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Canine Wharton's Jelly Derived Mesenchymal Stem Cells Isolation

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Abstract

Mesenchymal stem cells (MSCs) represent an attractive source of cells for cell therapy in veterinary medicine. Recent interest in stem cell biology and its therapeutic potential has led to search for accessible new sources of stem cells. Wharton`s Jelly-derived mesenchymal stem cells are multipotent with specific potential to differentiate into multiple lineages.

The aim of our study was the isolation, characterization and multilineage differentiation of Wharton`s Jelly derived mesenchymal stem cells obtained from canine umbilical cord following Caesarean section.

Our data confirmed that the isolated and cultivated mesenchymal stem cells have multipotent based on specific surface antigen expressing and differentiation capacity.

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Keywords: canine; stem cells; Wharton's Jelly; antigen expressing.

1. Introduction

Umbilical cord blood is an important source of hematopoietic stem cells (HSCs) in humans, being the most common source of mesenchymal cells (MSCs) for clinical applications (Cardoso et al., 2011, Francese et al., 2010, Kadam et al., 2009). The umbilical cord is formed from two arteries and one vein, which are surrounded by mucoid connective tissue, called the Wharton's jelly (WJ) (Wang et al., 2004). WJ is abundant in hyaluronic acid which

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forms a hydrated gel around the fibroblasts and collagen fibrils and maintains the tissue architecture and protecting the umbilical cord from pressure (Sobolewski et al., 1997; Wang et al., 2004). Recent studies demonstrate the presence of CD44 positive cells in WJ, with clonogenity and specific differentiation capacity (Wang et al., 2004). Mesenchymal stem cells (MSCs) from different sources are multipotent, capable of multilineage differentiation (Caplan and Bruder et al., 2001). MSCs have received extensive attention as a specific source of cells for veterinary regenerative medicine because possess valuable characteristics such as multipotency, paracrine activities, and immune modulation potential (Arthur et al., 2009, Caplan and Bruder et al., 2001).

The purpose of our study was the isolation and functional characterization of the Wharton jelly derived mesenchymal stem cells (WJ-MSCs) in dog.

2. Materials and Methods

Tissue samples were obtained from a healthy Cane Corso dog during cesarean section. Wharton's jelly is cord matrix, which could be easily distinguished from placenta by its macrostructure. The tissue was collected in sterile transport medium (DMEM supplemented with 10% fetal calf serum (FCS), 1% antibiotic-antimycotic) and transported to the cell laboratory in sterile condition. For isolation the cells, the blood vessels were removed, the mesenchymal tissue was scraped off, minced and incubated with collagenase solution (2 mg/ml) for 3 hours at 37ºC. The cells suspension were filtered and centrifuged. The supernatant were removed and the cells were seeded into T75 flasks with normal propagation media DMEM/F12 supplemented with 10% FCS, 2 mM glutamine, 1% Non Essential Amino-Acids (NEAA), 1% antibiotic-antimycotic (Sigma). The cultures were maintained in a humidified atmosphere containing 5% CO2 at 37C°. The minced tissue was incubated at 37°C for approximately 3–4 h. After incubation, the suspension was washed with phosphate-buffered saline (PBS; Cellgro, USA) and centrifuged at 350 × *g* for 5 min. The pellet was resuspended in the basal culture medium, which is low-glucose Dulbecco's modified Eagle's medium (LG-DMEM; Gibco BRL, USA) with 10% fetal bovine serum (FBS; Gibco BRL, USA). The resuspended cells were seeded into T75 polystyrene cell culture flasks (Nunc, USA) with basal culture medium. The basal culture medium was replaced three times per week until the adherent cells reached 70–80% confluency. After 72h the medium was replaced, the cells were grown until confluence (70-80%) and then passed (1:2).

After 5 passages the immunophenotypic analysis were performed with the FACS CantoII flow cytometer using the following antibodies: CD34, CD44, CD90, CD29. Data from 10,000 events were recorded. The multipotency was confirmed by the ability of cells to differentiate into osteocytes, adipocyte.

For the evaluation of osteogenic differentiation potential, 0.5×10^5 cells /well were cultured until subconfluencein the normal propagation medium. The medium was replaced by osteogenic medium consisting in DMEM (Gibco Life Technologies, Paisley, UK) supplemented with 20% FCS (Sigma), 0.2 mM L-ascorbic acid-2-phosphate (Sigma), 100 nM dexamethasone (Sigma), 10 mM ß-glicerophosphate (Sigma), 1%antibiotic antimycotic (Gibco). The medium was replaced every 3 days. After 21 days the culture were fixed and calcium deposition, were detected using Alizarin Red staining, the Alizarin Red was solubilized in cetylpyridinium chloride (Sigma) and absorbance were measured at 550 nm.

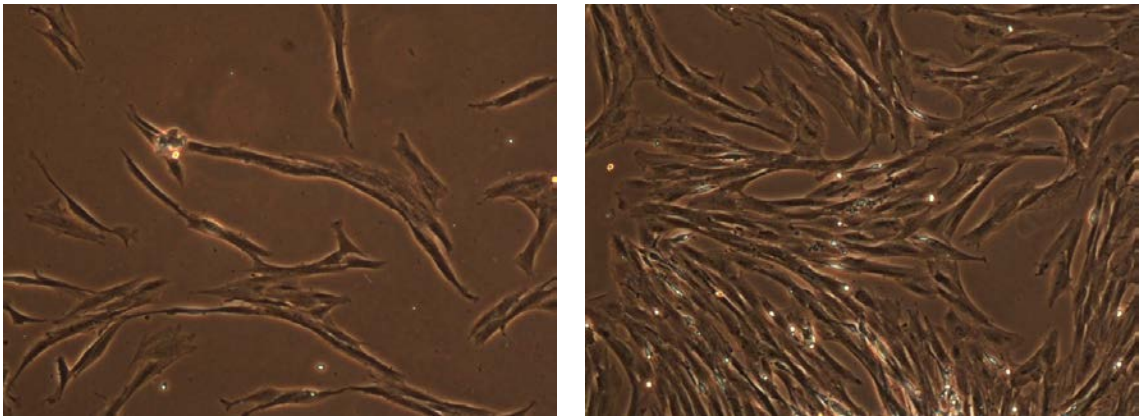
For the evaluation of adipogenic differentiation potential 0.5×10^5cells/well were seeded and cultured until 70% confluence in standard propagation medium. The medium was replaced with adipogenic induction medium: DMEM 4.5g/l glucose (Sigma) supplemented with 10% FCS (Sigma), 10^{-6} M dexamethasone (Sigma), 0.5mM isobutylmethylxanthine (Sigma), 10 µg/ml insulin (Sigma), 1% L-glutamine and 1% antibiotic/antimycotic (Gibco). The induction medium was replaced every 3 days. After 21 days of culture the cells were fixed with 10% formalin and stained with Oil Red O (Sigma-Aldrich, St. Louise, USA) for 10 min. The Oil Red was solubilized with 100% isopropanol, and the absorbance was measured at 450 nm.

Statistical analysis was performed 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.

3. Results and Discussion

Canine MSCs were isolated from Wharton jelly during cesarean section. MSCs were cultured in normal propagation medium and the cells were selected based on plastic adherence. After 72h the level of attachment and the cell morphologies were assessed. After 10 days the cells presented heterogeneous morphology and were formed

a monolayer consisting. After 1st passages the morphology of cells was predominant spindle-shaped (Fig.1) with cytoplasmic extensions and with capacity to forming clusters.



a **b**

Fig. 1. (a, b) Morphological features of dog WJ-MSC culture

Fluorescence-activated cell sorting analysis was used to identify cell surface markers after 5 passages. The cells were positive for CD44, CD90 and CD29 but negative for hematopoietic marker CD34. To evaluate the *in vitro* osteogenic differentiation potential the WJ derived MSCS were maintained in osteogenic induction medium for 21 days. The matrix calcification was detected using Alizarin red staining. MSCs grown in normal propagation medium were used as a negative control. The Alizarin Red was solubilized with cetylpyridinium chloride and the absorbance was measured. The average of absorbance of negative control was 0.135±0.01 and 2.086±0.10 for WJ-MSCs (Fig. 2).

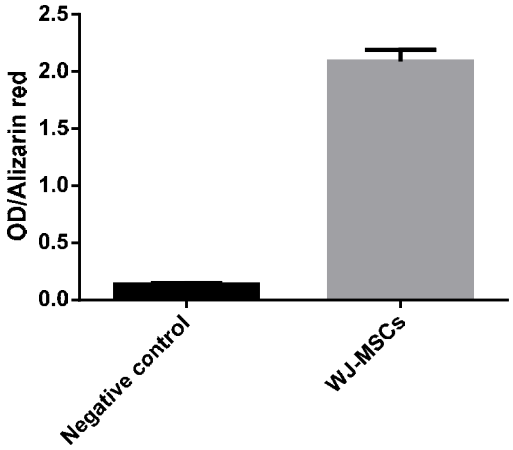


Fig. 2. Optical density or absorbance of Alizarin Red

In cultures treated with adipogenic induction medium the cells' morphology changed from a spindle-shaped towards a round morphology and after 15 days the presence of intracellular lipid droplets was highlighted using Oil Red staining. The cells were maintained with normal culture medium were represented the negative control. For evaluation, the Oil red was solubilized with 100% isopropanol and the absorbance was measured. The average of absorbance of negative control was 0.276±0.60, 0.619±0.04 for reMSCs and 0.667±0.05 for deMSCs (Fig.3).

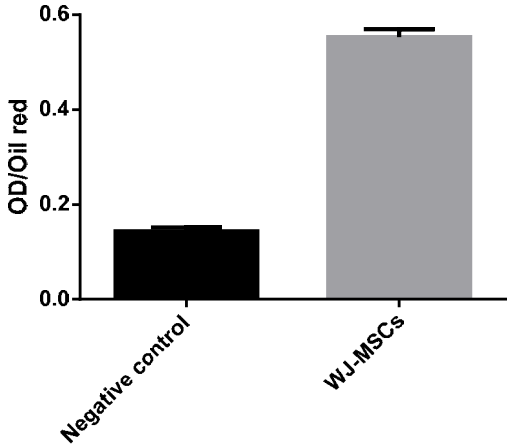


Fig. 3. Optical density or absorbance of Oil red

The cells morphology and plastic adherence property is not sufficient to characterize the tissue specific MSCs. For this reason is necessary a systematic analysis of some cell surface molecules of MSCs. Wharton's jelly derived stem cells express specific mesenchymal stem cell markers (CD73, STRO-1, CD90 and CD105 (Sreekumar et al., 2014). The plasticity of WJ-MSCS was assessed after directed differentiation into osteogenic and adipogenyc lineages. The differentiation was demonstrated using specific staining methods for lipid droplets and calcium deposits. Using flow cytometric analysis, we demonstrated that MSCs derived from umbilical cord, express matrix receptors and integrins markers, but not hematopoietic lineage markers. MSCs with similar characteristics can be isolate from different tissue. Vieira et al. (2010) isolated MSCs from subcutaneous adipose tissue after lipectomy with comparable characteristics; they concluded that these cells may represent an important stem cell source for veterinary cell therapy and also for preclinical studies. These data are in accordance with Neupane et al. (2008), they isolated MSCs from adipose tissue collected from subcutaneous, omental, and inguinal fat depots of dogs, using standard surgical procedures. These cells expressed stemness markers (OCT4, Nanog and SOX2) demonstrated by RT-PCR, and with trilineage differentiation capacity. The stemness markers OCT4, Nanog, and SOX2 are are usually described only for embryonic stem cells, but their expression has been documented in also in somatic stem cells (Neupane et al. 2008, Kucia et al., 2006, Izadpanah et al., 05).

4. Conclusion

Our study provides a protocol for isolation and characterization of MSCs from dog umbilical cord Wharton's jelly. Our results indicate that these cells have the potential to be a good source of cells in veterinary medicine.

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