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ADIPOSE DERIVED CELLS

Comparison between pediatric and adult adipose mesenchymal stromal cells

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Abstract

Background. Adipose-derived mesenchymalstromal cells (ASC) are currently tested in regenerative medicine to promote tissue reconstruction after injury. Regardingautologous purpose, the possible loss of therapeutic function and cell properties during aging have been questioned in adults. To date no reliable information is available concerning ASC from pediatric patients and a better knowledge is required for clinical applications. *Methods.* Subcutaneous adipose tissue was collected from 27 donors (0–1 years old) and 50 donors (1–12 years old) and compared with adult ASC for *in vitro* characteristics. ASC were then tested in a mouse model of limb ischemia. *Results.* Cells from the stromal vascular fraction (SVF) and subsequent cultured ASC were prepared. Only a greater amount in SVF cell number and ASC proliferative rate were found. Cell phenotype, colony formingunit-fibroblast (CFU-F) content, immunomodulation effect and adipogenic, osteoblastic and angiogenic potentials were not significantly different. *In vivo*, pediatric ASC induced an increase in microangiographic score in a mouse model of limb ischemia, even though improvement in vascular density was not significantly correlated to limb rescue. Finally messengerRNA (mRNA) analysis using a microarray approach identified that only 305 genes were differentially expressed (217 down- and 88 up-regulated) in pediatric versus adult ASC, confirming that ASC from both age groups shared very close intrinsic properties. *Conclusion.* This is the first study reporting a comparative analysis of ASC from a large number of donors and showing that their *in vitro* and *in vivo* properties were similar and maintained during aging.

Key Words: *adipose mesenchymalstromal cells, cell therapy, pediatric cells*

Introduction

Reconstructive surgery is a key feature of pediatric surgery, whatever the organ involved [1]. During the last century, the management of neonates and infants with congenital malformations or acquired disabilities has considerably improved, thanks to the development of both anesthetic and surgical techniques. However, multiple procedures are sometimes required during the growth of the child to achieve an acceptable, stable and functional outcome. As an example, the management of labio-alveolar cleft requires a long-term follow-up after the initial surgical procedure. Healing complications such as cutaneous scar retraction, secondary acquired palatal fistulae and other orthodontic abnormalities have to be detected rapidly to ensure their early correction. To overcome these limits, alternative therapies have been proposed to better restore initial anatomical and functional properties of the wounded tissue and then avoid subsequent procedures. Stem cell–based therapy has been pointed out as a good candidate to reach this purpose [1].

Adipose mesenchymal stromal cells (ASC) are well known within the field of reconstructive surgery, with lots of pre-clinical and now clinical applications [2,3]. Due to specific properties, such as differentiation

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potential into different tissue lineages, secretion and immunomodulation characteristics [4], their abundance, with little invasive access, and the absence of ethical issue, they have been widely studied in adults [5]. However, to date, very little information concerning pediatric ASC properties has been reported and it is insufficient to consider their clinical use in children. Maiorana et al. first reported the isolation and characterization of ASC from omental adipose tissue in 13 children (6.5 ± 4.3 years old) and 2 newborns describing their adipogenic differentiation potential and immunophenotype [6]. Guasti et al. focused on the plasticity of pediatric ASC that express pluripotency markers such as c-Myc, OCT4, Nanog, KLF4 and DNMT3B but not Sox2 and their multipotency, as pediatric ASC also express many lineage markers and differentiate into adipose, chondrogenic, osteoblastic and neuronal cell types [7]. Due to such skeletogenic differentiation potential of ASC from the 16 children tested, they concluded that adipose tissue could be a potential source of stem cells for cartilaginous or bone replacement. Wu et al. performed the only comparative study in infants (age < 1 year), adults and elderly ASC and concluded that infant ASC have long telomeres and exhibit enhanced angiogenic and osteogenic capabilities, based on four samples [8]. Moreover, the *in vivo* therapeutic potential of children ASC has never been evaluated. If the goal of an ideal therapy is not to repair but to regenerate a limb or organ, a more extent characterization of cells from younger donors is required as well as efficacy data in a model.

The aim of our study was, therefore, to assess the potentiality of pediatric ASC obtained from 27 donors from Group 0–1 years old and 50 donors from Group 1–12 years old to study sampling and phenotyping of the crude stromal vascular fraction (SVF) of adipose tissue, culture, differentiation, immunomodulation, gene expression profile and *in vivo* beneficial effect in ischemic tissue, depending on the age of the donor.

Methods

Adipose tissue sample collection

Pediatric subcutaneous adipose tissue (1 g) was obtained from donors aged from 0 to 1 year (Group 0–1; 0.3 ± 0.5 year old; n = 27; male = 23; female = 4) and from 1 to 12 years (Group 1–12; 6.6 ± 6.1 years old; n = 50; male = 47; female = 3) undergoing elective inguinal surgeries (inguinal hernia repair and orchidopexy). The investigation was approved by the local ethics committee (AS Child 1 no. 08-082-03 and AS Child 2 no. 11-228-02), national agency (CPP-ID-RCB no. 2008-A01469-46 and no. 2011-A01469-32) and written informed consent from both parents. Adult subcutaneous adipose tissue (10 g) was obtained from donors undergoing elective abdominal dermolipectomy (age 20–35 years, body mass index [BMI] < 28; n = 85; male = 4; female = 81). No objection certificate was obtained according to bioethics law number 2004-800 of August 6, 2004.

ASC isolation and culture

Adipose tissue samples were digested in alpha - Minimum Essential Medium (α-MEM, Invitrogen) supplemented with 0.4 U/mL NB4 collagenase (Serva electrophoresis) for 45 min at 37°C under agitation. Cellular suspension was filtrated through 25 µm nylon membrane and centrifuged at 600*g* for 8 min to separate floating mature adipocytes from SVF. SVF was incubated in erythrocyte lysis buffer (ammonium chloride solution, StemCell Technologies) for 5 min at 4°C and washed in phosphate-buffered saline (PBS). SVF cells were re-suspended in culture medium α-MEM for viability and cell numeration (Thoma hemocytometer).

SVF cells were then seeded at 4000 cells/cm^2 in flasks treated for cell culture (TPP, D. Dutscher) in ASC expansion medium that consisted of α-MEM supplemented with 2% human plasma enriched with human platelet growth factors (EFS-PM Toulouse), 1 U/mL heparin Choay (Sanofi Aventis), 0.25 µg/mL amphotericin, 100 µg/mL streptomycin and 100 U/mL penicillin (Invitrogen). Cells were incubated at 37°C under 5% CO_2 and the medium was changed twice a week. After 7 days, ASC were harvested with trypsin-ethylenediaminetetraacetic acid (EDTA; LifeTechnologies). The number of viable cells was determined using Trypan blue exclusion on a Countess cell counter. Cells were then used (ASC P0) or plated at a density of 2000 cells/cm^2 and cultured until use at passage P1 (ASC P1). For long-term culture ASC were successively passaged at 80% of confluency until passage 10 (ASC P10).

Colony forming unit-fibroblasts assay

Freshly prepared SVF cells or ASC P0 were seeded in 25 cm^2 flasks at 16 cells/cm^2 in ASC expansion medium. The medium was renewed every 2 or 3 days. The cultures were ended at day 14 for ASC P0 and day 10 for ASC P1. The flasks were stained with the kit RAL stainer MCDh (RAL Diagnostics) to score the fibroblast colonies using an optical microscope.

Cell phenotyping

SVF cells were incubated with PBS supplemented with FcR Block reagent (Miltenyi Biotec). Sextuplet staining were performed by incubating cells for 30 min at 4°C with the following conjugated primary antibodies or appropriate immunoglobulin (Ig) G isotype