Benefit of combining curcumin, harpagophytum and bromelain to reduce inflammation in osteoarthritic synovial cells

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Research Article

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Abstract

Background: Osteoarthritis is the most common cause of arthritis affecting millions of people worldwide, characterized by joint pain and inflammation. It is a complex disease involving inflammatory factors and affecting the whole joint including synovium. Since drug combination is widely used to treat chronic inflammatory diseases, a similar strategy may be worth of interest to design plant-derived natural products to reduce inflammation in OA joint. Here, we characterized the response of OA synovial cells to lipopolysaccharide (LPS) and investigated the biological action of the combination of curcumin, harpagophytum and bromelain in this original in vitro model of osteoarthritis.

Methods: Primary, human synovial cells from OA patients were stimulated with LPS and proteomic analysis was performed. Bioinformatics analysis were performed using Cytoscape App and SkeletalVis databases. Additionally, cells were treated with curcumin, harpagophytum and bromelain alone or the three vegetal compounds together. The expression of genes involved in inflammation, pain or catabolism were determined by RT-PCR. The release of the encoded proteins by these genes and of prostaglandin E2 (PGE2) were also assayed by ELISA.

Results: Proteomic analysis demonstrated that LPS induces the expression of numerous proteins involved in OA process in human OA synovial cells. In particular, it stimulates inflammation through the production of pro-inflammatory cytokines (Interleukin-6, IL-6), the catabolism through an increase of metalloproteases (MMP-1, MMP-3, MMP-13), and the production of pain-mediating neurotrophin (Nerve Growth Factor, NGF). These increases were observed at level of mRNA levels and of protein release. LPS also increases the amount of PGE2, another inflammation and pain mediator. At doses tested, vegetal extracts had little effects: only curcumin slightly counteracted the effects of LPS on NGF and MMP13 mRNA, and PGE2, IL-6 and MMP13 release. In contrast the association of curcumin with harpagophytum and bromelain reversed lots of effects of LPS in human OA synovial cells. It significantly reduced the gene expression and/or the release of proteins involved in catabolism (MMP3 and 13), inflammation (IL-6) and pain (PGE2 and NGF).

Conclusion: We show that the stimulation of human OA synovial cells with LPS permit to induce protein changes similar to an inflamed OA synovial tissues. In addition, using this model, we demonstrate that the combination of three vegetal compounds, namely curcumin, harpagophytum and bromelain have anti-inflammatory and anti-catabolic action in synovial cells and may thus reduce OA progression and related-pain.

Introduction

Osteoarthritis (OA) is a debilitating and painful disease characterized by inflammation of the synovial membrane and the progressive destruction of articular cartilage [1, 2]. It is one of the top ten causes of physical disability [3]; however, its etiology and pathogenesis are still not fully understood. Long
considered as a simple cartilage degenerative disease, OA is now described as a global joint chaos [4]. To date, no treatment has been able to reverse or alter OA progression.

Although OA is not classified as an inflammatory disease, many reports suggested that inflammation could be a major driver of OA development. Actually, elevated joint inflammation has been correlated with progression to the disease [5]. Thus, although OA pathogenesis remains unclear, inflammation is widely regarded as an extremely important factor for progression of this disease [2, 6–8].

Synovitis, i.e. inflammation of synovial tissues, is common in OA [9], and is mediated, in part, by fibroblast like synoviocytes (FLS). These cells play an important factors in OA inflammation and joint destruction, primarily by secreting a wide range of proinflammatory mediators, such as IL-6 and prostaglandin E2 (PGE2) [9], which leads the release of neurotrophins, such as NGF, participating to pain during OA, as well as the secretion of various type of proteases, including MMPs and a disintegrin and MMP with thrombospondin motifs (ADAMTS) [10], promoting the degradation of extracellular cell matrix (ECM), and further aggravating the progression of OA. Therefore, alleviating synovial inflammation may prevent the onset or minimize the progression of OA and symptoms [2, 11–13]. Conventional anti-inflammatory drugs are nonsteroidal anti-inflammatory drugs (NSAIDs) [14], however, they have several side effects and drug interactions including the risk of gastrointestinal, cardiovascular, and kidney problems. Used natural compounds may be a relevant alternative.

Herbal medicine has been used from ancient times to the present day for healing purposes. Curcumin (CUR) which is extracted from the rhizome of *Curcuma longa* L. is one of the most ancient medicinal herbs and is broadly used in human health due to its various therapeutic effects such as anti-inflammatory, antioxidant, anticancer and antimicrobial [15]. In patients with OA, oral administration of curcumin ameliorates the clinical manifestations of the disease [16–19], improves quality of life and enables a decrease in the consumption of NSAIDs [20]. This beneficial effect of curcumin is associated to its ability to reduce OA inflammation in cells, animal models, and human studies [21, 22]. The action of curcumin may be reinforced by combination with other natural compounds [15, 23].

The purpose of the present study was to investigate the effects of the combination of curcumin (CUR), bromelain (BRO), a food obtained from pineapple and having analgesic properties [24], and harpagophytum (HAR), a traditional remedy for articular diseases [25]), on inflammation in an original experimental *in vitro* model of osteoarthritis, using human synovial cells treated with lipopolysaccharide (LPS).

**Material And Methods**

**Reagents**

Lipopolysaccharide (LPS) from *E.Coli*, Sigma Aldrich, Saint Louis, USA) was dissolved in phosphate buffer saline without Calcium and Magnesium (DPBS, Lonza, Bâle, Suisse), to reach a concentration of 1 mg/ml and used at a final concentration of 1 µg/ml. Curcumin (Tumeric extract granule, 95%
curcuminoids, Natural, St Sylvain d’Anjou, France) was resuspended in dimethylsulfoxide (DMSO, Dutscher, Bernolsheim, Belgium). Bromelain (Bromelain 2500 GDU, Cambridge Commodities Ltd, Ely, UK) and Harpagophytum (Harpagophytum procumbens, Biosearch Life, Granada, Spain) extracts, the suspension was carried out in DPBS.

**Culture cells and treatments**

Human synoviocytes were recovered from the hip synovial membrane of 6 patients undergoing hip replacement surgery (age mean = 75 years). The cells were released by digestion of the synovial membrane with type I collagenase (2 mg/ml, 12 hours; ThermoFisher, Waltham, USA). The cells were incubated in Dulbecco's modified Eagle medium high glucose with glutamine and sodium pyruvate (DMEM, Dutscher), supplemented with 10% Fetal Bovine Serum (FBS, Dutscher), penicillin-streptomycin (Lonza) then incubated at 37 ° C in a humid atmosphere, containing 5% CO2.

To achieve the desired number of cells, passages were performed. The cells were rinsed with DPBS, then detached with 0.05% trypsin (ThermoFisher). The cells were recovered in culture medium and seeded at approximately 7500 cells / cm². The absence of mycoplasmas were checked by PCR.

The cells were processed at confluence. Treatments were diluted in new culture medium to the desired concentration. Each molecule was tested alone or in the presence of LPS. The three molecules were also tested together in order to see the effects of the combination of these three extracts, in the presence or not of LPS.

**Protein extraction**

Cells were lysed and protein extracted using Radio Immuno Precipitation Assay (RIPA) Buffer (50mM Tris-HCl pH 7.5; 1% Igepal CA-630; 150mM NaCl; 1mM EGTA; 1mM NaF; 0.25% Na-deoxycholate; Distilled water) supplemented with protease inhibitor (Leupeptine 1mg / ml; Phenyl methyl sulfonyl fluoride 200mM; pepstatin A 1mg/ml) and phosphatase inhibitor (sodium orthovanadate 200mM) as previously described [26].

**Proteomic experiment**

Five µg of each protein extract were prepared using a modified Gel-aided Sample Preparation protocol [27]. Samples were digested with trypsin/Lys-C overnight at 37°C. For nano-LC fragmentation, protein or peptide samples were first desalted and concentrated onto a µC18 Omix (Agilent) before analysis.

The chromatography step was performed on a NanoElute (Bruker Daltonics) ultra-high-pressure nano flow chromatography system. Approximatively 200ng of each peptide sample were concentrated onto a C18 pepmap 100 (5mm x 300µm i.d.) precolumn (Thermo Scientific) and separated at 50°C onto a reversed phase Reprosil column (25cm x 75µm i.d.) packed with 1.6µm C18 coated porous silica beads (Ionopticks). Mobile phases consisted of 0.1% formic acid, 99.9% water (v/v) (A) and 0.1% formic acid in 99.9% ACN (v/v) (B). The nanoflow rate was set at 400 nl/min, and the gradient profile was as follows:
from 2 to 15% B within 60 min, followed by an increase to 25% B within 30 min and further to 37% within 10 min, followed by a washing step at 95% B and reequilibration.

MS experiments were carried out on an TIMS-TOF pro mass spectrometer (Bruker Daltonics) with a modified nano electrospray ion source (CaptiveSpray, Bruker Daltonics). The system was calibrated each week and mass precision was better than 1 ppm. A 1600 spray voltage with a capillary temperature of 180°C was typically employed for ionizing. MS spectra were acquired in the positive mode in the mass range from 100 to 1700 m/z. In the experiments described here, the mass spectrometer was operated in PASEF mode with exclusion of single charged peptides. A number of 10 PASEF MS/MS scans was performed during 1.25 seconds from charge range 2–5.

Before post-process, the samples are analysed using Preview software (ProteinMetrics) in order to estimate the quality of the tryptic digestion and predict the post-translational modifications present. The result, below, is used for the “bank research / identification” part. The fragmentation pattern was used to determine the sequence of the peptide. Database searching was performed using the Peaks X + software. A UniProt Homo sapiens database (October 2020) was used. The variable modifications allowed were as follows: Nterm-acetylation, methionine oxidation, Deamidation (NQ), Methylation (KR) and Carbamylation. In addition, C-Propionoamide was set as fix modification. “Trypsin” was selected as Specific. Mass accuracy was set to 30 ppm and 0.05 Da for MS and MS/MS mode, respectively. Data were filtering according to a FDR of 0.5% and the elimination of protein redundancy on the basis of proteins being evidenced by the same set or a subset of peptides.

**Identification of differentially expressed proteins**

To quantify the relative levels of protein abundance between different groups, samples were analysed using the label-free quantification feature of PEAKS X + software. Feature detection was separately performed on each sample by the expectation-maximization based algorithm. The features of the same peptide from all replicates of each sample were aligned through the retention time alignment algorithms. Mass error tolerance was set at 30 ppm, Ion Mobility Tolerance (1/k0) at 0.07 and retention time tolerance at 10 min. Normalization factors of the samples were obtained by the total ion current (TIC) of each sample. Quantification of the protein abundance level was calculated using the sum area of the top three unique peptides. A 1.5-fold increase in relative abundance and a significance ≥ 5 using ANOVA as significance method were used to determine those enriched proteins

**Enrichment analysis et comparison with datasets related to skeletal biology**

Heatmap was performed with a Spearman clustering method using ComplexHeatmap package from R.

Enrichments in molecular process, cellular process and pathways (KEGG and Reactome) were performed using ClueGo App from Cytoscape software. Network specificity was set to medium; GO tree interval was set between 2 and 4. Clusters were performed using a selection set to 3 min genes in addition to 4% of genes. Enrichments were performed using a Bonferroni step down method.
Additionally, differentially expressed proteins were compared to existing gene expression datasets related to skeletal biology using SkeletalVis application (http://skeletalvis.ncl.ac.uk/skeletal/,[28]). Proteins encoded by genes associated with osteoarthritis joint damage in animals were also identified using OATargets databases [29].

**RNA extraction and RT-PCR**

RNA was extracted from the cell layer using the kit RNeasy mini kit (Qiagen, Hilden, Germany) according to the supplier's protocol. DNase treatment and the reverse transcription were, then, carried out using the kit DNase I (Sigma Aldrich) and the reverse transcriptase M-MLV (Invitrogen, Carlsbad, USA) as previously described [30]. Next, cDNA was amplified by real-time PCR using PCR master Mix (Power Syber Green, Applied biosystems, Courtaboeuf, France) and read on Step One Plus Real Time PCR system (Applied Biosystems) with the following primers: RPL13A Forward: 5’-GAGGTATGCTGCCCCACAAA-3’ and Reversed: 5’-GTGGGATGCCGTCAAACAC-3’; NGF Forward: 5’-AGCGCAGCGAGTTTTGG-3’ and Reversed: 5’-AGAAAGCTGCTCCCTTGTA-3’; IL-6 Forward: 5’-CACACAGACAGCCACTCACC-3’ and Reversed: 5’-TTTCACCAGGAAGTCTCCT-3’; MMP1 Forward: 5’-GAAGCTGCTTACGAATTTTGCGG-3’ and Reversed: 5’-CCAAAGGAGCTGTAGTGTCTT-3’; MMP3 Forward: 5’-TAAAGACAGCCACTT TTGGCGC-3’ and Reversed: 5’-TTGGGTATCCAGCTCGTACCTC-3’; MMP13 Forward: 5’-AAGGAGCATGCGACTTCTTCT-3’ and Reversed: 5’-TGGCAGGAAAGAC-3’. The relative mRNA level was calculated with the $2^{-\Delta\Delta CT}$ method. RPL13a was used as the invariant housekeeping gene internal control. The choice of this gene is based on our previously experience on the field.

**ELISA**

PGE2 and MMP release into conditioned media was quantified using commercially available enzyme immunoassay kit (R&D Biosystem) as previously [26]. For IL-6, we proceeded in the same way but using the Human beta-NGF ELISA Kit and Human IL-6 ELISA kit (Sigma Aldrich). The immunoassays were all carried out following manufacturer protocol. Absorbance was determined at 450 nm with a wavelength correction set at 540 nm using Multiskan GO spectrophotometer (Thermo Scientific).

**Statistical analyses**

All results are expressed as the mean of 3 or 4 patients (biological replicates) ± standard error of the mean (SEM). Statistical analyzes are carried out on GraphPad prism 8 software. After checking the normal distribution of samples, two-way ANOVA's tests were used for multiple comparisons. In significant cases, Tukey's multiple comparison test for matched samples were performed as post-hoc analysis. P-values < 0.05 were considered significant.

**Results**

**Stimulation of human OA synovial cells with LPS, an efficient OA model in vitro.**
Lipopolysaccharide (LPS) is recently considered as a trigger for the pathology of OA, and is used to model inflammatory component of OA. So, we planned to test the effects of curcumin, harpagophytum and bromelain in human OA synovial cells stimulated with LPS. Before, we wanted to validate the model and its ability to model OA inflammation. So, we did proteomic analysis to define differential expressed proteins between unstimulated OA synovial cells and LPS-stimulated OA synovial cells. 2917 proteins were identified in the control group, and 3011 in the LPS treated-group. Among them, 106 proteins were differentially expressed between the two groups (Peaks Sign >5, Fold-change > 1.5, figure 1 and table 1). More precisely, 66 proteins (i.e. 62%) were significantly downregulated by LPS, and 40 (i.e. 38%) were upregulated by LPS. ClueGo analysis revealed that these differentially expressed proteins are mainly involved in the biological processes of oxidative stress-induced cell death (45%, p-value < 0.01) and in the molecular processes of intramolecular oxidoreductase activity (25%, p-value < 0.01) and collagen binding (12.5%, p-value < 0.01) (figures 2, tables 2 and 3). Furthermore, pathway enrichment by KEGG (figure 3A, table 4) showed the presence of proteins involved in protein digestion and absorption, fructose and mannose metabolism and antigen processing and presentation (33% for each, p-value < 0.01).

Enrichment using Reactome (figure 3B, table 5) shows also the presence of proteins involved in assembly of collagen fibrils and other multimeric structures (24%, p-value < 0.05).

The comparison by signature of the proteomic profile between control and LPS stimulated synovial cells using skeletalvis database, which permits to explore skeletal biology related expression datasets [28], suggests that deregulated proteins were encoded by genes which are also differentially expressed in several other OA models (suppl data 1), namely “Synovial cells from inflammatory and normal areas of osteoarthritis synovial membrane” (signed jaccard (sig) = 0.015; zscore = 5.08) and observed in “Rat model of surgically induced knee osteoarthritis” (signed jaccard (sig) = 0.0118; zscore = 3.98). Besides, using OATargets databases [29], we could observe that several identified proteins were encoded by genes associated to OA, such as Thrombpondin-1 (THBS1), collagen alpha-1(VI) chain (COL6A1), superoxide dismutase [Mn] mitochondrial (SOD2) and Nicotinamide phosphoribyltransferase (NAMPT) (table 1). In addition, about half of these gene was also found at least once as a human OA DEG, and around 90% are known to interact with OA genes (table 1).

Together this proteomic analysis clearly confirms that LPS-stimulated synovial cells from OA human patients are good model to study osteoarthritis process in vitro.

**LPS increases the expression of genes associated to inflammation, catabolism and pain**

Next, using a most targeted strategy, we investigated the effect of LPS treatment in human OA synovial cells. After 24 hours of treatment, LPS stimulated inflammation through the production of pro-inflammatory cytokines (Interleukin-6, IL-6), the catabolism through an increase of metalloproteases (MMP-1, MMP-3, MMP-13), and the production of pain-mediating neurotrophin (Nerve Growth Factor, NGF). These increases were observed at level of mRNA levels and of protein release. LPS also increased the amount of PGE2, another pain mediator (figure 4).
The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to catabolism

Having validated our model, we continued by studying the effect of vegetal extracts (curcumin bromelain, and harpagophytum) on OA associated genes. At doses tested, vegetal extracts had little effects on the expression of catabolic genes. Only curcumin slightly counteracted the effects of LPS on MMP13 mRNA and protein release. However, the association of curcumin with harpagophytum and bromelain reversed effects of LPS on the mRNA levels of MMP1, MMP3 and MMP13, and on the release of MMP3 and MMP13 proteins (figure 5). These data suggest that the combination of curcumin, bromelain and harpagophytum may reduce cartilage degradation during OA process.

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain

Next, we investigated the effect of these vegetal compounds on the expression of genes involved in inflammation and pain (figure 6). We observed that only curcumin was able to slighty reduce the LPS-induced expression of NGF and the release of PGE2 and IL-6. Interestingly, the association of the three vegetal compounds (curcumin, harpagophytum and bromelain) significantly reduced the gene expression of IL-6 and NGF mRNA expression. It also decreases the IL-6 release and the production of PGE2. This suggest that the combination of the three compound may reduce inflammation and pain.

Discussion

To date, no efficient treatment exists to treat osteoarthritis. Consequently, the identification of strategies able to slow down OA progression and usable in the long term is crucial. Some natural compounds are known to present anti-oxidative and anti-inflammatory actions. So, they may be an alternative to pharmacological drugs. Herein, after a proteomic characterization of the in vitro OA model which was used in the study and validation that it is able to induce changes in gene expression profile similar to that is observed during OA, we showed that the combination of curcumin, harpagophytum and bromelain is efficient to counteract numerous LPS-induced effects in human OA synovial cells.

First, we evaluate the potential of lipopolysaccharide to induce changes in gene/protein expression mimicking some features of OA. LPS is an endotoxin and a classical activator of the innate immune system. Because of its pathophysiological properties, LPS has been used to induce arthritis in conjunction with collagen in animal models [31, 32]. More recently, researchers have started to connect LPS with the pathogenesis of OA [33]. LPS is released by gut microbiota and is correlated with the pathophysiology of osteoarthritis, in part through the activation of macrophages. In addition, local LPS administration to joints induce synovitis and is used as a model to evaluate potential treatments for acute synovitis [34].

Since LPS is now considered as a trigger for the pathology of OA, especially by activating synovial cells, we have proposed that stimulated human OA synovial cells may induce inflammation and reproduce in


vitro some changes observed during OA process. Using proteomics, we demonstrate here that treatment of human OA synovial cells with LPS induces the expression of signature genes of OA, and in particular reproduces some gene expression changes observed in synovial cells from inflammatory and normal areas of osteoarthritis synovial membrane. A more targeted strategy shows for instance that LPS induces the expression of MMPs, IL-6, PGE2 and NGF, which are mainly markers of catabolism, inflammation and pain in joints. Consequently, the stimulation of human OA synovial cells by LPS appears a good in vitro model to study inflammation during OA. Knowing that alleviating inflammation may prevent the onset or minimize the progression of OA [2, 11, 12, 33], we propose to use this in vitro model to test the ability of several natural substances to reduce inflammation.

First, we show that curcumin have some anti-catabolic and anti-inflammatory action in human OA synovial cells. This is in agreement with literature which demonstrate that curcumin reduces MMP-3 and MMP-13 expression in rabbit chondrocytes and in the articular cartilage of estrogen-deficient rats, preventing collagen degradation [35, 36]. Also, curcumin prevents the activation of nuclear factor kappaB (NF-κB), the major mediator of inflammation [36, 37]. Another study shows that curcumin favors cartilage anabolism by increasing type II collagen synthesis [22, 38].

We also investigated the effect of Harpagophytum, commonly known as devil’s claw, a plant used worldwide as a traditional remedy for joint pain associated with OA and mild rheumatic ailments [25, 39, 40]. Moreover, it has been described to have analgesic effects on neuropathic pain in rats [41]. We also studied the effect of bromelain, a food supplement that is sometimes describes as an alternative treatment to nonsteroidal anti-inflammatory drug (NSAIDs) [42]. Bromelain has analgesic properties [43, 44] and relieves OA [24]. However, at dose tested, neither Harpagophytum nor bromolain show significant effect on the expression of studied genes, including NGF or PGE2, which are known to be related to joint pain. However, the association of these vegetal components with curcumin permits to counteract numerous effect of LPS in human OA stimulated cells. The combination of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain, but also catabolism. This reinforced action of curcumin by combination with other natural compounds was already described [15]. For instance, the combination treatment with Lactobacillus acidophilus LA-1, vitamin B, and curcumin ameliorates the progression of osteoarthritis by inhibiting the pro-inflammatory mediators [23]. However, at our knowledge, this paper is the first to show the benefice to associated curcumin with bromelain and Harpagophytum.

In conclusion, we describe the changes in protein expression induced by LPS in human OA synovial cells and demonstrating that they are characteristic to inflamed OA synoviocytes, suggesting that this in vitro model may be useful to evaluate inflammation during OA. In addition, we showing that the combination of three natural vegetal components reduced expression of genes involved in catabolism, inflammation and pain, suggesting that together, they may present a beneficial effect on OA patients by alleviating OA pain and synovial inflammation, and reducing cartilage degradation.

Declarations
Ethics approval and consent to participate

The experimental protocol was approved by the local ethical committee “Comité de Protection des Personnes Nord-Ouest III” (agreement # A13-D46-VOL.19). The consent of each participant was obtained prior to surgery. They all signed agreement forms, in accordance with local law. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional file.

Competing interests

Thierry Conrozier received fees from LABRHA for scientific consultant and speaker services.

The other authors declare that they have no competing interests.

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This study was founded from Labhra laboratory, which supplied also vegetal compounds. The funding lab had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors’ contributions

CB participated in the conception, design of the study, analysis of data and drafted the manuscript. SB carried out the experiments and analysis data. BB and JP did proteomic experiments and analysis. TC participated to the coordination and conception of the study and design. KB participated in the conception and design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: List of deregulated proteins in LPS-stimulated synovial cells
| Accession | Group Profile (Ratio) | Gene names (primary) | Description | OA associated | human induced DEG | induced OA DEG | OA gene interaction | skeletal phenotype |
|-----------|-----------------------|---------------------|-------------|---------------|-------------------|---------------|---------------------|-------------------|
| Proteins down-regulated by LPS |
| Q92598    | 0.23                  | HSPH1               | Heat shock protein 105 kDa | false          | 2                 | 8             | 14                  | false             |
| P49327    | 0.25                  | FASN                | Fatty acid synthase          | false          | 3                 | 7             | 17                  | false             |
| Q9Y3C0    | 0.26                  | WASHC3              | WASH complex subunit 3       | false          | 1                 | 1             | 3                   | false             |
| Q9H4B7    | 0.32                  | TUBB1               | Tubulin beta-1 chain         | false          | 5                 | 1             | 3                   | false             |
| P45877    | 0.36                  | PPIC                | Peptidyl-prolyl cis-trans isomerase C | false     | 8                 | 9             | 0                   | false             |
| Q12768    | 0.43                  | WASHC5              | WASH complex subunit 5       | false          | 0                 | 0             | 2                   | false             |
| P07996    | 0.45                  | THBS1               | Thromboponin-1               | true           | 4                 | 2             | 19                  | true              |
| Q8WWI1    | 0.45                  | LMO7                | LIM domain only protein 7    | false          | 3                 | 5             | 7                   | false             |
| Q8WX93    | 0.47                  | PALLD               | Palladin                     | false          | 3                 | 2             | 5                   | false             |
| Q8NHPP    | 0.48                  | PLBD2               | Putative phopholipase B-like 2 | false     | 0                 | 0             | 2                   | false             |
| Q8NE86    | 0.49                  | MCU                 | Calcium uniporter protein mitochondrial | false     | 0                 | 0             | 0                   | false             |
| O60831    | 0.51                  | PRAF2               | PRA1 family protein 2         | false          | 1                 | 3             | 0                   | false             |
| Q9UMX0    | 0.52                  | UBQLN1              | Ubiquilin-1                   | false          | 1                 | 1             | 15                  | false             |
| Q9NR12    | 0.52                  | PDLIM7              | PDZ and LIM domain protein 7 | false     | 5                 | 3             | 7                   | false             |
| O00154    | 0.52                  | ACOT7               | Cytolic acyl coenzyme A thioester hydrolase | false     | 0                 | 3             | 1                   | false             |
| Q9Y305    | 0.53                  | ACOT9               | Acyl-coenzyme A thioesterase 9 mitochondrial | false     | 0                 | 4             | 6                   | false             |
| Q71U36    | 0.53                  | TUBA1A              | Tubulin alpha-1A chain       | true           | 1                 | 1             | 31                  | false             |
| P15374    | 0.53                  | UCHL3               | Ubiquitin carboxyl-terminal hydrolase isozyme L3 | false     | 0                 | 7             | 5                   | false             |
| Q04760    | 0.53                  | GLO1                | Lactoylglutathione lyase      | false          | 0                 | 3             | 3                   | true              |
| P30419    | 0.53                  | NMT1                | Glycylpeptide N-tetradecanoyltransferase 1 | false     | 1                 | 1             | 4                   | false             |
| P55809    | 0.54                  | OXCT1               | Succinyl-CoA:3-ketoacid coenzyme A transferase 1 mitochondrial | false     | 2                 | 4             | 3                   | false             |
| O43504    | 0.55                  | LAMTOR5             | Ragulator complex protein LAMTOR5 | false     | 0                 | 1             | 4                   | false             |
| P62841    | 0.55                  | RPS15               | 40S ribosomal protein S15    | false          | 1                 | 5             | 9                   | false             |
| P36776    | 0.55                  | LONP1               | Lon protease homolog mitochondrial | false     | 4                 | 3             | 7                   | false             |
| Q12849    | 0.55                  | GRSF1               | G-rich sequence factor 1      | false          | 0                 | 1             | 2                   | false             |
| Q5JRX3    | 0.55                  | PITRM1              | Presequence protease mitochondrial | false     | 2                 | 3             | 2                   | false             |
| Q8TDQ7    | 0.55                  | GNPDA2              | Glucamine-6-phphate isomerase 2 | false     | 0                 | 1             | 1                   | false             |
| P34932    | 0.56                  | HSPA4               | Heat shock 70 kDa protein 4   | false          | 0                 | 2             | 39                  | false             |
| Q15691    | 0.56                  | MAPRE1              | Microtubule-associated protein RP/EB family member 1 | false     | 0                 | 0             | 13                  | false             |
| P24539    | 0.56                  | ATP5PB              | ATP synthase F(0) complex subunit B1 mitochondrial | false     | 0                 | 0             | 6                   | false             |
| P00491    | 0.56                  | PNP                 | Purine nucleide phosphorylase | false     | 6                 | 3             | 1                   | false             |
| gene ID | score | gene symbol | gene name | alternatively spliced | start | end | frame | phase | splicing event flag |
|--------|-------|-------------|-----------|----------------------|-------|-----|-------|-------|-------------------|
| P69905 | 0.56  | HBA1; HBA2  | Hemoglobin subunit alpha | false   | 7    | 0   | 3     | false |                   |
| Q15008 | 0.57  | PSMD6       | 26S proteasome non-ATPase regulatory subunit 6 | false   | 0    | 5   | 4     | false |                   |
| P02768 | 0.57  | ALB         | Albumin   | false   | 2    | 0   | 9     | false |                   |
| Q9UHB6 | 0.57  | LIMA1       | LIM domain and actin-binding protein 1 | false   | 2    | 4   | 9     | true  |                   |
| Q15843 | 0.57  | NEDD8       | NEDD8     | false   | 0    | 3   | 9     | false |                   |
| P15848 | 0.58  | ARSB        | Arylsulfatase B | false   | 3    | 6   | 1     | true  |                   |
| O95202 | 0.58  | LETM1       | Mitochondrial proton/calcium exchanger protein | false   | 0    | 2   | 3     | false |                   |
| P12109 | 0.59  | COL6A1      | Collagen alpha-1(VI) chain | true   | 6    | 12  | 9     | false |                   |
| Q9UNZ2 | 0.59  | NSFL1C      | NSFL1 cofactor p47 | false   | 0    | 1   | 8     | false |                   |
| Q9Y5L4 | 0.59  | TIMM13      | Mitochondrial import inner membrane translocase subunit Tim13 | false   | 0    | 3   | 3     | false |                   |
| P55795 | 0.61  | HNRNPH2     | Heterogeneous nuclear ribonucleoprotein H2 | false   | 0    | 2   | 5     | true  |                   |
| Q9H008 | 0.61  | LHPP        | Phosphotyrosine phosphohistidine inorganic pyrophosphate phosphatase | false   | 4    | 5   | 0     | false |                   |
| P12111 | 0.62  | COL6A3      | Collagen alpha-3(VI) chain | false   | 6    | 16  | 2     | true  |                   |
| O14745 | 0.62  | SLC9A3R1    | Na(+/)+H(+) exchange regulatory cofactor NHE-RF1 | false   | 1    | 4   | 11    | true  |                   |
| P98179 | 0.62  | RBM3        | RNA-binding protein 3 | false   | 1    | 1   | 5     | false |                   |
| Q14318 | 0.62  | FKBP8       | Peptidyl-prolyl cis-trans isomerase FKBP8 | false   | 1    | 2   | 10    | true  |                   |
| O94826 | 0.62  | TOMM70      | Mitochondrial import receptor subunit TOM70 | false   | 0    | 2   | 4     | false |                   |
| P48509 | 0.62  | CD151       | CD151 antigen | false   | 0    | 1   | 0     | false |                   |
| O75348 | 0.62  | ATP6V1G1    | V-type proton ATPase subunit G 1 | false   | 1    | 2   | 0     | false |                   |
| P60953 | 0.63  | CDC42       | Cell division control protein 42 homolog | true    | 0    | 0   | 19    | true  |                   |
| Q9BRA2 | 0.63  | TXNDC17     | Thioredoxin domain-containing protein 17 | false   | 1    | 1   | 0     | false |                   |
| Q9BRF8 | 0.63  | CPPED1      | Serine/threonine-protein phosphatase CPPED1 | false   | 2    | 0   | 0     | false |                   |
| Q07666 | 0.64  | KHDRBS1     | KH domain-containing RNA-binding signal transduction-associated protein 1 | false   | 0    | 2   | 16    | true  |                   |
| Q16795 | 0.64  | NDUFA9      | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9 mitochondrial | false   | 0    | 2   | 6     | false |                   |
| O75431 | 0.64  | MTX2        | Metaxin-2 | false   | 0    | 1   | 0     | false |                   |
| Q9C0H2 | 0.64  | TTYH3       | Protein tweety homolog 3 | false   | 1    | 4   | 0     | false |                   |
| Q5JPE7 | 0.64  | NOMO2       | Nodal modulator 2 | false   | 0    | 0   | 1     | false |                   |
| P69849 | 0.64  | NOMO3       | Nodal modulator 3 | false   | 0    | 0   | 1     | false |                   |
| P00966 | 0.65  | ASS1        | Argininosuccinate synthase | false   | 4    | 2   | 4     | false |                   |
| O75531 | 0.65  | BANF1       | Barrier-to-autointegration | false   | 0    | 2   | 2     | false |                   |
| P14324 | 0.66 | FDPS | Farnesyl pyrophosphate synthase | false | 0 | 2 | 3 | false |
| P60983 | 0.66 | GMFB | Glia maturation factor beta | false | 1 | 2 | 1 | false |

**Protein up-regulated by LPS**

| Q96AT9 | 51.49 | RPE | Ribulose-phosphate 3-epimerase | false | 0 | 0 | 3 | false |
| Q14684 | 4.09 | PTGES | Prostaglandin E synthase | false | 8 | 4 | 0 | false |
| P43490 | 3.35 | NAMPT | Nicotinamide phosphoribyltransferase | true | 8 | 0 | 4 | false |
| Q43776 | 2.66 | NARS1 | Asparagine–tRNA ligase cytoplasmic | false | 0 | 0 | 3 | false |
| Q5VYK3 | 2.56 | ECPAS | Proteasome adapter and scaffold protein ECM29 | false | 1 | 0 | 8 | false |
| P12955 | 2.47 | PEPD | Xaa-Pro dipeptidase | false | 0 | 5 | 5 | true |
| P10301 | 2.33 | RRAS | Ras-related protein R-Ras | false | 3 | 1 | 5 | false |
| P19827 | 2.27 | ITIH1 | Inter-alpha-trypsin inhibitor heavy chain H1 | false | 0 | 1 | 1 | false |
| P15121 | 2.04 | AKR1B1 | Aldo-keto reductase family 1 member B1 | false | 0 | 4 | 3 | false |
| Q14828 | 1.96 | SCAMP3 | Secretory carrier-associated membrane protein 3 | false | 1 | 1 | 4 | false |
| P07711 | 1.95 | CTS | Cathepsin L1 | false | 4 | 0 | 3 | false |
| Q6IBS0 | 1.92 | TWF2 | Twinfilin-2 | false | 0 | 5 | 0 | false |
| P04179 | 1.9 | SOD2 | Superoxide dismutase [Mn] mitochondrial | true | 7 | 7 | 8 | false |
| P54709 | 1.85 | ATP1B3 | Sodium/potassium-transporting ATPase subunit beta-3 | false | 1 | 3 | 3 | false |
| Q9Y3Z3 | 1.84 | SAMHD1 | Deoxynucleide triphosphate triphohydrolase SAMHD1 | false | 1 | 2 | 3 | false |
| Q9Y3A6 | 1.84 | TMED5 | Transmembrane emp24 domain-containing protein 5 | false | 3 | 3 | 0 | false |
| Q13501 | 1.79 | SQSTM1 | Sequestome-1 | false | 3 | 1 | 42 | true |
| Q96JL7 | 1.79 | TMX3 | Protein disulfide-isomerase TMX3 | false | 0 | 0 | 0 | false |
| O75828 | 1.78 | CBR3 | Carbonyl reductase [NADPH] 3 | false | 2 | 5 | 3 | false |
| P35613 | 1.78 | BSG | Basigin | false | 2 | 0 | 7 | false |
| P26599 | 1.76 | PTBP1 | Polypyrimidine tract-binding protein 1 | false | 2 | 0 | 11 | false |
| Q9Y295 | 1.76 | DRG1 | Developmentally-regulated GTP-binding protein 1 | false | 0 | 1 | 2 | false |
| Q7L523 | 1.74 | RRAGA | Ras-related GTP-binding protein A | false | 0 | 3 | 2 | false |
| P61009 | 1.7 | SPCS3 | Signal peptidase complex subunit 3 | false | 2 | 1 | 1 | false |
| Q96HE7 | 1.69 | ERO1A | ERO1-like protein alpha | false | 5 | 1 | 1 | false |
| PDB ID  | Score | Protein                         | Description                                                                 | Enriched? | Gene1 | Gene2 | Gene3 | Enriched? |
|---------|-------|---------------------------------|-----------------------------------------------------------------------------|-----------|-------|-------|-------|-----------|
| Q9UL46  | 1.66  | PSME2                           | Proteasome activator complex subunit 2                                      | false     | 0     | 0     | 2     | false     |
| Q13724  | 1.63  | MOGS                            | Mannyl-oligaccharide glucidase                                              | false     | 0     | 2     | 5     | false     |
| Q9Y5P6  | 1.63  | GMPPB                           | Manne-1-phphate guanyltransferase beta                                       | false     | 2     | 2     | 1     | false     |
| P19525  | 1.61  | EIF2AK2                         | Interferon-induced double-stranded RNA-activated protein kinase             | false     | 1     | 1     | 18    | false     |
| P02794  | 1.59  | FTH1                            | Ferritin heavy chain                                                        | false     | 3     | 1     | 5     | false     |
| P18085  | 1.58  | ARF4                            | ADP-ribylation factor 4                                                     | false     | 2     | 10    | 10    | false     |
| P23381  | 1.57  | WARS1                           | Tryptophan--tRNA ligase cytoplasm                                           | false     | 0     | 0     | 3     | false     |
| P63244  | 1.56  | RACK1                           | Receptor of activated protein C kinase 1                                    | false     | 1     | 0     | 22    | false     |
| Q9NZ08  | 1.56  | ERAP1                           | Endoplasmic reticulum aminopeptidase 1                                     | false     | 1     | 1     | 3     | false     |
| O95747  | 1.55  | OXSR1                           | Serine/threonine-protein kinase R1                                          | false     | 1     | 1     | 6     | false     |
| P17858  | 1.54  | PFKL                            | ATP-dependent 6-phophofructokinase liver type                                | false     | 1     | 3     | 6     | false     |
| P08195  | 1.53  | SLC3A2                          | 4F2 cell-surface antigen heavy chain                                        | false     | 6     | 0     | 7     | false     |
| Q9UNN8  | 1.53  | PROCR                           | Endothelial protein C receptor                                              | false     | 4     | 8     | 0     | false     |
| P04439  | 1.51  | HLA-A                           | HLA class I histocompatibility antigen A alpha chain                        | false     | 1     | 0     | 5     | false     |
| P50991  | 1.5   | CCT4                            | T-complex protein 1 subunit delta                                           | false     | 0     | 3     | 11    | false     |

**Table 2: Enrichment in biological processes**
| GOID      | GOTerm                                                                 | Term PValue | % Associated Genes | Nr. Genes | Associated Genes Found                     |
|-----------|------------------------------------------------------------------------|-------------|--------------------|-----------|--------------------------------------------|
| GO:0006521| regulation of cellular amino acid metabolic process                    | 0.007       | 4.48               | 3         | [BSG, PSMD6, PSME2]                        |
| GO:0016667| oxidoreductase activity, acting on a sulfur group of donors            | 0.005       | 5.00               | 3         | [ERO1A, TMX3, TXNDC17]                     |
| GO:0071230| cellular response to amino acid stimulus                               | 0.001       | 5.56               | 4         | [ASS1, COL6A1, LAMTOR5, RRAGA]             |
| GO:0072523| purine-containing compound catabolic process                           | 0.004       | 5.36               | 3         | [ACOT7, PNP, SAMHD1]                       |
| GO:1901569| fatty acid derivative catabolic process                                | 0.000       | 18.75              | 3         | [ACOT7, LYPLA2, OXCT1]                    |
| GO:1901661| quinone metabolic process                                             | 0.001       | 8.11               | 3         | [AKR1B1, CBR3, NDUFA9]                    |
| GO:1990928| response to amino acid starvation                                     | 0.003       | 5.88               | 3         | [EIF2AK2, FASN, RRAGA]                    |
| GO:0034198| cellular response to amino acid starvation                            | 0.003       | 6.25               | 3         | [EIF2AK2, FASN, RRAGA]                    |
| GO:0070671| response to interleukin-12                                            | 0.005       | 5.08               | 3         | [CDC42, PSME2, SOD2]                      |
| GO:0035722| interleukin-12-mediated signaling pathway                             | 0.004       | 5.36               | 3         | [CDC42, PSME2, SOD2]                      |
| GO:0040019| positive regulation of embryonic development                          | 0.002       | 6.98               | 3         | [AKR1B1, OXSR1, RACK1]                    |
| GO:0071470| cellular response to osmotic stress                                   | 0.004       | 5.66               | 3         | [AKR1B1, LETM1, OXCT1]                    |
| GO:0051181| cofactor transport                                                    | 0.005       | 5.17               | 3         | [BSG, OXSR1, SLC9A3R1]                    |
| GO:0072337| modified amino acid transport                                         | 0.001       | 10.00              | 3         | [BSG, OXSR1, SLC9A3R1]                    |
| GO:0061245| establishment or maintenance of bipolar cell polarity                 | 0.004       | 5.66               | 3         | [ARF4, CDC42, SLC9A3R1]                   |
| GO:0035088| establishment or maintenance of apical/basal cell polarity            | 0.004       | 5.66               | 3         | [ARF4, CDC42, SLC9A3R1]                   |
| GO:0045197| establishment or maintenance of epithelial cell apical/basal polarity | 0.003       | 6.25               | 3         | [ARF4, CDC42, SLC9A3R1]                   |
| GO:0007006| mitochondrial membrane organization                                    | 0.000       | 4.05               | 6         | [ATP5PB, HSPA4, LETM1, MTX2, NMT1, TIMM13] |
| GO:0051205| protein insertion into membrane                                       | 0.007       | 4.48               | 3         | [HSPA4, NMT1, TIMM13]                     |
| GO:0090151| establishment of protein localization to mitochondrial membrane       | 0.003       | 5.88               | 3         | [HSPA4, NMT1, TIMM13]                     |
| GO:0051204| protein insertion into mitochondrial membrane                          | 0.003       | 6.38               | 3         | [HSPA4, NMT1, TIMM13]                     |
| GO:1902882| regulation of response to oxidative stress                            | 0.000       | 4.90               | 5         | [BSG, NONO, RACK1, SOD2, UBQLN1]          |
| GO:1902883| negative regulation of response to oxidative stress                  | 0.000       | 6.67               | 4         | [BSG, NONO, RACK1, SOD2]                  |
| GO:0036473| cell death in response to oxidative stress                            | 0.000       | 5.00               | 5         | [BSG, NONO, RACK1, SOD2, UBQLN1]          |
| GO:1900407| regulation of cellular response to oxidative stress                  | 0.000       | 5.38               | 5         | [BSG, NONO, RACK1, SOD2, UBQLN1]          |
| GO:1900408| negative regulation of cellular response to oxidative stress         | 0.000       | 6.90               | 4         | [BSG, NONO, RACK1, SOD2]                  |
| GO:0008631| intrinsic apoptotic signaling pathway in response to oxidative stress | 0.003       | 6.25               | 3         | [NONO, SOD2, UBQLN1]                      |
| GO:1903201| regulation of oxidative stress-induced cell death                    | 0.000       | 6.33               | 5         | [BSG, NONO, RACK1, SOD2, UBQLN1]          |
| GO:0036475| neuron death in response to oxidative stress                          | 0.001       | 9.09               | 3         | [BSG, NONO, RACK1]                        |
### Table 3: Enrichment in molecular functions

| GOID           | GOTerm                                      | Term PValue | % Associated Genes | Nr. Genes | Associated Genes Found                     |
|----------------|---------------------------------------------|-------------|--------------------|-----------|--------------------------------------------|
| GO:0005518     | collagen binding                            | 0.009       | 4.05               | 3         | [COL6A1, CTS]                              |
| GO:0016790     | thiolester hydrolase activity               | 0.000       | 10.00              | 4         | [ACOT7, ACOT9, FASN, LYPLA2]               |
| GO:0019210     | kinase inhibitor activity                    | 0.008       | 4.29               | 3         | [GMFB, RACK1, WARS1]                       |
| GO:0042169     | SH2 domain binding                          | 0.002       | 6.52               | 3         | [KHDRBS1, RACK1, SQSTM1]                   |
| GO:0042805     | actinin binding                             | 0.002       | 6.98               | 3         | [LM07, PALLD, PDLM7]                      |
| GO:0051117     | ATPase binding                              | 0.002       | 4.30               | 4         | [AKR1B1, ATP1B3, ATP6V1G1, NSFL1C]         |
| GO:0016667     | oxidoreductase activity, acting on a sulfur | 0.005       | 5.00               | 3         | [ERO1A, TMX3, TXNDC17]                     |
|                 | group of donors                             |             |                    |           |                                            |
| GO:0016860     | intramolecular oxidoreductase activity       | 0.000       | 6.67               | 4         | [ERO1A, GNPDA2, PTGES, TMX3]               |

### Table 4: Enrichment using KEGG

| GOID           | GOTerm                                      | Term PValue | % Associated Genes | Nr. Genes | Associated Genes Found                     |
|----------------|---------------------------------------------|-------------|--------------------|-----------|--------------------------------------------|
| KEGG:00051     | Fructose and mannose metabolism            | 0.002       | 9.09               | 3         | [AKR1B1, GMPPB, PFK]                       |
| KEGG:04612     | Antigen processing and presentation        | 0.003       | 5.13               | 4         | [CTSL, HLA-A, HSPA4, PSME2]                |
| KEGG:04974     | Protein digestion and absorption           | 0.005       | 4.21               | 4         | [ATP1B3, COL6A1, COL6A3, SLC3A2]           |

### Table 5: Enrichment using reactome
| GOID       | GOTerm                                                                 | Term PValue | % Associated Genes | Nr. Genes | Associated Genes Found                      |
|------------|-------------------------------------------------------------------------|-------------|--------------------|-----------|---------------------------------------------|
| R-HSA:1268020 | Mitochondrial protein import                                           | 0.001       | 6.25               | 4         | [MTX2, PITRM1, TIMM13, TOMM70]              |
| R-HSA:917937 | Iron uptake and transport                                              | 0.009       | 5.17               | 3         | [ATP6V1G1, FTH1, NEDD8]                     |
| R-HSA:9639288 | Amino acids regulate mTORC1                                            | 0.008       | 5.45               | 3         | [ATP6V1G1, LAMTOR5, RRAGA]                  |
| R-HSA:210991 | Basigin interactions                                                    | 0.001       | 12.00              | 3         | [ATP1B3, BSG, SLC3A2]                      |
| R-HSA:2173782 | Binding and Uptake of Ligands by Scavenger Receptors                    | 0.000       | 9.52               | 4         | [ALB, FTH1, HBA1, HSPH1]                   |
| R-HSA:447115 | Interleukin-12 family signaling                                        | 0.009       | 5.26               | 3         | [CDC42, PSME2, SOD2]                       |
| R-HSA:8950505 | Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation | 0.003       | 7.89               | 3         | [CDC42, PSME2, SOD2]                       |
| R-HSA:9020591 | Interleukin-12 signaling                                               | 0.005       | 6.38               | 3         | [CDC42, PSME2, SOD2]                       |
| R-HSA:1442490 | Collagen degradation                                                    | 0.012       | 4.69               | 3         | [COL6A1, COL6A3, CTSL]                     |
| R-HSA:1474290 | Collagen formation                                                     | 0.005       | 4.44               | 4         | [CD151, COL6A1, COL6A3, CTSL]              |
| R-HSA:186797 | Signaling by PDGF                                                      | 0.009       | 5.17               | 3         | [COL6A1, COL6A3, THBS1]                    |
| R-HSA:2022090 | Assembly of collagen fibrils and other multimeric structures            | 0.001       | 6.56               | 4         | [CD151, COL6A1, COL6A3, CTSL]              |
| R-HSA:216083 | Integrin cell surface interactions                                     | 0.004       | 4.71               | 4         | [BSG, COL6A1, COL6A3, THBS1]               |
| R-HSA:1632852 | Macroautophagy                                                         | 0.001       | 4.41               | 6         | [LAMTOR5, RRAGA, SQSTM1, TOMM70, TUBA1A, TUBB1] |
| R-HSA:2995410 | Nuclear Envelope (NE) Reassembly                                       | 0.019       | 4.00               | 3         | [BANF1, TUBA1A, TUBB1]                     |
| R-HSA:389957 | Prefoldin mediated transfer of substrate to CCT/TriC                   | 0.001       | 10.71              | 3         | [CCT4, TUBA1A, TUBB1]                      |
| R-HSA:389958 | Cooperation of Prefoldin and TriC/CCT in actin and tubulin folding     | 0.002       | 9.38               | 3         | [CCT4, TUBA1A, TUBB1]                      |
| R-HSA:389960 | Formation of tubulin folding intermediates by CCT/TriC                  | 0.001       | 12.00              | 3         | [CCT4, TUBA1A, TUBB1]                      |
| R-HSA:5626467 | RHO GTPases activate IQGAPs                                            | 0.002       | 9.38               | 3         | [CDC42, TUBA1A, TUBB1]                     |
| R-HSA:8852276 | The role of GTSE1 in G2/M progression after G2 checkpoint              | 0.000       | 6.49               | 5         | [MAPRE1, PSMD6, PSME2, TUBA1A, TUBB1]       |
| R-HSA:9663891 | Selective autophagy                                                    | 0.003       | 4.94               | 4         | [SQSTM1, TOMM70, TUBA1A, TUBB1]            |
Figure 1

Heatmap showing differentially expressed proteins by LPS in human synovial cells. Human synovial cells from three different patients were treated with LPS (1µg/ml) for 48h. At the end of experiments, proteins were extracted and proteomics analysis performed. Differentially expressed proteins between control group and LPS group is shown (n=3).
Enrichment in biological process and molecular function. From differentially expressed proteins between group control and LPS (figure 1), enrichments in biological process (A) and molecular process (B) were performed. Diagrams shows part of each GO Terms which were statistically enriched. *: p-value<0.05, **: p-value<0.01.
Enrichment in functional pathways. From differentially expressed proteins between group control and LPS (figure 1), enrichments pathways using KEGG (A) or Reactome (B) datasets were performed. Diagrams shows part of each GO Terms which were statistically enriched. *: p-value<0.05, **: p-value<0.01.
Figure 4

LPS induces gene expression and release in medium of markers of catabolism, inflammation and pain. Human synovial cells were treated with LPS (1µg/ml) for 24h. At the end of experiments, RNA was extracted. Relative mRNA expression of MMP-1, MMP-3, MMP-13, NGF and IL-6 was determined by RT-PCR. Values are compared to untreated cells and presented as log Fold Change (compared to control group). Culture medium were collected and ELISA performed to assayed MMP, IL-6 and PGE2.
concentration in medium. Values are expressed as µg/ml medium (n=4). *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Figure 5

The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to catabolism. Human synovial cells were treated with LPS (1 µg/ml) for 24h in the presence of curcumin (13 µM) with bromelain (14.7 µg/ml), and harpagophytum (36 µg/ml),

Figure 5
or altogether. At the end of experiments, RNA were extracted and medium collected. Relative mRNA expression of MMP1, MMP3, MMP13 were determined by RT-PCR. Culture media were also collected and ELISA performed to assayed MMP release in medium. Values were compared to LPS-treated cells and presented as relative expression (compared to LPS group). n=3. *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

Figure 6
The association of curcumin with bromelain and harpagophytum significantly reduced the LPS-induced expression of genes associated to inflammation and pain. Human synovial cells were treated with (1 µg/ml) for 24h in the presence of curcumin (13 µM) with bromelain (14.7 µg/ml), and harpagophytum (36 µg/ml), or altogether. At the end of experiments, RNA were extracted and medium collected. Relative mRNA expression of NGF and IL-6 were determined by RT-PCR. Culture media were also collected and ELISA performed to assay IL-6 and PGE2 release in medium. Values were compared to LPS-treated cells and presented as relative release (compared to LPS group). n=3. *: p-value<0.05, **: p-value<0.01, ***: p-value<0.001.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supp1data1SkeletalVis.xls