

PHENOTYPIC CHARACTERIZATION OF OSTEOBLASTS FROM THE SCLEROTIC ZONE OF HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE

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Aim of the study: There is a consensus that osteoarthritis (OA) is characterized by subchondral bone thickening, accompanied by an increased osteoid volume and a low mineralization. Until now, phenotypic changes occurring in osteoblasts from the sclerotic subchondral bone remain unexplored. This work was designed to compare gene expression in osteoblasts coming from the sclerotic and non sclerotic zones of human OA subchondral bone.

Methods: Human osteoblasts were isolated from sclerotic or non sclerotic areas of OA subchondral bone. They were cultured for 12 days in monolayer in a differentiation medium composed of 2% Ultrosor G as serum substitute 20 µg/ml proline, 50 µg/ml ascorbic acid, 10⁻⁸ M 1,25 dihydroxycalciferol. During this differentiation period, gene expression in sclerotic or non sclerotic osteoblasts was compared. Tissue non specific alkaline phosphatase (TNAP), osteocalcin (OC), interleukin (IL)-6, IL-8, transforming growth factor (TGF)-beta1, osteopontin (OPN), bone sialoprotein (BSP), vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-13, parathormone receptor (PTH-R), transglutaminase (TG)-2, factor XIIIa (FXIIIa) mRNA levels were quantified using real time RT-PCR. Transglutaminase activity was also quantified by enzymatic assays.

Results: MMP-13 (22-fold; p<0.001), OC (6.37-fold, p<0.001), OPN (3.87-fold; p<0.001), TNAP (1.98-fold, p<0.001), IL-6 (2.53-fold, p<0.001), IL-8 (1.81-fold, p<0.001), TGF-beta1 (1.41-fold, p<0.01) and VEGF (1.97-fold; p<0.001) gene expression was significantly higher in sclerotic osteoblasts than in non sclerotic cells. In contrast, PTH-R (0.63-fold, p<0.001) gene was depressed in sclerotic osteoblasts compared with non sclerotic cells. Finally, BSP, TG2 and FXIIIa mRNA levels were similar in sclerotic and non sclerotic osteoblasts. Transglutaminase activity was increased 1.78-fold in sclerotic osteoblasts (p<0.001).

Conclusions. Osteoblasts from the sclerotic subchondral bone showed an altered phenotype characterized by the overexpression of genes limiting bone mineralization (e.g OPN), and genes promoting osteoid matrix accumulation (e.g.TGF-beta1). These findings suggest that osteoblasts may contribute to subchondral bone sclerosis and constitute a potential target for future OA therapies.

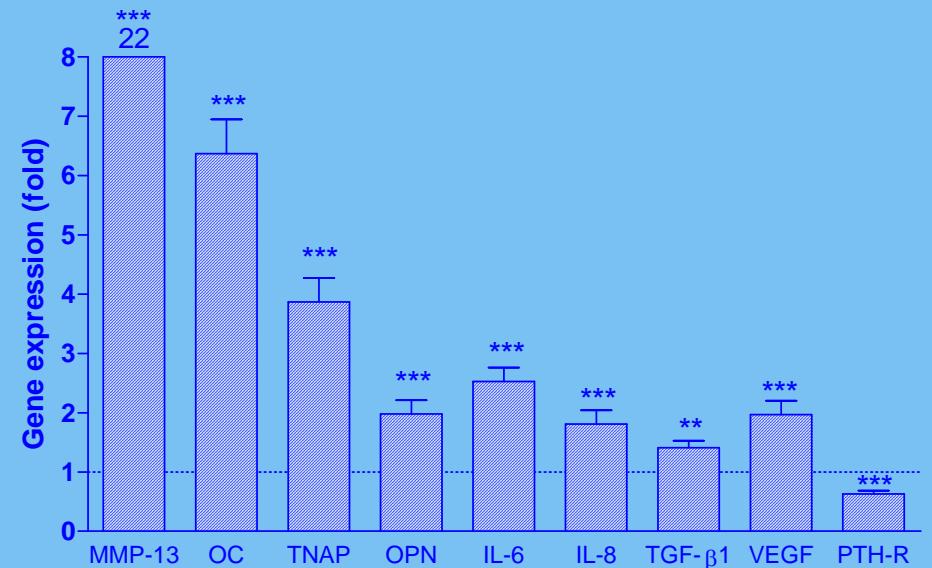


Figure 1: Fold-induction of genes expression by sclerotic osteoblasts compared to not sclerotic osteoblasts. Gene expressions are GAPDH normalized and are represented by the mean of 3 independent cultures ± SEM. Each experimental condition was performed in triplicate (n=9). Significant statistical differences are represented by ** = p<0,01 and *** = p<0,001 between NSC and SC osteoblasts.