related genes precedes lumen formation, implicating their roles in the luminal clearance of mammary glands.

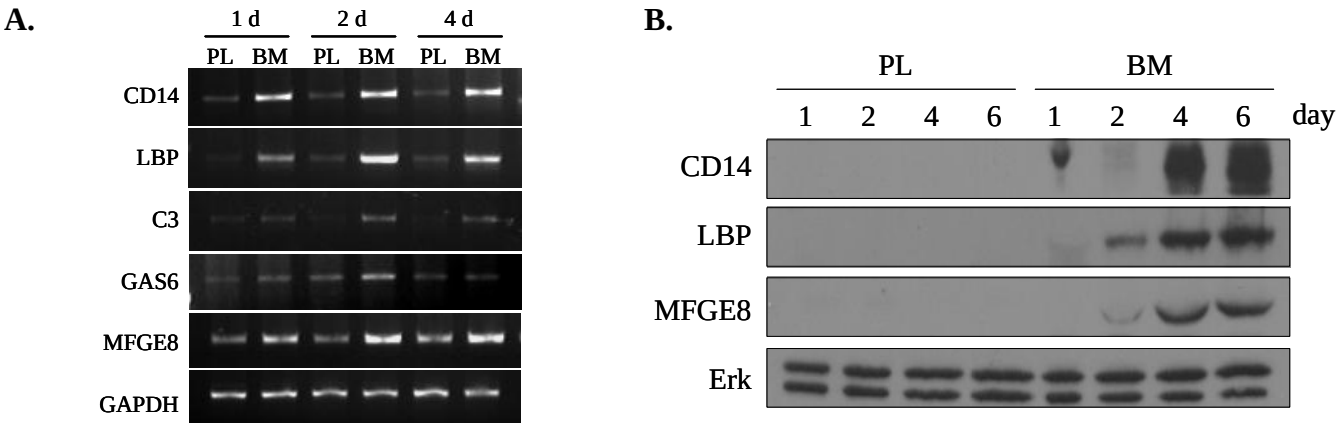

Figure 2. Kinetic analysis of clearance- and immune-related gene expression. Cells were cultured on plastic or BM for 1-6 days. Total RNA was analyzed by RT-PCR (A), and total lysates were analyzed by immunoblotting (B).

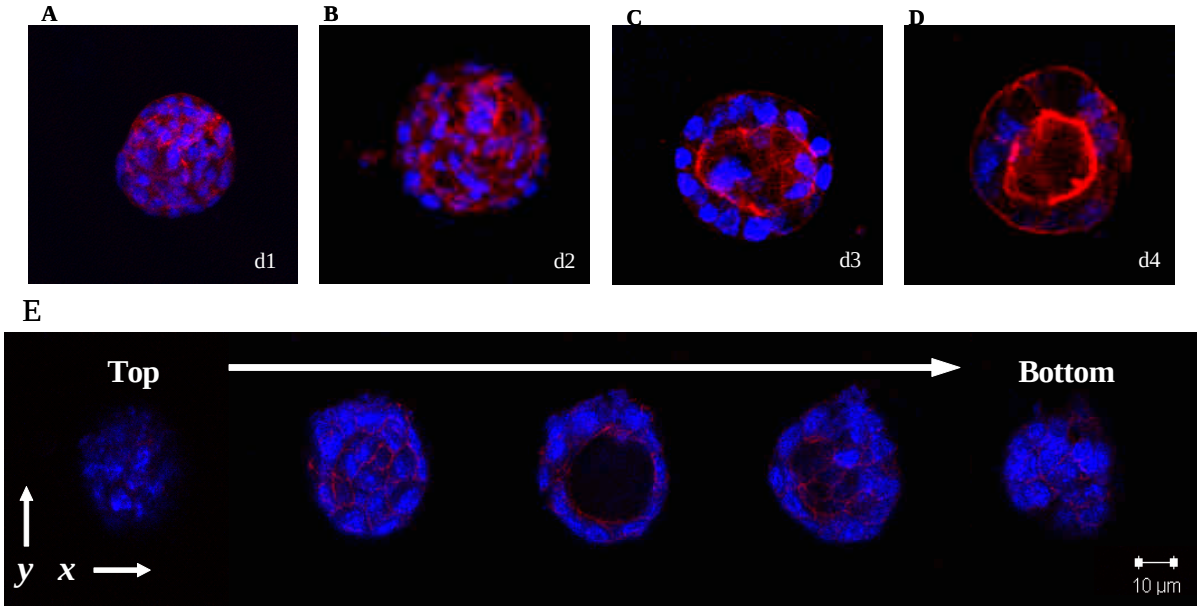

Figure 3. Kinetic analysis of lumen formation in mammary cells cultured on 3D BM.

ECM regulates the expression of clearance- and immune-related genes.

To further confirm that the expression of clearance- and immune-related genes is regulated by ECM, mammary cells were either cultured on BM for 4 d, or initially cultured on BM for 3 d, then trypsinized, and replated onto collagen I for 0-24 h. Total RNA was collected for RT-PCR analysis. Despite at different rates, re-adhesion of mammary cells onto collagen I led to a decrease in levels of CD14, LBP, C3, GAS6 and MFGE8, confirming the critical role of ECM in gene regulation (Fig. 4).

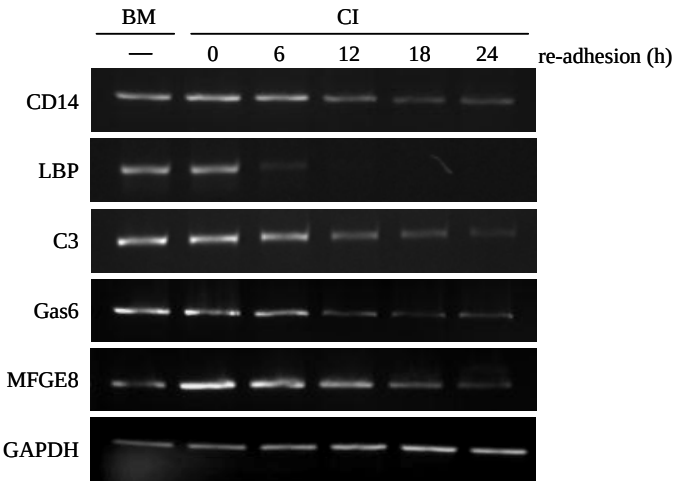

Figure 4. Re-adhesion of mammary cells to 2D collagen I decreases levels of clearance- and immune-related genes.

Increasing RhoA or decreasing Rac activity hampers the expression of clearance- and immune-related genes.

Our previous results shows that the RhoA/Rok/myosin II pathway is detrimental for prolactin signaling in mammary cells, whereas others demonstrate that Rac1 activity is essential for it (Akhtar et al., 2009; Akhtar and Streuli, 2006; Du et al., 2012; Lee et al., 2009). We would like to know if these pathways also regulated the expression of clearance- and immune-related genes. Cells cultured on BM were infected with adenovirus carrying constitutively active RhoA (Ad-L63RhoA) or treated with the Rac inhibitor NSC23766. Cells

cultured on plastic were treated with the Rok inhibitor Y27632. Total RNA were collected for RT-PCR analysis. Except for GAS6, other genes are regulated in a manner similar to that for prolactin signaling (Fig. 5). Thus, these ECM-elicited pathways also control the expression of clearance- and immune-related genes.

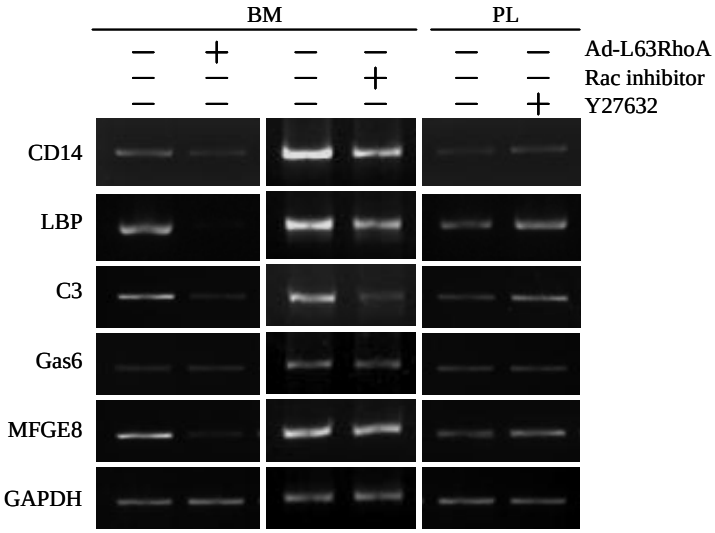

Figure 5. Increasing RhoA or decreasing Rac activity thwarts the expression of clearance- and immune-related genes.

Stat3 levels and activity are higher in cells cultured on BM.

Many immune-related genes that are upregulated in primary mammary cells cultured on BM, as revealed from microarray data, are acute phase genes. Stat3 is a key factor to induce acute phase genes. It is thus interesting to find out if Stat3 expression and activity are regulated by matrix as well. Mammary cells were cultured on plastic or BM, and total cell lysates were analyzed by immunoblotting. Levels of Erk were used as a loading control. Our results showed that Stat3 expression and phosphorylation levels were higher in cells cultured on BM (Fig. 6). Thus, upregulation of acute phase genes or even other clearance- or immune-related genes might be ascribed to the higher Stat3 activity.

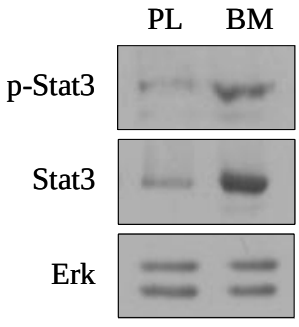

Figure 6. Mammary cells cultured on BM exhibit higher Stat3 levels and activity.

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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：

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本計畫的工作著重在釐清細胞外基質調節正常乳腺細胞構形及功能的機制，我們發現這有可能是透過影響免疫相關基因的表現所致。免疫相關基因參與凋亡細胞的清除，促進腺泡內腔的形成。此過程調控失當時可能會產生內腔充填、分化能力喪失以及不必要的發炎反應；這些情形反而有利於腫瘤的進展。我們希望這些研究能使我們對乳腺的正常發育有更深入的了解，並進而找出治療乳癌的策略。

國科會補助計畫衍生研發成果推廣資料表

日期:2013/10/26

國科會補助計畫	計畫名稱: 免疫相關基因對基質調控乳腺結構和功能所扮演的角色
	計畫主持人: 李宜儒
	計畫編號: 101-2320-B-040-005- 學門領域: 醫學之生化及分子生物

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：李宜儒	計畫編號：101-2320-B-040-005-
計畫名稱：免疫相關基因對基質調控乳腺結構和功能所扮演的角色	

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	0%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	1	1	100%		Du, J.-Y. and Lee, Y.-J. 2013. Upregulation of immune-related genes during luminal clearance of the mammary gland. The 28th Joint Annual Conference of Biomedical Sciences, Taipei, Taiwan. March 23-24, 2013. p127.
		專書	0	0	0%		
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（本國籍）	碩士生	2	2	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	0%		
		專任助理	0	0	0%		
國外	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	0	0	0%		
		專書	0	0	0%	章/本	
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	

	參與計畫人力（外國籍）	碩士生	0	0	0%	人次	
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		博士後研究員	0	0	0%		
		專任助理	0	0	0%		
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

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。

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論文：□已發表 □未發表之文稿 ■撰寫中 □無

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