Neuroscience Letters xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Up-regulation of stromal cell-derived factor-1 enhances migration of transplanted neural stem cells to injury region following degeneration of spiral ganglion neurons in the adult rat inner ear

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HIGHLIGHTS

- SGN-degenerated cochlear microenvironment plays a beneficial role for NSC migration.
- The role is mediated by the SDF-1.
- The increased SDF-1 protein was derived from the satellite Schwann cells of the spiral ganglion.
- Optimizing SDF-1 expression may improve the migration of stem cells in the cochlea.

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form 15 October 2012
Accepted 4 November 2012

Keywords:
Stromal cell-derived factor-1
Cell migration
Degeneration
Spiral ganglion neuron
Cochlea

ABSTRACT

Neural stem cell (NSC) transplantation into the cochlea is widely used for the treatment of spiral ganglion neuron (SGN) degenerative disease and injury in the animal models, but the migration of the transplanted NSCs to the injury region is difficult and the mechanism is still unclear. In this study, we aimed to validate whether the SGN-degenerated cochlear microenvironment plays a role in the NSC migration and investigated whether stromal cell-derived factor-1 (SDF-1) was involved in the NSCs migration. Using a rat SGN degeneration model, we demonstrated that the transplanted NSCs are more likely to migrate to the injury region during the early post-injury (EPI) than the late post-injury (LPI) stage and the control cochlea. We found that the expressions of SDF-1 increased transiently after SGN degeneration. Additionally, we showed that the NSCs express CXCR4, a receptor for SDF-1. We observed that the region to which the transplanted NSC localized coincides with the region where the SDF-1 is highly expressed following the degeneration of SGNs. Finally, we observed that the increased SDF-1 is derived from the Schwann cells in the SGN-degenerated model. These results suggest that SDF-1, which is derived from cochlear Schwann cells and up-regulated in the early injury microenvironment, plays a beneficial role in the NSC migration to the injury region. Optimizing SDF-1 expression in the host microenvironment or increasing the CXCR4 expression of the donor stem cells may improve the migration efficiency of transplanted cells toward the injury region in the cochlea.

1. Introduction

The majority of the spiral ganglion neurons (SGNs) in the cochlea are primary auditory afferent neurons. Thus, loss of the SGNs will lead to severe sensorineural hearing loss (SNHL). Unlike degeneration of the peripheral motor neurons and some sensory neurons, SGNs do not regenerate to any clinically significant extent [12,13]. Therefore, the repair and replacement of SGNs would be one of the essential steps in any attempt to restore auditory function.

Cell replacement therapy is considered to be a promising approach to replace damaged cells within the mammalian cochlea in recent years. Researchers in the area of otology performed these studies by transplanting stem cells into the animals' SGN-degenerated cochlea [3–5,9]. Interestingly, the data show that the SGN-degeneration provides a more permissible microenvironment than the normal cochlea for stem cells to survive [9,11], migrate into the injured tissue [16], and differentiate into neurons [4], but the mechanism is still unclear.

Chemokine stromal cell derived factor-1 (SDF-1, also known as CXCL12) signaling through CXCR4 has previously been identified as

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0304-3940/$ – see front matter © 2012 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.neulet.2012.11.018

a key step in the homing of stem cells [1,2,6,10]. Meanwhile, investigations have revealed that SDF-1 is often up-regulated within injuries in areas including the brain [6], heart [15] and kidney [18]. A recent study showed that the expression of SDF-1 is up-regulated in the setting of cochlear injury [7]. However, whether the up-regulated SDF-1 enhances the migration of transplanted neural stem cells (NSCs) toward the injured area of the cochlea is still unknown.

The purpose of this study was to examine the role of the injury microenvironment on the migration of transplanted NSCs, the expression of SDF-1 in the SGN degeneration model, the relationship between SDF-1 and NSC migration and the source of the SDF-1 in the injured spiral ganglion (SG).

2. Materials and methods

2.1. Animals and ethics statement

Randomly selected adult male SD rats weighing 300–350 g and exhibiting a normal Preyer's reflex ears were used. The treatment groups were as follows: 16 for the cochlea SGN degeneration model and 24 for the cell transplantation group. In the cochlea SGN degeneration model group, the animals that were allowed to recover for 3 days were designated as the early post-injury (EPI) model ($n = 8$), whereas the animals allowed to recover for 7 days were designated as the late post-injury (LPI) model ($n = 8$). In the cell transplantation group, the animals that were allowed to recover for 3 days after the ouabain exposure prior to the transplantation were designated as the early post-injury (EPI) group ($n = 8$), whereas the animals allowed to recover for 7 days were designated as the late post-injury (LPI) group ($n = 8$); the same procedures for cell transplantation were executed in normal rats as a control ($n = 8$). In addition, 25 rats were used for RT-PCR assay and 5 rats were used from each set. Embryos from C57BL/6-GFP mice (E14.5) were used to initiate the olfactory NSC culture. The animals were provided by the Fourth Military Medical University. All procedures concerning animals in this study were approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

2.2. Procedures for the SGN degeneration model

The protocols were performed according to Schmiedt et al. [14]. Briefly, the animals were anesthetized using 2.5 ml/kg 2% pentobarbital sodium. A conventional retroauricular approach in the lateral recumbent position was used. To induce the SGN degeneration model, 10 μl of a 10 mM ouabain solution in normal saline was placed in the RW niche of the left otic bulla for 10 min by a plastic pipe using a 10 μl syringe (Shanghai GAOGE Industrial and Trading co., Ltd, Shanghai, China) with a microinjection system (TJ-1A/L0107-1A, longer pump, Longer Precision Pump Co, Hebei, China). After the injection, the incisions were closed with sutures. Meanwhile, the right otic bulla also underwent the same protocol but using normal saline instead of the ouabain as a control.

2.3. Isolation and culture of the olfactory NSCs

GFP+ NSCs were obtained from the olfactory bulbs of C57BL/6-GFP mice at embryonic day 14.5, considering the day on which the vaginal plug formed to be E0.5. The cells were isolated and cultured using a previously described procedure with minor modifications [19]. The cells were resuspended in medium and incubated at 37 °C in a 5% CO_2 atmosphere. In this study, cells of 20 passages were harvested at a cell density of $2–3 \times 10^6$ cells/ml for transplantation.

2.4. Procedures for the cells transplantation

Transplantation of the NSCs was performed at 3 days (EPI model) and 7 days (LPI model) after the ouabain treatment, respectively, and the same procedures were executed in normal rats as a control. The left cochleae were surgically exposed, 0.5 μl of the NSC suspension was infused into the cochlear lateral wall via a small hole made in the lateral osseous wall of the basal turn using a microdissecting gimlet (without invading the scala media) with the microinjection system, over a period of 5 min. All animals were sacrificed one week after cell transplantation.

2.5. Tissue preparation and immunological studies

For the tissue preparation, rats were anesthetized and perfused through the heart with normal saline followed by 4% paraformaldehyde. The cochleae were post-fixed in the same solution overnight. After decalcification in 10% ethylene diamine tetraacetic acid for 7 days followed by dehydration in 30% sucrose for 24 h, the cochleae were sectioned on a cryostat at a 10-μm thickness across the modiolus.

For immunofluorescence, the sections were blocked with 5% normal goat serum and incubated at 4 °C overnight with the primary antibodies, then, incubated with the appropriate secondary antibodies for 1 h. To stain the nucleus, the sections were incubated with Hoechst 33342 dye (Sigma Chemical Co., St. Louis, USA).

The primary antibodies used in this study included rabbit anti-SDF-1 (ab25117; Abcam, Hong Kong, China), rabbit anti-CXCR4 (ab2074; Abcam), mouse anti-neuron-specific beta-three-tubulin (ab78078; Abcam), and mouse anti-myelin basic protein (MBP, ab62631; Abcam).

2.6. Reverse transcription polymerase chain reaction (RT-PCR)

For RT-PCR, ouabain-treated rats after 12 h and 1, 3 and 7 days and control rats were decapitated under deep anesthesia. The SGs were carefully dissected out of the osseous central modiolus. Total RNA isolated from SG samples was collected with Trizol reagent (Invitrogen) following the manufacturer's protocol. cDNA was synthesized and used for PCR with a kit (SYBR Premix EX Taq, Takara, Kyoto, Japan). Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as the reference control. The total RNA of cultured NSCs was isolated using the same protocol. The primers used for RT-PCR are summarized in Table 1.

2.7. Statistical analysis

The differences were analyzed with the Student's t-test or one-way ANOVA followed by least significant difference (LSD) post hoc test. Values of $p < 0.05$ were considered statistically significant. The data were expressed as the means ± SD. The statistical analyses were performed using software (version 13.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Establishment of the SGN degeneration animal model

To establish the SGN degeneration animal model, we locally administered a 10 mM ouabain solution into the round window niche of rats (Fig. 1A and B). The animals were sacrificed, respectively, at 3 and 7 days after the ouabain treatment. HE-stained sections showed an empty space in the area normally occupied by the SGNs, with only a few SGNs surviving compared with the control (Fig. 1C–E), which received the same surgical approach but were treated with normal saline without ouabain. A similar result

Please cite this article in press as: P.-z. Zhang, et al., Up-regulation of stromal cell-derived factor-1 enhances migration of transplanted neural stem cells to injury region following degeneration of spiral ganglion neurons in the adult rat inner ear, Neurosci. Lett. (2012), http://dx.doi.org/10.1016/j.neulet.2012.11.018