OsteoArthritis and Cartilage (2005) **13,** 697–708

doi:10.1016/j.joca.2005.04.004

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Comparison of the chondrosarcoma cell line SW1353 with primary human adult articular chondrocytes with regard to their gene expression profile and reactivity to IL-1β[1]

M. Gebauer Ph.D.†, J. Saas Ph.D.‡, F. Sohler M.Sc.§, J. Haag M.Sc.‖, S. Söder M.D.‖, M. Pieper Ph.D.‡, E. Bartnik Ph.D.‡, J. Beninga Ph.D.‡, R. Zimmer Ph.D.§ and T. Aigner M.D., D.Sc.‖*

† *Department of Genomic Sciences, Sanofi-Aventis, Frankfurt, FRG*

‡ *Therapeutic Department Thrombosis & Angiogenesis, Sanofi-Aventis, Frankfurt, FRG*

§ *Practical Informatics and Bioinformatics, Computer Science Department, University of Munich, FRG*

‖ *Osteoarticular and Arthritis Research, Department of Pathology, University of Erlangen-Nürnberg, FRG*

Summary

Objective: In this study, the human chondrosarcoma cell line SW1353 was investigated by gene expression analysis in order to validate it as an *in vitro* model for primary human (adult articular) chondrocytes (PHCs).

Methods: PHCs and SW1353 cells were cultured as high density monolayer cultures with and without 1 ng/ml interleukin-1β (IL-1β). RNA was isolated and assayed using a custom-made oligonucleotide microarray representing 312 chondrocyte-relevant genes. The expression levels of selected genes were confirmed by real-time polymerase chain reaction and the gene expression profiles of the two cell types, both with and without IL-1β treatment, were compared.

Results: Overall, gene expression profiling showed only very limited similarities between SW1353 cells and PHCs at the transcriptional level. Similarities were predominantly seen with respect to catabolic effects after IL-1β treatment. In both cell systems matrix metalloproteinase-1 (MMP-1), MMP-3 and MMP-13 were strongly induced by IL-1β, without significant induction of MMP-2. IL-6 was also found to be up-regulated by IL-1β in both cellular models. On the other hand, intercellular mediators such as leucemia inhibitory factor (LIF) and bone morphogenetic protein-2 (BMP-2) were not induced by IL-1β in SW1353 cells, but significantly up-regulated in PHCs. Bioinformatical analysis identified nuclear factor kappa-B (NFκB) as a common transcriptional regulator of IL-1β induced genes in both SW1353 cells and PHCs, whereas other transcription factors were only found to be relevant for individual cell systems.

Conclusion: Our data characterize SW1353 cells as a cell line with only a very limited potential to mimic PHCs, though SW1353 cells can be of value to study the induction of protease expression within cells, a phenomenon also seen in chondrocytes.

Key words: Chondrocytes, Cartilage, Cell line, Expression analysis, MMPs.

Introduction

Investigations of primary human (adult articular) chondrocytes (PHCs) are limited by the lack of a sufficient number of chondrocytes from operative procedures and the fact that after isolation, the biological and functional characteristics of PHCs differ considerably between donors and preparations[1,2]. Proliferative activity of PHCs *in vitro* implicates dedifferentiation, with loss of the typical chondrocytic cell morphology, as well as a significant change in the pattern of gene expression[3]. Long-term investigations of physiological characteristics of PHCs are hampered due to the rapid phenotypic destabilization of these cells.

For analytical assays chondrocytic cell lines are used as a substitute for PHCs. In this situation, chondrosarcoma-derived cell lines are a promising substitute for a chondrocytic experimental system, displaying sufficient proliferative activity and the ability to display a consistent response to phenotype modulating stimuli. The following study investigated the gene expression profile of the chondrosarcoma-derived cell line SW1353 using DNA microarray technology. SW1353 cells have been used in many studies to look at chondrocytic transcription factors (TFs) such as SOX9[4,5], to test chondrosarcoma-targeted gene therapy[6], and to study protease expression and regulation in chondrocytic cells[7–10]. The SW1353 expression profiles obtained in this study were correlated with data from PHCs in order to analyze the suitability of this chondrosarcoma-derived cell line as a substitute of PHCs for experimental research.

Methods

CULTURING OF SW1353 CELLS AND STIMULATION WITH IL-1β

SW1353 cells were obtained from American Type Culture Collection (ATCC) (no. HTB-94) and cultured in Dulbecco's

[1]Supported by Aventis Pharma GmbH Deutschland and the Ministry of Research (grant 01GG9824).

*Address correspondence and reprint requests to: Thomas Aigner, Osteoarticular and Arthritis Research, Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstrasse 8-10, D-91054 Erlangen, FRG. Tel: 49-9131-8522857; Fax: 49-9131-8524745; E-mail: thomas.aigner@patho.imed.uni-erlangen.de

Received 23 December 2004; revision accepted 12 April 2005.

Modified Eagle Medium (DMEM)/F12 (Gibco BRL, Germany) with 10% FCS (fetal bovine serum; Gibco BRL, Germany) in humidified atmosphere 5% CO_2 in air at 37°C. Monolayer cells were washed in phosphate buffered saline (PBS) three times and stimulated with 1 ng/ml interleukin-1β (IL-1β) (Roche Diagnostics, Germany) in DMEM/F12 containing 0.5% lactalbumin enzyme hydrolysate (Sigma, Germany).

ISOLATION, CULTURING, AND STIMULATION OF PHCs

Macroscopically normal human knee articular cartilage (from femoral condyles and tibial plateaus) was obtained from six donors at autopsy, within 48 h of death (46 years male, 52 years male, 64 years female, 64 years male, 66 years female, 79 years male). Cartilage pieces were finely chopped and chondrocytes were enzymatically isolated from the associated matrix: sliced cartilage pieces were first digested with 1 mg/ml pronase (Roche, Switzerland) in DMEM/F12 with 10% FCS (Biochrom, Berlin, FRG) for 30 min prior to incubation with 1 mg/ml collagenase P (Boehringer Mannheim, FRG) overnight in Hams-F12 (Gibco BRL, Germany) with 10% FCS. Cells were then washed several times in Hams-F12 and counted and checked for viability using trypan blue staining.

After isolation, chondrocytes were seeded at 2×10^6 cells/well in 6-well tissue culture plates and maintained for 48 h in DMEM/F12 medium supplemented with 10% FCS, 50 μg/ml penicillin/streptomycin solution (Gibco BRL, Germany) and 50 μg/ml ascorbate (Sigma, Germany).

Thereafter, (the non-passaged) chondrocytes were stimulated with 1 ng/ml recombinant human IL-1β (Biomol, Germany) in DMEM/F12 medium containing 10% FCS (Biochrom, Berlin, FRG) or cultivated in medium containing 10% FCS alone for 3 days (except the enzyme-linked immunoabsorbant assay (ELISA) experiments which were performed using 0.1 ng/ml IL-1β). The medium was changed everyday. At the end of the stimulation period the cells were washed in sterile PBS, lysed in 350 μl lysis RLT buffer/10^6 cells (Qiagen GmbH, Germany) and stored at −80°C.

RNA ISOLATION AND QUALITY CONTROL

RNA isolation from isolated chondrocytes was performed as described previously[11]. RNA was isolated from cultured cells using the RNeasy mini-kit (Qiagen, Germany) (with an on-column DNAse digestion step according to the manufacturer's instructions). Briefly, lysates of cells were passed through a Qiashredder (Qiagen, Germany) and the eluted lysates mixed 1:1 with 70% ethanol. The lysates were applied to a mini-column and after washing and DNAse I digestion, the RNAs were eluted in 30−50 μl of RNAse-free water. The quantity and quality of total RNA samples was checked by agarose-gel-electrophoresis and using the Bioanalyzer RNA 6000 Nano assay (Agilent, Waldbronn, Germany).

cDNA SYNTHESIS

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

TAQMAN PCR

TAQMAN polymerase chain reaction (PCR) was used to detect mRNA expression levels of human anabolic (aggrecan, Col2) and catabolic (MMP-1, -2, -3, -13, ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs) gene products as well as important mediators (IL-1β, IL-6, LIF) and TFs (SOX5/6/9). The primers (MWG Biotech, Germany) and TAQMAN probes (Eurogentec, Belgium) were designed using PRIMER EXPRESS™ software (Perkin Elmer). 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 previously[12]. For the standard curves 10^1, 10^2, 10^3, 10^4, 10^5, as well as 10^6 molecules per assay were used (all in triplicate).

For the analyses of the different genes, a separate master-mix was made up for each of the primer pairs and contained a final concentration of 200 μM NTPs, 600 nM Roxbuffer and 100 nM TAQMAN probe. For all genes the final reaction mix contained [besides cDNA and 1 U polymerase (Eurogentec, Belgium)] forward and reverse primers, the corresponding probes, and $MgCl_2$ at concentrations given in Table I. All experiments were performed in triplicate.

CONSTRUCTION OF THE SENSICHIP CARTILAGE MICROARRAY

The SensiChip technology (Qiagen, Zeptosens) is a two-color microarray platform using the Planar Wave Guide technology for microarray detection[13], which increases signal to noise ratios and thereby sensitivity of hybridization experiments. The arrays were spotted in duplicates with 70-mer oligonucleotides representing the 3′-UTR of 312 housekeeping and human cartilage-relevant genes. Every single gene was represented by one 70-mer oligonucleotide (the list of genes is available online http://www.bio.ifi.lmu.de/publications/SW1353). The gene-specific oligonucleotide sequences were designed by Operon (Cologne, Germany) using respective GenBank accession numbers and proprietary algorithms.

SENSICHIP MICROARRAY EXPERIMENTS − TIME COURSE STUDIES WITH IL-1β

The SW1353 time course study consisted of three independent culture series of SW1353 monolayers treated with 1 ng/ml IL-1β (Roche Diagnostics, Germany) in DMEM/F12 (Gibco BRL, Germany) containing 0.5% lactalbumin enzymatic hydrolysate (Sigma, Germany) or control medium (DMEM/F12/0.5% lactalbumin enzymatic hydrolysate) for 30 min, 6 h, 16 h, 24 h and 48 h. From each of the 30 cultures RNA was isolated using the RNeasy Kit (Qiagen). Total RNA (1 μg) from IL-1β-treated and control cultures was reverse transcribed in the presence of Alexa-labeled or Cy5-labeled dUTP nucleotides using the Omniscript Kit (Qiagen) and generated cDNA was subsequently purified using the QIAquick Kit (Qiagen).

Due to limited amounts of RNA starting material, 250 ng total RNA from IL-1β-stimulated PHCs and unstimulated controls from each time point (30 min, 6 h, 16 h, 24 h and 48 h) was amplified and thereby labeled with Cy3-UTP and Cy5-UTP, respectively (Amersham Pharmacia), using the MessageAmp aRNA Kit (Ambion). After cRNA clean-up using the RNeasy kit (Qiagen), 5 μg of Cy3-labeled cRNA from IL-1β-stimulated chondrocytes was mixed with 5 μg of Cy5-labeled cRNA from the respective unstimulated control. cRNA was fragmented by incubation with 40 mM TRIS-acetate, pH 8.1, 100 mM KOAc, 30 mM MgOAc for 15 min at 95°C and desalted using a Microcon YM-10 concentrator (Millipore).

Table I
Sequences and concentrations of primers and probes as well as magnesium conditions for quantitative real-time PCR experiments

	Accession no.	Primer	nM	Probe (all 100 nM)	Mg (mM)
GAPDH	NM_002046	fw: GAAGGTGAAGGTCGGAGTC	50	CAAGCTTCCCGTTCTCAGCC	5.5
		rv: GAAGATGGTGATGGGATTTC	900		
Aggrecan	NM_013227	fw: ACTTCCGCTGGTCAGATGGA	50	CCATGCAATTTGAGAACTGGCGCC	6
		rv: TCTCGTGCCAGATCATCACC	50		
COL2A1	NM_001844	fw: CAACACTGCCAACGTCCAGAT	50	ACCTTCCTACGCCTGCTGTCCACG	5.5
		rv: CTGCTTCGTCCAGATAGGCAAT	300		
MMP-1	NM_002421	fw: CTGTTCAGGGACAGAATGTGCT	300	ACGGATACCCCAAGGACATCTACAGCTCC	6.5
		rv: TCGATATGCTTCACAGTTCTAGGG	900		
MMP-2	NM_004530	fw: TCACTCCTGAGATCTGCAAACAG	300	TTGATGGCATCGCTCAGATCCGTG	6
		rv: TCACAGTCCGCCAAATGAAC	900		
MMP-3	NM_002422	fw: TTTTGGCCATCTCTTCCTTCA	900	AACTTCATATGCGGCATCCACGCC	4
		rv: TGTGGATGCCTCTTGGGTATC	300		
MMP-13	NM_002427	fw: TCCTCTTCTTGAGCTGGACTCATT	900	TCCTCAGACAAATCATCTTCATCACCACCAC	7
		rv: CGCTCTGCAAACTGGAGGTC	50		
ADAMTS-4	AF148213	fw: TGCCCGCTTCATCACTGA	900	ACAGTGCCCATAGCCATTGTCCAGGA	6
		rv: CAATGGAGCCTCTGGTTTGTC	50		
IL-1β	NM_000576	fw: CTTTGAAGCTGATGGCCCTAAA	300	TCCAGGACCTGGACCTCTGCCCTCT	5
		rv: AGTGGTGGTCGGAGATTCGTA	900		
IL-6	NM_000600	fw: TGACAAACAAATTCGGTACATCCT	50	CAGCCCTGAGAAAGGAGACATGTAACAA	5.5
		rv: TCTGCCAGTGCCTCTTTGCT	900		
LIF	NM_002309	fw: TGAACCAGATCAGGAGCCAACT	300	CAATGGCAGTGCCAATGCCCTCTTTATT	6
		rv: CCACATAGCTTGTCCAGGTTGTT	300		
SOX9	Z46629	fw: ACACACAGCTCACTCGACCTTG	900	TTAGGATCATCTCGGCCATCGTCGC	7
		rv: GGAATTCTGGTTGGTCCTCTCTT	50		
SOX5	NM_006940	fw: CAACCTTGGTGCTGCTGTATCT	300	CACAAACAGCCCACCACCCAAAAGC	6
		rv: ATCAGAGGTCTTGGGTTTAGCTGAT	900		
SOX6	NM_033326	fw: TGAGGAGCTACCAACACTTGTCA	900	CCATTCAACAAGATGCTGACTGGGACAG	6.5
		rv: TCGGAAGGAATATAGGGAACATAACT	900		
TNFα	NM_000594	fw: CCCCAGGGACCTCTCTCTA	50	AGTCAGATCATCTTCTCGAACCCCGAGTG	6
		rv: GGTTTGCTACAACATGGGCTACA	900		

Six hundred nanograms of either mixed Cy-dye labeled cRNA or purified cDNA sample was hybridized for 16 h on a SensiChip microarray (Qiagen, Zeptosens). Hybridization was repeated with inversely labeled material generated by exchanging Alexa- and Cy5-labeled dUTP nucleotides for IL-1β-treated and control sample. Inverse labeling was performed to compensate for differential labeling efficiency and fluorescence intensity associated with the two dyes.

After washing steps following the manufacturer's standard protocol, arrays were scanned by the SensiChip Reader. The resulting array images were analyzed using the SensiChip View 2.1 software (Qiagen, Zeptosens) to quantify gene-specific signal intensities.

EXPRESSION DATA ANALYSIS

All microarray scans were inspected visually and checked for quality based on the performance of negative, housekeeping and externally added Cy3/Cy5-prelabeled spiking controls. Raw signal intensities from each scan were imported into the gene expression analysis software Resolver version 4.0 (Rosetta Biosoftware, Seattle, USA). The software employs an error modeling approach for analysis of microarray data[14]. An error model specific for the SensiChip microarray platform was designed by Rosetta Biosoftware to determine the variance of signal intensities, based on expression data from repeated hybridizations of the same RNA material. The complete description of the used statistical methods is available in the technology section of the Rosetta Biosoftware website (http://www.rosettabio.com/tech/default.htm).

All scans were pre-processed and normalized using the SensiChip error model to calculate *P*-values and error bars for every gene expression profile. The *P*-value for a gene estimates the probability that the observed differential gene expression was due to a measurement error. Gene regulation was considered as statistically significant if the calculated *P*-value was below a threshold of 0.05. For normalization of expression data, the average brightness of the Cy3 and Cy5 channels was used, calculated from spots within a range from 30% to 85% of the signal intensity distribution of all spots. Scans from multiple experiments (replicates) were combined by averaging expression data using an error-weighted algorithm (also described in the statistical methods document available on the above-mentioned website).

BIOINFORMATICS ANALYSIS OF TRANSCRIPTION FACTORS (TFs)

In order to analyze TF activity from the expression data, groups of potentially regulated target genes were determined for each TF. The required knowledge on TFs and their target genes are partially contained in databases like TRANSFAC[15] and the scientific literature. In this study, a combination of three types of evidence for the regulation of a target gene by the binding of a TF to its promoter was used: predicted binding sites, known binding sites, and co-occurrences of TFs and target genes extracted from the scientific literature. Binding site predictions for target genes were computed with the TRANSFAC tool MATCH on sequences starting 3 kb upstream from the transcription start site and ending 1 kb downstream. Known binding sites were extracted from TRANSFAC's SITE table[15]. Co-occurrences in the literature were computed using the text-mining tool ProMiner[16]. A gene was added to the group of potential target genes of a TF if the gene contained

a TRANSFAC binding site for TF or if the gene contained both a predicted binding site of TF and a literature co-occurrence with that TF. Target gene groups were checked for enrichment of significantly regulated genes (P-value $<$ 0.01 for at least one time point) in either cell type. This enrichment was quantified by P-values for all the TF target gene groups using Fisher's exact test[17]; the corresponding "group P-value" is the probability of finding the observed number of significantly regulated genes in the target gene group by selecting genes randomly. A group P-value lower than 0.05 was considered significant.

ANALYSIS OF MMP-1, -3 AND -13 RELEASE IN IL-1-STIMULATED SW1353 CHONDROSARCOMA CELLS

5×10^4 SW1353 cells/well were seeded into a 96-well flat-bottom plate in serum-free DMEM medium (Gibco) and cultured at 37°C and 5% CO_2. MMP expression was induced by stimulation with 0.1 ng/ml IL-1β (Roche) for 24 h. The cell culture supernatant was harvested, centrifuged and stored at −20°C until further analysis. Amounts of MMP-1, -3 and -13 (activated and inactive pro-forms) in the culture supernatant were determined with commercially available enzyme linked immunoabsorbent assays from Amersham Life Science, according to the manufacturer's protocol.

Results

SIMILARITIES AND DIFFERENCES IN GENE EXPRESSION BETWEEN SW1353 AND PHCs

In order to characterize more extensively the SW1353 cell line gene expression profiling was performed. PHCs were analyzed in parallel in order to check for similarities between the two cell types. Overall, three independent cultures and stimulation experiments with SW1353 cells were performed and analyzed separately. Subsequently, the expression profiles were merged bioinformatically. The PHCs derived from the cartilage of three normal donors were also cultured independently, but pooled directly after RNA isolation. The 20 most strongly expressed genes of both cellular model systems are listed in Table II. All further data are available as supplementary material (http://www.bio.ifi.lmu.de/publications/SW1353).

In general, most components of the extracellular cartilage matrix (i.e., anabolic genes) such as collagen types II, III, and XI, aggrecan, and COMP were hardly detectable in SW1353 cells in the microarray experiments, whereas they were well detected in the PHCs (10−100× more than in SW1353: see supplementary material). This was confirmed by real-time PCR for collagen type II and aggrecan (data not shown). Also, although expressed by the SW1353 cells, fibronectin was considerably reduced compared to PHCs. Collagen type VI was significantly expressed and COL6A3 enhanced compared to PHCs (3.3×). The catabolic genes were expressed either at considerably reduced levels (MMP-1: 0.03× and MMP-13: 0.07×) or to a similar or slightly enhanced extent (MMP-2: 1.4×; ADAMTS-4: 2.6×). Of the cartilage-relevant SOX genes (SOX5, SOX6, SOX9), all were expressed at very low levels and significantly lower than in PHCs (both in microarray experiments and real-time PCR analysis). Of note, SW1353 expressed no significant levels of IL-1β or TNF (tumor necrosis factor)-alpha (both $<$0.0001 molecules/molecules glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in real-time PCR analysis).

REACTIVITY OF SW1353 CELLS TO IL-1β

Overall, out of the 312 investigated genes, IL-1β significantly up-regulated 11 genes and significantly down-regulated eight genes ($P < 0.05$ and 1.2× absolute fold-change in at least two time points) in SW1353 cells [Table III(a)].

COMPARISON OF IL-1β REACTIVITY OF SW1353 CELLS AND PHCs

Overall, IL-1β regulated more genes in PHCs than in the SW1353 cells (Table III). The different responsiveness of both cellular systems to IL-1β was also apparent from comparison of the expression data and dendrograms resulting from two-dimensional hierarchical clustering of genes that were significantly regulated by IL-1β in SW1353 cells and PHCs, respectively. As shown in Table III and Fig. 1, the two gene sets overlapped by only a few genes and resulted in completely different dendrograms. Furthermore, groupings for genes based on the observed expression data could not be transferred from one system to the other and were very different if comparing the same set of genes between the respective systems [Fig. 1(a vs b, c vs d)].

In order to understand in more detail specific similarities and differences between SW1353 cells and PHCs, three major gene sets were analyzed individually (Table III): anabolic genes, catabolic genes and intercellular mediators.

Among the *anabolic* genes, not much overlap was found between the SW1353 cells and the PHCs. After IL-1 treatment, PHCs showed classical down-regulation of anabolic activity, in particular the expression of collagen type II (<0.1×; $P < 0.001$) and aggrecan (0.24×; $P < 0.001$). In contrast, no significant changes were observed in the SW1353 cells in the microarray experiments, although very low expression levels were also found in the non-treated SW1353 cells. Real-time PCR demonstrated a moderate reduction of collagen type II (down to 50% after 16 h), but not of aggrecan (data not shown). The only gene significantly down-regulated in both SW1353 cells and PHCs was COMP, one of the anabolic genes expressed at high enough levels in both cell types to allow accurate quantitation.

In terms of important inducible intercellular *mediators* we found basically no expression of IL-1β, IL-6 and LIF in unstimulated SW1353 cells. After stimulation with IL-1β, we observed a strong induction of IL-6, but not of LIF, IL-1β itself or bone morphogenetic protein-2 (BMP-2). All four mediators were, however, strongly induced in PHCs after IL-1β stimulation. This was also confirmed by real-time PCR [Fig. 2(a)]. Again, IL-6 was strongly induced by IL-1β in SW1353 cells, whereas much less induction was found for leucemia inhibitory factor (LIF).

Analysis of the *catabolic* genes showed that SW1353 cells reacted in a broadly similar manner to PHCs. Thus, genes such as MMP-1, MMP-3, and MMP-13 were all significantly up-regulated by IL-1β, both in SW1353 cells and PHCs. MMP-2 was expressed (real-time PCR: 0.04/GAPDH), but not regulated in both SW1353 cells and PHCs, which is in line with previous results[18].

CONFIRMATION OF MMP EXPRESSION AND REGULATION BY QUANTITATIVE PCR AND ELISA

Quantitative PCR measurements, using RNAs from all triplicate stimulation experiments performed for the array

Table II
Table showing the 20 most strongly expressed genes in SW1353 cells and PHCs. The genes were ranked by the mean of normalized expression levels ("int": intensity). Genes that are listed for both cellular model systems are shown in bold

SW1353			PHC		
Gene	Accession no.	int	Gene	Accession no.	int
ADAM17	NM_021832	16.5	MMP-3	X05232	16.1
TGFB1	NM_000660	12.7	FN1	M10905	15.4
SERPINE1	M16006	11.6	**RPL13A**	X56932	14.7
ADAMTS-4	NM_005099	10.7	SPP1	NM_000582	14.4
TUBA3	AF141347	9.8	**TIMP1**	X03124	12.2
RPL13A	X56932	6.9	FMOD	NM_002023	11.0
TIMP1	X03124	4.5	COMP	NM_000095	9.7
UBC	M26880	3.9	AGC1	NM_001135	9.2
RPS9	U14971	3.6	CHI3L2	NM_004000	7.8
GNB2L1	NM_006098	3.3	ATF4	M86842	7.1
COL6A3	NM_004369	2.9	**RPS9**	U14971	6.7
ITGA5	NM_002205	2.2	SERPINA1	NM_000295	6.6
CTSB	NM_001908	2.2	**TGFB1**	NM_000660	6.0
MMP-2	J03210	2.2	**UBC**	M26880	5.5
ATF4	M86842	2.0	MMP-1	X05231	4.6
TXN	NM_003329	1.9	**ADAM17**	NM_021832	4.3
SLC25A6	BC007850	1.5	**ADAMTS-4**	NM_005099	4.0
GPX1	NM_000581	1.5	**SERPINE1**	M16006	3.6
CTSD	NM_001909	1.5	**COL6A1**	X15880	3.1
COL6A1	X15880	1.4	COL2A1	X16468	2.8

studies, confirmed the induction of MMPs-1, -3 and -13 as major catabolic cartilage enzymes by IL-1β [Fig. 2(b)], whereas MMP-2 was not induced. Using ELISAs, SW1353 cells (Fig. 3) showed a strong increase in MMP (-1, -3, -13) secretion after stimulation by IL-1β (MMP-1: 9×; $P < 0.05$; MMP-3: 45×; $P < 0.005$; MMP-13: 33×; $P < 0.0001$). No significant increase in MMP-2 expression was found (data not shown), confirming our findings at the mRNA level.

ANALYSIS OF TFs

Since the activation of TFs can be regulated transcriptionally as well as by protein interaction and modification (e.g., phosphorylation), it is difficult to validate the activity of a TF by its gene expression level alone. Therefore, we inferred activation of a TF from its effect on the expression levels of its potential target gene group.

The analysis of target gene groups showed IL-1β-mediated induction of five common target genes by the TF nuclear factor kappa-B (NFκB) in both cell types. Both Rel proteins, c-Rel (REL) and RelA (RELA), had significant group *P*-values below 0.05 for PHCs and for SW1353 cells. Their predicted group of target genes (represented on the microarrays) was IL-6, TNF, NFKBIE (NFκB inhibitor epsilon), intercellular adhesion molecule 1 (ICAM1) and NFKB1 (Table IV). Figure 4 shows the expression profiles of RelA (c-Rel was not represented on the microarrays) and the predicted target genes of RelA and c-Rel, which were similar in the two cell types. The intercellular similarity for each gene of the NFκB target gene group and RelA was measured by Pearson correlation coefficients of the log-ratio expression levels. The overall similarity in the gene group is higher than that expected by chance. Using the rank sum test[19] to compare the intercellular correlations (of the five genes) with all other genes, significance at the 5% level was shown, i.e., the probability of picking five genes at random that are similarly correlated between the two cellular model systems is below 5%. NFKBIE is contained only in the predicted NFκB gene target group of PHCs cells but not in the NFκB target group for SW1353 cells. NFKBIE regulation distinguishes the activity of the TF NFκB in the two cell types. Consistent with this finding, NFKBIE showed no correlation of expression levels between the measurements.

For SW1353 cells, two other significant TFs were RUNX2 (regulating MMP-13, SMAD 2, MYC, RUNX1 and RUNX2 itself) with a group *P*-value of 0.05 and RARA (regulating BGLAP, ICAM1 and MYC) with a group *P*-value of 0.04. A second relevant TF for PHCs was ATF2 (regulating MAPK8, IL-1β, FN1, PLAT and TNF) with a group *P*-value of 0.02. Figure 5 shows the expression profiles of the predicted ATF2 target genes. Except for IL-1β, we did not observe any correlations between the two cell types. In fact, ATF2, PLAT and FN1 were anti-correlated, confirming the different roles of ATF2 in the two cell lines.

Discussion

One highly relevant result of our study is that relative to PHCs, the SW1353 cell line shows much reduced expression levels of anabolic genes of chondrocytes, i.e., constituents of the extracellular cartilage matrix. The only exception was collagen type VI and to some extent fibronectin, both constituents of the pericellular matrix. Whether this reflects that chondrocytic cells try primarily to build up the pericellular matrix, or whether it is chondrocyte-unrelated is unclear, but a similar feature has previously been observed in other chondrocytic cell lines[2]. Although high absolute expression levels of molecules of interest are not a prerequisite for successful studies (as discussed below), it does appear that SW1353 cells are not *a priori* a very good candidate *in vitro* system for studying chondrocyte anabolism. This is further supported by the fact that major TFs relevant for the expression of collagen type II, such as SOX9, SOX5, and SOX6, are hardly expressed by SW1353 cells compared to PHCs[5,20] (own unpublished results). The notion that SW1353 cells exhibit only a limited chondrocytic phenotype is in line with previous reports showing an epithelial phenotype of SW1353 cells after long-term culture[5]. Similar to others

Table III

Genes which are significantly regulated ($P < 0.05$ and 1.2× absolute fold-change in at least two time points) by stimulation with 1 ng/ml IL-1β in SW1353 cells (a) and PHCs in vitro (b; the values for MMP-3 were beyond the linear range of the measurement and were, therefore, not included in b). Given are the ratios IL-1β-stimulated vs controls. Genes that are listed for both cellular model systems are shown in bold. (R: ratio; ns: not significant)

Gene	Accession no.	30 min		6 h		16 h		24 h		48 h	
		R	*P*-value	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value
(a)											
MMP-3	X05232	0.92	ns	26	<0.001	100	<0.001	100	<0.001	96	<0.001
MMP-1	X05231	0.91	ns	6	<0.001	32	<0.001	33	<0.001	18	<0.001
MMP-13	X75308	1	ns	3.1	<0.001	38	<0.001	45	<0.001	10	<0.001
TNFAIP6	NM_007115	1.4	ns	4	<0.001	7.5	<0.001	8.9	<0.001	4	<0.001
HLA-B	M81798	0.92	ns	1.7	<0.001	2.6	0.004	2.9	<0.001	2.7	<0.001
IL-6	NM_000600	1.4	ns	28	<0.001	72	<0.001	13	<0.001	2.5	<0.001
CHI3L2	NM_004000	0.74	0.031	1	ns	2.3	0.006	1.5	0.019	1.9	<0.001
MADH1	NM_005900	1.2	0.054	2	<0.001	1.7	0.078	1.7	<0.001	1.6	<0.001
MRS3/4	AF327402	1	ns	5.7	<0.001	2	0.045	1.8	<0.001	1.4	0.05
NFKB1	M58603	1	ns	2.9	<0.001	1.8	0.055	1.8	<0.001	1.2	ns
PPARG	NM_138712	0.93	ns	0.69	0.027	0.66	ns	0.67	0.036	0.89	ns
RELA	Q04206	1.03	ns	1.92	<0.001	1.05	ns	1.03	ns	0.84	0.003
MADH3	NM_005902	0.89	ns	1.4	<0.001	0.59	ns	0.83	<0.001	0.74	<0.001
ATF2	NM_001880	1	ns	0.86	ns	0.84	ns	0.7	0.001	0.72	<0.001
DLX5	NM_005221	0.8	ns	0.75	ns	1	ns	0.71	0.003	0.63	<0.001
BMP4	NM_001202	0.9	ns	0.8	0.041	0.18	<0.001	0.52	0.001	0.62	<0.001
CAT	NM_001752	0.56	0.08	0.51	0.084	0.04	0.013	0.42	0.013	0.6	0.015
Link protein	NM_001884	0.64	ns	0.67	ns	0.16	ns	0.28	<0.001	0.35	<0.001
PRKDC	NM_006904	1	ns	0.86	ns	0.04	0.032	0.42	0.029	0.32	<0.001
(b)											
LIF	NM_002309	1.5	ns	24	<0.001	23	<0.001	8.1	<0.001	30	<0.001
TNFAIP6	NM_007115	1	ns	4.9	<0.001	9.2	<0.001	6.4	<0.001	11	<0.001
IL-6	NM_000600	2	0.018	2.8	<0.001	11	<0.001	13	<0.001	11	<0.001
IL-1B	K02770	1.3	ns	5.9	<0.001	17	<0.001	20	<0.001	9.4	<0.001
DUSP6	BC003143	1.2	ns	4	<0.001	6.8	<0.001	5	<0.001	7.6	<0.001
MMP-13	X75308	0.91	ns	4.6	<0.001	13	<0.001	11	<0.001	6.8	<0.001
MMP-1	X05231	0.93	ns	3.3	<0.001	4.1	<0.001	3.1	<0.001	5.2	<0.001
NOS2A	AB022318	1.1	ns	11	<0.001	9	<0.001	7.1	<0.001	4.9	<0.001
IL-1A	X02851	1.3	ns	2.6	0.002	6.7	<0.001	4.8	<0.001	4.6	<0.001
JUN	J04111	2.7	<0.001	2.1	0.008	3.7	<0.001	2.7	<0.001	4	<0.001
BMP-2	NM_001200	1	ns	3.16	<0.001	6.6	<0.001	1	ns	4	<0.001
RUNX1	NM_001754	1.1	ns	1.5	ns	3.5	<0.001	3.3	<0.001	3.7	<0.001
NFKBIE	U91616	1	ns	2	0.016	2.9	<0.001	2	0.012	2.9	<0.001
NFKB1	M58603	0.86	ns	8.1	<0.001	3	<0.001	2.3	0.004	2.8	<0.001
CD44	NM_000610	1	ns	2.17	0.006	3.3	<0.001	1.9	0.019	2.6	<0.001
TXNRD1	NM_003330	1.1	ns	4.6	<0.001	2.5	0.001	2.4	0.002	2.4	0.002
RELA	Q04206	1.5	ns	2	0.018	1.5	ns	2.3	0.004	1.4	ns
FOS	V01512	2.4	0.002	0.55	0.07	0.5	0.028	0.55	0.042	0.76	ns
PTHR1	NM_000316	0.87	ns	0.38	0.002	0.5	0.008	0.43	0.003	0.55	0.036
HF1	NM_000186	0.79	ns	0.46	0.006	0.48	0.009	0.42	0.002	0.51	0.018
SPP1	NM_000582	0.49	0.011	0.51	0.017	0.68	0.17	0.51	0.016	0.37	<0.001
COL1A2	X55525	0.79	ns	0.35	<0.001	0.43	0.002	0.4	0.001	0.31	<0.001
COMP	NM_000095	0.55	0.032	0.31	<0.001	0.31	<0.001	0.36	<0.001	0.31	<0.001
FGFR2	NM_023028	0.86	ns	0.16	<0.001	0.26	<0.001	0.42	0.002	0.3	<0.001
SCIN	NM_033128	1.2	ns	0.49	0.024	0.31	<0.001	0.3	<0.001	0.29	<0.001
COL10A1	NM_000493	0.6	0.068	0.15	<0.001	0.14	<0.001	0.09	<0.001	0.26	<0.001
A2M	M11313	0.91	ns	0.48	<0.001	0.35	<0.001	0.33	<0.001	0.23	<0.001
Link protein	NM_001884	1	ns	0.59	0.063	0.34	<0.001	0.29	<0.001	0.21	<0.001
GDF5	NM_000557	1	ns	0.4	0.001	0.26	<0.001	0.48	0.009	0.2	<0.001
MATN2	NM_002380	1.2	ns	0.39	<0.001	0.27	<0.001	0.19	<0.001	0.18	<0.001
GDF10	NM_004962	1	ns	0.17	<0.001	0.08	<0.001	0.09	<0.001	0.13	<0.001
MATN3	NM_002381	1.3	ns	0.55	0.033	0.21	<0.001	0.14	<0.001	0.11	<0.001
CKTSF1B1	NM_013372	0.83	ns	0.4	0.001	0.11	<0.001	0.06	<0.001	0.07	<0.001
COL2A1	X16468	0.9	ns	0.31	<0.001	0.11	<0.001	0.09	<0.001	0.06	<0.001

we found that despite the low expression levels there was some indication of down-regulation of collagen type II after treatment with IL-1β[5]. This is interesting as it documents that basic aspects of gene regulation are at least in part also active in cells in which the genes of interest are minimally expressed. In this respect, other chondrocytic cell lines such as immortalized human chondrocyte cell lines[21] are suitable *in vitro* systems, despite the lack of significant expression levels of many cartilage-specific gene products[22]. Overall, however, one needs to check carefully for

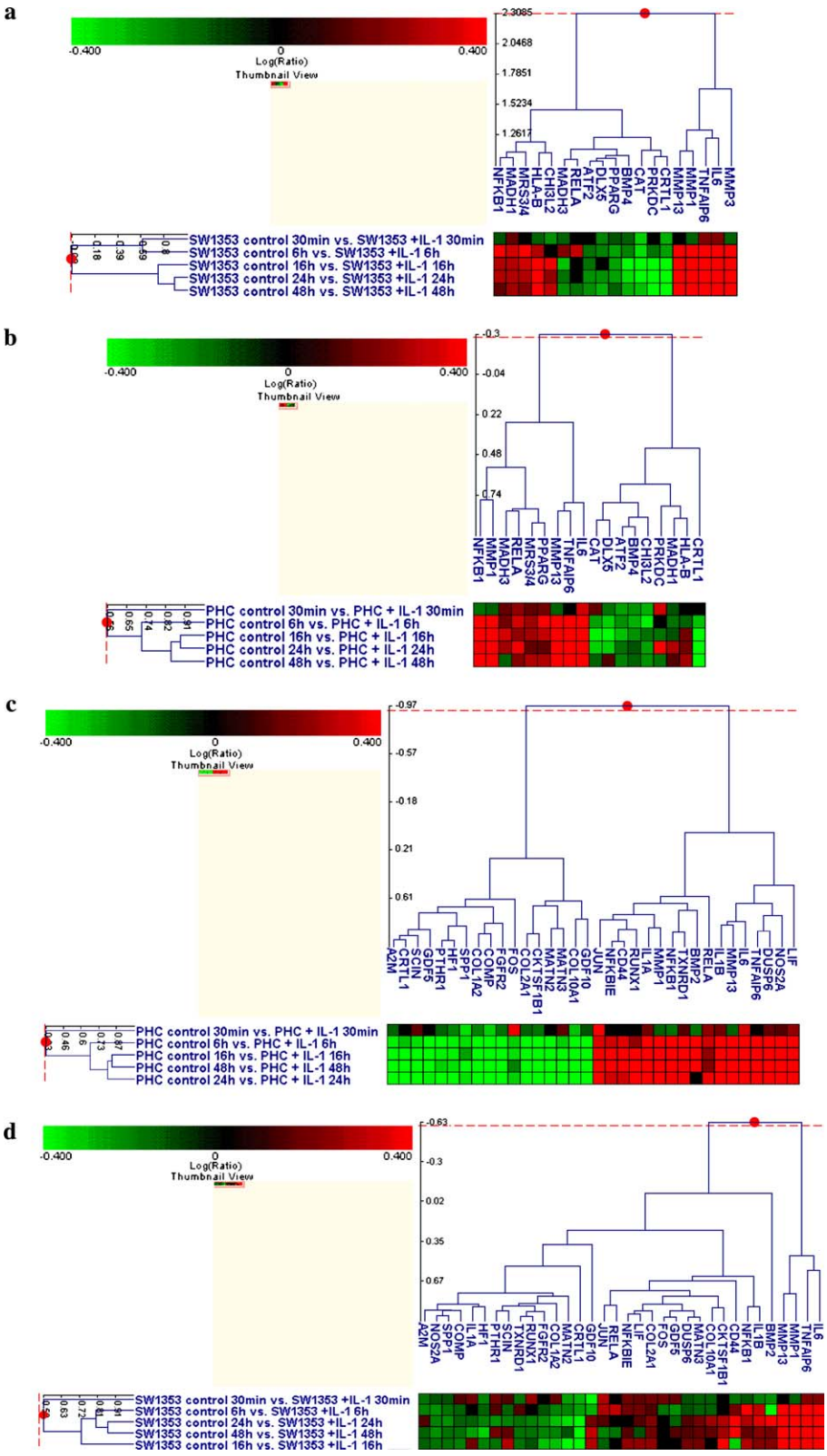
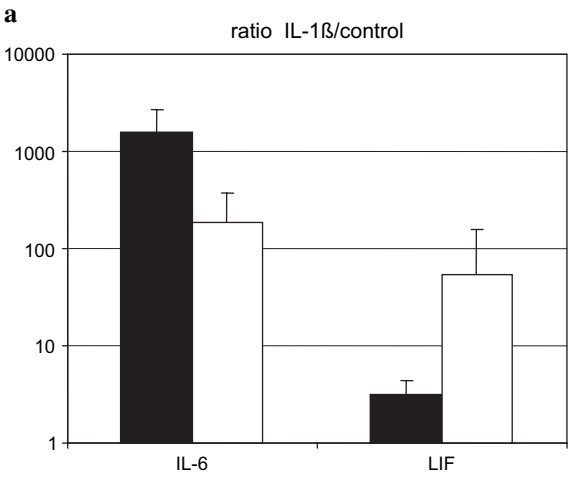
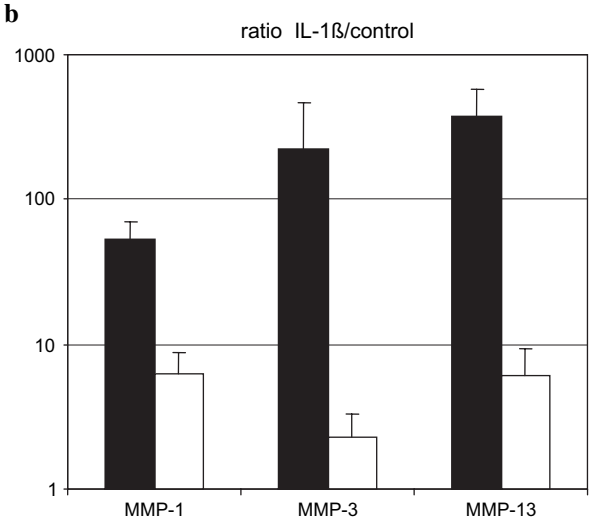


Fig. 1. Two-dimensional hierarchical clustering of hybridization experiments from human SW1353 cells and PHCs stimulated in triplicate with 1 ng/ml IL-1β at 30 min, 6 h, 16 h, 24 h and 48 h. For the five time intervals of IL-1β stimulation, expression profiles of treated samples were compared to control samples (triplicates each). Dendrograms illustrate SW1353 expression values (a) and PHCs expression values (b) with genes that were significantly regulated by IL-1β in SW1353 cells (1.2× absolute fold-change and P-value < 0.05 in at least two time points). Dendrograms (c) and (d) show the corresponding expression data for genes that were significantly regulated by IL-1β in PHCs (same criteria as above).



	/GAPDH			
	SW1353		PHCs	
	control	IL-1ß	control	IL-1ß
IL-6	0.00009	0.066	0.05	2.8
LIF	0.00013	0.0004	0.003	0.06



	/GAPDH			
	SW1353		PHCs	
	control	IL-1ß	control	IL-1ß
MMP-1	0.0006	0.03	0.36	2.7
MMP-3	0.0030	0.31	42.2	111.1
MMP-13	0.0015	0.45	0.07	0.24

Fig. 2. Real-time PCR analysis of the IL-1β induced expression of IL-6 and LIF (a) and MMP-1, -3, and -13 (b) in SW1353 cells (solid bars) and PHCs (open bars) after 48 h of stimulation with 1 ng IL-1β (SW1353: three independent experiments; PHCs: six independent experiments).

reactivity in these circumstances and in fact in this study aggrecan expression in SW1353 cells was not influenced at all by IL-1β.

mRNA expression levels of matrix-degrading enzymes were also partly lower in SW1353 cells than those observed in adult articular chondrocytes. The expression levels of

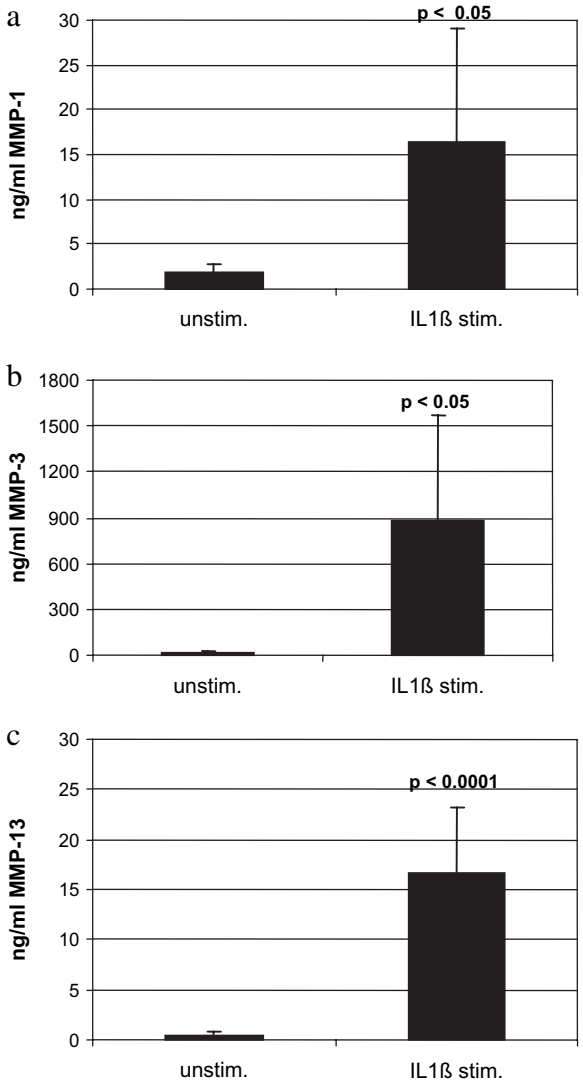


Fig. 3. Representation of protease levels (a: MMP-1; b: MMP-3; c: MMP-13) secreted into the culture medium by SW1353 cells without and with stimulation by 0.1 ng/ml IL-1β (for 24 h). Total protease levels (i.e., active and pro-forms) were measured by the ELISA (given as ng/ml medium).

MMPs detected in our study are in line with previous work in which the expression and regulation pattern of especially MMP-1 and MMP-13 in SW1353 cells was investigated[7–10]. In terms of reactivity of MMP expression to IL-1β stimulation, we found in SW1353 cells a pattern already known from PHCs *in vitro*: strong induction of MMP-1[9,12,23–25], MMP-3[12] and MMP-13[9,12,23,26,27] and no significant influence on the expression of MMP-2[18]. This highlights the merits of studying these phenomena in this cell type[7–10]. The same is true for induction of IL-6[28-32]. However, not all genes reacted in a similar way in SW1353 cells as in PHCs. For example, LIF was hardly induced and BMP-2 was not found to be up-regulated by IL-1β as would be expected[33,34]. Again, in the context of investigating the regulation of catabolic events one also needs to ensure that the cell line model adequately mimics the regulation pattern within the physiological cell type of interest. Activation of MMP expression by IL-1β is potentially a general effect within cells and may be independent of their origin. If so, the

Table IV
Predicted targets of RelA and c-Rel, the evidence used for the prediction and supporting literature references that were found by browsing through the list of co-occurrences (automatically generated from text mining) or the references in the TRANSFAC database

Transcription factor	Target gene	Predicted binding site	TRANSFAC binding site	Literature co-occurrence	Literature reference
RelA	IL-6		+	+	39,40
	ICAM1		+	+	39
	NFKB1	+		+	–
	TNF		+	+	41
c-Rel	IL-6	+		+	42
	ICAM1	+		+	43
	NFKB1	+		+	–
	NFKBIE	+		+	–

use of SW1353 cells as a substitute for chondrocytes is challenged once more and requires additional experimental evidence and validation. The selection of chondrocytes taken for the study might obviously also influence gene expression levels. Different donor ages or joints, different donor sites (e.g., femoral vs tibial) can potentially influence gene expression patterns. Overall, variability between individuals is a major problem in primary cultures *per se*. Primary cells may change their phenotypes dramatically in culture. Thus, 48 h give only limited insight in what these cell do. In fact it is possible that they become more similar to SW1353 cells after prolonged culture. In particular after losing their differentiated phenotype they certainly lose chondrocyte-typical gene expression[35,36].

A study similar to ours was performed previously[37], but with only one early data point measured after stimulation with IL-1β (2 h). This study concentrated on early response genes, namely TFs, which are regulated at this early stage and which were not within the focus of our investigation. In a very recent study paralleling some aspects of ours, comparable results were found after IL-1β stimulation, although the cells were much less responsive to TNF-alpha[38]. In this study, however, neither phenotypic characterization of the cell line nor bioinformatics analysis of the relevant intracellular gene regulatory pathways was performed.

Our bioinformatics analysis identified relevant regulation contexts, one of which was regulation through the TF NFκB (c-Rel, RelA), in which the similarity of the expression of the predicted target genes between the two cell types was significant (correlation P-value < 0.05). All other predicted regulation contexts were relevant for only one cell type, but not for both (e.g., TFs ATF2 for PHCs, and RUNX2 and RARA for SW1353 cells). However, even in the predicted regulation context for NFκB, expression data between the two cellular model systems were quite different, especially

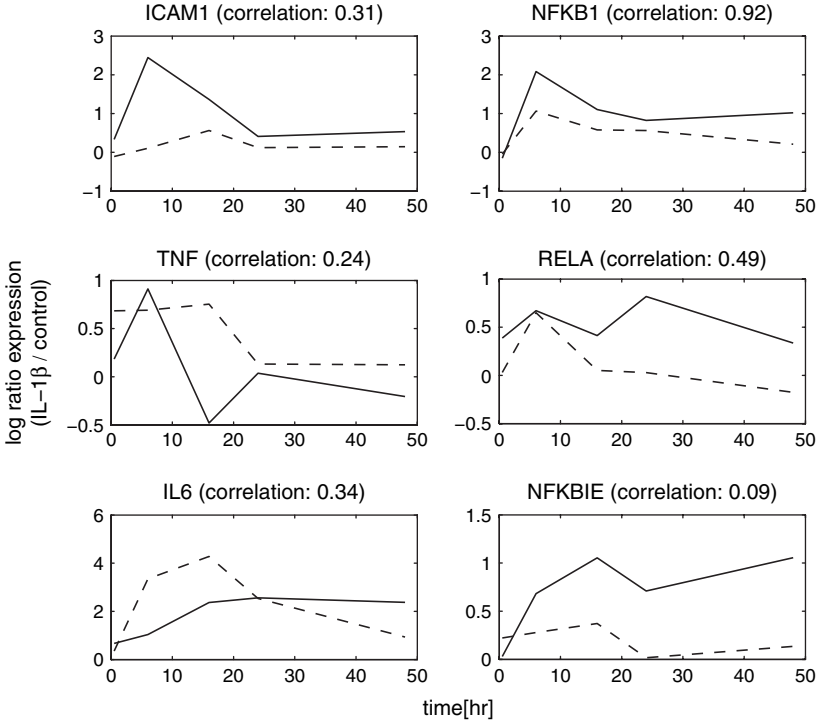


Fig. 4. Expression profiles of genes in the predicted NFκB regulation context (the only regulation context common to both systems according to our analysis) as log-ratios of expression levels between IL-1β stimulated cells and untreated cells against time in hours after the treatment. Profiles for SW1353 cells are shown as dashed lines, profiles for PHCs as solid lines. Correlation values quantify the similarity between the two systems for each gene. Statistical analysis showed that the correlation of the NFκB targets is significantly higher than expected by chance (details are given in the text).

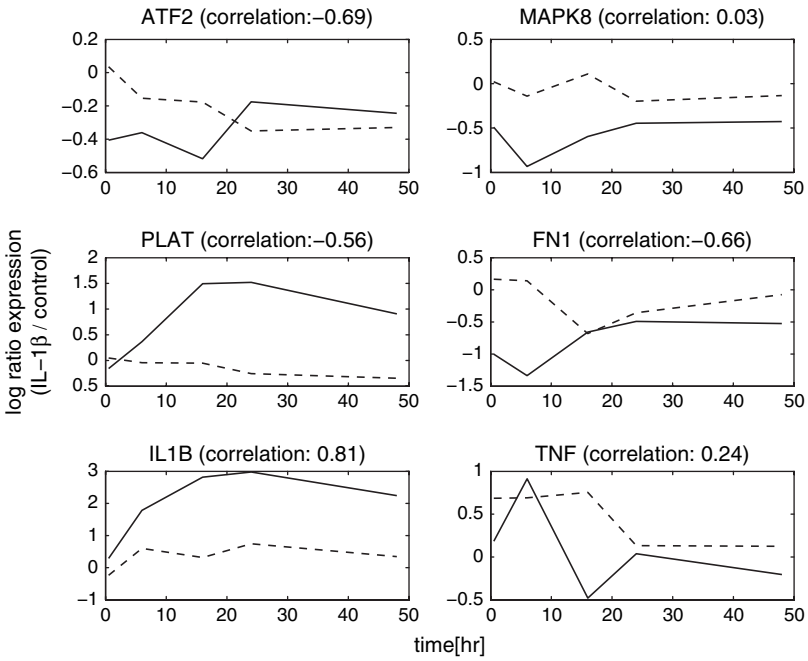


Fig. 5. Expression profiles of genes in the predicted ATF2 regulation context as log-ratios of expression levels between IL-1β stimulated cells and untreated cells against time in hours after the treatment. Profiles for SW1353 cells are shown as dotted lines, profiles for PHCs as drawn lines. Correlation values quantify the similarity between the two systems for each gene. Statistical analysis showed that the correlation of the ATF2 targets is not significant (details are given in the text). (*The down-regulation of TNF-alpha after 16 h in PHCs might represent a calculation error due to very low expression levels detected.)

for NFKBIE. Reasons for the observed differences could be due to different activation or abundance of the NFκB subunits, or additional relevant TFs in the respective cell types.

Taken together, our data characterize SW1353 cells as a cell line with only a limited potential to mimic PHCs, since SW1353 cells barely express typical marker genes of chondrocytic differentiation. In addition, the overlap in similar gene expression for the 312 genes measured on the SensiChip was quite small, indicating that conclusions drawn based on measurements with the SW1353 cell line cannot be used as general predictions for the behavior of PHCs. Despite this, SW1353 cells appear to be a valuable *in vitro* system for investigating catabolic gene regulation by IL-1β (and presumably also TNF-alpha[10] and FGFs (fibroblast growth factor)[5]) in chondrocytic cells. In addition, our bioinformatic study of more general signaling mechanisms indicates that the involvement of important TFs such as NFκB can be predicted correctly. This implies that with appropriate methods some predictions from the SW1353 data can be made for the mechanisms in PHCs, despite the fact that the actual quantitative expression data within the two systems are quite diverse. Further studies have to prove or disprove the further potential of the SW1353 cell line, which in terms of culturing properties is a very suitable *in vitro* model system.

Acknowledgments

We acknowledge Brigitte Bau (University of Erlangen) and Beatrice Schumann (Sanofi-Aventis, Frankfurt) for expert technical assistance and Klaus Lindauer, Ph.D. (Sanofi-Aventis, Frankfurt), for bioinformatical support and Chris Barnes, Ph.D. (Sanofi-Aventis, Frankfurt), for critical reading of the manuscript.

Appendix A. Supplementary information

Supplementary information for this manuscript can be downloaded at doi: 10.1016/j.joca.2005.04.004.

References

1. Goldring MB, Birkhead JR, Suen L-F, Yamin R, Mizuno S, Glowacki J, *et al*. Interleukin-1-modulated gene expression in immortalized human chondrocytes. J Clin Invest 1994;94:2307–16.
2. Finger F, Schörle CM, Zien A, Gebhard PM, Goldring MB, Aigner T. Molecular phenotyping of human chondrocyte cell lines T/C-28a2, T/C-28a4, C-28/I2. Arthritis Rheum 2003;48:3395–403.
3. Bonaventure J, Khadom N, Cohen-Solal L, Ng KH, Bourguignon J, Lasselin C, *et al*. Re-expression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. Exp Cell Res 1994;212:97–104.
4. Tsuda M, Takahashi S, Takahashi Y, Asahara H. Transcriptional co-activators CREB-binding protein and p300 regulate chondrocyte-specific gene expression via association with Sox9. J Biol Chem 2003;278:27224–9.
5. Schaefer JF, Millham ML, de Crombrugghe B, Buckbinder L. FGF signaling antagonizes cytokine-mediated repression of Sox9 in SW1353 chondrosarcoma cells. Osteoarthritis Cartilage 2003;11:233–41.
6. Ketola A, Maatta AM, Pasanen T, Tulimaki K, Wahlfors J. Osteosarcoma and chondrosarcoma as targets for virus vectors and herpes simplex virus thymidine kinase/ganciclovir gene therapy. Int J Mol Med 2004;13:705–10.

7. Tardif G, Dupuis M, Reboul P, Geng CS, Pelletier JP, Ranger P, *et al*. Identification and differential expression of human collagenase-3 mRNA species derived from internal deletion, alternative splicing, and different polyadenylation and transcription initiation sites. Osteoarthritis Cartilage 2003;11:524–37.
8. Cowell S, Knauper V, Stewart ML, D'Ortho MP, Stanton H, Hembry RM, *et al*. Induction of matrix metalloproteinase activation cascades based on membrane-type 1 matrix metalloproteinase: associated activation of gelatinase A, gelatinase B and collagenase 3. Biochem J 1998;331:453–8.
9. Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 2000;43:801–11.
10. Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M, *et al*. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res 2003;288: 208–17.
11. McKenna LA, Gehrsitz A, Soeder S, Eger W, Kirchner T, Aigner T. Effective isolation of high quality total RNA from human adult articular cartilage. Anal Biochem 2000;286:80–5.
12. Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T. Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes *in vivo* and *in vitro*. Arthritis Rheum 2002;46:2648–57.
13. Duveneck GL, Abel AP, Bopp MA, Kresbach GM, Ehrat M. Planar waveguides for ultra-high sensitivity of the analysis of nucleic acids. Anal Chim Acta 2002;469: 49–61.
14. Rajagopalan D. A comparison of statistical methods for analysis of high density oligonucleotide array data. Bioinformatics 2003;19:1469–76.
15. Wingender E, Chen X, Fricke E, Geffers R, Hehl R, Liebich I, *et al*. The TRANSFAC system on gene expression regulation. Nucleic Acids Res 2001;29: 281–3.
16. Hanisch D, Fluck J, Mevissen HT, Zimmer R. Playing biology's name game: identifying protein names in scientific text. Pac Symp Biocomput 2003:403–14.
17. Casella G, Berger RL. Statistical Interference. Pacific Grove: Duxbury 399, 2002.
18. Duerr S, Stremme S, Soeder S, Bau B, Aigner T. MMP-2/gelatinase A is a gene product of human adult articular chondrocytes and increased in osteoarthritic cartilage. Clin Exp Rheumatol 2004;22: 603–8.
19. Rohatgi VK, Saleh AKME. An Introduction to Probability and Statistics. New York: Wiley, 2001.
20. Aigner T, Gebhard PM, Schmid E, Bau B, Harley V, Pöschl E. SOX9 expression does not correlate with type II collagen expression in adult articular chondrocytes. Matrix Biol 2003;22:363–72.
21. Kokenyesi R, Tan L, Robbins JR, Goldring MB. Proteoglycan production by immortalized human chondrocyte cell lines cultured under conditions that promote expression of the differentiated phenotype. Arch Biochem Biophys 2000;383:79–90.
22. Gelse K, Pöschl E, Aigner T. Collagens – structure, function and biosynthesis. Adv Drug Deliv Rev 2003; 55:1531–46.
23. Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, *et al*. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 1996;97:761–8.
24. Kandel RA, Petelycky M, Dinarello CA, Minden M, Pritzker KPH, Cruz TF. Comparison of the effect of interleukin 6 and interleukin 1 on collagenase and proteoglycan production by chondrocytes. J Rheumatol 1990;17:953–7.
25. Stephenson ML, Goldring MB, Birkhead JR, Krane M, Rahmsdorf HJ, Angel P. Stimulation of procollagenase synthesis parallels increases in cellular procollagenase mRNA in human articular chondrocytes exposed to recombinant interleukin 1β or phorbol ester. Biochem Biophys Res Commun 1987;144(2): 583–90.
26. Nguyen Q, Mort JS, Roughley PJ. Preferential mRNA expression of prostromelysin relative to procollagenase and *in situ* localization in human articular cartilage. J Clin Invest 1992;89:1189–97.
27. Reboul P, Pelletier JP, Tardif G, Cloutier J-M, Martel-Pelletier J. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. J Clin Invest 1996;97: 2011–9.
28. Lotz M, Moats T, Villiger PM. Leukemia inhibitory factor is expressed in cartilage and synovium and can contribute to the pathogenesis of arthritis. J Clin Invest 1992;90:888–96.
29. Villiger PM, Geng Y, Lotz M. Induction of cytokine expression by leukemia inhibitory factor. J Clin Invest 1993;91:1575–81.
30. Guerne P-A, Carson DA, Lotz M. IL-6 production by human articular chondrocytes. J Immunol 1990;144: 499–505.
31. Guerne P-A, Vaughan JH, Carson DA, Terkeltaub R, Lotz M. Interleukin-6 (Il-6) and joint tissues. Ann NY Acad Sci 1989;557:558–61.
32. Bender S, Haubeck HD, van de LE, Dufhues G, Schiel X, Lauwerijns J, *et al*. Interleukin-1 beta induces synthesis and secretion of interleukin-6 in human chondrocytes. FEBS Lett 1990;263:321–4.
33. Fukui N, Zhu Y, Maloney WJ, Clohisy J, Sandell LJ. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-alpha in normal and osteoarthritic chondrocytes. J Bone Joint Surg 2003;85-A(Suppl 3):59–66.
34. Fan Z, Bau B, Yang H, Aigner T. Il-beta induction of Il-6 and LIF in normal articular human chondrocytes involves the ERK, p38 and NFkB signaling pathways. Cytokine 2004;28:17–24.
35. Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. Cell 1978;15:1313–21.
36. von der Mark K, Gauss V, von der Mark H, Müller PK. Relationship between cell shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. Nature 1977;267:531–2.
37. Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1beta. Arthritis Res 2001; 3:381–8.

38. Shi J, Schmitt-Talbot E, DiMattia DA, Dullea RG. The differential effects of IL-1 and TNF-alpha on proinflammatory cytokine and matrix metalloproteinase expression in human chondrosarcoma cells. Inflamm Res 2004;53:377–89.
39. Catron KM, Brickwood JR, Shang C, Li Y, Shannon MF, Parks TP. Cooperative binding and synergistic activation by RelA and C/EBPbeta on the intercellular adhesion molecule-1 promotor. Cell Growth Differ 1998;9:949–59.
40. Kawashima T, Murata K, Akira S, Tonozuka Y, Minoshima Y, Feng S, *et al*. STAT5 induces macrophage differentiation of M1 leukemia cells through activation of IL-6 production mediated by NF-kappaB p65. J Immunol 2001;167:3652–60.
41. Xu Z, Dziarski R, Wang Q, Swartz K, Sakamoto KM, Gupta D. Bacterial peptidoglycan-induced tnf-alpha transcription is mediated through the transcription factors Egr-1, Elk-1, and NF-kappaB. J Immunol 2001;167:6975–82.
42. Civil A, Rensink I, Aarden LA, Verweij CL. Functional disparity of distinct CD28 response elements toward mitogenic responses. J Biol Chem 1999;274:34369–74.
43. Spiecker M, Darius H, Liao JK. A functional role of I kappa B-epsilon in endothelial cell activation. J Immunol 2000;164:3316–22.