Proteomic analisys of human articular chondrocytes treated with glucosamine sulphate

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Introduction

Osteoarthritis is the most common joint disease in the world, and it is rapidly becoming a major public health issue among the aged population. The initiating event of osteoarthritis is still unknown and believed to be multifactorial. It is characterized by quantitative and qualitative destructive changes in the architecture and composition of all the joint structures. Chondrocytes are the only cell type that is present in mature cartilage, and is responsible for repairing the damaged tissue.

In vitro and in vivo studies on chondrocytes and experimental animal model treated with symptomatic slow-acting drugs, such as glucosamine compounds, have suggested their capability to inhibit cartilage destruction and to promote disease suppression (Scotto d'Abusco et al., 2007). However, their exact mechanisms have not been completely elucidated. In order to describe more clearly the effects of glucosamine compounds on cartilage biology, we have analyze whether glucosamine sulphate (GS) is able to modify the protein expression profile of normal chondrocytes stimulated with interleukin-1β (IL-1β) and/or to modify the protein expression profile of osteoarthritic chondrocytes in basal condition.

Materil and methods

Chondrocytes were obtained from 3 osteoarthritic patients undergoing joint replacement, and from 1 healthy donor. Normal chondrocytes were treated with GS 10mM and IL-1β 10ng/mL, while osteoarthritic chondrocytes were treated with GS 10mM alone. Whole cell proteins were isolated 24 hours after cellular stimulation and resolved by two-dimensional electrophoresis (2-DE) as previously described (Ruiz-Romero et al; 2005). The gels were stained with SYPRORuby and digitized using a CCD. The images analysis was performed using the PDQuest 7.3.1 computer software. Using PDQuest tools, protein spots were enumerated, quantified and characterized with respect to their molecular mass and isoelectric point by bilinear interpolation between landmark features on each image. Protein expression data from each gel were normalized for the total density present in the gel images. Some of the altered proteins were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry.

Results

We examined a mean of 500 protein spots that were present in each gel. Both qualitative and quantitative changes in protein expression patterns between controls (normal chondrocytes stimulated with IL-1β and osteoarthritic chondrocytes in basal condition) and treated cells (normal chondrocytes stimulated with IL-1β + GS 10 mM and osteoarthritic chondrocytes stimulated with GS 10 mM alone) were studied. In normal chondrocytes, 39 protein spots were modulated by GS treatment compare to the control condition. In osteorthritic chondrocytes, 11 protein spots were found to be statistically altered in GS treated cells compare to the untreated cells ($p<0.05$). Identified proteins are listed in the Table. The most of them (36,8%) are involved in cellular metabolism (in particular glycolysis), protein folding (36,8%) and stress response (10,5%).

Conclusions

This preliminary study describes the differences between the protein expression profiles of normal and osteoarthritic chondrocytes treated with glucosamine sulphate. We have identified novel molecular targets that maybe could explain the beneficent effects of glucosamine sulphate in osteoarthritic treatment.

Protein identified in normal chondrocytes			
NAME	Protein ID	Ratio IL-1β +GS/IL-1β	SSP (PDQuest)
Protein disulfide-isomerase precursor	PDIA1	6.39	611
78 kDa glucose-regulated protein precursor	GRP78	7.89	802
Heat shock cognate 71 kDa protein	HSP7C	7.57	1820
Protein disulfide-isomerase A3 precursor	PDIA3	10.89	2610
Protein disulfide-isomerase A3 precursor	PDIA3	10.00	3601
Superoxide dismutase [Mn], mitochondrial precursor	SODM	0.17	7002
Annexin A2	ANXA2	4.01	7201
Annexin A2	ANXA2	2.63	7210
Alpha-enolase	ENOA	0.50	7402
Pyruvate kinase isozymes M1/M2	KPYM	0.61	8607
Protein identified in osteoarthritic chondrocytes			
NAME	Protein ID	Ratio GS/untreated cells	SSP (PDQuest)
14-3-3 protein theta (14-3-3 protein tau)	1433T	6.53	206
Calumenin precursor (Crocalbin)	CALU	-2.27	602
Endoplasmin precursor (GRP94)	ENPL	2.70	1701
Endoplasmin precursor (GRP94)	ENPL	-2.1	1702
L-lactate dehydrogenase B chain	LDHB	2.73	4302
Isocitrate dehydrogenase [NADP] cytoplasmic	IDH1	-1.4	7605
Inosine-5'-monophosphate dehydrogenase 2+ Glucose-6-phosphate 1-dehydrogenase	IMDH2+ G6PD	No in GS	7706
Destrin (Actin-depolymerizing factor)	DEST	-14.3	9002

Bibliografía

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Alteraciones en proteínas mitocondriales de condrocitos articulares humanos descritas mediante técnicas proteómicas

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Introducción

La mitocondria esta involucrada en numerosos procesos celulares, y se ha demostrado que disfunciones mitocondriales están asociadas con apoptosis, envejecimiento y numerosas condiciones patológicas, incluyendo la osteoartritis (OA). El objetivo de este trabajo es analizar los cambios en las proteínas mitocondriales características de condrocitos OA mediante técnicas de proteómica.