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Does the maternal age affect the mesenchymal stem cell markers and gene expression in the human placenta? What is the evidence?

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ABSTRACT

Background: Although the human placenta is considered medical wastes, it has become a main source of stem cells. Due to their easy isolation, ability to resist immune rejection and ability to differentiate into different types of adult cells, placental stem cells are considered superior to other stem cells.
Objectives: This study aimed to assess the impact of the maternal age on the expression of mesenchymal stem cell (MSC) markers CD105 and CD29 in different areas of a term human placenta and to identify the differential expression of these markers in different placental areas.
Subjects and methods: In this comparative cross sectional study, one hundred term placentas were collected after delivery from healthy mothers divided into five groups according to their age. Placentas were processed to assess both immune- and gene-expression of CD105 and CD29 surface antigen markers. Data of the different studied age groups was compared using the Statistical Package of Social Science (SPSS) software.
Results: CD105 and CD29 immunoexpression in decidua basalis, fetal membrane and placental villi showed significant negative correlations with the maternal age. CD105- and CD29-positive MSCs were significantly abundant in the decidua basalis and placental villi. Real-time polymerase chain reaction results were consistent with those of the immunohistochemical study.
Conclusion: Labeling the placenta-driven MSCs with the specific area from which the cells were taken as well as the mother's age is advised and could be helpful in controlling the quality of the cell banks as well as the favorable outcome of the therapeutic applications.

1. Introduction

The extra-embryonic tissues, such as the amnion, placenta, and amniotic fluid, are rich in fetal stem cells (FSCs), which are considered the 'half way house' between embryonic stem (ES) cells and adult stem cells. These FSCs are characterized by having expansion potential and a lack of tumorigenicity (Marcus and Woodbury, 2008). Because placenta can be noninvasively harvested, used with few ethical reservations, and is a voluminous organ with average weights of more than 590 g, it is considered an attractive source of stem cells that can be used for cell therapy in the field of regenerative medicine (Bolisetty et al., 2002; Miki and Strom, 2006; Matikainen and Laine, 2005; Abdulrazzak et al., 2010).

The human placenta is an important source of particularly the mesenchymal stem cells (MSCs), which represent less than one percent of its cells. These can be isolated from first-, second-, and third-trimester placental compartments, including the amnion, chorion, decidua parietalis, decidua basalis, and placental villi (Zhao et al., 2005; Alviano et al., 2007; Soncini et al., 2007; Pasquinelli et al., 2007; Parolini et al., 2010). The following hypothesis has been postulated "Because MSCs can inhibit the action of T-lymphocytes as well as the differentiation and proliferation of monocytes, they are not immunogenic" (Zhang et al., 2006a,b; Moore et al., 2003). These features have led to the recognition of placental MSCs as an excellent option in cell therapy (Barker and Wagner, 2003; Zhang et al., 2006a,b). Amniotic epithelial cells (AECs) are another type of placental stem cell that is commonly isolated from the amniotic membrane using digestive enzymes (Miki et al., 2010). Particular attention has been directed toward human amnion mesenchymal stem cells (HAMCs) due to their accessibility, availability,

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and immunomodulatory properties (Fatimah et al., 2013). Human chorionic trophoblastic cells and hematopoietic stem cells have also been isolated from the placenta (Parolini et al., 2008).

Many previous studies have been performed to identify the stemness makers of the human placenta. Fukuchi et al. (2004), Igura et al. (2004), Li et al. (2005), and Mihu et al. (2008) have isolated placental SCs that exhibited strong immune-expression of MSC makers CD29, CD44, CD73, CD105, and CD166 and did not express any of the Hematopoietic stem cell (HSC) markers (CD31, CD34, and CD45). In addition, Abumaree et al. (2013) detected CD105-positive stem cells in the fetal part of the placenta using real-time flow cytometry and polymerase chain reaction (PCR). In this study, the MSCs markers CD105 and CD29 were particularly investigated in the human placental tissue.

Many layers of regulation in response to local, systemic, and environmental factors govern stem cell behavior (Morrison and Spradling, 2008). Evidence from several systems also suggests that the stem cell functional output is altered during aging (Sharpless and DePinho, 2007). The effect of aging on the regenerative ability of living organisms and its impact on biological activities have been the focus of many previous studies (Janzen et al., 2006; Sperka et al., 2012), but few, if any, studies have investigated the effect of age on neither fetal stem cells in general nor placental stem cells in specific. Thus, this study aimed to assess the impact of the maternal age on the expression of MSCs surface antigen markers CD105 and CD29 in different areas of the term human placenta and to identify the differential expression of these markers in different placental areas. We hypothesized that MSCs marker expression is reduced in placenta of older mothers compared to the younger ones and this reduction is attributed to reduced gene expression as well as cell amount.

2. Materials and methods

2.1. Patient selection and grouping

The present study was approved by the Biomedical Research Ethics Committee of the Faculty of Medicine of King Abdulaziz University in Jeddah, Saudi Arabia, and informed consents signed by all of the participants were obtained. A total of 100 full-term pregnant Saudi mothers who delivered either normally or through Cesarean sections in the department of Obstetrics and Gynecology at King Abdulaziz University Hospital, Jeddah between June, 2012 and May, 2013 were included in this study. The inclusion criteria for the cases were the following: full term (≥C37 weeks) pregnant female aged twenty years or higher. The gestational age was confirmed using the date of the last menstrual period and through an ultrasound examination performed 11–13 weeks after gestation (Hadlock et al., 1992) obtained from the patient's medical records. To ensure that only normal pregnancies were included in the study, the exclusion criteria were the following: pregnancies complicated with gestational diabetes, preeclampsia, or any other chronic diseases. A total of 20 full-term, freshly delivered placentas were collected from each of the following maternal age-dependent groups: A (20–25) years, B (26–30) years, C (31–35) years, D (36–40) years, and E (40 or more) years. In this study, the maternal age was the predictor, immune- and gene-expressions of stem cell markers were the outcome while the potential confounders were eliminated through the exclusion criteria and no effect modifiers were spotted.

2.2. Processing of the placenta

Each placenta collected at the time of delivery was weighed and washed with phosphate buffered saline (PBS, pH 7.4, BDH Limited Poole, England). For the routine histopathological processing, the placental samples were obtained through a standard procedure: a full-depth cube of the placental tissue (fetal through to maternal) from a macroscopically normal placental disk approximately 5 cm from the umbilical cord insertion was obtained. Each cube of tissue was processed to obtain paraffin blocks, cut serially into 4-μm-thick sections, mounted on glass slides, and stained with hematoxylin and eosin (Bancroft and Stevens, 2002). In the microscopic examination of the placenta, the stem villi were determined by their large fetal vessels surrounded by identifiable media, thick trophoblastic covering, rare fetal capillaries, and missing sinusoids, and the intermediate and terminal villi were determined by their sinusoidally dilated fetal capillaries that occupy more than 50% of the villous stroma (Schweikhart and Beck, 1986) and by their diameter of less than 80 μm (Egbor et al., 2006).

2.3. Immunohistochemistry

Human endoglin/CD105 antibody (Leica, Bio Systems; diluted to 15 μg/ml), human integrin β1/CD29 antibody (Leica, Bio Systems; diluted to 15 μg/ml), human CD34 antibody (Cell Marque, Tool of Biology Research; diluted to 25 μg/ml), and HRP-conjugated primary antibody dilution buffer (R&D System, USA) were used in this study.

The immunohistochemical stains were performed on paraffin-embedded tissue sections (4 μm). A standard immunohistochemistry staining procedure was performed (Thomasse, 2001; Miller, 2001; Castrechini et al., 2010). Briefly, the tissues were deparaffinized using xylene and ethanol. Antigen retrieval was achieved by boiling the tissue slides with 0.01 M citric buffer in a microwave for 5 min. Hydrogen peroxide was used to quench the endogenous peroxidase activity. After blocking with 10% serum–Tris buffer for 20 min at room temperature, the sections were incubated with the primary antibody at the dilution mentioned above at room temperature for 120 min. The corresponding biotinylated conjugated secondary antibody from a Dako staining system was used. Slides stained with a non-specific IgG were used as negative controls. The nuclei were counterstained with hematoxylin. An Olympus BX-51 microscope with a digital camera connected to a computer was used to photograph the images. A question was raised; what is behind the changes observed in markers immunoexpression, is it the change in the expression level of the individual cells or the change in the cell amount. In order to answer this question the area percentage (AP) of the brown label was assessed as an indicator of the cell number while the mean intensity (MI) of the reaction was assessed as an indicator of the expression level. Both parameters were assessed by an investigator blind to the age group examined, in 20 non-overlapping fields in each sample with a 40× objective lens and a 10× ocular lens using the Pro Plus image analysis software version 6.0 produced by Cybernetics company. In each time, the region of interest (ROI) was identified based on the area of the UC examined as was described by Decaestecker et al. (2009).

2.4. Gene expression analysis

Quantitative Real time RT-PCR was performed at the Molecular Biology Lab, Zagazig University, Egypt to detect the expression level of human CD105 and CD29 genes in the different studied placental areas in all examined groups (A–E) relative to the housekeeping gene, β-actin. Placentas were collected within a maximum 1 h after the delivery and were rapidly dissected by one of the trained investigators who use a sterile technique to sample the delivered placenta. She first separated the amniotic membrane and washed it from the blood cells with PBS as was previously described by Marongiu et al. (2010). Regarding the decidua basalis, it was taken from the maternal side of the placenta (the basal plate). Three random, 5–8 mm samples from the most superficial