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Polydatin induces bone marrow stromal cells migration by activation of ERK1/2

ZhenQiu Chen[a,b,1], QiuShi Wei[a,b,1], GuoJu Hong[b], Da Chen[b], Jiang Liang[a], Wei He[a,b,*], Mei Hui Chen[b,c,*]

[a] Department of Orthopaedics, The First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Baiyun District, Guangzhou, Guangdong 510405, China
[b] Key Laboratory of Orthopaedics & Traumatology, Guangzhou University of Chinese Medicine, Guangzhou 510405, China
[c] Guangdong Provincial Acadamy of Chinese Medical Sciences, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China

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ABSTRACT

Bone marrow stromal cells (BMSCs) have proven to be useful for the treatment of numerous human diseases. However, the reparative ability of BMSCs is limited by their poor migration. Polydatin, widely used in traditional Chinese remedies, has proven to exert protective effects to BMSCs. However, little is known about its role in BMSCs migration. In this study, we studied the effects of polydatin on rat BMSCs migration using the scratch wound healing and transwell migration assays. Our results showed polydatin could promote BMSCs migration. Further experiments showed activation of ERK 1/2, but not JNK, was required for polydatin-induced BMSCs migration, suggesting that polydatin may promote BMSCs migration via the ERK 1/2 signaling pathways. Taken together, our results indicate that polydatin might be beneficial for stem cell replacement therapy by improving BMSCs migration.

1. Introduction

Bone marrow stromal cells (BMSCs), with the capacity for unlimited self-amplification and for terminal differentiation into cell types with specific functions, are most commonly used for cell replacement therapy for various diseases, including spinal cord injury (SCI) and osteonecrosis of the femoral head (ONFH) [1–4]. Successful BMSCs transplantation requires that the stem cells cross the endothelium and migrate to the target tissues and organs, a process called homing [5]. However, only a small proportion of the transplanted BMSCs reach the target tissues and organs during stem cell therapy in vivo, thus significantly hampering the clinical application of BMSCs [6,7]. Therefore, it is urgently needed to develop an improved approach to increase BMSCs migration and homing in order to enhance the therapeutic benefit [8,9].

Polydatin, widely used in traditional Chinese remedies, has been shown to exert multiple pharmacological actions, such as antioxidation, antiinflammation, immunoregulation, antitumor, and neuroprotection [10,11]. Previously, we have reported that polydatin could protect BMSCs from oxidative stress, thus improving the survival of BMSCs after transplatation [11]. However, little is known about its role in BMSCs migration, another important factor which affects their reparative capacity.

It has been reported that Extracellular signal-regulated kinase1/2 (ERK1/2) [12,13] and c-JUN NH2-terminal protein kinase (JNK) [13] are involved in the enhancement of migration of BMSCs. In this study, we demonstrated for the first time that polydatin might promote BMSCs migration via activation of ERK 1/2, but not JNK, suggesting that polydatin could be a promising approach to increase the cell migration in stem cell replacement therapy.

2. Meterials and methods

2.1. Materials

4-week-old male specific pathogen-free Sprague–Dawley (SD) rats (100 ± 20 g) were supplied by the Center of Experimental Animals, Guangzhou University of Chinese Medicine (Guangzhou,

* Corresponding authors at: Key Laboratory of Orthopaedics & Traumatology, Guangzhou University of Chinese Medicine, Guangzhou 510405, China.
E-mail addresses: hw13802516062@126.com (W. He), chenmeihuionly@163.com (M.H. Chen).
[1] These authors contributed equally to this study.

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China, Certificate No. 00100561). All procedures were performed according to animal guidelines of Guangzhou University of Chinese Medicine. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (MO, USA). Trypsin and Low-glucose Dulbecco's modified Eagle's medium (LG-DMEM) were obtained from Gibco-BRL (NY, USA). Fetal bovine serum (FBS) was obtained in Hyclone (Logan, UT). The JNK-specific inhibitor SP600125 and the extracellular signal-regulated kinase (ERK)-specific inhibitor SL327 were purchased from Santa Cruz (TX, USA). Polydatin (Aladdin) was dissolved in DMSO before dilution with the culture medium.The final concentration of DMSO was 0.1%.

2.2. Cell culture and treatment

BMSCs was obtained as previously described [11,14]. Briefly, bone marrow was flushed out with a 5–10 mL syringe using LG-DMEM. The marrow washouts were collected, centrifuged and cultured in LG-DMEM supplemented with 10% FBS in a standard incubator under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cells from 3 to 5 passages were used for the experiments.

2.3. MTT assay

An MTT assay was performed to evaluate the effect of polydatin on the proliferation of BMSCs. BMSCs were plated on 96-well plates at a density of 1×10^4 cells per well. When cells grew to 80% confluence, the cells were serum starved for 12 h. Cells were then treated with polydatin (10, 30, 100 μM) for 24 h 10 μL MTT (5 mg/mL) was then added to each well and the mixture was incubated for 3 h at 37 °C. MTT reagent was then replaced with DMSO (100 μL per well) to dissolve formazan crystals. After the mixture was shaken at 37 °C for 30 min, absorbance was determined at 570 nm. Results were expressed as the percentage of MTT reduction and the absorbance of control cells was set as 100%

2.4. Scratch wound healing assay

The scratch wound healing assay was used to determine cell migration ability. BMSCs at passage 3 were seeded in 6-well plates at a density of 1×10^6 cells per well. When cells grew to 90% confluence, the medium was aspirated away, and cells were serum starved for 12 h. A scratch was created by using a pipette tip on a uniform layer of cells. The wounds were photographed at 0, 24 h at the same area after wounding. The wound widths of different areas at different time points were measured with Image J software.

2.5. Transwell migration assay

Cell migration was assessed using a Transwell Boyden Chamber (pore diameter: 8 μm. Millipore, Billerica, MA) as previously reported [13]. BMSCs at passage 3 were serum starved for 12 h, and resuspended in 150 μL of medium with 1% FBS and adjusted to a density of 3×10^4 cells per well. The cells were then added into the upper compartment of the chamber. 700 μL of serum-free LG-DMEM, with or without polydatin, was added to the lower chamber. The cells were allowed to migrate at 37 °C in an atmosphere of 5% CO2 and 95% air for 24 h. A cotton-tipped swab was used to remove all the cells on the upper side of the filters, and the cells that migrated through the pores were strained with 0.05% crystal violet (Beyotime, Haimen, China) for 40 min. The BMSCs migrated to the underside of the filters were counted under a

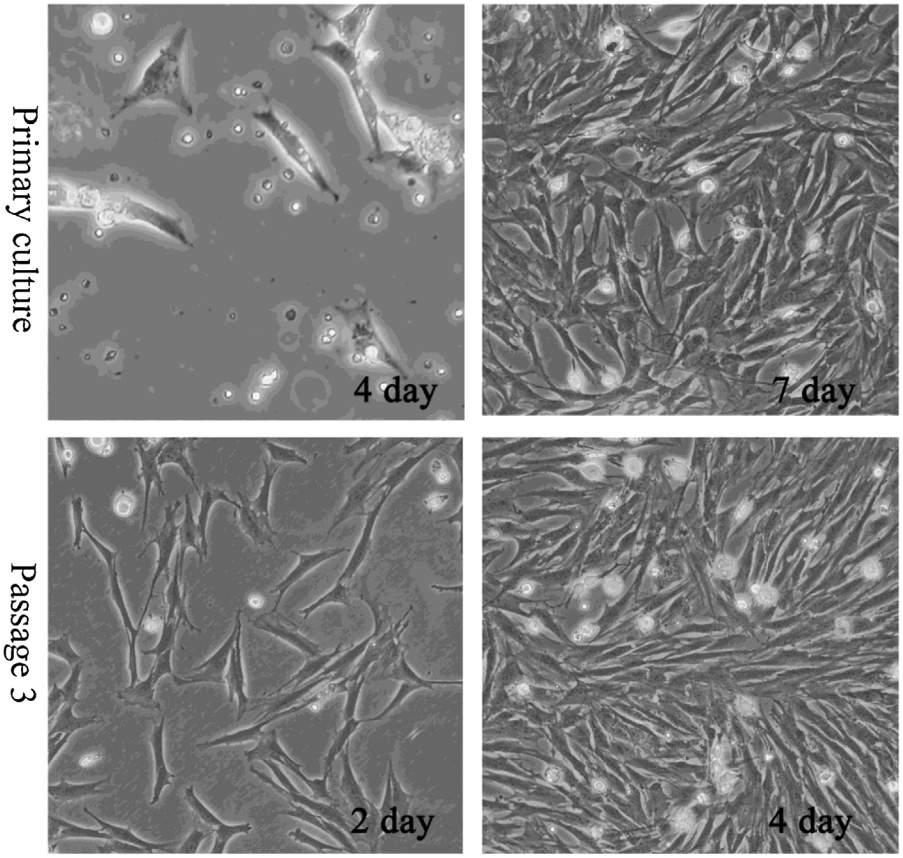

Fig. 1. Representative fields of BMSCs morphologies.