行政院國家科學委員會專題研究計劃成果報告

胰磷酯質水解酵素 A_2 對激發 NIH3T3 細胞入侵細胞外細胞母質之研究

The stimulatory effect of phosphorylase A_2 receptor in the extracellular matrix invasion by NIH3T3 cells

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一、中文摘要

本研究的主要目的在於瞭解胰磷酯質水解酵素促進小鼠胚胎纖維母細胞及癌細胞對細胞外細胞母質的入侵作用的機轉。我們希望研究含金屬蛋白解酵素在此一作用中所扮演的角色。首先我們利用 RT-PCR 證實我們所購得的細胞株即小鼠胚胎纖維母細胞及纖維瘤細胞確實含有胰磷酯質水解酵素受體。此一結果並進一步由西方墨點法證明。經由實驗，我們發現有兩種含金屬蛋白水解酵素、即 MMP-2 及 -9、的量在所研究的兩種細胞中皆會因胰磷酯質水解酵素的刺激而有所增加。有趣的是，此一增加的現象只見於細胞粗萃取液的水溶液相中，而未見於酯溶液相中和培養基質中。由於細胞粗萃取液的水溶液相中所含有的蛋白質可來自於細胞質中的可溶性蛋白及附著於細胞膜上的蛋白，我們希望利用生物素對細胞表面蛋白質標記的技術來區別這兩種來源的蛋白質。實驗結果證實，水溶液相中所增加的兩種含金屬蛋白水解酵素確實是由細胞膜上萃取得。由於含金屬蛋白水解酵素一般是屬於分泌型蛋白，唯有在參與細胞外細胞母質的入侵作用時會附著於細胞膜外部某些特定區域，因此我們的發現進一步證實胰磷酯質水解酵素在促進小鼠胚胎纖維母細胞及纖維瘤細胞對細胞外細胞母質的入侵作用的關聯性。

關鍵詞：
細胞外細胞母質、胰磷酯質水解酵素、含金屬蛋白水解酵素、纖維瘤細胞、小鼠胚胎纖維母細胞、入侵作用。

二、Abstract

It has been known that type-I phospholipase A_2 (PLA_2-I) induced cellular ECM invasion of NIH3T3 as well as some tumor cell lines through its receptor. To further understand the mechanism of PLA_2-I-induced ECM invasion the role of matrix metalloproteinases in both NIH3T3 and fibrosarcoma, NFSa, is studied. We first performed RT-PCR and western blotting to demonstrated that both cell lines did contain endogenous mouse PLA_2-I receptor. The increase of active matrix metalloproteinase-2 and -9 (MMP-2 and -9) in the fraction containing mostly soluble proteins of PLA_2-I-treated NIH3T3 and NFSa cells was demonstrated. However, the distribution of

MMPs in the conditioned medium and detergent phase from treated and untreated cells was not much different. A further study using cell surface biotinylation and phase partition has shown that the activated MMP-2 and -9 did associated with the plasma membrane of both cell lines, which is consistent with the observation that MMP-2 and -9 may localize at the certain area of cell surface during ECM invasion. TIMPs, the tissue inhibitors for metalloproteinase, were not affected by the treatment of PLA_2-I. In summary, this study has shown that membrane-associated MMP-2 and -9 did increase in NIH3T3 and NFSa cells upon the treatment of PLA_2-I. A further study is needed to elucidate the molecular mechanism of PLA_2-I-induced MMP activation and membrane association.

Keyword: Extracellular matrix, Invasion, Pancreatic phospholipase A_2, PLA_2-I receptor, Fibrosarcoma, Metalloproteinase.

三、緣由與目的

Invasiveness is a phenomenon describing the ability of cells to cross an anatomic barrier, extracellular matrix (ECM),. ECM is a complex and dynamic meshwork of collagens, glycoproteins and proteoglycans that are assembled outside the cells (1-4). ECM plays important roles in separating different cell types and tissue compartments and providing structural and mechanical support to organisms. Besides, ECM also has a profound influence on many biological functions such as cell migration and adhesion, angiogenesis, as well as cell proliferation and differentiation.

Cellular ECM invasion occurs in the embryo as well as adult organism, including embryonic implantation (5,6), wound healing (7-9), tumor metastasis, and angiogenesis (10). A common feature of invasive processes is the degradation of macromolecules of the extracellular matrix which is required for invasive cells to migrate into adjacent tissues. A large and growing body of evidence suggests that matrix metalloproteinases (MMPs) especially gelatinases, MMP-2 and MMP-9, may play an important role in the ECM invasion (11,12). Interestingly, the activation of gelatinases requires membrane association and the presence of membrane-type 1 metalloproteinase (MT1-MMP) (13-15).

The regulation of cellular ECM invasion is not yet clear understood so far, although some receptor-mediated pathways are implicated to participate in this important cellular event (6,16). Interestingly, the pancreatic phospholipase A_2 (PLA_2-I) was shown to induce cellular ECM invasion on NIH3T3 and some cancer cell lines via PLA_2-I receptor (17). The main aim of this study is to understand the role and molecular mechanism of PLA_2-I in the induction of cellular ECM invasion in NIH3T3 as well as fibrosarcoma cells.

四、結果與討論

The presence of PLA2-I receptor in both NIH3T3 and NFSa cells: To find out the involvement of matrix metalloproteinases in PLA_2-I induced ECM invasion mouse embryonic fibroblast, NIH3T3, and mouse fibrosarcoma, NFSa, were used in this study. The RT-PCR of the total RNA isolated from both cell

lines was performed by using a primer set specific to the C-terminal region of the mouse PLA_2-I receptor gene, 4486-4674 bp. The result showed the both NIH3T3 and NFSa cell lines contain PLA_2-I receptor mRNA. The Western blotting also confirmed the functional expression of the receptor.

Determination of matrix metalloproteinases involved in the ECM invasion of NIH3T3 and fibrosarcoma: Both NIH3T3 and NFSa at their 80% confluence were treated with 100 nM porcine pancreatic phospholipase A_2 (p PLA_2-I) at a 37°C humidified incubator containing 5% CO_2 for about 24 hours. After incubation, conditioned media were collected and the cells were subjected to phase separation as described by Monsky and colleagues (18). The resulting aqueous and detergent phases from the phase separation along with the conditioned medium were subjected to gelatin zymography analysis (19,20). The result showed that the amount of both MMP-2 and -9 in aqueous phase of the cell extract, i.e., the fraction mainly containing cytosolic and membrane associated proteins, increased due to the treatment of PLA_2-I. The conditioned medium and detergent phase, i.e., a fraction containing mainly integrated membrane proteins, however, exhibited no difference in the distribution of both MMPs. The result did demonstrate that PLA_2-I may induce cellular ECM invasion through increasing active MMPs especially MMP-2 and -9.

It has been suggested that TIMP-2, the tissue inhibitors for metalloproteinase, may help recruiting MMP-9 to the cell surface for activation (15). Hence, we want to find out whether TIMP involve in PLA_2-I-induced MMPs activation. According to the result from reverse gelatin zymography (21), we found that the amount of TIMPs in medium was not affected by the treatment of PLA_2-I in both cell lines. This result suggests that TIMPs may not involve in the PLA2-I-induced MMPs activation and membrane association.

Cell surface biotinylation: Since the MMPs in the aqueous phase could be either cytosolic form or membrane-associated form, the biotinylation of cell surface proteins (21) was performed to clarify these possibilities. Both NIH3T3 and NFSa cells, after treated with 100 nM PLA_2-I for 24 hours, were further incubated with sulfo-NHS-biotin at 4°C for 30 min. Subsequently, cells were lysed and subjected to phase partitioning. After phase partitioning, aqueous phase proteins was collected and separated on a SDS-polyacrylamide gel. After transblotting, the biotinylated proteins were detected using streptavidin-conjugated horseradish peroxidase and substrate, TMB. The result clearly showed that PLA_2-I did induce the association of MMP-2 and -9 on the surface of NIH3T3 and NFSa cells.

五、計劃成果自評

It has been demonstrated that PLA_2-I could induce ECM invasion of normal as well as tumor cells via its receptor. The molecular mechanism for this process is , however, not well understood. Hence the major aim of this project is to elucidate the molecular mechanism of PLA_2-I-induced ECM invasion. We found that PLA_2-I induced cellular ECM invasion of both NIH3T3 and NFSa cells by activating and cell surface localization of two metrix metalloproteinases, MMP-2 and -9. This finding

is significant because MMP-2 and -9 have long been to link to the angiogenesis and the metastasis of tumor cells (10,22,23). Our results suggest that PLA_2-I may somewhat play a role in angiogenesis and tumor metastasis in the tissues that express it. In the future, we will consult these results and develop new experiments to further delineate the molecular mechanism of PLA_2-I-induced ECM invasion.

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