Chemotactic migration of bronchial smooth muscle cells to fibronectin for the process of airway remodeling in asthma

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The asthma patients have been led to airway narrowing as a result of tissue structural change called airway remodeling. Remodeling is one of the pathological conditions in which tissue components such as epithelial cells, fibroblasts, smooth muscle cells (SMCs), and myofibroblasts are involved in the repair process after airway injury. Myofibroblasts increase in asthmatic airway and play a crucial role in the remodeling of bronchi and paid our attention to the mechanism of the chemotactic migration of the bronchial SMCs (bSMCs).

We found that chemotactants for bSMCs were produced in bSMC-conditioned medium using the modified Boyden chamber assay. Fibronectin was determined as a chemotactant for bSMCs in bSMC-conditioned medium by Western blotting analysis and neutralization test using anti-fibronectin antibody. We also found that the migration of bSMCs in bSMC-conditioned medium was inhibited by addition of anti-β1 integrin antibody. In addition, we found by Gelatin zymography that metalloproteinases (MMPs) were secreted into bSMC-conditioned medium and these MMPs were determined to be MMP-1, 2 and 3 by Western blotting analysis.

These findings suggest that bSMCs produce MMPs containing MMP-1, 2 and 3 to degrade connective tissue and migrate into the degraded connective tissue by producing fibronectin as a chemotactant, indicating that migration of bSMCs into connective tissue may be an important process for airway remodeling of asthma.

Analysis of glycosyltransferases involved in chondroitin sulfate synthesis in cartilage.

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Cartilage contains a large amount of chondroitin sulfate proteoglycans (CSPGs). The major CSPG, aggrecan, contains more than a hundred chondroitin sulfate (CS) chains, which contribute to water retention essential for the cartilage function. Recently, several glycosyltransferases involved in the synthesis of CS chains have been identified, including chondroitin synthase-1/CS synthase-1 (CSS-1), CS synthase-2 (CSS-2), -3 (CSS-3), CS glucuronyltransferase (CSGlcAT), CS N-acetylgalactosaminyltransferase-1 (CSGalNAcT-1), and -2 (CSGalNAcT-2). However, individual roles of these enzymes have not been understood yet. Here, we determined the enzymes mainly involved in CS synthesis in cartilage. In situ hybridization revealed gene expression of CSS-1, CSS-2, CSGlcAT and CSGalNAcT-1 in articular cartilage and growth plate, whose patterns were colocalized with that of aggrecan core protein. When their expression levels during differentiating chondrogenic ATDC5 and N1511 cell lines were examined by quantitative real-time RT-PCR, expression of both CSGlcAT and CSGalNAcT-1 increased, correlated with that of aggrecan core protein. In contrast, the levels of all the other enzymes remained constant during the differentiation.

Overexpression of CSGlcAT and CSGalNAcT-1 in chondrocytes (LTC cells) caused increase of CS synthesis up to 2- and 5-fold, respectively, compared with the mock transfectant. The synthesized CS chains were mainly 4O-sulfated and similar in size. These results indicate that CSGalNAcT-1 plays main role in CS chain synthesis in cartilage.