International Journal of Pediatric Otorhinolaryngology 91 (2016) 72–81

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: http://www.ijporlonline.com/

Regenerative efficacy of mesenchymal stromal cells from human placenta in sensorineural hearing loss

Kicheol Kil [a, 1], Mi Young Choi [b, c, 1], Ji Sun Kong [c], Woo Jin Kim [c], Kyoung Ho Park [c, *]

[a] Department of Obstetrics and Gynecology, Seoul St. Mary's Hospital, The Catholic University of Korea School of Medicine, Seoul, South Korea
[b] Department of Medical Cell Biology, The Catholic University of Korea School of Medicine, Seoul, South Korea
[c] Department of Otolaryngology-Head & Neck Surgery, Seoul St. Mary's Hospital, The Catholic University of Korea School of Medicine, Seoul, South Korea

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form
7 October 2016
Accepted 9 October 2016
Available online 11 October 2016

Keywords:
Amnion
Chorion
Mesenchymal stromal cell
Hearing loss

ABSTRACT

Introduction: Hearing loss is a common chronic disorder characterized by decline of auditory function. The global population have suffered from deafness and the transplantation of stem cells is regarded as a therapeutic strategy for this disease.
Methods: We collected placenta from a total of 13 samples of full term pregnant women and isolated MSCs derived from human placenta and transplanted MSCs on deaf animal model. The normal group and the sensorineural hearing loss (SNHL) group and the experimental (transplanted MSCs) group were compared and estimated hearing level using auditory brainstem response (ABR) recordings and the otoacoustic emission (OAE) test.
Results: ABR threshold value and DPOAE level showed that MSCs transplantation groups was improved than the SNHL group. And the number of spiral ganglion neurons were increased in all turn of the cochlea. And there was no evidence of acute immunological rejection and inflammation response was not observed.
Discussion: This study is to evaluate regenerative efficacy of hearing loss by transplanting mesenchymal stromal cells (MSCs) derived from human placenta (amnion and chorion) in deaf animal model. We identified that MSCs transplantation restored auditory impairment and promoted cell regeneration. We hope to overcome sensorineural hearing loss by transplanting stem cells such as mesenchymal stromal cells (MSCs) from easily accessible adult stem cell source in placenta.

1. Introduction

There are over 250 million patients worldwide suffering from incurable neurological disease, including deafness. Among patients suffering from deafness, almost 90% have some form of sensorineural dysfunction, such as sensory hair cell loss. It is estimated that the number of patients with neuron-related disease will double by the year 2030. Recently, the transplantation of stem cells has emerged as a promising therapeutic strategy for the treatment of permanent hearing loss [1,2]. Furthermore, articles and broadcasts have reported that many incurable neurological diseases have been influenced by the use of in vivo therapeutic methods.

Mesenchymal stromal cells (MSCs) are stem cells with a capacity for self-renewal and can differentiate into mesoderm- and non-mesoderm-derived tissues. From a therapeutic perspective, MSCs are promising therapeutic agents for tissue regeneration due to their ease of preparation and immunologic privilege. Many other studies have isolated adult mesenchymal stromal cells from bone marrow, adipose tissue, umbilical cord blood (UCB), lung tissue, and Wharton's jelly [3–7]. Additionally, in our previous study, we demonstrated that UCB-derived mesenchymal stromal cells can differentiate into auditory hair cells and neuronal cells in vitro when exposed to a neuronal-developmental microenvironment. These cells can be differentiated into all three germ cell layers (neuron, supporting cells, and hair cells). Several studies have shown that mesenchymal stromal cells can be collected from

* Corresponding author. Department of Otolaryngology Head & Neck Surgery, Seoul St. Mary's Hospital, The Catholic University of Korea, 222, Banpo-daero, Seocho-gu, Seoul, South Korea.
E-mail address: khpent@catholic.ac.kr (K.H. Park).
[1] Kicheol Kil & Mi Young Choi were co-first author & equally contributed to this article.

http://dx.doi.org/10.1016/j.ijporl.2016.10.010
0165-5876/

placenta and umbilical cord blood as a derivative and used in the field of regenerative medicine [7,8]. However, the amount of MSCs derived from UCB was very small, and it was difficult to achieve a proper environment for the differentiation of MSCs; consequently, they did not proliferate well. Recently, the isolation of MSCs from adult stromal cells has demonstrated that these cells are a potential source of mesenchymal stromal cells with therapeutic potential. Also, our previous studies and other articles have shown that many types of adult stem cells can differentiate into various cell types. In addition, these cells have been used in therapeutic pathways that promote wound-healing and effect incurable diseases and nerve disorders [7–13]. Consequently, the aim of this article was to evaluate the therapeutic regeneration and treatment effects with respect to hearing loss using mesenchymal stromal cells derived from human placenta. Also, it is mandatory to develop novel techniques for isolating, culturing, and mass producing MSCs with proliferative capacity.

Therefore, in this study, we have shown that human amniotic membrane-derived MSCs (AM-MSC) and chorionic membrane-derived MSCs (CM-MSC) can be differentiated into neurons, glial cells, and hair cells. In addition, we transplanted AM-MSC into an animal model of deafness and evaluated their therapeutic efficacy with respect to hearing.

2. Material & methods

2.1. Isolation of MSCs derived from human placenta

Placentas were collected after birth from women who carried to full-term after obtaining informed consent and were placed in heparin for storage. The average age of the pregnant women whose placentas were used in this study was 30–40 years. Placentas were isolated from a total of 13 women. These samples were washed three times with PBS and stored in serum-free α-MEM (Alpha Minimum Essential Medium, Gibco, Grand Island, NY). Samples were carefully divided into amnion membrane and chorionic membrane using micro pincers and scissors in a highly sophisticated technique. Each membrane was then minced with fine iris scissors, and 10 ml of 0.25% trypsin-EDTA (Gibco) was added to the tissue maintained in a water bath for 1 h at 37 °C. After the incubation, an equal volume of complete medium (containing 10% FBS and 1% P/S) was used to halt the enzymatic activity, and samples were incubated for 3 min. The obtained samples were centrifuged at 1000 rpm for 5 min at 4 °C. The pellets were re-suspended in 1 ml of culture medium and transferred to a fresh culture dish. The culture medium was replaced at 3-day intervals, and bFGF was added to the culture medium to achieve a concentration of 20 ng/ml. The proliferating cells were used in this research and cells did not exceed three passages.

2.2. FACS analysis

To confirm whether any MSCs were derived from the placenta, we evaluated the attached cells using FACS analysis. Flow cytometry of the cell surface markers CD34, CD45, CD73, CD90, and HLA-DR was conducted on the MSCs derived from placenta. All MSCs present at the third passage from the originating placenta were plated in a test tube (BD, Franklin Lakes, NJ, USA) at 1×10^5 cells/ml and washed three times with wash buffer (PBS and 3% FBS). The cells were incubated with primary antibody for 60 min using saturating concentrations of the monoclonal antibodies noted above. After the cells were washed with wash buffer and centrifuged at 1000 rpm for 5 min, they were re-suspended in ice cold PBS and incubated with a FITC-labeled or PE-labeled secondary antibody for 20 min in the dark at 4 °C. Cell fluorescence was evaluated by flow cytometry in a FACS Caliber instrument (BD), and the data were analyzed using Cell Quest software (BD). An isotype control was included in each experiment, and specific staining was measured from the cross point of the isotype with a specific antibody graph.

2.3. Neurosphere and neural differentiation of human placenta

After primary culture in complete medium (α-MEM; containing 10% FBS and 1% P/S), each of the MSC samples was cultured for up to three generations. The cells were then trypsinized and re-plated on 60 mm tissue culture plates at a density of 1×10^5 cells per well. Subsequently, differentiation into neuronal progenitor cells and neuronal lineage cells, including hair cells, glial cells, and neurons, was induced with neurotrophin factors. The MSCs were divided into three groups: a proliferation-only group without growth factors, which was termed the normal group; a differentiated into progenitor cell group termed the sphere group; and a neural differentiated cell group termed the neuron group. The sphere group was cultured in DMEM:F12 medium (Gibco) containing 1 ml of b27 supplement, 20 ng/ml EGF, and 10 ng/ml bFGF for about 21 to 25d. The neuron group was cultured in neuro-basal medium (including b27 supplement) treated with 10 ng/ml GDNF, BDNF, and NT3 for 14 days each (all medium and growth factors used were purchased from Invitrogen). At this time, in order to accelerate neural growth, each of the MSC samples was additionally treated with 20 ng/ml bFGF at 3-day intervals. Detection of neurosphere and neural cells was performed using immunocytochemistry.

2.4. Confirmation of sphere and neuronal lineage cells

Twenty-one days after differentiation into neurospheres and 14 days after the initiation of neural induction, cells were characterized by immunocytochemistry. Furthermore, to characterize the differentiated cells in more detail, we used antibodies specific to the pre-synaptic marker VGLUT3 (vesicular glutamate transporter type 3) and the post-synaptic marker VDSS (L-type of voltage gated Ca^{2+} channel). This assumption is supported by the detection of the intermediate filament protein nestin [13,14] in neuronal cells, as indicated by the presence of nestin antibody staining, and as a functional pre-synaptic and post-synaptic marker of double staining. Analyses of neuronal cells were performed on the cells 14 days after differentiation. Neurospheres were identified in cultures 21–25 days after differentiation. Initially, each of the placenta MSC samples was cultured separately and aliquoted into a Lab-Tec 4-well Chamber Slide (Nalgene Nunc International, Rochester, NY) at a concentration of 1×10^4 cells/well. All dissociated cells were fixed for 10 min on ice in 4% paraformaldehyde in PBS or left to differentiate further into neuronal lineage cells. Cells were then treated with 0.5% Triton X-100 (Promega, Madison, WI) at room temperature for 20 min, rinsed twice with PBS, and incubated with synaptic marker antibodies diluted 1:200. The secondary antibodies used were Alexa Fluor 488-conjugated goat anti-rabbit/mouse (1:300; Molecular Probes Eugene, OR). The specimens were then blocked using 5% normal goat serum. Each sample was double-stained using antibodies to specific cellular markers, including the glial cell markers GFAP (glial fibrillary acidic protein) and S-100; the neuronal cell markers βIII-tubulin, NF (neurofilament), and MAP2 (microtubule-associated protein 2); the neuronal progenitor marker nestin; and the hair cell markers myosin-VIIA and TRPA1 (Transient Receptor Potential Cation). Unless described above, all unmentioned antibodies were purchased from Abcam, Cambridge, UK. All specimens subject to double staining were processed overnight and rinsed with PBS containing 3% BSA. The secondary antibody, Alexa 555-conjugated goat anti-rabbit/mouse,