[Frontiers in Bioscience 10, 2027-2035, September 1, 2005]

THE HUMAN CHONDROSARCOMA HCS-2/8 CELL LINE IS RESPONSIVE TO BMP-7, BUT NOT TO IL-1BETA

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1. ABSTRACT

Cultures of primary chondrocytes as *in vitro* model systems for studying the cellular behavior of chondrocytes are notoriously difficult to cultivate and propagate. One way to circumvent these problems appears to be the use of immortalized/immortal chondrocytic cell lines. In the present study, we were interested whether the chondrosarcoma derived HCS-2/8 cells are suitable for studying major cellular reaction pattern in response to key anabolic (BMP-7) and catabolic (IL-1beta) factors. Therefore, we used cDNA array and real-time PCR technology in order to evaluate gene expression triggerd by stimulation with IL-1beta (0,1-100 ng/ml) and BMP-7 in confluent monolayer cultures. HCS-2/8 cells hardly responded to IL-1beta, but showed good responsiveness to BMP-7. We found 12 genes up- and 17 significantly down-regulated by BMP-7 (out of 340 investigated genes). Besides the expected activation of anabolic genes chondrocytic cells after BMP-stimulation try to neutralize activation of the BMP-signalling cascade by expressing intra- and extracellular BMP-antagonists. Chondrosarcoma derived cell lines are a potential substitute for primary articular chondrocytes promising consistent expression of a differentiated chondrocyte phenotype with sufficient proliferative capacity. However, as shown by this study one needs to carefully select the cell line depending on the effects which one intends to study. In this respect, HCS-2/8 cells are a validated tool for studying BMP-effects on chondrocytes, but not e.g. effects of interleukin-1.

2. INTRODUCTION

Cultures of primary chondrocytes as *in vitro* model systems for studying the cellular behavior of chondrocytes are notoriously difficult to cultivate and propagate. Especially human chondrocytes are difficult to obtain for a variety of reasons including the difficulty to isolate from a single joint a significant number of cells. Thus, there exists a high need for well available cellular systems in order to simulate and investigate cellular reaction patterns under defined conditions in chondrocytic cells *in vitro*.

One way to circumvent these problems appears to be the use of immortalized/immortal chondrocytic cell lines which allow to perform investigations in a largely unlimited and standardized manner. In this respect, Takigawa and colleagues established one of the first chondrocytic cell lines from a well differentiated, human chondrosarcoma (1). These cells were shown in several studies to retain a chondrocytic morphology and continue to proliferate in long-term culture. These cells have since then been used in many studies in order to investigate cellular reaction patterns of chondrocytic cells with regard to loading (2), analysis of intracellular signal transduction pathways (3), anabolic factors (4,5), and in particular effects of CTGF (6-9) and TNF-alpha (10). Recently, we established a basic gene expression profile of the HCS-2/8 cells depending on the culture modalities used (11).

Table 1. Sequences of primers and probes for real-time PCR experiments

Gene	Acc.No.	Primers	nM	Probe	$MgCl_2$
GAPDH	NM_002046	fw: GAAGGTGAAGGTCGGAGTC rv: GAAGATGGTGATGGGATTTC	50 900	CAAGCTTCCCGTTCTCAGCC	5.5
ADMATS-4	AF148213	fw: TGCCCGCTTCATCACTGA rv: CAATGGAGCCTCTGGTTTGTC	900 50	ACAGTGCCCATAGCCATTGTCCAGGA	4
Aggrecan	NM_013227	fw: ACTTCCGCTGGTCAGATGGA rv: TCTCGTGCCAGATCATCACC	50 50	CCATGCAATTTGAGAACTGGCGCC	6
Col 2A1	NM_001844	fw: CAACACTGCCAACGTCCAGAT rv: CTGCTTCGTCCAGATAGGCAAT	50 300	ACCTTCCTACGCCTGCTGTCCACG	5.5
MMP-1	NM_002421	fw: CTGTTCAGGGACAGAATGTGCT rv: TCGATATGCTTCACAGTTCTAGGG	300 900	ACGGATACCCCAAGGACATCTACAGCTCC	6.5
MMP-3	NM_002422	fw: TTTTGGCCATCTCTTCCTTCA rv: TGTGGATGCCTCTTGGGTATC	900 300	AACTTCATATGCGGCATCCACGCC	4
MMP-13	NM_002427	fw: TCCTCTTCTTGAGCTGGACTCATT rv: CGCTCTGCAAACTGGAGGTC	900 50	TCCTCAGACAAATCATCTTCATCACCACCAC	7
IL-6	NM_000600	fw: TGACAAACAAATTCGGTACATCCT rv: TCTGCCAGTGCCTCTTTGCT	50 900	CAGCCCTGAGAAAGGAGACATGTAACAA	5,5
LIF	NM_002309	fw: TGAACCAGATCAGGAGCCAACT rv: CCACATAGCTTGTCCAGGTTGTT	300 300	CAATGGCAGTGCCAATGCCCTCTTTATT	6
SOX5	NM_006940	fw: CAACCTTGGTGCTGCTGTATCT rv: ATCAGAGGTCTTGGGTTTAGCTGAT	300 900	CACAAACAGCCCACCACCCAAAAGC	6
SOX6	NM_033326	fw: TGAGGAGCTACCAACACTTGTCA rv: TCGGAAGGAATATAGGGAACATAACT	900 900	CCATTCAACAAGATGCTGACTGGGACAG	6,5
SOX9	Z46629	fw: ACACACAGCTCACTCGACCTTG rv: GGAATTCTGGTTGGTCCTCTCTT	900 50	TTAGGATCATCTCGGCCATCGTCGC	7
TNFalpha	NM_000594	fw: CCCCAGGGACCTCTCTCTA rv: GGTTTGCTACAACATGGGCTACA	50 900	AGTCAGATCATCTTCTCGAACCCCGAGTG	6

In the present study, we were interested whether the HCS-2/8 cells are suitable for studying major cellular reaction patterns in response to established anabolic and catabolic stimuli. For this, we tested the effects of two potent anabolic and catabolic stimulators of articular chondrocytes, BMP-7 (12,13) and IL-1beta (14,15).

3. MATERIALS AND METHODS

3.1. Culture of HCS-2/8 cells - stimulation with IL-1BETA and BMP-7

The human HCS-2/8 chondrosarcoma cell line was cultured in DMEM medium (PAA, Austria) supplemented with 20% foetal bovine serum (Gibco BRL, Germany) in humidified atmosphere 5% CO_2 in air at 37 °C as described by Takigawa *et al.* (16). To obtain HCS-2/8 cells at different growth conditions cells were seeded at densities of 1x $10^5/cm^2$ and grown for 3 days to obtain sub-confluent stage cultures, 2x $10^5/cm^2$ and cultured for 7 days to obtain confluent stage cultures, and 6x $10^5/cm^2$ and grown for 10 days for over-confluent stage cultures. For the IL-1beta-stimulation experiments, chondrocytes were either stimulated with 0,1-1-10 ng/ml rhIL-1beta (Biomol, Germany) 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. In the BMP-7 time course experiments confluent chondrocytes were either stimulated with BMP-7 (600 ng /ml BMP-7 (W. Sebald, Würzburg) in DMEM (PAA, Austria), 1% foetal bovine serum) or control medium (DMEM, 1% foetal bovine serum) for 30 min, 6 hr, 16 hr, 24 hr and 48 hr. At the end of the stimulation period cells were washed in sterile PBS and lysed in 350 µl lysis RLT buffer/10^6 cells (Qiagen, Hilden, Germany) and stored at -80°C.

3.2. RNA-isolation

At the end of the culture period, cells were washed in DEPC-PBS and lysed in Qiagen RNA lysis buffer (100 µl/10^6 cells). RNA was isolated according to the manufacturer's protocol (Qiagen Mini RNeasy total RNA isolation kit; Qiagen GmbH, Germany). The quality of the isolated RNA was assessed by ethidium bromide staining on agarose gels and the concentration was determined by spectrophotometry (260/280nm).

3.3. Reverse transcription

First strand cDNA was synthesized using 1 µg of total RNA, 400U M-MLV Reverse Transcriptase, RNase H Minus (Promega, Germany), 2 mM dNTPs (Roth) and 200 ng random primers (Promega, Germany) in a total volume of 40 µl.

3.4. TAQMAN-PCR

TAQMAN-PCR (real-time PCR) was used to detect human COL2, aggrecan, MMP-1, -3, -13 as well as ADAMTS-4, IL-1beta, TNF-alpha, IL-6, LIF, SOX5, SOX6, SOX9 and GAPDH. The primers (MWG Biotech, Germany) and TAQMAN-probes (Eurogentech, Belgium) were designed using PRIMER EXPRESS TM software (Perkin Elmer) (17,18). A separate master-mix was made up for each of the primer pairs and contained a final concentration of 200µM NTPs, 450 nM Roxbuffer and 100 nM TAQMAN probe. For all genes, the final reaction mix contained besides cDNA and 0.5U polymerase (Eurogentech, Belgium) forward and reverse primers, the corresponding probes, and $MgCl_2$ at concentrations given in table 1. All experiments were performed in triplicates.

3.5. Construction of the SensiChip cartilage microarray

The SensiChip technology is a two-color microarray platform using the Planar Wave Guide technology for microarray detection (19) which increases signal to noise ratios and thereby sensitivity of hybridization experiments. The array was compiled for the measurement of 340 genes relevant for cartilage (containing matrix genes, catabolic genes, differentiation markers and all known genes related to the BMP-pathway). For each gene one 70-mer oligonucleotide directed against

the 3´-UTR of the target transcript was designed and spotted in duplicate onto the microarray.

3.6. Microarray experiments - time course studies with BMP-7

The time course study consisted of 3 independent culture series of confluent HCS-2/8 monolayers treated with BMP-7 (600 ng /ml BMP-7 in DMEM (PAA, Austria, 1% foetal bovine serum) or control medium (DMEM, 1% foetal bovine serum) for 30 min, 6 hrs, 16 hrs, 24 hrs and 48 hrs. From each (of the 30) cultures, RNA was isolated using the RNeasy Kit (Qiagen). 1µg total RNA from BMP-7-treated and control cultures were reverse transcribed in the presence of Alexa-labeled and Cy5-labeled dUTP nucleotides using the Omniscript Kit (Qiagen), respectively. After cDNA purification, labeled cDNAs were mixed and hybridized for 16 hrs on SensiChip microarrays (Qiagen). Hybridizations were repeated with inversely labeled material generated by exchanging Alexa- and Cy5- labeled nucleotides for BMP-7-treated and control samples. Inverse labeling was done to compensate for differential labeling efficiency and differences in fluorescence intensity associated with the two dyes. Microarrays were washed according to standard procedures (Qiagen), scanned using the SensiChip Reader and analyzed by SensiChip View 2.1 software to obtain signal intensities and control the hybridization performance. Raw intensity data files of quality controlled scans were imported into Resolver software version 4.0 (Rosetta) for further analysis.

3.7. Analysis of microarray data

After import of intensity data files into the Resolver software version 4.0 (Rosetta) data are processed by an error model specifically developed for the Sensichip technology to estimate the error of intensity measurements and to calculate p-values and error bars. The error modelling approach is described in Rajagopalan D. 2003 (20). The description of the error model and the used statistical methods are available under the technology section from the Rosetta Biosoftware website (http://www.rosettabio.com/tech/default.htm). Intensity files were normalized and all 6 replicates for each time point were merged to obtain more robust data. The ratios and p-values of control versus BMP-7 treated samples were calculated for each time point by averaging the data using an error weighted algorithm. The p-value assigned to each gene measurement reflects the probability that the observed regulation is due to measurement error and a regulation with a calculated p-value below a threshold (e.g. 0,05) is considered statistically significant.

3.8. Measurement of glycosaminoglycan (GAG)-content

HCS-2/8 cells were seeded in 24-well plates (5x104 cells/well) and cultured for 24 hrs in DMEM medium (PAA, Austria) supplemented with 10% foetal bovine serum (Hyclone). Medium was then exchanged in 4 wells with DMEM/1% FCS for 24 hrs and cells exposed to 600 ng/ml BMP-7 in DMEM/1% FCS (or medium DMEM/1% FCS in 4 wells as control) for three days. The supernatant was harvested and an aliquot taken for the quantification of GAGs by photometry (OD650nm) using the dimethylmethylene blue method (kit provided by Biocolor Ltd., Belfast, Northern Ireland). Plates containing cell monolayers were stored at –80°C until measurement of DNA for standardization. Fluorometric quantification of DNA was done using the Fluo ReporterBlue Fluorometric dsDNA Quantification Kit (Molecular Probes, Oregon, USA) following the instructions of the manufacturer. Samples were measured in 96-well plates using the plate reader Tecan Genios (Tecan, Crailsheim Germany) and data were analysed in GraphPadPrism 4.0 (Graph Pad Software Inc., San Diego, CA, USA).

4. RESULTS

4.1. Culturing of HCS-2/8 cells - general gene expression profiling

Gene expression profiling using the cDNA array technology as well as real-time PCR confirmed previous Affymetrix-GeneChip data (11): HCS-2/8 cells expressed rather high amounts of aggrecan, but hardly any collagen type II, at least not in the passage used in this study. In general, many constituents of the extracellular matrix were very much down-regulated in the HCS-2/8 cells compared to primary articular chondrocytes: this included molecules such as collagen type III, COMP, osteopontin, and fibronectin. Others, in particular collagen type VI (alpha 1 and 3), collagen types IX and XI as well as biglycan were significantly stronger expressed in HCS-2/8 cells compared to physiological chondrocytes. Together with many matrix components, also cartilage matrix degrading proteases were down-regulated, notably MMPs-1, -3, and -13, as well as ADAMTS-5.

The overall chondrocytic phenotype of the cells was confirmed not only by the expression of chondrocyte-typical gene products such as collagen types IX and XI as well as aggrecan, but also by the expression of transcription factors SOX-9 (0,02 molecules/molecules GAPDH) and SOX-5 (0,03 molecules/molecules GAPDH) and -6 (0,05 molecules/molecules GAPDH), which are important mediators of the chondrocytic phenotype *in vivo* (21,22). Of interest, HCS-2/8 expressed no detectable amounts of BMP-7, a major anabolic growth factor, nor IL-1beta and TNFalpha, major catabolic cytokines of adult articular cartilage.

4.2. HCS-2/8 cells are hardly responsive to IL-1BETA

First, we tested whether the HCS-2/8 cells are reactive to IL-1beta as a major catabolic inductor of articular chondrocytes. Because a first attempt using 10 ng/ml IL-1beta and confluent cultures conditions revealed largely negative results for important marker genes usually repressed or induced in chondrocytes by IL-1beta, we performed a more extensive analysis using three different concentrations of IL-1beta (0,1 ng/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml) at all (or part of the) three growing conditions, which were described in previous work to be relevant for the phenotype of the cells (1): sub-confluent, confluent and over-confluent culture of the HCS-2/8 cells (results are summarized in table 2). In these experiments, only a minor reduction of the expression of anabolic genes aggrecan and collagen type II at unphysiologically high concentrations of IL-1beta (10 ng/ml, 100 ng/ml) as well

Table 2. Quantitative gene expression analysis of anabolic and catabolic genes as well as mediators in HCS-2/8 cells cultured in different densities and stimulated with different concentrations of IL-1beta (0,1, 1, 10, 100 ng/ml).

	Sub-confluent			**Confluent**				**Over-confluent**			**PHC**
IL-1beta (ng/ml)	**0,1**	**1**	**10**	**0,1**	**1**	**10**	**100**	**0,1**	**1**	**10**	**10**
aggrecan	1,1	1,1	0,8	1,9	1,6	1,1	0,8	1,1	1,0	0,8	0,3
Col 2	1,1	0,9	0,6	0,8	1,0	1,0	1,1	1,0	1,2	1,4	0,05
MMP-1	nd	nd	2,1	nd	nd	1,0	1,0	nd	nd	1,4	7,4
MMP-3	nd	nd	1,4	nd	nd	1,0	0,9	nd	nd	1,5	2,5
MMP-13	nd	nd	2,5	nd	nd	0,9	1,1	nd	nd	1,1	6,6
ADAMTS-4	nd	nd	1,0	nd	nd	1,0	1,1	nd	nd	1,6	8,9
IL-6	nd	nd	1,2	nd	nd	0,7	1	nd	nd	1,2	220
LIF	nd	nd	0,7	nd	nd	0,5	0,9	nd	nd	0,9	6,2

Assays were always performed for three independent culture experiments. Shown are the ratios (stimulated vs. unstimulated) after Il-1 incubation. For comparison, values for normal adult articular chondrocytes are given in the right column (43). nd: not determined

as a minor non-significant induction of collagenases MMP-1 and -13 were observed. Most interestingly, IL-6 and LIF, two genes strongly induced by IL-1beta in articular chondrocytes (23-29), were not induced at all in HCS-2/8 cells.

4.3. HCS-2/8 cells are responsive to BMP-7

Next, we were interested whether HCS-2/8 cells are responsive to BMP-7. For this, we tested the reactivity of aggrecan and collagen type II mRNA expression to this agent using real-time PCR. This showed the induction of both genes (aggrecan: 100%; collagen type II: 70%), with a magnitude comparable to primary human adult articular chondrocytes (30). The increased synthesis of proteoglycans was confirmed by measurements of the proteoglycan content (increase of 40%; $p<0,05$; figure 1).

4.4. Gene expression profiling of HCS-2/8 cells after stimulation with BMP-7

The responsiveness of HCS-2/8 cells to BMP-7 by real-time PCR analysis prompted us to investigate the general expression pattern of these cells using the SensiChip-technology, because of its characteristic high sensitivity. Genes specifically relevant for cartilage (matrix genes, catabolic genes and chondrocyte phenotype genes) as well as genes assigned to the BMP pathway were chosen for the construction of a custom-made SensiChip. A time-course experiment (0, 6, 16, 24, and 48 hrs) with independent triplicates was performed using confluent HCS-2/8 cells cultured as monolayers. Two-dimensional clustering of profiles and regulated genes revealed two main clusters (transcripts induced and repressed by BMP-7) and showed the relatedness of the profiles during the stimulation period (figure 2). Profiles from the early timepoints (30 min, 6 hrs) were more similar to each other than the profiles from later time points with stronger and more numerous effects on gene expression (visualized in figure 2). Overall, 12 genes were significantly up- and 14 were significantly down-regulated out of 340 genes investigated (figure 3) (dataset available online under www.bio.ifi.lmu.de/publications/FBS2005). Besides the activation of anabolic genes, in particular genes linked to the BMP/TGF-beta pathway were modulated (figure 3, table 3). Of note, among these genes the two BMP-inhibitors Noggin and Smad-6 were increased.

The data obtained by cDNA-array technology were validated by real-time PCR (figure 4) showing also an up-regulation of collagen type II and aggrecan as well as Smad 6 and TIMP-3, whereas BMP-2 was down-regulated and TNFalpha and Il-1beta not expressed or induced at all (not shown)

5. DISCUSSION

Adult articular chondrocytes are regarded as terminally differentiated cells that maintain their phenotype under conditions of low to negligible cellular turnover in vivo (31). However, they are difficult to isolate and even more difficult to keep in culture preserving their phenotype. Proliferation largely implicates the loss of the differentiated phenotype of the cells. Chondrosarcoma derived cell lines might be a potential substitute promising consistent expression of a differentiated chondrocyte phenotype with sufficient proliferative capacity (4,31,32). However, as shown by this study one needs to carefully select the cell line depending on the effects which one intends to study. Thus, HCS-2/8 cells are largely non-reactive to IL-1beta. Also, no major influence of the culture density of the HCS-2/8, which was reported to be important for the phenotype of these cells (1), was observed. This was true for both, anabolic and catabolic genes, which are usually strongly regulated by IL-1beta *in vitro* (14,17,33,34). Our findings are in line with a previous study showing a non-responsiveness of MMP-13 expression to IL-1beta in HCS-2/8 cells (35). Also tested intercellular mediators such as IL-6 and LIF, which are strongly switched on by IL-1beta in adult chondrocytes (24,26), were not induced in HCS-2/8 cells under any condition. The reason for the missing Il-1 responsiveness of HCS-2/8 cells is unclear. Interestingly, the mRNA expression levels of the functional IL-1 receptor was very much reduced compared to (responsive) physiological chondrocytes. However, other members of the intracellular signaling cascade were expressed so, overall, the non-responsiveness of HCS-2/8 cells to IL-1 requires further investigations. Of note, also other chondrosarcoma-derived cell lines such as the Swarm rat chondrosarcoma cells (36) are not responsive to Il-1beta stimulation, whereas others such as the SW1353 cells are (14,37).

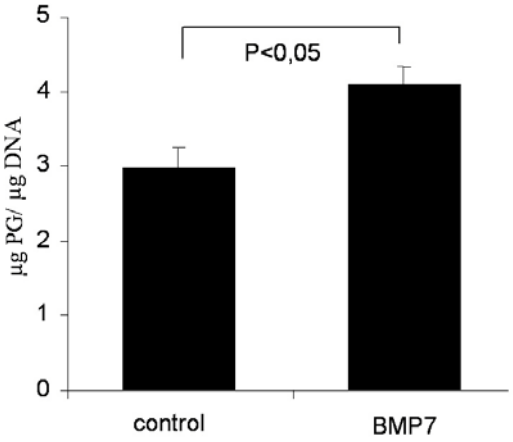

Figure 1. Quantification of glycosaminoglycans (µg/µg DNA) in the supernatant of HCS2/8 cells after stimulation with BMP-7. Cells were cultured in control medium or stimulated with BMP-7 (600 ng/ml) for 3 days and GAGs were quantitated in the supernatant and DNA in the cell pellet, respectively. The amount of GAGs (± standard deviation) is given relative to the amount of DNA. A non-parametric Mann-Whitney test (software GraphPadPrism 4.0) was used for statistical analysis.

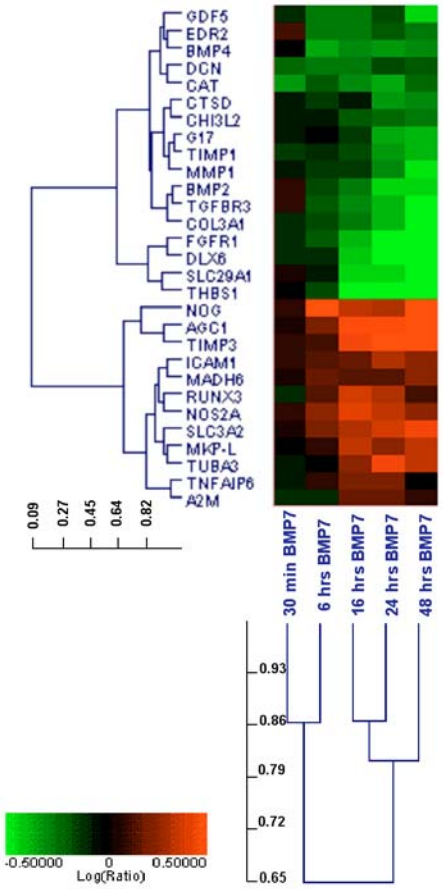

Figure 2. Clustering of regulated genes and stimulation experiments in HCS-2/8 cells treated with BMP-7. For the five time intervals of BMP-7 stimulation, expression profiles of treated samples were compared to control samples (triplicates each) and genes with a 1,5 fold change (and a corresponding p-values of less than 0,001) were considered regulated and were used for clustering. Hierarchical clustering groups the two most similar objects into a cluster (similarity was determined using Euclidean distance) and than keeps joining the most similar object to the newly formed cluster. The figure shows a dendrogram for gene profiles (columns) and for experiments (rows) respectively, as the result of a two dimensional clustering. The scale with correlation values for clusters is plotted for both dimensions. The direction and magnitude of gene regulation is indicated by colour type/saturation (green: down-regulated genes, red: up-regulated genes).

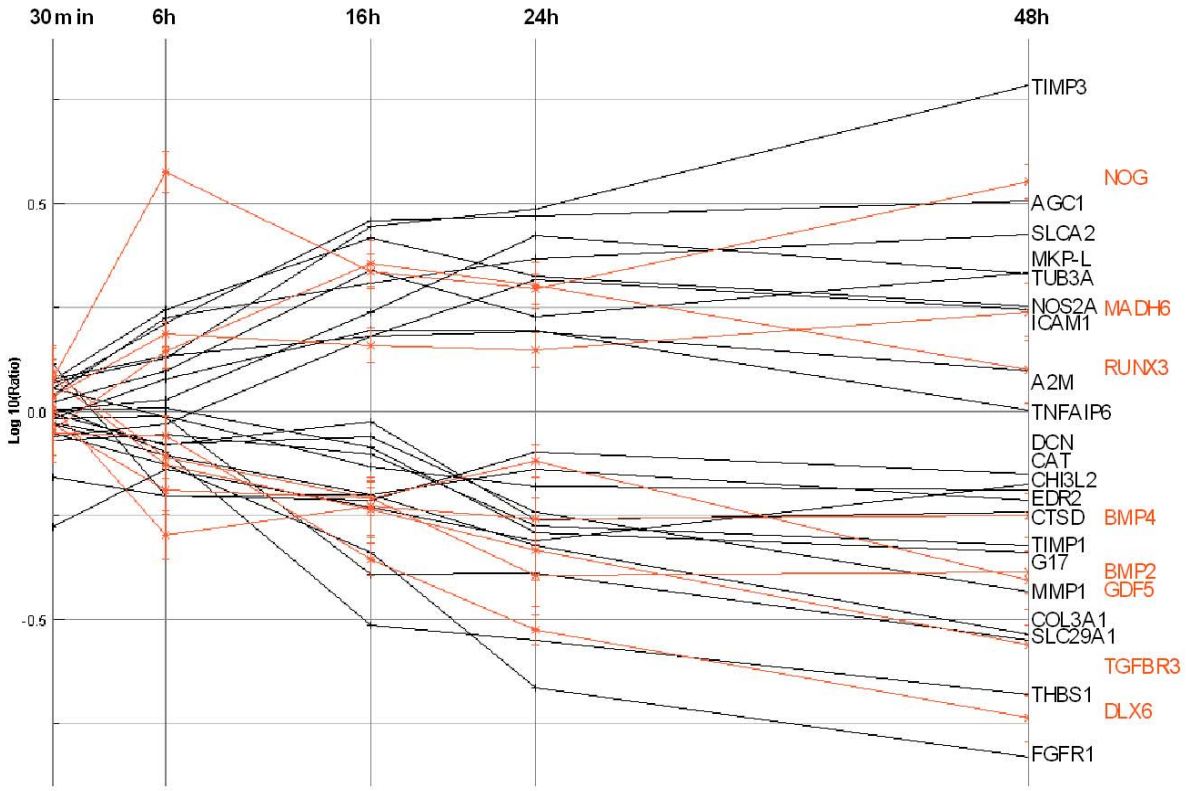

Figure 3. Temporal regulation of genes in HCS-2/8 cells after stimulation with BMP-7. (a) Graphical representation of genes showing a regulation at two (or more) time points within the timecourse (30 min, 6 , 16 , 24 and 48 hours) of at least 1,5 fold and a corresponding p-value less than 0,001. Genes of the BMP/TGF- family and BMP/TGF-signalling molecules are marked in red.

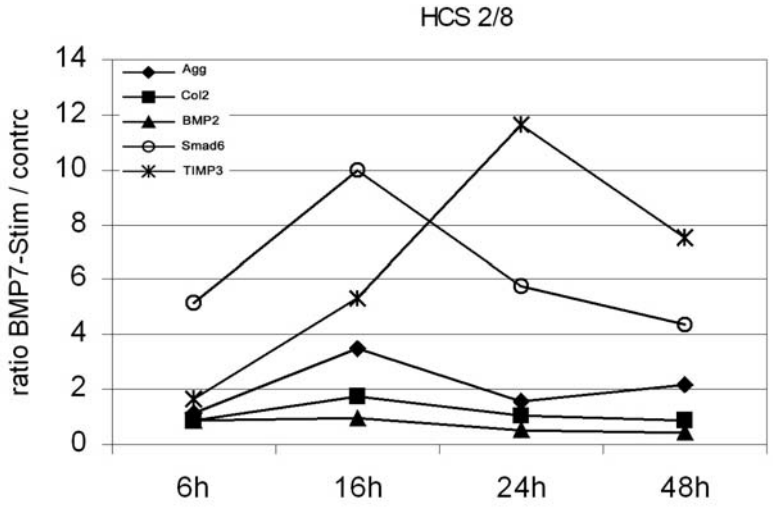

Figure 4. Validation of changes of gene expression levels in the cDNA-array experiments using quantitative PCR: shown are ratios (stimulated versus un-stimulated) for Col2, aggrecan, BMP-2, TIMP-3 and Smad 6 for the different time points (6 hrs, 16 hrs, 24 hrs, 48 hrs).

The lack of responsiveness of the HCS-2/8 cells to IL-1 is in contrast to the good responsiveness of the HCS-2/8 cells to BMP-7 as well as TNF-alpha and FGF. The first was investigated for the first time in this study, whereas TNF-alpha (10) and FGF (35) have been looked at previously. Thus, BMP-7 stimulation lead to a significant

Table 3. Overview of numerical values of BMP-7 regulated genes (given are ratios compared to control levels).

		30 min		6 hrs		16 hrs		24 hrs		48 hrs	
Gene	**Acc.No.**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**
TIMP3	U02571	1,16	ns	1,33	< 0,01	2,77	< 0,001	3,05	< 0,001	6,03	< 0,001
NOG	NM_005450	1,19	ns	3,72	< 0,001	2,16	< 0,001	1,96	< 0,001	3,54	< 0,001
AGC1	NM_001135	1,13	ns	1,61	< 0,05	2,85	< 0,001	2,93	< 0,001	3,19	< 0,001
SLC3A2	NM_002394	1,08	ns	1,66	< 0,001	2,02	< 0,001	2,31	< 0,001	2,64	< 0,001
MKP-L	NM_007026	1,04	ns	1,24	< 0,001	2,16	< 0,001	1,68	< 0,001	2,14	< 0,001
TUBA3	AF141347	1,00	ns	1,06	ns	1,72	< 0,001	2,63	< 0,001	2,12	< 0,001
NOS2A	AB022318	1,18	ns	1,74	< 0,001	2,58	< 0,001	2,10	< 0,001	1,77	< 0,001
ICAM1	NM_000201	1,19	ns	1,35	< 0,05	1,50	< 0,001	2,06	< 0,001	1,74	< 0,001
MADH6	NM_005585	1,08	ns	1,52	< 0,001	1,43	< 0,001	1,39	< 0,001	1,73	< 0,001
RUNX3	NM_004350	0,90	ns	1,38	< 0,001	2,25	< 0,001	1,99	< 0,001	1,25	ns
A2M	M11313	0,86	< 0,01	0,92	< 0,05	1,50	< 0,001	1,54	< 0,001	1,24	< 0,001
TNFAIP6	NM_007115	0,96	ns	1,19	ns	1,56	< 0,001	1,56	< 0,001	1,00	ns
DCN	M14219	0,69	< 0,05	0,62	< 0,001	0,61	< 0,001	0,80	ns	0,70	ns
CAT	NM_001752	0,52	ns	0,72	< 0,01	0,59	< 0,001	0,49	< 0,001	0,66	0,01
CHI3L2	NM_004000	0,95	ns	0,97	ns	0,73	< 0,05	0,66	< 0,001	0,64	< 0,001
EDR2	NM_004427	1,29	< 0,05	0,62	< 0,001	0,63	< 0,001	0,72	< 0,01	0,61	< 0,001
CTSD	NM_001909	0,98	ns	0,82	< 0,05	0,94	ns	0,55	< 0,001	0,57	< 0,001
BMP4	NM_001202	1,02	ns	0,50	< 0,001	0,59	< 0,001	0,55	< 0,001	0,56	< 0,001
TIMP1	X03124	0,85	ns	0,88	ns	0,79	< 0,05	0,53	< 0,001	0,47	< 0,001
G17	NM_006841	1,00	ns	1,01	ns	0,82	ns	0,51	< 0,001	0,46	< 0,001
BMP2	NM_001200	1,23	ns	0,77	< 0,01	0,62	< 0,001	0,40	< 0,001	0,41	< 0,01
GDF5	NM_000557	0,92	ns	0,64	< 0,001	0,62	< 0,001	0,75	< 0,001	0,39	< 0,001
MMP1	X05231	0,93	ns	0,83	ns	0,86	ns	0,57	< 0,001	0,37	< 0,001
COL3A1	X14420	0,93	ns	0,78	ns	0,63	< 0,05	0,47	< 0,001	0,29	< 0,001
SLC29A1	NM_004955	1,13	ns	0,96	ns	0,40	< 0,001	0,41	< 0,001	0,28	< 0,001
TGFBR3	NM_003243	1,19	ns	0,74	< 0,05	0,58	< 0,001	0,46	< 0,001	0,27	< 0,001
THBS1	NM_003246	1,02	ns	0,79	ns	0,30	< 0,001	0,28	< 0,001	0,21	< 0,001
DLX6	NM_005222	0,88	ns	0,87	ns	0,44	< 0,001	0,30	< 0,001	0,18	< 0,001
FGFR1	NM_015850	0,88	ns	0,74	< 0,05	0,45	< 0,001	0,22	< 0,001	0,15	< 0,001

(see legend for figure 3). R=ratio (treatment vs. control)

induction of collagen type II and aggrecan in HCS-2/8 cells, the two main anabolic gene products of articular chondrocytes. This chondrocyte-typical reaction pattern is very comparable to the effects of BMP-7 seen in primary adult articular chondrocytes (12,30). This is not surprising, because HCS-2/8 cells show in many respects a chondrocytic phenotype (11) and do e.g. express major chondrocyte-typical transcription factors such as SOX9 (38,39) and SOX5 and SOX6 (21).

In our array experiments, looking at 340 genes, 12 genes were up- and 17 were down-regulated by BMP-7. Besides the activation of anabolic genes, in particular genes linked to the BMP/TGF-beta pathway were modulated: this included a significant up-regulation of Noggin, a classical extracellular BMP-antagonist (40) known to be induced by BMPs (41), indicating a negative feed-back loop after BMP-stimulation. Also up-regulated was Smad 6, which belongs to the family of inhibitory Smad-signalling molecules and is known to inhibit BMP-signalling intracellularily (42). At the same time other members of the TGFbeta/BMP-family were down-regulated. Altogether, our data further suggest that chondrocytic cells after BMP-stimulation try to counteract via expressing intra- and extracellular BMP-antagonists.

Overall, our study shows how important it is not only to select a cell line for their general cellular phenotype, but also for its ability to react in specific circumstances in a physiological way: in this respect the HCS-2/8 cells appear to be very suitable for investigating effects of BMP-activity on chondrocytes, but not of IL-1.

6. ACKNOWLEDGEMENTS

This work was supported by the BMBF (grant 01GG9824) and Aventis Pharma Deutschland GmbH. We thank Stefanie Weidauer and Anika Becker for expert technical assistance.

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Key Words: Chondrocyte, Cell Line, Growth Factors, Chondrosarcoma**,** Cartilage, IL-1, BMP, Microarray, DNA array

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