Influence of p38MAPK inhibition on IL-1ß-stimulated human chondrocytes: A microarray approach

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Abstract. Articular chondrocytes respond to extracellular influences by activating signaling pathways which change gene expression. One key signal transduction pathway of inflammatory joint disease is mediated by the p38MAPK which is known to be activated by the pro-inflammatory cytokine IL-1ß. We used the p38MAPK inhibitor SB203580 and a whole human genome microarray in an *in vitro* inflammation model to identify genes regulated by this pathway in human chondrocytes. We found that 1,141 genes were regulated by IL-1ß, and 646 genes were regulated by the inhibitor whereas 116 genes were co-regulated by both substances. To elucidate the overall effect of SB203580, a GoMiner pathway analysis was performed which revealed involvement of versatile biological processes. Predominantly affected terms were 'response to stimulus', 'oxygen metabolism' and 'ligase activity'. We discuss herein the relevance and function of affected fields including the involved genes and unexpected effects of p38MAPK inhibition as it relates to the context of cartilage. Our results do not predict a pro-apoptotic or cancer promoting effect and markedly extend the knowledge on p38MAPK inhibition in chondrocytes beyond primary target genes.

Introduction

In arthritis, signaling pathways are induced by mechanical stress and/or inflammatory cytokines (1). An important mediator of inflammation in rheumatoid and osteoarthritis is the cytokine IL-1ß which is tightly associated with joint destruction, e.g. by inducing cartilage-degrading enzymes or

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further pro-inflammatory factors (2). One of the involved signaling pathways is the p38MAPK pathway, a complex cascade of protein kinases that mediates multiple functions including cytokine production and cell death. The family of p38MAPK at the center of the pathway is composed of α, ß, γ and δ isoforms. The ubiquitously expressed α and ß2 forms are key enzymes in inflammatory signaling (3). The MAPK p38α transduces signals through downstream effectors such as transcription factors, elongation factors, and other MAPKs. It contributes to inducible cytokine gene expression at least partially by stabilizing mRNA (3). This kinase represents a point of convergence for multiple signaling processes which impact on a broad range of inflammatory events. The p38MAPK is therefore a prime target for the development of anti-inflammatory drugs. By now, diverse inhibitors have been identified. The pyridinyl imidazole inhibitor SB203580 was reported on in 1994 (4) and is often used as a reference p38 inhibitor. It is equipotent against p38α and p38ß, binds competitively with respect to ATP and inhibits the kinase activity of the activated and unactivated forms of the kinase (3). Numerous studies implicated p38MAPK inhibition, and its efficacy was demonstrated in a variety of models for selected aspects of cellular response. A comprehensive knowledge of genome-wide effects on gene expression and cell metabolism, however, has not yet been achieved. As the p38MAPK has a key function in cell signaling, a broad approach could shed light on the versatile effects of p38MAPK inhibition. Recently, Namdari *et al* reported on transgenic mice with reduced p38MAPK activity in chondrocytes showing higher grades of osteoarthritis in the knee joint (5). This finding clearly demonstrates the need for further investigations on cellular effects beyond well-known target genes. We, therefore, conducted a whole human genome array analysis to identify further downstream genomic targets of p38MAPK inhibition possibly relevant for therapeutic application in an established inflammation cell culture model using IL-1ß-stimulated human chondrocytes.

Materials and methods

Cartilage samples. Human osteoarthritic cartilage was obtained from 18 donors undergoing total knee replacement

due to osteoarthritis with informed consent of the patients according to the terms of the Ethics Committee of the University of Ulm. The age of the donors ranged from 58 to 72 years.

Cell culture. Well-preserved cartilage from femoral condyles was used for chondrocyte isolation as described previously (6). The cartilage was minced and digested 45 min with 9 U/ml pronase (Sigma-Aldrich, Munich, Germany) and 14 h with 80 U/ml collagenase (Sigma). After washing and filtering, the isolated cells were cultivated in complete medium consisting of 1:1 DMEM/Ham's F12 supplemented with 10% fetal bovine serum, 0.5% penicillin/streptomycin, 0.5% L-glutamine and 10 μg/ml 2-phospho-L-ascorbic acid trisodium salt (Sigma-Aldrich, Fluka, Seelze, Germany). After 24 h of incubation, adhered chondrocytes were frozen in complete medium containing 5% DMSO (dimethyl sulfoxide; Roth, Karlsruhe, Germany). All chemicals were obtained from Biochrom, Berlin, Germany, unless indicated otherwise.

Cell stimulation and treatment with p38MAPK inhibitor. After thawing and recovering in complete medium, cells of 6 different donors were pooled and seeded at a density of $5x10^4$ cells/cm². After 24 h of adherence in complete medium, cells were silenced for 24 h in serum-free medium (DMEM containing 0.5% penicillin/streptomycin, 0.5% L-glutamine, 1% non-essential amino acids 100-fold concentrate, 1 mM Pyruvat, 0.1% SES1 Solution A and 0.1% freshly added SES1 Solution B (all from Biochrom). Subsequently, cells were stimulated for 24 h with 10 ng/ml rhIL-1ß (tebu-bio, Offenbach, Germany) in serum-free medium, and inhibitor-treated cells were pre- (15 min) and co-incubated with 10 μM SB203580 (provided by RPatiopharm GmbH, Ulm, Germany) in addition to stimulation. Cells were lysed in 600 μl lysis buffer RLT (Qiagen, Hilden, Germany) per 10^6 cells.

Microarray. After cell lysis, a whole human genome oligomicroarray (Human Genome Oligo Set Version 2.0, Operon, Germany) was conducted at the Chip Facility of Ulm according to Buchholz *et al* (7). All experiments were performed in triplicate with 6 different donors each. The hybridization signals were analyzed with GenePix Pro 4.0 imaging software (Axon Instruments, Union City, CA, USA). Data normalization was performed with the LIMMA software package.

GOMiner analysis. Genes which showed at least a 2-fold regulation and a significance level of p<0.05 in the microarray analysis were assigned to Gene Ontologies by a pathway analyzing tool called GoMiner (8,9). We used the three ontologies produced by the Gene Ontology (GO) consortium, namely 'biological process', 'cellular component' and 'molecular function'. In brief, given a set of genes and one ontology, the set of all unique GO terms within the ontology were first identified which were associated with one or more of the genes of interest. Next, the number of selected differentially expressed genes and the number of genes that were assayed (all the genes represented on the microarray) were annotated at each term (8).

mRNA isolation. mRNA was isolated with the RNeasy Mini Kit (Qiagen) in accordance with the manufacturer's instructions.

Statistical analysis. For the microarray results, a Print-tip LOESS-normalization according to Buchholz *et al* (7) and a moderated t-test (10) were performed at the Chip Facility of Ulm. In the GoMiner analysis, the two-sided Fisher's exact test evaluated whether there were more genes of interest at the term than one might expect by chance (8).

Results

To understand the molecular basis of osteoarthritis (OA) cartilage degeneration and to develop novel approaches to therapeutic options, a thorough knowledge of the involved cell-biological processes is important. The p38MAPK, a key enzyme in pathway signaling, represents a promising pharmaceutical target. Therefore, we investigated the effects of the p38MAPK inhibitor SB203580 in an *in vitro* OA model using a microarray approach.

Cell phenotype and viability. For the microarray experiment, OA cartilage was used which macroscopically had a smooth surface and no severe osteoarthritic changes. As described elsewhere (6), the chondrocytes showed a stable differentiation stage during the experimental period and comparable cell viability with and without IL-1ß-stimulation.

Microarray experiment. Our whole human genome microarray represented ~23,000 genes. Genes were defined as differentially expressed between the probe and associated reference if first, the difference between the mean normalized expression values was at least 2-fold between the sample sets and second, a two-sided t-test yielded a p-value <0.05. All of the microarray data have been deposited on ArrayExpress (http:// www.ebi. ac.uk/microarray-as/aer; Accession no. E-MEXP-1434).

Chondrocyte treatment with IL-1ß and the p38MAPK inhibitor SB203580 induced numerous changes in the chondrocyte gene expression profile. Overall, 1,141 genes were regulated by IL-1ß compared to control cells. Co-incubation of IL-1ß-stimulated cells with SB203580 revealed a total of 646 genes regulated by SB203580 compared to cells treated with IL-1ß but not inhibited. By comparison of IL-1ß- and SB203580-regulated genes, we found 116 common genes that were co-regulated by both treatments. Most of the co-modulated genes were regulated in opposite directions; only 13% moved unidirectional and from these most genes were up-regulated. Among the analyzed genes of the microarray, some are hypothetical or unknown (42 of the 116 co-regulated genes). The intersections of the number of all IL-1ß- and SB203580-regulated genes and the list of known co-regulated genes with their accession numbers, fold change and p-values for both IL-1ß and SB203580 regulation are shown in Fig. 1 and Table I.

GoMiner analysis of co-regulated genes. Genes co-regulated by IL-1ß and SB203580 were further analyzed with the GoMiner software tool and classified into biological coherent categories. A Fisher's exact test evaluated Gene ontology

Table I. List of genes co-regulated by IL-1ß and SB203580 in human chondrocytes.

Gene symbol	Gene description	Gene Bank Accession no.	Influence of IL-1ß		Influence of SB203580	
			Fold change	p-value	Fold change	p-value
Down-regulated by IL-1ß						
HOMER-3	Homer, neuronal immediate early gene, 3	NM_004838	0.071	0.0043	3.701	0.0147
HOXD12	Homeo box D12	NM_021193	0.122	0.0003	3.292	0.0459
SHARP	SMART/HDAC1 assoc. repressor protein	NM_015001	0.168	0.0051	3.391	0.0349
EEF1A1	Eukar. translation elongation factor 1 α 1	AJ420488	0.183	0.0009	0.478	0.0413
TUBB	Tubulin, ß polypeptide	NM_001069	0.187	0.0000	3.213	0.0136
FKBP9	FK506 binding protein 9 (63 kDa)	BC011872	0.215	0.0040	2.077	0.0245
CPSF1	Cleavage and polyadenylation specific factor 1, 160 kDa subunit	NM_013291	0.239	0.0029	6.449	0.0137
PDCD2	Programmed cell death 2	AK055180	0.242	0.0321	10.112	0.0297
LETM1	Leucine zipper-EF-hand containing transmembrane protein 1	NM_012318	0.249	0.0004	2.138	0.0138
GSTA4	Glutathione S-transferase A4	NM_001512	0.290	0.0246	7.268	0.0043
RPL18	Ribosomal protein L18	NM_000979	0.295	0.0304	2.046	0.0439
TRIM36	Tripartite motif-containing 36	NM_018700	0.303	0.0452	3.795	0.0188
BARX1	BarH-like homeobox 1	NM_021570	0.309	0.0003	2.867	0.0263
RAB27A	RAB27A, member RAS oncogene family	U38654	0.309	0.0173	3.543	0.0101
MT-ACT48	Mitochondrial acyl-CoA thioesterase	NM_012332	0.336	0.0328	4.660	0.0045
PDLIM1	PDZ and LIM domain 1 (elfin)	NM_020992	0.355	0.0253	2.228	0.0280
MKKS	McKusick-Kaufman syndrome	NM_018848	0.387	0.0051	4.347	0.0055
HDAC8	Histone deacetylase 8	NM_018486	0.394	0.0012	2.021	0.0183
LASP1	LIM and SH3 protein 1	NM_006148	0.408	0.0007	2.328	0.0180
EVC	Ellis van Creveld syndrome	NM_014556	0.422	0.0018	2.158	0.0107
NARS	Asparaginyl-tRNA synthetase	NM_004539	0.423	0.0023	2.143	0.0184
ANXA2P3	Annexin A2 pseudogene 3	M62895	0.428	0.0063	2.735	0.0222
C2orf9	Chromosome 2 open reading frame 9	NM_032309	0.434	0.0116	9.320	0.0015
CCT6A	Chaperonin contain. TCP1, subunit 6Aζ1	NM_001762	0.439	0.0177	3.094	0.0299
HYA22	HYA22 protein	NM_005808	0.451	0.0018	3.328	0.0343
TPST1	Tyrosylprotein sulfotransferase 1	NM_003596	0.458	0.0332	2.304	0.0332
LOC51031	CGI-150 protein	NM_016080	0.475	0.0042	2.823	0.0097
TR (PRDX2)	Thioredoxin peroxidase reductase	NM_005809	0.487	0.0029	2.179	0.0407
NYD-SP27	Testis-development related NYD-SP27	NM_033123	0.488	0.0285	2.521	0.0252
GPR58	G protein-coupled receptor 58	NM_014626	0.494	0.0333	2.364	0.0365
Up-regulated by IL-1ß						
SOD2	Superoxide dismutase 2, mitochondrial	NM_000636	74.988	0.0000	0.329	0.0276
MMP13	Matrix metalloproteinase 13 (collagenase 3)	NM_002427	67.185	0.0008	0.074	0.0003
RAB5C	RAB5C, member RAS oncogene family	NM_004583	26.556	0.0000	0.042	0.0014
NOS2A	Nitric oxide synthase 2A (induc., hepatoc.)	NM_000625	23.638	0.0022	0.182	0.0027
SLC9A3R1	Solute carrier family 9 (sodium/hydrogen	NM_004252	16.833	0.0042	0.135	0.0127
KRTHA7	Keratin, hair, acidic, 7 exchanger, isoform 3 reg. factor 1	NM_003770	16.807	0.0036	0.194	0.0151
GBP1	Guanylate binding protein 1, interferon-inducible, 67 kDa	NM_002053	14.361	0.0000	0.079	0.0279
PHT2	Peptide transporter 3	NM_016582	13.125	0.0000	0.232	0.0104
MPL	Myeloproliferative leukemia virus oncogene	NM_005373	9.517	0.0006	0.209	0.0082
HLA-A	Major histocompatibility complex, class I, A	NM_002116	8.587	0.0002	0.301	0.0030
HUMMHCW1A	Cw1 antigen	M12679	8.171	0.0150	0.294	0.0259
NUP160	Nucleoporin 160 kDa	D83781	7.291	0.0031	0.207	0.0049
G1P3	Interferon, α-inducible prot. (clone IFI-6-16)	NM_022873	6.541	0.0169	0.444	0.0398
ITGB3BP	Integrin ß 3 binding prot. (ß3-endonexin)	NM_014288	6.144	0.0369	2.262	0.0195
PLIN	Perilipin	NM_002666	4.766	0.0032	2.448	0.0191

Table I. Continued.

Gene symbol	Gene description	Gene Bank Accession no.	Influence of IL-1ß		Influence of SB203580	
			Fold change	p-value	Fold change	p-value
SF3B3	Splicing factor 3b, subunit 3, 130 kDa	NM_012426	4.477	0.0465	2.462	0.0233
WARS	Tryptophanyl-tRNA synthetase	NM_004184	4.379	0.0097	0.365	0.0342
ITGA2B	Integrin, α 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)	NM_000419	4.028	0.0009	0.319	0.0265
GRIK5	Glutamate receptor, ionotropic, kainate 5	AJ249209	3.989	0.0010	0.463	0.0360
BDKRB1	Bradykinin receptor B1	NM_000710	3.794	0.0027	4.178	0.0365
ACAS2L	Acetyl-coenzyme A synthetase 2 (AMP forming)-like	AK024396	3.784	0.0001	0.377	0.0030
NXF2	Nuclear RNA export factor 2	NM_017809	3.705	0.0088	0.171	0.0106
PSMB8	Proteasome (prosome, macropain) subunit, b-type, 8 (large multifunct. Prot. 7)	NM_004159	3.690	0.0058	0.357	0.0333
PLK	Polo-like kinase (*Drosophila*)	NM_005030	3.192	0.0273	0.382	0.0254
HCK	Hemopoietic cell kinase	NM_002110	3.172	0.0003	0.312	0.0207
GPRC5B	G protein-coupled receptor, (C1B)	NM_016235	3.124	0.0214	3.544	0.0241
HLA-E	Major histocompatibility complex, class I, E	NM_005516	3.123	0.0084	0.277	0.0387
LOC51133	NY-REN-45 antigen	NM_016121	3.089	0.0029	3.284	0.0054
FACVL1	Fatty-acid-coenzyme A ligase, very long-chain 1	NM_003645	2.842	0.0087	0.303	0.0401
RHEB2	Ras homolog enriched in brain 2	NM_005614	2.717	0.0114	0.456	0.0495
PRKAG3	Protein kinase, AMP-activated, γ 3 non-catalytic subunit	NM_017431	2.715	0.0040	0.261	0.0023
COL4A6	Collagen, type IV, α 6	NM_033641	2.703	0.0081	0.350	0.0227
UGT8	UDP glycosyltransferase 8 (UDP-galactose ceramide galactosyltransf.)	NM_003360	2.419	0.0034	0.474	0.0068
HAL	Histidine ammonia-lyase	NM_002108	2.291	0.0009	0.287	0.0468
GRO1	GRO1 oncogene (melanoma growth stimulating activity, α)	NM_001511	2.258	0.0012	0.447	0.0419
LOC51030	CGI-148 protein	NM_016078	2.237	0.0014	4.225	0.0348
CHRNA5	Cholinergic receptor, nicotinic, α polypept. 5	NM_000745	2.225	0.0048	0.381	0.0292
SCTR	Secretin receptor	NM_002980	2.198	0.0011	0.277	0.0017
RANBP3	RAN binding protein 3	NM_003624	2.158	0.0242	0.364	0.0120
RANGAP1	Ran GTPase activating protein 1	AB058738	2.156	0.0017	0.452	0.0178
C1S	Complement component 1, s subcomp.	NM_001734	2.143	0.0260	0.240	0.0078
ACATN	Acetyl-coenzyme A transporter	NM_004733	2.065	0.0084	2.064	0.0495
TST	Thiosulfate sulfurtransferase (rhodanese)	NM_003312	2.059	0.0287	0.447	0.0227
TNRC4	Trinucleotide repeat containing 4	NM_007185	2.035	0.0046	0.408	0.0145

(GO) terms with a significant accumulation of changed genes on all levels of the hierarchical GO tree structure and revealed far more than 100 terms significantly affected by the applied cell treatment. In the gene ontology 'biological process' we found 54 terms, in 'molecular function' 49 terms and in 'cellular component' 14 terms. To further concentrate these results we combined subsequent terms of subcategories under the highest parent GO term, which was affected significantly and could detect a couple of main fields. Fig. 2 shows the main GO terms of the different categories ranked according to the number of subsequent terms that could be assigned to them.

The category 'biological process' was reduced to 11 main terms (Fig. 2). The highest ranked terms 'response to stimulus' and 'organic acid metabolism' subsumed 16 subsequent terms, including 17 and 6 co-regulated genes,

respectively. The term 'oxygen and reactive oxygen species metabolism', which was highly significantly affected (p=0.0007), was partly involved in the term 'response to stimulus'. The total number of involved terms shows the great dimension of the 'response to stimulus' term. Certainly, not all of its aspects are influenced by the applied cell treatment, but the number of 17 co-regulated genes indicates a substantial involvement of the p38MAPK pathway in this biological process, playing a pivotal role in the context of inflammation. Therefore, these two terms were further analyzed in the next paragraph as an example of detailed interpretations of GO term results.

In the category 'molecular function', 7 main terms were identified (Fig. 2). The highest ranked parent term 'ligase activity' included 15 of 18 possible child terms and involved 5 regulated genes.
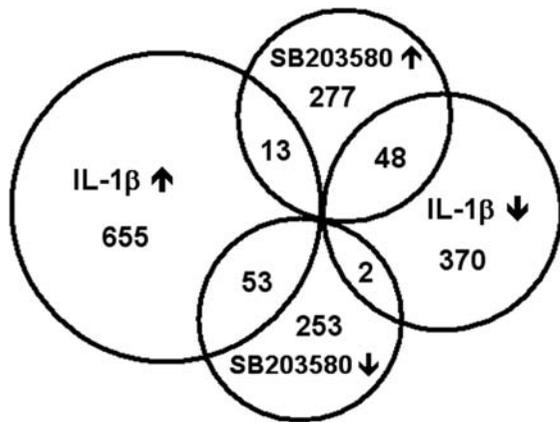
Figure 1. Number of all IL-1ß- and SB203580-regulated genes found in the microarray analysis. Arrows indicate up- and down-regulation in comparison to reference cells. IL-1ß-stimulated cells were compared to untreated cells, SB203580-treated and IL-1ß-stimulated cells were compared to stimulated cells without inhibition.
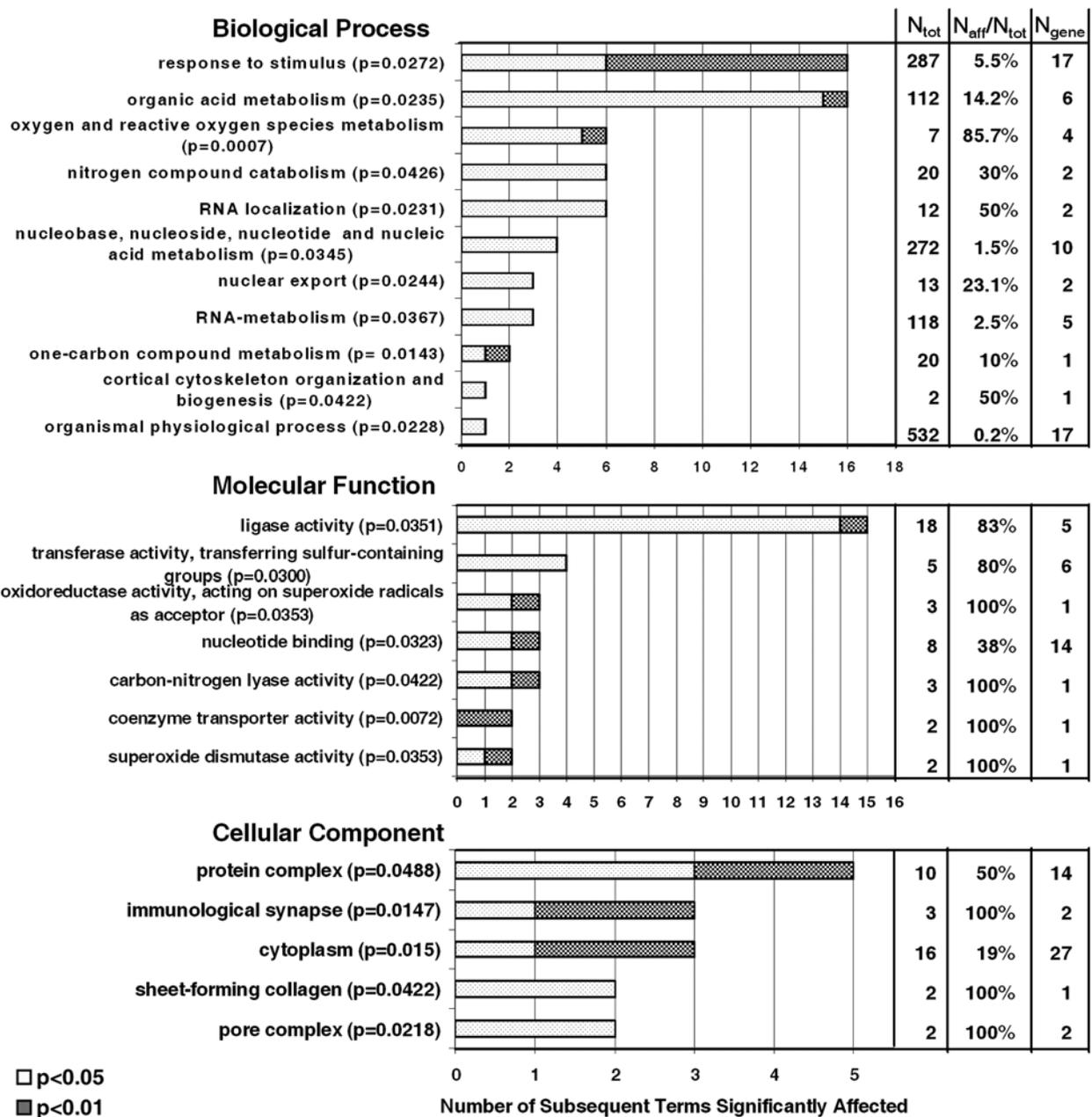

Figure 2. Main GO terms of the different GO categories that were significantly enriched in genes co-regulated by IL-1ß and SB203580. The main terms are ranked according to the number of affected child terms which could be assigned to them. The p-value of the main terms is given in parentheses. The affiliated table shows the total number of possible child terms (N_{tot}), the percentage of affected to total child terms (N_{aff}/N_{tot}) and the number of involved co-regulated genes (N_{gene}).
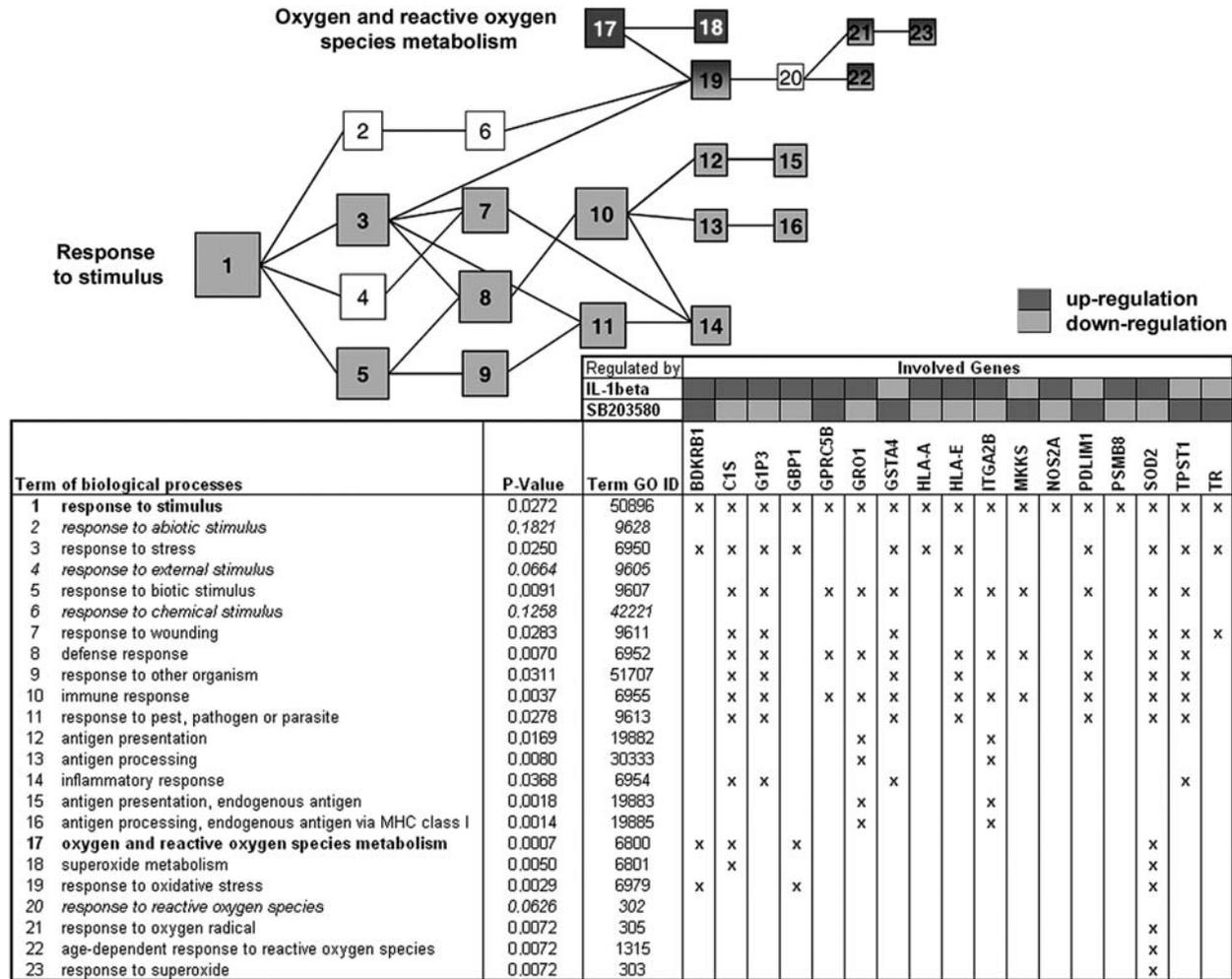
Figure 3. Directed acyclic graph (DAG) representation of the terms 'response to stimulus' and 'oxygen and reactive oxygen species metabolism' with all affected child terms and involved regulated genes. The associated terms that were not significantly affected are in italics. The p-values, the identification numbers (GO ID) of the terms and the direction of the gene regulation by IL-1ß and SB203580 are provided.

Beside the other listed main terms in 'molecular functions' of Fig. 2, 17 further GO terms of this category were significantly affected but could not be summarized in parent terms. These single terms were not ranked in the plot shown in Fig. 2.

The category 'cellular component' comprised 5 main terms. The highest ranked main term 'protein complex' subsumed 5 of 10 possible child terms that were significantly enriched in regulated genes.

Detailed analysis of two GO terms. As an example, the terms 'response to stimulus' and 'oxygen and reactive oxygen species metabolism' were assembled in a directed acyclic graph (DAG) representation in Fig. 3. All enriched child terms, the involved genes and their regulation by IL-1ß and SB203580 are given. A detailed analysis of the function of the according proteins and the direction of regulation helps to interpret the cellular response. The term 'oxygen and reactive oxygen species metabolism', which is also partly involved in the 'response to stimulus' term, involved the 4 genes *Nos2a*, *Pdlim1*, *Sod2* and *Tr*. Subsuming all given subterms, the term

'response to stimulus' involved 17 different genes, of which 12 genes were up-regulated and 5 genes were down-regulated by IL-1ß. In 15 cases, SB203580 counteracted these modulations, whereas for the IL-1ß-stimulated genes *Bdkrb1* and *Gprc5b*, the inhibition of p38MAPK further enhanced gene expression. Many of these genes are known to play a role in cartilage biology or even the pathogenesis of arthritis and will be considered in the discussion.

Pathway and oncogene analysis of all SB203580-regulated genes. For therapeutical interventions in cellular processes, the question of unexpected effects is an important issue. We, therefore, further analyzed all 646 genes that were regulated by the p38MAPK inhibitor SB203580 irrespective of an IL-1ß regulation. A GoMiner pathway analysis in the category 'biological process' of the list of SB203580-regulated genes revealed a significant enrichment of regulated genes in 41 different GO terms. Table II gives 6 main terms subsuming 24 significantly affected child terms. Just as for the GoMiner pathway analysis of co-regulated genes, we found the terms 'oxygen and reactive oxygen species metabolism' and

Table II. GO terms of the category 'biological process' significantly enriched in SB203580-regulated genes.[a]

Main term	p-value	Number of affected child terms	Number of involved genes
Gas transport	0.0491	2	2
Macromolecule biosynthesis	0.0258	3	15
Negative regulation of vasodilation	0.0330	1	1
Oxygen and reactive oxygen species metabolism	0.0393	2	5
Positive regulation of oxidoreductase activity	0.0330	2	1
Response to stimulus	0.0050	14	39
→ Subgroup: regulation of blood coagulation	0.0411	2	2

[a]Affected child terms are subsumed.

Table III. List of SB203580-regulated genes that are also listed in the Tumor Gene Family of Databases, their function in tumor biology and the effect of the regulation on cancer development.

Gene symbol	Regulation by SB203580	Function in tumor biology	Accession no. OMIM[a]	Predicted effect of SB203580 on cancer development
CDC25B	Down	Overexpressed in breast cancer, upregulates cyclin-dependent kinases	116949	Repression
GRO1	Down	Chemokine, overexpressed in ovarian cancer	155730	Repression
HCK	Down	Expression prominent in cells of myeloid lineage	142370	Repression
IL2RA	Down	Cytokine receptor, oncogene family member, activation of Raf-1	147730	Repression
JUN	Down	Transcription factor, proto-oncogene	165160	Repression
MDM2	Up	Oncogene, major regulator of the tumor suppressor p53	164785	Enhancement
MPL	Down	Cytokine-receptor, oncogene	159530	Repression
NF2	Up	Tumor suppressor	101000	Repression
NME1	Up	Metastasis-suppressor gene	156490	Repression
SOS1	Down	May act as a positive regulator of RAS	182530	Repression
TCF3	Down	Transcription factor, PRL-fusion protein may contribute to the acute lymphoblastic phenotype	147141	Repression

[a]Accession number for the 'Online Mendelian Inheritance in Man' (OMIM).

'response to stimulus' significantly involved. The subgroup 'regulation of blood coagulation' may be surprising in the context of chondrocytes concerning unexpected effects and is additionally indicated. It involves the regulation of plasma kallikrein gene expression, an activator of fibrinolysis. Other alarming terms like apoptosis were not significantly affected by p38MAPK inhibition.

Further data interpretation included a comparison of the SB203580-regulated genes with a list of more than 300 genes that are known to play a role in tumor biology [Tumor Gene Family of Databases (11)]. Among the 646 regulated genes, we found 11 matches listed in Table III. The regulation by p38MAPK inhibition, the gene functions in tumor context and the OMIM Accession nos. (12) are indicated. Except for Mdm2 regulation (Table III), SB203580 regulation predicts a cancer repressive effect.

Discussion

The whole human genome microarray analysis of IL-1ß- and p38MAPK inhibitor-treated chondrocytes revealed a huge number of genes whose expression was affected by the cell treatment. Many typical effects previously described in the literature were confirmed (6). Comparing the effects of IL-1ß and SB203580, we found that more than 80% of the SB203580-regulated genes were unaffected by IL-1ß pointing to the importance of p38MAPK in many different pathways (13).

The condensation of these results in a GoMiner Pathway Analysis of co-regulated genes showed a number of mainly affected fields in the different GO categories. Especially the terms 'response to stimulus' and 'oxygen and reactive oxygen species metabolism' of the category 'biological process'

were predominantly involved and analyzed in detail (Fig. 3). The regulation of the genes involved in oxygen metabolism causes a consistent effect. The up-regulation of *Nos2a* (nitric oxide synthase 2A) and the down-regulation of *Tr* (thioredoxin reductase) by IL-1ß both aggravate the oxidative stress in the cell by enhanced synthesis of NO and by reduced regeneration of the antioxidant thioredoxin (14-16). The up-regulation of *Sod2* (superoxide dismutase 2) counteracts this process as it is a protector from ROS-mediated cell damage (17). The effect of p38MAPK inhibition by SB203580 treatment is directly opposed to the stimulation effect. This means a lesser extent of oxygen species formation but also a lower protection by SOD2.

For some of these factors, a role in cartilage and joint biology is already described and may shed light on their function in inflammatory processes. The already described events in oxygen species metabolism by *Nos2a* and *Tr* regulation may contribute to inflammation in joint diseases (17). The down-regulation of the glutathione S-transferase gene *Gsta4* can also be added to this metabolism field as this enzyme is involved in cellular detoxification mechanisms of electrophile compounds like hydroperoxides (18). Recently, Vaillancourt *et al* found that GSTA4-4 expression is critically important for cellular defense against oxidative stress-induced cell death in osteoarthritic cartilage (19).

The up-regulation of *Hla-a*, assigned to the term 'response to stimulus', is somehow surprising in the cartilage context. HLA-A, a major histocompatibility antigen class I, is actually expressed on chondrocytes (20) and normally responsible for antigen presentation of endogenous peptides. In tissues not primarily involved in immune response, possible roles in other functions such as immune surveillance for the development of tumor cells are discussed (20). Notably, an association of the HLA system and osteoarthritis of the hand was suggested previously (21).

The Bradykinin receptor BDKRB1 is a G protein-coupled receptor and is co-induced with COX2 and NOS2A by inflammatory stimuli (22). It was shown that this receptor mediates effects of the inflammatory peptide bradykinin in porcine chondrocytes like PGE$_2$ generation (23).

The serine protease C1s is part of the first complement complex and initiates the complement cascade which actually is a central component in host defense. More important in the cartilage context may be the report of a collagenase activity of C1s (24). A role of C1s was also suggested in degenerative joint diseases (25).

Overall, the detailed interpretation of IL-1ß- and SB203580-induced regulations of genes assigned to the term 'response to stimulus', shows a strong anti-inflammatory effect of p38MAPK inhibition in versatile fields of cellular response. One has to keep in mind, however, that individual results have to be verified with a larger sample size. Another point to be taken into account is an inhibitory effect of SB203580 on other kinases, although weaker than on p38α (3), that could additionally affect the gene expression profile.

The GO category 'molecular function' also revealed a predominantly affected term called 'ligase activity'. This term comprises enzymes of EC class 6, that catalyze mainly ligations of nucleotide fragments. These reactions are most important in DNA replication and repair which was also necessary in cells that suffer from DNA damage. Such damage can be caused by reactive oxygen species (ROS) and NO (15), induced by cytokines, for example. This reflects the conditions in our *in vitro* cell culture model. Probably, the chondrocytes initiate protective processes like DNA repair mechanisms (26) for which ligase activity is an important function (27). A second aspect of ligase activity is provided by some of the subsumed child terms that represent aminoacyl-tRNA ligase functions. Enzymes of this term are not only housekeeping genes involved in protein synthesis. New findings have shown that they play non-catalytic roles in diverse biological processes and in signal transduction (28).

The question regarding unexpected effects of SB203580 was addressed with the pathway analysis of all inhibitor-regulated genes. Among the listed terms, oxygen metabolism might be a critical point concerning unwanted effects of SB203580. Most of the genes involved were already discussed in the previous passage and revealed a rather attenuating effect of SB203580 on oxidative stress in the cell. The term 'regulation of blood coagulation', mainly reflects the regulation of plasma kallikrein gene expression. In fact, an association of kallikrein and the fibrinolytic system with cartilage degradation has been suggested (29,30). Other alarming terms like apoptosis are not significantly affected by p38MAPK inhibition. Recent findings indicated that genetic inhibition throughout the lifespan leads to worsened osteoarthritis in adult mice (5). Therefore, the timing of the therapeutical application of p38 inhibitors was considered to be critical. Our results do not provide an indication that short-term exposure of chondrocytes to SB203580 implicate important alarming effects.

Similarly, the search for oncogene regulation in chondrocytes by inhibitor treatment revealed a rather cancer repressing effect of SB203580. This is in agreement with reports of cytotoxicity enhancing effect of p38MAPK inhibition in myeloma cells (31).

In summary, we present initial data of a genome-wide analysis of p38MAPK inhibition in an inflammation model of human chondrocytes. Besides well-described effects we found new aspects of cellular reactions in response to p38MAPK inhibition such as alterations in 'ligase activity'. Additional information on the expected involvement of biological areas such as 'response to stimulus' or 'reactive oxygen species' further contributes to our fragmentary knowledge on these cellular processes in chondrocytes which has been based on primary target genes so far.

The great number of SB203580-regulated genes further emphasizes the pivotal role of this signaling pathway in human chondrocytes which is not restricted to IL-1ß induction. Finally, a whole genome array is a valuable tool in unravelling unexpected effects which may contradict a therapeutic application. In our analyses, we found no markedly alarming gene regulation predicting, for example, chondrocyte apoptosis which might aggravate joint degeneration.

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