膠原蛋白酵素於正常人類角質細胞之免疫電顯研究

Ultrastructural Localization of Procollagenase/Collagenase in Normal Human Keratinocytes by Immunoelectron Microscopy

中文摘要

本實驗室過去的研究結果發現：（一）體外培養中的正常人類角質細胞(normal human keratinocyte, NHK)可藉 12-O-tetra-decanoyl phorbol-13-acetate (TPA)誘導其合成與分泌纖維母細胞型膠原蛋白酵素(fibroblast-collagenase, matrix metallo-proteinase-1, MMP-1)。（二）NHK 細胞種植於重建的第一型膠原蛋白膜片上，經 TPA 誘導且具有胰蛋白酵素的存在下，會經由分泌膠原蛋白酵?& 漆悍垂塥熔臚@ 型膠原蛋白膜片；此細胞為媒介的膠原蛋白分解活性(cell-mediated collagen breakdown)會被抗纖維母細胞型膠原蛋白酵素之抗血清抗體或單株抗體所抑制。（三）在細胞為媒介的膠原蛋白分解活性實驗中，位於細胞下及細胞周圍的膠原蛋白膜片很快被分解，但是膠原蛋白膜片的其餘部分則仍然完好，此一現象無法以單純的膠原蛋白酵素之擴散現象加以解釋。NHK 細胞經 TPA 誘導後，以抗纖維母細胞型膠原蛋白酵素之抗血清抗體或單株抗體為一級抗體，進行間接免疫螢光染色時，其陽性螢光反應主要分佈在細胞核周圍，但此免疫反應究竟確切位於細胞內之何種超微結構，至今仍未有報告。本研究將 NHK 細胞以免疫組織化學反應法處理後，再以穿透式電子顯微鏡觀察，以了解此酵素於合成及分泌過程中，在 NHK 細胞內所位居之超微結構。本研究首先根據標本大小建立二種不同的 NHK 細胞初代培養方法。在先驅實驗中以穿透式電子顯微鏡觀察 NHK 細胞之超微結構。發現 NHK 細胞具有型態上不同之次族群(subpopulation)，其共通之特徵是細胞內具有成束的張絲(tonofilament)。小型 NHK 細胞的胞內膜性結構及張絲較不發達，表面有細長之微突起。大型 NHK 細胞之內質網較發達，張絲更為明顯，細胞核周圍有大量之次級溶小體(secondary lysosome)及多泡體(multivesicular body)，細胞表面則呈不規則之皺摺，偶有細胞間接合(intercellular junction) 。另有些細胞之型態界於大小兩型細胞之間，稱之為中間型細胞。再以免疫細胞化學染色法作 NHK 細胞內膠原蛋白酵素之定位，其膠原蛋白酵素之免疫反應標示主要分布在細胞核周圍之粗糙內質網及高基氏體中，細胞膜外表面亦可見散在性之免疫反應標示，且較常分布於細胞互相粘接之交界處。以 TPA 處理之角質細胞有更發達之粗糙內質網及高基氏體，且免疫反應較強，而細胞膜外表面上之免疫反應的密度也比未以 TPA 處理者為高。此研究結果顯示，在培養的正常人類角質細胞內，膠原蛋白酵素合成及分泌的途徑，可能與一般的分泌性蛋白質之

合成及分泌的途徑相似。而細胞膜外表面上之膠原蛋白酵素標示，暗示角質細胞細胞膜之外表面可能有膠原蛋白酵素之接受體，可藉以進一步提高膠原蛋白酵素在近細胞空間(pericellular space)的濃度，提高此酵素被活化之效率，並且在細胞周圍形成局部的蛋白質酵素瀑布。此膠原蛋白酵素之接受體具有重要的生理及病理意義，且將來可以做爲發展治療藥物的目標。

英文摘要

Fibroblast-collagenase (collagenase-1, matrix metalloproteinase-1, MMP-1) is an unique enzyme for the initial cleavage of interstitial collagen types I and III. It is thought to play a major role in the degradation of extracellular matrix during the pathogenesis of many important human diseases such as cancer, rheumatoid arthritis, and periodontitis. Our previous studies applied with enzymatic hydrolysis of rat tail tendon collagen, Western blotting and others have proved that TPA-treated normal human keratinocytes (NHKs) de novo synthesis and secret procollagenase/collagenase-1 in culture. Studies with indirect immunofluorescent technique have also demonstrated that the cultured NHKs with TPA treatment produce procollagenase/ collagenase-1 mainly distributing in cytoplasm especially around perinuclear region, but definite localization could not be determined by this immunofluorescent technique. The purpose of the present research is to find the intracellular localization of synthesized procollagenase and its possible secretory pathway. For this, immunoelectron microscopic studies in the ultrastreptoavidin-biotin method to localize procollagenase/ collagenase intracellularly were performed in cultured NHKs with or without TPA treatment. In the preliminary research, the cultured NHKs without immunolabeling were studied in the transmission electron microscopy. The cultured NHKs consisted of heterogeneous subpopulations morphologically. The smaller and less differentiated cells contain small villous projections on the surfaces, while the larger ones contain broad and irregular processes. The Golgi apparatus, rough endoplasmic reticulum and multivescular bodies of the smaller cells are less prominent than those of the larger ones. The tonofilaments of cytokeratins are more abundant in the larger ones. Then immunoelectron

microscopic study was performed and showed that procollagenase/collagenase were located in rough endoplasmic reticulum, Golgi apparatus and outer surface of cell membrane. The pattern of subcellular localization in cultured NHKs was the same as that in the synovial fibroblasts. The immunolocalization of procollagenase/collagenase in the outer surface of cell membrane suggested that procollagenase/collagenase may be involved in the proteinases cascade around the pericellular space of NHKs.