Original Article

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The cytotoxicity of advanced glycation end products was attenuated by UCMSCs in human vaginal wall fibroblasts by inhibition of an inflammatory response and activation of PI3K/AKT/PTEN

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SUMMARY Pelvic organ prolapse (POP) occurs when the pelvic organs (bladder, bowel or uterus) herniate into the vagina, causing incontinence, voiding, and bowel and sexual dysfunction, negatively impacting upon a woman's quality of life. Intermediate intermolecular cross-links and advanced glycation cross-links increase in prolapsed tissue. Stem cells are able to participate in tissue repair due to their ability to differentiate into multiple lineages, and thus into various types of connective tissue cells, so they therefore hold great promise for treating pelvic floor dysfunction. The current study found that advanced glycation end products (AGEs) inhibited the viability and proliferation of human vaginal wall fibroblasts (VWFs), were cytotoxic to VWFs, and also induced the apoptosis of VWFs. In contrast, umbilical cord-derived mesenchymal stem cells (UCMSCs) secreted anti-inflammation cytokines to protect against the cytotoxic effects of fibroblasts induced by AGEs and attenuated the cytotoxic effect of AGE on fibroblasts by activation of the PI3K/Akt-PTEN pathway. This study demonstrated that UCMSCs inhibited the cytotoxic effect of AGE in cells from patients with POP by inducing an anti-inflammatory reaction and activating the PI3K/AKT/PTEN signaling pathway. The current results provide important insights into use of stem cells to treat POP.

Keywords pelvic organ prolapse; advanced glycation end products; umbilical cord-derived mesenchymal stem cells; cytokines; PI3K-AKT

1. Introduction

Pelvic floor dysfunction (PFD) is the term for a group of clinical conditions, including stress urinary incontinence (SUI), pelvic organ prolapse (POP), overactive bladder syndrome, and fecal incontinence (*1,2*). In the general population, POP is an exceedingly common condition for mature women, with an estimated 41% presenting to their primary gynecologist with prolapse (*3*). PFD is primarily caused by aging and parity, and there are bimodal peaks of POP in these women at the ages of 46 and 71 (*4*). Treatments for this condition are still conservative and symptom-based. Women with symptoms who failed to respond to or who chose not to receive conservative treatment are candidates for surgery. Traditionally, surgeries include anterior, posterior, or total repair of the vagina, with concomitant hysterectomy, but the rate of recurrence can be as high as 20-30 % (*5,6*). Synthetic and biomaterial meshes have recently been used during surgery to provide improved long-term outcomes; however, about one-third of meshes cause scarring, erosion, and pain (*7*). Alternative methods are therefore needed to promote the repair and regeneration of damaged tissues.

In the supportive system of the pelvic floor, fibrous connective tissues surrounding the pelvic organs form fascia and ligaments to provide mechanical strength to support the vagina and its adjacent organs. Due to their specific anatomical location, these tissues are subjected to constant mechanical tensile loading from abdominal pressure and gravity (*8*). The fascia and ligaments of the pelvic floor mainly consist of dense connective tissues containing fibroblasts and extracellular matrix (ECM) secreted by fibroblasts.

Advanced glycation end products (AGEs), the products of nonenzymatic glycation and oxidation of proteins and lipids, accumulate in diverse biological settings including: diabetes, inflammation, renal failure, and aging (*9*). In a study examining the actual role of AGEs in the pathological physiology of POP, Jackson *et al.* found that both intermediate intermolecular cross-links and advanced glycation cross-links increased in

prolapsed tissue (*10,11*). AGEs can affect the metabolism of collagen through the receptor for AGEs (RAGE) but not directly through changes in expression or structure. AGEs activate the p-p38 MAPK and NF-κB-p-p65 pathways, thereby regulating collagen metabolism, although other pathways may also be involved (*12*). Taken together, these findings provide an enhanced understanding of the mechanism through which AGEs contribute to collagen metabolism in pelvic tissue of POP and the pathophysiology of POP.

Stem cells are able to participate in tissue repair due to their ability to differentiate into multiple lineages, and thus into various types of connective tissue cells, so they therefore hold great promise for treating PFD (*13*). Bone marrow-derived mesenchymal stem cells (BMSCs) are one of the most well-characterized stem cell sources, have great differentiation capability, and secrete bioactive factors that facilitate tissue repair (*14,15*). In animal models of SUI, periurethral injection of BMSCs restored the damaged external urethral sphincter and significantly alleviated SUI symptoms (*16*). Tissue engineering (TE) approaches have been used in different areas of medicine to improve long-term outcomes of surgical interventions (*17*). BMSCs are believed to regulate the repair process at sites of injured tissue by interacting with essential endogenous cells involved in the healing process: fibroblasts, endothelial cells, and epithelial cells (*18,19*). Umbilical cord-derived mesenchymal stem cells (UCMSCs) are isolated from the human umbilical cord and have better cell content and greater ability to proliferate than BMSCs. UCMSCs have lower immunogenicity than BMSCs, are easy to obtain, and cause no ethical controversy, so they have attracted increasing attention from researchers (*20*).

The current study investigated the anti-inflammatory role of UCMSCs and signaling pathways to inhibit the cytotoxic effect of AGEs in POP. Those findings were analyzed to determine if UCMSCs could serve as a potential treatment that reduces cell damage.

2. Materials and Methods

2.1. Culture of human UCMSCs and human vaginal wall fibroblasts (VWFs)

Human UCMSCs were purchased from the Shanghai Branch of Chinese Academy of Science and cultured in Gibco Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a 5% CO_2 atmosphere.

Human fibroblasts derived from the vaginal wall were obtained from patients suffering from POP or other diseases who underwent a hysterectomy at the Obstetrics and Gynecology Hospital of Fudan University. Ethical approval was obtained from the Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University. Briefly, fresh vaginal wall tissue specimens from the surgical margin of the free womb were rinsed 3 times with phosphate-buffered saline (PBS) (containing 1% penicillin, streptomycin, amphotericin B) at 4˚C for 5 min and digested at 37˚C for 30 min in PBS containing 2% collagenase. After separation, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (containing 10% fetal bovine serum, 1% penicillin, streptomycin, and amphotericin B) at 37.5˚C in a 5% CO_2 atmosphere, with replacement of the culture medium every 2-3 days. VWFs were identified using anti-vimentin antibody staining and subsequently stored in liquid nitrogen for further study.

2.2. Co-culture of human UCMSCs with VWFs

Human UCMSCs were co-cultured with VWFs by seeding UCMSCs (5×10^4 cells/dish) and fibroblasts (1×10^5 cells/dish) onto DMEM/F-12 culture medium.

2.3. Cell treatment and chemicals

VWFs or co-cultured cells were treated with AGEs at various concentrations (0, 25, 50, 100, and 200 μg/mL) in DMEM/F-12 containing 10% FBS for 2 days and then used for subsequent experiments. Untreated cells served as the control group (con). The PI3K inhibitor LY294002 and the Akt inhibitor GSL 690693 were purchased from Selleckchem (Houston, TX, USA).

2.4. Annexin V/PI double-staining

The fibroblasts from each treatment group were harvested and washed with PBS twice before being labeled with Annexin V/PI double-staining (KeyGen Biotech, China) in the dark, as described previously. All samples were analyzed with flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA) using the analytical software Cell Quest (BD, USA).

2.5. Real-time cell analyzer (RTCA) system

The xCELLigence RTCA DP System (ACEA Biosciences, San Diego, California, USA) allows label-free and real-time monitoring of cellular processes, such as cell proliferation, cytotoxicity, adhesion, viability, invasion, and migration, using electronic cell sensor array technology.

In brief, 50 μL of cell culture medium at room temperature was added to each well of E-plate 16 plates for analysis with the xCELLigence RTCA DP System. The E-plate 16 was then connected to the cell culture incubator and electrical contacts were checked. Background impedance was measured for 24 hours. UCMSCs were resuspended in cell culture medium and

adjusted to 5,000 cells/well. The cell suspension (100 μL) was added to wells containing 50 μL of medium on the E-plate 16 in order to determine the optimum cell concentration. After incubation at room temperature for 30 minutes, the E-plate 16 was placed in the cell culture incubator. Cell adhesion, growth, and proliferation were monitored every hour for a period of up to 24 hours *via* the incorporated sensor electrode arrays of the E-plate 16. After 24 hours, different concentrations of AGEs were added to 200 μL of cell culture medium, and live cells were monitored every 15 minutes for a period of up to 96 hours. Electrical impedance was measured with the RTCA-integrated software of the xCELLigence system as a dimensionless parameter termed CI.

2.6. RNA extraction and real-time RT-PCR

For PCR analysis, total RNA was extracted with an RNA extraction Kit (Axygen, CA, USA) according to the manufacturer's protocol, and the purity and concentration of RNA were measured with a NanoDrop 2000c (Thermo, Fisher, MA, USA). RNA (1 μg) was converted into cDNA using reverse transcriptase (Promega, Madison, USA). The normalization controls for mRNA and miRNA were GAPDH and U6 RNA, respectively. Threshold cycle (Ct) values were calculated using the software supplied with the Applied Biosystems 7900 Real-time PCR system.

2.7. Cytokine analysis

A Bio-Rad Bio-plex 200 suspension array system was used to measure the cytokine levels in cell culture medium. This bead-based Luminex technology allows for analysis of multiple proteins in a single sample. The experimental protocol allows for simultaneous reporting of standards, controls, blanks, and cytokines of interest in duplicate. The human cytokine 15-plex kit included the following cytokines: IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40L, and TNF-α. Bio-Rad Bio-plex Data Pro software was used for data analysis to identify the extreme values, data distribution, and to select the range.

2.8. Cell Counting Kit-8 (CCK-8) Assay

Following the protocol for the CCK8 assay (Do-jindo Laboratories, Kumamoto, Japan), cell growth by transfected cells in 96-well plates was assessed at 48 hours. A spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the absorbance at 450 nm.

2.9. Statistical analyses

Data were analyzed using the software GraphPad Prism (Version 7). Experimental results were expressed as the mean ± standard error of the mean (SEM). Each value is the mean of the data from an assay performed in triplicate. Data were subjected to analysis of variance (ANOVA), and the Tukey test was used to separate the means. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. AGEs inhibited the viability and proliferation of VWFs from patients with PFD

To analyze the effect of AGEs on PFD, human fibroblasts were obtained from the vaginal wall of patients with POP. These fibroblasts were isolated, cultured, and treated with different concentrations of AGEs. Changes in biological function including cell viability, cell apoptosis, and cell proliferation were detected. A CCK-8 assay was used to detect cellular dehydrogenase activity to evaluate the viability of fibroblasts. AGEs were found to significantly decrease the viability of fibroblasts in a dose-dependent manner in all treated groups compared to the control group (Figure 1A). Annexin V/PI double-staining was performed to examine cell apoptosis. AGEs significantly induced the apoptosis of fibroblasts in a dose-dependent manner compared to control cells (Figure 1B). The xCELLigence RTCA DP System was used to monitor cell proliferation in real time. AGEs markedly inhibited the growth of treated cells in a time- and concentration-dependent manner (Figure 1C).

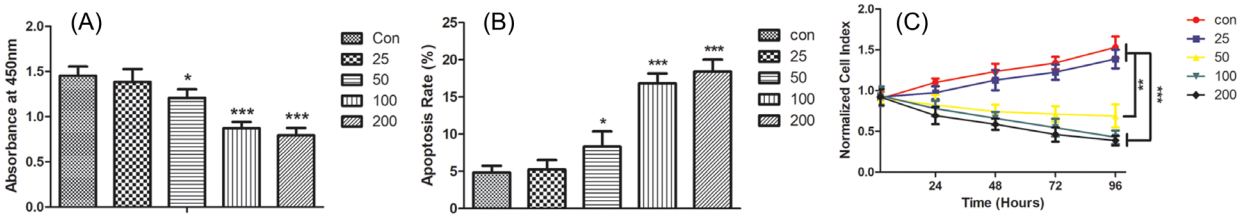

Figure 1. Effect of different concentrations of AGEs on cell viability, apoptosis, and proliferation of vaginal wall fibroblasts from patients with pelvic floor dysfunction. (A) AGEs decreased the viability of fibroblasts in a dose-dependent manner in all treated groups compared to the control group. **(B)** AGEs induced the apoptosis of fibroblasts in a dose-dependent manner compared to control cells. **(C)** AGEs inhibited the growth of treated cells in a time- and concentration-dependent manner. $^{*}p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

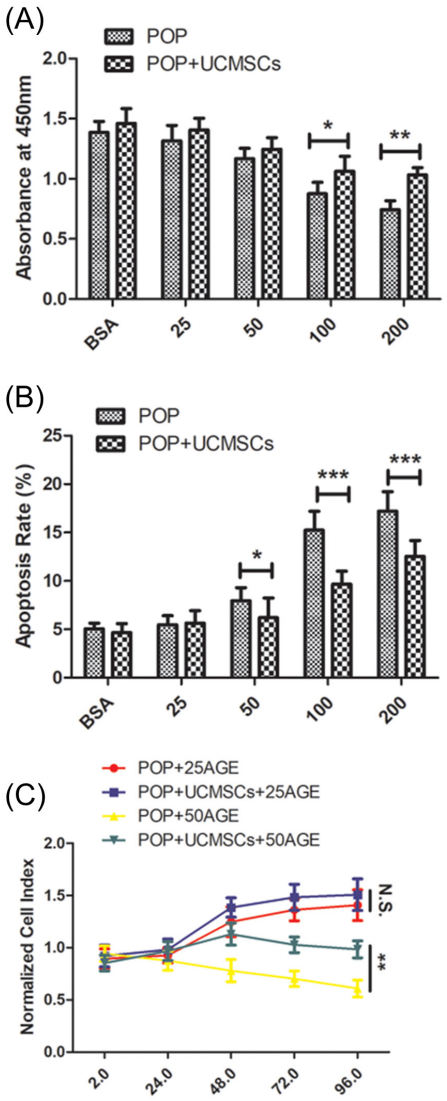

Figure 2. UCMSCs inhibited the cytotoxic effects of fibroblasts induced by AGEs. (A) The viability of fibroblasts co-cultured with UCMSCs and treated with AGEs increased at the concentrations of 100 μg/mL and 200 μg/mL compared to the control. **(B)** UCMSCs decreased the rate of apoptosis in POP treated with AGEs. **(C)** UCMSCs attenuated the inhibitory effect of AGEs on cell proliferation. $^{*}p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

3.2. UCMSCs protect fibroblasts against cytotoxic effects induced by AGEs

To determine whether UCMSCs protected against the cytotoxic effects of AGEs, fibroblasts were co-cultured with human UCMSCs and then treated with different concentration of AGEs. Directly cultured fibroblasts treated with different concentration of AGEs served as the corresponding control group. UCMSCs significantly increased the viability of fibroblasts until treatment with AGEs at a concentration of 100 μg/mL compared to the control (Figure 2A). Compared to the control group, cells co-cultured with UCMSCs exhibited a significant reduction in the rate of apoptosis starting at the concentration of 50 μg/mL when treated with AGEs

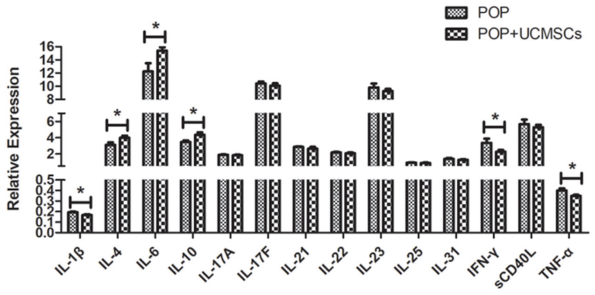

Figure 3. UCMSCs secrete anti-inflammation cytokines to attenuate the cytotoxic effect of AGE in POP. Cytokines from the supernatant of UCMSCs co-cultured with fibroblasts were treated with AGEs at a concentration of 100 μg/mL. $^{*}p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

(Figure 2B), and co-culturing attenuated the inhibitory effect of AGEs on cell proliferation (Figure 2C).

3.3. UCMSCs secrete anti-inflammation cytokines to attenuate the cytotoxic effect of AGEs

To explore whether UCMSCs secreted cell factors that affect the cytotoxic effect of AGEs, cytokines from the supernatant of UCMSCs co-cultured with fibroblasts that were treated with AGEs at a concentration of 100 μg/mL were analyzed. The anti-inflammatory cytokines IL-4, IL-6, and IL-10 increased in UCMSCs while the pro-inflammatory cytokines IL-1β, IFN-γ, and TNF-α decreased in UCMSCs compared to fibroblasts cultured alone (Figure 3).

3.4. UCMSCs attenuate the cytotoxic effect of AGE on fibroblasts by activating the PI3K/Akt/PTEN pathway

Compared to the control, co-culturing with UCMSCs significantly enhanced the expression of PI3K and Akt mRNA in fibroblasts that were treated with 100 μg/mL of AGEs. The signaling pathway for that effect was analyzed. PEN modulates apoptosis, and the expression of PTEN mRNA decreased in co-cultured cells (Figure 4A).

Fibroblasts were pre-treated with the PI3K inhibitor LY294002 and the Akt inhibitor GSK 690693. An enhanced cytotoxic effect of AGEs at a concentration of 100 μg/mL was noted in UCMSCs co-cultured with fibroblasts; the rate of apoptosis increased compared to that in untreated cells (Figure 4B). Treatment with these inhibitors had an additive inhibitory effect on proliferation (Figure 4C) and cell viability (Figure 4D).

4. Discussion

The current study investigated whether UCMSCs could attenuate the cytotoxic effect of AGE-induced cell apoptosis in POP and the possible molecular mechanisms allowing UCMSCs to resist AGE-induced cytotoxicity

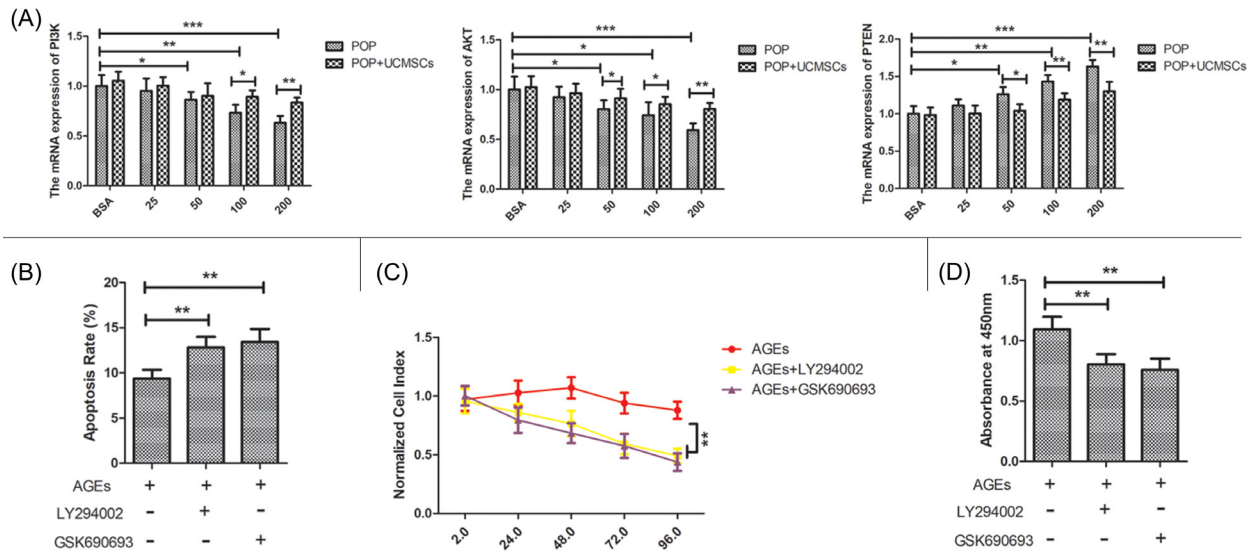

Figure 4. UCMSCs attenuate the cytotoxic effect of AGE on fibroblasts by activating the PI3K/Akt/PTEN pathway. (A) The levels of expression of PI3K, AKT, and PTEN mRNA in POP and co-cultured UCMSCs treated with different concentrations of AGEs. **(B)** The PI3K inhibitor LY294002 and the Akt inhibitor GSK 690693 increased the rate of apoptosis in co-cultured UCMSCs treated with 100 μg/mL of AGEs. **(C)** The PI3K inhibitor LY294002 and the Akt inhibitor GSK 690693 inhibited proliferation in co-cultured UCMSCs treated with 100 μg/mL of AGEs. **(D)** The PI3K inhibitor LY294002 and the3 Akt inhibitor GSK 690693 inhibited cell viability in co-cultured UCMSCs treated with 100 μg/mL of AGEs. $^{*}p < 0.05$. All results are expressed as the mean ± SEM, and data are representative of at least three experiments.

in POP. The current study found that AGEs inhibit the proliferation of VWFs and have dose-dependent cytotoxic effects on those cells. AGEs also induce the apoptosis of VWFs. In contrast, UCMSCs protect fibroblasts against the cytotoxic effects of AGEs by secreting anti-inflammatory cytokines to improve cell proliferation and cell viability and decrease the rate of apoptosis. Moreover, the PI3K/AKT/PTEN pathway is involved in UCMSCs inhibiting the cytotoxic effect of AGE-mediated cell apoptosis.

Human fibroblasts have the advantages of being easily harvested, cultured, and expanded *in vitro*, which make them an ideal cell source for regenerative medicine. VWFs play an important role in the pathophysiology of POP, which controls the integrity of collagen, and thereby impacts the mechanical properties of the pelvic floor (*21,22*). Primary culture of VWFs is commonly used to evaluate connective tissue in POP (*23*). Previous studies have described the impacts of AGEs on fibroblast proliferation. One study reported that AGEs promote the proliferation of fibroblasts, but another reported that AGEs induced the apoptosis of or inhibited the proliferation of fibroblasts (*24,25*). In the current study, the proliferation of fibroblasts from patients with POP was significantly inhibited by increasing concentrations of AGEs, suggesting that fibroblasts are more likely to be inhibited in POP. These results explain the smaller number of fibroblasts in the pelvic floor of patients with POP.

MSCs have been extensively used as cell-based therapies predominantly for their anti-inflammatory and immunomodulatory non-stem cell properties (*26*). They have also potential for tissue engineering purposes for regenerating new tissues or promoting the activity of endogenous stem cells (*27*). MSC populations have the capacity for self-renewal, a high proliferative potential, and differentiate into a variety of mesodermal and other lineages. Recent advances in cellular identification using more specific markers has shown that MSCs can be extracted from most tissues including bone marrow, the umbilical cord, the placenta, adipose tissue, and the endometrium, although not all of these sources have demonstrated clonogenicity for their MSC populations (*28,29*).

Typically, MSCs actively respond to stress or injury in a similar manner to the way cells of the innate immune system respond to pathogen exposure. When supplied systemically, exogenous MSCs home in on sites of injury in response to inflammation (*30,31*). There, MSCs operate in a paracrine manner secreting large amounts of diverse proteins, growth factors, cytokines, and chemokines that promote a variety of actions including neo-angiogenesis, tissue regeneration and remodeling, immune cell activation, suppression of inflammation, and cellular recruitment (*32*).

The potential of MSCs to serve as a cell-based therapy has recently been explored in numerous clinical applications. The ability to direct BMSCs to differentiate into other cell types and lineages has shown that these cells maintain a phenotype lacking tissue-specific characteristics until they are exposed to signals in damaged tissues (*33,34*). MSCs obtained from dental pulp have been used to repair related tissues such as the periodontal ligament, dental papilla, and dental follicle (*35*). The ability of adipose tissue and bone marrow MSCs to act as precursor cells has also

been exploited by directing their differentiation toward the chondrogenic lineage in order to produce cartilage-synthesizing chondrocytes (*36*). Although MSCs show promise as cell-based therapies, greater understanding of their mechanism of action and their potential is needed. Early use of MSCs has not always met expectations, often leading to inconsistent results. This may be due to lesser refined methods of isolating and cultivating MSCs resulting in the administration of fibroblasts and myofibroblasts rather than undifferentiated MSCs. Production of significant numbers of MSCs posed a challenge until recently since the regenerative potential of MSC declined during culture expansion, which is required due to the small numbers of perivascular MSC present within tissues (*37*).

POP is a common hidden disease burden for large numbers of women. Compounding this burden is the inadequacies of current surgeries with or without mesh. Recent advances in cellular phenotyping and gene profiling suggest endometrial MSCs as a possible complement to mesh-based POP treatment (*38*). The capacity of eMSCs to regenerate tissue is exemplified during a woman's reproductive life, where they regenerate at least one centimeter of endometrial lining each menstrual cycle for over 400 menstrual cycles. Seeding eMSCs onto polyamide/gelatin composite mesh and implanting them into the vaginal wall allow favorable modulation of the innate immune response and accelerate organized tissue repair. The first attempt at combining eMSCs and mesh to treat a fascial defect was successful in rodent models. This is encouraging, suggesting that further development of this approach using an ovine model is warranted (*39-41*).

In a recent *in vivo* study on PFD, transplantation of BMSCs resulted in new tissue growth and collagen deposition in a wound healing model. In the context of PFD, an appropriate amount of elastic fibers in the connective tissue is extremely crucial to functionally restoring pelvic floor support. Simple deposition of collagen would cause formation of dense connective tissues and eventually scar tissues (*42,43*).

UCMSCs have shown great potential in regenerative medicine for their extensive sources, potential to differentiate into multiple lineages, low immunogenicity, and self-renewal ability (*44*). The safety and therapeutic potential of human UCMSCs have been increasingly studied in the context of regenerative medicine and immune modulation. The immunosuppressive and anti-inflammatory properties of cultured/expanded UCMSCs have led these cells to be tested for their therapeutic potential in preclinical animal models since the mid-2000s, and their differentiation characteristics and responses to external environment have been extensively documented in *in vitro* single and co-culture setups (*45-47*). They have multiple advantages such as easy isolation and harvesting, no posing of ethical concerns, no tumor susceptibility, and low immunogenicity (*48*). As a result, UCMSCs hold significant promise for tissue engineering and regenerative medicine applications (*49*). To date, UCMSCs have been widely used in multiple studies to treat conditions such as acute lung injury, insulin-resistant diabetes, Alzheimer's disease (AD), acute myocardial infarction, graft-versus-host diseases (GVHD), aplastic anemia, arthritis, liver disease, spinal cord injury, systemic lupus erythematosus, and stroke (*50-52*).

Previous studies demonstrated that phosphatase and tensin homolog (PTEN) can negatively regulate the PI3K/AKT pathway, which in turn influences the nuclear factor kappa-light- chain-enhancer of activated B cells (NF-κB) signalling to modulate cell survival, migration, and proliferation. AGEs adjust the metabolism of target proteins through RAGE and activate an array of signal transduction cascades, such as MAPK, ROS, p38, NO, and NF-κB (*53,54*). The current authors hypothesized that the PI3K/Akt/PTEN pathway may be involved in governing the observed effects of UCMSCs to resist the cytotoxic effect of AGEs in POP. PI3K is a lipid kinase that induces cell cycle progression, cell survival, and cell migration, and many pieces of evidence have indicated that PI3K/AKT signaling is constitutively activated in many tumors with PTEN dysfunction.

The current findings further substantiate the contention that UCMSCs play a beneficial anti-inflammatory role by inhibiting the cytotoxic effect of AGEs. PI3K-AKT signaling, which is closely related to cell proliferation, is firmly considered to be involved in inflammatory action as well (*55*). In the current study, the anti-inflammatory conditions created by cells co-cultured with UCMSCs inhibited the expression of PTEN to reverse the apoptosis and improve cell proliferation in POP.

In conclusion, the anti-inflammatory role of UCMSCs may help to reverse the cytotoxic effect of AGEs in patients with POP and activate the PI3K/AKT/PTEN signaling pathway to increase proliferation and decrease apoptosis. These roles might provide important insights into the use of UCMSCs to treat POP.

Acknowledgements

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References

1. Arnouk A, De E, Rehfuss A, Cappadocia C, Dickson S, Lian F. Physical, Complementary, and alternative medicine in the treatment of pelvic floor disorders. Curr

Urol Rep. 2017; 18:47.
2. Easley DC, Abramowitch SD, Moalli PA. Female pelvic floor biomechanics: Bridging the gap. Curr Opin Urol. 2017; 27:262-267.
3. Tinetti A, Weir N, Tangyotkajohn U, Jacques A, Thompson J, Briffa K. Help-seeking behaviour for pelvic floor dysfunction in women over 55: Drivers and barriers. Int Urogynecol J. 2018; 29:1645-1653.
4. Cardenas-Trowers O, Meyer I, Markland AD, Richter HE, Addis I. A Review of phytoestrogens and their association with pelvic floor conditions. Female Pelvic Med Reconstr Surg. 2018; 24:193-202.
5. Weintraub AY, Glinter H, Marcus-Braun N. Narrative review of the epidemiology, diagnosis and pathophysiology of pelvic organ prolapse. Int Braz J Urol. 2020; 46:5-14.
6. Wallace SL, Miller LD, Mishra K. Pelvic floor physical therapy in the treatment of pelvic floor dysfunction in women. Curr Opin Obstet Gynecol. 2019; 31:485-493.
7. Taithongchai A, Sultan AH, Wieczorek PA, Thakar R. Clinical application of 2D and 3D pelvic floor ultrasound of mid-urethral slings and vaginal wall mesh. Int Urogynecol J. 2019; 30:1401-1411.
8. Hong S, Hong L, Li B, Wu D, Liu C, Min J, Guo W, Hu M, Tang J, Li Y. The role of GPX1 in the pathogenesis of female pelvic organ prolapse. PLoS One. 2017; 12: e0181896.
9. Deluyker D, Evens L, Bito V. Advanced glycation end products (AGEs) and cardiovascular dysfunction: Focus on high molecular weight AGEs. Amino Acids. 2017; 49:1535-1541.
10. Jackson S, James M, Abrams P. The effect of oestradiol on vaginal collagen metabolism in postmenopausal women with genuine stress incontinence. BJOG. 2002; 109:339-344.
11. Sferra R, Pompili S, D'Alfonso A, Sabetta G, Gaudio E, Carta G, Festuccia C, Colapietro A, Vetuschi A. Neurovascular alterations of muscularis propria in the human anterior vaginal wall in pelvic organ prolapse. J Anat. 2019; 235:281-288.
12. Chen YS, Wang XJ, Feng W, Hua KQ. Advanced glycation end products decrease collagen I levels in fibroblasts from the vaginal wall of patients with POP *via* the RAGE, MAPK and NF-κB pathways. Int J Mol Med. 2017; 40:987-998.
13. Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science. 2014; 346:1248012.
14. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal stem cell migration and tissue repair. Cells. 2019; 8:784.
15. Jin M, Chen Y, Zhou Y, Mei Y, Liu W, Pan C, Hua X. Transplantation of bone marrow-derived mesenchymal stem cells expressing elastin alleviates pelvic floor dysfunction. Stem Cell Res Ther. 2016; 7:51.
16. Du XW, Wu HL, Zhu YF, Hu JB, Jin F, Lv RP, Sun S, Wang HY, Xu JW. Experimental study of therapy of bone marrow mesenchymal stem cells or muscle-like cells/ calcium alginate composite gel for the treatment of stress urinary incontinence. Neurourol Urodyn. 2013; 32:281-286.
17. Mangir N, Aldemir Dikici B, Chapple CR, MacNeil S. Landmarks in vaginal mesh development: Polypropylene mesh for treatment of SUI and POP. Nat Rev Urol. 2019; 16:675-689.
18. Wang YJ, Yan J, Zou XL, Guo KJ, Zhao Y, Meng CY, Yin F, Guo L. Bone marrow mesenchymal stem cells repair cadmium-induced rat testis injury by inhibiting mitochondrial apoptosis. Chem Biol Interact. 2017; 271:39-47.
19. Han S1, Wang B2, Li X1,3, Xiao Z1, Han J1, Zhao Y1, Fang Y4, Yin Y1, Chen B1, Dai J1. Bone marrow-derived mesenchymal stem cells in three-dimensional culture promote neuronal regeneration by neurotrophic protection and immunomodulation. J Biomed Mater Res A. 2016; 104:1759-1769.
20. Płusa T, Baranowska A, Baranowski P. [Stem cells in contemporary medicine]. Pol Merkur Lekarski. 2019; 46:5-8.
21. Lu Y, Chen HY, Wang XQ, Wang JX. Correlations between mitofusin 2 expression in fibroblasts and pelvic organ prolapse: An *in vitro* study. Chin Med J (Engl). 2017; 130:2951-2959.
22. Ruiz-Zapata AM, Kerkhof MH, Ghazanfari S, Zandieh-Doulabi B, Stoop R, Smit TH, Helder MN. Vaginal fibroblastic cells from women with pelvic organ prolapse produce matrices with increased stiffness and collagen content. Sci Rep. 2016; 6:22971.
23. Medel S, Alarab M, Kufaishi H, Drutz H, Shynlova O. Attachment of primary vaginal fibroblasts to absorbable and nonabsorbable implant materials coated with platelet-rich plasma: Potential application in pelvic organ prolapse surgery. Female Pelvic Med Reconstr Surg. 2015; 21:190-197.
24. Dai J, Chen H, Chai Y. Advanced glycation end products (AGEs) induce apoptosis of fibroblasts by activation of NLRP3 inflammasome *via* reactive oxygen species (ROS) signaling pathway. Med Sci Monit. 2019; 25:7499-7508.
25. Nonaka K, Kajiura Y, Bando M, Sakamoto E, Inagaki Y, Lew JH, Naruishi K, Ikuta T, Yoshida K, Kobayashi T, Yoshie H, Nagata T, Kido J. Advanced glycation end-products increase IL-6 and ICAM-1 expression *via* RAGE, MAPK and NF-κB pathways in human gingival fibroblasts. J Periodontal Res. 2018; 53:334-344.
26. Hejretová L, Čedíková M, Dolejšová M, Vlas T, Jindra P, Lysák D, Holubová M. Comparison of the immunomodulatory effect of single MSC batches versus pooled MSC products. Cell Tissue Bank. 2019; 21:119-129.
27. Marolt Presen D, Traweger A, Gimona M, Redl H. Mesenchymal stromal cell-based bone regeneration therapies: From cell transplantation and tissue engineering to therapeutic secretomes and extracellular vesicles. Front Bioeng Biotechnol. 2019; 7:352.
28. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011; 9:12.
29. Toh WS, Lai RC, Zhang B, Lim SK. MSC exosome works through a protein-based mechanism of action. Biochem Soc Trans. 2018; 46:843-853.
30. Vinci P, Bastone A, Schiarea S, Cappuzzello C, Del Prete A, Dander E, Biondi A, D'Amico G. Mesenchymal stromal cell-secreted chemerin is a novel immunomodulatory molecule driving the migration of ChemR23-expressing cells. Cytotherapy. 2017; 19:200-210.
31. Saparov A, Ogay V, Nurgozhin T, Jumabay M, Chen WC. Preconditioning of human mesenchymal stem cells to enhance their regulation of the immune response. Stem

Cells Int. 2016; 2016:3924858.
32. Rackham CL, Amisten S, Persaud SJ, King AJF, Jones PM. Mesenchymal stromal cell secretory factors induce sustained improvements in islet function pre- and post-transplantation. Cytotherapy. 2018; 20:1427-1436.
33. Brown C, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, Svinarich D, Dodds R, Govind CK, Chaudhry GR. Mesenchymal stem cells: Cell therapy and regeneration potential. J Tissue Eng Regen Med. 2019; 13:1738-1755.
34. Atashi F, Modarressi A, Pepper MS. The role of reactive oxygen species in mesenchymal stem cell adipogenic and osteogenic differentiation: A review. Stem Cells Dev. 2015; 24:1150-1163.
35. Sharpe PT. Dental mesenchymal stem cells. Development. 2016; 143:2273-2280.
36. Liu Y, Lin L, Zou R, Wen C, Wang Z, Lin F. MSC-derived exosomes promote proliferation and inhibit apoptosis of chondrocytes *via* lncRNA-KLF3-AS1/miR-206/GIT1 axis in osteoarthritis. Cell Cycle. 2018; 17:2411-2422.
37. Oryan A, Kamali A, Moshiri A, Baghaban Eslaminejad M. Role of mesenchymal stem cells in bone regenerative medicine: What is the evidence? Cells Tissues Organs. 2017; 204:59-83.
38. Edwards SL, Ulrich D, White JF, Su K, Rosamilia A, Ramshaw JA, Gargett CE, Werkmeister JA. Temporal changes in the biomechanical properties of endometrial mesenchymal stem cell seeded scaffolds in a rat model. Acta Biomater. 2015; 13:286-294.
39. Emmerson SJ, Gargett CE. Endometrial mesenchymal stem cells as a cell based therapy for pelvic organ prolapse. World J Stem Cells. 2016; 8:202-215.
40. Darzi S, Deane JA, Nold CA, Edwards SE, Gough DJ, Mukherjee S, Gurung S, Tan KS, Vashi AV, Werkmeister JA, Gargett CE. Endometrial mesenchymal stem/stromal cells modulate the macrophage response to implanted polyamide/gelatin composite mesh in immunocompromised and immunocompetent Mice. Sci Rep. 2018; 8:6554.
41. Su K, Edwards SL, Tan KS, White JF, Kandel S, Ramshaw JAM, Gargett CE, Werkmeister JA. Induction of endometrial mesenchymal stem cells into tissue-forming cells suitable for fascial repair. Acta Biomater. 2014; 10:5012-5020.
42. Zhao B, Hu M, Wu H, Ren C, Chen J, Zhang X, Cui S. Peroxisome proliferator-activated receptor-γ and its related pathway in bone marrow mesenchymal stem cell differentiation co-cultured with mechanically stretched ligament fibroblasts. Int J Mol Med. 2018; 42:219-227.
43. Dandia H, Makkad K, Tayalia P. Glycated collagen – A 3D matrix system to study pathological cell behavior. Biomater Sci. 2019; 7:3480-3488.
44. Chandravanshi B, Bhonde RR. Human umbilical cord-derived stem cells: Isolation, characterization, differentiation, and application in treating diabetes. Crit Rev Biomed Eng. 2018; 46:399-412.
45. Zheng Q, Fu X, Jiang J, Zhang N, Zou L, Wang W, Ding M, Chen H. Umbilical cord mesenchymal stem cell transplantation prevents chemotherapy-induced ovarian failure *via* the NGF/TrkA pathway in rats. Biomed Res Int. 2019; 2019:6539294.
46. De Witte SFH, Peters FS, Merino A, Korevaar SS, Van Meurs JBJ, O'Flynn L, Elliman SJ, Newsome PN, Boer K, Baan CC, Hoogduijn MJ. Epigenetic changes in umbilical cord mesenchymal stromal cells upon stimulation and culture expansion. Cytotherapy. 2018; 20:919-929.
47. Wang P, Zeng F, He L, Wang J, Zhang T, Zhang D. Alteration of the immune status of umbilical cord mesenchymal stem cells stimulated by TLR1/2 agonist, Pam3Csk. Mol Med Rep. 2016; 14:2206-2212.
48. Meng M, Liu Y, Wang W, Wei C, Liu F, Du Z, Xie Y, Tang W, Hou Z, Li Q. Umbilical cord mesenchymal stem cell transplantation in the treatment of multiple sclerosis. Am J Transl Res. 2018; 10:212-223.
49. Zhang Y, Xing Y, Jia L, Ji Y, Zhao B, Wen Y, Xu X. An *in vitro* comparative study of multisource derived human mesenchymal stem cells for bone tissue engineering. Stem Cells Dev. 2018; 27:1634-1645.
50. Guan LX, Guan H, Li HB, Ren CA, Liu L, Chu JJ, Dai LJ. Therapeutic efficacy of umbilical cord-derived mesenchymal stem cells in patients with type 2 diabetes. Exp Ther Med. 2015; 9:1623-1630.
51. Xu Z, Nan W, Zhang X *et al.* Umbilical cord mesenchymal stem cells conditioned medium promotes Aβ25-35 phagocytosis by modulating autophagy and aβ-degrading enzymes in BV2 cells. J Mol Neurosci. 2018; 65:222-233.
52. Alshareeda AT, Alsowayan B, Almubarak A, Alghuwainem A, Alshawakir Y, Alahmed M. Exploring the potential of mesenchymal stem cell sheet on the development of hepatocellular carcinoma *in vivo*. J Vis Exp. 2018;139.57805
53. Kim J, Park JC, Lee MH, Yang CE, Lee JH, Lee WJ. High-mobility group box 1 mediates fibroblast activity *via* RAGE-MAPK and NF-κB signaling in keloid scar formation. Int J Mol Sci. 2017; 19:76.
54. Chen XJ, Wu WJ, Zhou Q, Jie JP, Chen X, Wang F, Gong XH. Advanced glycation end-products induce oxidative stress through the Sirt1/Nrf2 axis by interacting with the receptor of AGEs under diabetic conditions. J Cell Biochem. 2018; doi: 10.1002/jcb.27524.
55. Jin QS, Huang LJ, Zhao TT, Yao XY, Lin LY, Teng YQ, Kim SH, Nam MS, Zhang LY, Jin YJ. HOXA11-AS regulates diabetic arteriosclerosis-related inflammation *via* PI3K/AKT pathway. Eur Rev Med Pharmacol Sci. 2018; 22:6912-6921.

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