ORIGINAL RESEARCH—BASIC SCIENCE

Intracavernous Delivery of Clonal Mesenchymal Stem Cells Restores Erectile Function in a Mouse Model of Cavernous Nerve Injury

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ABSTRACT

Introduction. Recently, much attention has focused on stem cell therapy; bone marrow-derived stem cells (BMSCs) are one of the most studied mesenchymal stem cells used in the field of erectile dysfunction (ED). However, a major limitation for the clinical application of stem cell therapy is the heterogeneous nature of the isolated cells, which may cause different treatment outcomes.

Aim. We investigated the effectiveness of mouse clonal BMSCs obtained from a single colony by using subfractionation culturing method (SCM) for erectile function in a mouse model of cavernous nerve injury (CNI).

Methods. Twelve-week-old C57BL/6J mice were divided into four groups: sham operation group, bilateral CNI group receiving a single intracavernous (IC) injection of phosphate-buffered saline (20 μL) or clonal BMSCs (3×10^5 cells/20 μL), and receiving a single intraperitoneal (IP) injection of clonal BMSCs (3×10^5 cells/20 μL).

Main Outcome Measures. The clonal BMSC line was analyzed for cell-surface epitopes by using fluorescence-activated cell sorting and for differentiation potential. Two weeks after CNI and treatment, erectile function was measured by electrically stimulating the cavernous nerve. The penis was harvested for histologic examinations and Western blot analysis.

Results. Clonal BMSCs expressed cell surface markers for mesenchymal stem cells and were capable of differentiating into several lineages, including adipogenic, osteogenic, and chondrogenic cells. Both IC and IP injections of clonal BMSCs significantly restored cavernous endothelial and smooth muscle content, and penile nNOS and neurofilament content in CNI mice. IC injection of clonal BMSCs induced significant recovery of erectile function, which reached 90–100% of the sham control values, whereas IP injection of clonal BMSCs partially restored erectile function.

Conclusion. We established a homogeneous population of mouse clonal BMSCs using SCM; clonal BMSCs successfully restored erectile function in CNI mice. The homogeneous nature of clonal mesenchymal stem cells may allow their clinical applications. **Ryu J-K, Kim D-H, Song KM, Yi TG, Suh J-K, and Song SU. Intracavernous delivery of clonal mesenchymal stem cells restores erectile function in a mouse model of cavernous nerve injury. J Sex Med 2014;11:411–423.**

Key Words. Erectile Dysfunction; Cavernous Nerve Injury; Stem Cell Therapy; Clonal Mesenchymal Stem Cell; Mouse Model of Erectile Dysfunction

Introduction

Erectile dysfunction (ED) is a frequent complication of radical prostatectomy (RP) following the treatment of prostate cancer despite advances in surgical techniques, such as nerve-sparing techniques and robotic procedures [1,2]. Partial cavernous nerve injury (CNI) or neurapraxia is inevitable, even with bilateral nerve-sparing RP because cavernous nerves run close to

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the prostate capsule and are microscopic in size [3,4]. Because nerve regeneration is a slow process, long-term denervation of the penis results in structural changes in erectile tissue, including loss of endothelial and smooth muscle content and deposition of extracellular matrix and cavernous fibrosis, which lead to irreversible damage to erectile tissue [5–8]. Although oral phosphodiesterase-5 inhibitors are generally effective for treating ED, men with ED resulting from RP are less responsive to these therapies than the general ED population [9,10]. Therefore, new treatment strategies correctly underlying pathologic processes in the corpus cavernosum tissue are required for effectively treating ED.

Recently, significant attention has focused on stem cell therapy for treating ED at the preclinical level. A variety of stem cells, including adult stem cells isolated from bone marrow, adipose tissue, skeletal muscle, and umbilical cord blood, as well as embryonic stem cells, have been used for neural, vascular, endothelial, or smooth muscle regeneration in animal models of ED [11–15]. Mesenchymal stem cells (MSCs) are adult stem cells; bone marrow-derived stem cells (BMSCs) are the most extensively examined MSCs in preclinical studies in the field of ED. Previous studies in animal models of ED due to diabetes, aging, or CNI have shown that intracavernous (IC) injection of BMSCs alone or genetically modified BMSCs have a beneficial effect on erectile function by enhancing the regeneration of nerves, endothelial cells, or smooth muscle cells in the penis [16–19].

The most popular method for isolating MSCs involves fractionation of mononuclear cells from donor tissues through gradient centrifugation, which utilizes adherent cells and removes non-adherent floating cells. Most preclinical trials in the field of ED have used adherent cell-culture techniques after gradient centrifugation to isolate MSCs [16–19]. However, recent evidence suggests that MSCs isolated using the conventional gradient centrifugation method are heterogeneous and have different differentiation potentials, which may lead to unpredictable therapeutic outcomes [20,21], thereby significantly limiting their clinical application. To obtain a homogeneous subpopulation of clonal MSCs, we recently established a new protocol, referred to as subfractionation culturing method (SCM), to generate single-cell-derived clonal BMSCs from a relatively small amount of bone marrow aspirate [22]. Clonal BMSCs significantly reduced inflammatory responses in rats with acute pancreatitis [23] and ameliorated radiation-induced salivary gland damage in mice through an anti-apoptotic effect [24].

In the present study, we isolated a homogeneous population of mouse clonal BMSCs by SCM and investigated their effectiveness for treating ED in a mouse model of ED induced by bilateral cavernous nerve crushing.

Methods

Isolation and Establishment of Clonal BMSC Lines

Mouse bone marrow samples were harvested from the tibiae and femurs of 5-week-old C3H mice by flushing out the bone marrow cavity with 5-mL culture medium composed of Dulbecco's modified Eagle's Medium (DMEM)-low glucose (Gibco-BRL, Life Technologies, Gaithersburg, MD, USA), 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin. Next, 15 mL of complete growth medium was added to the mixture. This mixture was then incubated in a 100-mm culture dish. After incubation for 1 hour at 37°C with 50 mL/L CO_2, the supernatant was transferred to a clean intermediate 100-mm dish (Figure 1). After the second 1-hour incubation, the supernatant was transferred from the intermediate dish into a new dish (D1) and incubated for an additional 1 hour. The supernatant was then transferred from D1 into a new dish (D2), incubated for 1 day, and then transferred from D2 into a new dish (D3) and incubated for 1 day. This process was repeated two more times with 1 and 2 days of incubation (for D4 and D5, respectively). After incubation for 7–14 days, well-separated, single-cell-derived colonies on the culture dishes were detached and isolated after treatment for 2–3 minutes with trypsin/ethylene diamine tetraacetic acid (EDTA) in cloning cylinders (Gibco-BRL). Cultures were transferred to a six-well plate and then to large culture flasks where the cells continued to expand. Once the cells reached approximately 70–80% confluence, they were recovered using trypsin/EDTA and passaged at 50–100 cells/cm^2. We obtained between 10 and 20 colonies on D1 through D3 dishes using bone marrow samples from 5 mice and were able to establish several clonal BMSC lines, of which one (D101) was selected for use in this study.

Immunocytochemistry and Flow Cytometry

For phenotyping of cell surface antigens, established clonal BMSCs at five to seven passages were harvested from the 175-cm^2 flasks by treatment with trypsin/EDTA and were washed twice with phosphate-buffered saline (PBS). Cells were