Tissue and Cell 48 (2016) 370–382

Contents lists available at ScienceDirect

Tissue and Cell

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

Human umbilical cord blood-mesenchymal stem cells transplantation renovates the ovarian surface epithelium in a rat model of premature ovarian failure: Possible direct and indirect effects

Amr K. Elfayomy[a,b], Shaima M. Almasry[c,d,*], Shereen A. El-Tarhouny[e,f], Magda A. Eldomiaty[c,g]

[a] Department of Obstetrics and Gynecology, Taibah University, Almadinah Almunawarah, Saudi Arabia
[b] Department of Obstetrics and Gynecology, Zagazig University, Zagazig, Egypt
[c] Department of Anatomy, Taibah University, Almadinah Almunawarah, Saudi Arabia
[d] Department of Anatomy, Mansoura University, Mansoura, Egypt
[e] Department of Clinical Biochemistry, Taibah University, Saudi Arabia
[f] Department of Medical Biochemistry, Zagazig University, Egypt
[g] Department of Anatomy, Tanta University, Tanta, Egypt

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 4 May 2016
Accepted 7 May 2016
Available online 10 May 2016

ABSTRACT

This study aimed to isolate mesenchymal stem cells (MSC) from human umbilical cord blood (HCB) and to explore their influence on the ovarian epithelium after paclitaxel-induced ovarian failure. Ninety-five rats were divided into 6 groups: control, paclitaxel, paclitaxel and saline, HCB-MSC-treated for 2 weeks, HCB-MSC-treated for 4 weeks, and HCB-MSC-treated for 6 weeks. HCB cells were studied for CD34, CD44, and Oct ¾ using flow cytometry. Serum levels of FSH and E2 were measured using ELISA, RT-PCR analysis for human gene; beta-actin (ACTB), immunohistochemical analysis for CK 8/18, TGF-ß, PCNA and CASP-3 were performed. We found that ACTB gene was expressed in all rats' ovaries received HCB-MSC. After 4 weeks of transplantation, there was significant reduction in FSH, elevation in E2 levels, stabilization of the surface epithelium morphostasis, an increase in the antral follicle count and increase in integrated densities (ID) of CK 8/18, TGF-ß, and PCNA expressions and decrease in ID of CASP-3 expression. We concluded that HCB-MSC can restore the ovarian function after paclitaxel injection through a direct triggering effect on the ovarian epithelium and/or indirect enrichment of ovarian niche through regulating tissue expression of CK 8/18, TGF-ß and PCNA. These molecules are crucial in regulating folliculogenesis and suppressing CASP-3-induced apoptosis.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Premature ovarian failure (POF) is a syndrome characterized by a lack of folliculogenesis and ovarian estrogen production and elevated gonadotropins. POF has been associated with infertility and early menopause in young women. The syndrome occurs in 1% of menopausal women and in 0.1% of women younger than 30-years-old (Gosden et al., 2013). The long-term side effects of exposure to chemotherapeutic treatment in women often lead to damaged ovarian function, with a high-level impact on patient self-esteem and quality of life. Chemotherapy exposure can even induce amenorrhea and infertility (Terraciano et al., 2014; Wan et al., 2015).

Chemotherapy-induced ovarian damage has been directly correlated with the dose of therapy, type of the used therapeutic agents, duration of therapy and age of the patient undergoing treatment (Beerendonk and Braat, 2005). Paclitaxel is one of the most commonly used chemotherapeutic medications for the treatment of epithelial cancers of the breast, ovary, brain, neck, and lung. Paclitaxel is also preferred in the treatment of other malignancies that are refractory to conventional chemotherapy, including previously treated lymphoma; small-cell lung cancers; esophageal, gastric, and endometrial and bladder cancers; malignant germ cell tumors; and AIDS-associated Kaposi's sarcoma (Gücer et al., 2001; Yucebilgin et al., 2004). A limited number of studies have proven that paclitaxel damages healthy, mature oocytes and affects short-

* Corresponding author at: Department of Anatomy, Taibah University, Almadinah Almunawarah, Saudi Arabia.
E-mail address: almasry.shaima@gmail.com (S.M. Almasry).

http://dx.doi.org/10.1016/j.tice.2016.05.001
0040-8166/© 2016 Elsevier Ltd. All rights reserved.

term reproductive potential in a dose-dependent manner (Ozcelik et al., 2010).

Human umbilical cord blood (HCB) has emerged as an attractive tool for cell-based therapy. Although the clinical application of HCB is currently restricted to the subject areas of hematology and oncology, an increasing number of surveys have demonstrated potential for further application in the treatment of non-hematopoietic diseases. Given its ready availability, ease of collection and lack of serious ethical dilemmas, HCB represents a valuable alternative for bone marrow-mesenchymal stem cells (MSC) as it contains multiple types of immature cells derived from the developing fetus (Koblas et al., 2005; Wang et al., 2009). HCB-MSC possesses immunophenotype and functional properties close to the characteristics applied to bone marrow-derived mesenchymal progenitor cells. These cells can differentiate into a variety of cell types. Pham et al. (2014) concluded that clonally expanded MSC from umbilical cord blood (UCB) exhibit multilineage differentiation potential differentiation for adipocytes, osteoblast, and chondrocytes. It has been shown that UCB-MSC also can differentiate into cardiomyocytes (Ishimine et al., 2013), hepatocytes (Chagraoui et al., 2003), and neural cells (Jiang et al., 2010**).**

MSC: mediated repair mechanisms also require the production of cytokines, such as vascular endothelial growth factor, hepatocyte growth factor, insulin-like growth factor-1 and basic fibroblast growth factor, for angiogenesis, anti-apoptosis and mitogenesis (Kinnaird et al., 2004).

Cytokeratins are cytoskeletal intermediate filament proteins that originate from a family of homologous proteins, forming six classes of molecules (Townson et al., 2010). Cytokeratins were notable in human epithelial cells (Maurizii et al., 1997), granulosal cells of follicles at various stages of growth and atresia, in luteal cells and in oocytes from both fetal and adult ovaries and were described to have a key role in folliculogenesis, function of corpus luteum and in oocyte efficiency (Hummitzsch et al., 2015). Townson et al. (2010) have proposed that CK 8/18 could convey the resistance to apoptosis in ovarian cells during folliculogenesis.

This work proposed to explore the possible isolation of MSC from the HCB and to determine their therapeutic influence on the restoration of ovarian function in a paclitaxel-induced ovarian failure rat model through action on the surface epithelium and/or adaptation of the expression pattern of CK 8/18 and some growth factors in the ovarian tissue.

2. Materials and methods

2.1. Animal

All of the animal procedures were conducted in accordance with the principles of laboratory animal care (National Institute of Health [NIH] Publication No. 85-23, revised 1996 or European Communities Council Directive of 1986, 86/609/EEC). The experiments were performed with the permission of the ethics committee of Taibah University. Ninety-five adult, female, albino Wistar rats, weighing 180–220 g, were housed in groups of 4 rats per wire cage and were maintained under standard laboratory conditions (14 h of light, 10 h of dark; 25 °C) for 10 days to acclimatize the animals to the laboratory conditions. During acclimatization, standard rat chow and water were available *ad libitum*. Vaginal smears were obtained daily. Only those rats showing at least two consecutive normal 4-day vaginal estrus cycles were included in the experiments.

2.2. Experimental design

Ninety-five rats were included in this study. Twenty four control rats were euthanized at the end of the experiment to identify normal ovarian histology. To establish animal models of chemotherapy-induced ovarian failure, 71 rats received intraperitoneal injections of a single dose of paclitaxel (7.5 mg/kg; intaxel, Fresenius Kabi Oncology, India) using a sterile technique (Yucebilgin et al., 2004; Ozcelik et al., 2010). One week after paclitaxel therapy, the rats were subdivided into the following groups: group 1 (paclitaxel group, 13 rats, euthanized after 6 weeks without treatment); group 2 (paclitaxel and saline group, 12 rats, euthanized after 6 weeks without treatment); group 3 (HCB-MSC-treated, 15 rats, euthanized after 2 weeks); group 4 (HCB-MSC-treated, 15 rats, euthanized after 4 weeks); and group 5 (HCB-MSC-treated, 16 rats, euthanized after 6 weeks). The last three groups received a bulk population of the adherent HCB-MSC (2×10^6) in 20 μl of PBS by direct injection into the bilateral ovaries (Fu et al., 2008).

Blood samples were collected from the retro-orbital venous plexus immediately before the sacrifice. After centrifugation, the serum was stored at −20 °C until determination of basal FSH and E2 levels by ELISA (LEGEND MAX Mouse ELISA Kit) according to the manufacturer's instructions.

The incorporation of the transplanted HCB-MSC into ovarian tissues was examined by the presence or absence of the human housekeeping β actin gene using polymerase chain reaction (PCR).

2.3. Collection and isolation of the mononuclear cell layer from HCB

Sterile collection tubes (50 ml) containing 5 ml of citrate phosphate dextrose adenine-1 as an anticoagulant was used for HCB collection. The mononuclear cell layer was separated by Ficoll density gradient centrifugation using Ficoll-Hypaque (Biochrom AG, Germany), which was added to the cord blood sample at a 1:2 ratio. The samples were centrifuged for 20 min at 1800–2000 RPM at 20 °C, and then the upper layer (plasma) was aspirated and discarded, leaving the mononuclear cell layer undisturbed at the interphase stage (lymphocytes, monocytes and thrombocytes). The cell viability was determined by mixing 100 μl cell suspension with 100 μl of 0.4% trypan blue solution. Cells were counted using a haemocytometer microchamber under a light microscope

2.4. Culture of separated mononuclear cells

To obtain single cell-derived, clonally expanded MSC, approximately 4.0×10^5 cells were re-suspended in a T75 flask at 37 °C in a humid CO^2 incubator containing 20 ml of low glucose (5.6 mmol/L) pre-warmed MSC-complete medium, Dulbecco's modified Eagle's medium, 10% fetal calf serum (FCS), penicillin (100 U/ml), amphotericin B (5 μg/ml), and streptomycin (100 μg/ml). The culture medium was removed every 3 days and replaced with 20 ml of pre-warmed complete medium. The cells were subcultured when they reached 80–90% confluence. The separated fibroblast-like colonies were identified at a mean interval of 1–3 weeks after initial plating. The adherent stromal bone-marrow fibroblast-like cells were fixed and stained with Trypan blue staining. The growth curves of the HCB-MSC were also evaluated (Harris, 2008).

2.5. Flow cytometry analysis of cultured cells

Flow cytometry was used to characterize stem cell preparations by analyzing the expression of cell surface CD markers, namely, CD34 and CD44 and intracellular molecule, Oct 3/4 (Rebelatto et al., 2008). Before cytometry testing, 2 μl IgG1- fluorescein isothiocyanate (FITC) was added into negative control. Flow cytometry was performed as previously described by Preffer et al. (2002). Briefly, the cells were dissociated into a single cell suspension by trypsin digestion. Then, the aliquots of the expanded cells

Download English Version:

https://daneshyari.com/en/article/2203492

Download Persian Version:

https://daneshyari.com/article/2203492

Daneshyari.com