Journal of Genetic Engineering and Biotechnology xxx (2017) xxx–xxx

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Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Differentially expressed genes: *OCT*-4, *SOX*2, *STAT*3, *CDH*1 and *CDH*2, in cultured mesenchymal stem cells challenged with serum of women with endometriosis

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ARTICLE INFO

Article history:
Received 24 April 2017
Accepted 5 October 2017
Available online xxxx

Keywords:
Endometriosis
Mesnchymal stem cells
OCT-4
*SOX*2
*STAT*3
E-cadherin
N-cadherin

ABSTRACT

Endometriosis is a common chronic gynecological disorder defined as the presence of ectopic functional endometrial tissues, outside uterine cavity, primarily on the pelvic peritoneum and the ovaries. Several studies revealed a correlation between aberrant stem-cell activity in the endometrium and endometriosis. Yet the molecular and cellular behaviors of mesnchymal stem cells in development of endometriosis are hampered by lack of invitro experiments. Our aim was to explore morphological and molecular changes associated with mesenchymal stem cells (MSCs) exposition to serum derived from women with severe endometriosis. Two cell cultures of MSCs isolated from endometrial tissues of two endometriosis-free women. Each cell culture was treated individually with the serum of women with endometriosis (experimental group/n = 7), and serum of women without endometriosis (control group/ n = 4) for 14 days. Quantitative Real-Time PCR was performed later to reveal expression of *OCT*-4, *CDH*1 and *CDH*2, *STAT*3 and *SOX*2 genes. Morphologically, cells showed no significant changes. However from molecular point of view, we found increased expression in *OCT*-4, *CDH*1 and *CDH*2. For *STAT*3 and *SOX*2 we did not find a significant difference. This study shows that endometriosis serum induced molecular changes in human endometrial MSCs (EnMSCs) that might be related to altered cell behavior which may be a step in differentiation that may be completed invivo by other factors to complete the process of transition. Further researches are needed for optimization to reach differentiation.

1. Introduction

Endometriosis is the growth of endometrial tissue outside the uterine cavity. It is a common gynecological disease that causes chronic pelvic pain and infertility. It affects 8–10% of women. Several theories explain the development of endometriosis. Retrograde menstrual reflux [52], presence of ectopic endometrial stem cells [48] and genetic factors [25] in the etiology of endometriosis have been the main issues in the pathogenesis of endometriosis nowadays [4].

Several factors may play role in development of endometriosis as including abnormal endometrium, altered peritoneal environment, reduced immune surveillance [53], increased angiogenic capacity [14] and endometrium inducing factor(s) [33]. Long-term endometriotic lesions may develop from endometrial stem/progenitor cells, those that may have been established by more mature transient amplifying progenitor cells. Genes encoding proteins involved in cell adhesion, extracellular matrix remodeling, migration, proliferation, immune system regulation, and inflammatory pathways may work by different mechanisms to establish ectopic endometrial implants [12].

Peer review under responsibility of National Research Center, Egypt.
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https://doi.org/10.1016/j.jgeb.2017.10.006
1687-157X/

Stem cells are undifferentiated cells, featured by their ability to self-renew and differentiate into various specialized cells. EnMSCs derived from ectopic endometriotic lesions exhibit elevated proliferative, migratory, and angiogenic activities. Accordingly, it was reported that EnMSCs represent a major player in endometriosis pathogenesis [11,45].It was also suggested that the stem cell theory, may account for an alternative endometriosis pathogenesis mechanism and can be involved in all conventional theories as well [49]. Endometrial stem cells are responsible for the rapid cellular proliferation and regeneration of the endometrium [3]. Years ago it was found that an intact endometrial lining could be reached from few endometrial cells regeneration [51].

Transcription Factors (TFs) are proteins that participate in DNA binding, protein–protein interactions, and transcriptional activation or repression. TFs interact with the basal transcriptional machinery and/or chromatin modifying proteins, via altering the rate of gene transcription [55]. Octamer-binding transcription factor 4 (*OCT*-4), sex determining region Y-box 2 (*SOX*2) and signal transducer and activator of transcription 3 (*STAT*3) are three major transcription pluripotency factors. Normally, they contribute to regulation of proliferation and differentiation in stem cells [43,48]. These factors were reported to be aberrantly expressed in endometriotic tissues [21,2,20,1]. *OCT*-4 was found to stimulate endometrial cell migration activity leading to ectopic endometrial development [20]. *SOX*2, together with *NANOG*, was suggested to enhance cell survival in ovarian endometrial tissues which may further promote endometriotic ectopic growth [29]. *STAT*3 activation was found to play an important role in the pathogenesis of endometriosis. It contributes to the inflammatory phenotype of eutopic endometriotic tissue [41]. Cadherins are a family of calcium-dependent cell adhesion molecules. They are transmembrane glycoproteins that account for cell-cell contact through adherens junctions. Cadherin family comprises several members such as *E-cadherin (CDH*1) and *N-cadherin (CDH*2) and others [50]. They modulate a wide variety of processes, including cell polarization, migration and cancer metastasis [9]. Cadherins were found to take part in the strong adhesion exhibited by the endometriotic cells in ectopic sites [8].

We established our study to understand the pathogenesis of stem cells in development of endometriosis. This will enable us to achieve a definitive treatment or even prophylaxis against this intractable disease in the future.

2. Materials and methods

2.1. Study population

This study accounts an experimental prospective case-control pilot study, including eleven women subjects. It was approved by the Medical Research Ethics committee of the National Research Centre, Cairo, Egypt. Written informed consents were obtained from all participants. The samples were recruited from the Obstetrics and Gynecology Department, Faculty of Medicine, Cairo University. Of the eleven participants, seven had severe endometriosis (the experimental group) and four were endometriosis free (the control group).

The following criteria were met in the study; (1) the endomtriotic women suffered from bilateral endometriomas >5 cm in diameter with peritoneal adhesions and underwent open or laparoscopic surgery for removal, (2) the control women with infertility and underwent diagnostic laparoscopy, (3) endometriosis laparoscopic diagnosis was confirmed by histopathological examination, while, the laparoscopy inspection in control subjects showed that they were clearly free from any endometriotic lesions, (4) all participants did not receive any hormonal therapy 6 months prior to the time of sample collection, as well, (5) they did not have a history of blood malignancies, chronic or immunological diseases.

2.2. Serum collection

Peripheral blood samples, from endometriotic (n = 7) and control (n = 4) women were collected. Whole blood of each participant was obtained into vacutainers without anticoagulant then centrifugation of blood sample at 1800 g for 10 min was done followed by separation of resulting supernatant (filtered through 0.2 mm pore size membrane). Collected sera were then stored at −80 °C for later use.

2.3. Tissue sample collection

Endometrial tissue samples were collected under sterile conditions from endometriosis free women (aged 20–49 years), who underwent a diagnostic hysteroscopy as a step of their work up for subfertility. Women who did not receive any type of hormonal therapy six months before sample collection and without history of immunological diseases, malignancies or chronic diseases were eligible for this study.

Full thickness biopsies were taken from healthy endometrium during hysteroscopy. A part of the biopsy is sent for pathological study to confirm healthy endometrium. Endometrial tissue samples were immediately immersed in Dulbecco's modified Eagle's medium (DMEM) (Lonza, Belgium) low glucose media containing antibiotic/antifungal mix and then transferred to the laboratory to undergo mesenchymal stem cell isolation.

2.4. Isolation and culture of endometrial mesenchymal stem cells (EnMSCs)

Endometrial tissue samples (n = 2) were washed with phosphate buffer saline (PBS), minced into tiny pieces then digested with 1 mg/ml type 1A collagenase (Gibco, life technologies, USA) for 60 min at 37 °C. Afterwards, cells and cell aggregates were filtered through 100 μm pore size cell strainer (Greiner Bio-one, Germany). Obtained cell suspensions were centrifuged, resuspended and cultured in DMEM low glucose medium (Lonza, Belgium) supplemented with 10% FBS, 100 units/ml penicillin (Gibco), 100 μg/ml streptomycin (Pen-Strep, Lonza) and 2 mM/L glutamax (Gibco) [11]. Two cell cultures were obtained, each from a separate endometrial biopsy. Cultured cells were then incubated at 37 °C and humidified atmosphere with 5% CO_2 concentration in a CO_2 incubator (Sartorius stedim biotech, GmbH, Germany). Media was exchanged every 2–3 days. Subsequent subculture was done at approximately 80% confluence till passage 3.

2.5. Serum application

Previously collected sera (7 endometriotic and 4 control serum samples) were added to the culture media generating an experimental and a control culture group. Two different serum concentrations in the culture medium were investigated, one was 0.2% and the other was 10%. Media containing serum was applied to 30% confluent EnMSCs cultures at passage three for a period of 14 days.

2.6. Microscopic examination and Photo-documentation

The morphology of EnMSCs treated with sera of normal and women with endometriosis was periodically examined, under inverted microscope (Nikon eclipse TS 100, Japan) and cells was

Please cite this article in press as: Salama E et al. Differentially expressed genes: *OCT*-4, *SOX*2, *STAT*3, *CDH*1 and *CDH*2, in cultured mesenchymal stem cells challenged with serum of women with endometriosisOCT-4, *SOX*2, *STAT*3, *CDH*1 and *CDH*2[Error cc:query|Please check the inserted running head and correct if necessary.[Error /cc:query] −>. Journal of Genetic Engineering and Biotechnology (2017), https://doi.org/10.1016/j.jgeb.2017.10.006