Identification of novel biological markers of osteoarthritis by a proteomic approach

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Aim of the study

A proteomic approach was used to discover novel osteoarthritis specific biological markers by comparing the protein profile of urines of healthy subjects, or patients with osteoarthritis or osteoporosis.

Methods

Urine samples were collected from 10 women (76 ± 5 years) undergoing knee replacement surgery due to severe OA, four age-matched women with severe osteoporosis (OP) and five young healthy women (CTRL) (25.6 ± 2.6 years) without clinical signs of joint disorders. Proteins were separated by two-dimensional difference gel electrophoresis (2D-DIGE), and the proteins with significantly increased or decreased expression in the OA sample were subject to identification by tandem mass spectrometry.

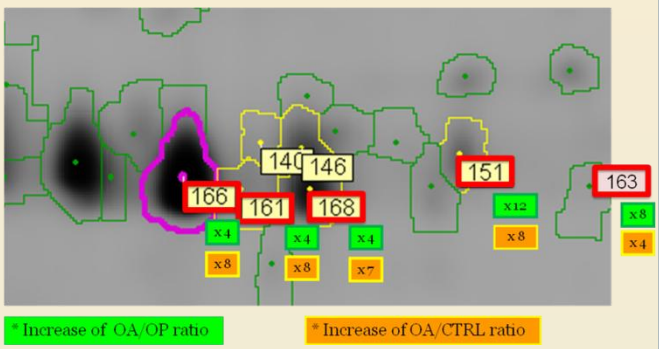
Results

Protein abundance modifications OA *VS* CTRL

OA/CTRL ratio	Protein description	Swiss-Prot accession
4.18	Beta-actin	P60709
2.28	AMBP protein precursor	P02760
2.2	Fibulin-3	Q12805
	Apoptosis-inducing factor 2	Q9BRQ8
2.01	Zn-α-2-glycoprotein precursor	P25311
	Fibulin-3	Q12805
2	Zn-α-2-glycoprotein precursor	P25311
1.83	Poly-Ig receptor (PIGR)	P01833
1.6	Poly-Ig receptor (PIGR)	P01833
1.54	GP36b	Q12907
-1.64	Kininogen-1 precursor	P01042
	A1AT	P01009
-1.68	Transferrin	P02787
-1.7	Kininogen-1 precursor	P01042
	Alpha 1 anti-trypsin (A1AT)	P01009
-1.83	Kininogen-1 precursor	P01042
-1.89	Kininogen-1 precursor	P01042
	A1AT	P01009
-1.91	Kininogen-1 precursor	P01042
	A1AT	P01009
-2.3	Mannan-binding lectin serine protease 2	E.C 3.4.21.104
-2.44	Serpin B3	P29508
-5.84	Serpin B1	P30740

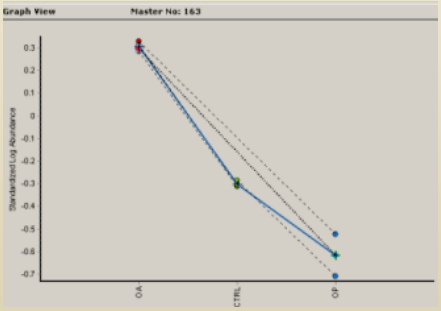
List of identified proteins with abundance change in osteoarthritis (OA) compared to control (C). Spots intensity was increased or decreased at least 1.5 times in OA compared to CTRL urine samples (t-test: $p < 0.05$).

Enhanced portion of 2D-DIGE gel of proteins extracted from OA or OP patients and CTRL. Selected spots (166,161,168,151,163) contain fragments of fibulin-3 identified by mass spectrometry.



Five spots containing fibulin-3 sequences were increased by 4 to 8 fold according the isoform in urine of patients with OA compared with those of CTRL, but decreased by 4 to 12 fold according the isoform in OP urine compared with OA.

Particularly, two forms of fibulin 3 (spots 151 and 163) presented a stronger decrease in OP/OA ratio than in CTRL/OA ratio.



Graph view of fibulin-3 abundance modification based on spot 163 volume decrease in OP and CTRL urine compared to OA.

Conclusion

Some of the proteins identified are known to be implicated in the inflammatory process, for example, the kininogen precursor or alpha-1-antitrypsin. This observation coincides with the pathology of osteoarthritis. A significant increase in the concentration of specific fibulin-3 fragments was observed in OA subjects compared to CTRL and OP subjects. Fibulin-3 is expressed in cartilage and bone structures during development and may play a role in the skeletal system. It is known to be intimately associated with TIMP-3, an inhibitor of metalloproteinase involved in the pathogenesis of OA. These data suggest that fibulin-3 fragments could be useful biomarkers of OA.