PROTEOMIC ANALYSIS OF OSTEOBLASTS SECRETOME

provides new insights in mechanisms underlying osteoarthritis SUBCHONDRAL BONE SCLEROSIS

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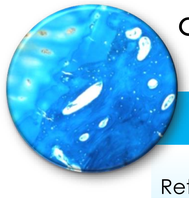
PURPOSE. Osteoarthritis (OA) is characterized by cartilage degradation but also by other joint tissues modifications like subchondral bone sclerosis. In this study, we used a proteomic approach to compare secretome of osteoblast isolated from sclerotic (SC) or non sclerotic (NSC) area of OA subchondral bone.

METHODS. Secretome was analyzed using differential quantitative and relative label free analysis on nanoUPLC G2 HDMS system. mRNA of the more differentially secreted proteins were then quantified by RT-PCR and the most relevant proteins identified using western-blotting and immunoassays.

RESULTS



OSTEOBLASTS SECRETOME → MS/MS → 175 PROTEINS IDENTIFIED

Confirmed by mRNA (5 other patients)

Decreased in sclerosis (ratio SC/NSC)	Increased in sclerosis (ratio SC/NSC)	NSC only	SC only
SPARC (0.8, p<0.001, n=5)	SERPIN F1 (1.3, p=0.01, n=5)		**CSF1** (n=3)
Reticulocalbin 1 (0.7, p=0.01, n=4)	TIMP-1 (1.4, p<0.001, n=5)		
Periostin (0.7, p<0.001, n=5)	IGFBP3 (1.5, p<0.001, n=4)		
TSP4 (0.6, p=0.04, n=3)	TIMP-2 (1.7, p=0.01, n=4)		
IGFBP2 (0.6, p=0.01, n=4)	**a-2-HS-glycoprotein** (1.9, p<0.001, n=3)		
Calumenin (0.6, p<0.001, n=5)	Reticulocalbin3 (1.8, p=0.01, n=5)		
Versican (0.6, p<0.001, n=5)	**SERPIN E1** (1.9, p<0.001, n=5)		
78 kDa glucose-regulated protein (0.5, p<0.001, n=4)	**SH3BGRL3** (1.9, p<0.001, n=5)		
IGF2 (0.5, p<0.001, n=4)	**IGFBP6** (2, p<0.001, n=5)		
VCAM1 (0.4, p<0.001, n=5)	**SERPIN E2** (2.4, p<0.001, n=3)		
Osteomodulin (0.4, p=0.021, n=5)	**Fibulin-3** (2.5, p<0.001, n=5)		
IGFBP5 (0.4, p<0.001, n=5)	**CHI3L1** (7.1, p<0.001, n=4)		

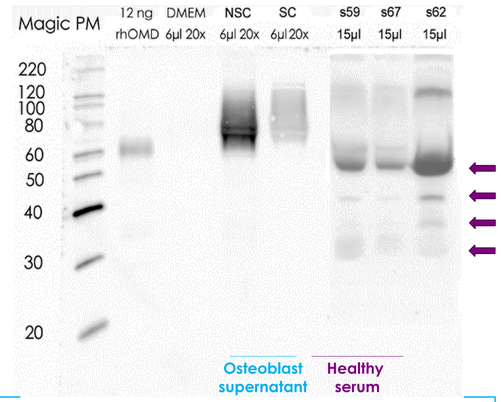


Fig. 1 : Osteomodulin (OMD) western-blot
OMD found in osteoblasts culture supernatants or in human serum

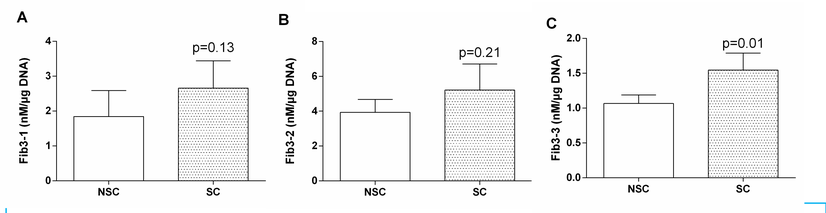
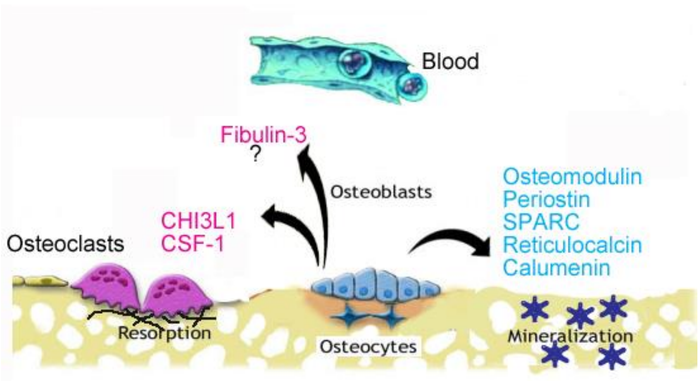


Fig. 2 : Fibulin-3 ELISA
Fibulin-3 epitopes found in osteoblasts culture supernatants



CONCLUSION

We highlighted some **proteins differentially secreted by the osteoblasts coming from OA subchondral bone sclerosis**. These changes contribute to explain some features observed in OA subchondral bone, like the **increase of bone remodeling** or **abnormalities in bone matrix mineralization**. Among identified proteins, **osteomodulin** was found decreased and **fibulin-3** increased in serum of OA patients. These findings suggest that osteomodulin, CHI3L1 and fibulin-3 could be biomarkers to monitor early changes in subchondral bone metabolism in OA.