

Expression and significance of SDF-1 and its receptor CXCR4 in the retina of pregnant rats after optic nerve injury

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Abstract

Stromal cell–derived factor 1 (SDF-1) and its receptor CXCR4 have shown to play a role in embryonic development process, regulation of hematopoiesis, mediating immunology response, inflammatory reaction, and metastasis of malignant tumor. Recently, SDF-1 and CXCR4 are also closely related to retinal neovascularization. This study was to investigate the expression of SDF-1 and CXCR4 in the retina after optic nerve injury in pregnant rats so as to reveal its significance. A total of 12 pregnant rats were randomly divided into normal group and experimental group (after 5 days of optic nerve injury), six rats in each group; expressions of SDF-1 as well as CXCR4 in rat retina were detected by immunofluorescence staining and western blot assay. The result of immunofluorescence staining showed that the relative gray scale values of SDF-1 and CXCR4 in the experimental group were significantly higher than those in the normal group ($P < 0.05$), and the result of Western blot assay showed that the expression levels of SDF-1 and CXCR4 in the experimental group were significantly higher than those in the normal group ($P < 0.05$). In conclusion, SDF-1 and its receptor CXCR4 have abnormal expression in the retina of pregnant rats after optic nerve injury, which may be involved in the occurrence and development of optic nerve injury.

Keywords

CXCR4, optic nerve injury, pregnant rats, retina, SDF-1

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Introduction

Many ocular diseases, such as glaucoma, some retinopathy, and other final pathological processes such as optic nerve injury, ocular trauma, and optic neuritis directly damage the optic nerve, eventually leading to blindness or serious visual impairment.¹ The death of retinal ganglion cells after optic nerve injury and irreversible visual impairment caused by reduced visual functional units are important factors leading to blindness, and there is no effective treatment at present.²

After optic nerve injury, nerve structure and peripheral microenvironment are destroyed, cell axons are severed, and edema and death occur. At the same time, there are some unfavorable factors,

such as free radical formation, retinal ganglion cell superoxide increase, caspase-2 activation, and c-Jun N-terminal kinase (JNK) signal pathway activation.³ After optic nerve injury, due to its

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inherent repairing ability, there are some favorable factors for optic nerve repair, such as upregulation of self-phagocytosis to protect neuron survival, significant expression of ciliary neurotrophic factor in astrocytes, and upregulation of thymosin beta-4, which all enhance the survival and promotion of retinal ganglion cells.⁴ In addition, many unknown factors remain to be found. Stromal cell-derived factor 1 (SDF-1) is a member of α -chemoattractant family and CXCR4 is its only specific receptor widely expressed in various tissues and organs, and SDF-1 plays biological role by combining with CXCR4.⁵ A recent study has found that except for the role in embryonic development process, regulation of hematopoiesis mediates immunology response, inflammatory reaction, and the invasion as well as metastasis of malignant tumor, SDF-1, and its receptor CXCR4 is also closely related to retinal neovascularization.⁶

Pregnancy can cause changes in function of healthy eyes and also affect previously existing retinopathy such as diabetic retinopathy. Pregnancy gives rise to changes in hormones, metabolism, blood rheology, cardiovascular and immune functions in pregnant women, all of which can make damages to the retina.⁷ At present, there is no information about the expression of SDF-1 and CXCR4 in the retina of pregnant rat after optic nerve injury. A pregnant rat model of partial optic nerve injury was established in order to understand the expression of SDF-1 and CXCR4 in pregnant retina after optic nerve injury and to lay a foundation for the study of the role of SDF-1 and CXCR4 in the regeneration and repair of optic nerve injury.

Materials and methods

Animals and grouping

A total of 12 pregnant SD rats were purchased from Nanjing Junke Biological Engineering Co., Ltd., and they were fed enough food and water at 8:00–20:00 with light exposure environment and at the room temperature of 18°C–22°C. They were randomly divided into the normal group and the experimental group (after 5 days of optic nerve injury), six rats in each group.

Reagents and instruments

Reagents and instruments used are medium noninvasive vascular clamp (Beijing Rui three minimally invasive medical technology Co Ltd), ophthalmic

operating microscope (Shanghai Hanfei Medical Instrument Co., Ltd), fluorescence microscopy (CAI Kang Shanghai optical instrument factory), laser scanning confocal microscope (Shanghai Optical Instrument No. 1 Factory), western blot kit (Shanghai Junrui Biotechnology Co., Ltd.), rabbit anti-SDF-1 (Jiangsu, Prosser Biotechnology Co., Ltd.), rabbit anti-CXCR4 (Beijing International Ruida Technology Co., Ltd), and goat anti-GFAP antibody (Shenzhen Xin Bo Sheng Biotechnology Co., Ltd.).

Establishment of optic nerve injury model

The rats during pregnancy were injected 10% chloral hydrate (0.5 mL/100 g) for anesthesia and fixed on the operating table under lateral position; local disinfection was given to the operation eyes in which forniceal conjunctiva was cut open to the angle of about 120°; the rats were given blunt dissection away from vortex vein to expose optic nerve followed by avoidance of blood vessels 2 mm back away from the ball; the medium noninvasive vascular clamp was used to clip optic nerve for 10 s, and dilated pupil was seen in the operation eyes with no ischemia in the eye ground followed by suturing of bulbar conjunctiva and antibiotic oculentum coating.

Immunofluorescence staining

The right eye was randomly taken from three rats in both normal group and experimental group (after 5 days of optic nerve injury), and after anesthesia by intraperitoneal injection and 4% paraformaldehyde perfusion through the heart for fixation, the right eyeball was removed and cornea as well as refractive system of eye was cut followed by 4% paraformaldehyde fixation for 2 h, dehydration of gradient sucrose, embedding, and preservation at freezing compartment of refrigerator at 20 below zero and the specimen was given frozen section with the thickness of 10 μ m and pasted with anti-falling slide followed by restoration at refrigerator under –80°C. Section of optic plane was selected, and co-expression of SDF-1 as well as GFAP, CXCR4 immunofluorescence staining, phosphate-buffered saline (PBS) rinsing, and 10 min 0.1% Triton X-100 permeation were carried out; after being sealed, it was incubated with the working liquid of primary antibody containing goat anti-GFAP (1:200), rabbit anti-SDF-1 (1:100), and rabbit

anti-CXCR4 (1:100), at 4°C for the overnight; then it was incubated with donkey anti-goat Cy3 (1:800), donkey anti-rabbit fluorescein isothiocyanate (FITC; 1:100), goat anti-rabbit FITC (1:100) for 2 h at room temperature followed by 4',6-diamidino-2-phenylindole (DAPI) incubation for 5 min at room temperature after and mounting with the mounting medium of anti-fluorescent quenching with PBS rinsing conducted prior to each step. Expression of SDF-1, GFAP, and CXCR4 was given localization examination by confocal laser scanning and the relative gray values of SDF-1 and GFAP fluorescence intensity were semi quantitatively measured by ImageJ software.

Western blot

The right eye was randomly taken from another 3 rats in both normal group and experimental group (after 5 days of optic nerve injury) after the anesthesia by intraperitoneal injection, the left eyeball was removed rapidly followed by removal of and the entire retina under a microscope. The removed retina was quickly placed in a numbered centrifuge tube, sealed and preserved at -80°C in refrigerator, all these operations were carried out on ice. The expression of SDF-1, GFAP, and CXCR4 in retina of rats with partial optic nerve injury was determined by western blot assay, and retinal tissue was collected to extract cellular total protein, which was then quantified and denaturated followed by electrophoresis and transmembrane, and after being closed, it was incubated with corresponding primary antibody at 4°C for the overnight (rabbit anti-SDF-1 1:200, goat anti-GFAP, 1:500, rabbit anti-CXCR4, 1:200, GAPDH, 1:500). After the end of incubation, it was given full phosphate-buffered saline with Tween® 20 (PBST) washing and then incubated with corresponding second antibody at room temperature for 2 h followed by film developing. The band was assessed by Quantity One image software to analyze optical density value with the ratio of optical density value of a detected protein brand to that of GAPDH band of the same lane as the result.

Statistical processing

SPSS 221 statistical software was used to analyze the data with the application of t-test for assessment; $P < 0.05$ suggested there was difference of statistical significance.

Results

Location expression of SDF-1 and CXCR4 in the retina of pregnant rats after partial optic nerve damage

A low level of GFAP expression was seen in normal retinal nerve fiber layer and outer plexiform layer in the normal group, while in the experimental group, the expression level of GFAP gradually increased with prolongation of the time after the injury, and there was also visible line-shaped expression in inner nuclear layer and inner plexiform layer and outer nuclear layer.

The expression level of retinal SDF-1 in the normal group was low and located at nerve fiber layer and outer plexiform layer; while in the experimental group, it both increased and enhanced with several positive line-shaped expression seen in inner nuclear layer and outer nuclear layer, and the SDF-1 expression was also seen in inner nuclear layer and external limiting membranes.

In the normal group, the retinal GFAP/SDF-1 double-labeled cell was mainly found in the nerve fiber layer and outer plexiform layer; in the experimental group, it had significantly increasing cell number with small amounts of positive line-shaped expression seen in inner nuclear layer and outer nuclear layer.

In the normal group, the expression of retinal CXCR4 was mainly found in ganglion cell layer, outer nuclear layer, as well as inner nuclear layer and mainly distributed in the cell membrane as well as inside the cytoplasm; while in the experimental group, there was no significant changes in CXCR4 expression location and level as shown in Figures 1 and 2.

Changes in expression of SDF-1 and CXCR4 in the retina of rat after partial optic nerve damage

The semi-quantitative measurement of SDF-1 and GFAP fluorescence intensity by ImageJ software showed that the relative gray value of SDF-1 immunofluorescence in the experimental group was significantly higher than that in the normal group ($P < 0.05$; as shown in Figure 3(a)) and that the relative gray value of CXCR4 immunofluorescence in the experimental group was significantly higher than that in the normal group ($P < 0.05$; as shown in Figure 3(b)).

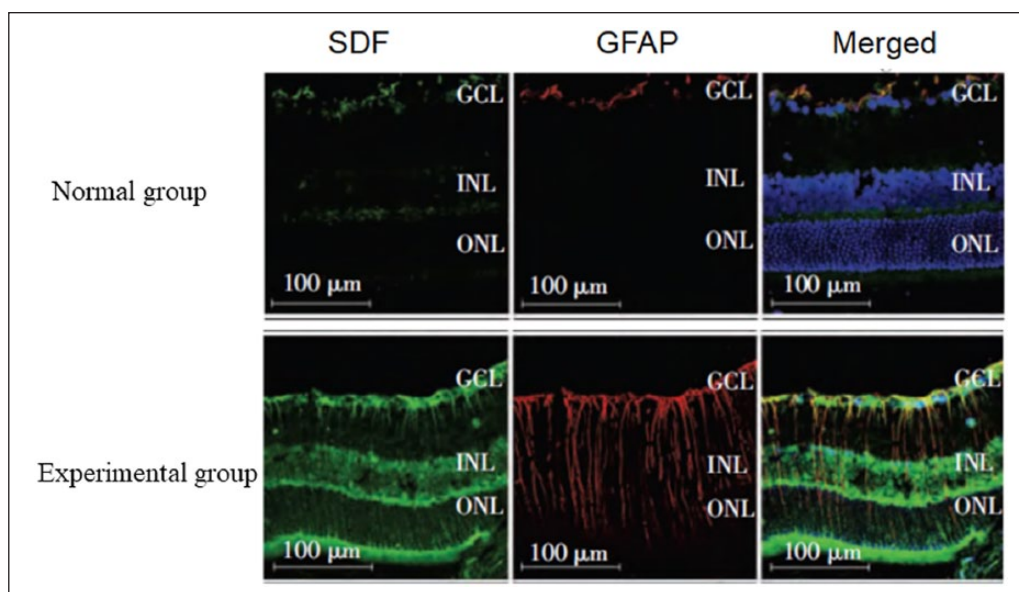


Figure 1. Location expression of SDF-1 in the retina after optic nerve clamp injury in pregnant rats. GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer.

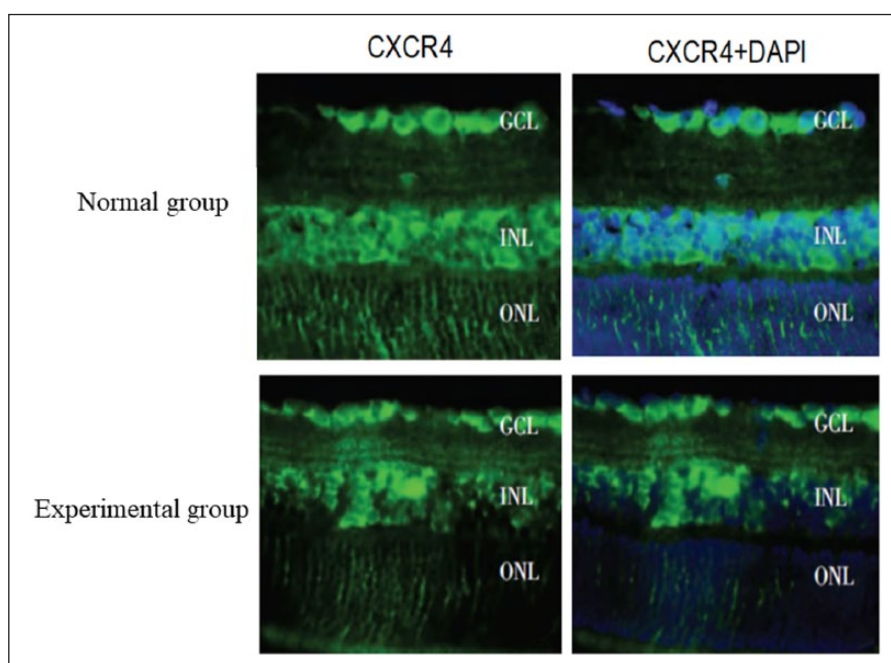


Figure 2. Location expression of CXCR4 in the retina after optic nerve clamp injury in pregnant rats. GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer.

The determination of expression of SDF-1 and CXCR4 protein by western blot

The results of western blot assay showed that the expression levels of SDF-1 and CXCR4 in the experimental group were significantly higher than those in the normal group ($P < 0.05$), as shown in Figure 4.

Discussion

Optic nerve injury is a common complication of brain external injury and maxillofacial trauma. The main cause for visual loss is that the external force acting on the orbit results in optic canal fracture and optic nerve compression. Optic nerve injury is a common kind of ocular trauma with moderately

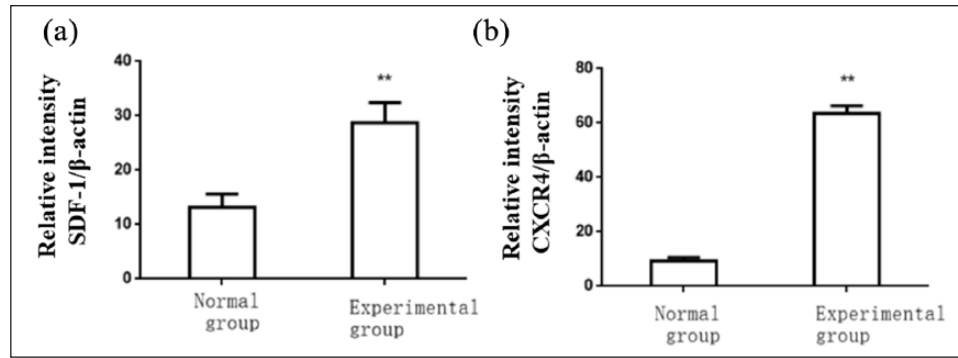


Figure 3. Relative gray value of (a) SDF-1 and (b) CXCR4 immunofluorescence staining in the retina of pregnant rats after optic nerve clamp injury (compared with the normal group, ** $P < 0.05$).

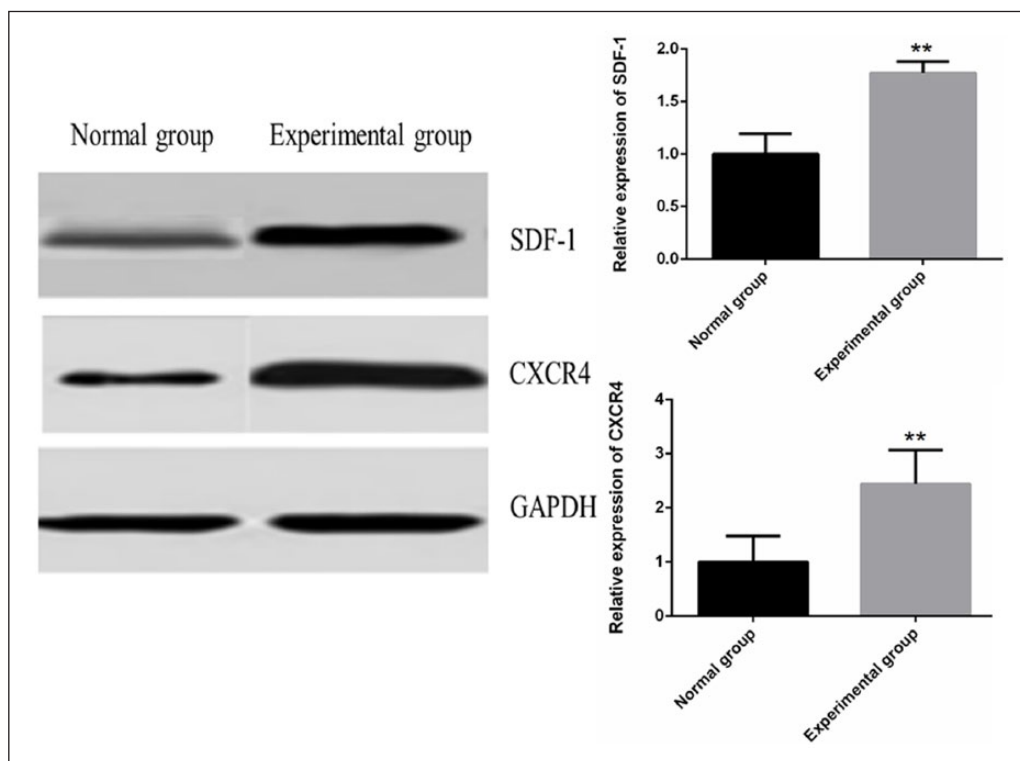


Figure 4. Relative expression of SDF-1 and CXCR4 proteins in the retina after optic nerve clamp injury in pregnant rats.

poor prognosis.^{8,9} At present, there is no effective treatment in clinical trials and the study of optic nerve injury has become one of research hotspots.

Injury of neuronal axons affects the soma of neurons, including dysplasia of axonal transport, degeneration, apoptosis, and necrosis of neurons.¹⁰ Optic nerve injury is also bound to affect ganglion cell and the pericaryon of optic nerve and may also exert transneuronal effect on retinal layers of vision signal conduction path to change retinal morphology. Depending on the injury extent, type, age, and distance from the injury site

to ganglion cells, 50%–90% retinal ganglion cells may get lost after optic nerve injury.¹¹ About 5%–10% of the retinal ganglion cells survived 12–15 months after the intraorbital optic nerve transection. These cells may represent a subset of the retinal ganglion cells and may have different conditions for survival or may be supported by endogenous retinal nutrients. If the optic nerve is crushed or severed, the retinal ganglion cell would inevitably die and be engulfed by settled microglia. The death rate and time of retinal ganglion cells depends on the site of axis resection. If the

site where the optic nerve is crushed or severed is near the eye and not in the distal part, the retinal ganglion cell would die moderately early and quickly.¹²

Model of optic nerve crush is a common experimental model of optic nerve contusion. In this study, a medium-sized artery clamp was used to clip the optic nerve for 10 s at 2 mm away from the eyeball in rats for successful establishment rat model, which is simple to operate with stable injury condition. SDF-1 is one of CXC chemokines secreted by marrow stroma cell and stroma cells of other tissues, and its only known specific receptor is CXCR4. SDF-1 can induce bone marrow-derived endothelial progenitor cells (EPCs) to migrate into ischemic hypoxic tissues and participate in the formation of new blood vessels by binding with CXCR4.¹³ The more severe the eye lesions in diabetic retinopathy, the higher the SDF-1 concentration in the vitreous body.¹⁴ However, there are few reports about the expression of SDF-1 and its receptor CXCR4 in the retina of the pregnant rats after optic nerve injury as well as limited understanding of changes in their localization and expression levels. We studied the distribution and expression of SDF-1 and CXCR4 in the retina of pregnant rats with the methods of immunofluorescence and western blot and found that after optic nerve injury, SDF-1 and GFAP were co expressed, and SDF-1 was expressed in astrocytes as well as Müller cells. By ImageJ semi-quantitative measurement of SDF-1 fluorescence intensity and western blot detection, it was found that the expression level of SDF-1 in experimental group was higher than that in the normal group ($P < 0.05$), which suggests that after optic nerve injury, the expression of SDF-1 is related to the activation of astrocytes and Müller cells. Activation of colloid cells plays a “double-edged sword” role in nerve repair: they help to fight injury and promote the repair of neurons, but hinder tissue repair and nerve regeneration.¹⁵ However, the mechanism of how SDF-1 is involved in the repair of optic nerve injury through its participation in microglia activation is still unknown. The study found that CXCR4 is expressed in retinal ganglion cell layer, inner nuclear layer, and outer nuclear layer with no obvious changes in expression sites between before and after injury, but the ImageJ semi-quantitative measurement of SDF-1 fluorescence intensity and

western blot detection turned out that the expression of CXCR4 in the experimental group was significantly higher than that in the normal group ($P < 0.05$), suggesting that CXCR4 may take a part in the process of pathological damage in the retina of pregnant rats after optic nerve injury, which is one of the important causes for secondary damages of retinal ganglion cells in pregnant rats. Therefore, reducing retinal SDF-1 and CXCR4 in pregnant rat under pathological conditions may play a certain role in delaying or lowering retinal injury, which creates a new idea for prevention and treatment of pregnancy complications of optic nerve injury and provides novel method of medical rescue as well as a theoretical basis for clinical development of corresponding drugs.

Declaration of conflicting interests

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