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Original article

Telomerase reverse transcriptase genetically modified adipose tissue derived stem cells improves erectile dysfunction by inhibiting oxidative stress and enhancing proliferation in rat model

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ABSTRACT

Erectile dysfunction (ED) is considered to be incapable of obtaining or/and keeping a sufficient erection function to receive the satisfactory during the sexual intercourse. This study aims to investigate the effects of telomerase reverse transcriptase (hTERT) modified adipose tissue derived stem cells (ADSCs) autologously injected into cavernosa of the ED rats on erectile function. The ADSCs were isolated form the rat subcutaneous adipose tissue sample, and identified by examining the CD29 and CD44 molecule. The ED model was established by using 100 μg/kg apomorphine (APO). The adenovirus expressing rat hTERT (Ade-hTERT vector) was established, and transfected into ADSCs and injected into ED rat model, respectively. Telomerase activity, cell growth, cell apoptosis were analyzed by using TRAP ELISA assay, CCK8 assay and flow cytometry assay, respectively. The trophic growth factors were examined by using enzyme-linked immunosorbent assay (ELISA). The mRNA and proteins were detected by using semi-quantitative PCR and western blot assay, respectively. Ade-hTERT vector was highly expressed in both ADSCs and ED rat mode. The hTERT expression enhanced the telomerase activity, inhibits cell apoptosis and enhances proliferation of ADSCs ($P < 0.05$). hTERT expression triggers the secretory function of ADSCs and induces differentiative potential of ADSCs. hTERT expression inhibits apoptosis and increases eNOS and nNOS levels in older ED rats compared to the Ade-vector injected ED rats ($P < 0.05$). In conclusion, the hTERT modification could enhance the ADSCs proliferation, and hTERT modified ADSCs could increase the anti-oxidative stress capacity in the ED rat model.

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1. Introduction

Erectile dysfunction (ED) is considered to be incapable of obtaining or/and keeping a sufficient erection function to perform satisfactory sexual intercourse [1]. ED is a main health disorder that significantly affects on the life quality of both men and their partners [2]. The ED is one of the most important complications of the diabetes mellitus (DM), 20%–75% of the patients develop ED [3]. The occurrence of the ED in diabetic patients is often 10–15 years earlier and the symptoms in diabetic patients are severe compared to the non-diabetic men [4]. Recently, Garcia, et al. [2] investigated the pathophysiologic molecular mechanisms involved in ED processes, and suggested that ED is a disorder of the predominantly neuro-vascular etiology. Meanwhile, the cause for the ED in men is very complex involved the impairments of the tissues, such as the muscle, blood vessel (VEGF signaling system) and the nerve system [5]. Furthermore, the hyperglycemia could also cause the smooth muscle dysfunction by increasing the production of free-oxygen radical species and triggering the oxidation of the low-density lipoprotein [6].

Till now, several therapeutic methods are available for the ED patients. The first-line drug for majority of ED patients is the selective phosphodiestersase type-5 inhibitors (PDE-5I), such as Tadalafil, Vardenafil, Sildenafil, which are convenient to oral and with a relatively high efficacy (more than 70%) [2]. However, the oral drug are associated with low efficacy in the diabetic ED patients, because the endothelial dysfunction caused by the diabetes reduces the nitric oxide (NO) production in clinics [7,8]. The other therapeutic strategies, such as alprostadil or papaverine injection, implanting with the penile prosthesis, application of the

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vacuum constriction, all of which have their own advantages and limitations [3]. Therefore, the therapeutic method to improve the cavernous smooth muscle cells functions and restore the impaired endothelial cells (ECs) is critical and significant for the ED patients.

In past decades, the stem cell therapy strategy has been extensively used as a potential method for ED treatment. The mesenchymal stem cells are considered to differentiate into ECs, which are crucial for the ED therapy [9,10]. The previous study [11] reported that the transplantation of the bone stem cells beneficially improve the erectile function for the diabetic rats by triggering levels of smooth muscle and endothelium. In the recent years, the adipose-tissue derived stem cells (ADSCs), which with similar differentiated characteristics to the bone marrow-derived stromal cells (BMSCs), were widely used in the ED therapy [1,3,12]. The ADSCs treatment has been become a valuable strategy due to their abundant sources and ease of the isolation [13]. Nowadays, various methods have been reported to enhance the therapeutic effects of ADSCs in the previous studies [14–16]. The hypoxic preconditioning (HP) was added to the ADSCs, and was illustrated to comprehensively enhance the endogenously regenerative/defense genes expression in processes of ADSCs application [14–16]. Gokce et al. [17] found that the transplantation of genetically modified (by using IFNα-2b) ADSCs could enhance the erectile function in ED rats. Liu et al. [3] also reported that the ADSCs expressing vascular endothelial growth factor (VEGF) gene could trigger a therapeutic effect and improve the erectile function in the diabetic ED rats.

The hTERT is a catalytic component for the telomerase, which has the reverse transcriptase activity [18]. The previous studies reported that the hTERT overespressed stem cells could maintain the proliferation capacity and enhance the differentiation ability into multiple cells [19,20], and speculated that the hTERT may be helpful to the proliferation of ADSCs. Therefore, we used the hTERT to generate the modified ADSCs in this study.

This study aims to investigate the efficacy of the telomerase reverse transcriptase (hTERT) genetically modified ADSCs in improving erectile function, restoring endothelial function and decreasing fibrosis in a rat model of ED.

2. Materials and methods

2.1. ADSCs isolation

The rat ADSCs were isolated from the healthy rats according to the previously described [21]. This study was approved by the ethics committee of the Southwest Hospital, Third Military Medical University, Chongqing, China. The isolation processes and the usage of rat ADSCs were conducted in accordance with the approved guidelines. Briefly, the rat subcutaneous adipose tissue samples were extracted from the healthy rats. The fat in the tissues was dissociated enzymatically at 37 °C for 1 h by utilizing the 0.2% collagenase type 2 (Sigma-Aldrich, St. Louis, Missouri, USA). The digested tissues were neutralized by incubating with the Dulbecco's modified Eagle's media (DMEM, Gibco, Grand Island, NY, USA) supplementing with 20% fetal bovine serum (FBS Gibo, Grand Island, NY, USA), and then centrifuged for 5 min at 2000 r/min. The obtained stromal cell pellet was suspended in the DMEM and cultured in the 25 cm^2 plate with 5% CO_2 at 37 °C. In order to reach the confluent, the isolated cells were passaged by utilizing the 0.25% trypsin (Sigma-Aldrich, St. Louis, Missouri, USA) supplementing 0.1% ethylenediaminetetraacetic acid (Sigma-Aldrich, St. Louis, Missouri, USA).

2.2. Identification for ADSCs

In this study, the ADSCs specific surface antigens, including CD29, CD44 and CD31, were identified by employing the flow cytometry assay. The ADSCs were cultured and re-suspended in the DMEM containing 10% FBS to adjust the cell concentration of 1×10^7 cells/ml. Then, the ADSCs were incubated with the mouse anti-CD29, mouse anti-CD44 and mouse anti-CD31 antibodies for 30 min on the ice. Finally, the ADSCs were identified and analyzed on the FACS flow cytomery (BD Biosciences, San Jose, CA, USA) by using flow cytometry analysis. The collected data was analyzed by utilizing the Cytometry Analysis Software (BD, Franklin Lakes, NJ, USA).

2.3. Establishment of erectile dysfunction rat model

The rats (250–300 g) were purchased from the Experimental Animal Center of Third Military Medical University, Chongqing, China. The rats were housed in the environment with a 12 h ligh/dark cycle at the room temperature. The erectile dysfunction was induced in the rats by 100 μg/kg apomorphine (APO) according to the Heaton's published methods [22]. Post the APO subcutaneous injection, 100 rats were assigned as older ED rats (20 months old) and 40 rats were determined as young ED rats (2 months old).

All of the experiments were conducted according to the American guidance of the Ethical care of animals. All of experiments were approved by the Southwest Hospital, Third Military Medical University, Chongqing, China.

2.4. Construction for the adenovirus expressing rat hTERT

In this study, the replication defective adenovirus that expresses the rat hTERT was established due to the previously published study [23]. The gene sequence of the rat hTERT was amplified by using the PCR, and then sub-cloned into the IRES2-EGFP plasmid. The primer sequences were listed as the followings: sense primer, 5′-GGATCCCGGAAGAGTGTCTGGAGCAA-3′, anti-sense primer, 5′-GGATGAAGCGGAGTCTGGAGGAATTCC-3′. The IRESE-EGFP plasmid expressing the hTERT sequence was co-transfected with the pBHGfrtdelE13FLP adenovirus vector (Quantum Biotech. Montreal, Canada) into the ADSCs to establish the adenovirus expressing the hTERT (ADSCs-Ade-hTERT).

2.5. Trial grouping

For the in vitro experiments, the ADSCs were randomly divided into three groups, including ADSCs group, ADSCs-Ade-vector group (transfected with blank adenovirus vector) and ADSCs-Ade-hTERT group (transfected with pBHGfrtdelE13FLP adenovirus).

For the in vivo experiments, the rats were divided into three groups for both young (2 months) and older rats (20 months), including ADSCs-young (ADSCs-older, n = 20), ADSCs-Ade-vector-young (ADSCs-Ade-vector-older, n = 20) and ADSCs-Ade-hTERT-young (ADSCs-Ade-hTERT-older, n = 20) group.

2.6. Telomerase activity examination

The telomerase activity was examined by utilizing the TRAP-ELISA kit (Catalogue No. 185466, Boehringer Mannheim Co. Mannheim, Germany) according to the instructions of the manufacturer. The specific processes for the TRAP ELISA assay was performed as the previous study described [24]. The relative telomerase activity for every one sample was calculated as the following: (sample absorbance-heat-treated sample absorbance)/the internal standard sample absorbance.

2.7. Cell growth assay

The ADSCs cell growth was detected by employing the Cell Counting Kit 8 (CCK8) assay due to the instructions of the

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