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SHORT COMMUNICATION

A method of isolation and characterization of canine endometrial-derived mesenchymal stem cells

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Endometrial tissue is a known source of mesenchymal stem cells (MSCs). We isolated canine endometrial stem cells from canine endometrial tissues using an enzymatic method and confirmed the immunophenotype of mesenchymal stem cells and multilineage differentiation. Canine endometrial tissues were obtained from canine ovariohysterectomy surgery and isolated using 0.2% collagenase type I. We measured the immunophenotype of stem cells using flow cytometry. To confirm the differentiation ability, a trilineage differentiation assay was conducted. In this study, canine endometrial-derived MSCs (cEM-MSCs) were isolated by enzyme treatment and showed a spindle-shaped morphology under a microscope. Moreover, cEM-MSCs showed a trilineage differentiation ability. In this study, the canine endometrium was a good source of MSCs.

Key Words: Dog, Endometrium, Mesenchymal stem cells, Isolation, Characterization

INTRODUCTION

Mesenchymal stem cells (MSCs) can self-renew and undergo multilineage differentiation. MSCs can be isolated from various tissues such as adipose tissue, bone marrow, umbilical cord, amniotic membrane, and amniotic fluid (Ding et al, 2011). Furthermore, MSCs from human endometrial tissues have been identified, and investigated extensively (Gargett et al, 2016). MSCs are known to exhibit tissue regeneration, immunomodulation, and anti-inflammatory effects (Iyer and Rojas, 2008). Currently, MSCs are gaining significant attention in the veterinary medicine field and extensive research is being conducted on stem cell therapy (Quimby, 2018; Prisilin et al, 2022). However, research on canine endometrial stem cells in veterinary medicine is insufficient. In this study, we isolated canine endometrial-derived MSCs (cEM-MSCs) and showed their immunophenotypes and multilineage differentiation ability. Our results suggest that canine endometrial tissue may be a good source of MSCs and that cEM-MSCs could be beneficial as a therapeutic agent for dogs.

MATERIALS AND METHODS

Isolation of MSCs

Canine endometrial tissues were collected during ovariohysterectomy (OHE) surgery from healthy dogs. The protocols for this study followed the guidelines of the Animal Care and Use Committee of Chungnam National University. Endometrial tissue was scraped using a sterile blade and subjected to enzyme treatment. To obtain the cells, the tissue was scrapped, minced, and digested with 0.2% collagenase type I (Sigma-Aldrich, USA), and shaking every 15 min for 30 min at 37℃ in a humidified atmosphere of 5% CO_2. After incubation, the cell suspensions were filtered through a 40 μm cell strainer. After washing by centrifugation, the pellet was resuspended in culture medium containing Dulbecco's

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modified Eagle's medium supplemented with 10% Fetal Bovine Serum (Gibco), 1% GlutaMax (Gibco), and 1% penicillin/streptomycin (Welgene). Cells were cultured in T-75 flasks under a humidified atmosphere of 5% CO_2 at 37℃ for 7 days. The culture medium was changed twice a week until the adherent cells reached 70~80% confluence.

Flow cytometry

The immunophenotype of MSCs was evaluated at passage 3 (P3) by flow cytometry. The cells were stained with FITC- and PE-conjugated monoclonal antibodies as follows: CD9, CD14, CD34, CD44, and CD45 (Thermo Fisher, USA). The cells were washed with phosphate-buffered saline (Welgene), and analyzed using a BD Canto II flow cytometer (BD Biosciences, USA). The results were analyzed using FlowJo software (Tree Star, Inc., Oregon Corporation, USA).

Trilineage differentiation assay

Trilineage differentiation ability was evaluated in at P3. Cells were confirmed to be multipotent based on their osteogenic, adipogenic, and chondrogenic differentiation abilities using commercial differentiation kits (StemPro Osteogenesis Differentiation Kit, StemPro Adipogenesis Differentiation Kit, and StemPro Chondrogenic Differentiation Kit; Thermo Fisher Scientific) according to the manufacturer's instructions. After differentiation, cells with osteogenic, adipogenic, and chondrogenic differentiation were stained with Alizard Red S, Oil Red O, and Alcian Blue, respectively.

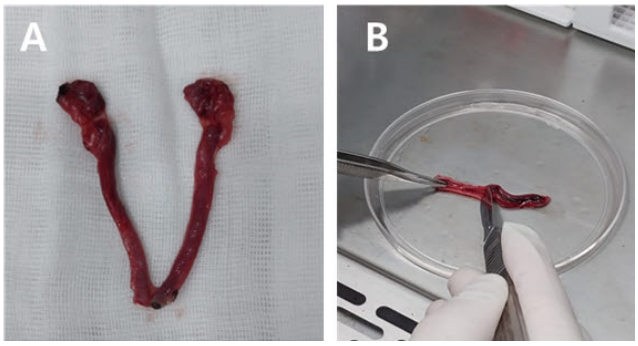

Fig. 1. (A) Canine endometrial tissue. (B) Scrapping of endometrial tissue using sterile blade.

RESULTS AND DISCUSSION

Isolation and morphology of MSCs

Canine endometrial tissue was obtained after OHE surgery (Fig. 1A) and scraped using a sterile blade (Fig. 1B). Isolation of MSCs was successful using enzymatic treatment. Cells of P3 generally showed plastic adherence in vitro and a spindle-shaped morphology (Fig. 2).

Immunophenotype

To confirm that the canine endometrial stem cells were MSCs, their immunophenotypes were analyzed using flow cytometry. The cells were positive for CD9 and CD44 as mesenchymal cell markers and negative for CD14, CD34, and CD45 as hematopoietic cell markers (Fig. 3).

Identification of trilineage differentiation capability

To characterize cEM-MSCs, we performed trilineage differentiation assays. MSCs differentiate into adipocytes, chondroblasts, and osteoblasts. To confirm osteogenesis, MSCs were cultured in osteogenic differentiation media for 2 weeks. Calcium mineralization formation was identified on the culture dish and we used Alizarin Red S staining, which detects the presence

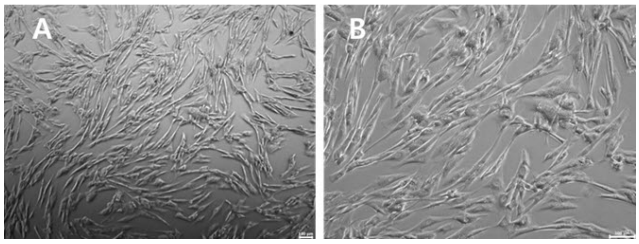

Fig. 2. (A) cEM-MSCs (P3) morphology; ×50 magnification. (B) ×100 magnification.

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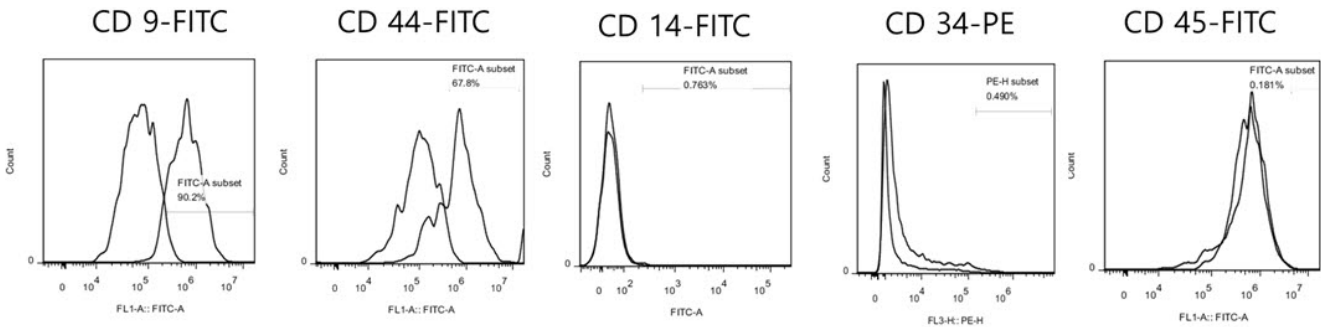

Fig. 3. Immunophenotype of cEM-MSCs.

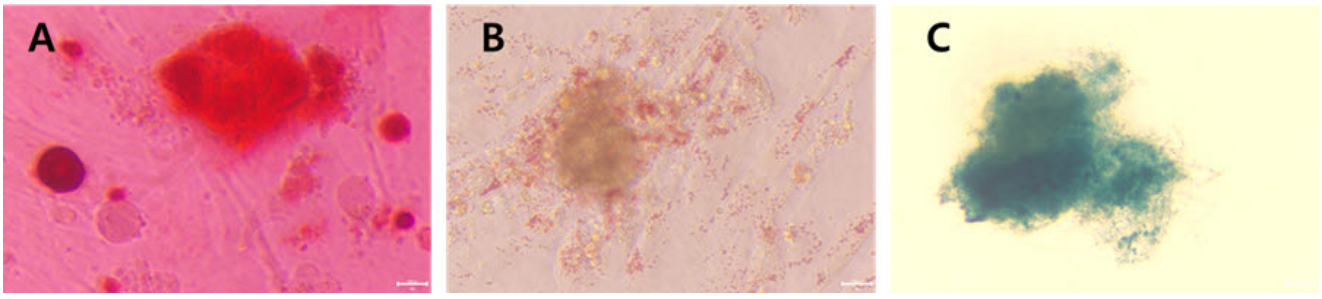

Fig. 4. (A) Osteogenic differentiation stained by Alizarin Red S. (B) Adipogenic differentiation stained by Oil Red O. (C) Chondrogenic differentiation stained by Alcian Blue.

of calcium deposits in the MSCs. As a result, Alizarin Red S positive stained cells were confirmed. To identify adipogenesis, MSCs were cultured in adipogenic differential media for 2 weeks. To confirm the presence of lipid droplets, we stained the cells with Oil Red O and confirmed the presence of Oil Red O-positive cells in the medium. To investigate chondrogenic ability, MSCs were cultured by the hanging drop method using chondrogenic differentiation medium for 2 weeks. The spheroids were collected and stained with Alcian Blue to check the synthesis of proteoglycans by chondrocytes. Alcian Blue stained cells were then confirmed (Fig. 4).

In this study, canine endometrial stem cells were isolated from canine endometrial tissues by enzymatic method (0.2% collagenase type I) spindle-shaped cells adhering to plastic were observed in vitro. Immunophenotypic analysis of the cEM-MSCs revealed typical patterns of MSCs. Moreover, a trilineage differentiation assay showed that cEM-MSCs can differentiate into adipocytes, osteoblasts, and chondroblasts. We propose that canine endometrial tissue is a good source of MSCs and has the potential to be a source for cell therapy.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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