Biophysical Chemistry 229 (2017) 84–92

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

A biophysical approach to quantify skeletal stem cells trans-differentiation as a model for the study of osteoporosis

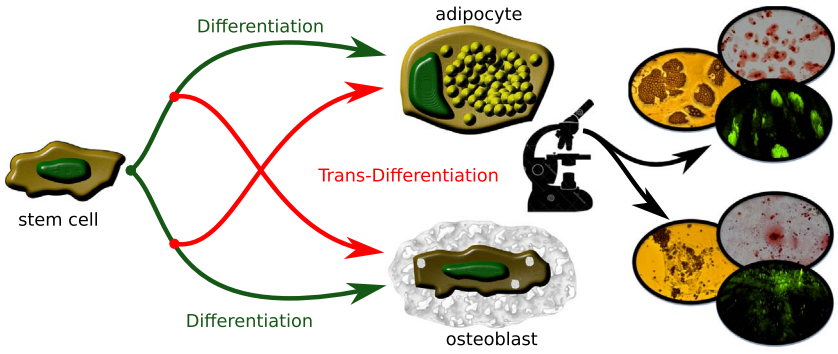
L. Petecchia, F. Viti, F. Sbrana, M. Vassalli, P. Gavazzo*

Institute of Biophysics, National Research Council, Via De Marini 6, 16149 Genova, Italy

HIGHLIGHTS

- Direct and trans-differentiation of pre-osteoblast in adipocyte behave similarly.
- Trans-differentiation of pre-osteoblasts into adipocytes is extremely efficient.
- Along the initial stages of differentiation hBM-MSCs maintain original plasticity.
- QPI can support quantitative cell morphology studies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Quantitative phase imaging
Real time qPCR
Morphometry
Osteoblast
Adipocyte

ABSTRACT

The stroma of human bone marrow contains a population of skeletal stem cells (hBM-MSC) which are common ancestors, among the others, of osteoblasts and adipocytes. It has been proposed that the imbalance between hBM-MSC osteogenesis and adipogenesis, which naturally accompanies bone marrow senescence, may contribute to the development of bone-associated diseases, like osteoporosis. The possibility to reproduce this mechanism *in vitro* has been demonstrated, providing a good model to disclose the details of the complex bone-fat generation homeostasis. Nevertheless, the lack of a simple approach to quantitatively assess the actual stage of a cellular population hindered the adoption of this *in vitro* model.

In this work, the direct differentiation of hBM-MSCs towards a single (osteo or adipo) lineage was characterized using quantitative biophysical and biological approaches, together with the parallel process of trans-differentiation from one lineage to the other. The results confirm that the original plasticity of hBM-MSCs is maintained along the initial stages of the differentiation, showing that *in vitro* conversion of pre-osteoblasts into adipocytes and, *vice versa*, of pre-adipocytes into osteoblasts is extremely efficient, comparable with the direct differentiation. Moreover, a method based on digital holography is proposed, providing a quantitative indication of the phenotype stage along differentiation.

1. Introduction

Human bone marrow harbors a population of stromal mesenchymal stem cells (hBM-MSC, human bone marrow-derived mesenchymal stem cells), which are adherent, fibroblast-like, and pluripotent progenitor cells. Several authors prefer the denomination “skeletal stem cells” to highlight the remarkable potential of hBM-MSC in healing large bone defects and in bone regeneration [1,2].

* Corresponding author at: Biophysics Institute, National Research Council, Via De Marini 6, 16149 Genova, Italy.
E-mail address: gavazzo@ge.ibf.cnr.it (P. Gavazzo).

http://dx.doi.org/10.1016/j.bpc.2017.05.011
Received 12 April 2017; Received in revised form 9 May 2017; Accepted 22 May 2017
Available online 26 May 2017

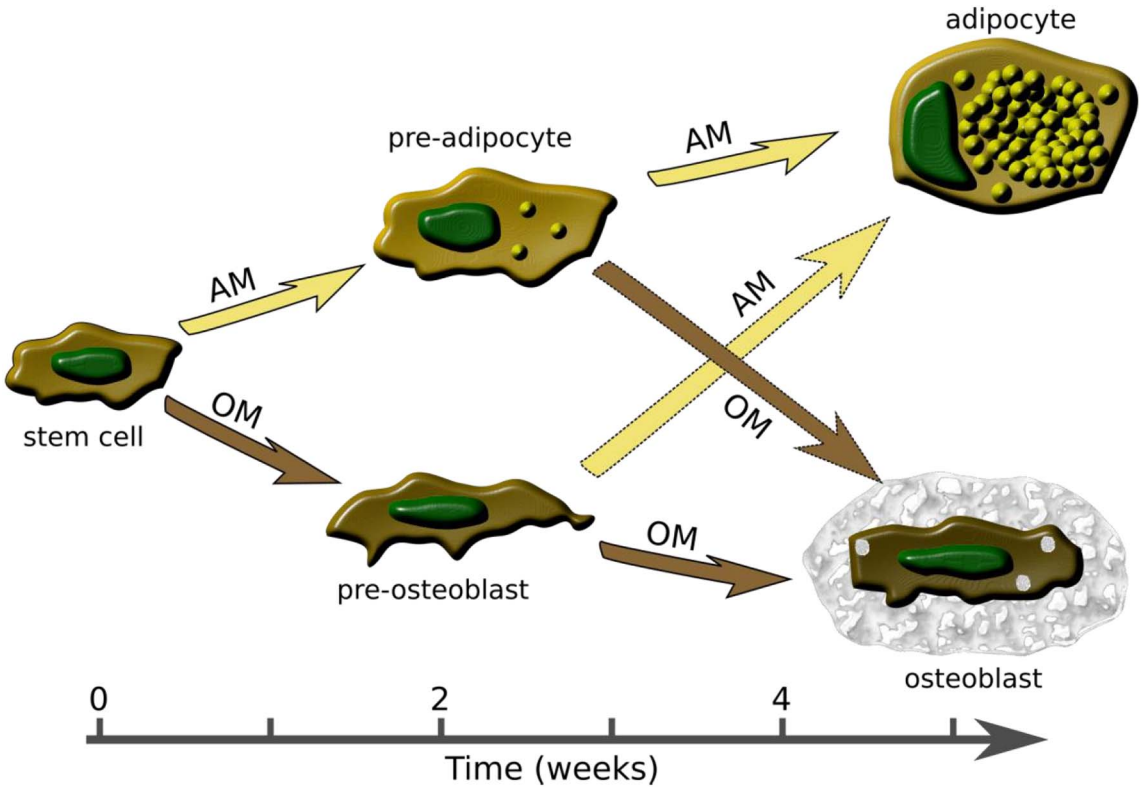


Fig. 1. Cartoon of direct/trans-differentiation experimental design. hBM-MSCs are partially committed towards a specific lineage using AM (adipogenic) or OM (osteogenic) culture medium. Once achieved the intermediate stage (at about 2 weeks) they are stimulated to fully differentiate along the same lineage or, alternatively, are subjected to trans-differentiation by switching the medium, and addressed towards the opposite lineage until full differentiation.

In physiological condition, hBM-MSCs usually differentiate into mature osteoblasts and contribute to maintain bone homeostasis in the adults. Alternatively, mesenchymal stem cells can be committed to become mature adipocytes and be stored in the marrow cavities not indispensable for hematopoiesis. The differentiation into osteoblasts or adipocytes is tightly regulated, but the mechanisms promoting this choice and the pathways involved are not fully elucidated. Nevertheless, a remarkable role seems to be played by the composition and the dynamic changes occurring in the bone marrow (BM) microenvironment, where the products of autocrine, paracrine and endocrine activities are poured. For example, TGF-beta1, BMPs, IGF, Wnt-signalling pathway components, and transcriptional regulators constitute the typical protein pool present in the healthy bone marrow microenvironment, while a pathological situation is usually enriched of inflammatory factors such as reactive oxygen species (ROS) and interleukins [3]. The equilibrium between adipogenesis and osteogenesis is unbalanced towards the former during senescence, and a similar tendency can arise under pathological conditions [4], contributing to the development of bone-associated diseases like osteoporosis and osteopenia [5–8]. Moreover, the unbalance between osteogenesis and adipogenesis is also claimed to have an active role in the pathophysiology of various metabolic disorders such as diabetes mellitus, atherogenesis, multiple myeloma [9]. Many clinical and *in vivo* studies have shown that bone loss and trabecular bone deterioration are associated to an increase in marrow adipose tissue in elder, osteoporotic or bone-disease affected mammalian organisms. This could be due to a drop in the competence of hBM-MSC to form osteoblasts or to an alteration in the trans-differentiation mechanism linking one lineage to the other. While the process of *in vivo* bone and fat trans-differentiation is not unequivocally proven [10], Song and Tuan [11] demonstrated the occurrence of *in vitro* trans-differentiation at the single cell level in hMSC-derived osteoblasts, adipocytes and chondrocytes [11]. Whether this takes place through a dedifferentiation phase followed by a re-differentiation towards the new destination, as they proposed, or more simply through a direct switch between lineage-specific biochemical pathways is still debated [10]. Cell-based approaches demonstrated to be very helpful in elucidating the molecular mechanisms underlying skeletal stem cell differentiation and trans-differentiation progression, and provided many insights into the involved biochemical pathways. Protocols for hBM-MSC isolation and maintenance in culture are well established and widely employed. hBM-MSC can be grown on monolayer and, in response to specific "*in vitro*" culture conditions, these cells can proliferate, thus generating daughter cells, which in turn can become restricted and committed to one specific lineage, giving rise to a fully differentiated phenotype. Since their multipotency is preserved also *in vitro*, hBM-MSC can be addressed to differentiate in osteoblasts, chondrocytes, adipocytes, and myoblasts [12,13].

Several protocols of trans-differentiation (or reprogramming) have been set and phenotype switches of committed cell types have been described by various groups [10,14,15] even though the molecular mechanisms involved in these processes have not been fully elucidated [16].

Therefore, shading light on the basis of hBM-MSC maturation stages is a priority for a better comprehension of bone related diseases pathogenesis, also in view of future applications of personalized cell therapy.

In this work we performed a morphological and functional analysis of hBM-MSC along their differentiation and trans-differentiation progressions, exploiting a composite set of traditional and innovative approaches able to investigate functional and morphological features peculiar of these events. Morphometric 2D and 3D cell parameters (area, perimeter, thickness, roughness, volume, *etc.*) were evaluated from images obtained with quantitative phase imaging techniques, while staining and qPCR experiments were implemented to assess the enriched presence of proteins typical of diverse lineages, and in the transcriptional activity of different target genes depending on the designed commitment.