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Original Article

Improved fat transplantation survival by using the conditioned medium of vascular endothelial growth factor transfected human adipose-derived stem cells

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Abstract Autologous fat transplantation has been applied widely in clinic. However, the low survival rate is still a problem to be solved. Studies shows that the human adipose-derived stem cells (ADSCs) transfected by vascular endothelial growth factor (VEGF) can improve the survival rate of autologous fat transplantation. Our study is to evaluate the effects of the conditioned medium of VEGF-transfected human adipose-derived stem cells (VEGF-ADSCs-CM) on fat transplantation. ADSCs were isolated and transfected with MOI = 40. The study was divided into three groups, VEGF-ADSCs-CM group, normal-ADSCs-CM group and control group. The conditioned media for VEGF-ADSCs-CM group and normal-ADSCs-CM group were collected, and then mixed with fat, with the mixtures being injected into the back of nude mice. On 4, 7, 15, 30, 60 days after transplantation, the grafts were evaluated on the wet weight, histology, ELISA and western blot. As the results revealed, the survival rate of VEGF-ADSCs-CM group was highest with the best fat cell morphology, and the VEGF secretion of VEGF-ADSCs-CM group was also highest. Therefore, our study demonstrates that VEGF-ADSCs-CM can improve the survival rate of fat transplantation effectively, and VEGF-ADSCs-CM can be regarded as an effective assisted method for fat transplantation.

Conflicts of interest: All authors declare no conflicts of interests.

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Introduction

Nowadays, autologous fat transplantation has been more widely applied because of its advantages, such as easy access, little damage, no reject reaction and no conspicuous postoperative scar. However, the long-term effect of fat transplantation was not ideal. Early blood vessel formation and adequate blood supply after fat transplantation were essential for improving the fat graft survival. Therefore, a variety of cell assisted therapies have been applied to fat transplantation and achieved effective results, for example, adipose-derived stem cells (ADSCs) [1,2], mesenchymal stem cells (MSC) [3], platelet-rich plasma (PRP) [4], and stromal vascular cells (SVF) [5]. Since Matsumoto et al. [1] put forward the concept of cell assisted lipo-transfer (CAL) and achieved success, ADSCs are universally considered as a good assistant method to improve the fat survival rate. In addition, vascular biology studies have showed that some cytokines was essential to improve the survival rate of ischemic tissue. Vascular endothelial growth factor (VEGF) is one of the most important growth factors to promote angiogenesis in vivo. VEGF works on the early stage of blood vessel formation, promotes the proliferation of vascular endothelial cells, and improves the permeability of blood vessels [6]. Due to the short half-life of exogenous VEGF [7], we use ADSCs as a carrier for sustained release of endogenous VEGF. It has been successfully used to improve the survival rate of fat transplantation [8]. Because of the strong paracrine effect of ADSCs, the conditioned medium of ADSCs is also rich in VEGF. Therefore, we hypothesized that the conditioned medium of VEGF-transfected human adipose-derived stem cells (VEGF-ADSCs-CM) can also promote the survival rate of fat transplantation.

Methods

Human tissue sampling

We obtained adipose tissue two times successively from two healthy female donors (age 20 years and age 28 years) undergoing liposuction of the abdomen from the First Affiliated Hospital of Jinan University, China. The donors were informed consent, and the study was reviewed and approved by the institutional review board of Jinan University. The adipose tissue from first abdomen liposuction was processed to culture ADSCs, and the adipose tissue from second abdomen liposuction was used for transplantation.

ADSCs isolation and culture

Briefly, adipose tissue was minced into 1 mm^3 pieces and washed with equal volumes of phosphate buffered saline, then treated with 0.1% type I collagenase for 40—60 min at 37 °C with evenly agitation. The collagenase was neutralized with 5 mL complete medium after centrifuging at 1200 rpm for 10 min and remove adipocytes layer. After lysing red blood cells, the suspension was filtered through a 200 μm nylon mesh to remove connective tissue debris. The cells were collected by centrifugation at 1200 rpm for 5 min and resuspended in complete medium and cultured at 37 °C and 5% CO_2. And the third-passage of ADSCs were used for transfection.

VEGF transfection

The third-passage of ADSCs were plated in 6-well plates. When the ADSCs growth to 80% of the hole, 1×10^8 TU/mL lentiviral vector-VEGFA liquid (Genechem, Shanghai, China) was added to each hole according to the multiplicity of infection (MOI) = 0, 10, 20, 30, 40, 50 individually. After 4 h infection, supplementing complete culture medium. The culture medium containing virus was discarded after 24 h, and changed the fresh medium, continued to culture at 37 °C and 5% CO_2. The fluorescence intensity was observed every 2 days. The culture medium was changed and collected every 2 days until tenth days.

Collecting the conditioned medium

When VEGF-ADSCs and normal-ADSCs covered about 80% of flasks, the conditioned medium was removed and the pure DMEM was added into flasks. Three days later, the conditioned medium was collected to a centrifuge tube and centrifuged at 1000 rpm for 5 min, then the supernatant was filtered using 0.22 μm syringe filter, and storage in −80 °C for fat transplantation.

Mouse models for fat transplantation

BALB/C-nunu nude mice (5-week-old, female) were purchased from Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China). Animals were housed in groups of six in a room with an artificial 12 h light/dark schedule and had free access to food and water at all times. Three dorsal subcutaneous sites of each mouse were taken as recipient areas for fat transplantation. Mice were injected with 0.3 mL of aspirated fat and 0.2 mL corresponding CM, and mice of control group were injected with 0.3 mL aspirated fat and 0.2 mL DMEM. The mixtures were injected using an 18-gauge angiocatheter needle and a 1.0-mL syringe. Weighing syringe before and after injection and recording and calculating the average fat weight.

Mice (n = 6) were sacrificed separately on 4, 7, 15, 30 and 60 days after fat transplantation. The transplanted fat was carefully dissected from their back, and were measured immediately with electronic balance. According to the formula "Fat retention rate (%) = (post transplant fat wet weight/pre transplant fat wet weight) × 100%", the survival ratio for transplanted fat was calculated. Then each transplanted fat was divided into two equal portions. One portion was used to determine the expression levels of VEGF, and the other portion was placed in 4% paraformaldehyde for histological examination.

Histology

Paraffin-embedded fat graft histological sections (5 μm) were prepared and stained with hematoxylin and eosin using standard procedures. Then the structure changes of the transplanted fat were observed and photographed by electron microscope.