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IL-1β promotes the migration of olfactory epithelium neural stem cells through activating matrix metalloproteinase expressions

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ABSTRACT

Background: To investigate the effects of IL-1β on the migration of olfactory epithelium neural stem cells (OENSCs), and to assess the mechanisms.

Methods: The effects of different concentrations of IL-1β on cell proliferation, apoptosis and migration were evaluated by cell counting assay, flow cytometry and transwell migration assay, respectively. Matrix metalloproteinase (MMP)-2 and MMP-9 expression in both protein and mRNA levels were detected. Small interfering RNA (siRNA) technique was employed to knockdown MMP-2 and MMP-9 expression. Additionally, c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) inhibitors were applied to assess the potential signaling pathways involved in the effects of IL-1β on cell migration.

Results: IL-1β promoted cell migration of OENSCs in a concentration-dependent manner at the concentration range of 0–80 ng/ml, but did not affect cell proliferation and apoptosis. Mechanically, IL-1β promoted MMP-2 and MMP-9 expressions. Knockdown of MMP-2 or MMP-9 could significantly reduce IL-1β-induced cell migration. IL-1β activated JNK, NF-κB, Extracellular Signal-Regulated Kinase (ERK) and p-65 phosphorylation. Finally, we evidenced that inhibition of JNK or NF-κB significantly inhibited cell migration.

Conclusion: Our study demonstrated that IL-1β promoted the migration of OENSCs through activating MMP expression. Moreover, JNK and NF-κB signaling pathways were involved in the regulation. This study provides important experimental evidence for the application of OENSCs in the transplantation therapy.

1. Introduction

Neural stem cells (NSCs) are a kind of undifferentiated cells with the ability of self-replication and multi-differentiation into neurons, glial cells and oligodendrocytes in a suitable environment. With the characteristics of unlimited proliferation and multi-directional differentiation, NSCs may be good donors for cell transplantation [1]. NSCs were initially found in the embryo tissue. Interestingly, recent studies also suggest that NSCs exist in the adult brain tissue and could be successfully cultured *in vitro*. Therefore, these cells have the proliferative potential and could differentiate to repair injured nerve upon induction [2,3].

The injured mammalian olfactory mucosa can repair itself and the olfactory receptor neurons are sustainable to self-update throughout the whole life cycle [4]. It was found that *in vitro* cultured olfactory epithelium neural stem cells (OENSCs) could repair the damaged hippocampal neurons when delivered to hippocampus or cerebrospinal fluid [5]. These results point toward the nerve repairing effect of OENSCs. Our previous study also found that OENSCs implantation into cochlea could repair the rat hearing function in noise-induced deafness [6]. Further study demonstate that OENSCs can migrate from the lateral wall of the cochlea to the ganglion to exert their function [6] through replacing the injured cells.

Cell migration is related to the hydrolysis of extracellular matrix and remodeling of cell structure [7]. Matrix metalloproteinase (MMPs) family is a kind of proteolytic enzyme dependent on zinc and calcium to activate [8]. MMPs degrade all protein components in extracellular matrix, destruct the tissue barrier and contribute to cell differentiation, migration and invasion. MMP-2 and MMP-9 were reported to correlate with tumor size, depth of invasion and lymph node metastasis [9], and the expression of MMP-9 in gastric cancer was positively correlated with the invasion and metastasis ability [10]. A large number of studies have also shown that a variety of MMPs were also expressed in stem cells. For example, MMP-2 and MMP-3 regulate the migration ability of

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human bone marrow mesenchymal stem cells (BMSCs) [11]. The expression of MMP-2 can promote BMSCs migration [11]. Meanwhile, it is found that cytokines/chemokines are involved in the regulation of cell migration by MMPs [12]. Inflammatory factors could induce the migration of BMSCs to injure or inflammation site to repair the tissue. After the addition of exogenous inflammatory factors, the activity of MMP-2 could be up-regulated to increase the migration ability of human BMSCs [13]. Research has also found that IL-1β could activate extracellular regulated protein kinases (ERK), P38-Mitogen Activated Protein Kinase (MAPK), c-Jun N-terminal kinase (JNK) and NF- κB signaling pathways to upregulate MMP-9 expression and enhance cell migration, whereas MMP-2/9 inhibitors reduced the cell migration [14–16]. Among all the MMPs, MMP-2 and MMP-9 are more closely related to cell migration.

Many studies show that cochlear injury leads to increase of IL-1β [17,6]. We previously found that implantation of OENSCs into cochlea of the rats with noise-induced deafness could repair the hearing function [6]. Therefore, cytokines, especially IL-1β likely takes part in the regulation of migration of OENSCs. This study aims to explore the potential pathways involved in IL-1β-induced cell migration of olfactory epithelium neural stem cells.

2. Materials and methods

2.1. Preparation and culture of olfactory epithelium neural stem cells

Sprague-dawley rats (100–200 g) were purchased from the Animal Center of Changhai Hospital of Second Military Medical University (Shanghai, China) and housed in a temperature-controlled room with a standard 12-h light/12-h dark cycle and ad libitum access to food and water. This study followed < The regulation of experimental animals > (2011). Fresh olfactory mucosa tissue was collected from nasal and nasal septum. Briefly, the adult rats were killed by cervical dislocation. Under aseptic conditions, bilateral turbinate and nasal septum were collected and washed in PBS for 3 times. Under the anatomical microscope, the olfactory mucosa was carefully peeled off by micro instruments, and then put into the PBS solution containing 2.4 U/mL neutral protease, and then digested at 37 ˚C for 45–50 min. The epithelial layer and lamina propria were separated under microscope, and the lamina propria was placed in PBS solution containing 0.25% I collagenase, and digested at 37 ˚C for 10 min. The intrinsic layer is digested and mixed with epithelium. After repeated pipetting, the solution was centrifuged and the supernatant was removed. The cells were resuspended in DMEM/F12 with 15% FBS and penicillin-streptomycin Solution (Penicillin: 100 U/ml, streptomycin: 0.1 mg/ml). The upper solution was taken out and cultured in non- poly-L-Lysine (PLL) coated culture-flask for 2-3 days. In the absence of PLL coating, the olfactory epithelial neural stem cells adhered to the wells rapidly. After one-week culture, the cells covered the bottom of the flask. Thereafter, the cells were digested and resuspended in penicillin and streptomycin containing DMEM/F12, supplying with 1% Insulin-Transferrin-Selenium, bFGF20 ng/ml, EGF40 ng/ml. The cell medium was changed every 2 days. One week later, the cells formed visible ball. After termination of digestion (0.25% pancreatin for 1 min), the proliferation ball was blown into a suspension state with 10% DMEM/F12. The nerve spheres were digested with 2.4U/ml neutral protease and 10 μg/ml DNase37 for 15 min, and then dispersed into cell suspension.

The isolated cells were identified by immunohistochemical staining with CK5 and Nestin antibody. Briefly, the medium was discarded and cells were fixed in 4% PFA. After several rounds of washing with PBS, the cells were incubated with cytokeratin 5 antibody (Novus, 1: 400) at 4 ˚C overnight. After washing with PBS, a second antibody conjugated with fluorescent labeling was applied for 2 h at room temperature. The images were taken under a microscope (Olympus, Japan)

The cells in logarithmic growth phase (3×10^3) were treated with different concentrations of IL-1β (0, 10, 20, 40 and 80 ng/ml) for 24 h. After the treatments, cell migration, apoptosis and proliferation were detected. In some of the experiments, MMP-2 or MMP-9 was silenced to study the mechanisms.

2.2. Cell transfection

When cell confluence reached about 50–70%, Lipofectamine™2000 was applied to assist the transfection of scrambled control (SC) and siRNAs. 6 h later, the medium was changed back into DMEM. MMP-2 siRNAs and MMP-9 siRNAs were designed by Shanghai GenePharma Co., Ltd (Shanghai, China). The sequences of siRNAs were listed as following:

MMP2-553 sense (5′-3′): GACCUUGACCAGAACACCATT,
MMP2-553 antisense (5′-3′):UGGUGUUCUGGUCAAGGUCTT;
MMP2-762 sense (5′-3′): ACGCUUUUCUCGAAUCCAUTT,
MMP2-762 antisense (5′-3′): AUGGAUUCGAGAAAAGCGUTT;
MMP2-2004 sense (5′-3′): CUGCCUUUAACUGGAGUAATT,
MMP2-2004 antisense (5′-3′): UUACUCCAGUUAAAGCAGTT;
MMP9-354 sense (5′-3′): CCAACUAUGACCAGGAUAATT,
MMP9-354 antisense (5′-3′):AUAGGUGAUGUUAUGAUGGTT;
MMP9-956 sense (5′-3′): CCAACUAUGACCAGGAUAATT,
MMP9-956 antisense (5′-3′): UUAUCCUGGUCAUAGUUGGTT,
MMP9-1595 sense (5′-3′): GCGCUCUGCAUUUCUUCAATT,
MMP9-1595 antisense (5′-3′): UUGAAGAAAUGCAGAGCGCTT.

2.3. Cell migration assay

The cells in logarithmic growth phase were cultured in serum-free medium for 24 h. The medium was discarded and the cells were digested by trypsin. The cells underwent washing, centrifuging, and resuspension in serum free DMEM/F12 culture medium. 100 μL (1×10^6/ml) suspended cells was added into each Transwell chamber. 600 μL DMEM/F12 containing 10% FBS was added into each well in a 24-well plate. The experiments were divided into five groups (control, 10 ng/ml, 20 ng/ml, 40 ng/ml and 80 ng/ml IL-1β). The prepared transwell chambers were hanged in the 24-well plate. The transwell chamber was completely contacted with the well without a bubble. The culture plate was incubated for 20 h at 37 ˚C and 5% CO_2.

The cells in down chamber were fixed in paraformaldehyde and stained with crystal violet. Four fields in upper and lower, left and right positions were selected in the counting. The experiment was repeated for 3 times and mean value was calculated.

2.4. Flow cytometry

After treatments, the cells were collected for Annexin V/PI staining (Beyotime, Ningbo, China) and the apoptosis was detected within 1 h by FACSCalibur (BD, Franklin Lakes, New Jersey, USA). Based upon Annexin V and PI staining, the upper left quadrant was damaged cells, the upper right quadrant was late apoptosis or necrotic cells, the lower left quadrant was negative normal cells, and the right lower right was the early apoptotic cells. The sum of the two quadrants of the right upper and lower quadrants was used as the apoptosis rate.

2.5. CCK-8 assay

The cells were seeded in 96-well plates. After treatments, cell counting kit-8 (CCK-8) assay was applied to detect the cell proliferation as previously described (15). The optical density (OD) was determined by Microplate Reader (Bio-Tek, USA) at 570 nm. OD value represented the cell viability.

2.6. Real time − polymerase chain reaction

The cells in logarithmic growth phase were cultured in serum-free medium for 24 h. After treatments, total RNA was extracted according