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Isolation and Functional Characterization of Equine Adipos Tissue Derived Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) isolated from different sources are self-renewing progenitor cells with multipotent differentiation capacity. MSCs posed the capacity to proliferate extensively in vitro in optimal culture conditions, and represents the key factors in regenerative medicine. Our objective was to isolate and characterize equine MSCs from adipose tissue. Samples were obtained during castration. The adipose tissue samples were enzymatically digested and the isolated cells were cultured in DMEM/F12 medium. MSCs were characterized by morphology, multilineage differentiation capacity and expression of specific markers. Equine adipose tissue derived stem cells displayed mostly fibroblastic–like morphology, capable of trilineage diffentiation capacity and showed significant expression of CD105, CD44, CD90, CD49 while were negative for CD43 and CD73. This study demonstrated the characteristics of equine adipose tissue derived stem cells, ideal candidate for veterinary regenerative medicine.

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Keywords: horse; stem cells;isolation; characterization.

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1. Introduction

Mesenchymal stem cells (MSCs) are considered one of the most promising cell types for regenerative therapy, due to their extensive proliferative ability and differentiation potential into various mesenchymal lineages and capacity to secrete bioactive molecules (Chamberlain et al., 2007, Nöth et al., 2010, Barberini et al., 2014). MSCs are the multipotent precursor cells of connective tissues and play an important role in tissue regeneration. Cellular therapy and tissue engineering in veterinary medicine are rapidly developing areas (Carvalho et al., 2013).

Adipose tissue represents a good and accessible source of stem cells in equine medicine and not only (Schnabel et al., 2009, Barberini et al., 2014). In equine medicine bone marrow is one of the most studied and used sources for obtaining adult stem cells (Gutierrez-Nibeyro 2011; Sole et al., 2012; Barberini et al., 2014). Amniotic membrane and umbilical cord (Lange-Consiglio et al., 2013) also represents a promising source of MSCs.

These cells are preferred because they are less immunogenic, their collection is non-invasive (Rogers et al., 2004; Guest et al., 2008). MSCs isolated from equine tissues are identified by their adherence to plastic, their morphology and their ability and multilineage differentiation capacity (Barberini et al., 2014). immunophenotyping in horses is limited because the availability of the monoclonal anti-horse antibodies are few and other species do not cross-react with equine species (Pascuccia et al., 2011). Several markers have been tested and used, based on minimal criteria established by the International Society for Cellular Therapy (ISCT) to define human MSCs (Dominici et al., 2006) and adipose-tissue derived stem cells (Bourin et al., 2013; Paebst et al., 2014). Our work focused on isolation and functional characterization of the adipose-derived mesenchymal stem cells (AT-MSCs) in horses.

2. Material and methods

Tissue samples were obtained from a healthy adult stallion during castration. Adipose tissue (approximately 1 g) were collected from the scrotal sac and transported in DMEM medium (Sigma) supplemented with 10% fetal calf serum (FCS, Sigma), 1% antibiotic-antimycotic (Gibco) at room temperature in a sterile 50 mL conical tube. The adipose tissue sample was washed three times with PBS, minced into 1 to 2 mm pieces and digested with collagens type II 0.075% solution (Sigma) in Hank's Balanced Salt Solution (HBSS) (Sigma), for 1 h at 37°C and in slow agitation.

The cells suspension was filtered and centrifuged. After centrifugation were resuspended in normal propagation medium were represented by DMEM/F12 (Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham) (Sigma) supplemented with 10% FCS (EuroClone), 2 mM glutamine, 1% Non Essential Amino-Acids (NEAA) (Sigma). The cultures were maintained in a humidified atmosphere containing 5% CO2 at 37°C. After 3 days of culture, the medium was replaced. MSC-like cell were growth until confluence (70-80%) and then sub-cultured (1:2). For evaluation the CFU-F the cells were plated at 1 cell/cm in 100 cm^2 Petri dishes, and incubated in a humidified atmosphere with 5% CO_2 at 37°C for 10 days. After fixation with methanol the cultures were stained with 0.5% crystal violet (Sigma) in 100% methanol for 10 minute. The cultures were examined under phase contrast inverted light microscope (Nikon) and the colonies (> 50 cells) were counted.

The study was performed in triplicate. CFU-F efficiency was estimated using the formula: CFU-F efficiency = (counted CFU-F/cells originally seeded) × 100. In order to investigate the proliferation potential of equine mesenchymal stem derived from adipose tissue a total number of 10^5 cells/ well were seeded in 24-well cell culture plates.

After 24 h (t24h) the non-adherent and the adherent cells (N0) were counted. 24 h later (t48h) the adherent cells from three wells were counted (N48h) (and repeated eight times in 48h intervals). The doubling time (tD) was calculated according to the formula: tD = (log 2 × t)/ (log N48h - log N0). The experimet were performed in triplicate. Immunophenotypic analysis of AT-MSCs was performed at P3 with the FACS CantoII flow cytometer using the following antibodies: mouse anti-human CD34, mouse anti-human CD105, mouse anti-rat CD90, mouse anti-human CD44 and mouse anti-human CD73.

Data from 10,000 events were recorded. For the statistical analysis, the binomial dependent variables were evaluated using GraphPad Prism 6 software (Manual Graph Pad Prism 5.0, GraphPad Software). A value of $p < 0.05$ was considered statistically significant. Data were reported as the mean ± SD.