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Effects of *chondroitin sulfate* on the gene expression profile in *IL-1β stimulated* synovial fibroblast cells cultures

C Lambert[1], J-E Dubuc[2], E Montell[3], J Vergés[3] and and Y E Henrotin[1]

[1]Bone and Cartilage Research Unit, University of Liège, Liège, Belgium, [2]Orthopaedic Department, Cliniques Universitaires St Luc, Brussels, Belgium, [3]Pre-Clinical R&D Area, Pharmascience Division, Bioibérica, S.A, Barcelona, Spain

P U R P O S E. Chondroitin sulfate (CS) is one the most used molecules in the management of osteoarthritis (OA). In this study, we performed a microarray analysis and identified a differential expression profile between control and IL-1β stimulated synovial fibroblast cells cultures. In a second step, we investigated the effects of CS on this gene expression profile. ■

M E T H O D S. OA synovial specimens were obtained from 12 patients undergoing knee replacement. Synovial fibroblast cells (SFC) were enzymatically isolated and used after four passages (P4). SFC were pre-treated 1 hour with highly purified bovine CS (200 μg/ml, Bioibérica S.A., Barcelona, Spain) before treatment with IL-1β (1 ng/ml) for 24 hours. Gene expression profiling was performed using Illumina's multi-sample format Human HT-12 BeadChip (Illumina Inc.). Differential analysis was performed with the BRB array tools software. Class comparison test between control (Ctl) and interleukin (IL)-1β conditions, Ctl and Ctl/CS and IL-1β and IL-1β/CS conditions was based on paired t-test where Ctl and IL-1β, Ctl and Ctl/CS and IL-1β and IL-1β/CS were paired for each patient. Probes with a p-value below 0.001 were chosen and classified as up- or down-regulated ones. ■

Category	Up-regulated genes (Fold change)
Cytokines	IL-8 (156.25); IL-6 (58.82); **TNFAIP6** (16.39); **TNFAIP3** (5.55); **IL-32** (4.54); **TNFRSF11B** (2.70); **IL-1β** (2.70); **IL-26** (2.38); **TNFAIP8** (2.22); **TNFSF9** (2.13); **TNFRSF1B** (2.04); **TNFRSF12A** (1.58); **TNFAIP2** (1.53)
Chemokines	**CXCL6** (62.5); **CCL20** (58.82); **CXCL1** (50); **CXCL2** (18.18); **CCL5** (14.92); **CCL2** (9.09); **CCL8** (7.14); **CCL13** (5.26); **CXL12** (2.56); **CCL7** (1.92); **CCL3** (1.78)
Enzymes	**PTGES** (4.54); **PLD1** (2.85); **PLCB1** (1.87); **PI3** (1.88); **PLA2G4A** (1.88)
Metallothioneins	**MT1G** (14.28); **MT2A** (10.86); **MT1A** (10.20); **MT1F** (10); **MTE** (7.69); **MT1X** (6.66); **MT1M** (5.88); **MT1H** (5.88); **MT1E** (5); **MT3** (1.69)

Table 1

Up-regulated inflammatory intermediates between control (Ctl) and IL-1β conditions. The most up-regulated *cytokines* were IL-8 and IL-6. Several chemokines, enzymes and metallothioneins were also identified.

Category	Differentially expressed genes (Fold change)	
	Up-regulated	*Down-regulated*
Angiogenesis	STC1 (9.09); **BDKRB1** (5.8); **RCAN1** (4.16); **ECGF1** (4); **HBEGF** (3.33); **PF4V1** (3.125); **EPAS1** (3.03); **DNER** (2.6); **ESM1** (2.5); **PLAT** (2.2); **BDKRB2** (2.04); **VEGFA** (1.8)	**ANGPTL5** (-1.14); **RNH1** (-1.31)

Table 2

Angiogenesis intermediates between Ctl and IL-1β conditions. Stanniocalcin 1 (STC1) is the most up-regulated with a fold change of 9,09.

Category	Differentially expressed genes (Fold change)	
	Up-regulated	*Down-regulated*
Anabolism	**HAS1** (3.84); **HAS2** (2.13); **BMP1** (1.26)	**HABP4** (-1.25); **HAS3** (-1.48); **CILP** (-1.29); **GPC4** (-1.6); **ACAN** (-1.34)
Catabolism	**MMP3** (62.5)	

Table 3

Anabolism and catabolism intermediates between Ctl and IL-1β conditions. The differential expression of intermediates involved in both cartilage anabolism and catabolism was revealed by the IL-1β stimulation, showing an imbalance in favour of catabolism.

Ctl/CS versus Ctl	IL-1β/CS versus IL-1β
CCL2 (1.59); **IL-6** (1.52); **ALPL** (1.47); **Alpha-tubulin2** (1.45); **BIRC3** (1.37); **BMPER** (1.35); **RDH5** (1.33); **AQP1** (1.33); **SRPX2** (1.32); **CCL13** (1.30); **PDPN** (1.30); **TUBB2C** (1.30); **LOXL4** (1.30); **IL32** (1.28); **MGLL** (1.28); **DRAM1** (1.28); **PAMR1** (1.27); **GAS6** (1.27); **ETV5** (1.25); **ATP5G1** (1.25); **TUBG1** (1.25); **TM4SF1** (1.25); **AIF1L** (1.25); **NTN4** (1.25); **SERPINE1** (1.25); **GAS6** (1.25); **WISP1** (1.23); **TUBA1B** (1.23); **NME1** (1.23); **FJX1** (1.23); **WDR1** (1.23); **GAS6** (1.23); **DKK1** (1.23); **PHLDA1** (1.22); **JUN** (1.22); **MARCH4** (1.22); **TUBA1C** (1.22); **MT2A** (1.22); **HSD3B7** (1.22); **IL8** (1.22); **TUBA1C** (1.20); **TUBA1A** (1.20); **TMEM158** (1.20); **NT5E** (1.20); **PODXL** (1.20); **RERG** (1.20); **LOC345041** (1.20)	**TMEM158** (1.59); LOXL4 (1.52); CLDN11 (1.45); **RDH5** (1.41); **CCL5** (1.33); **CCL5** (1.32); **CCL13** (1.30); **KRT34** (1.30); **DNAJB4** (1.30); **GAS6** (1.28); **MGLL** (1.27); **GLIPR1** (1.27); **CCL3L3** (1.25); **FJX1** (1.23); **ELOVL6** (1.22); **MRGPRF** (1.20); **NT5E** (1.20)

Table 4

Up-regulated genes between Ctl/CS versus Ctl and IL-1β/CS versus IL-1β. *660* genes were identified as differentially expressed between Ctl and Ctl/CS conditions while *241* genes were identified between IL-1β and IL-1β/CS. Among them, our attention was focused on two genes up-regulated in the presence of CS: *LOXL4*: lysyl oxidase-like 4 and *CDLN11*: claudin 11, two genes that negatively regulate cell invasion.

C O N C L U S I O N. We here evidenced in synovial fibroblast cells the modulation of gene expression following IL-1β stimulation. We also demonstrated the modulatory effects of CS on gene expression and isolated several CS-modulated genes of interest such as LOXL4 and CDLN11, which could constitute new mechanisms of action of the molecule and contribute to explain the symptomatic efficacy of CS in the treatment of OA. ■

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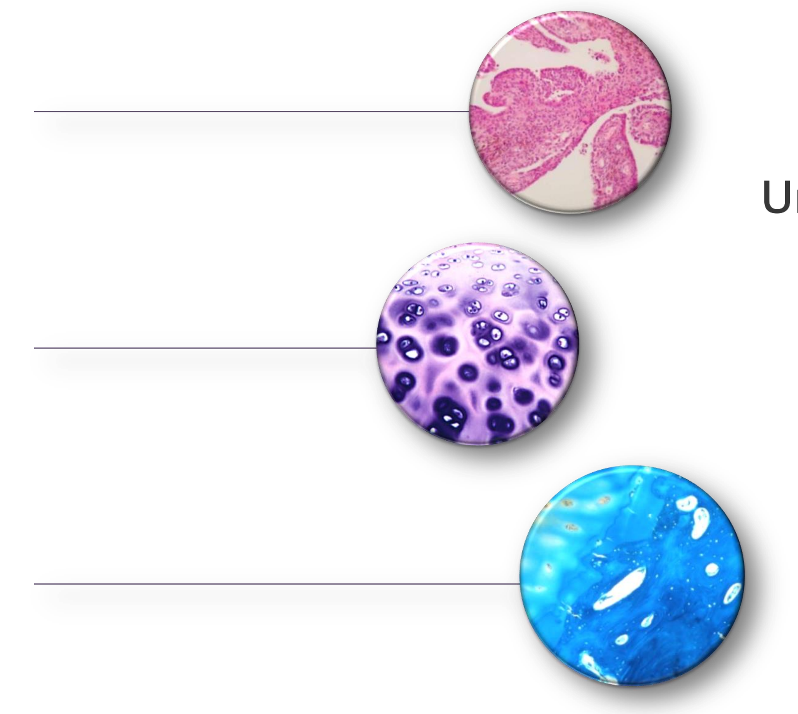

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