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Fat harvesting site is an important determinant of proliferation and pluripotency of adipose-derived stem cells

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ABSTRACT

To define the optimal fat harvest site and detect any potential differences in adipose-derived stem cells (ASCs) proliferation properties in camels, aspirates from the abdomen and hump sites were compared. Obtained results revealed that ASCs from both abdomen and hump exhibited spindle-shaped and fibroblast-like morphology with hump-derived ASCs being smaller in size and narrower in overall appearance than abdominal ASCs. Abdominal ASCs required a greater time for proliferation than the hump-derived cells. These results were further confirmed with a tetrazolium-based colorimetric assay (MTT) which showed a greater cell proliferation rate for hump ASCs than for the abdomen. Under inductive conditions, ASCs from both abdominal and hump fat deposits maintained their lineage differentiation potential into adipogenic, chondrogenic, and osteogenic lineages during subsequent passages without any qualitative difference. However, expression of alkaline phosphatase was higher in osteogenic differentiated cells from the hump compared with those of the abdomen. Moreover, the increase in calcium content in hump-derived stem cells was higher than that in abdominal-derived stem cells. In conclusion, our findings revealed that ASCs can be obtained from different anatomical locations, although ASCs from the hump fat region may be the ideal stem cell sources for use in cell-based therapies.

1. Introduction

Stem cells are identified by their unique capability to self-renew, as well as to generate progenitor cells, which then give rise to an inexhaustible supply of differentiated cell types [1]. In this respect, adult mesenchymal stem cells (MSCs) have received great interest and are currently the most appealing cell subsets, due mainly to their extensive self-renewal and differentiation potential [2]. Several adult tissues have been shown to contain rare populations of stem cells with mesenchymal characteristics. In our previous studies, as well as in studies from other laboratories, MSCs have been isolated and cultured from bone marrow [3], umbilical cord blood [4], muscle tissue [5], neuronal tissue [6], periosteum [7], periodontal ligament [8], dental pulp [9], deciduous teeth [10], dental follicle [11], and pancreatic [12] and hepatic tissues [13].

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Adipose tissue is another alternative source for MSCs [14] that can be isolated by a less invasive method and in large quantities [15] than from other sources. These MSCs, which are appropriately named adipose-derived stromal cells (ASCs), are isolated with higher efficiency, have a great expansion potential, and also appear to differentiate efficiently into the same cell lineages as other sources do [16–18]. This source of multipotent cells has been obtained and expanded efficiently from humans [19,20], but also from species such as mouse [21], rat [22], rabbit [23], sheep [24], horse [25], dog [26], as well as non-human primate [27].

Differences in differentiation potential have been observed among ASCs obtained from various species [28]. Our recent experimental findings [29] have provided some support for the hypothesis that camel ASC populations from abdominal tissue like human lipoaspirate [3,19,20] were able to maintain their unique properties during multi passages and that those cells had potential to differentiate into multilineage cells.

Another important aspect to be determined is whether the diversity existing in depots of adipose tissue within the same organism could lead to different outcomes of differentiation. A recent study in dogs demonstrated that the anatomical origin of the adipose tissue has an evident effect in the differentiation potential of the ASCs [30]. In this regard, it has been shown that ASCs from the neck region may be the ideal stem cell sources for tissue engineering approaches for the regeneration of nervous tissue in rat [31]. Therefore, it seems that settling preferred donor origins and sites for lipoaspiration and isolation of ASCs will certainly contribute to a more successful use of fat transplantation enriched with mesenchymal cells. To the best of our knowledge, no previous study has evaluated the effect of anatomical origin of the adipose tissue on proliferation of ASCs and the extent of their pluripotency in camel. A camel model was selected for this study largely due to its different fat depot physiologies.

Therefore, in this study we isolated camel ASCs from abdominal and hump tissues to compare their ease of isolation and to characterize their proliferation ability along the cultured time. The multipotency of these cells was assessed by inducing their differentiation into different cellular lineages, including adipogenic, chondrogenic, and osteogenic and analyzing the production of specific extra cellular matrix and cytoskeletal elements.

2. Materials and methods

All chemical reagents were obtained from Sigma (Sigma, USA) unless otherwise noted.

2.1. Harvesting of camel adipose tissue

Adipose tissues were collected from abdominal and hump depots from 5 adult dromedary camels, between 2 and 5 years of age. For sampling, using sterile scalpel blades, forceps, and scissors, adipose tissues were harvested from abdominal and hump tissues after slaughter of each of the five animals.

2.2. Isolation and expansion of camel ASCs

Camel ASCs were isolated by enzymatic digestion as previously performed in our laboratory [29]. Briefly, adipose tissues were washed extensively by phosphate buffer saline (PBS) and dissected into 1 mm pieces, followed by treating with 0.2% collagenase type I in Dulbecco's modified Eagle's medium (DMEM). The cell suspension was centrifuged at 350 *g* for 5 min, and the supernatant was discarded. Cell pellet was suspended in DMEM (low glucose) with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin 100 U/ml, streptomycin 0.1 mg/ml) at 37 °C atmosphere of 5% CO_2. Cells were seeded in tissue culture polystyrene plates and grown in monolayer culture under above conditions. When the cells reached 80% of confluence, they were detached from the culture dish using trypsin/EDTA. Cells were then divided into two aliquots; the first aliquot was passaged into fresh culture flask upon reaching confluence and the second was frozen in 90% FBS and 10% DMSO and stored in liquid nitrogen for later use. The survival rate, evaluated by trypan blue staining exclusion test, of all thawed MSC lots was >85% before use in this study. To study the variability of cryopreserved progenitor cells, all ASCs thawed cells were subjected to the trypan blue test and examined for proliferative capacity. Cells from passage 2 to passage 3 were used for all experiments.

Population doubling time (PDT) was calculated according to the equation PDT = culture time (CT)/population doubling number (PDN). To determine PDN, the formula PDN = log N/N0 × 3.31 was used. In this equation, N stands for the cell number at culture end and N0 the number of the cells at culture initiation. To determine the culture time and N and N0 in each passage, cells were counted and plated at 10^4 cells/cm^2 in 25-cm^2 culture flasks for a period when one of the cultures reached confluence. At this time, the cells were trypsinized and counted. Using the data, PDT was calculated for ASCs from abdominal and hump fat depot in each passage until passage 8 (P8).

ASCs at passage 2 (P2) were seeded with an initial cell density of 5000 cells/well in 24-well tissue culture polystyrene plates and were cultured for 5 days. The proliferation of these cells was evaluated via tetrazolium-based colorimetric assay (MTT test). Thus on each day, 50 μl of MTT solution (5 mg/ml in DMEM) was added to each well, to evaluate the conversion of MTT to formazan crystals by the mitochondrial dehydrogenases of the living cells. The plate was incubated at 37 °C, 5% CO_2. After 3.5 h incubation, the supernatant was removed to examine the dissolution of the dark-blue intracellular formazan, and 250 μl dimethyl sulfoxide (DMSO) as an appropriate solvent was added. The optical density was read at a wavelength of 570 nm in a micro-plate reader (ELx-800, BIOTEK instruments, Winooski, VT, USA).

2.3. Multilineage differentiation potential of ASCs

Different batches of cryopreserved cells from passage 0 were thawed and expanded to the second passage (P2). When 80% confluent, the cells were used for induction of differentiation into three lineages.

2.3.1. Adipogenic

Induction of adipogenic differentiation was accomplished as previously described [29]. Briefly, the cells were seeded at a density of 2 × 10^4 cells/cm^2 into four-chamber slides and cultured in basal media (DMEM with 10% FBS) supplemented with 0.5 mM hydrocortisone, 60 mM indomethacine, and 0.5 mM isobutylmethylxanthine. To assess adipogenic capacity, lipid accumulation was identified in differentiated cells with *in situ* Oil Red O (ORO) staining 21 days after induction. An undifferentiated batch of cells was allocated to the control group without differentiating supplements.

2.3.2. Chondrogenic

Chondrogenic differentiation of ASCs was induced by a 21-day culture in micropellet, as described elsewhere [29,32]. Briefly, MSCs (2.5 × 10^5 cells) were pelleted by centrifugation in 15 ml conic tubes and cultured in DMEM supplemented with 0.1 μM dexamethasone (Sigma), 50 μg/ml ascorbic acid 2-phosphate (Sigma), 1% insulin–transferrin–sodium selenite supplement (Gibco), and 10 ng/ml TGF-β (Peprotech).