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EVALUATION OF THE OSTEOGENIC AND ENDOTHELIAL DIFFERENTIATION POTENTIAL OF SSEA-4 SUBPOPULATION OF ADIPOSE DERIVED STEM CELLS

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ABSTRACT
Human Adipose-Derived Stem Cells (hASCs), an underappreciated source of stem cells, might consist in a new cell poll for tissue engineering (TE) applications, including bone TE. Furthermore, these cells can be harvested in a large number with low morbidity and their developmental plasticity was already proven both in vivo and *in vitro*. SSEA-4, an early stem cell marker is known to be expressed by hASCs though in a low percentage. Considering the pluripotency of SSEA-4 cells, we examined whether the SSEA-4$^+$hASCs could differentiate into the endothelial and the osteogenic lineages, aiming to develop a new strategy for promoting the vascularization of bone TE constructs.
Analysis by immunocytochemistry and flow cytometry demonstrated the ability of SSEA-4$^+$hASCs cells to rapidly acquire stable endothelial features (CD31, CD34, CD105 and vWF expression), whilst formation of tubular structures resembling capillary like organization when seeded in Matrigel confirmed their *in vitro* functional competence. Also, the presence of scavenger receptors for Dil-ac-LDL is consistent with the characteristic of endothelial cells. Furthermore, SSEA-4$^+$hASCs subpopulation was found suitable for osteogenic differentiation. An upregulation of osteogenensis related genes (osteocalcin-OPN and osteopontin-OCN) was observed which was confirmed also by means on immunocytochemistry. Matrix mineralization was also revealed by Alizarin Red.

INTRODUCTION

Several studies suggest that human adipose-derived stem cells (hASCs) are an underappreciated source of cells for promoting therapeutic angiogenesis and vascularization of engineered constructs (A. Miranville, 2004). The concept of adipose tissue (AT) as a stem cell source is emerging since the discovery of a subpopulation of MSCs residing in the stromal fraction (SVF) of the AT (P.A.Zuk, 2002). Furthermore, AT as a postnatal source of cell for regenerative medicine is appealing: it is an abundant and accessible source since subcutaneous fat can be harvested following minimally invasive procedures, with low donor site morbidity and without gender restriction (M. Vallée, 2009). Moreover, their developmental plasticity, often referred as the inherent ability retained within stem cells to cross lineage barriers and to adopt the phenotypic, biochemical and functional properties of cells unique to other tissues, was already proven both *in vitro* and *in vivo* (B.A. Bunnell, 2008). The stem cell population within the adipose tissue stroma resembles bone marrow mesenchymal stem cells (MSCs) and can differentiate towards osteogenic, chondrogenic, adipogenic, myogenic and neurogenic lineages, rendering them a high potential for future TE applications (H Tapp, 2005). Moreover, SVF harbours more than 2% of cells featuring potential for multilineage differentiation towards cell types compared to 0.002% for bone marrow-derived MSCs (B.M.Strem, 2005). Therefore, these adipose-derived stem/ stromal cells (ASCs) bear great potential for organ regeneration, in particular for the production of a wide range of autologous TE substitutes (M. Vallée, 2009). Despite hASCs great potential for differentiation towards different lineages, studies have revealed that endothelial differentiation cannot be achieved with high efficiency. The presence of SSEA-4$^+$subpopulation in the adipose stroma raises interest for therapeutic application, since it is expected to present a high differentiation potential (U. Riekstina, 2009). The exploitation of this potential could offer important cues for understanding the hASCs developmental plasticity. Considering the pluripotency of these cells, the present work is focused on the evaluation of the differentiation potential of SSEA-4$^+$hASCs towards endothelial and osteogenic lineages, in order to evaluate their potential for bone vascularization applications.

MATERIALS AND METHODS

Human lipoaspirates were obtained from individuals undergoing plastic surgery after informed consent. Stromal vascular fraction (SVF) was obtained using a modified procedure from (Zuk *et al.*, 2002) The SSEA-4$^+$cells were selected from the SVF by using an immunomagnetic sorting technique and cultured in either EGM 2-MV or expansion medium. A fraction of the SVF was cultured under the same conditions for comparison. The identity of the cells under different conditions was established by immunocytochemistry.

and flow cytometry for the expression of mesenchymal (CD105, CD90, CD73), endothelial (CD31, vWF), haematopoietic (CD45, CD34) and embryonic stem cell (SSEA-4) related markers. Endothelial phenotype was further assessed based on the ability of the cells to scavenge the acetylated Dil-Low Density Lipoprotein (LDL) complex and on the capacity to form capillary-like structures on Matrigel. The osteogenic differentiation potential was assessed by the analusis of matrix proteins deposition (immunocycotchemistry) and matrix mineralization (Alizarin Red Staining). RT-PCR was used to confirm at the molecular level the expression of endothelial and osteogenic phenotype-related genes.

RESULTS

In the SSEA-4$^+$hASCs cultures in EGM-2 MV it was observed the presence of endothelial-like colonies characterized by cobblestone morphology (Fig. 1A). Cells with these characteristics were not observed in the SVF cells cultured under the same conditions. Pure endothelial cultures were stable up to 5 passages. Moreover, they were characterized by the expression of endothelial markers (Fig. 1B), ability to scavenge LDL and to form capillary-like structures when seeded on Matrigel, demonstrating their *in vitro* functional competence. The endothelial differentiation was further confirmed by the gradual decreased expression of SSEA-4 along with the increase of CD105, CD34, but not CD45 expression. Upregulated expression of CD31 and vWF was confirmed at molecular level.

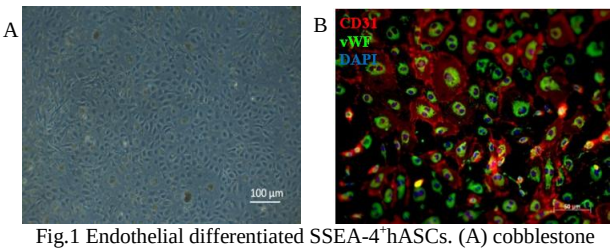

Fig.1 Endothelial differentiated SSEA-4$^+$hASCs. (A) cobblestone morphology; (B) vWF (green) coexpressed with CD31 (red)

The osteogenic differentiation potential of SSEA-4$^+$ hASCs was clearly demonstrated by an intense matrix mineralization (Fig. 2A) and the expression of OCN and OPN genes (Fig. 2B).

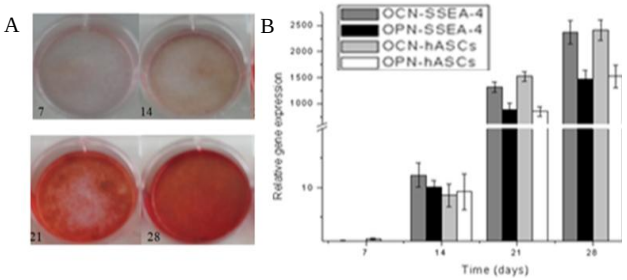

Fig.2 Osteogenic differentiation of SSEA-4$^+$hASCs. (A) Gradual mineralization observed by Alizarin Red staining along 28 days; (B) Upregulation of osteogenesis related genes: OCN and OPN

CONCLUSIONS

We have established a protocol for the isolation and differentiation of SSEA-4$^+$hASCs. These cells differentiate towards the endothelial lineage, maintaining consistent endothelial phenotype along passages, when cultured in EGM-2 MV. Furthermore, SSEA-4$^+$hASCs showed a strong osteogenic potential. Therefore, hASCs allow selection, *in vitro* culture and expansion of cells with high angiogenic and osteogenic potential. Thus, SSEA-4$^+$ subpopulation exhibits a promising potential for the development of vascularized bone TE constructs.

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REFERENCES

Bunnell B.A. (2008), "Adipose-derived stem cells: isolation, expansion and differentiation", *Methods* **45**: 115–120

Miranville A. (2004), "Improvement of postnatal neovascularization by human adipose tissue-derived stem cells", *Circulation* **110**: 349-355

Riekstina U. (2009), "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis", Stem *Cell Reviews and Reports* **5**: 378–386

Strem B.M. (2005) "The growing importance of fat in regenerative medicine", *Trends Biotechnology* **23**: 64–66

Tapp H. (2009), "Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair,*Experimental Biology andMedicine* **234**: 1-9

Vallée M. (2009), "Adipose-tissue engineering: Taking advantage of the properties of human adipose-derived stem/stromal cells ", *Pathologie Biologie* **57**: 309–317

Zuk P.A. (2002) "Human adipose tissue is a source of multipotent stem cells", *Molecular Biology of the Cell* **13**: 4279–4295

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