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The mechanically activated p38/MMP-2 signaling pathway promotes bone marrow mesenchymal stem cell migration in rats

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ABSTRACT

Objective: The aim of the present study was to investigate the effect of static strain on bone marrow mesenchymal stem cell (BMMSC) migration and whether the p38/matrix metalloproteinase-2 (MMP-2) axis plays a role in induction of BMMSC migration under mechanical strain.
Design: Both *in vivo* and *in vitro* investigations were performed. Twelve adult male Sprague-Dawley rats were randomly divided into 2 groups (n = 6 per group). Rats in the experimental group underwent right mandibular distraction osteogenesis, whereas rats in the control group were subjected to osteotomy in the mandible without distraction. Immunohistochemistry and immunofluorescence were performed to evaluate phospho-p38 (p-p38) and Nestin expression. BMMSCs were isolated from rat mandibles. BMMSCs in the experimental group were subjected to static mechanical strain for 2 h, whereas those in the control group underwent no strain. The biological roles of static strain and the p38/MMP-2 axis in BMMSC migration were evaluated by Transwell assays and western blotting by inhibiting p38 phosphorylation.
Results: There were significantly more $Nestin^+$ cells in the bone calluses of the experimental group than in those of the control group. In addition, $Nestin^+/p\text{-}p38^+$ cell numbers were significantly higher in the experimental group than in the control group, indicating that static strain activated p38 signaling in BMMSCs *in vivo*. In accordance with *in vivo* results, static strain *in vitro* stimulated phosphorylation of p38 in BMMSCs. Furthermore, expression of MMP-2 was elevated in BMMSCs under static strain compared with the control, and strain-induced MMP-2 expression was abolished by inhibition of p38 phosphorylation in BMMSCs. Moreover, Transwell assay results showed that static strain promoted BMMSC migration, which was abolished by inhibition of p38 phosphorylation.
Conclusions: The present study demonstrated that static strain can promote the migration ability of BMMSCs via p38/MMP-2 signaling. To the best of our knowledge, this study is the first report demonstrating that the p38/MMP-2 axis governs BMMSC migration under static mechanical strain.

1. Introduction

Biomechanical stimuli are critical factors in development and maintenance of the skeleton as well as the cell phenotype and function (Goodman & Aspenberg, 1993; Luu et al., 2009). Accumulated evidence suggest that the stem cell niche in bone marrow provides a significant pool of mesenchymal stem cells (MSCs) (Kfoury & Scadden, 2015; Mendez-Ferrer et al., 2010). The biology of bone marrow mesenchymal stem cells (BMMSCs) is inevitably affected by mechanical strain loaded on the bone, particularly in the treatment of distraction osteogenesis (DO) and orthodontics, which make use of static strain for bone

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regeneration. It has been documented that static strain exerts significant regulatory control over the fate of BMMSCs. (Engler, Sen, Sweeney, & Discher, 2006). In addition, static strain had been demonstrated to promote BMMSC proliferation *in vitro* (Kim, Song, & Hwang, 2010).

The migration of BMMSCs is one of the most critical processes during BMMSC responses to mechanical strain. As pericytes of a perivascular niche, BMMSCs must escape the stem cell niche and migrate to the bone forming areas for bone regeneration and repair (Du et al., 2014). This process involves secretion of matrix metalloproteinases (MMPs) by BMMSCs to degrade the surrounding extracellular matrix (ECM) (Kasper et al., 2007; Mott & Werb, 2004). Matrix metalloproteinase-2 (MMP-2) is one of the critical MMPs mediating BMMSC migration (Karadag & Fisher, 2006). However, to date, little is known about the mechanotransduction governing BMMSC migration under static mechanical strain.

p38 is a member of the mitogen-activated protein kinase superfamily, which is composed of 3 main members providing regulatory control of diverse cellular activities such as cell differentiation and apoptosis (Chang & Karin, 2001; Sui et al., 2014). It has been well documented that p38 is capable of regulating cellular responses to mechanical signals (Rosenzweig, Quinn, & Haglund, 2014; Wu et al., 2014). Recent studies demonstrated that p38 is also involved in regulation of migration ability in various cells including cancer cells and smooth muscle cells (Liu et al., 2015; Rousseau et al., 2006). It has been reported that activated p38 induces MMP-2 and MMP-9 expression to degrade the ECM and facilitate cell motility (Huang et al., 2014).

This study aimed to explore whether p38-mediated increases in level of MMP-2 regulated the migration ability of BMMSCs under static strain. To test our hypothesis, a rat mandibular DO model was established according to our previous studies, and static strain was applied for both *in vivo* and *in vitro* assays. We found that the p38/MMP-2 axis may play a significant role in regulation of BMMSC migration ability.

2. Materials and methods

2.1. Animals and surgical procedure

The animal experimental protocol was approved by the Committee on Use of Live Animals for Teaching and Research of the Fourth Military Medical University. Twelve adult male Sprague-Dawley rats (280–300 g) were randomly divided into 2 groups. Rats in the experimental group (n = 6) underwent right mandibular DO as described in our previous studies (Wang et al., 2012). Briefly, rats were anaesthetized with 1% pentobarbital sodium (30 mg/kg) injected intraperitoneally. A vertical osteotomy was created in the retromolar area using a diamond disk. Afterwards, a custom-made distraction device (Zhongbang Titanium Biomaterials Co., Xi'an, China) was placed and fixed with 2 screws on each side. Following a latency period of 5 d, the rat mandible was gradually distracted for 10 d at a rate of 0.2 mm/12 h. Rats in the control group (n = 6) were subjected to the same surgical procedure as the experimental group, but no distraction was performed. Rats in both groups were killed on day 15, and mandible samples were harvested for laboratory analysis.

2.2. Isolation of rat BMMSCs

The rat mandibles were harvested, and attached teeth and soft tissues were separated carefully. The mandibles were cut into small pieces, and individual cells were obtained through digestion using 3 mg/mL collagenase type I (Worthington, Lakewood, NJ, U.S.) and 4 mg/mL Dispase (Roche, Indianapolis, IN, U.S.) for 60 min at 37 °C. A single-cell suspension was obtained by passing cells through a 70-μm cell strainer (BD Bioscience, San Jose, CA, U.S.). All of the single cells obtained were seeded onto 100-mm culture dishes (Corning Inc., Corning, NY, U.S.), and incubated at 37 °C and 5% CO_2 in alpha minimum essential medium (α-MEM, Hyclone Laboratories Inc., Logan, UT, U.S.) supplemented with 10% FBS (Hyclone Laboratories Inc., Logan, UT, U.S.) and antibiotics (100 units/mL penicillin G and 100 g/mL streptomycin, Hyclone Laboratories Inc., Logan, UT, U.S.). After 48 h, the medium was replaced to remove the floating cells. Passages were performed every 3 d when cells approached confluence.

2.3. Mechanical strain application

A total of 3×10^5 to 5×10^5 BMMSCs were seeded onto collagen I-coated silicone membrane plates (Flexcell international, Hillsborough, NC, U.S.) and cultured for 72 h before mechanical strain. Cells in the experimental group underwent static strain (6%, 2 h). In contrast, no mechanical strain was applied in the control group. After 2 h, all cells were harvested for subsequent experiments.

2.4. Immunohistochemistry and immunofluorescence analysis

The right mandibles and the bone callus specimens from the osteotomy zone were harvested and fixed with 4% paraformaldehyde at 4 °C for 48 h. The specimens were decalcified for 4 weeks, embedded in paraffin, and then sliced longitudinally into 3 μm sections for immunohistochemistry and immunofluorescence analysis. The protocols performed were described in our previous study (Du et al., 2014). For immunohistochemistry, the primary antibody was anti-Nestin (1:2000, Abcam, Cambridge, UK). Brown particles in the cytoplasm were considered indicative of positive staining. Images were photographed in 5 randomly selected high magnification fields (400×) per slide under a microscope (Olympus Corporation, Tokyo, Japan). The numbers of $Nestin^+$ cells were counted manually. All of the experiments were performed in triplicate.

For immunofluorescence, the tissue sections were incubated with anti-Nestin (1:100, Abcam, Cambridge, UK) and anti-phospho-p38 (p-p38) (1:800, Cell Signaling Technology, Danvers, MA, U.S.) primary antibodies. Secondary antibodies were as follows: Cy3, goat anti-mouse IgG (1:500, Abbkine, CA, U.S.), which specifically binds to the anti-Nestin primary antibody, and Dylight 488, goat anti-rabbit IgG (1:800, Abbkine, CA, U.S.), which specifically binds to the anti-p-p38 primary antibody. Nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI, Fanbo, Beijing, China). Integrated optical density of the positive cells was blindly assessed with Image-Pro Plus analysis software (Media Cybernetics, Inc., Rockville, MD, U.S.). All of the experiments were performed in triplicate.

2.5. Western blot analysis

Total cell protein was separated by 10% SDS-PAGE (Pall Corporation, NY, U.S.) and transferred to ImmobilonTM-P membranes (EMD Millipore, Billerica, MA, U.S.). Afterwards, membranes were blocked with 5% nonfat milk for 1 h and then immunoblotted with anti-p-p38 (1:1000, Affinity Biosciences, Cincinnati, OH, U.S.), anti-p38 (1:1000, Affinity Biosciences, Cincinnati, OH, U.S.), anti-MMP-2 (1:1000, Affinity Biosciences, Cincinnati, OH, U.S.), and anti-rat β-tubulin (Proteintech Group, Inc., Rosemont, IL, U.S.). Protein bands were visualized with a chemiluminescence reagent (Thermo, Rockford, IL, U.S.).

2.6. Migration and invasion assay

BMMSCs were subjected to mechanical strain for 2 h with or without SB203580 pretreatment (20 μM, Selleck, Houston, TX, U.