Subcutaneous adipocytes may become osteoblasts

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Summary

Commonly, mesenchymal stem cells derived from bone marrow (BMSCs) are mainly utilized in regenerative medicine field. BMSCs are able to differentiate into several lineages, showing immunosuppressive properties, and they are genetically stable in long-term cultures. In the last years, another mesenchymal stem cells population, obtained from adipose tissue, defined adipose-derived stem/stromal cells (ASCs), it is under assessment of scientific research, as alternative to BMSCs. In fact, ASCs show similar capacity to BMSCs, but unlike BMSCs can be harvested more easily with an higher yield and with less invasive manipulation. In this review the abilities of ASCs to differentiate in osteoblasts cells are shown.

KEY WORDS: adipose-derived stem/stromal cells; adipose tissue; osteoblast; osteogenic differentiation.

Introduction

The adipose tissue derives from the mesoderm layer and it is one of the most abundant human tissue types. Initially, it was simply considered a metabolic reservoir of high-energy substrates (1). Subsequent studies have shown that this tissue can secrete many hormones, growth factors and cytokines (2, 3), and it also contains a large amount of mesenchymal stem cells. These cells have been defined adipose-derived stem/stromal cells (ASCs) (4-6). These cells can be easily harvested with non invasive technique and in great amounts from fat tissue, i.e. with cosmetic liposuction, approximately ~5000 cell/ml fat can be obtained (7). Moreover, ASCs are characterized by their ability of self-renews and generating multilinage cells (8, 4, 5, 9), included osteoblasts cells. In fact, if seeded in osteogenic medium, they express typical markers of osteoblast phenotype including alkaline phosphatase (ALP), type I collagen, Osteopontin (OPN), Osteonectin (ON), Runt-related transcription factor 2 (RUNX2), mothers against decapentaplegic homolog1 (SMAD1), Bone Morphogenetic Proteins (BMP) 2, BMP-4, BMP receptors I and II (5, 2, 10-14) and calcium deposits (15). In addition, some *in vitro* studies show that ASCs seeded on titanium allows in presence of osteogenic medium, are able to adhere, to proliferate and to acquire an osteoblastic-like phenotype (15, 16). These results suggest that these cells, together with their immunosuppressive capacities (17), could be a real and important tool for bone tissue repair, and bone tissue engineering. Nevertheless, again many studies have to be conducted for assess safety of ASCs. The aim of this review is to examine the osteogenic differentiative capacity of ASCs.

Mesenchymal stem cells

Stem cells are divided in two groups: embryonic stem cells (ESCs) and adult stem cells (18). Both are capable to duplicate themselves indefinitely while maintaining toti/multipotency and to differentiate into cells of several lineages (19, 20, 8), but contrary to adult stem cells, ESCs utilization is limited for ethical issues and for their higher tumorigenicity (21, 22). The first stem cells described were the hematopoietic cells (HSCs) (23). These cells are present in the bone marrow and can differentiate into all blood cell lineages (24). Subsequent studies have shown the existence of another type of stem cells, including in bone marrow, with spindle-shaped morphology (25), able to adhere on plastic surface, and with multilineage mesodermal potentials (26, 27). These cells were defined mesenchymal stem cells (MSCs) (28). Subsequent researches have demonstrated that MSCs can be obtained from many other tissues (29-32).

Mesenchymal stem cells from adipose tissue

In early 2000, a research group published a paper in which was reported the existence of stem cell populations within adipose tissue (4, 5), defined by the term preadipocytes or ASCs (33, 34). These cells, that can be easily obtained, and in great amounts from fat tissue (7, 35), have a spindle form, the ability to adhere to plastic surface and the capacity to differentiate into many multilineage cell types (4, 5, 8), both of mesodermal, but also of endodermal and ectodermal origin (36-38). *In vitro*, they express typical mesenchymal stem linage markers as: CD13, CD29, CD44, CD73, CD90, CD105, CD133 and CD166, while they not express hematopoietic cell-surface markers as: CD14, CD11b, CD34, CD45, CD19, CD79 (5). Therefore, ASCs matches the criteria for the identification of mesenchymal stem cells, proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (39): they have to be plastic-adherent when seeded in specific medium; they must have the ability to osteogenic, adipogenic, and chondrogenic differentiation; they must express typical mesenchymal markers, but they must lack the expression of hematopoietic markers.

ASCs can be isolated from adipose tissue coming from plastic biopsies or surgeries. In our lab, after the obtainment of informed consent in accordance with the Institutional Review Board protocol, the adipose tissue biopsies were minced into small pieces (0.2–0.5mm) and the fragments were washed with McCOY'S 5A medium, centrifuged at 200g for 10 min, resuspended in Ham's F12 Coon's modification medium supplemented with 20% FBS and 3 mg/ml collagenase type I, digested for 3 h at 37°C, mechanically dispersed and passed through a sterile 230-mm stainless steel tissue sieve. The undigested tissue trapped in the sieve was discarded, while the infranatant containing the preadipocyte fraction was collected and the cells were sedimented by centrifugation at 300g for 5 min. The pellet was incubated with an erythrocytes lysis buffer for 2 min at room temperature and the remaining cells were cultured in 100mm culture plates in growth medium: Ham's F12 Coon's modification medium supplemented with 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin and 1 ng/ml basic fibroblast growth factor and incubated at 37°C in humid atmosphere with 5% CO2. The medium was refreshed twice a week (15).

Bone tissue and osteoblast cells

Bone tissue is constituted by cells embedded in an mineralized extracellular matrix made of collagen and not-collagen proteins. These cells are represented by osteoclasts, osteoblasts and osteocytes. The osteoblast cells have the role to secerne the organic components of the bone extracellular matrix and promote its mineralization. These cells derive from stromal progenitors present in bone marrow (BMSCs) and their differentiation process is regulated by specific hormones and growth factors. Two major signaling pathways that regulate the differentiation of these cells are represented by WNT and TGF-β signaling.

WNT signaling: this pathway is constituted by WNT proteins that build a complex with Frizzled transmembrane receptors and low density lipoprotein receptor-related protein 5/6 (LRP 5/6), inducing a cascade of signals that bring to the activation of RUNX2, a key transcription factor involved in the osteogenic differentiation (35, 37, 40).

TGF-β signaling: TGF-β superfamily consists of several molecules, the ones important for osteogenic differentiation are the BMP (41). The BMP work in two different manners, one SMAD-dependent and one SMAD-independent. In the first case, specific BMP bind to specific SMAD proteins activating the expression of genes that have a key role in osteogenic differentiation as RUNX2 and OSTERIX (OSX) (42, 43). In the second case, other BMP involving MAP-kinases signaling, regulate the phosphatasis alkaline and osteocalcin (OCN) expression in osteoblastic cells (44).

Among the most important transcription factors that participate to the osteogenic differentiation there are RUNX2 and OSX. RUNX2 is a member of the runt-relate factor family and it is considered the key switch of the osteoblastic differentiation (34); in fact, it regulates the time-dependent activation and/or the inhibition of essential genes involved in this process; his lack indeed causes the formation of a cartilage skeleton in the RUNX2 knock out mices (38). RUNX2 is a necessary but not enough factor for the osteoblastic differentiation; in fact, in knock out mices for OSX, but not for RUNX2, the cells are not able to differentiate in osteoblast and to deposit bone matrix (35).

ASCs can differentiate in osteoblasts cells

The title of this section does not end with a question mark, but if we want to assign a punctuation, we could put an exclamation point. In fact, as described above, many studies report results that clearly show how ASCs can differentiate in osteoblasts cells (2, 5, 8, 45). In fact, these cells *in vitro* can differentiate into the osteoblast lineage, utilizing specifics culture mediums supplemented with appropriate factors that stimulate osteogenesis. We use in our laboratory, Ham's F12 Coon's modification medium supplemented with 10% FBS, 100 IU/ml penicillin, 100 mg/ml streptomycin, 10 nM dexamethasone, 0.2mM 2-phosphate ascorbate, and 10mM β-glycerolphosphate (15). Other factors that can be utilized to induce ASCs osteogenic differentiation are 1,25 vitamin D_3 and BMP-2 (8, 12, 46-48). In the presence of these factors, ASCs in time-dependent manner express genes and proteins associated with the osteoblast phenotype, including ALP, Type I Collagen, OPN, ON, RUNX2, BMP-2, BMP-4 and BMP receptors I and II (2, 5, 10-14). Also, during the time between 2 and 4 weeks of culture, the extracellular matrix mineralization begins and proceeds through the activity of ALP, which hydrolyze phosphate esters making available inorganic phosphate to form hydroxyapatite (4, 5). The expression of these phenotypic traits are regulated by signaling pathways practically identical to those involved in the regulation of BMSCs differentiation in mature osteoblasts. In fact, many studies show that TGF-β (49, 50) and WNT (51) signaling are heavily involved in this differentiative process.

Conclusions

Going back to the title of the last paragraph, if a question should had been formulated: can the preadipocytes differentiate in osteoblasts? The answer would be obvious. In fact, as described above, ASCs in presence of specific osteoinductive medium express time-dependent typical markers of osteoblastic lineage and share with the progenitors of these cells, BMSCs, signaling pathways that regulate their osteodifferentiation. Moreover, in our laboratory, we have studied the growing and the osteogenic differentiation ability of ASCs on titanium allows (Ti6Al4V), usually used in prothesic replacements (15). Results show an excellent cell adhesion on Ti6Al4V allow surface, and when seeded in presence of osteogenic medium, ASCs express typical osteoblastic phenotype markers. All these features, easy cells collection with minimal invasive procedures and large yields, differentiative and transdifferentiative potentiality, and immunosuppressive capacity, make ASCs a real alternative to BMSCs for tissue engineering applications.

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