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Effect of PDGF-Rb antagonist imatinib on endometrial injury repairing in mouse model

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ABSTRACT

Objective: To study the effects of PDGF-Rb antagonists imatinib on endometrial injury repairing in the mouse model.

Methods: The cultured MSCs cells from male mice were marked with BrdU *in vitro*, and then transplanted to the female mice which suffered from radiation injury through tail vein, PDGF-Rb antagonists imatinib was injected through abdominal cavity. Four groups were arranged, which were radiation transplantation group, normal control group, imatinib intervention group and radiation control group. BrdU incorporation, *SRY* expression and MVD status were detected in uterus of mice.

Results: *SRY* gene was negative expressed in normal control group and radiation control group. *SRY* gene presented positive in radiation transplantation group and imatinib intervention group; BrdU incorporation showed negative in radiation control group and normal control group which died in the early stage in mice; the incorporation of BrdU was higher in radiation transplantation group compared with imatinib intervention group; CD34 was positive on the uterus of all the four groups, which showed highest in radiation control group and lowest in radiation control group; The MVD in imatinib intervention group was lower than radiation control group; the difference of MVD was significantly compared with normal control group ($P < 0.05$).

Conclusions: PDGF-Rb antagonists imatinib could inhibit the repairing function of MSCs in the endometrial lesions in mice.

1. Introduction

In recent years, with the development of assisted reproductive technology such as embryo freezing, patients have got multiple opportunities for embryo transfer, which makes the role of the receptivity of endometrium more important in pregnancy. Although endometrium has a strong ability of proliferation, it is easily injured by intrauterine operation and tuberculosis infection, thus leading to the thinning of endometrium and reducing of ability to conceive. Currently, due to reasons such as ethics, surrogacy is banned in many countries [1], and uterus transplant exists risks such as immunological rejection, therefore more attention is drawn to researches of endometrial repair after injury. In previous studies, scientists found the phenomena such as exogenous mesenchymal stem cell and embedded endometrial cell in the female patients with bone marrow transplantation, among which mesenchymal stem cell can probably develop to endometrial stem cells and plays an important role in periodic proliferation of endometrium. It is believed that CD146 and platelet-derived growth factor-receptor beta (PDGF-Rb) are expressed in endometrial stem cells and is the marker of endometrial stem cells [2]. PDGF is important promote mitosis and chemotactic agent in the body, which can promote proliferation, transfer and chemotaxis of a variety of histocytes and is helpful to the tissue repair of different kinds of injuries [3]. In previous studies, we found that PDGF-Rb has a certain correlation with the thickness of endometrium. In order to further explore the effect of PDGF-Rb on ectomesenchymal stem cells in the process of endometrial injury repairing, bone marrow mesenchymal stem cell was carried out in mice with endometrial injury induced by radiation. Through intraperitoneal injection of PDGF-Rb receptor

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antagonist imatinib, we explored the effect of imatinib on mesenchymal stem cell in mice in the process of endometrial injury repairing.

2. Materials and methods

2.1. Reagents

PDGF-Rb antagonist imatinib (Glivec, 100 mg/tablet) was purchased from Beijing Novartis Pharma Ltd. BrdU powder and the antibody were provided by Wuhan Boster Biological Engineering Co., Ltd. Rabbit anti-mouse polyclonal CD34 antibody and rabbit anti-mouse polyclonal CD29 antibody were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

2.2. Experimental animals

Adult KM mice of clean degree, aging 6–7 weeks, weighting 25–30 g were provided by Laboratory AnimaI Centre of Luye Pharma Group Ltd. [license no. SCXK (Lu) 20090013]. The genetic relationship of male and female mice was marked, and brothers and sisters were selected for bone marrow transplantation.

2.3. Primary culture and identification of mesenchymal stem cell by whole bone marrow adherent method and BrdU marking method

Six week-old mice were selected and killed by dislocation. The thighbone and tibia bone marrows were collected for cell culture and were transplanted with purification of mesenchymal stem cells [4,5]. In the process of part of the purification of MSCs cell passages, complete medium containing 30 μg/mL BrdU was added and cultured for 48 h. BrdU antibody was used to detect the BrdU marking positive rate of MSCs [6]. Marking positive rate = BrdU positive cell/total MSCs × 100%. According to the biological characteristics of MSCs, CD34 and CD29 antibodies were used for immunohistochemical analysis to decide whether it was mesenchymal stem cell [7]. The purified MSCs marked with BrdU was brushed by cytobrush, repeatedly beat into cell suspension and centrifuged for further use.

2.4. Transplantation of mesenchymal stem cell

The lethal dose of receptors in mice was calculated with body surface area and volume of the mice. Rotary cobalt-60 therapy unit was used for the irradiation of gamma rays in receptor body of female mice with the dosage rate of 1.0 Gy/min and the total dose of 8 Gy [8]. Within 2 h after radiotherapy, mesenchymal stem cell was transplanted in female mice (the brood male and female mice were strictly selected for the transplantation of mesenchymal stem cell to avoid immunological rejection), and serum-free DMEM medium was used to adjust the cell density to 5×10^7 cells/mL and 0.1 mL cell sap was injected to receptor mice through caudal vein [4,5]. PDGF-Rb receptor antagonist imatinib was diluted to 1% aqueous solution with normal saline and was intraperitoneally injected with 0.2 mL according to the mice weight (75 mg/kg) [9]. A total of 32 female mice were used in this study with 8 mice in each group. The mice were divided into groups for intervention according to Table 1.

2.5. Observation of post-transplant, trace of BrdU and trace detection of Y-chromosome

Female mice of each group were fed in sterile laminar flow cabinet and given 2×10^5 U/L sterile water. The survival time of mice was recorded. After 30 d, mice were killed by dislocation. One side of the uterus was fixed with 4% paraformaldehyde and performed HE staining and BrdU immunohistochemistry to observe the endometrial injury repairing. The other side of the ovary and uterus were placed at −70 °C to detect *SRY* gene by PCR. qRT-PCR was used to detect the gene expression of the sex determining region Y in the uterus of mice. Primer sequences are as follows: F: 5′-GCTGGGATGCAGGTGGAAAA-3′, R: 5′-CCCTCCGATGAGGCTGATATT-3′, length: 150 bp.

2.6. Detection of microvessel density

Microvessel density of uterus tissue in each group of mice were detected with the primary antibody CD34 according to the method of Weidner *et al* [2].

2.7. Statistical analysis

BrdU cell nucleus with claybank particle deposition was positive cell. The section was observed under 200× light microscope. IMAGE-PROPLUS was used for the analysis of rate of positive cells. Each group selected 30 sections and two different horizons were selected for quantitative analysis in each section. Furthermore, the staining intensity of positive cell was transferred to quantitative indicator to calculate its average optical density. Results were expressed as mean ± sd. SPSS17.0 statistical software was used for the variance analysis and t test.

3. Results

3.1. Culture and identification of MSCs

The culture medium was changed after 48 h of the culture of primary mesenchymal stem cell, and many adherent cells were observed under the microscope. After 3–4 day of culture, more cells were polygonal or fusiform and grew to clone shape. After

Table 1
Groups of female mice and intervention measures.

Groups	Radiotherapy dose	Tail vain injection	Intraperitoneal injection
Radiation transplantation group	8 Gy	0.1 mL MSCs cell suspension	0.2 mL normal saline
Imatinib intervention group	8 Gy	0.1 mL MSCs cell suspension	0.2 mL 1% imatinib
Radiation control group	8 Gy	0.1 mL DMEM	0.2 mL normal saline
Normal control group	0	0.1 mL DMEM	0.2 mL normal saline

10 day of culture, Cell fusion rate reached 90% and cells were passaged (Figure 1). Immunohistochemistry technology was used to authenticate CD29 (+) and CD34 (−) cells. Results showed that the obtained cells had character of mesenchymal stem cell (Figure 2). Meanwhile, BrdU marking positive rate was as high as 80% (Figure 3).

3.2. Survival situation of mice in different groups

After the irradiation of γ ray, mental atrophy, loss of weight and hair loose began to be appeared in mice. In radiation control group, mice successively died 4 days after radiotherapy; 14 days after radiotherapy, dead mice were eight. In radiation transplantation group, a total of 2 mice died after 14 days of transplantation, and before death, mice arched backs and refused food and water. After 14 days of transplantation, the activity and feeding of the survival mice was increased compared with that before 2 weeks. The body weight was also gradually increased. The survival time of two mice was about 20 days, while that of four mice could reach 30 days. No death was found in the mice of normal control group. The mice dying in radiation transplantation group were dissected. Results showed that the mice died in the early stage of radiotherapy (within 14 day of radiotherapy) became to have the symptoms of intestinal contents decrease and intestinal canal flatulence. Mice died at other time did not find the obvious lesions of celiac intestinal canal, but the uterus was small and ovary was pale and smooth. Three mice were dead in imatinib intervention group within 14 days, among which 2 were dead in 22nd and 25th day, respectively, and 3 were survived to 30 days.

3.3. Detection of pathology of the uterus

HE staining results showed that uterus in radiation control group was smaller than that in normal control group. The endometrial layer became thinner and the incidence rate of endometrial layer gland sag was decreased. The endometrium of mice in radiation transplantation group and imatinib intervention group was scattered in endometrial glands (Figure 4).

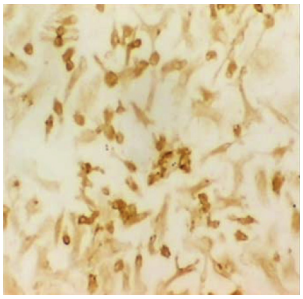

Figure 3. MSCs marked with BrdU (200×).

3.4. BrdU quantification of the uterus of mice

In the early death mice of normal control, radiation control and radiation transplantation groups, BrdUs of uteruses were negative. In the uterus of survival mice in radiation transplantation and imatinib intervention groups, BrdU mainly expressed in capillary endothelial cell and the small amount of stromal cells (Figure 4), and BrdU expression in radiation transplantation group was stronger than that in imatinib intervention group ($P < 0.05$).

3.5. Trace detection of Y-chromosome in the uterus tissue of mice

In female mice (negative control), *SRY* expression was negative, while *SRY* expression of male mice (positive control) was positive. qPCR results showed that *SRY* gene expression was not detected in both radiation control and normal control groups, but the *SRY* gene expressions were positive in radiation transplantation and imatinib intervention groups (Figure 5).

3.6. Microvessel density of uterus of mice

CD34 positive cells could be found in the uterus of mice in different groups of which the normal control was the highest and radiation control was the lowest. MVD in imatinib intervention group was lower than that in radiation transplantation group. Compared with normal control group, the changes of MVD in other three groups were significantly different ($P < 0.05$) (Table 2).

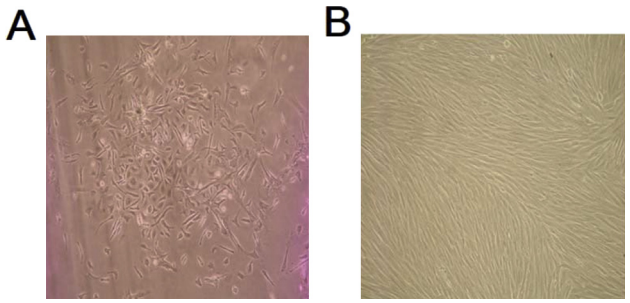

Figure 1. Primary culture of MSCs (200×) (A) and MSCs of the third generation (200×) (B).

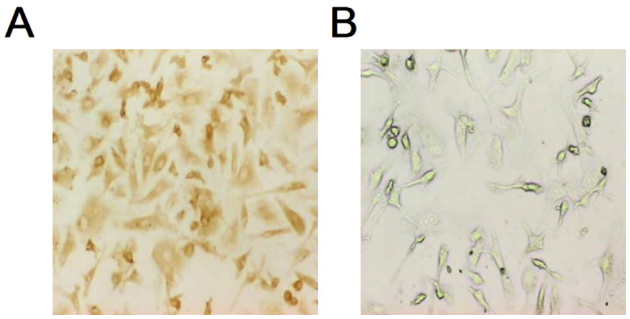

Figure 2. MSCs marked with CD29 (200×) (A) and with CD34 (200×) (B).

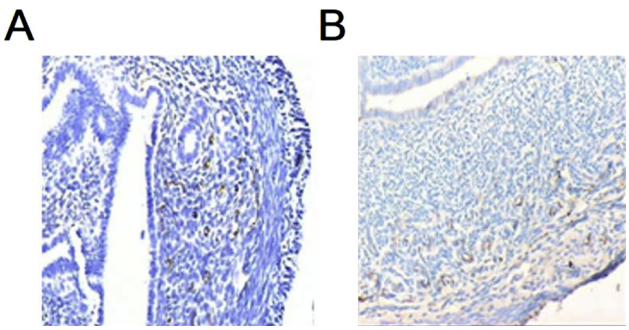

Figure 4. BrdU mark of uterus in radiation transplantation group (100×) (A) and in imatinib intervention group (100×) (B).

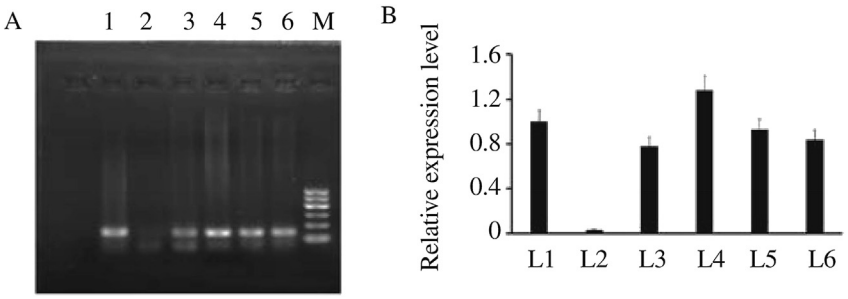

Figure 5. Expression of *SRY* gene by general PCR detection (A) and qRT-PCR detection (B).
1. adult male mice (positive control); 2. normal control group; 3, 4: radiation transplantation group; 5, 6: imatinib intervention group; M: PCR marker.
L1: adult male mice (positive control); L2: normal control group; L3, L4: radiation transplantation group; L5, L6: imatinib intervention group.

Table 2
Comparision of BrdU, *SRY* and MVD in uterus tissue of mice in different groups.

Groups	Brdu	*SRY*	MVD
Normal control	–	Negative	8.38 ± 2.34
Radiation control	–	Negative	2.06 ± 1.12#
Radiation transplantation group	0.214 ± 0.072	Positive	3.94 ± 1.49#
Imatinib intervention group	0.134 ± 0.053*	Positive	2.71 ± 0.92#

*Compared with radiation transplantation group, the difference was significant ($P < 0.05$). #Compared with normal control group, the difference was significant ($P < 0.05$).

4. Discussion

Bone marrow mesenchymal stem cells have strong proliferation ability and have multipotential differentiation [10]. Domestic and overseas scholars have studied the transplantation of bone marrow mesenchymal stem cells and proved that mesenchymal stem cell can migrate to a variety of tissues and organs and may provide cell origination for tissue injury repairing [11]. Taylor *et al* detected the HLA antigen in the endometrium of 4 female patients with bone marrow transplantation and found gene of the donor, which indicated that bone marrow stem cell of donor was helpful to the regeneration of endometrial in recipient [12]. We choose radiotherapy to injury the endometrium because radiotherapy injury has no drug residues compared with chemical injury, and has no inhibition to cells after transplantation. Radiological dose reached sublethal dose, and no mouse was dead in radiation control group within 14 days. We cultured the bone marrow-derived MSC of male mice *in vitro* and transplanted to female mice after radiotherapy to establish the model of bone marrow-derived mesenchymal stem cells in mice with endometrial injury and explore the effect of PDGF-Rb receptor antagonist imatinib on endometrial injury repairing.

PDGF-Rb and its receptor pathway is one of the important pathways of specific regulating proliferation and migration of capillary pericytes. The newborn capillaries involve in the tissue repair process through angiogenesis. Imatinib is a kind of PDGF-Rb receptor antagonist that can effectively inhibit PDGF-Rb and receptor pathway. In the *in vitro* study of Griffith *et al*, it showed that imatinib can reduce transmembrane transport and proliferation of matrix cell in extracellular matrix culture system via inhibiting the effect of endometrial proliferation and can be used in the treatment of endometriosis [13]. Therefore, we believed that imatinib can interdict the role of PDGF-Rb pathway and inhibit the generation of microvascular, thus inhibit tissue repair process [10]. The results in the present study showed that in radiation control group, 87.5% mice were dead within the 14 days of radiotherapy, with the less endometrial glands which showed that the purpose of endometrium to injury was achieved. No mouse was dead in normal control group and the endometrial glands and thickness were normal. Gene expressions of *BrdU* and *SRY* in radiation transplantation group and imatinib intervention group were positive, while BrdU in radiation control group and normal control group were positive, and *SRY* gene expression was highly expressed in the two groups, which indicated that *in vitro* MSCs could migrate to uterus and mainly locate in vascular endothelial cell of uterus and a small amount of matrix cells. BrdU and microvessel density in imatinib intervention group were lower than that in radiation transplantation group, which indicated that PDGF-Rb antagonists imatinib could inhibit the repair effect of MSCs on endometrial injury in mice. The results in the present study were consistent with the research of Griffith *et al* [13].

In conclusion, endometrial receptivity is one of the important factors of embryo transfer. More and more researchers pay attention to the form and function of endometrium before transplantation. Studying the mechanism of endometrial injury repairing is beneficial to promote the growth of endometrium and provides a favorable environment for embryo growth. Through animal model, we verify that blocking PDGF-Rb pathway will inhibit the regeneration of endometrium, which provides us a theoretical basis in the clinical work.

Conflict of interest statement

We declare that we have no conflict of interest.

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