Role and interaction of basic fibroblast growth factor with connective tissue growth factor in skin fibrosis.

Sonoko Chujo, Yuka Igawa, Miki Kondo, Fumiaki Shirasaki, Kazuhiko Takehara

Department Dermatology, Kanazawa University Graduate School of Medical

Skin fibrotic disorders such as systemic sclerosis (SSc) are characterized by an excessive production of extracellular matrix (ECM) and understood to develop under the influence of certain growth factors. Basic fibroblast growth factor (bFGF) is the prototype of the FGF family of structurally related proteins and a potent chemotactic factor for fibroblasts and endothelial cells. Previous studies have shown that bFGF suppresses collagen synthesis in vitro. On the other hand it has been reported that bFGF stimulates collagen synthesis in vivo. Thus the effects of bFGF on collagen production is not yet clear. To better understand the effects of bFGF on skin fibrosis in vivo, we established an animal model of skin fibrosis induced by exogenous application of growth factors. In this model, bFGF transiently induced subcutaneous fibrosis. Simultaneous injections of bFGF and connective tissue growth factor (CTGF) increased skin fibrosis compared with single injection of bFGF. Serial injection of bFGF for the first 3 days followed by CTGF for the next 4 days or bFGF after CTGF did not cause skin fibrosis. The simultaneous injections increased macrophage chemoattractant (MCP-1) mRNA expression levels. To further define the mechanisms of skin fibrosis in vivo, bFGF plus CTGF was simultaneously injected into MCP-1 knockout mice. Collagen levels in granulation tissues were decreased by simultaneous injections of bFGF and CTGF on day 8 in MCP-1 knockout mice. The number of inflammatory cells, such as mast cells, macrophages and lymphocytes, was significantly decreased in MCP-1 knockout mice compared with wild type mice. These results suggest that bFGF associates with CTGF and increases collagen production. MCP-1 and inflammatory cells may also contribute to the increase of fibrosis.

Splicing Factor 3b binds BMPR-IA and negatively regulates osteochondral differentiation

Hiroki Watanabe, Masafumi Shionyu, Koji Kimata, Tomoatsu Kimura, Hideo Watanabe

Institute of Molecular Science of Medicine, Aichi Medical University; Department of Orthopaedic Surgery, Toyama University; Faculty of Bio-Science, Nagahama Institute of Bio-Science and Technology

BMP2/4 play critical roles in early embryogenesis and in skeletal development. BMP2/4 signals conduct into the cell via two types of serine/threonine kinase receptors, known as BMPR-I (IA and IB) and BMPR-II. Here we identified a molecule that interacts with the intracellular domain (ICD) of BMPR-IA, using a yeast two-hybrid system. Out of 700,000 in human fetal brain cDNA library, 17 colonies bound to BMPR-IA ICD were identified to be splicing factor 3b subunit 4 (SF3b4). Reporter gene assays using C2C12 cells revealed that SF3b4 inhibits Smad 1/5/8 pathway but not Smad 2/3. Overexpression of SF3b4 showed little effect on ERK1/2, JNK/SAPK, p38 MAPK pathways as assessed by both ELISA and western blot. When SF3b4-expressing stable transfectants of ATDC5 and C2C12 cells were prepared and analyzed, the ATDC5 transfectants showed lower intensity of alcin blue staining and the C2C12 transfectants had reduced ALP activity. These observations indicate that SF3b4 inhibits osteochondral differentiation. Functional analysis demonstrated that proline-rich domain is required for both interaction with BMPR-IA and the inhibitory activity. These results suggest that SF3b4, originally identified as a molecule essential for assembly of prespliceosome, has another function to inhibit osteochondral differentiation through BMP-mediated signal transduction.