

Fibulin-3 fragments (Fib3-1 and Fib3-2) : potential new biomarkers for the diagnosis of osteoarthritis

Y. Henrotin¹, M. Gharbi², G. Mazzucchelli³, J.-E. Dubuc⁴, E. De Pauw³, M. Deberg²

¹ Bone and Cartilage Research Unit, University of Liege, Belgium, ²Artialis SA, Liege, Belgium, ³Mass Spectrometry Laboratory, University of Liege, Belgium, ⁴Cliniques Universitaires St Luc, Brussels, Belgium

Background

Fibulin-3 is a member of the fibulin family protein, newly recognized as extracellular matrix protein that negatively regulates chondrocyte differentiation. Using proteomic analysis, we have found elevated levels of two fragments of fibulin-3 (Fib3-1 and Fib3-2) in the urine of osteoarthritic (OA) patients compared to those of healthy or osteoporotic subjects.

Objectives

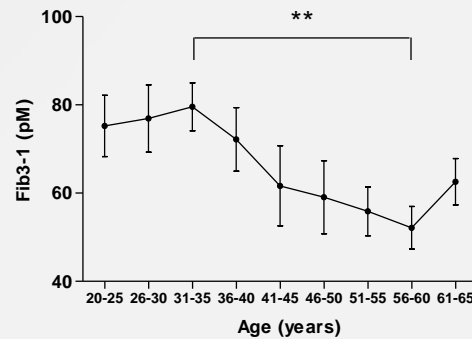
The aim of this study was to measure Fib3-1 and Fib3-2 in serum of healthy subjects and OA patients.

Methods

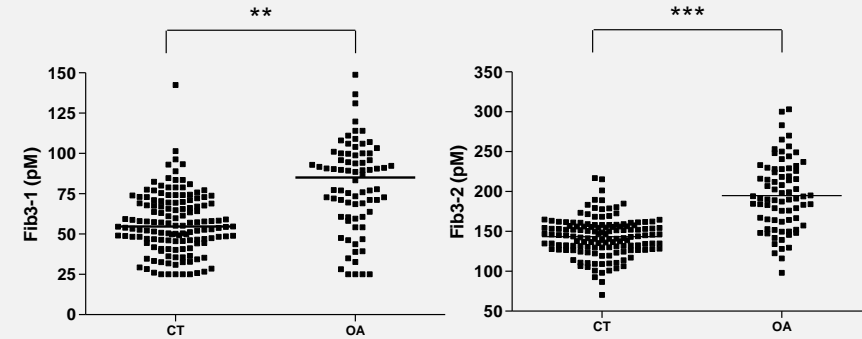
Specific immunoassays were developed using two rabbit antisera with high specificity for Fib3-1 and Fib3-2 respectively, ELISAs were validated in serum population collected from healthy subjects and patients with knee OA. Antisera did not recognize native fibulin-3 and did not cross-react with each fragment. Immunohistochemistry analysis was performed on articular cartilage from tibial plateaus

Fib3-1 and Fib3-2 in healthy population

Median of concentration for Fib3-1 in serum of normal subjects was 58.5 pM. Linear regression showed a modification of Fib3-1 concentration in women serum with age ($r=0.72$). Considering Fib3-2 fragment, we observed that neither the age, nor the gender modified the serum level. The median concentration of Fib3-2 in serum of normal subjects was 140.8 pM ($n = 236$).

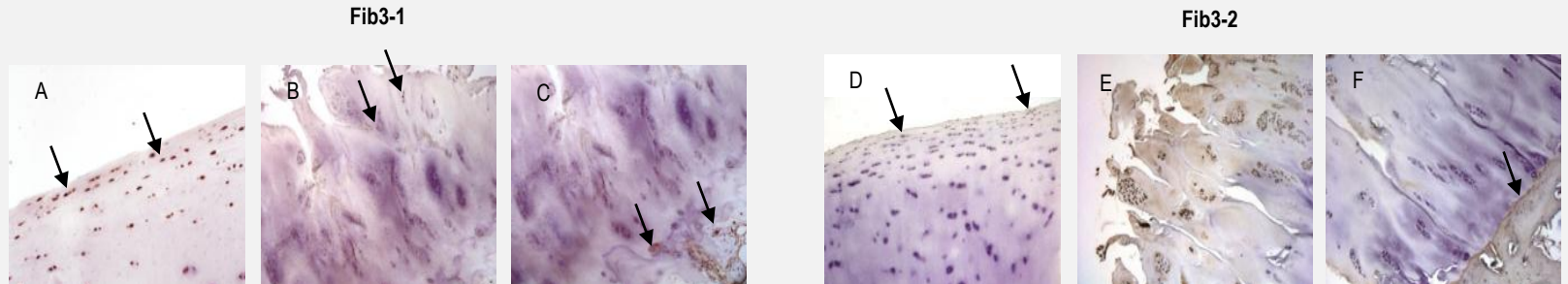


Fib3-1 and Fib3-2 in OA population



In comparison with age-matched healthy subjects, **Fib 3-1 and Fib3-2 were higher in OA ($p<0.0001$)**. Medians of Fib3-1 concentration was 54.6 pM in healthy subjects and 85.1 pM in OA; medians of Fib3-2 concentration was 144.4 pM in healthy subjects and 191.4 pM in OA ($n_{CT} = 140$; $n_{OA} = 76$).

Immunolocalization of Fib3-1 and Fib3-2



In healthy cartilage, antibodies labeled a thin layer of flattened chondrocytes at the surface of cartilage. A weak staining of superficial zone of extracellular matrix was also observed. There was a similar pattern of staining with both antisera (A, D).

In OA cartilage, a strong labeling was found with Fib3-2 antibodies in extracellular matrix and in cell clusters (E). A discrete labeling was found with Fib3-1 antibodies (B). Inner zone of cartilage appeared not labeled. A clearly distinct limit appears between trabecular bone zone which is strongly stained and the overlaying cartilage (C, F).

Conclusion

By comparison with healthy subjects, Fib3-1 and Fib3-2 serum levels are found elevated in patients with knee OA. Furthermore, the immunohistochemical findings indicate that fibrillated cartilage is a major source of fibulin-3 fragments.