

## SHORT REPORT

## Effects of ibuprofen on gene expression in chondrocytes from patients with osteoarthritis as determined by RNA-Seq

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**ABSTRACT**

Non-steroidal anti-inflammatory drugs are a widely used symptomatic treatment in osteoarthritis (OA), but their effects on cartilage remain controversial. We studied the effects of ibuprofen on gene expression in chondrocytes from patients with OA using RNA-Seq. Chondrocytes were isolated from cartilage samples of patients with OA undergoing knee replacement surgery, cultured with ibuprofen, and total mRNA was sequenced. Differentially expressed genes were identified with edgeR using pairwise comparisons. Functional analysis was performed using ingenuity pathway analysis (IPA). Ibuprofen did not induce statistically significant changes in chondrocyte transcriptome when the cells were cultured in the absence of added cytokines. In inflammatory conditions (when the cells were exposed to the OA-related cytokine interleukin (IL)-1 $\beta$ ), 51 genes were upregulated and 42 downregulated by ibuprofen with fold change >1.5 in either direction. The upregulated genes included anti-inflammatory factors and genes associated with cell adhesion, while several mediators of inflammation were among the downregulated genes. IPA analysis revealed ibuprofen having modulating effects on inflammation-related pathways such as integrin, IL-8, ERK/MAPK and cAMP-mediated signalling pathways. In conclusion, the effects of ibuprofen on primary OA chondrocyte transcriptome appear to be neutral in normal conditions, but ibuprofen may shift chondrocyte transcriptome towards anti-inflammatory phenotype in inflammatory environments.

**INTRODUCTION**

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat osteoarthritis (OA) pain but there are some concerns on their effects on chondrocyte biology.<sup>1</sup>

OA is characterised by constant low-grade joint inflammation and transient inflammatory exacerbations. The inflammatory nature of the disease is evidenced by the increased production of proinflammatory cytokines, particularly interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6) and tumour necrosis factor  $\alpha$

**Key messages**

- The current evidence about the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on osteoarthritis (OA) cartilage is conflicting.
- We investigated the effects of ibuprofen on gene expression in OA chondrocytes by using RNA-Seq.
- In neutral conditions (in the absence of added inflammatory factors), ibuprofen had no statistically significant effects on gene expression in OA chondrocytes.
- In inflammatory conditions mimicked by the presence of interleukin (IL)-1 $\beta$ , ibuprofen upregulated several anti-inflammatory factors while downregulating inflammatory mediators such as IL-6 and IL-23. Ibuprofen also inhibited phosphatase and tensin homolog (PTEN) signalling.
- The findings support the assumption that NSAIDs are safe for cartilage when treating OA pain. They also may shift chondrocyte transcriptome towards an anti-inflammatory phenotype in OA exacerbations.

(TNF $\alpha$ ). They drive the production of catabolic enzymes such as matrix metalloproteinases (MMPs), accelerating joint destruction.<sup>2</sup>

NSAIDs exert their effects by inhibiting the synthesis of prostanoids, particularly prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by cyclo-oxygenase (COX) enzymes. By altering the balance of proinflammatory and anti-inflammatory mediators in the joint, they have been hypothesised to affect OA pathogenesis. These effects, if any, are however controversial, as both potential benefits (eg, alleviation of joint inflammation and reduction of cartilage catabolism) and harms (eg, impairment of cartilage anabolism and accelerated radiographic joint destruction) have been reported.<sup>1,3</sup>

We carried out a genome-wide expression analysis on the effects of the NSAID ibuprofen on gene expression in OA chondrocytes in

**Table 1** All genes upregulated or downregulated by ibuprofen in the presence of IL-1 $\beta$  with FC  $\geq 1.5$  in either direction

Gene	Name	Function	Mean (IL1)	Mean (IL1 +ibu)	FC	adj. P
PPARG	Peroxisome proliferator activated receptor gamma	Carbohydrate and lipid metabolism, inflammation	0.3	0.9	<b>2.87</b>	5.0E-06
UMODL1	Uromodulin like 1	Regulation of apoptosis?	0.3	0.7	<b>2.39</b>	0.0011
XIRP1	Xin actin binding repeat containing 1	Actin binding	5.7	13.4	<b>2.38</b>	< 1.0E-06
DACT1	Dishevelled binding antagonist of beta catenin 1	Regulation of cell cycle and tissue development	4	8.4	<b>2.1</b>	< 1.0E-06
CSF2/GM-CSF	Colony stimulating factor 2=Granulocyte-macrophage colony stimulating factor	Leucocyte differentiation, immune response	5.2	11.2	<b>2.09</b>	< 1.0E-06
PPARGC1B	PPARG coactivator 1 beta	Regulation of transcription	0.4	0.8	<b>2.07</b>	0.00024
FAM186B	Family with sequence similarity 186 member B	?	0.4	0.7	<b>1.92</b>	0.0035
SOX17	SRY-box 17	Cell proliferation, tissue development	3.5	6.8	<b>1.91</b>	< 1.0E-06
MTSS1	MTSS1, I-BAR domain containing	Cell adhesion	6.9	12.6	<b>1.9</b>	< 1.0E-06
AKAP6	A-kinase anchoring protein 6	Regulation of cell proliferation, cAMP signalling	2.1	3.9	<b>1.89</b>	< 1.0E-06
PDE5A	Phosphodiesterase 5A	Regulation of NO signalling	1.2	2.3	<b>1.85</b>	7.0E-06
RGS2	Regulator of G protein signalling 2	Regulation of G protein signalling	52.1	95.9	<b>1.85</b>	< 1.0E-06
CAMK2A	Calcium/calmodulin dependent protein kinase II alpha	Wnt and TGF $\beta$ signalling, NF- $\kappa$ B activation	1.8	3.3	<b>1.81</b>	< 1.0E-06
MAP1LC3C	Microtubule associated protein 1 light chain 3 gamma	Autophagy	0.7	1.2	<b>1.79</b>	3.3E-05
NRG1	Neuregulin 1	Cell differentiation, signal transduction	0.7	1.3	<b>1.78</b>	5.9E-05
SELE	Selectin E	Inflammation	41.3	73.5	<b>1.78</b>	< 1.0E-06
FCRLA	Fc receptor like A	Immunoglobulin binding	4.2	7.4	<b>1.76</b>	< 1.0E-06
DENND3	DENN domain containing 3	Autophagy	14.6	25.3	<b>1.75</b>	< 1.0E-06
FCRLB	Fc receptor like B	Immunoglobulin binding	0.8	1.4	<b>1.73</b>	3.3E-05
MOXD1	Monoxygenase DBH like 1	Monoamine metabolism	123.1	210.3	<b>1.72</b>	< 1.0E-06
SPNS2	Sphingolipid transporter 2	Lipid transport	2.4	4.0	<b>1.72</b>	< 1.0E-06
PODXL	Podocalyxin like	Cell adhesion	11.2	19.0	<b>1.71</b>	< 1.0E-06
RP1	RP1, axonemal microtubule associated	?	0.4	0.6	<b>1.67</b>	0.023
IDO1	Indoleamine 2,3-dioxygenase 1	Modulation of inflammation and cartilage development	1.3	2.1	<b>1.65</b>	0.0012
SCUBE3	Signal peptide, CUB domain and EGF like domain containing 3	TGF $\beta$ signalling	42.5	72.5	<b>1.64</b>	< 1.0E-06
KCNJ15	Potassium voltage-gated channel subfamily J member 15	Potassium transport	1.3	2.2	<b>1.63</b>	1.0E-06
SERPINE1	Serpin family E member 1	Inhibition of proteolysis	455.9	748.6	<b>1.62</b>	< 1.0E-06
PSD2	Pleckstrin and Sec7 domain containing 2	?	0.4	0.6	<b>1.62</b>	0.035
LINGO1	Leucine rich repeat and Ig domain containing 1	?	1.9	3.1	<b>1.61</b>	< 1.0E-06
AKNAD1	AKNA domain containing 1	?	0.6	1.0	<b>1.60</b>	0.0048
STRA6	Stimulated by retinoic acid 6	Retinol and adipokine binding	1.9	2.9	<b>1.59</b>	0.00058
ITGAX	Integrin subunit alpha X	Cell adhesion	11.0	17.4	<b>1.58</b>	< 1.0E-06
KCNN3	Potassium calcium-activated channel subfamily N member 3	Potassium transport	2.8	4.3	<b>1.58</b>	< 1.0E-06
ICAM5	Intercellular adhesion molecule 5	Cell adhesion	4.5	7.2	<b>1.58</b>	< 1.0E-06
FGD4	FYVE, RhoGEF and PH domain containing 4	Cytoskeleton organisation	21.9	34.0	<b>1.57</b>	< 1.0E-06
KCNN4	Potassium calcium-activated channel subfamily N member 4	Potassium transport	6.4	10.0	<b>1.57</b>	< 1.0E-06
LRRRC55	Leucine rich repeat containing 55	Potassium transport	0.8	1.3	<b>1.56</b>	0.0013
CXCR3	C-X-C motif chemokine receptor 3	Inflammation	0.7	1.1	<b>1.55</b>	0.017
CD24	CD24 molecule	Wnt and MAPK signalling, regulation of inflammation	5.4	8.5	<b>1.55</b>	< 1.0E-06
FGR	FGR proto-oncogene, Src family tyrosine kinase	PI3K-Akt signalling, regulation of inflammation	3.4	5.2	<b>1.54</b>	< 1.0E-06

Continued

**Table 1** Continued

Gene	Name	Function	Mean (IL1)	Mean (IL1 +ibu)	FC	adj. P
PEG10	Paternally expressed 10	Inhibition of TGFβ signalling	23.9	36.4	<b>1.54</b>	< 1.0E−06
SIGLEC15	Sialic acid binding Ig like lectin 15	Regulation of bone resorbtion	1.6	2.4	<b>1.54</b>	0.00024
CPNE2	Copine 2	Bone erosion	18.7	28.9	<b>1.54</b>	< 1.0E−06
WNK4	WNK lysine deficient protein kinase 4	Ion transport	4.6	7.0	<b>1.53</b>	< 1.0E−06
RTL3	Retrotransposon Gag like 3	Regulation of collagen production	3.3	5.0	<b>1.53</b>	< 1.0E−06
RGS3	Regulator of G protein signalling 3	Inhibition of MAPK signalling	65.1	99.2	<b>1.52</b>	< 1.0E−06
AOC2	Amine oxidase, copper containing 2	Amine metabolism	68.0	102.3	<b>1.51</b>	< 1.0E−06
IL10RA	Interleukin 10 receptor subunit alpha	Regulation of inflammation	1.0	1.6	<b>1.51</b>	0.0018
RGS16	Regulator of G protein signalling 16	?	60.1	90.0	<b>1.51</b>	< 1.0E−06
PCDH17	Protocadherin 17	Cell adhesion	0.9	1.4	<b>1.51</b>	0.028
GPR158	G protein-coupled receptor 158	?	1.3	1.9	<b>1.50</b>	0.00017
IL23A	Interleukin 23 subunit alpha	Inflammation	15.2	4.7	<b>−3.24</b>	< 1.0E−06
HAS1	Hyaluronan synthase 1	Extracellular matrix production	0.8	0.3	<b>−2.77</b>	< 1.0E−06
IGFBP4	Insulin-like growth factor binding protein 4	Cell proliferation and metabolism	213.8	79.7	<b>−2.73</b>	< 1.0E−06
IL6	Interleukin 6	Inflammation	958.4	403.8	<b>−2.49</b>	< 1.0E−06
PDE3A	Phosphodiesterase 3A	Lipid metabolism	0.9	0.3	<b>−2.48</b>	0.00013
STAT4	Signal transducer and activator of transcription 4	Inflammation, regulation of cell proliferation	2.5	1.0	<b>−2.36</b>	< 1.0E−06
PCSK1	Proprotein convertase subtilisin/kexin type 1	Metabolism	7.2	3.2	<b>−2.19</b>	< 1.0E−06
ADAMTS6	ADAM metallopeptidase with thrombospondin type 1 motif 6	Extracellular matrix catabolism	10.5	4.9	<b>−2.18</b>	< 1.0E−06
HAL	Histidine ammonia-lyase	Histidine catabolism	1.7	0.8	<b>−2.12</b>	< 1.0E−06
DNAH17	Dynein axonemal heavy chain 17	Cytoskeleton component	1.0	0.5	<b>−2.06</b>	2.00E−06
CSF3	Colony stimulating factor 3	Inflammation, regulation of cell proliferation	19.8	9.9	<b>−2.02</b>	< 1.0E−06
AREG	Amphiregulin	EGF signalling, regulation of cell proliferation	2.3	1.2	<b>−2.01</b>	< 1.0E−06
CA12	Carbonic anhydrase 12	Acidity regulation, Regulation of proliferation	20.9	10.5	<b>−2.00</b>	< 1.0E−06
INSC	Inscuteable homolog (Drosophila)	Cell differentiation	0.6	0.3	<b>−1.98</b>	0.0011
KCNE5	Potassium voltage-gated channel subfamily E regulatory subunit 5	Regulation of potassium transport	1.3	0.6	<b>−1.94</b>	6.00E−06
LDB2	LIM domain binding 2	Regulation of transcription	0.5	0.3	<b>−1.92</b>	0.005098
DOK6	Docking protein 6	?	0.9	0.5	<b>−1.80</b>	0.000598
DAW1	Dynein assembly factor with WD repeats 1	Dynein assembly	0.9	0.5	<b>−1.78</b>	0.000565
TMEM71	Transmembrane protein 71	?	1.8	1.0	<b>−1.77</b>	2.00E−06
MAMSTR	MEF2 activating motif and SAP domain containing transcriptional regulator	Regulation of transcription	0.5	0.3	<b>−1.72</b>	0.021819
KNDC1	Kinase non-catalytic C-lobe domain containing 1	?	0.8	0.5	<b>−1.70</b>	0.002773
EFHC2	EF-hand domain containing 2	Cell proliferation	0.8	0.5	<b>−1.69</b>	0.004747
MEX3A	Mex-3 RNA binding family member A	PI3K-Akt signalling	0.9	0.5	<b>−1.69</b>	0.001905
TGFBI	Transforming growth factor beta induced	ECM organisation, chondrocyte differentiation	127.8	80.7	<b>−1.64</b>	< 1.0E−06
C3AR1	Complement C3a receptor 1	Inflammation	3.5	2.2	<b>−1.63</b>	< 1.0E−06
EFEMP1	EGF containing fibulin like extracellular matrix protein 1	Inhibition of chondrocyte differentiation	72.6	45.4	<b>−1.63</b>	< 1.0E−06
NAMPT	Nicotinamide phosphoribosyltransferase / visfatin	Cartilage catabolism	596.1	368.8	<b>−1.60</b>	< 1.0E−06
FOXF1	Forkhead box F1	Morphogenesis	1.2	0.8	<b>−1.60</b>	0.000928
AVP11	Arginine vasopressin induced 1	MAPK signalling	39.7	24.8	<b>−1.60</b>	< 1.0E−06
SEMA3A	Semaphorin 3A	Regulation of inflammation and apoptosis	98.0	61.6	<b>−1.59</b>	< 1.0E−06
STC1	Stanniocalcin 1	Regulation of cartilage development	2.0	1.3	<b>−1.59</b>	0.002967

Continued

Table 1 Continued

Gene	Name	Function	Mean (IL1)	Mean (IL1 +ibu)	FC	adj. P
TSKU	Tsukushi, small leucine rich proteoglycan	?	14.9	9.4	-1.58	< 1.0E-06
SMOC1	SPARC related modular calcium binding 1	ECM organisation	199.0	126.1	-1.57	< 1.0E-06
ARAP2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	Cell adhesion, inhibition of Akt signalling	12.5	8.0	-1.56	< 1.0E-06
BEND5	BEN domain containing 5	Negative regulation of transcription	0.9	0.6	-1.54	0.01673
EPB41L3	Erythrocyte membrane protein band 4.1 like 3	Cortical cytoskeleton organisation	34.6	22.5	-1.53	< 1.0E-06
EPB41L4B	Erythrocyte membrane protein band 4.1 like 4B	Regulation of cell adhesion and gene expression	2.8	1.8	-1.53	0.000106
ACSL4	Acyl-CoA synthetase long-chain family member 4	Lipid metabolism	428.1	280.7	-1.52	< 1.0E-06
NR4A2	Nuclear receptor subfamily 4 group A member 2	Wnt signalling, regulation of apoptosis	19.8	13.1	-1.51	< 1.0E-06
PID1	Phosphotyrosine interaction domain containing 1	Oxidative metabolism	285.1	190.6	-1.51	< 1.0E-06
RSPO3	R-spondin 3	Wnt signalling, morphogenesis	82.9	53.8	-1.50	< 1.0E-06

Red = upregulated genes; blue = downregulated genes.

adj. P, False discovery rate (FDR) -adjusted P value; IL, interleukin; Mean, trimmed mean of M-values (TMM) normalized counts.

normal and inflammatory conditions in vitro by using RNA-Seq.

## METHODS

Cartilage samples were obtained from 10 patients with OA (mean age 67 years (SEM 3.8 years), 8 females, Kellgren-Lawrence grade 3.7 (SEM 0.15)) undergoing knee replacement surgery in Coxa Hospital for Joint Replacement, Tampere, Finland.

Chondrocytes were isolated by enzyme digestion and seeded on 24-well plates for 24 hours. Thereafter the experiments were started, and the cells were cultured either alone, with ibuprofen (10 µM), with IL-1β (100 pg/mL), or with a combination of ibuprofen and IL-1β for 24 hours. Cell culture, RNA sequencing, RT-PCR and data analysis are described in online supplemental data S1.

## RESULTS

### The effects of ibuprofen on OA chondrocytes in neutral conditions

In the absence of exogenous cytokines, no genes were found to be differentially expressed between chondrocytes cultured with or without ibuprofen when the results were adjusted by false discovery rate.

### The effects of ibuprofen on OA chondrocytes in inflammatory conditions

In inflammatory conditions (ie, in the presence of the OA-related cytokine IL-1β), ibuprofen induced the upregulation of 51 genes while 42 were downregulated in a statistically significant manner with a fold change >1.5 into either direction (table 1). All differentially expressed genes are listed in online supplemental tables S2 and S3.

The upregulated genes included anti-inflammatory factors such as peroxisome proliferator-activated receptor gamma (*PPARG*) and its coactivator *PPARGC1B*

as well as IL-10 receptor subunit alpha. In addition, some genes associated with inflammation, including C-X-C motif chemokine receptor 3 (*CXCR3*), selectin E (*SELE*) and granulocyte-macrophage colony stimulating factor (*CSF2/GM-CSF*) were also upregulated (table 1).

On the other hand, several mediators of inflammation (such as *IL23A*, *IL6* and *NAMPT* (nicotinamide phosphoribosyltransferase aka visfatin)) were downregulated, as was the catabolic enzyme *ADAMTS6* (ADAM metalloproteinase with thrombospondin type 1 motif 6). Insulin-like growth factor-binding protein 4 (*IGFBP4*), which sequesters IGF and regulates chondrocyte proliferation,<sup>4</sup> was also downregulated. Hyaluronan synthase 1 (*HAS1*) and stanniocalcin-1 (*STCI*), previously shown to be upregulated in inflamed OA synovium,<sup>5</sup> were also downregulated by ibuprofen (table 1).

Differential expression of selected inflammation and cartilage-related genes (*PPARG*, *PPARGC1B*, *CSF2*, *IL23*, *HAS1*, *IGFBP4*, *ADAMTS6* and *IL6*) was confirmed with RT-PCR using chondrocytes from a different set of 10 patients (online supplemental figure S4). As expected, IL-1β was shown to strongly increase the synthesis of prostanoids, and this increase was inhibited by ibuprofen (online supplemental figure S5).

When all genes affected by ibuprofen in a statistically significant manner in the presence of IL-1β were analysed with ingenuity pathway analysis (IPA), activated canonical pathways included several associated with inflammation and cell adhesion such as IL-8, integrin, ERK/MAPK and cAMP-mediated signalling pathways (table 2). Conversely, phosphatase and tensin homolog (PTEN) signalling was inhibited (table 2). Differentially expressed genes included in the significantly activated/inhibited pathways are listed in online supplemental table S6.

**Table 2** Canonical IPA pathways significantly upregulated or downregulated (z-score  $\geq 2.5$  or  $\leq -2.5$ ) by ibuprofen in the presence of IL-1 $\beta$

Canonical pathway	adj. P	z-score
Integrin signalling	4.37E-08	<b>4.95</b>
Actin cytoskeleton signalling	0.0022	<b>4.24</b>
PI3K signalling in B lymphocytes	0.00032	<b>3.44</b>
Agrin Interactions at neuromuscular junction	0.0037	<b>3.32</b>
IL-8 signalling	7.08E-07	<b>3.29</b>
ERK5 signalling	0.0083	<b>3.16</b>
Glioblastoma multiforme signalling	1.32E-06	<b>3.14</b>
Paxillin signalling	4.27E-06	<b>3.05</b>
ErbB2-ErbB3 signalling	0.029	<b>3.00</b>
Fc $\gamma$ RIIB signalling in B lymphocytes	0.025	<b>3.00</b>
Renal cell carcinoma signalling	0.0016	<b>3.00</b>
Bladder cancer signalling	6.31E-06	<b>3.00</b>
14-3-3-mediated signalling	0.0058	<b>2.89</b>
PKC $\theta$ signalling in T lymphocytes	0.030	<b>2.84</b>
Calcium signalling	0.0083	<b>2.84</b>
Thrombin signalling	0.0025	<b>2.83</b>
CREB signalling in neurons	0.0019	<b>2.83</b>
HGF signalling	1.15E-06	<b>2.83</b>
Non-small cell lung cancer signalling	0.0029	<b>2.83</b>
$\alpha$ -Adrenergic signalling	5.37E-06	<b>2.71</b>
Endothelin-1 signalling	0.0098	<b>2.68</b>
Mouse embryonic stem cell pluripotency	0.0052	<b>2.67</b>
NF- $\kappa$ B activation by viruses	0.00089	<b>2.67</b>
Macropinocytosis signalling	4.27E-07	<b>2.67</b>
CXCR4 signalling	0.0048	<b>2.67</b>
p70S6K signalling	0.0026	<b>2.67</b>
cAMP-mediated signalling	0.0034	<b>2.56</b>
ErbB4 signalling	0.014	<b>2.53</b>
Chemokine signalling	0.013	<b>2.53</b>
Actin nucleation by ARP-WASP complex	0.00078	<b>2.53</b>
Regulation of cellular mechanics by calpain protease	5.25E-05	<b>2.53</b>
Synaptic long-term potentiation	0.00011	<b>2.52</b>
Cardiac hypertrophy signalling	0.00015	<b>2.50</b>
ERK/MAPK signalling	1.91E-05	<b>2.50</b>
fMLP signalling in neutrophils	0.0012	<b>2.50</b>
PAK signalling	0.00013	<b>2.50</b>
Rac signalling	0.026	<b>2.50</b>
IL-3 signalling	0.0018	<b>2.50</b>
Acute myeloid leukaemia signalling	0.0017	<b>2.50</b>

Continued

**Table 2** Continued

Canonical pathway	adj. P	z-score
Telomerase signalling	0.0011	<b>2.50</b>
Wnt/Ca+pathway	5.25E-05	<b>2.50</b>
PTEN signalling	0.00087	<b>-2.67</b>

adj.P, False discovery rate (FDR) -adjusted P value; CREB, cAMP response element-binding protein; IL-1 $\beta$ , interleukin 1 $\beta$ ; IPA, ingenuity pathway analysis.

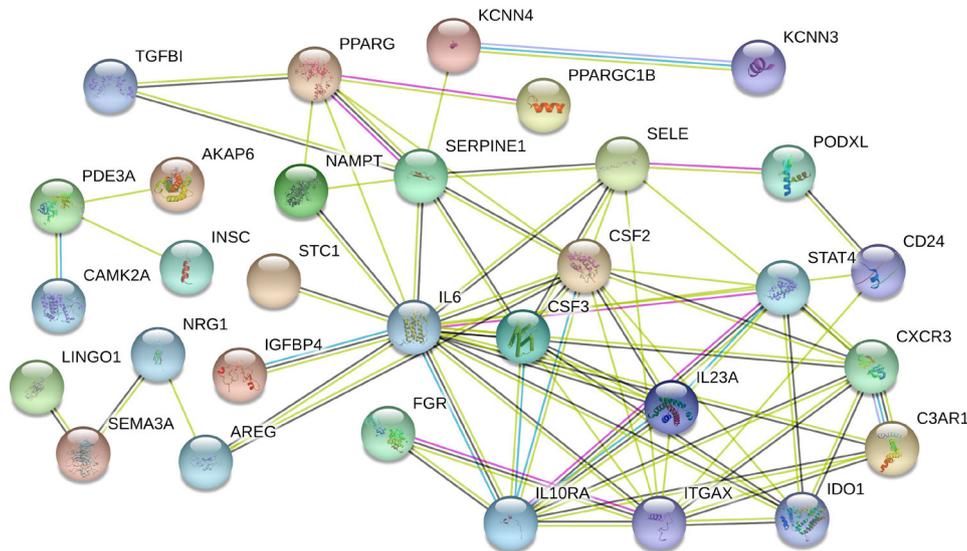
Among the genes with FC >1.5 in either direction, STRING analysis identified *IL6* (which was downregulated by ibuprofen) as a central node in the interaction network (figure 1). Other genes occupying central places include *PPARG*, granulocyte-macrophage colony-stimulating factor and selectin E (*PPARG*, *CSF2* and *SELE* respectively, all upregulated by ibuprofen).

## DISCUSSION

Ibuprofen did not have any significant effects on gene expression in primary OA chondrocytes cultured in the absence of added cytokines. This implies that ibuprofen has a neutral effect on chondrocyte transcriptome in non-inflamed joints. In cells treated with IL-1 $\beta$ , ibuprofen regulated the expression of both proinflammatory and anti-inflammatory factors and seemed to shift the balance to favour the latter.

Ibuprofen is a widely used non-selective NSAID. Like other NSAIDs, it exerts its effects by inhibiting prostanoïd, particularly PGE<sub>2</sub>, synthesis by COX-1 and COX-2 enzymes. In addition to their role as mediators of a pain, prostanoids such as PGE<sub>2</sub> mediate various inflammatory responses. Prostanoids have also been implicated in the pathogenesis OA by affecting cartilage matrix integrity and proteoglycan degradation as well as chondrocyte dedifferentiation and apoptosis.<sup>1-6</sup> Cellular effects of prostanoids are mediated through G-protein coupled receptors; many prostaglandin receptor subtypes, particularly DP<sub>1</sub>, EP<sub>2</sub>, EP<sub>4</sub> and IP,<sup>7</sup> activate adenylate cyclase leading to increased intracellular levels of the multifunctional second messenger cAMP. By activating protein kinase A and transcription factors such as cAMP response element-binding protein, cAMP also regulates the expression of a number of genes.<sup>8</sup> This pathway offers a possible prostanoid-dependent mechanism for the changes in gene expression seen in the present study. In addition, the IPA analysis showed that ibuprofen regulates several other inflammatory pathways which may mediate its effects on chondrocyte transcriptome by prostanoid dependent or independent manner.

In our data, ibuprofen increased the expression of *PPARG* and its coactivator 1 beta (*PPARGC1B*). *PPARG* expression has been shown to be downregulated in OA cartilage,<sup>9</sup> and *PPARG* may affect the pathogenesis of OA by suppressing joint inflammation, downregulating the production of catabolic enzymes and inhibiting



**Figure 1** Interactions among the genes that were upregulated or downregulated by ibuprofen with an FC 1.5 or greater (in either direction) in IL-1 $\beta$ -treated cells. Genes with no identified interactions are excluded from the graph. Colours of the edges: green=activation, blue=binding, black=chemical reaction, red=inhibition, violet=catalysis, pink=posttranslational modification, yellow=transcriptional regulation, grey=other interaction.

chondrocyte apoptosis.<sup>10</sup> Induction of some proinflammatory factors such as *CSF2*/GM-CSF by ibuprofen can be regarded as a potentially deleterious effect, as GM-CSF has been shown to promote OA development and pain.<sup>11</sup> To our knowledge, this is the first study linking NSAIDs to GM-CSF production in chondrocytes.

*IL6* and *IL23A* as well as *ADAMTS6* (ADAM metalloproteinase with thrombospondin type 1 motif 6) are examples of proinflammatory/catabolic factors that were suppressed by ibuprofen. Ibuprofen downregulated also hyaluronan synthase 1 (*HAS1*) and stanniocalcin-1 (*STC1*) both of which have been shown to be upregulated in inflamed OA joints.<sup>5</sup> These data suggest that ibuprofen can, to some extent, ‘normalise’ the phenotype of OA tissue under inflammatory conditions. Notably, *IL23A* was the most strongly downregulated gene in our data. The potential local roles of this proinflammatory cytokine in OA cartilage appear relatively understudied, but its serum levels in patients with OA have been found to be higher compared with controls.<sup>12</sup> IL-6 is considered a central proinflammatory mediator in OA.<sup>13</sup> *HAS1* is one of the three principal enzymes participating in the synthesis of hyaluronan, a central extracellular matrix (ECM) component. It may also promote inflammation by producing pericellular, monocyte-attracting hyaluronan coats.<sup>14</sup> *STC1* is a calcium-regulating and phosphate-regulating protein whose effects on cartilage appear to be complex. It may inhibit cartilage development,<sup>15</sup> but its expression in synovial cells has also been linked to slower OA progression.<sup>16</sup>

Integrin signalling was the IPA pathway most strongly activated by ibuprofen. This is interesting, as dysregulated integrin signalling has been implicated in OA pathogenesis.<sup>17</sup> Other significantly upregulated pathways include several linked to inflammation (such as

IL-8, NF- $\kappa$ B and MAPK/ERK signalling). Looking at the specific genes included in these pathways and affected by ibuprofen (online supplemental table S6) reveals that these can be mostly considered negative feedback genes rather than the major proinflammatory mediators/ effectors of these pathways. Examples include several integrins (*ITGAM*, *ITGAX*, *ITGB2*, *ITGB3* and *ITGB5*) in the IL-8 and NF- $\kappa$ B pathways, growth factors and their receptors (*VEGFA*, *VEGFC*, *HBEGF* and *FGFR3*) in IL-8 signalling as well as anti-inflammatory MAPK phosphatases and PPAR pathway constituents (*DUSP1*, *DUSP2*, *DUSP4*, *PRKARIA*, *PRKAR1B*, *PRKAR2B* and *PPARG*) in MAPK/ERK signalling.

Intriguingly, PTEN signalling was inhibited by ibuprofen. PTEN is a modulator of phosphoinositide 3-kinase/Akt (PI3K/Akt) signalling with various potential effects including promotion of apoptosis, regulation of cell adhesion and inhibition of cell proliferation. *PTEN* is upregulated in OA chondrocytes, where it inhibits the production of ECM components,<sup>18</sup> and interventions that inhibit PTEN slow the development of osteoarthritic changes in cartilage.<sup>19</sup> To our knowledge, PTEN has not previously been linked to NSAIDs in cartilage.

Previous studies have investigated the effects of NSAIDs and COX-2 selective inhibitors on cartilage/synovial explants.<sup>6, 20</sup> Both prostaglandin-mediated and prostaglandin-independent effects have been observed; these include, for example, inhibition of chondrocyte apoptosis, reduction of nitric oxide synthesis as well as reduced production of catabolic MMPs on IL-1 $\beta$  stimulation.<sup>1</sup> Our study expands these results by investigating the whole transcriptome of ibuprofen-treated OA chondrocytes and provides a starting point for future studies.

In conclusion, ibuprofen alone had no significant effects on gene expression in chondrocytes supporting

cartilage safety of COX inhibitors in the treatment of OA pain. When used in a setting of joint inflammation, ibuprofen seems to shift chondrocyte transcriptome towards an anti-inflammatory phenotype.

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