

Co-expression of adipogenic and osteoblastic proteins in MSC-derived osteoblasts following co-culture with MSC-derived adipocytes

Aline Clabaut¹, Meryem Tardivelle², Céline Grare¹, Pierre Hardouin¹, Odile Broux¹

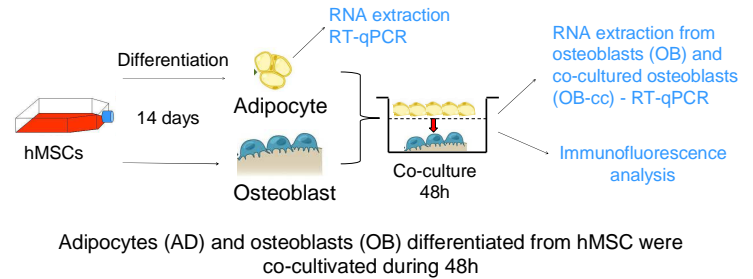
1) PMOI, ULCO, Boulogne-sur-Mer, France 2) BiCel, Biocenter, Université de Lille, France

Background

In osteoporosis and aging, bone loss is accompanied by an increase of adiposity in the marrow. Marrow adipocytes and osteoblasts compete in their differentiation from mesenchymal stem cells (MSC), and adipocytes are suspected to modify osteoblasts phenotype.

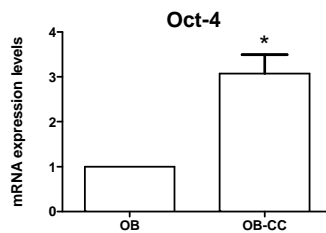
Indeed, in our lab, we have previously showed with *in vitro* coculture model based on human primary MSCs, that MSC-derived osteoblasts showed appearance of adipocyte and decrease of late osteogenic mRNA markers. Thus, MSC-derived adipocytes seem to be capable of inducing MSC-derived osteoblasts to differentiate towards an adipocyte-like phenotype. These results support the hypothesis of a transdifferentiation of osteoblasts in the vicinity of adipocytes. The aim of this study was to confirm this transdifferentiation.

Experimental design



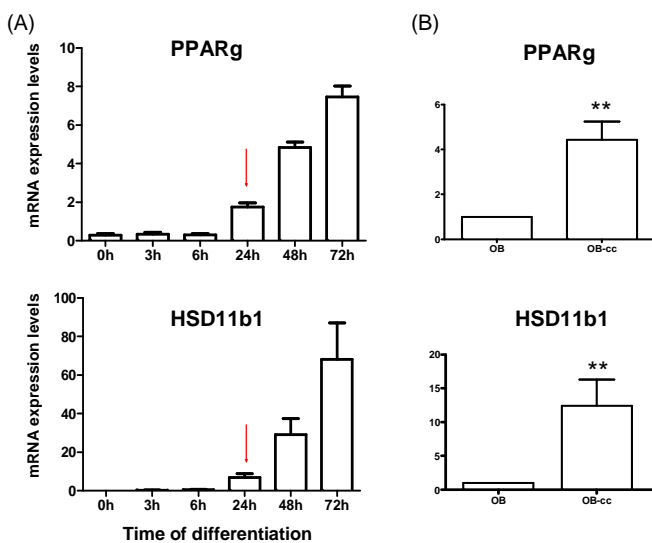
Results

1. The higher expression levels of reprogramming gene Oct-4 observed in OB-cc compared to OB, confirmed the phenotypic conversion of OB after co-culture



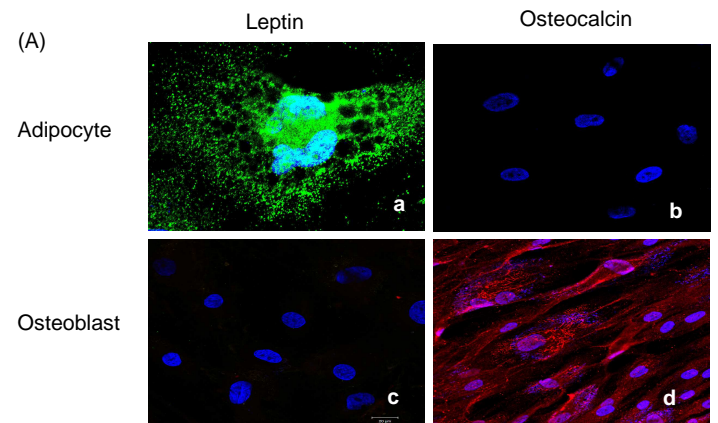
Quantitative RT-PCR analysis of the Oct-4 gene in OB and OB-cc. Graphs were representative of 4 independent experiments, performed in duplicate.

2. Adipocyte-specific gene expression levels in OB-cc were comparable to MSC-derived adipocytes precociously differentiated and confirmed the commitment of OB-cc towards an adipocyte-like phenotype

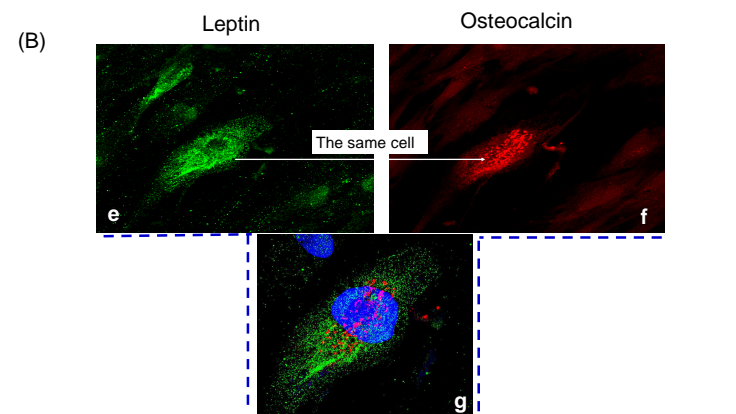


PPARγ and HSD11b1 mRNA levels in MSC-derived adipocytes at 0, 3, 6, 24, 48 and 72h of differentiation (A) and in OB-cc (B). Graphs were representative of at least 4 independent experiments, performed in duplicate.

3. Immunofluorescence microscopic analysis confirmed the co-localization of adipogenic and osteoblastic proteins in OB-cc



(A) Simple immunofluorescence confocal analysis showed that leptin and osteocalcin are specific markers for adipocyte and osteoblast, respectively. The green fluorescence revealed the leptin expression (A 488) and the red fluorescence revealed the osteocalcin expression (A 568). Nuclei were stained with DAPI.



(B) Double immunofluorescence microscopic analysis showed a co-localization of leptin (e) and osteocalcin (f) in the same OB-cc cell. (g) Overlap of e and f images. Nuclei were stained with DAPI.

Conclusion

In this study, we showed MSC-derived osteoblasts can transdifferentiate into another lineage under the influence of secreted products release by MSC-derived adipocytes. Thus, the increase of bone marrow adiposity observed during aging or osteoporosis is not a passive phenomenon;

adipocyte can perturbate OB phenotype and so are highly suspected to be involved in bone loss. The understanding of the mechanisms of this phenotypic conversion could open new approach for osteoporosis treatment.