Middle East Fertility Society Journal (2014) **19**, 156–170

Middle East Fertility Society

Middle East Fertility Society Journal

www.mefsjournal.org
www.sciencedirect.com

ORIGINAL ARTICLE

Comparative characteristics of amniotic membrane, endometrium and ovarian derived mesenchymal stem cells: A role for amniotic membrane in stem cell therapy

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Received 7 November 2013; accepted 25 January 2014
Available online 29 April 2014

KEYWORDS

Adult stem cells;
Amniotic stem cells;
Differentiation;
Endometrial stem cell;
Multipotent/progenitor cell;
Female reproductive tract;
Human endometrium;
Mesenchymal stem cell

Abstract *Objectives:* Isolation and comparison of cellular and molecular characteristics of MSCs derived from full term amniotic membrane (AM), endometrium and ovarian tissues.

Investigation of a possible therapeutic potential for AM MSCs in the female reproductive tract.

Study design & methods: Human amniotic membranes (AMs) from term placentas ($N = 8$), endometrial/stromal (by scrapping endometrium) and ovarian tissues from premenopausal hysterectomies ($N = 6$) after mincing and enzymatic digestion were included in the study.

Immunophenotyping by flow cytometry, Karyotyping, differentiation to mesoderm, RT-PCR analysis for transcription factors, WNT signaling pathways, pluripotency markers and HOXA genes were performed.

Results: Generated cells showed morphologically and phenotypically the characteristics of MSCs from other sources with fairly comparable results. The cumulative population doubling

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Peer review under responsibility of Middle East Fertility Society.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.mefs.2014.01.002

was significantly higher in endometrium MSCs 25 ± 5.2 compared to 17.2 ± 4.5 for AM MSCs and 10.7 ± 2.4 for ovarian MSCs ($P < 0.001$). They differentiated into osteogenic, adipogenic and chondrogenic lineages till passage 9. They all possessed normal karyotype by passage 6. They all expressed HOXA genes and differentially pleuripotency markers.

Intrauterine injection of GFP transfected AM MSCs into female Wistar rats ($N = 10$), demonstrated the presence of AM cells with GFP expression after 14 days within several mice uterine and ovarian cryosections. AM MSCs highly expressed genes involved in patterning of the female reproductive tract.

Conclusions: AM, endometrium and ovarian derived MSCs share fairly similar morphological, cellular and molecular characteristics. They possess mesenchymal stemmness potentials and endometrial MSCs possess the highest expansion capacity. AM MSCs may have a potential for stem cell therapy in the female reproductive tract.

1. Introduction

MSCs represent an important stem cell population with multipotent capabilities; they are considered candidates for stem cell therapy.

The amniotic membrane (Am) has considerable advantageous characteristics. It has low immunogenicity, anti-inflammatory properties and lacks ethical problems and has been suggested for clinical trials (1).

Investigating the role of endometrial stem/progenitor cells in uterine dysfunction and the pathogenesis of common gynecological diseases is mandatory. Such knowledge will enhance the understanding of endometriosis, adenomyosis, endometrial hyperplasia, and endometrial cancer, and will help in finding proper therapeutic solutions (2). Studies showed that ovarian stem cells can be cultured in vitro into matured oocytes. A case of ovarian failure following chemotherapy was transplanted with cryopreserved tissue and resulted in a live birth (3). Therefore, potential implications of stem cells to reproductive biology and medicine are apparent.

Uterine and ovarian insufficiency could be rejuvenated by stem cell therapy. Adult stem cells are considered for the regeneration and remodeling of the female reproductive tract, still their full potential remains to be determined.

Our aim is to isolate and study cellular (phenotypic, proliferative, differentiation) potentials together with molecular characteristics of MSCs derived from AM, endometrium and ovarian tissues.

We investigated a possible therapeutic potential for AM MSCs in the female reproductive tract by IU injection of AM transfected MSCs into female rats, and assessing their presence in uterine and ovarian cryosections 2 weeks later.

2. Materials and methods

Term human placentas ($N = 8$) were obtained with informed consent from healthy pregnant women at delivery (maternity hospital, Cairo University). Endometrial/Stromal and ovarian tissue pieces were obtained from premenopausal patients ($N = 6$) undergoing pan hysterectomy at the obstetric gynecology department, Cairo University. The study was approved by the Institutional Review Board of the Faculty of Medicine, Cairo University.

2.1. Isolation of human mesenchymal stem cells derived from amniotic membrane (AM), endometrium and ovarian tissues

The fresh amnion was mechanically separated from the loose chorion and decidua, washed with phosphate-buffered saline (PBS) to remove cellular debris, incubated in 0.25% trypsin–EDTA (ethylene amine tetra acetic acid; GIBCO-Invitrogen, Carlsbad, CA, USA) at 37 °C for 30 min. Full thickness endometrium was collected in a Heppes-buffered medium (DMEM) with 5% fetal calf serum and antibiotics. A piece of ovarian tissue was collected the same way. Human endometrial tissue was scraped from the myometrium. Ovarian tissues were further dissected by a gynecologic surgeon into cortex (the outermost layer) and medulla (the innermost layer).

Each tissue was minced with a scalpel into pieces not bigger than 2 mm. Enzymatic digestion by 1 mg/ml collagenase type I (Sigma Aldrich) and 5 ml Accutase (Sigma Aldrich) was performed for 60 min at 37 °C with permanent shaking, followed by filtration through a 100 μm mesh filter (BD FALCON). Some tissue pieces were used as explants.

Cell suspensions were centrifuged at 400*g* for 10 min, cell pellets were washed and then cultured at a density of 1×10^5 -cells/cm^2 in T75 culture flasks in a Dulbecco modified Eagle medium, DMEM (Hyclone, Thermoscientific) supplemented with 10% Fetal calf serum (FCS, Euroclone, Germany), penicillin (100 units/ml), and streptomycin (100 μg/ml). When cultures reached 80–90% confluence; MSCs were trypsinized using trypsin EDTA 0.25% (Hyclone, Thermofisher Scientific), and replated for passaging. MSCs adherent to plastic dishes and having a fusiform appearance were established.

2.2. Proliferation capacity and morphological characteristics

Morphology evaluated each passage for different generated cells.

Passage population doubling and the cumulative population doubling were calculated over 50 days using the formula: Population doubling = log (NH)−log (Ni)/log 2 where: (Ni) = initial number of cells; (NH) = number of cells in the confluent layer, with high seeding density 10×10^3 cell/cm^2 (4).

2.3. Karyotype evaluation

Cells (passage 6)were treated with culture medium containing colchicine solution 0.1 ml (Invitrogen) for 30 min and treated with hypotonic buffer 0.075 M KCl (Potassium Chloride, Invit-