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S1P/S1PR3 signaling mediated proliferation of pericytes via Ras/pERK pathway and CAY10444 had beneficial effects on spinal cord injury

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ABSTRACT

Pericytes have long been regarded merely to maintain structural and functional integrity of blood-brain barrier (BBB). Nevertheless, it has also been identified as a component of scar-forming stromal cells after spinal cord injury (SCI). In process of enlargement of spinal cavity after SCI, the number of pericytes increased and outnumbered astrocytes. However, the mechanism of proliferation of pericytes remains unclear. Sphingosine-1-phosphate (S1P) has been reported to play important roles in the formation of glia scar, but previous studies had paid more attention to the astrocytes. The present study aimed to observe the effects of S1P and S1P receptors (S1PRs) on proliferation of pericytes and investigate the underlying mechanism. By double immunostaining, we found that the number of PDGFRβ-positive pericytes was gradually increased and sealed the cavity, which surrounded by reactive astrocytes. Moreover, the subtype of S1PR3 was found to be induced by SCI and mainly expressed on pericytes. Further, by use of CAY10444, an inhibitor of S1PR3, we showed that S1P/S1PR3 mediated the proliferation of pericytes through Ras/pERK pathway. Moreover, CAY10444 was found to have the effects of enhancing neuronal survival, alleviating glial scar formation, and improving locomotion recovery after SCI. The results suggested that S1P/S1PR3 might be a promising target for clinical therapy for SCI.

1. Introduction

Most previous studies involving scar tissue after spinal cord injury (SCI) have mainly focused on astrocytes, which had been regarded as the major source of the glial scar after injury [1]. Indeed, apart from ensuring proper blood-brain barrier (BBB) structure and function [2,3], pericytes has also been identified to give rise to scar-forming stromal cells and outnumber astrocytes [4], implying important roles in pathological mechanism of SCI. The number of pericytes was gradually increased during the process of gilal scar formation. However, the mechanism of proliferation of pericytes after SCI remains unclear. A better understanding of this mechanism will provide a new perspective to treat SCI.

Sphingosine-1-phosphate (S1P), a bioactive phospholipid, mediates numerous cell-intrinsic and extrinsic effects through activation of its cell-surface S1P receptors (S1PRs) [5,6]. As G-protein-coupled receptors, S1PRs are composed of five subtypes (S1PR1-S1PR5), and different receptors are expressed by diverse cells to play important roles in cell survival, proliferation and differentiation [7,8]. It was reported that four out of the five S1PRs (S1PR1, S1PR2, S1PR3, S1PR5) can be expressed on neurons, astrocytes, oligodendrocytes and microglia in central nervous systems [9,10]. However, whether S1PRs were involved in proliferation of pericytes after SCI and the underlying mechanism has never been elucidated.

We therefore explored the effect of S1PRs on proliferation of pericytes and its mechanism during cavity enlargement after SCI. We found that S1P/S1PR3 mediated proliferation of pericytes via Ras/pERK pathway. Moreover, CAY10444 was found to have the effects of enhancing neuronal survival, alleviating glial scar formation, and improving locomotion recovery after SCI. The results suggested that the strategy of modulating S1P/S1PR3 signaling can be translated into clinical therapy for SCI.

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2. Materials and methods

2.1. Animal model of SCI

Sprague-Dawley rats were anesthetized with 1% sodium pentobarbital (60 mg/kg), and laminectomy was made to expose the spinal cord. The crush model of SCI was made by our modified mechanical device as described [11]. The width of forceps tips is 0.5 mm, and the duration of the injury lasts for 20 s. The bladder was emptied twice daily by manual expression after injury until reestablishment of micturition reflex. For proliferative detection, intraperitoneal injections of bromodeoxyuridine (BrdU, 100 mg/kg, Sigma) were given once daily for 14 days after SCI. All animal experiments were carried out in accordance with the recommendations of 'Animal Care and Use, Committee of Xi'an Jiaotong University'.

2.2. Immunohistochemistry

At different times after SCI, rats were sacrificed and cryostat sections of the spinal cord were cut at a thickness of 12 and prepared for immunostaining.

After blocking nonspecific binding by incubating in 3% BSA for 10 min, sections were incubated overnight at 4 °C with primary antibodies. The primary antibodies used in this study were goat anti-PDGFRβ (1:500, Millipore), rabbit anti-GFAP (1:1000, DAKO), rabbit anti-Iba-1(1:1000, WAKO), mouse anti-S1P (1:200, Lpath), rabbit anti-S1P1 (1:200, Cayman Chemical), mouse anti- S1P3 (1:300, Sigma), rabbit anti-BrdU (1:400, Abcam). After washing three times with PBS, sections were incubated with secondary antibodies conjugated with Alexa Fluor 594 or Alexa Fluor 488 (1:1000, Jackson ImmunoResearch) in a dark environment for at room temperature (RT) 4 h. Nuclei were counterstained with Hoechst 33342 (1:5000, Molecular Probes). Sections were then photographed under a confocal laser scanning microscope (FV1000, Olympus).

2.3. Nissl staining

Neuronal survival was performed by Nissl staining. Briefly, the sections were immersed in 1% thionine solution at 50 °C for 40 min, followed by differentiation with 70% alcohol for about 3 min.

2.4. S1P concentration

S1P concentration was measured as described previously

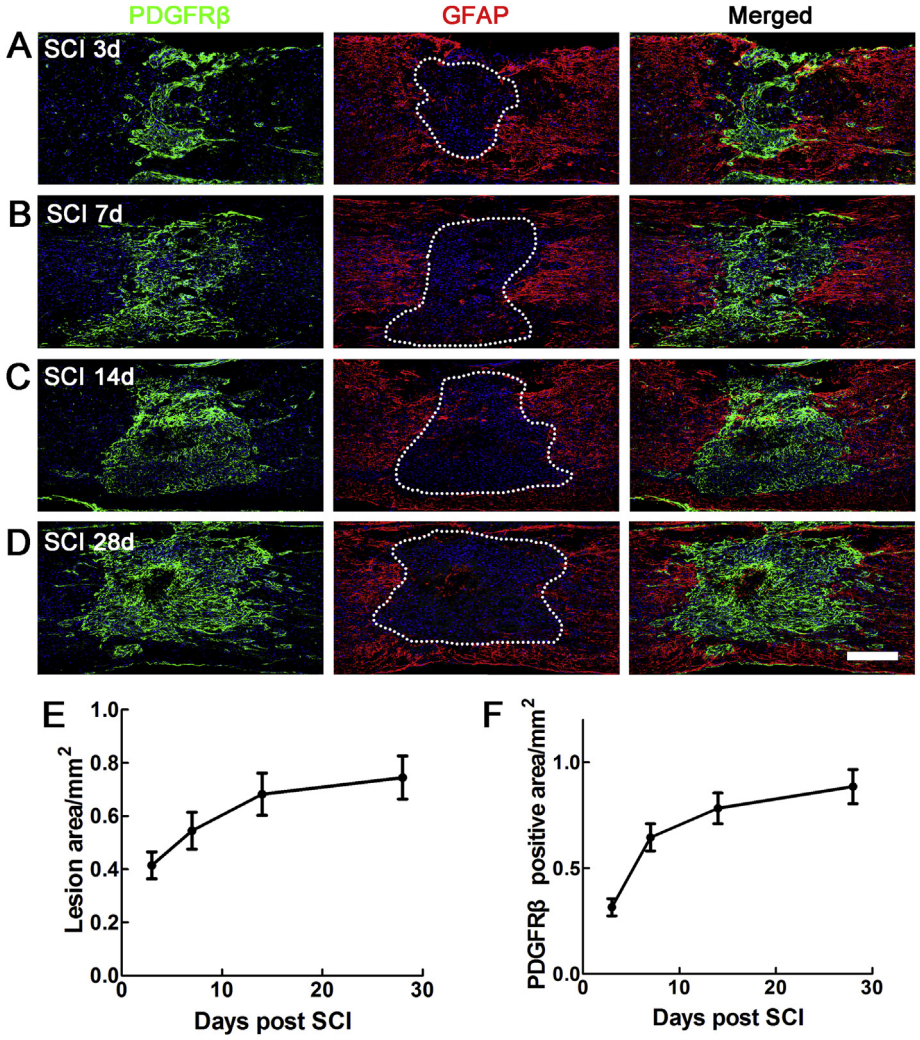

Fig. 1. The numbers of pericytes increased during enlargement of spinal cavity. Double staining of PDGFRβ and GFAP at 3 (A), 7 (B), 14 (C), 28 days (D) after SCI. (E–F) Quantification of the size of spinal cavity and the numbers of PDGFRβ-positive pericytes at different time points after SCI (n = 6). Scale bar = 200 μm.