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Lab Note

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Human umbilical cord mesenchymal stromal cells promote the regeneration of severe endometrial damage in a rat model

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Due to the influence of various social and environmental factors, the incidence of infertility is increasing, and the application of assisted reproductive technology (ART) is becoming more and more widespread. At present, the clinical pregnancy rate of *in vitro* fertilization (IVF) is 40% ~ 60%, and decreased endometrial receptivity is one of the reasons for implantation failure. Endometrial receptivity refers to the capacity of the endometrium to accept embryos. Intrauterine adhesions (IUA), also known as Asherman syndrome (AS), is one of the most common causes of decreased endometrial receptivity after intrauterine surgery or inflammation. At present, most experts believe that IUA should be defined as intrauterine adhesion or fibrosis, which is accompanied by one or more of the following clinical symptoms: menstrual reduction, amenorrhea, repeated abortion, infertility and abnormal placental formation. The treatment of AS needs to restore the structure of the uterine cavity, restore menstruation, and promote the repair and regeneration of fibrosis and dysplasia of the endometrium. Stem cell therapy is becoming a new and effective treatment method to promote the repair and regeneration of the endometrium.

The types of stem cells currently used in animal experiments and clinical trials include bone marrow mesenchymal stromal cells (BMSCs) [1], adipose-derived mesenchymal stromal cells (ADSCs) [2], and menstrual blood-derived stromal cells (MBSCs) [3], *etc.* These stem cells have a certain endometrial regeneration effect in related experiments. However, BMSCs and ADSCs need to be extracted by invasive separation, and MBSCs may spread human papilloma virus (HPV) of vaginal cervix infection iatrogenicly. Human umbilical cord mesenchymal stromal cells (hUCMSCs) may overcome these limitations, and these cells will not face the problem of reduced differentiation potential as the donor ages. Additionally, hUCMSCs can be easily isolated from a readily available medical waste product with low risks of viral infection and high proliferative potential in the *in vitro* culture [4]. These aspects are superior to invasively extracted bone marrow mesenchymal stem cells. MSCs derived from the umbilical cord can be obtained from the amniotic membrane, cord lining, Wharton's jelly, and perivascular region. Most researchers use hUCMSCs from Wharton's jelly. The hUCMSCs used in this study are also derived from Wharton's jelly. Wharton's jelly-derived MSCs not only share advantages with UCMSCs from other regions, but also have the advantages of shorter and more stable doubling time and better differentiation potential in biological characteristics [5]. Our goal was to explore the pathological changes of severe endometrial injury in rats, clarify the regeneration effect of transplanted hUCMSCs on severely damaged endometrial, and explore an effective and feasible method for the treatment of endometrial injury.

This study was carried out in accordance with the "Guidelines for the Protection and Application of Experimental Animals" issued by the National Institute of Health in the United States. The use of laboratory animals strictly followed the corresponding regulations of the Animal Experimental Management Committee of the Shanghai University of Traditional Chinese Medicine. The hUCMSCs used in this study were isolated from the fourth generation umbilical cord Wharton's jelly cultured in the same batch of primary cells obtained from Shandong Stem Cell Group (Jinan, China), and conformed to the standards of the International Society for Cell Therapy to define pluripotent MSCs. The surface antigen markers of hUCMSCs were identified by flow cytometry.

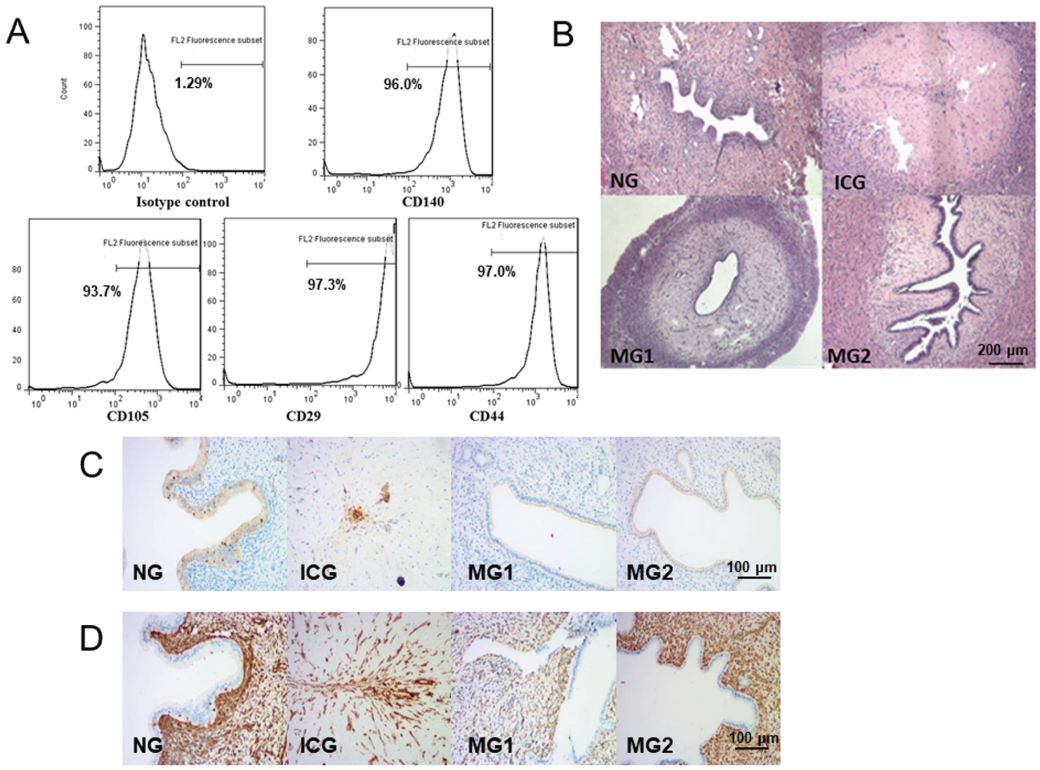

Figure 1. Characterization of hUCMSCs (A) Expression levels of CD140, CD105, CD29 and CD44 in hUCMSCs. Flow cytometry showed that the expression levels of CD140, CD105, CD29 and CD44 were 96%, 93.7%, 97.3% and 97% respectively. (B) The results of rat endometrial HE staining. Scale bar=200 μm. (C) The results of CK immunohistochemical staining. Scale bar=100 μm. (D) The results of vimentin immunohistochemical staining. Scale bar=100 μm.

Thirty-two Sprague-Dawley (SD) rats were randomly divided into 4 groups (8 rats in each group): normal group (NG), injury control group (ICG), MSC group 1 (MG1) and MSC group 2 (MG2). The normal group rats were not subjected to any processing. In the ICG, MG1, and MG2 groups, a severe rat endometrial injury model was established by injecting absolute ethanol into the uterine cavity. In the MG1 group, under isoflurane inhalation anesthesia, hUCMSCs (1×10^7 cells) were injected into the tail vein of each rat within 24 h after the model was established. In the MG2, within 24 h after the model was established each rat was treated in the same way as the MG1 rats. Then, on the 7th day after the model was established, rats in the MG2 were again subjected to laparotomy under abdominal anesthesia. During the operation, hUCMSCs (1×10^7 cells) were injected into each side of the uterine cavity with a 1-mL syringe. On the 15th day after modeling, all rats were sacrificed by cervical dislocation. Histological analysis was performed on rat endometrium and the mRNA expressions of cytokines *bFGF*, *TNF-α*, *IL-6* and *IL-1β* were determined by real-time PCR (q-PCR). **Materials and Methods** are included in the **Supplementary data**.

Mesenchymal stromal cells (MSCs) express marker genes of CD105, CD73, CD44, CD166, CD54, CD102, CD49, CD29, and CD140, but do not express the marker genes of CD14, CD34, CD45, CD11a or HLA-DR, and lack the expressions of the defining markers of red blood cells, platelets and endothelial cells such as CD31 [6]. Due to the low expression of major histocompatibility complex (MHC) I molecules and lack of MHC II molecule expression in MSCs, cytotoxic T lymphocytes and natural killer cells are unable to recognize and destroy allogeneic MSCs [7]. In this study, hUCMSCs highly express the marker genes of CD140, CD29, CD44 and CD105 (Figure 1A), which is consistent with MSCs characterization markers. The low immunogenicity of hUCMSCs is the major advantage of its utilization in allogeneic transplantation. This makes it possible to achieve allogeneic transplantation without using immunosuppressive drugs.

The rat endometrium was examined by hematoxylin-eosin staining (Figure 1B). In NG, the endometrium was fold-like. In ICG, the uterine cavity was closed. There was no endometrial epithelium, glandular or endometrial stroma, but fibrovascular proliferation and some scattered inflammatory cells were observed. In MG1, epithelial cells can be observed in the uterine cavity, and the folds were reduced. The endometrial stromal cells, individual glands and scattered inflammatory cells can be observed. In MG2, the endometrial epithelium was cubic columnar in morphology, the intima was wrinkled, and the endometrial glands and interstitiaum can be observed under the epithelium. Compared with that in MG1, the staining results of rat endometrium in MG2 showed an increase in the number of endometrial wrinkles and an increase in the number of glands. Cytokeratin (CK) is expressed in epithelial cells to maintain the integrity and continuity of epithelial tissue. Vimentin is expressed in various cell types such as mesenchymal cells, vascular endothelial cells, and smooth muscle cells. In this study, CK immunohistochemical staining was used to observe endometrial and glandular epithelial cells; while Vimentin immunohistochemical staining was used to observe endometrial stromal cells. The immunohistochemical staining results showed that there was negative expression of CK in ICG, weak expression of CK in MG1, and

positive expression of CK in MG2 (Figure 1C). The distribution of vimentin in ICG was loose and scattered, while the distribution of vimentin in MG1 was evener than that of ICG. However, the expression in MG1 was lower than that in NG, and the vimentin in MG2 was densely distributed under the endometrial epithelium, which was similar to that in NG (Figure 1D). Therefore, our results demonstrated that hUCMSCs can promote the repair of severe endometrial damage in rats, and the effect of intravenous injection combined with local application in the uterine cavity is better than intravenous injection alone.

At present, it is believed that the relevant mechanism of MSC to promote tissue regeneration includes homing differentiation, paracrine function, and immune regulation. Most researchers believe that stem cell homing is similar to leukocyte migration behavior and is a multi-step- and multi-factor-coordinated process involving many cytokines, receptors, adhesion factors, and extracellular matrix-degrading proteases [8]. The stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR-4) axis is an important biological axis that promotes the homing of mesenchymal stem cells to injured tissues [9]. Inflammation, tissue ischemia, liver drug damage, and damage to the body with chemotherapy drugs, as well as pro-inflammatory factors such as TNF-α or IL-1, may cause an increase in SDF-1 expression. The model of severe endometrial injury induced by intrauterine infusion of absolute ethanol was utilized. In this study, the expression levels of IL-1β and TNF-α mRNA in ICG were higher than those in NG (Figure 2), and it had the conditions to induce the homing of hUCMSCs. The expression of TNF-α mRNA was lower in MG1 with intravenously transplanted hUCMSCs than in ICG (Figure 2). Therefore, we believe that hUCMSCs are induced in MG1 to home to the damaged tissue of the endometrium and further play a role in inflammation inhibition and tissue repair.

The paracrine function of MSCs helps repair damaged tissues through a variety of mechanisms. MSCs are a rich source of growth factors such as bFGF, vascular endothelial growth factor (VEGF), hepatocyte growth factor, and insulin-like growth factor-1, which can promote angiogenesis, inhibit cell apoptosis and stimulate cell proliferation [10]. Compared with those in ICG, the expression levels of TNF-α in MG1 and MG2 transplanted with hUCMSCs were reduced. The expression level of bFGF in MG2 (hUCMSCs transplanted into both vein and uterus) was higher than that in ICG (Figure 2). The decrease in TNF-α expression and the increase in bFGF expression were related to hUCMSCS transplantation, indicating that hUCMSCS may inhibit inflammation, and induce tissue regeneration and repair through a paracrine model.

In summary, our study demonstrated that transplantation of hUCMSCs promotes the repair of severe endometrial damage in rats, and that the effect of venous combined intrauterine transplantation is better than that of simple vein grafting (Figure 3). The homing and paracrine mechanisms are potentially involved in the endometrial repair process. Further studies are required to understand the underlying mechanisms of this process and its potential impact on the fertility of rats.

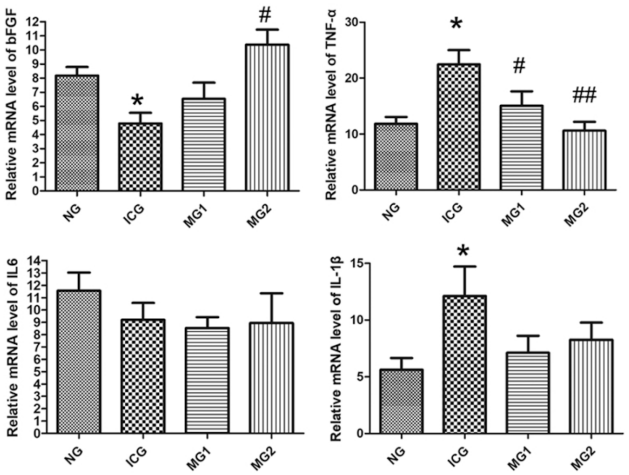

Figure 2. mRNA expression levels of bFGF, TNF-α, IL-6 and IL-1β in the rat endometrium The expression was measured by q-PCR. **P*<0.05 vs NG. #*P*<0.05, ##*P*<0.01 vs ICG.

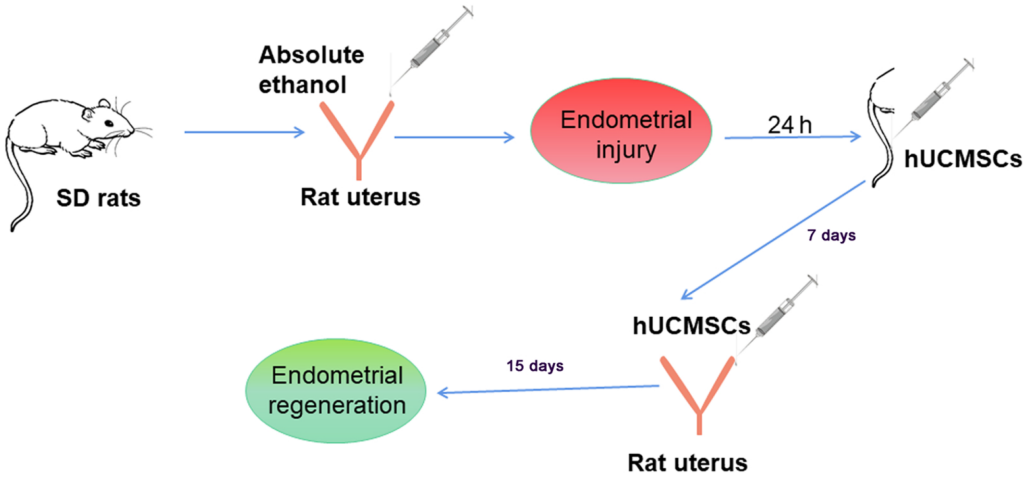

Figure 3. hUCMSCs promote the recovery of severe endometrial damage in rats Intrauterine perfusion of absolute ethanol caused severe damage to the endometrium of rats. Under isoflurane inhalation anesthesia, hUCMSCs (1×10^7 cells) were injected into the tail vein within 24 h after the model was established. Then, on the 7th day after the model was established, rats were again subjected to laparotomy under abdominal anesthesia. During the operation, hUCMSCs were injected into the uterine cavity (1×10^7 cells per side of the uterine cavity) with a 1-mL syringe. On the 15th day after modeling, the effect of hUCMSCs on endometrial regeneration was studied.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Jing Z, Qiong Z, Yonggang W, Yanping L. Rat bone marrow mesenchymal stem cells improve regeneration of thin endometrium in rat. *Fertility Sterility* 2014, 101: 587–594.e3
2. Kilic S, Yuksel B, Pinarli F, Albayrak A, Boztok B, Delibasi T. Effect of stem cell application on Asherman syndrome, an experimental rat model. *J Assist Reprod Genet* 2014, 31: 975–982
3. Tan J, Li P, Wang Q, Li Y, Li X, Zhao D, Xu X, ***et al.*** Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome. *Hum Reprod* 2016, 31: 2723–2729
4. Li T, Xia M, Gao Y, Chen Y, Xu Y. Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. *Expert Opin Biol Ther* 2015, 15: 1293–1306
5. Ishige I, Nagamura-Inoue T, Honda MJ, Harnprasopwat R, Kido M, Sugimoto M, Nakauchi H, ***et al.*** Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. *Int J Hematol* 2009, 90: 261–269
6. Rostovskaya M, Anastassiadis K. Differential expression of surface markers in mouse bone marrow mesenchymal stromal cell subpopulations with distinct lineage commitment. *PLoS ONE* 2012, 7: e51221
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, ***et al.*** Multilineage potential of adult human mesenchymal stem cells. *Science* 1999, 284: 143–147
8. Mannello F. Commentary: multipotent mesenchymal stromal cell recruitment, migration, and differentiation: what have matrix metalloproteinases got to do with it? *Stem Cells* 2006, 24: 1904–1907
9. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? *Blood* 2005, 106: 1901–1910
10. Chen L, Tredget EE, Wu PYG, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS ONE* 2008, 3: e1886