Stromelysin Synthesizing Cells in the Synovial Tissues of Rheumatoid Arthritis Demonstrated by In Situ Hybridization and Immunohistochemical Methods

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Department of Pathology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ, USA, *Department of Pathology, Tohoku University School of Medicine, Sendai 980, †Department of Surgery, Keio University School of Medicine, Tokyo 160, and ‡Department of Medicine, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ, USA

Sawai, T., Murakami, K., Ohtani, Y., Kurkinen, M., Kyogoku, M. and Hayashi, M. Stromelysin Synthesizing Cells in the Synovial Tissues of Rheumatoid Arthritis Demonstrated by In Situ Hybridization and Immunohistochemical Methods. Tohoku J. Exp. Med., 1990, 160 (3), 285-286 — We identified the cells synthesizing stromelysin to be synovial cells in the synovial tissue from four biopsy cases with rheumatoid arthritis (RA) by in situ hybridization and immunohistochemical studies. In the cases which showed severe inflammation of synovia such as well developed lymphoid follicle and diffuse inflammatory infiltration, synovial cells located only in the superficial layer of synovial tissues showed abundant mRNA and enzymic protein of stromelysin.

— metalloproteinase; stromelysin; in situ hybridization; immunohistochemistry; rheumatoid arthritis

Two kinds of enzymes, collagenase and gelatinase, had been well known as members of metalloproteinase family. A third metalloproteinase named stromelysin has recently been reported. This newly defined enzyme has two characteristic functions, firstly ; stromelysin degrades collagen type IV, type V, fibronectin, laminin and cartilage proteoglycan, secondly it acts as an endogenous proactivator for procollagenase. In vivo, therefore, this enzyme is considered to play an important role for deteriorating bones and cartilages in rheumatoid arthritis. In this report, by employing immunohistochemical and in situ hybridization methods, we examined which types of cells synthesize stromelysin in rheumatoid synovia.

All synovial tissues used in this study were obtained from surgery for total knee replacement of four RA patients and 2 osteoarthritis patients. The stromelysin probe for
in situ hybridization was a 0.3 kbp Eco R1-PuvII stromelysin cDNA fragment from H25A clone (Saus et al. 1988). The DNA fragment was labeled with $^{3}H$-dTTP by nick translation to a specific activity of $2.0 \times 10^{5}$ cpm/$\mu$g. The hybridization procedure used in this study was essentially the same as that described previously (Hayashi et al. 1986). Since the recent analysis revealed stromelysin gene shared 54% homology to collagenase genes, we performed the control study by employing the prehybridization mixture which contains ten times excess non-labeled stromelysin cDNA and non-labeled collagenase cDNA, respectively, compared with the ones which are ordinarily applied, while sections were digested with RNase (2 mg/ml, room temperature) before in situ hybridization with labeled stromelysin cDNA. These experiments revealed that stromelysin cDNA used in this study was specific for stromelysin mRNA. For immunohistochemical detection of stromelysin, we used polyclonal antibody produced in the sheep directed against human stromelysin (Okada et al. 1989). Two of the 4 RA cases demonstrated many cells which had abundant silver grains. These cells may correspond to the cells rich in stromelysin mRNA. These cases exhibited severe inflammatory features such as well developed lymphoid follicles and diffuse inflammatory infiltration. The stromelysin mRNA positive cells were located only in the superficial layer of synovia and no cells in the deep region had many grains (Fig. 1). In these two cases, immunohistochemical study revealed that stromelysin protein was located in the cytoplasm of the cells in the same superficial layer (Fig. 2). In the remaining two cases with fibrotic synovia of RA and two cases of OA which were used for control study, there were no cells having stromelysin mRNA and protein in it. Endothelial cells and vascular myocytes and interstitial fibroblasts synthesized slight amounts of silver grains (data not shown), though there was no positive cell reactant for anti-stromelysin antibody.

References

