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Integrin α1β1 mediates collagen induction of MMP-13 expression in MC615 chondrocytes

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Abstract

During endochondral ossification, type I collagen is synthesized by osteoblasts together with some hypertrophic chondrocytes. Type I collagen has also been reported to be progressively synthesized in degenerative joints. Because Matrix Metalloproteinase-13 (MMP-13) plays an active role in remodeling cartilage in fetal development and osteoarthritic cartilage, we investigated whether type I collagen could activate MMP-13 expression in chondrocytes. We used a well-established chondrocytic cell line (MC615) and we found that MMP-13 expression was induced in MC615 cells cultured in type I collagen gel. We also found that α1β1 integrin, a major collagen receptor, was expressed by MC615 cells and we further assessed the role of α1β1 integrin in conducting MMP-13 expression. Induction of MMP-13 expression by collagen was potently and synergistically inhibited by blocking antibodies against α1 and β1 integrin subunits, indicating that α1β1 integrin mediates the MMP-13-inducing cellular signal generated by three-dimensional type I collagen. We also determined that activities of tyrosine kinase and ERK and JNK MAP kinases were required for this collagen-induced MMP-13 expression. Interestingly, bone morphogenetic protein (BMP)-2 opposed this induction, an effect that may be related to a role of BMP-2 in the maintenance of cartilage matrix.

Keywords: Chondrocyte; Collagen; Integrin; MMP-13; BMP-2; Osteoarthritis

1. Introduction

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that play multiple roles in biology of the extracellular matrix (ECM), such as release of cryptic fragments and neo-epitopes from ECM macromolecules, release of growth factors and modification of cell–ECM interface [1]. Because MMPs degrade most components of the ECM, they have been particularly studied in the context of ECM remodeling. For instance, during endochondral ossification, MMPs are associated with chondrocyte hypertrophy [2,3] and with matrix vesicles implicated in cartilage mineralization [4–7]. More precisely, proteolysis involving MMP-13 (collagenase-3) is required for hypertrophic chondrocyte differentiation in vitro [3,8], and this is consistent with reports that MMP-13 is expressed in hypertrophic and calcifying cartilage of mammalian growth plate [9–15]. MMP-13 is also expressed by early osteogenic cells of the bone collar and by osteoblasts invading the growth plate [16]. These findings together suggest that MMP-13 plays an active role in remodeling cartilage and bone during long bone development. Accordingly, biochemical characterization of this enzyme has revealed that it degrades very efficiently fibrillar collagens such as type II collagen, major protein in cartilage, and type I collagen, major protein in bone [17]. MMP-13 can also degrade type X collagen [18] and aggrecan [19], two other cartilage macromolecules.

In addition to its role in skeletal development, MMP-13 is involved in cartilage pathology. MMP-13 is expressed in adult articular cartilage in human osteoarthritis (OA) [20] and experimental OA [21–23], and a direct correlation between excess production of MMP-13 and cartilage destruction has been demonstrated by the cleavage of type II collagen in human OA [20].

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Despite the importance of MMP-13 in cartilage biology, the molecular signaling pathways that regulate MMP-13 expression in chondrocytes have been only partially identified. The 120-kDa fibronectin fragment (FN-f) stimulates production of MMP-13 by human chondrocytes through the α5β1 integrin receptor and activation of ERK, JNK, and p38 mitogen-activated protein (MAP) kinases [24]. Recently, it has been shown that this FN-f-induced signaling involves activation of protein kinase C (PKC) [25]. In addition, MAP kinase activation has been linked to MMP-13 expression in response to interleukin-1β in chondrocytes [26–28] in response to TGF-β1 in breast cancer cells [29], transformed keratinocytes [30] and fibroblasts [31,32] and in response to fibroblast culture in collagen gels [33].

Type II collagen is the major protein of cartilage but a switch occurs from type II collagen to type I collagen at sites where MMP-13 is involved in cartilage degradation, like in hypertophic cartilage [34,35]. Furthermore, the presence of type I collagen has also been reported in osteoarthritic cartilage [36–39]. In this context, we investigated whether type I collagen signals chondrocytes to activate MAP kinase pathway and subsequent expression of MMP-13. We used the well-established chondrocytic cell line (MC615) that has been shown to express several biochemical markers of the articular cartilage [40–44]. We first found that three-dimensional (3-d) collagen gel promotes MMP-13 expression in MC615 cells. We also found that α1β1 integrin, a major collagen receptor expressed at the surface of chondrocytes [45], is expressed in MC615 cells, and we further showed that this integrin mediates 3-d collagen signaling responsible for MMP-13 expression. We determined that activities of tyrosine kinase and ERK and JNK MAP kinases were required for this collagen-induced MMP-13 expression. These results demonstrate for the first time that type I collagen via α1β1 integrin and MAPK signaling can mediate MMP-13 expression in chondrocytes. Lastly, we showed that BMP-2 opposes this collagen induction of MMP-13 expression. This interesting finding raises the possibility that type I collagen and BMP-2 signaling act in balance in cartilage homeostasis, suggesting a chondroprotective capacity for BMP-2.

2. Materials and methods

2.1. Cell culture

The MC615 cell line was characterized previously [40] and routinely maintained in 1:1 high-glucose DMEM/Ham's F12 containing 10% FBS and supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all products from GIBCO-Invitrogen, Cergy-Pontoise, France).

2.2. Type I collagen gels

Collagen was extracted and purified as described [46] from the skin of young calves (kindly provided by Symatèse Biomatériaux Lyon, France). Four volumes of this collagen in 7 mM acetic acid solution, characterized as containing 98% type I collagen and 2% type III collagen, were mixed with one volume of 5× concentrated DMEM/Ham's F12 supplemented with 200 U/ml penicillin and 200 μg/ml streptomycin for a final concentration of 1.25 mg/ml, neutralized with 0.5 M NaOH and kept on ice. MC615 cells were trypsinized, resuspended in DMEM/Ham's F12 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, and mixed with neutralized collagen solution to a final concentration of 2×10^6 cells/ml. One milliliter of this mixture was transferred into each well of a 24-well plate and incubated at 37 °C in the presence of 5% CO_2 for collagen polymerization. After 1 h, 1 ml DMEM/Ham's F12 containing 1% FBS and supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin was added in each well, and the incubations continued for different periods of time. Alternatively, MC615 cells were embedded in agarose gel as previously described [41], and with the same seeding conditions as in collagen gel. The culture medium was replaced each day. In experiments involving BMP-2, tyrosine kinase or MAPK inhibitors, or blocking antibodies against different collagen-binding integrin subunits, these were added to the cell suspension prior to mixing it with neutralized collagen solution. These modulators were replaced with the culture medium each day. In experiments involving two-dimensional (2-d) cell cultures, MC615 cells were plated on Corning dishes or Biocoat type I collagen-coated dishes (BD BioSciences, Le Pont de Claix, France) and cultured in the same medium as the cells embedded in collagen gel.

2.3. Reagents and antibodies

Recombinant human BMP-2 was a gift of Wyeth Research (Cambridge, MA, USA). Recombinant human MMP-13 was obtained from R and D Systems (Lille, France) and goat polyclonal anti-mouse MMP-13 antibodies were a kind gift from C. Peeters-Joris and were previously described [47]. Monoclonal Hamster antibodies raised against mouse integrin β1 chain (CD29), rat/mouse integrin α1 chain (CD49a), rat integrin α2 chain (CD49b), and Hamster IgG were obtained from BD Pharmingen (Le Pont de Claix, France). The CD29, CD49a and CD49b antibodies have been shown to recognize the respective integrin subunits by flow cytometry [48]. Antibodies anti-PYK2 were from Upstate Biotechnology (Charlottesville, VA, USA) and anti-phospho-PYK2 (Y402) were from Cell Signaling (Beverly, MA, USA). The tyrosine kinase inhibitor Genistein, the MEK inhibitor PD 98059 and the p38 inhibitor SB 203580 were obtained from Calbiochem (San Diego, CA, USA). The JNK inhibitor SP 600125 was purchased from Sigma-Aldrich (L'Isle d'Abeau, France). Preliminary experiments indicated that BMP-2 and inhibitors have no effect on multiplication of MC615 cells cultured for 4 days in collagen gel (data not shown).

2.4. Flow cytometry

MC615 cells were released from collagen gels by a 15 min treatment with 1.5 mg/ml collagenase (type IA, Sigma) in phosphate-buffered saline (pH 7.4) with 1 mM $CaCl_2$. For integrin detection, cells were washed and resuspended in PBS containing saturating amounts of mAbs against integrins. After 20-min incubation at 4 °C, cells were washed and resuspended in PBS containing FITC-mouse anti-hamster IgG (BD Pharmingen). Controls were carried out using isotype-matched antibodies or by omission of primary antibody. Cells were analysed with a FACS Calibur™ cytofluorometer.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

MC615 cells were released from collagen gels by a brief treatment with collagenase, as described above. Total RNA was isolated from cells with the RNeasy kit (Qiagen, Courtaboeuf, France), and digested with DNase to remove any contaminating genomic DNA. For RT, a 40 μl reaction contained 1 μg total RNA, 12.5 ng/μl oligo(dT_{12}VN) primers (V represents A, G, or C and N represents A, T, G or C), 500 μM each dNTP, 100 ng/μl BSA, 10 mM DTT, 4 U of RNasin (Promega, Charbonnières les Bains, France) and 200 U SuperScript II RNase H^- (GIBCO-Invitrogen). Reactions were carried out at 42 °C for 50 min followed by an inactivation of the enzyme at 70 °C for 15 min. The cDNAs were then incubated with 4 U RNase H (GIBCO-Invitrogen) at 37 °C for 30 min. For PCR amplification, a 50 μl reaction contained 2 μl RT aliquot, 50 μM each dNTP, 0.2 μM each primer, 1.5 mM $MgCl_2$ and 2.5 U AmpliTaq DNA polymerase (Applied Biosystems, Villebon sur Yvette, France). Following an initial denaturation step of 2 min at 95 °C, amplification consisted of 30–35 cycles of 30 s at 95 °C, 30 s at optimal temperature (see Table 1), and 30 s at 72 °C, followed by a final extension step of 5 min at 72 °C. Amplification was performed in a GeneAmp PCR system 2400 (Perkin-Elmer, Courtaboeuf,