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CX3CL1/fractalkine 在糖尿病腎病變致病角色之研究

(1/2)

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計畫名稱：CX3CL1/fractalkine 在糖尿病腎病變致病角色之研究 (第一年期中報告)

在第一年計畫中，我們假設高糖或第二型血管張力素能夠刺激腎臟細胞表現 fractalkine, 且其機轉和 MAPKs 以及 NF-κB 等核轉錄因子有關，因此擬探討高糖或第二型血管張力素是否會刺激腎小球膈細胞、腎小管上皮細胞、以及腎間質纖維母細胞表現 fractalkine， 以及 MAPKs 和 NF-κB 的訊息傳遞角色。

Primary cultures of **rat mesangial cells** were performed by sequential sieving methods in our laboratory as described previously. A rat proximal tubular cell line (**NRK-52E**) and a rat renal interstitial fibroblast cell line (**NRK-49F**) were obtained from American Type Culture Cellection (ATCC, Rockville, MD, USA).

These cells were screened first by RT-PCR for the presence of CX3CL1 gene expression. The result showed that mesangial cells expressed CX3CL1 mRNA before and after TNF-α stimulation. In contrast, neither NRK-49F or NRK-52E cells expressed significant amount of CX3CL1 mRNA at basal state (**Figure 1**). We then incubated the three types of cells with DMEM containing 0.1% fetal calf serum, and either low (5.6 mM) or high (30 mM) glucose for 24 hours. The Northern blot results show that only mesangial cells exhibited appreciable amount of CX3CL1 mRNA (**Figures 2 & 3**).

Rat mesangial cells were used to test whether high glucose can stuimulate the expression of CX3CL1 gene. Our results show that high glucose stimulated CX3CL1 gene expression in a time-dependent manner, starting at 24-48 hours. This effect was specific since stimulation with mannitol at the same osmolality (30 mM) did not induce the same effect as high glucose (**Figures 2 & 3**). When the mesangial cells were pretreated with various pharmacological protein kinase inhibitors (NF-κB, protein kinase C, and p42/44 MAPK), the stimulatory effect of high glucose on CX3CL1 gene expression at day 5 was either blocked or attenuated. However, inhibition of p38 MAPK using SB 203580 failed to affect high glucose-stimulated CX3CL1 mRNA expression (**Figure 4**).

In summary, our results so far show that (1) rat mesangial cells, but not NRK-49F or NRK-52E cells, expressed CX3CL1 mRNA at basal experimental conditions; (2) high glucose stimulated CX3CL1 gene expression by rat mesangial cells in a time-dependent manner; and (3) high-glucose-stimulated CX3CL1 gene expression in rat mesangial cells is mediated cooperatively by protein kinase C, p42/44 MAPK, and NF-κB pathways.

Figure 1. RT-PCR of CX3CL1 gene expression in rat mesangial cells, NRK 49F cells, NRK 52E cells, and vascular smooth muscle cells. Abbreviations: STD: 100-bp DNA ladder; VSMC: vascular smooth muscle cells; 49F: NRK 49F; 52E: NRK 52E; MC: mesangial cells; T: TNF-α (2.5 ng/mL) treatment for 8 hours.

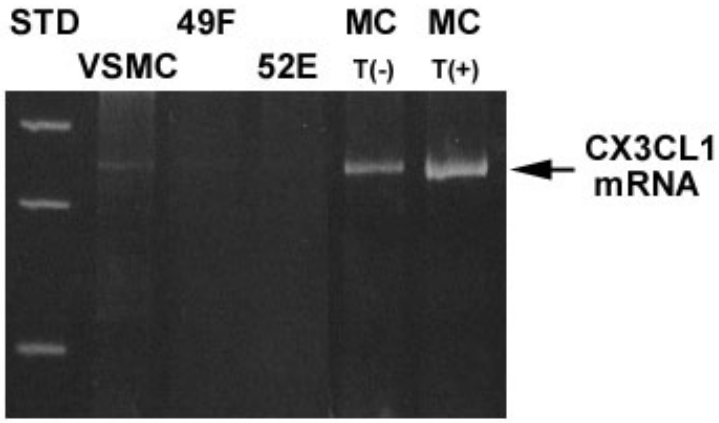

Figure 2. Time-course of CX3CL1 gene expression following high glucose stimulation in rat mesangial cells. Abbreviations: L: low glucose 5.6 mM; M: mannitol with the same osmolarity as that of high glucose (30 mM); H: high glucose 30 mM.

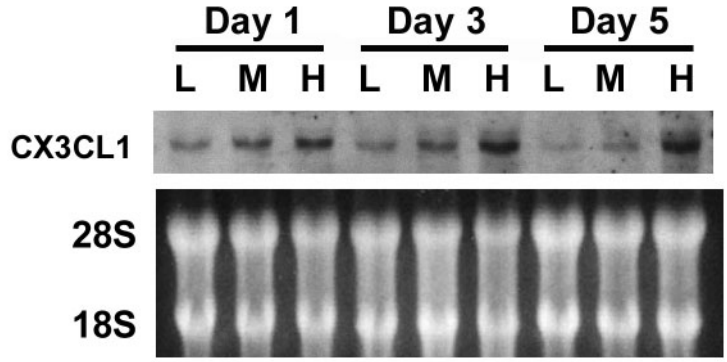

Figure 3. Time-course of CX3CL1 gene expression following high glucose stimulation in rat mesangial cells. Abbreviations: L: low glucose 5.6 mM; M: mannitol with the same osmolarity as that of high glucose (30 mM); H: high glucose 30 mM.

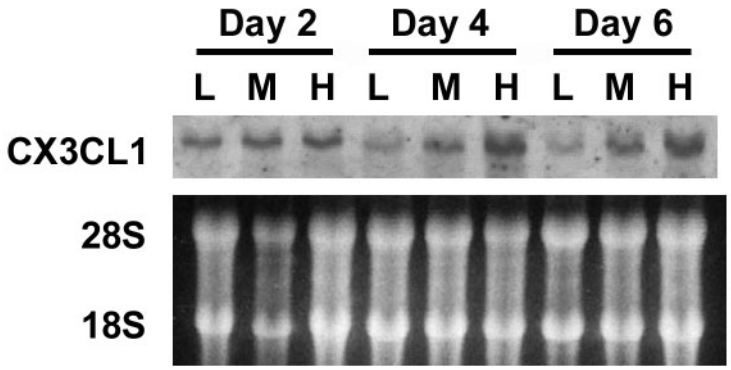

Figure 4. Effects of various pharmacological inhibitors on CX3CL1 gene expression stimulated by high glucose at day 5 in rat mesangial cells. Abbreviations: MG132: a selective inhibitor of NF-κB; Cal C: a selective inhibitor of protein kinase C; PD98059: a selective inhibitor of p42/44 MAPK; SB203580: a selective inhibitor of p38 MAPK.