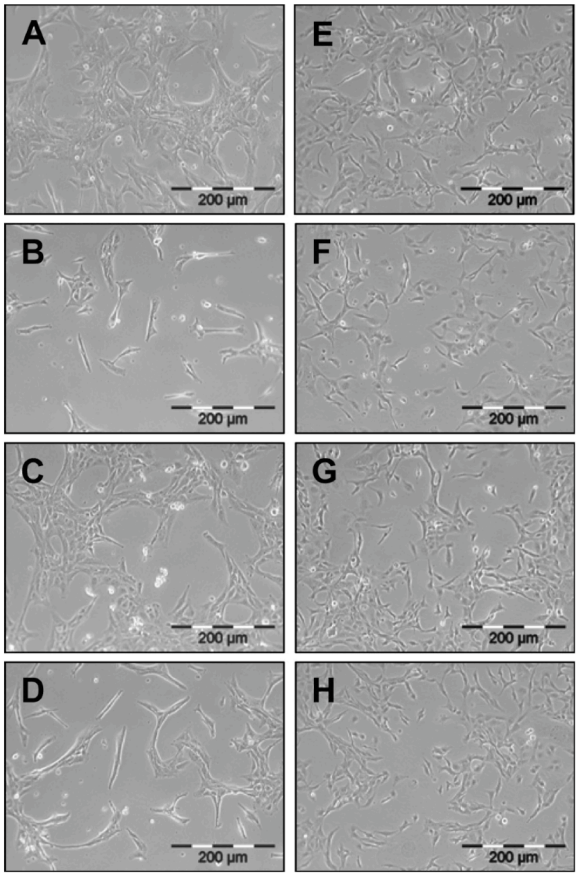


FIG. 1. Morphology of immortalized synovial fibroblasts treated with antirheumatic drugs. (**A**) Rheumatoid arthritis synovial fibroblasts (HSE) demonstrated a typical fibroblast-like morphology. (**B**) Treatment of HSE with antirheumatic drugs resulted in a spindle-like morphology and massive detachment of cells after application of methotrexate. (**C**) Prednisolone had minor effects on HSE cells with few cells floating in the medium. (**D**) After treatment with high doses of diclofenac, HSE cells elongated and detached from the cell culture surface. Normal synovial fibroblasts (K4IM) (**E**) maintained a fibroblast-like morphology after treatment with methotrexate (**F**), prednisolone (**G**), and diclofenac (**H**). Scale bars represent 200 µm.

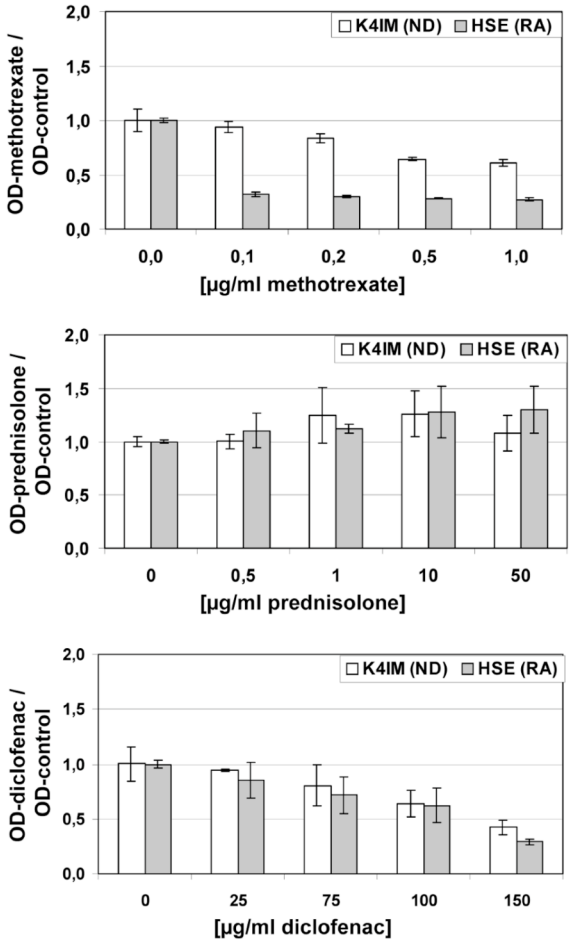


FIG. 2. Cytotoxic effects of antirheumatic drugs on synovial fibroblasts. MTS tetrazolium cytotoxicity assay of rheumatoid arthritis (HSE) and normal (K4IM) synovial fibroblasts treated with 0 to 1.0 µg/ml methotrexate, 0 to 50 µg/ml prednisolone, and 0 to 150 µg/ml diclofenac. Error bars represent the standard deviation.

using doses that had either an effect on cell morphology or cell viability.

Gene expression profiling of synovial cells treated with antirheumatic drugs

Gene expression analysis of synovial fibroblasts resulted in 916 probe sets representing 739 genes that were reproducibly differentially expressed between RASF and NDSF (data not shown). To identify genes that are regulated upon treatment with antirheumatic drugs and that may have an impact on RA, we focused on the 916 probe sets and determined genes that were differentially expressed in RASF treated with the respective antirheumatic drug (**Fig. 3**; **Table 2**). Treatment of RASF with 0.2 µg/ml MTX resulted in differential expression of 29 genes (**Fig. 3A**). Eleven of these genes were also differentially expressed in NDSF treated with MTX. Of special interest are 10 genes that were differentially expressed in RASF compared to NDSF and whose expression profile was reverted by treating RASF with MTX. These genes are known from cell growth and apoptosis-like *insulin-like growth factor binding protein-3* (*IGFB3*), *retinoic acid induced 3* (*RAI3*), *DNA-damage-inducible*

Table 2. Fold Changes of Rheumatoid Arthritis–Related Genes That Were Reverted by Antirheumatic Treatment as Determined by Microarray Analysis

Gene Identifier	*Gene Symbol*	*HSE vs. K4IM (mean FC)*	*HSE vs. K4IM (high FC)*	*HSE vs. K4IM (low FC)*	*Antirheumatic Drug*	*Treated HSE vs. HSE (mean FC)*	*Treated HSE vs. HSE (high FC)*	*Treated HSE vs. HSE (low FC)*	*Treated K4IM vs. K4IM (mean FC)*	*Treated K4IM vs. K4IM (high FC)*	*Treated K4IM vs. K4IM (low FC)*	*Annotation*
BF340228	**IGFBP3**	**–6.0**	**–3.7**	**–8.9**	**MTX**	**2.0**	**2.3**	**1.8**	**NC**	**NC**	**NC**	**Cell growth and apoptosis**
K01228	COL1A1	–5.2	–2.3	–8.6	MTX	–2.1	–1.9	–2.5	–1.8	–1.6	–2.0	Development
AV733308	**ITGA6**	**–4.5**	**–2.6**	**–7.0**	**MTX**	**1.7**	**1.9**	**1.6**	**NC**	**NC**	**NC**	**Cell adhesion**
BC004908	**MGC4655**	**–4.3**	**–3.0**	**–6.5**	**MTX**	**2.2**	**2.6**	**1.6**	**1.8**	**1.9**	**1.7**	**Metabolism**
NM_003979	**RAI3**	**–4.0**	**–2.6**	**–5.7**	**MTX**	**1.7**	**2.0**	**1.5**	**NC**	**NC**	**NC**	**Cell growth**
NM_019058	**DDIT4**	**–3.2**	**–2.1**	**–4.3**	**MTX**	**1.7**	**1.7**	**1.5**	**NC**	**NC**	**NC**	**Apoptosis**
NM_004403	**DFNA5**	**–2.9**	**–2.5**	**–3.2**	**MTX**	**1.5**	**1.5**	**1.4**	**NC**	**NC**	**NC**	**Metabolism**
L37882	FZD2	–2.8	–2.3	–3.5	MTX	–2.1	–1.7	–2.5	–2.6	–2.5	–2.6	Cell growth
NM_001233	**CAV2**	**–2.6**	**–2.1**	**–3.2**	**MTX**	**1.5**	**1.6**	**1.4**	**NC**	**NC**	**NC**	**Cell growth and apoptosis**
NM_003364	**UPP1**	**–2.6**	**–1.5**	**–3.7**	**MTX**	**1.8**	**2.1**	**1.5**	**NC**	**NC**	**NC**	**Metabolism**
NM_021242	MIG12	2.2	2.8	1.7	MTX	1.8	2.0	1.7	1.6	1.6	1.4	Unknown
X57348	SFN	2.2	3.0	1.4	MTX	4.2	4.6	3.7	1.9	2.0	1.7	Cell growth
NM_006404	PROCR	2.6	3.5	2.0	MTX	1.6	1.9	1.4	NC	NC	NC	Immune response
NM_024430	PSTPIP2	2.6	3.7	2.0	MTX	2.4	2.6	2.1	4.2	4.6	3.7	Unknown
M15330	IL1B	3.2	5.5	1.7	MTX	1.8	1.9	1.6	NC	NC	NC	Immune response
NM_001657	AREG	3.8	7.5	1.7	MTX	6.0	7.0	4.9	NC	NC	NC	Cell growth
NM_005261	GEM	3.8	5.7	2.6	MTX	2.0	2.3	1.9	NC	NC	NC	Immune response
BC005127	ADFP	4.1	6.5	3.0	MTX	1.6	1.7	1.4	NC	NC	NC	Development
NM_002090	CXCL3	4.3	12.1	2.0	MTX	2.0	2.3	1.7	8.4	13.0	5.3	Immune response
U77914	JAG1	4.7	7.7	2.8	MTX	2.0	2.5	1.7	3.2	3.4	3.0	Cell growth
M15329	IL1A	5.1	8.6	3.5	MTX	1.8	2.0	1.5	7.9	8.6	7.0	Immune response
NM_024883	**CDH4**	**5.7**	**9.2**	**2.8**	**MTX**	**–1.6**	**–1.4**	**–2.0**	**NC**	**NC**	**NC**	**Cell adhesion**
AF069681	KHDRBS3	6.8	11.3	2.8	MTX	1.7	2.0	1.4	NC	NC	NC	Development
NM_030965	**SIAT7E**	**7.8**	**21.1**	**2.8**	**MTX**	**–2.1**	**–1.9**	**–2.5**	**NC**	**NC**	**NC**	**Metabolism**
AF043337	IL8	9.3	21.3	2.4	MTX	1.6	1.7	1.6	8.1	9.0	7.3	Immune response
AF133207	HSPB8	16.2	36.8	2.6	MTX	2.9	3.7	2.1	NC	NC	NC	Unknown
NM_005213	CSTA	26.7	36.8	18.4	MTX	1.7	1.7	1.6	2.2	2.6	1.9	Unknown
NM_006227	PLTP	61.1	137.2	18.4	MTX	1.8	2.0	1.5	NC	NC	NC	Cell growth
NM_004867	ITM2A	73.5	166.2	17.4	MTX	1.9	2.1	1.6	NC	NC	NC	Unknown
NM_000346	SOX9	–2.9	–1.7	–4.3	PRD	–1.9	–1.7	–2.0	–2.9	–2.5	–3.5	Development
NM_001995	ACSL1	2.3	2.6	2.0	PRD	1.9	2.1	1.7	2.9	3.2	2.6	Metabolism
AL513583	GM2A	2.5	3.2	2.0	PRD	1.5	1.6	1.4	NC	NC	NC	Metabolism
M15330	**IL1B**	**3.2**	**5.5**	**1.7**	**PRD**	**–3.0**	**–2.9**	**–3.0**	**–5.3**	**–4.8**	**–5.7**	**Immune response**
L27624	**TFPI2**	**5.1**	**9.2**	**1.9**	**PRD**	**–2.1**	**–2.1**	**–2.1**	**–1.8**	**–1.6**	**–2.1**	**Immune response**
BC000915	PDLIM1	5.8	8.0	4.0	PRD	1.4	1.4	1.4	1.6	1.7	1.4	Metabolism
AF043337	**ÎL8**	**9.3**	**21.3**	**2.4**	**PRD**	**–2.4**	**–2.4**	**–2.6**	**–3.4**	**–2.9**	**–3.8**	**Immune response**
NM_015364	**LY96**	**14.8**	**27.9**	**5.7**	**PRD**	**–2.1**	**–1.7**	**–2.5**	**NC**	**NC**	**NC**	**Immune response**
AV699347	**XIST**	**41.3**	**157.6**	**6.1**	**PRD**	**–1.7**	**–1.5**	**–2.0**	**NC**	**NC**	**NC**	**Sex specific**
X57348	SFN	2.2	3.0	1.4	DIC	2.3	2.3	2.1	NC	NC	NC	Cell growth
NM_004199	P4HA2	2.5	3.0	1.6	DIC	1.5	1.6	1.5	NC	NC	NC	Unknown
NM_003238	**TGFB2**	**3.7**	**6.1**	**2.5**	**DIC**	**–1.7**	**–1.4**	**–1.7**	**NC**	**NC**	**NC**	**Cell growth**
AF043337	IL8	9.3	21.3	2.4	DIC	1.5	1.5	1.4	NC	NC	NC	Immune response
NM_002781	PSG3	12.7	19.7	8.0	DIC	1.9	2.1	1.7	NC	NC	NC	Pregnancy associated
NM_004867	ITM2A	73.5	166.2	17.4	DIC	1.5	1.5	1.4	NC	NC	NC	Unknown

FC = fold change; NC = no change; MTX = methotrexate; PRD = prednisolone; DIC = diclofenac. Genes that are related to rheumatoid arthritis as shown by differential expression between untreated HSE and K4IM cells, and those genes with expression profiles reverted by treatment with antirheumatic drugs are given in bold.

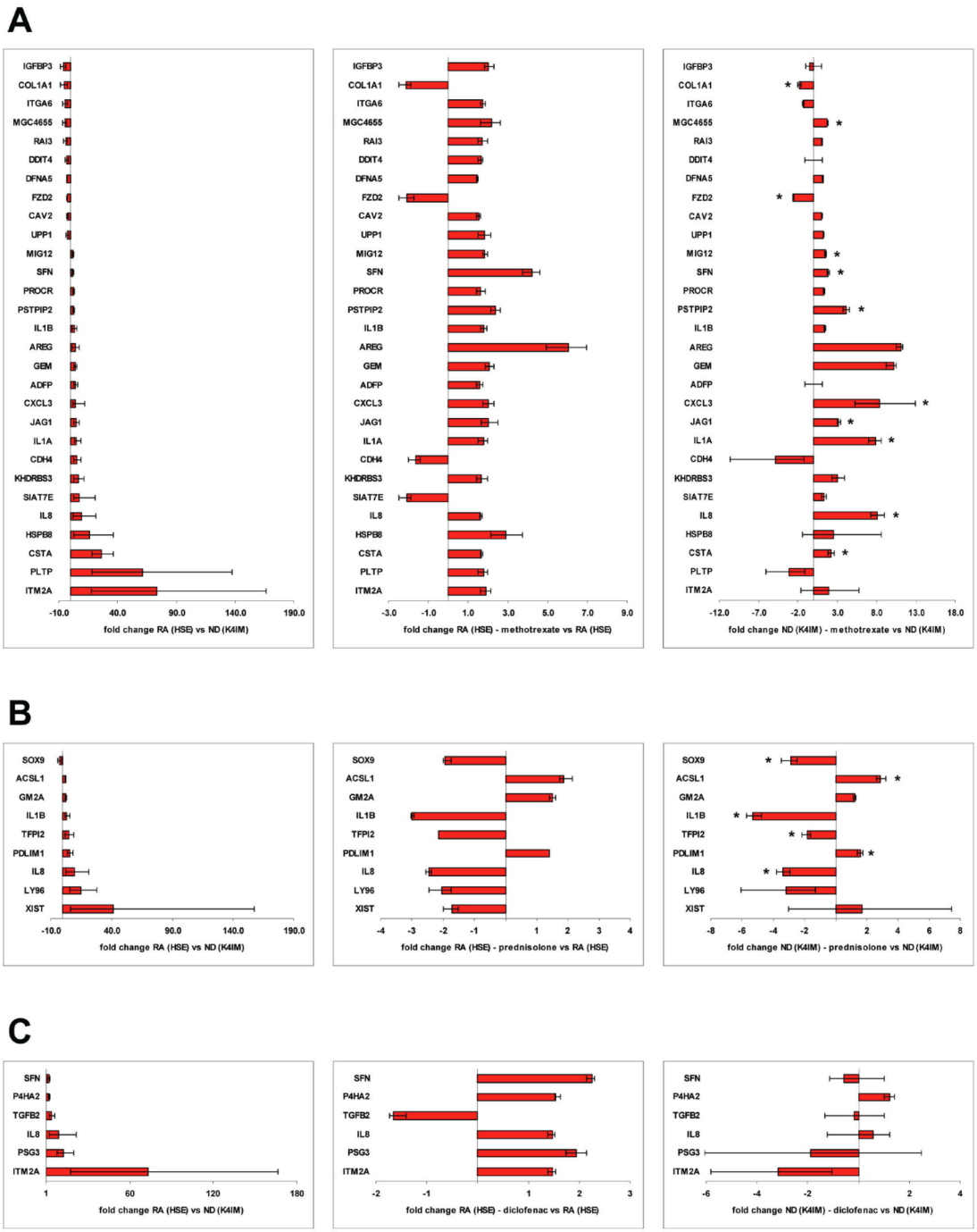


FIG. 3. Rheumatoid arthritis (RA) gene expression profiles reverted by antirheumatic treatment. Centroid view (fold change) of differentially expressed genes between RA (HSE) and normal (K4IM) synovial fibroblasts, which were reverted by antirheumatic drugs. Expression profiles of RA-related genes that are reverted by (**A**) 0.2 µg/ml methotrexate (MTX), (**B**) 1 µg/ml prednisolone, or by (**C**) 75 µg/ml diclofenac. (**Left**) Genes differentially expressed in untreated HSE versus K4IM cells and affected by antirheumatic treatment. (**Center**) Genes differentially expressed after antirheumatic treatment of HSE cells. (**Right**) Drug-affected profiles of RA-related genes in K4IM cells.

transcript 4 (*DDIT4*), and *caveolin 2* (*CAV2*). The genes *deafness, autosomal dominant 5* (*DFNA5*), *MGC4655*, *uridine phosphorylase 1* (*UPP1*), and *sialyltransferase 7* (*SIAT7E*) are associated with cell metabolism, and *integrin α6* (*ITGA6*) as well as *cadherin type 4* (*CDH4*) play a role in cell adhesion and motility. Using prednisolone (1 µg/ml), 9 genes were differentially expressed in treated compared to untreated RASF (**Fig. 3B**). Expression of *IL1β* and *IL8, tissue factor pathway inhibitor 2* (*TFPI2*), *lymphocyte antigen 96* (*LY96*), and the *X (inactive)-specific transcript* reverted after treatment with prednisolone. Six of these 9 genes were also prednisolone-dependently regulated in NDSF. Treatment of RASF with 75 µg/ml diclofenac resulted in 6 differentially expressed genes (**Fig. 3C**). One of these, *transforming growth factor-β2* (*TGFB2*), known from cell growth and differentiation, was reverted to normal. In NDSF, none of those genes were differentially expressed.

Validation of gene expression profiles by reverse transcriptase PCR and immunoassay

To confirm the expression profiles that were determined by microarray analysis, differential expression of selected genes was analyzed by reverse transcriptase (RT) PCR (**Fig. 4**). *IGFBP3*, *ITGA6*, *MGC4655*, *RAI3*, *DDIT4*, *DFNA5*, *CAV2*, and *SIAT7E* were confirmed as differentially expressed between RASF and NDSF and reverted, at least in part, upon application of MTX. The gene expression profiles of *CDH4* and *UPP1* could not be confirmed by RT-PCR (data not shown). *TFPI2* was induced in RASF compared to NDSF and was repressed by prednisolone. *LY96* was also induced by RASF, but its repression by prednisolone could not be confirmed. *TGFB2*, which was repressed in RASF after treatment with diclofenac as shown by microarray analysis, exhibited an increase in RASF compared to NDSF. RT-PCR did not show repression of *TGFB2* in RASF upon treatment with diclofenac.

For validation of the expression profiles of *IL1β* and *IL8*, we analyzed the presence of the respective ILs in the supernatant of RASF after treatment with prednisolone (**Fig. 5**). IL1β was not detected in the supernatant of NDSF and was elevated in supernatants of RASF. Prednisolone repressed the expression of *IL1β* and resulted in decreased levels of IL1β in the supernatants of RASF. The level of IL8 was low in supernatants of NDSF and rose up to 6400 pg/ml in supernatants of RASF. Application of prednisolone lowered the level of IL8, reaching 3300 pg/ml in supernatants of RASF. This confirmed the expression profile of *IL1β* and *IL8* as determined by gene expression analysis.

Screening for dominant pathways involved in drug action

The KEGG database was retrieved for the leading pathways with at least 5 genes changed upon drug exposure. Of all drugs investigated, MTX induced the most changes, with the highest number of genes ($n = 14$) changed in the cell cycle (hsa04110) and

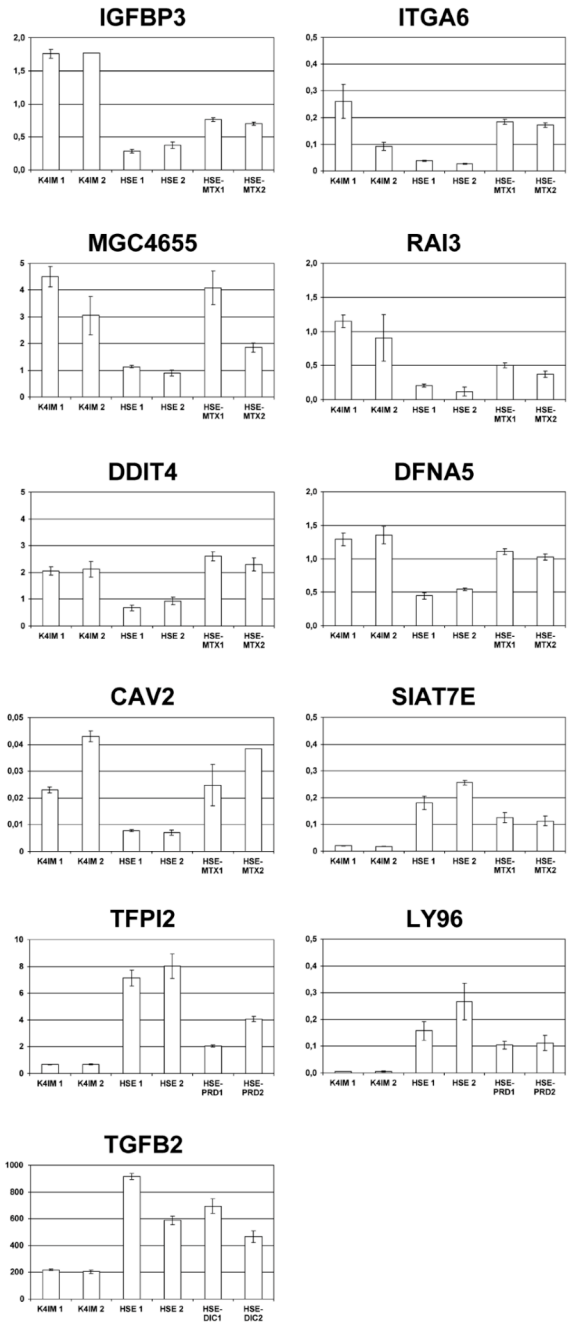


FIG. 4. Real-time PCR analysis of selected rheumatoid arthritis (RA)–related genes reverted by treatment. Real-time reverse transcriptase PCR confirmed MTX-dependent regulation of *IGFB3*, *ITGA6*, *MGC4655*, *RAI3*, *DDIT4*, *DFNA5*, *CAV2*, *SIAT7E*, and prednisolone (PRD)–dependent regulation of *TFPI2*. Regulation of *LY96* and *TGFB2* as determined by microarray analyses could not be confirmed by real-time expression analysis. The mean of each triplicate well is plotted, and the error bars represent the standard deviation.

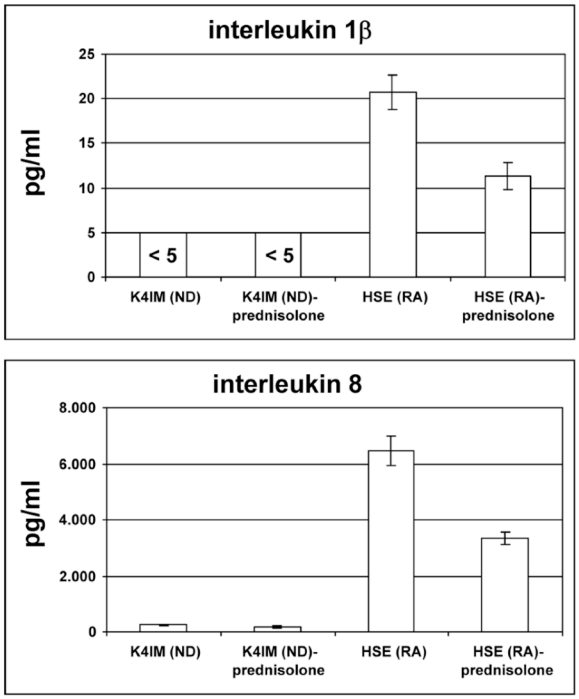


FIG. 5. Suppression of interleukin (IL)-1β and -8 release into the supernatant of rheumatoid arthritis (RA) synovial fibroblasts by prednisolone. Elevated levels of IL1β and IL8 in supernatants of RA HSE cells were reduced by prednisolone. The mean of each duplicate is plotted, and the error bars represent the standard deviation.

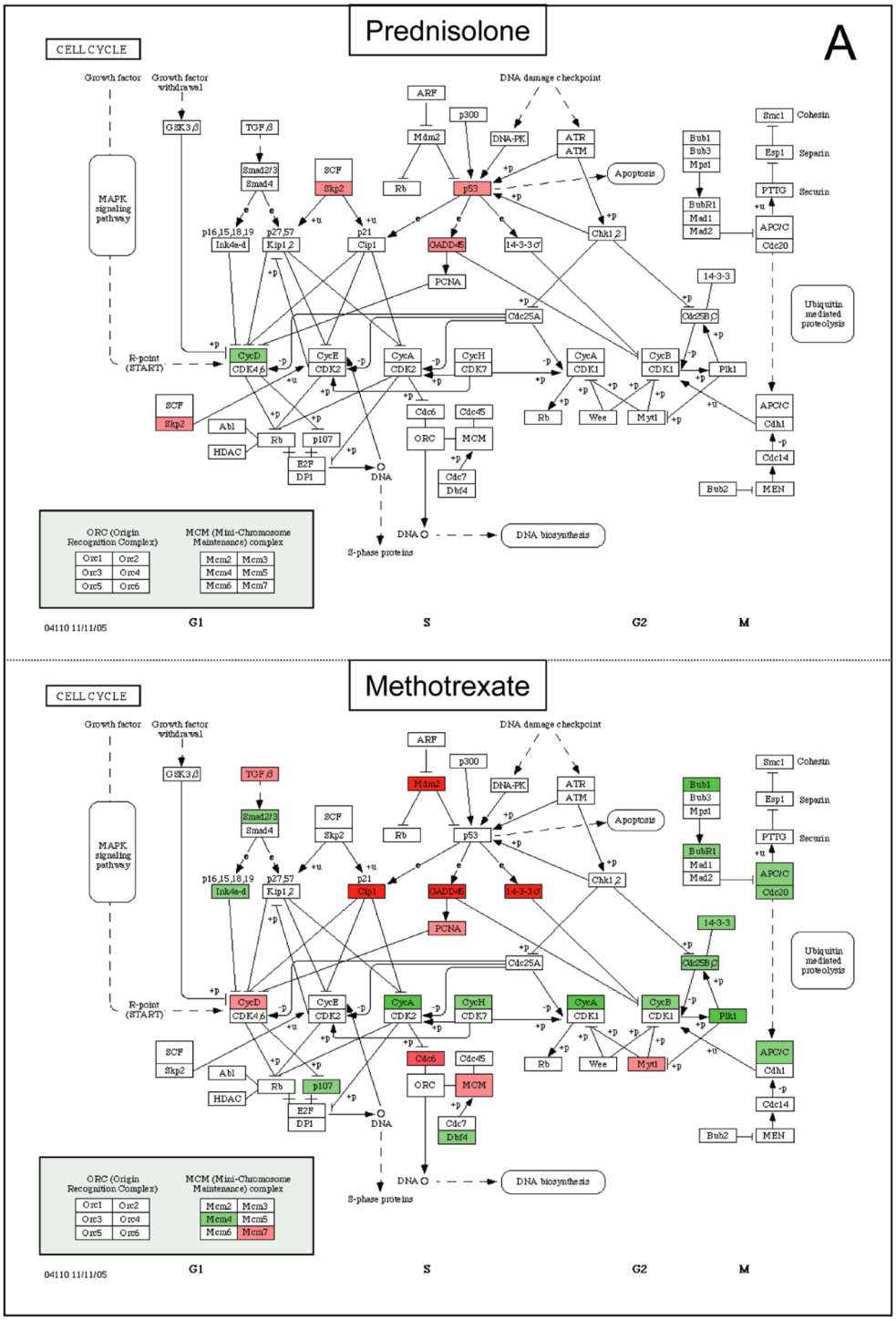
mitogen-activated protein kinase signaling (hsa04010), followed by cytokine-cytokine receptor interaction (hsa04060), pathways related to cell-cell and cell-matrix interaction (hsa04510, hsa01430, hsa04512), apoptosis (hsa04210), hematopoietic cell lineage (hsa04640,) and insulin signaling (hsa04910). Prednisolone changed the expression of up to 10 genes in the cytokine-cytokine receptor interaction pathway collection, focal adhesion (hsa04510), extracellular matrix–receptor interaction (hsa04512), and hematopoietic cell lineage (hsa04640). Incubation with diclofenac revealed the least changes, with fewer than 5 genes in any of the 3 leading pathways: cytokine-cytokine receptor interaction, peroxisome proliferator-activated receptor signaling, and cell cycle. Details of the prominent changes by prednisolone and MTX are shown in **Figure 6**, with only MTX-induced reduction of several cyclin genes known to regulate and promote cell proliferation (**Fig. 6A**). In contrast, the proinflammatory mediators IL1, IL8, and CXCL3 were suppressed by prednisolone but found to be upregulated by MTX (**Fig. 6B**). Transcripts for extracellular matrix products including collagens, laminin, and thrombospondin were increased after prednisolone and decreased after MTX exposure.

DISCUSSION

In the present study, we documented that antirheumatic drugs have distinct effects on the RA-related expression patterns in synovial fibroblasts. Treatment of RASF with MTX reversed the expression of disease-related genes known from cell growth, metabolism, apoptosis, and cell adhesion. Prednisolone repressed genes that were induced in RASF and that are related to inflammation. Diclofenac had no effect on the expression of genes differentially expressed in RASF compared to NDSF.

In RA, the inflamed synovial tissue plays an important role in joint inflammation and destruction. Global assessment of the gene expression profile of RA synovial tissue underlined its dual nature in RA. Recently, 2 groups of RA synovial tissues were reported with predominant expression of 1) genes of the adaptive immune system and 2) genes related to tissue remodeling with a low inflammatory gene expression signature.[17,18] Therefore, activated synovial fibroblasts may play a crucial role in the onset of synovitis and potentially promote RA and joint destruction. Isolated and propagated RASF show enhanced, tumor-like proliferation activity,[19] have the capacity to invade cartilage in an severe combined immunodeficient (SCID) mouse model,[20] and may reflect the disease status of the synovial tissue from which they originated.[21] In this work, we took advantage of these growth properties of RASF and their pathophysiological relevance. To ensure standardization and comparability between different drug-testing experiments, we used immortalized synovial fibroblasts and determined the RA gene expression profiles that are reverted by the treatment with commonly used antirheumatic drugs.

Treatment with MTX resulted in the reduction of cell viability that may correlate with a decrease in proliferation activity of the cells. Similar effects of MTX on cell proliferation were also shown for synovial cells from patients with RA, osteoarthritis (OA), and other arthritides.[22] This antiproliferative effect of MTX was substantiated in our study by the reversion of the expression of *IGFBP3*, *RAI3*, *DDIT4*, and *CAV2*, genes that are related to proliferation and apoptosis. IGFBP3 is a carrier for insulin-like growth factors (IGF)–1 and -2 and plays an important role in proliferation and apoptosis of various cell types including tumor cells.[23] IGFBP3 was found reduced in sera of patients with RA and other arthritides but was elevated in sera of patients treated with glucocorticoids, NSAIDs, or DMARDs.[24,25] Increased levels of IGF and IGFBP3 were also detected in the synovial fluid of patients with RA and OA compared to normal donors.[26,27] Interestingly, *IGFBP3* was repressed in RASF compared to NDSF and was induced upon treatment with MTX. Although it is unclear whether synovial fibroblasts contribute to the levels of IGFBP in synovial fluid or serum, the reduced expression of *IGFBP3* in RASF may favor an IGF-mediated proliferation of cells in the inflamed RA synovium. MTX may exert its antiproliferative effect by directly regulating the IGF system through IGFBP or by inducing apoptosis as shown for RA synovial tissue



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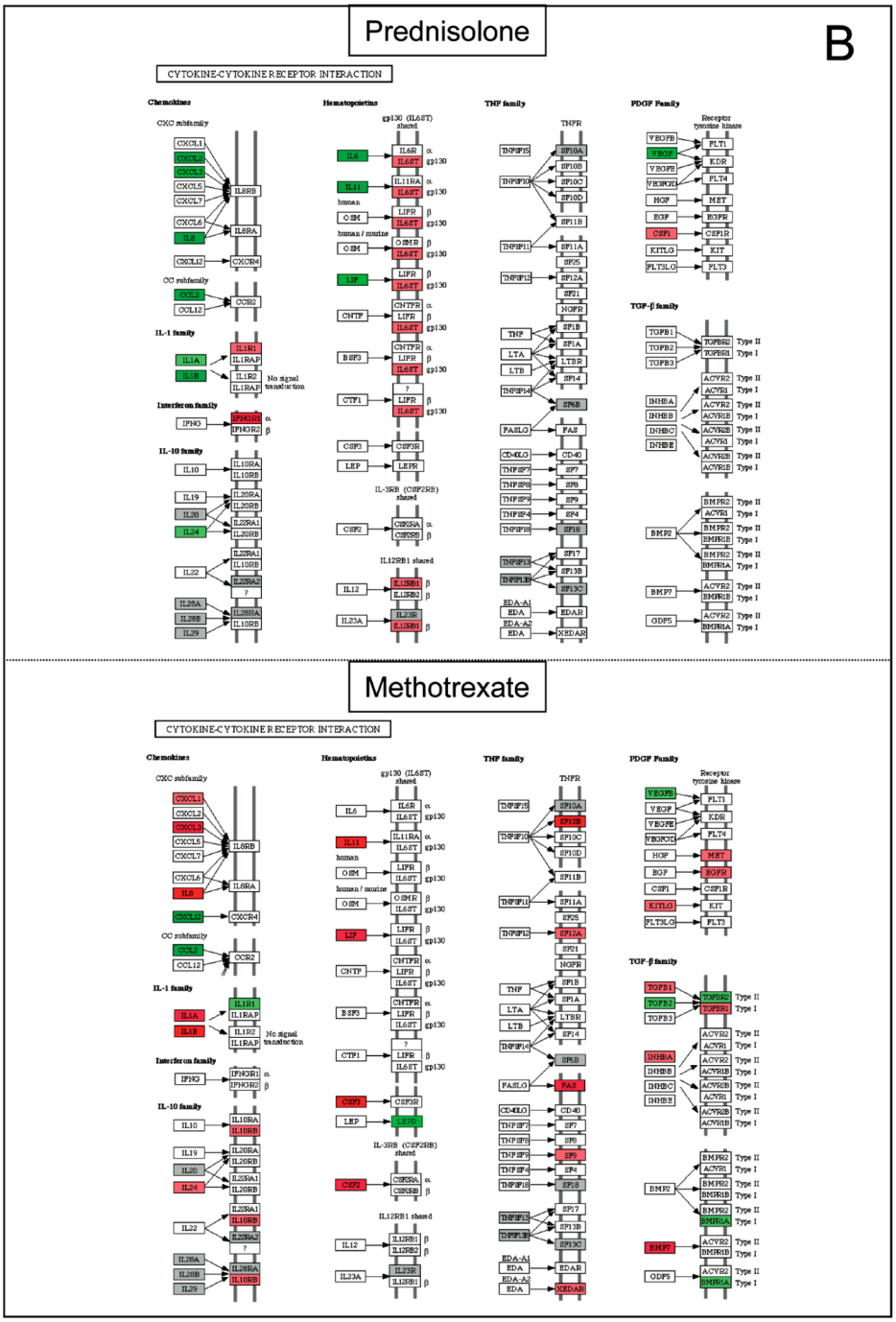

FIG. 6. Molecular pathways influenced by methotrexate and prednisolone. (**A**) In the Kyoto Encyclopedia of Genes and Genomes cell cycle pathway, various cyclin genes are down-regulated (<–1.3-fold) after methotrexate (MTX) exposure of HSE cells (green). Genes that inhibit cyclins and that are related to stress are up-regulated (>1.3-fold; red). (**B**) A selection of cytokine-cytokine receptor interactions was found to be changed after drug exposure. Prednisolone suppressed transcription of typical proinflammatory interleukins and chemokines. In contrast, MTX induced several of these proinflammatory mediators in synovial fibroblasts. Ligands of the tumor necrosis factor family were not influenced. FAS was induced by MTX, suggesting an increased susceptibility for death signaling.

in the SCID mouse model after oral treatment.[28] Treatment of immortalized RASF with MTX also affected regulation of the cell adhesion molecule *ITGA6*. *ITGA6* is repressed in RASF and is induced after application of MTX. This is consistent with the reduced expression of ITGA6 in highly inflamed synovial tissue of RA patients and its induction in OA synovial fibroblasts after stimulation with proinflammatory cytokines such as TNF-α. Remarkably, TNF-α repressed and MTX induced the expression of ITGA6 in long-term cultures of RASF.[29] Therefore, the loss of integrins in RASF may contribute to their evasion from inflamed synovial tissues prior to the formation of pannus tissue.

Glucocorticoids, like prednisolone, are commonly used in the treatment of RA, effectively reduce disease activity in the short term, and may exert their anti-inflammatory effects by inhibiting proliferation and function of different cell types including fibroblasts and macrophages.[30] Here, prednisolone repressed the proinflammatory interleukins IL1β and IL8, which were elevated in RASF. ILs play an important role in the pathophysiology of RA. IL1β is elevated in RA synovial fluid,[31] and injection of IL1 transgenic synovial fibroblasts into rabbit joints induces arthritis.[32] In the mouse collagen induced-arthritis model, prednisolone reduced the expression of IL1β.[33] Treatment of RA patients with the human IL1 receptor antagonist (Anakinra) reduces disease activity and defers bone destruction.[34] IL8 may play an important role in cell infiltration and fibroblast activation in RA. This IL is elevated in inflamed compared to normal synovial tissue[35] and induces arthritis after infusion into rabbit joints.[36] IL8 has been shown to be produced predominantly by synovial macrophages, is released by synovial fibroblasts, and is elevated in the sera of RA patients.[37] These data are consistent with our results that document the induction of IL1β and IL8 in RASF and their repression upon prednisolone treatment.

Comparing molecular changes induced by prednisolone and MTX in general, KEGG pathway analysis revealed complex influences of MTX dominated by antiproliferative effects and of prednisolone with typical anti-inflammatory effects. Also important is the induction of proinflammatory mediators in the synovial fibroblasts by MTX, possibly as a response to stress. Such effects were also reported in the context of MTX pneumonitis.[38] These findings could explain the different clinical effects of treatment with respect to the kinetics of response and side effects. Whereas prednisolone reduces clinical symptoms of pain and inflammation within less than 24 h, patients receiving MTX often complain about side effects within this time frame after application. Furthermore, improvement will occur not earlier but within several weeks of MTX therapy, which could well correlate with a gradual reduction of cells involved in synovitis. MTX may reduce cell proliferation or induce apoptosis not only in fibroblasts but also in T cells and monocytes.[39] However, in lymphocytes, molecular effects of MTX may also involve suppression of activation and adhesion molecules.[40]

NSAIDs, such as diclofenac, relieve pain of RA patients but have only limited effects on inflammation and disease progression.[41] Low doses of diclofenac had no effect on the growth properties of synovial fibroblasts or on the expression profile of disease-related genes. Although high doses of diclofenac had cytotoxic effects on RASF and NDSF, disease-related genes were not affected. An apoptotic effect on primary RASF has been reported for several NSAIDs, including high doses of diclofenac, which were accompanied by the activation of the peroxisome proliferator-activated receptor γ.[42] Moreover, diclofenac has been shown to target nonfibroblastic inflammatory cells by reducing the amount of polymorphonuclear cells in the synovial fluid in a rabbit antigen-induced arthritis model.[43] Thus, diclofenac may exert its RA-related effects on cell types infiltrating the synovium rather than on the synovial fibroblasts.

With respect to target validation, our in vitro system of synovial fibroblasts allows us to validate drug candidates that interfere with immune stimulation or cell proliferation or that affect tissue matrix formation. This validation could be performed using customized arrays with genes related to these pathways. Furthermore, for synovial fibroblast targeting, anti-inflammatory concepts seem to be more effective than antiproliferative mechanisms for a drug discovery approach. Metabolic inhibitors such as MTX may especially induce stress signaling and thus may increase acute side effects, which could be monitored in this system.

In conclusion, the expression of a distinct subset of RA-related genes in synovial fibroblasts is reverted by the treatment with frequently used antirheumatic drugs. Thus, gene expression profiling of immortalized synovial fibroblasts reflects pathophysiological mechanisms involved in rheumatoid synovitis and is a valuable tool in the study of antirheumatic drug effects. Furthermore, synovial fibroblasts are well suited for the screening of potential antirheumatic compounds that exhibit similar properties as DMARDs and SAIDs and may therefore support defining promising candidates for the treatment of RA.

ACKNOWLEDGMENTS

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3.3 Untersuchung von krankheitsspezifischen Stimulationseffekten auf Knorpelgewebe

Aus den vorangegangenen Untersuchungen zu einem isolierten Zelltyp, den Synovialfibroblasten, wird folgendes deutlich: Zytokine als Entzündungsvermittler haben nicht nur eine große Bedeutung für die Ausbreitung der Entzündung und für das Expressionsverhalten anderer Zellen, sondern sind auch zentraler Angriffspunkt für die stärksten bekannten Entzündungshemmer, die Glucocorticoide.

Um die Mechanismen der Ausbreitung besser zu verstehen und spezifischere Ansätze der Therapie zu erforschen, ist die Charakterisierung von Stimulationssignaturen essentiell. Es konnten jedoch nicht alle denkbaren Stimulationen als individuelle Signaturen in allen beteiligten Zelltypen auf einmal untersucht werden. Deshalb musste eine zunächst reduzierte Auswahl getroffen werden.

Synovialfibroblasten werden als ein zentraler Zelltyp in der Aufrechterhaltung der synovitischen Entzündungsprozesse bei rheumatoider Arthritis angesehen. Sie zeigen einen transformierten Phänotyp, synthetisieren verschiedene proinflammatorische Zytokine und sind nachweislich an der Invasion in den Knorpel mitbeteiligt. Mit dem Knorpelgewebe als der Zielstruktur der arthritischen Zerstörung stellt sich die Frage, welchen Einfluss Entzündungsmediatoren auf das Expressionsverhalten von Chondrozyten nehmen.

In der nachfolgenden Arbeit wurde deshalb der Kulturüberstand von rheumatoide Arthritis Synovialfibroblasten verwendet, um *in vitro* gezüchtetes Knorpelgewebe zu stimulieren. Die Transkriptomanalysen ergaben ein mit Matrixdestruktion assoziiertes Profil, bestehend aus Markergenen für Entzündung und für NFκB-Signaltransduktion, aus Zytokinen, Zytokinrezeptoren und Matrix zerstörenden Metalloproteasen. Neben diesen inflammatorischen und destruktiven Veränderungen kam es gleichzeitig zur verminderten Expression von Matrixmolekülen wie z.B. Cartilage Oligomeric Matrix Protein und Chondroitinsulfat Proteoglykan 2. Das verwendete Modell zeigt somit ein gestörtes Gleichgewicht von auf- und abbauenden Prozessen im Knorpel sowie Verstärkungen der Entzündung. Es zeigt relevante pharmakologische Zielstrukturen auf und kann als in vitro Krankheitsmodell zur Untersuchung der molekularen Effekte von verschiedenen Wirksubstanzen der antirheumatischer Therapie verwendet werden.

Research article

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Key regulatory molecules of cartilage destruction in rheumatoid arthritis: an *in vitro* study

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Abstract

Background Rheumatoid arthritis (RA) is a chronic, inflammatory and systemic autoimmune disease that leads to progressive cartilage destruction. Advances in the treatment of RA-related destruction of cartilage require profound insights into the molecular mechanisms involved in cartilage degradation. Until now, comprehensive data about the molecular RA-related dysfunction of chondrocytes have been limited. Hence, the objective of this study was to establish a standardized *in vitro* model to profile the key regulatory molecules of RA-related destruction of cartilage that are expressed by human chondrocytes.

Methods Human chondrocytes were cultured three-dimensionally for 14 days in alginate beads and subsequently stimulated for 48 hours with supernatants from SV40 T-antigen immortalized human synovial fibroblasts (SF) derived from a normal donor (NDSF) and from a patient with RA (RASF), respectively. To identify RA-related factors released from SF, supernatants of RASF and NDSF were analyzed with antibody-based protein membrane arrays. Stimulated cartilage-like cultures were used for subsequent gene expression profiling with oligonucleotide microarrays. Affymetrix GeneChip Operating Software and Robust Multi-array Analysis (RMA) were used to identify differentially expressed genes. Expression of selected genes was verified by real-time RT-PCR.

Results Antibody-based protein membrane arrays of synovial fibroblast supernatants identified RA-related soluble mediators (IL-6, CCL2, CXCL1–3, CXCL8) released from RASF. Genome-wide microarray analysis of RASF-stimulated chondrocytes disclosed a distinct expression profile related to cartilage destruction involving marker genes of inflammation (*adenosine A2A receptor*, *cyclooxygenase-2*), the NF-κB signaling pathway (*toll-like receptor 2*, *spermine synthase*, *receptor-interacting serine-threonine kinase 2*), cytokines/chemokines and receptors (*CXCL1–3*, *CXCL8*, *CCL20*, *CXCR4*, *IL-1β*, *IL-6*), cartilage degradation (*matrix metalloproteinase (MMP)-10*, *MMP-12*) and suppressed matrix synthesis (*cartilage oligomeric matrix protein*, *chondroitin sulfate proteoglycan 2*).

Conclusion Differential transcriptome profiling of stimulated human chondrocytes revealed a disturbed catabolic–anabolic homeostasis of chondrocyte function and disclosed relevant pharmacological target genes of cartilage destruction. This study provides comprehensive insight into molecular regulatory processes induced in human chondrocytes during RA-related destruction of cartilage. The established model may serve as a human *in vitro* disease model of RA-related destruction of cartilage and may help to elucidate the molecular effects of anti-rheumatic drugs on human chondrocyte gene expression.

ADORA2A = adenosine A2A receptor; BCL2A1 = BCL2-related protein A1; CMKOR = chemokine orphan receptor; COMP = cartilage oligomeric matrix protein; COX = cyclooxygenase; CSPG = chondroitin sulfate proteoglycan; ECM = extracellular matrix; GCOS = GeneChip Operating Software; Gro = growth-related oncogene; IFI-6–16 = interferon-α inducible protein-6–16; IL = interleukin; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; NDSF = synovial fibroblast cell line derived from normal donor; NDSFsn = supernatant of NDSF; NF = nuclear factor; OAS1 = 2',5'-oligoadenylate synthetase 1; PGES = prostaglandin E synthase; RA = rheumatoid arthritis; RASF = synovial fibroblast cell line derived from patient with RA; RASFsn = supernatant of RASF; RIPK = receptor-interacting serine/threonine kinase; RMA = Robust Multi-array Analysis; RT-PCR = polymerase chain reaction with reverse transcription; SF = synovial fibroblasts; SMS = spermine synthase; STAT = signal transduction and activators of transcription; STS = steroid sulfatase; THBS = thrombospondin; TLR = toll-like receptor; TNF = tumor necrosis factor; TXNIP = thioredoxin interacting protein.