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Regulation of anabolic and catabolic gene expression in normal and osteoarthritic adult human articular chondrocytes by osteogenic protein-1

Z. Fan[1], S. Chubinskaya[2], D.C. Rueger[3], B. Bau[1], J. Haag[1], T. Aigner[1]

[1]Cartilage Research, Department of Pathology, University of Erlangen-Nürnberg, Germany; [2]Department of Biochemistry and Section of Rheumatology, Rush Medical College at Rush-Presbyterian St. Luke's Medical Center, Chicago, Illinois; [3]Stryker Biotech, Hopkinton, Massachusetts, USA.

Zhiyong Fan, MD; Susan Chubinskaya, PhD; David C. Rueger, PhD; Brigitte Bau, MSc; Jochen Haag, MSc; Thomas Aigner, MD.

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Please address correspondence and reprint requests to: Thomas Aigner, MD, Cartilage Research, Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstr. 8-10, D-91054 Erlangen, Germany.
E-mail: Thomas.aigner@patho.imed.uni-erlangen.de

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ABSTRACT

Objective. *Osteoarthritis is characterized by dramatic changes in chondrocyte metabolism including the overexpression of catabolic enzymes, but also a lack of anabolic activity. In this respect, osteogenic protein 1 (OP-1) appears to be one of the most potent anabolic factors of chondrocytes. In this study, we were interested in: (1) whether recombinant human OP-1 exerts its anabolic effects also on osteoarthritic chondrocytes, (2) whether OP-1 modulates the expression of catabolic genes, and (3) whether the BMP effects are related to the expression levels of its intracellular mediators (R- and I-Smads).*

Methods. *Chondrocytes were isolated from cartilage of either normal (n=5) or osteoarthritic (n=8) human knee joints and cultured in short-term high-density monolayer cultures with and without recombinant OP-1. RNA was isolated and analyzed for mRNA expression levels of anabolic (aggrecan, collagen type II), catabolic (MMP-1, -3, -13, ADAMTS-4), and intracellular signaling mediators (Smad 1, 4, 5, 6, 7, and 8) by quantitative online PCR.*

Results. *After OP-1 stimulation, the anabolic genes were significantly upregulated in osteoarthritic chondrocytes in comparison to normal chondrocytes. Neither in normal nor osteoarthritic chondrocytes were significant changes observed for the matrix degrading enzymes. Smads were also expressed in both normal and osteoarthritic cells at roughly the same level with and without stimulation with OP-1.*

Conclusion. *Osteoarthritic chondrocytes are not hypo-responsive to anabolic stimulation by OP-1. Thus, human recombinant OP-1 could be a suitable anabolic activator of osteoarthritic chondrocytes. This might be of particular interest as chondrocytes themselves showed very low levels of OP-1 expression.*

Introduction

Osteoarthritis (OA) is characterized by dramatic changes in cartilage metabolism. This includes the overexpression of catabolic matrix degrading enzymes, but also a lack of anabolic activity of chondrocytes (1). The anabolic activity of chondrocyte is classically thought to be maintained by growth factors such as IGF-I and transforming growth factor ß (TGF-ß), but more recently bone morphogenetic proteins (BMPs), members of the transforming growth factor-ß superfamily (2), have become the focus of interest (3).

Osteogenic Protein-1 (OP-1), also known as bone morphogenetic protein-7 (BMP-7), is found in many embryonic tissues, notably at sites of mesenchymal condensations such as the teeth and limb buds, but also the kidney, brain and eye. Studies by Flechtenmacher *et al.* (1996) and Nishida *et al.*. (2000) showed that OP-1 can significantly enhance the anabolic activity of chondrocytes, mainly the expression of aggrecan, type II collagen, and hyaluronan (3,4). Thus, OP-1 appears to be one of the most potent anabolic factors for adult articular chondrocytes. However, it remains unclear whether OA chondrocytes are still responsive to this stimulus. In fact, a reduced responsiveness of OA chondrocytes to anabolic stimuli such as IGF-I was reported previously (5).

BMPs exert their actions on the cells via a rather well defined intracellular signaling pathway involving specific kinase receptors, which phosphorylate the Smad-signaling molecules upon BMP-binding. In a previous study, we were able to demonstrate that the intracellular mediators of BMP-signaling, the BMP-receptor-associated Smads (R-Smads 1, 5 and 8) as well as the common Smad (C-Smad 4), are also expressed in human adult articular chondrocytes (6) confirming that BMP-signaling is a potentially important signaling pathway within these cells.

In this study, we attempted to determine: (1) whether OP-1 also exerts its anabolic effects on OA chondrocytes, (2) whether OP-1 modulates catabolic gene expression either in normal or in OA chondrocytes and (3) whether the BMP effects are related to the expression levels of its intracellular mediators or their inhibitors.

Material and methods

Cell isolation, culture, and stimulation with OP-1

Normal human knee articular cartilages were dissected from 5 normal donors at autopsy, within 48 hours of death (2 males and 3 females, 39-79 years, mean age 57.8 yrs), and OAcartilage was obtained from 8 patients who had undergone knee arthroplasty for advanced osteoarthritis immediately after the endoprosthetic operation (4 males and 4 females, 67-76 years, mean age 71 yrs). All osteoarthritic samples therefore were taken from peripheral areas of late stage, advanced osteoarthritic joints.

Cartilage pieces were finely chopped and chondrocytes were enzymatically isolated as described (7) and seeded at 2 x 10^6 cells/well in 6-well tissue culture plates and maintained for 48 hours in DMEM/F12 medium (Gibco BRL, Karlsruhe Germany) supplemented with 10% FCS and 50 µg/ml penicillin/streptomycin solution (Gibco BRL, Karlsruhe, Germany) and 50 µg/ml ascorbate (Sigma, Taufkirchen, Germany).

Thereafter, chondrocytes were stimulated with 100 ng/ml OP-1 (Stryker Biotech, Hopkinton, MA) in DMEM/ F12 medium containing 10% FCS (Biochrom, Berlin, Germany) or cultivated in medium containing 10% FCS alone for 3 days. The medium was changed every day. At the end of the stimulation period the cells were washed in sterile PBS, and lysed in 350 µl lysis RLT buffer/10^6 cells (Qiagen, Hilden, Germany) and stored at -80ºC.

RNA isolation, cDNA synthesis and TAQMAN PCR

RNA was isolated and cDNA synthesized by reverse transcription as described previously (7,8). TAQMAN PCR was used to detect human aggrecan, collagen type2,MMP-1,-3,-13, ADAMTS-4 as well as Smad 1, 4, 5, 6, 7 and 8 in RNA samples of human articular cartilage. The primers (MWG Biotech, Germany) and TAQMAN probes (Eurogentec, Belgium) were designed using PRIMER EXPRESS TM software (Perkin Elmer) (Table I). In order to be able to obtain quantifiable results for all genes, specific standard curves using sequence specific control probes were performed in parallel to the analyses as described (7). All experiments were performed in triplicate.

Statistical analysis

For statistical analysis, the t-test for pair-wise comparisons was used and P-values below 0.05 were considered as significant.

Results

Expression of BMP-7 in vitro

First, we investigated the expression of OP-1 by normal and OA chondrocytes (short-term high-density monolayer cultures). Neither using conventional PCR (40 cycles) nor by quantitative RT-PCR could we detect significant

Table I. Sequences of primers and probes for quantitative online-PCR experiments.

Acc. No.	Primer	nm	Probe	Mg mM	
GAPDH	NM_002046	fw: GAAGGTGAAGGTCGGAGTC rv: GAAGATGGTGATGGGATTTC	50 900	CAAGCTTCCCGTTCTCAGCC	5.5
MMP-1	NM_002421	fw: CTGTTCAGGGACAGAATGTGCT rv: TCGATATGCTTCACAGTTCTAGGG	300 900	ACGGATACCCCAAGGACATCTACAGCTCC	6.5
MMP-3	NM_002422	fw: TTTTGGCCATCTCTTCCTTCA rv: TGTGGATGCCTCTTGGGTATC	900 50	AACTTCATATGCGGCATCCACGCC	4
MMP-13	NM_002427	fw: TCCTCTTCTTGAGCTGGACTCATT rv: CGCTCTGCAAACTGGAGGTC	900 50	TCCTCAGACAAATCATCTTCATCACCACCAC	7
ADAMTS-4	AF148213	fw: TGCCCGCTTCATCACTGA rv: CAATGGAGCCTCTGGTTTGTC	900 50	ACAGTGCCCATAGCCATTGTCCAGGA	6
Smad 1	U57456	fw: CACAAACATGATGGCGCCT rv: CATAGTAGACAATAGAGCACCAGTGTTTT	50 900	CCCTGCCCTCAGAAATCAACAGAGGAGA	6,5
Smad 4	NM_005359	fw: GCCCTCCAGCTCCTAGACG rv: TGTAGTCCACCATCCTGATAAGGTT	50 900	TACCATGCCGATTGCAGACCCACAA	6
Smad 5	NM_005903	fw: ACCAGCCCAACAACACTCCT rv: AGCTGAAATGGACTTCCTGGTC	50 900	TTCCCTTATCTCCAAACAGCCCTTATCCCC	4,5
Smad 6	AF037469	fw: TGAATTCTCAGACGCCAGCAT rv: AGTACGCCACGCTGCACC	50 900	TCTCCGGACGCCACCAAGCC	5
Smad7	NM_005904	fw: CTCAAACCAACTGCAGACTGTCC rv: AGTATGCCACCACGCACCA	50 900	TGCTGTGCCTTCCTCCGCTGAA	4,5
Smad8	NM_005905	fw: TCCTGGCCAAGTTCCGC rv: AGTGCAGAGCACGGAGGC	50 900	CCTCCCTGCACAGTGAGCCACTCA	4,5
Aggrecan	NM_013227	fw: ACTTCCGCTGGTCAGATGGA rv: TCTCGTGCCAGATCATCACC	50 50	CCATGCAATTTGAGAACTGGCGCC	6
Col2	NM_001844	fw: CAACACTGCCAACGTCCAGAT rv: CTGCTTCGTCCAGATAGGCAAT	50 300	ACCTTCCTACGCCTGCTGTCCACG	5,5

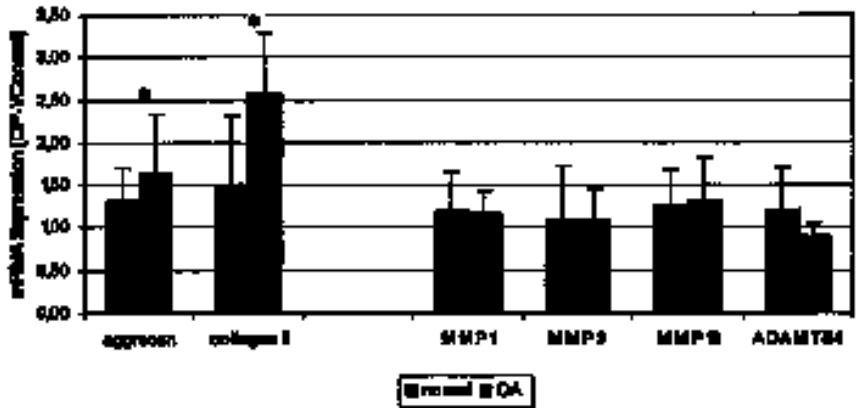

Fig. 1 Quantitative TaqMan analysis for expression of messenger RNA in normal and osteoarthritic cartilage before and after OP-1 stimulation for aggrecan, collagen type II and matrix degrading enzymes: MMP-1, MMP-3, MMP-13 and ADAMTS-4. (*P < 0.05 stimulation versus control). Bars show the means and standard deviation. Absolute values are shown below.

signals for OP-1 mRNA expression in normal nor OA chondrocytes (below 0.0001/GAPDH; normal: n = 4; osteoarthritic: n=4).

Effect of OP-1 on chondrocyte anabolism and catabolism in normal articular chondrocytes

For assessment of the anabolic activity of adult articular chondrocytes, the mRNA expression levels of the two major anabolic gene products collagen type II and aggrecan were measured (aggrecan: 1.74/GAPDH ± 1.25; collagen type II-normal: 4.18/GAPDH ± 2.70). After stimulation with OP-1 a slight (but not significant) up-regulation of both genes was found (Fig. 1).

To assess the catabolic activity, gene expression levels of the major proteases involved in collagen (MMP-1 and MMP-13) and aggrecan (stromelysin (MMP-3) and ADAMTS-4) degradation were analyzed (MMP-1: 1.5/GAPDH ± 1.50; MMP-3: 52/GAPDH ± 23; MMP-13: 0.22/GAPDH ± 0.17; ADATMS-4: 0.002/GAPDH ± 0,001). mRNA expression of none of the four enzymes was significantly changed by the treatment with OP-1 in normal articular chondrocytes (Fig. 1).

Effect of OP-1 on chondrocyte anabolism and catabolism in OA articular chondrocytes

Our first finding of interest was that both collagen type II (Col2) and aggrecan mRNA expression were lower in OA chondrocytes than in their normal counterparts (Col2: 9.1x; Agg: 3.4x). Inversely, the expression of the matrix degrading enzymes was increased in OAcells (MMP-1: 2.6x; MMP-3: 2.4x; MMP-13: 2x, ADAMTS-4: 10x). After OP-1 stimulation, the anabolic genes were significantly up-regulated in OA chondrocytes (Fig. 1) whereas again no significant changes were observed for the matrix degrading enzymes (Fig. 1).

Effect of OP-1 stimulation on intracellular mediators of BMP-signalling in normal and OA chondrocytes

All R-Smads (Smad 1, 5, and 8), C-Smad 4, and I-Smads (Smad 6 and 7) were expressed in human articular chondrocytes *in vitro*, both normal and OA, at roughly the same level (Table II). No significant differences were detectable after stimulation by OP-1 (Table II).

Discussion

The present study demonstrates the effect of OP-1 on normal and OAchondrocytes. The most interesting finding of our study was that OAchondrocytes are not hypo-responsive to OP-1 compared to their normal counterparts, which is in line with previous reports looking at sulfate incorporation (9). A hypo-responsiveness of OA chondrocytes was described previously for other anabolic growth factors such as IGF-I (5). In fact, OP-1 was more potent on OA than on normal adult chondrocytes which, however, in agreement with previous studies, also showed an anabolic response (3, 9, 10). Thus, OP-1 might be a good anabolic stimulator for the treatment of OA joint disease. This might be of particular interest as chondrocytes themselves showed only very minor expression of OP-1, confirming previous results using RNA from chondrocytes *in vitro* (9) and RNA directly isolated from articular cartilage (10, 11). One obvious caveat that must be kept in mind is the fact that late stage OA chondrocytes may not be entirely comparable with chondrocytes from OA cartilage with early or moderately advanced degeneration, which would be the real target of any conservative intervention. Due to material problems (i.e. the availability of chondrocytes from earlier OA stages) this question can hardly be addressed, at least not in human patients.

The second aim of our study was to identify whether OP-1 could modulate the expression of major collagen (MMP-1 and MMP-13) and aggrecan degrading enzymes (MMP-3 and ADAMTS-4). We found no significant regulation of these in cultured normal

Table II. The expression levels of Smad-proteins in cultured normal and osteoarthritic chondrocytes with and without stimulation with BMP-7.

	Smad1/GAPDH	Smad4/GAPDH	Smad5/GAPDH	Smad6/GAPDH	Smad7/GAPDH	Smad8/GAPDH
Normal	0.00167	0.0182	0.0138	0.0465	0.0189	0.0590
Normal + OP-1	0.00211	0.0195	0.0171	0.0512	0.0269	0.0717
OA	0.00153	0.0256	0.0145	0.0637	0.0142	0.0757
OA+ OP-1	0.00155	0.0300	0.0122	0.0708	0.0167	0.0965

and OA articular chondrocytes. This suggests that OP-1 selectively modulates the anabolic activity of chondrocytes, which is consistent with our unpublished data showing – based on oligo-chip expression analysis – a very limited range of genes that are influenced by OP-1 as compared to, for example, Il-1ß which has both catabolic and anti-anabolic effects.

Bone morphogenetic proteins mediate their effects within the cells by binding to specific receptors that initiate the Smad signaling pathway, namely R-Smads 1, 5, and 8 and C-Smad 4. I-Smads 6 and 7 inhibit intracellular signal transduction. As was shown previously (6), all Smads were expressed in both normal and OA chondrocytes on roughly the same level and OP-1 did not significantly modulate any of the expression levels of the signaling molecules. This suggests that OP-1 has no direct effect on its intracellular mediators in adult articular chondrocytes. This is particularly notable for the I-Smads, as in other cellular systems up-regulation of these inhibitors by BMPs was reported in a cellular negative feedback mechanism (12). This appears to be different in adult articular chondrocytes.

A very interesting result of our *in vitro* study was that OAchondrocytes showed reduced levels of the expression of anabolic genes as compared to normal genes. This is different to our previous findings *in situ* where collagen type II mRNA expression was up-regulated in OA chondrocytes *in situ* (13, 14). This might correspond to the reported increased fragility of OA chondrocytes after isolation from the surrounding cartilage matrix (15) and might reflect an overall derangement of OA chondrocytes. The increase of matrix degrading proteases in OA chondrocytes compared to normal ones might reflect the increased catabolic activity of these cells.

Overall, OA chondrocytes are not hyporesponsive to anabolic stimulation by OP-1. Thus, OP-1 might to be a suitable anabolic activator of OAchondrocytes. This may not be relevant for the majority of the late-stage OA chondrocytes, which are in any case metabolically activated (13), but may be of interest for the cells of the upper zones of ongoing cartilage destruction which show a failure of anabolic gene expression (1) and potentially also chondrocytes during the initial stages of cartilage degeneration.

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