

OSTEOARTHRITIC SCLEROTIC SUBCHONDRAL OSTEOBLASTS

secreted elevated concentrations of FIBULIN-3 FRAGMENTS *in vitro*

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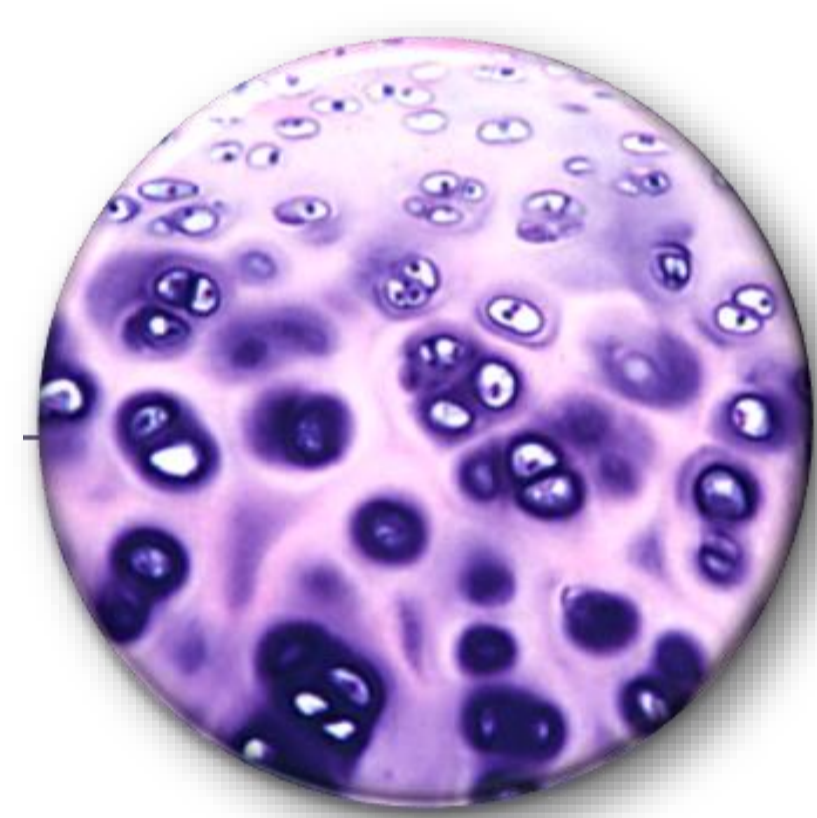


D-BOARD Consortium, an European Committee FP7-project

P U R P O S E. To determine the expression of fibulin-3 gene by human osteoarthritic (OA) subchondral osteoblasts and the concentration of three fibulin-3 epitopes (Fib3-1, Fib3-2 and Fib3-3) in OA osteoblasts culture supernatants. The fibulin-3 neo-epitopes, originally discovered by proteomics studies, are recently shown to be diagnostic and prognostic biomarkers of OA.

M E T H O D S. OA osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) zones of OA subchondral bone of nine men undergoing total knee replacement.

OA osteoblasts cultures were stopped at two different maturation stages, either pre-osteoblasts (after 3 days in 10% FBS) or mature osteoblasts (after further 14 days of culture in 2% ultrosor G, proline 20 µg/ml, ascorbic acid 50 µg/ml and 1.25 dihydroxycholecalciferol 10⁻⁸ M). Maturation level was assessed by alkaline phosphatase activity and mineralization capacity. We evaluated the expression of fibulin-3 gene by RT-PCR in SC and NSC osteoblasts, and the concentration of three neo-epitopes, Fib3-1, Fib3-2 and Fib3-3 by specific competitive ELISAs.



R E S U L T S. Fibulin-3 gene was 2.3-fold overexpressed in SC compared to NSC pre-osteoblasts ($p=0.0255$ $n=5$) and 1.3-fold in mature SC osteoblasts compared to NSC osteoblasts. The concentration of the three neo-epitopes were increased in SC pre-osteoblasts supernatants compared to NSC, but the difference was only significant for Fib3-3 [Fib3-1 (1.44-fold $p=0.13$, $n=4$) Fib3-2 (1.32-fold $p=0.21$, $n=7$) Fib3-3 (1.45-fold $p=0.01$, $n=9$)]. In contrast, the three fibulin-3 neo-epitopes levels were significantly higher in mature SC osteoblasts than in mature NSC osteoblasts culture supernatants [2.53- fold for Fib3-1 ($p=0.0069$, $n=7$), 2.41-fold for Fib3-2 ($p=0.0364$, $n=7$)

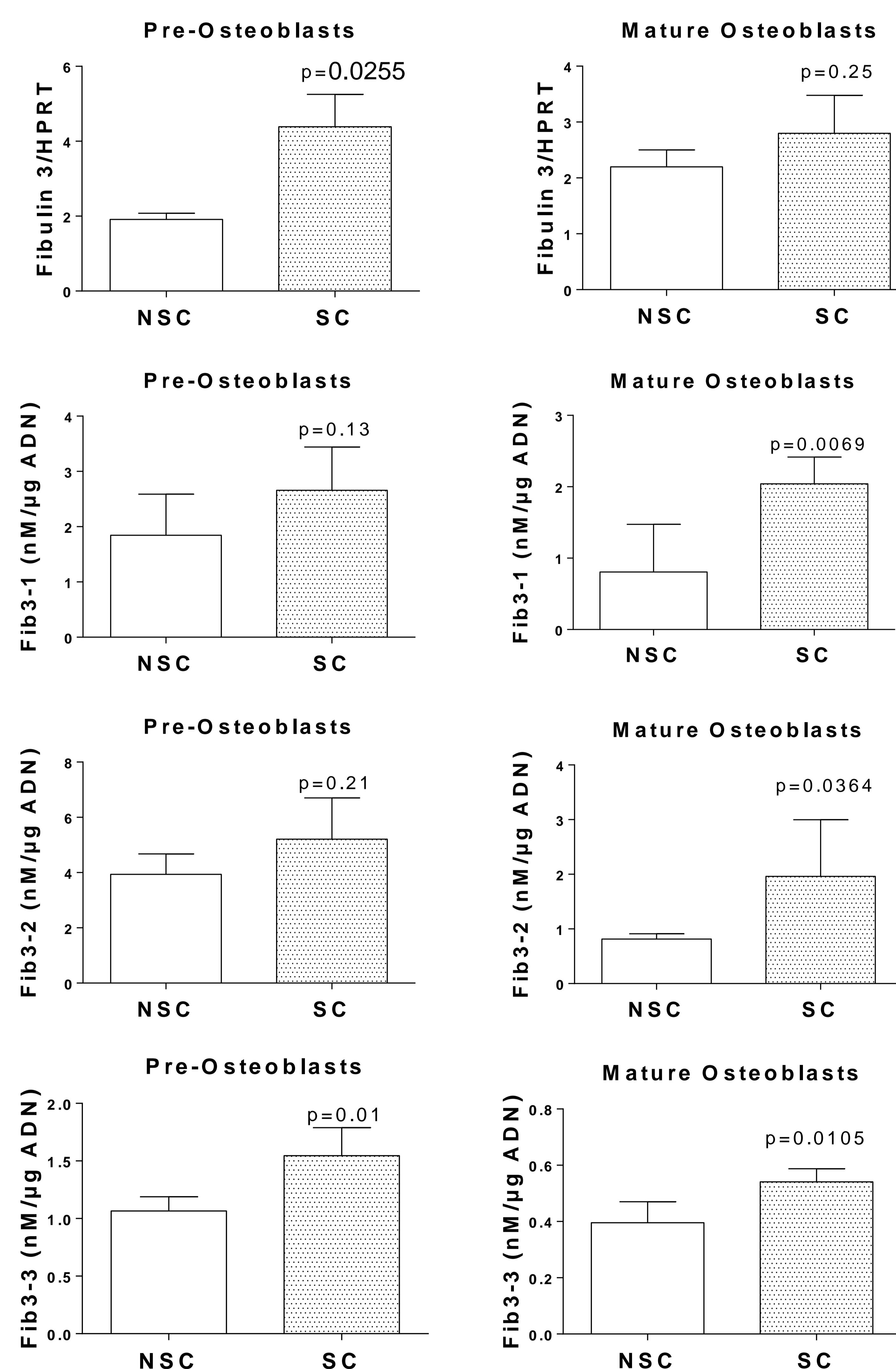


Fig. 1 : Fibulin-3 expression and secretion

Fibulin-3 fragments found in osteoblasts culture supernatant or expression HPRT normalized. Paired T-test.

C O N C L U S I O N

Fibulin-3 fragments, further to be associated immunohistochemically with cartilage superficial layer degradation/fibrillation, could be a marker of the osteochondral plate remodeling in osteoarthritic joints. These findings contribute to explain the increased concentration of fibulin-3 neo-epitopes in sera and urines of OA patients.

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