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EXPLORING THE THERAPEUTIC POTENTIAL OF UMBILICAL CORD MESENCHYMAL STEM CELLS IN MODULATING TROPHOBLAST CELL FUNCTION FOR PREGNANCY-RELATED PLACENTAL DISORDERS

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ABSTRACT
Preeclampsia and intrauterine growth restriction are disorders caused by dysfunctional trophoblast cells during pregnancy. Unfortunately, trophoblast cell function is little improved by treatments that target it. In addition to their ability to self-renew, to differentiate into a variety of cell types, and to possess paracrine properties, umbilical cord mesenchymal stem cells (hUCMSCs) have a number of immunomodulatory properties, making them a potential source for cell therapy. We used hUCMSC supernatants or cocultured hUCMSCs with trophoblast cells to determine whether hUCMSCs could influence trophoblast cell functions. A remarkable difference existed between the two treatments in terms of trophoblast cells' migration and invasion capacities, as well as their ability to proliferate. As well, hUCMSCs modulated the levels of hCG, PIGF, and sEndoglin in trophoblast culture medium when a concentration of hUCMSCs is applied. Through improving trophoblast cellular functions, hUCMSCs may be used to treat pregnancy-related placental diseases.

Keywords: Preeclampsia, Intrauterine Growth Restriction, Trophoblast Cells, Umbilical Cord Mesenchymal Stem Cells, Cell Therapy

INTRODUCTION
It is fundamental to the implantation and development of the embryo and the placenta that trophoblast cells are involved. An early stage of pregnancy is marked by the proliferation of extravillous trophoblasts that penetrate the decidua. altering the uterine spiral arteries. In pregnancy, disorders such as preeclampsia and intrauterine growth restriction (IUGR) are associated with dysfunctions of the trophoblast cells. Preeclampsia, for which there is no clear pathophysiology, may be connected to abnormal placentation. Due to inadequate tracheal penetration and vascular conversion, invasive trophoblasts lead to poor perfusion of the uteroplacenta. Clinical symptoms are associated with cellular ischemia, hypoxia, oxidative stress, and vascular endothelial injury as a result of inflammation, oxidative stress, and vascular endothelial injury. Pregnant women and their babies should be relieved of their clinical symptoms and prevented from developing complications. There are currently few treatment options that aim to improve organ perfusion or microcirculation. The underlying cause of this condition, dysfunctional trophoblast cells, is rarely addressed by effective etiotropic treatments.

MSCs have the ability to self-renew and are multipotent, in addition to secreting cytokines, possessing immunosuppressive capabilities, and not being very immunogenic. The use of MSCs for cell transplantation, gene therapy, and regenerative medicine has become increasingly popular in the past few decades. Decidua-derived MSCs are under investigation for their ability to alleviate hypertension and proteinuria in pregnant mice with preeclampsia-like symptoms, prevent glomerulonephritis, and facilitate placental development. Furthermore, it has recently been shown that trophoblast invasion and immune responses can be regulated by MSCs derived from chorionic plates. MSCs may be useful for restoring the functions of dysfunctional trophoblast cells, allowing placental development to take place normally.

Typically, umbilical cords are disposed of as medical waste. They are available in abundance. Human umbilical cord MSCs are more proliferative, have a greater expansion potential, and are immunosuppressive than MSCs from other sources. Aside from being easy to obtain and noninvasive, In addition to making osteoblasts, adipocytes, chondrocytes, endothelial cells and osteoblast-like cells from Wharton's jelly, hUCMSCs can be differentiated into adipocytes and chondrocytes as well. A variety of diseases have been investigated for the therapeutic potential of hUCMSCs as seed cells because of these reasons. While hUCMSCs have not yet been studied in relation to trophoblast cells, it is possible they might prove beneficial.

A study was conducted using three different lines of human MSC from umbilical cord tissue of three female neonates to determine if these human MSCs affect trophoblast cell growth and function. In most cases, villous trophoblasts are studied using the immortalized trophoblast cell line HTR-8/SVneo. In order to study the effects of hUCMSCs on trophoblast cell proliferation, migration, invasion, and secretion, SVneo cells were used as a model in this study. We analyzed the effects of hUCMSC supernatants and coculturing on these cells, as MSCs can do so through paracrine factors they secrete and cell-to-cell interactions. In order to determine whether the changes in hUCMSCs were specific or not, foreskin fibroblasts (hFF) were used as controls.

METHODS AND MATERIALS

This study made use of Dulbecco's Modified Eagle Medium (DMEM)/F12 medium (Gibco), fetal bovine serum (FBS) (Gibco), and penicillin and streptomycin solution. Along with trypsin, BD Biosciences also offers antibodies against CD73, CD90, and CD105, osteogenesis and adipogenesis differentiation kits, Alizarin Red S solution, Oil Red O, Matrigel, calciumein-AM, MTS, total RNA extraction kits, reverse transcription kits, SybrGreen qPCR kits, oligo (Sangon Biotech), hCG, PIGF, and sEndoglin ELISA kits, as well as trypsin. Upon cesarean delivery of three full-term female neonates, umbilical cord samples were collected within minutes and stored in cold saline aseptically. After rinsing the umbilical cords several times, short pieces of the cord were dissected and removed from the body. A small incision was made in the umbilical cord in order to expose Wharton's jelly and to remove its arteries and veins. Incubation was conducted at 37°C with 5% CO2 in T-75 flasks containing 10 mL medium (DMEM/F12 medium with 10% FBS and 1% penicillin and streptomycin solution) after the gel tissues had been chopped (small pieces of umbilical cord Wharton's jelly) were transferred into the flasks. and saturated humidity. After the remnants of cord fragments were removed, the cultures were fed for three days after a 7–10-day culture period. When cultures reach confluency, cells are trypsinized and passed onto a new flask. Surface marker expression was examined after three passages of hUCMSCs. Following trypsinization, cells were washed in PBS and resuspended at a density of 1 × 106 cells/mL. In 1.5 mL Eppendorf tubes, 100 liters of cell suspension were added. Positive control tubes (PBS) were used as positive controls, while CD73-FITC, CD90-FITC, CD105-FITC, CD14-FITC, CD34-FITC, and CD45-FITC are isotype control antibodies that were incubated for 30 minutes in the other tubes. In the following step, the cells were analyzed using flow cytometry. In 6-well culture plates, hUCMSCs were cultured for 2-3 weeks in an adipogenic or osteogenic differentiation medium. A new medium was replaced every 2-3 days. The osteogenic phenotype was demonstrated by staining cells with Alizarin Red S for 10 minutes after they had been fixed with 4% formaldehyde solution for 30 minutes. A microscope was used to take

photomicrographs. In parallel to Alizarin Red S staining, Oil Red O staining was used to demonstrate adipogenic differentiation. The first step was to examine whether trophoblast cells could increase their proliferation ability when they were exposed to conditioned medium. Twenty-four hours were spent incubating thirty thousand hUCMSCs or human fibroblasts in T-75 flasks containing 10 mL of DMEM/F12 medium containing 10% FBS and 1% penicillin/streptomycin solution, and then the culture medium was collected and filtered for use. To harvest HTR-8/SVneo trophoblast cells, hUCMSC or hFF supernatants were diluted in standard medium for 48 hours and then incubated for another 48 hours. Spectrophotometer measurements were conducted after 1 hour of incubation with 10 l MTS on the plates. In addition, trophoblasts cocultured with either hUCMSCs or hFFs were compared for their ability to proliferate. Cells from the HTR8/SVneo strain were cocultured with hUCMSCs or hFFs in the upper chamber of a 6-well Transwell plate. Trypsinized cells in the lower chamber were counted under a microscope after 48 hours of incubation. Transwell inserts (8 m pore size, BD Biosciences) were used to examine the migration ability of HTR8/SVneo trophoblast cells. A 300-liter medium chamber in the insert contained HTR8/SVneo cells that were seeded in the upper chamber. Lower chambers contained either the standard medium with 2, 5, or 10 104 hUCMSCs or hFF cells or the It is possible to condition medium (0, 25, 50, or 100% hUCMSC or hFF supernatants in standard medium) in several ways. Fluorescence microscope imaging was conducted after 16 hours of culture on the labeled cells. Using Transwell inserts with 24 wells, HTR8/SVneo trophoblast cells were tested for their invasion ability. A 1: 5 dilution of Matrigel in standard medium was precoated on the upper chamber of the insert for 30 minutes at 37°C. We then added 300 liters of 1% FBS medium to the upper chamber, along with HTR8/SVneo cells. As a control, the wells in the lower chambers were filled with 800 mL of hUCMSC supernatant in standard medium, or 104 hUCMSCs or hFF cells are used in each well. Following 24 hours in culture with 80 L fluorescent stain, the labeled cells were observed and photographed using a fluorescence microscope. Coculturing of two types of adherent cells was done with the Transwell insert system (9 m pore size). The lower chamber contained HTR-8/SVneo cells, which also contained 2 mL standard medium, and the upper chamber contained hUCMSCs or hFF at 0 g/well, 2.5 g/well, or 10 g/well, with 1 mL standard medium. The supernatant collected from the lower chamber after 48 hours of incubation was tested by enzyme-linked immunosorbent assays (ELISAs) and real-time PCR on HTR-8/SVneo cells. As a next step after coculturing HTR-8 and SVneo cells for 48 hours, we removed the upper chambers, and extracted RNA from the cells using TRIzol. There were 40 cycles of 95 degrees Celsius for 15 seconds and 60 degrees Celsius for 20 seconds in the PCR. Denaturation at 95°C for 30 seconds was followed by annealing at 60°C for 20 seconds. The primer sequences were as follows: β-actin forward primer, 5 -CCAACCGCGAGAAGATGA-3 ; β-actin reverse primer, 5 -CCAGAGGCGTACAGGGATAG-3 ; -hCG forward primer, 5-CCAGTACCACCCCGTCATCG-3; hCG reverse primer, 5 -CTACACGCGAAGCTC AGGTA-3; PIGF forward primer, 5□GCGGTACCCAAACTCATACACAATAGAC-3 ; PIGF reverse primer, 5-TTAAGCTTCCGTAGGTAAGGCTGTGGCT-3 ; sEndoglin forward primer, 5 -GTCTCACTTCATGCCTCCAGCT-3 ; sEndoglin reverse primer, 5 -ACTGCCTCAACATGGACAGCCT-3. In coculture experiments, hCG, PIGF, and sEndoglin levels were measured using commercial kits according to the manufacturer's protocol. A streptavidin-horseradish peroxidase-conjugated antibody was used in addition to the biotin-conjugated antibody. A spectrophotometer at 450 nm was used to determine absorbance within 30 minutes of labeling.

STATISTICS

A triplicate of each experiment was conducted. Analyzed the data using the SPSS software to determine its statistical significance. A mean and standard error of the mean are presented. ANOVA and DunnT tests were used to assess statistical significance, and independent t-tests were used to compare two groups. A statistically significant P value of 0.05 was considered significant, and a significant P value of 0.01 was considered very significant.

RESULTS

By using tissue block attachments, we were able to isolate hUCMSCs successfully. Following 4 days in culture, cells shaped like spindles dotted the tissue blocks. At a density of 10 104 cells/mL, these hUCMSCs were trypsinized 2 weeks after reaching 80% confluency. Each time the hUCMSCs were passaged, they grew extremely quickly and reached confluence in 3 days. In order to detect the surface markers of the cells, we used them after the third passage. CD73, CD90, and CD105 were strongly positive in these hUCMSCs, while CD14, CD34, and CD45 were negatively expressed. Three to four weeks of osteogenic medium exposure tested the differentiation abilities of fourth-passage hUCMSCs. According to Alizarin Red S, brown calcium deposits and osteoid formation were observed. Three weeks were needed for hUCMSC differentiation into adipocytes. Oil Red O stained lipid granules that appeared in induced hUCMSCs had adipocytotic characteristics. These lipid granules tended to unite to form adipocytes. Multipotent mesenchymal stem cells should meet the minimum criteria outlined above. In contrast, study found no obvious difference between three different lines of hUCMSC in our study. To study hUCMSCs' effects on human first trimester trophoblast cells, we used human HTR-8/SVneo trophoblast cells, a type of human trophoblast cell. The control group consisted of human foreskin fibroblasts. Initially, HTR8/SVneo trophoblast cells were cultured with conditioned medium. Proliferation of HTR-8/SVneo cells increased slightly in hUCMSC conditioned medium in comparison to medium alone and hFFs conditioned medium; however, this was not statistically significant. Undercoculturing conditions were also examined. Compared to a medium only control and a coculture of hFFs at the same density, coculturing $2.5x10^4$ hUCMSCs/well with HTR-8/SVneo cells markedly enhanced their proliferation. In comparison to the medium-only control, HTR8/SVneo cells cultured with hUCMSC supernatant continuously generated significant migration ($P>0.01$). A significant increase in migration ability was observed when HTR-8/SVneo cells were cultured in hUCMSCs supernatant compared to hFFs supernatant. A similar experiment was also conducted with coculturing. Compared with medium-only controls, migration ability of hUCMSCs cocultured with HTR-8/SVneo cells was significantly upregulated ($P = 0.01$). The increase demonstrated was also significant compared with the growth of cells cocultured with hFFs ($P>0.05$).

Migration and invasion results were similar. The effects of supernatants from hUCMSCs and hFFs were first compared. At 50 and 100%, A comparison of HTR-8/SVneo cells with and without hUCMSC supernatant indicated that hUCMSC supernatant significantly enhanced their invasive ability ($P = 0.01$). In addition, hUCMSC supernatant at 50% (P 0.05) and 100% (P 0.01) increased HTR-8/SVneo cells' invasion ability when compared with hFF supernatant at the same concentration. A coculturing experiment revealed that coculturing HTR-8/SVneo cells with 2.5 ($P = 0.05$), 5 ($P = 0.01$), or 10 ($P = 0.01$) hUCMSCs/well improved their invasive abilities over medium only cultures. As a preliminary experiment, Several cytokines were detected in coculture medium , and ultimately selected -hCG, PIGF, and sEndoglin to test hUCMSC effects on trophoblast secretion. The cells were seeded in 6-well plates with hUCMSC or hFF supernatant in medium and 0, 25, 50, or 100% supernatant was added. The supernatant of the cultured cells was collected for ELISA detection after 48 hours and total RNA was extracted for real-time PCR analysis. The level of -hCG secreted by -hUCMSC conditioned medium was not significantly different from that of -hCG mRNA conditioned medium, although the level was significantly lower at 100% than in hFF conditioned medium. As compared to hFF supernatant and the medium-only control, PIGF concentration is significantly increased with 50% and 100% hUCMSC supernatant. No significant change was observed in PIGF mRNA levels. In comparison to 100% hFF supernatant, it was found that sEndoglin concentrations were significantly reduced by 100% hUCMSC supernatant, but the medium-only control had no effect. A slight downward trend was also seen in the real-time results, but no significant change was observed.

Coculturing experiments were also conducted. At a concentration of 104 cells per well in the lower chamber of a 6-well Transwell insert system, HTR-8/SVneo cells were seeded in the upper chamber. and the upper chamber was seeded with either hUCMSCs or hFFs at a concentration of 0, 2.5, 5, or 10×10^4 cells/well. In accordance with the above description, samples were collected and assessed

after 48 hours in culture. There was no significant difference in -hCG concentration in the presence of 2.5 or 5 10^4 cells per well, but it decreased in the presence of $10X10^4$ hUCMSCs or hFFs per well. As compared to the medium-only control, There was a significant increase in the mRNA levels of -hCG at 5 and 10 x 104 cells per well The concentration of PIGF was significantly increased by coculturing with 2.5 or 10 10 4 hUCMSCs/well as compared with hFFs and, for the higher concentration, by controlling only with medium. However, there was no significant change in PIGF mRNA level. In cocultures with hUCMSCs, In comparison to hFF and medium-only controls, neither sEndoglin nor its mRNA were significantly different.

DISCUSSION

A trophoblast cell is a major resident cell at the fetal-maternal interface during early pregnancy and has a significant role in embryonic development. In multiple fields and diseases, hUCMSCs have proven to be an excellent source of mesenchymal stem cells. The purpose of this study was to examine the proliferative, migrational, invasional, and secretory functions of trophoblast cells when treated with hUCMSCs. Coculturing with hUCMSCs or adding them to hFFs can increase trophoblast cell function more than controls or hFFs with just medium. Proliferation of trophoblast cells is essential for the implanting of embryos. There was a significant increase in proliferation of HTR-8/SVneo trophoblast cells with cells/well hUCMSCs, but this increased proliferation was not seen with conditioned medium hUCMSCs. Due to a depletion of nutrients in the medium and a reduction in the secretion of mediating factors by hUCMSCs, cell numbers may have decreased gradually as hUCMSC supernatant concentrations increased. However, further research will be needed to clarify the specific mechanisms at work. Numerous studies have confirmed that preeclampsia occurs when trophoblast invasion is inadequate. Invasive and migration capabilities of HTR-8/SVneo cells were significantly influenced by hUCMSC supernatant as well as coculture with hUCMSCs. Conversely, hFF cells influenced these trophoblast cells' migration and invasion abilities positively although to a lesser extent. Our findings are in agreement with the results of a study that demonstrated that chorionic plate-derived mesenchymal stem cells cocultured with trophoblasts increased trophoblast invasion capacity, and MMP-2 activity increased significantly. Inflammatory cytokines such as TNF- upregulate MMP2, MT-1 MMP, and MMP-9 production in human MSCs, which are abundantly expressed and synthesized with matrix metalloproteinases (MMPs). Despite this, the complex mechanisms remained unclear. As well as cytokines and hormones, cytokines and hormones play a role in embryo implantation regulation. The dominant hormone during pregnancy is hCG, which plays a variety of important roles, including stimulating implantation and decidualization angiogenesis, facilitating trophoblast differentiation, producing progesterone, and regulating immune cells, all of which are vital to establishing the fetomaternal interface. Deficient placentation and higher risk of pregnancy disorders can occur as a result of reduced hCG production during the first trimester. Based on a recent study published by Pizarro, HCG changes extracellular matrix remodeling in endometrial stromal cells and allows HTR-8/SVneo trophoblast cells to invade in vitro. Based on the above data, no definite conclusion could be drawn, and more research is needed to determine what led to the decrease in hCG levels. Additionally, PIGF belongs to the same family as VEGF and is a secreted proangiogenic protein. As a result of its role in vascular development, trophoblast invasion, and inflammatory processes, PIGF contributes to fetal development. When maternal serum PIGF levels decrease during early gestation, In pregnancy, abnormalities and preeclampsia are more likely to occur. The medium concentration of PIGF in medium was significantly higher in hUCMSC supernatant and coculture than in medium alone or in hFF cells. Human UCMSCs are capable of facilitating PIGF synthesis and release, as well as increasing PIGF secretion, which is beneficial to trophoblast cells. During pregnancy, antiangiogenic proteins can also influence the pathology of many disorders. The syncytiotrophoblast expresses endoglin during pregnancy. The trophoblast differentiation process is negatively regulated by TGF- isoforms 1, 3 and endoglin in response to several TGF- superfamily ligands. As well as controlling vasomotor tone, endoglin binds and activates endothelial nitric oxide synthase. It has also been found that extravillian trophoblasts can invade more

easily following the loss of sEndoglin. hUCMSCs have been found to stimulate proliferation, migration, invasion, and secretion of While mRNA levels of sEndoglin were not significantly altered in 100% hUCMSC supernatant, sEndoglin concentrations were significantly lower than in 100% hFF supernatant, suggesting that hUCMSCs are beneficial to HTR-8/SVneo trophoblasts.

HTR-8/SVneo trophoblast cells in vitro, as a supernatant or coculture, which may provide new insights into potential therapeutic or preventative approaches for pregnancy-related disorders. Even if hUCMSCs alter HTR-8/SVneo biology, it is unclear how it would be translated into clinical practice since symptoms of pregnancy disorders like preeclampsia typically do not emerge until after 20 weeks of pregnancy, by which time trophoblast invasion (or failure) has been completed (usually by 8–10 weeks of gestation). It is possible to treat pregnant women with pregnancy disorders who have risk factors with hUCMSCs as a preventive measure. Since HTR-8/SVneo represents only one type of cell, we examined it separately from its microenvironment and tissue architecture. In vivo and on other kinds of placental cells, no information is available on the effects of hUCMSCs. However, we were only able to isolate three hUCMSC lines in our study, despite the fact that they did not vary in physiological effects, and future studies with more hUCMSC lines will be necessary. Additionally, all three hUCMSC lines were derived from female neonates, and many physiological differences could be due to sex differences. As a result, further study is needed to determine whether the sex of hUCMSC lines impacts trophoblast migration and invasion. These effects are also unknown in terms of their mechanisms. The mechanism could be mediated by miR-181a, which we detected in hUCMSCs, but we have not yet found a satisfactory result. There are still a lot of unknowns regarding the interaction between hUCMSCs and trophoblasts, and more research will be undertaken in the future.

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