PLEIOTROPHIN (PTN) AN EMBRYONIC GROWTH AN DIFFERENTIATION FACTOR IS EXPRESSED IN OSTEOARTHRITIC CARTILAGE AND IN CULTURED CHONDROCYTES

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Introduction

Pleiotrophin (PTN), is an 136-amino acid (15.3 kDa) secreted growth / differentiation cytokine that is developmentally regulated (1). PTN is a differentiation or growth factor for various cell types (therefore termed pleiotrophin); it promotes angiogenesis, stimulates neurite outgrowth from cultured neurons, and induces cell migration (1). Together with midkine (MK) it forms a family of heparin-binding proteins that are normally expressed during embryogenesis, but only at low levels in healthy adult tissues (2). However, PTN is re-expressed in some human tumors, eg meningiomas (2,3).

In fetal or juvenile cartilage, PTN is an abundant protein (1,2) and appears to be implicated in the regulation of proteoglycan synthesis by chondrocytes (1,2) and probably controls – together with other angiogenesis factors – the invasion of blood vessels into hypertrophic cartilage (1,2). Since genes expressed during development often re-appear in the disease state, we investigated the formation of PTN in OA cartilage, synovial fluid and cultured chondrocytes.

Materials and methods

Tissues and synovial fluids: Tibial plateaus were obtained from OA patients who underwent joint replacements. Normal control samples without morphological signs of OA were obtained during routine cadaver autopsies (Department of Anatomy). Synovial fluids were obtained from arthroscopies.

RT-PCR: RNA was isolated with the guanidium thiocyanate method, digested with DNase, cDNA generated and RT-PCR performed with the primers 5'-CCT-TCT-TGG-CAT-TCA-TTT-TCA-TAC-3' (sense) and 5'-GAG-GTT-TGG-GTT-TGG-TGG-TGA-3' (antisense) at an annealing temperature of 60°C with 40 cycles according to previous protocols.

Western blot and immunohistochemistry: Western blots from cartilage homogenates were obtained after separation in 15% SDS-PAGE gels, semi-dry transfer onto nitrocellulose membranes and chemoluminescence detection. For immunohistochemistry, cartilage samples were incubated with anti-PTN (1:500 in PBS overnight at 4°C).

Enzyme-linked immunosorbent assay (ELISA): The PTN-ELISA was performed with Nunc-Immuno Maxisorb plates coated with anti-PTN. Statistical significance was evaluated by the t-test.

Cell culture and stimulation of chondroctyes and 3T3 fibroblasts: Human chondrocytes and 3T3 fibroblasts were cultivated for 96 h at 37°C. The cells were exposed to human recombinant PTN for 72 h. After 72 h an anti-phospho-tyrosine-Western-blot was performed. Proliferation assays were performed with subconfluent cells for 24 h after that DNA was measured with the CyQuant method.

Results

PTN is expressed in osteoarthritic cartilage

We could detect PTN mRNA in homogenates of cartilage from the tibial plateau from OA patients, but not of normal articular cartilage of adults. Cartilage from OA patients with moderate (early OA) degenerative changes generally yielded a stronger signal than tissue from OA patients with severe ones. In Western blot experiments similar results were obtained for the expression PTN protein: Whereas homogenates of normal adult cartilage yielded no signal, an 18 kDa band was stained in the homogenates of cartilage from OA patients. Again, in homogenates from patients with moderate degenerative changes (early OA patient) the band was stronger than in those with more severe changes.

Most values from late OA were not or only weakly elevated as compared to the healthy samples.

PTN can be immunostained in chondrocytes of OA patients

In accordance with the above results, OA cartilage, but not normal adult cartilage from the tibial plateau could be immunostained for PTN.

PTN stimulates proliferation in 3T3 fibroblasts

As an example for fibroblastic synovial cells we stimulated 3T3 fibroblasts with PTN. PTN increased the proliferation rate of 3T3 fibroblasts app. 2 fold.

Discussion

We now show that PTN, a developmentally expressed growth and differentiation factor, is re-expressed and up-regulated in OA chondrocytes whereas it is absent in cartilage from healthy adults. In contrast, PTN is highly expressed in fetal epiphyseal cartilage where it appears to function as a differentiation factor (2).

In juvenile chondrocytes, recombinant PTN has been reported to inhibit on ³H-dihydmidine incorporation, and to stimulate extracellular matrix synthesis, in particular that of biglycan and collagen type II, but not of decorin and aggrecan (2). Re-expression of PTN in OA cartilage could be interpreted as a mechanism to restore cartilage integrity, especially of the extracellular matrix. This hypothesis is sustained by the higher PTN concentrations in the synovial fluid measured in early OA (with partially still intact chondrocytes) as compared to the values in late OA when cartilage destruction is too high to allow regenerative processes. Under this aspect, PTN measurements in the synovial fluid could be used as an additional parameter for the staging of OA.

Receptors for PTN and their signal transduction mechanisms have not yet been fully characterized. So far, interaction of PTN with the receptor-type protein tyrosine phosphatase ζ/β1, anaplastic lymphoma kinase (ALK) and syndecan-3 have been shown (2). By disruption of the normal balance between tyrosine kinase and phosphatase activities, phosphorylation of intracellular proteins was found to be increased (2).

So far, the clinical interest for PTN was in its expression in certain types of cancer. Chronic inflammatory diseases like OA have to be added to the list of pathological conditions with PTN expression since proliferation of synovial fibroblasts may be influenced by PTN via diffusion from the synovial fluid. Under this aspect PTN might function as an amplifier of inflammation.

References

1. Hsiu-Jeng et al. (1982);
2. Kurtz et al. (1995);
3. Li et al. (1992)

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