

## SDF-1/CXCR4 激活 ERK 和 PI3K/AKT 通路介导髓核致炎根性疼痛

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**摘要:**【目的】探讨基质细胞衍生因子1/趋化因子CXC受体4(SDF-1/CXCR4)通路在髓核致炎根性疼痛中的作用及机制。【方法】分为三部分:① 26只大鼠随机分为假手术组和模型组,分别行假手术或髓核自体移植。Von Frey 纤维丝测量大鼠机械撤足阈值(PWT)的变化; Western blot 法检测大鼠脊髓中 SDF-1、CXCR4、磷酸化细胞外信号调节激酶(pERK)和磷酸化蛋白激酶B(pAKT)表达水平;免疫荧光染色定位 SDF-1、CXCR4 的表达细胞。② 54只大鼠随机分为假手术组、模型组、溶剂组、AMD3100组、SDF-1中和抗体组、对照 Ig G 组。鞘内注射相应药物,测量大鼠 PWT 的变化和大鼠脊髓中 pERK 和 pAKT 的表达变化。③ 18只大鼠随机分为溶剂组、U0126组、LY294002组,鞘内注射相应药物,测量大鼠 PWT 的变化。【结果】① 髓核自体移植使大鼠 PWT 下降( $P < 0.001$ ),脊髓中 SDF-1、CXCR4、pERK、pAKT 表达上调( $P < 0.05$ ),SDF-1 主要与脊髓中神经元共表达,CXCR4 与神经元和星形胶质细胞共表达。② 鞘内注射 SDF-1 中和抗体或 CXCR4 抑制剂 AMD3100 升高髓核自体移植大鼠的 PWT( $P < 0.05$ ),并使髓核自体移植大鼠脊髓中 pERK 和 pAKT 表达下调( $P < 0.05$ )。③ 鞘内给予 MEK 抑制剂 U0126 或 PI3K 抑制剂 LY294002 可升高髓核自体移植大鼠 PWT( $P < 0.05$ )。【结论】SDF-1/CXCR4 通过激活 ERK 和 PI3K/AKT 通路介导髓核致炎根性疼痛。

**关键词:** 腰椎间盘突出症;髓核;根性疼痛;基质细胞衍生因子1;CXCR4

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## SDF-1/CXCR4 Activates ERK and PI3K/AKT Signaling Contributing to the Pathogenesis of Radicular Pain Induced by Autograft of Nucleus Pulposus

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**Abstract:**【Objective】To investigate the effect and mechanism of SDF-1/CXCR4 in radicular pain induced by autograft of nucleus pulposus.【Methods】Three parts were included. ① 26 rats were randomly divided into sham group and model group. Autograft of nucleus pulposus was done in model group. Paw withdrawal threshold (PWT) was tested by von Frey filaments. The expression of SDF-1, CXCR4, pERK and pAKT of spinal cord was tested by western blot. Immunofluorescence staining was used to locate the expression of SDF-1 and CXCR4. ② 54 rats were randomly and equally divided

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into sham group, model group, vehicle group, SDF-1 neutralizing antibody group, AMD3100 group, and isotype IgG group. Drug was administered intrathecally. PWT and the expression of pERK and pAKT of spinal cord were tested. ③ 18 rats were randomly and equally divided into model group, U0126 group and LY294002 group. Drug was administered intrathecally. PWT was tested.【Results】① Autologous nucleus pulposus transplantation in rats reduced PWT ( $P<0.001$ ) and increased the expressions of SDF-1, CXCR4, pERK and pAKT in spinal cord of rats ( $P<0.05$ ). SDF-1 was mainly co-expressed with neuron, while CXCR4 was co-expressed with neuron and astrocyte. ② SDF-1 neutralizing antibody and CXCR4 inhibitor AMD3100 reduced PWT ( $P<0.05$ ). The expression of pERK and pAKT in spinal cord of SDF-1 neutralizing antibody group and AMD3100 group was reduced ( $P<0.05$ ). ③ Intrathecally administration of MEK inhibitor U0126 or PI3K inhibitor LY294002 reduced PWT ( $P<0.05$ ).【Conclusion】SDF-1/CXCR4 activates ERK and PI3K/AKT signaling, which contributes to the pathogenesis of radicular pain induced by autograft of nucleus pulposus.

**Key words:** lumbar disc herniation; nucleus pulposus; radicular pain; SDF-1; CXCR4

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根性疼痛是指脊神经的背根和/或背根神经节(dorsal root ganglion, DRG)受到刺激而引起的疼痛。它是腰椎间盘突出症(lumbar disc herniation, LDH)最常见的临床症状<sup>[1]</sup>。LDH导致根性疼痛的机制有机械压迫学说、炎症免疫学说等,其中炎症免疫学说越来越受到重视。突出的髓核组织可引起化学刺激,并诱发炎症免疫反应产生IL-6、IL-1 $\beta$ 、TNF- $\alpha$ 等细胞因子,刺激传入神经纤维,导致痛觉的外周敏化;同时这些因子在脊髓中表达上调,导致痛觉的中枢敏化<sup>[2]</sup>。但目前LDH导致根性疼痛的确切机制并不完全清楚。基质细胞衍生因子1(stromal cell derived factor-1, SDF-1),因最初发现由骨髓基质细胞分泌而得名,它又称趋化因子CXC配体12(chemokine CXC ligand 12, CXCL12)。趋化因子CXC受体4(CXCR4)是SDF-1的特异性受体。SDF-1与CXCR4广泛地表达于多种细胞和组织中,并在炎症、肿瘤等疾病中起着至关重要的作用<sup>[3]</sup>。文献报道,在完全弗式佐剂诱导炎性痛模型<sup>[4]</sup>、癌痛模型<sup>[5-6]</sup>、术后急性切口痛模型<sup>[7]</sup>中,阻断SDF-1/CXCR4信号通路可以减轻模型的痛觉过敏,这提示SDF-1/CXCR4信号通路参与了多种类型疼痛的发生机制。研究表明,SDF-1/CXCR4是通过激活细胞外信号调节激酶(extracellular signal-regulated kinase, ERK)和磷脂酰肌醇3-激酶/蛋白激酶B(phosphoinositide 3-kinase/protein kinase B, PI3K/AKT)通路发挥作用:大鼠足跖切口急性疼痛模型中,SDF-1/CXCR4可激活ERK通路,但不激活PI3K/AKT通路<sup>[7]</sup>;蜜蜂毒注射痛敏预点燃模型中,SDF-1/CXCR4同时激活ERK和PI3K/

AKT通路<sup>[8]</sup>。可见在不同的疼痛模型中,SDF-1/CXCR4对ERK和PI3K/AKT通路的作用是不一样的。目前,在髓核致炎根性疼痛的发病机制中,SDF-1/CXCR4是否参与,其是否通过激活ERK和PI3K/AKT通路发挥作用尚无报道。本研究旨在探讨SDF-1/CXCR4通路在髓核致炎根性痛大鼠模型中的作用及其机制,为阐明LDH引起根性疼痛的发病机制提供新的实验依据。

## 1 材料与方法

### 1.1 主要试剂和仪器

SDF-1、GAPDH、Tubulin、GFAP、Neun、CD11b一抗,HRP标记的IgG二抗,对照IgG(abcam,美国);CXCR4、磷酸化ERK(Phospho-ERK, pERK)、磷酸化AKT(Phospho-AKT, pAKT)一抗(Affinity, 中国);FITC荧光二抗(Invitrogen, 美国);SDF-1中和抗体(US Biological, 美国);AMD3100、U0126、LY294002(MCE, 中国);二甲亚砜(dimethyl sulfoxide, DMSO, Sigma, 美国);Von Frey纤维丝(Stoelting, 美国);Biorad ChemiDoc MP凝胶成像系统(Biorad, 美国);倒置荧光显微镜(Leica, 美国);PE-10导管(Smiths medical, 英国)。

### 1.2 实验动物

SPF级雄性SD大鼠(200~280 g)由中山大学动物实验中心提供,许可证号SCXK(粤)2016-0029。分笼饲养,自由进食,室温(23 $\pm$ 2) $^{\circ}$ C,相对湿度55%~65%,维持大鼠12 h/12 h昼夜节律。本研究所有实验操作均符合中山大学动物实验中心伦理

委员会要求并按照实验动物使用原则进行。

### 1.3 实验分组

本研究分为三个部分。实验一:26只大鼠随机分为模型组和假手术组。检测每组6只大鼠手术前后的机械撤足阈值(paw withdrawal threshold, PWT)变化;假手术组术后第14天和模型组术后第3、7、14天各3只大鼠术后脊髓SDF-1、CXCR4、pERK、pAKT表达变化;模型组2只大鼠脊髓行免疫荧光化学染色将SDF-1、CXCR4分别与星形胶质细胞标志蛋白GFAP、小胶质细胞标志蛋白CD11b和神经元标志蛋白Neun共染。实验二:54只大鼠随机分为假手术组、模型组、溶剂组、AMD3100组、SDF-1中和抗体组、对照IgG组,分别行假手术、建模、建模后连续5d鞘内注射50 g/L DMSO 10  $\mu$ L、CXCR4抑制剂AMD3100 10  $\mu$ g、SDF-1中和抗体5  $\mu$ g,对照组IgG 5  $\mu$ g(给药剂量参考文献报道<sup>[9-10]</sup>和预实验结果)。检测每组6只大鼠手术前后的PWT变化;每组3只大鼠检测脊髓中pERK和pAKT表达变化。实验三:18只大鼠随机分为溶剂组、U0126组、LY294002组,分别建模后连续5d鞘内注射50 g/L DMSO 10  $\mu$ L、MEK的抑制剂U0126 10  $\mu$ g、PI3K抑制剂LY294002 10  $\mu$ g(给药剂量参考文献报道<sup>[9-11]</sup>和预实验结果)。检测每组6只大鼠手术前后的PWT变化。

### 1.4 鞘内置管

在实验二和实验三中,在假手术和建模前,参照Liu等<sup>[12]</sup>描述的方法对大鼠进行鞘内置管。戊巴比妥钠腹腔注射麻醉大鼠(50 mg/kg, ip.),切开皮肤暴露L3~4椎间隙,将PE-10导管向头侧置入约2 cm到达腰膨大水平。将导管经皮下隧道固定至大鼠头部两耳之间,关闭切口。术后剔除手术损伤致下肢运动功能障碍的大鼠,并于鞘内注射2%利多卡因10  $\mu$ L以验证导管位置,剔除导管位置错误的大鼠。鞘内置管手术后5d再进行后续实验。

### 1.5 模型建立

参考文献<sup>[13]</sup>建立动物模型。用戊巴比妥钠麻醉大鼠(50 mg/kg, ip.),在髂嵴附近做正中纵切口,顿性分离左侧椎旁肌,切除行L5和L6关节突关节和L5椎板,暴露L5 DRG。取尾椎2个节段自体髓核。模型组将获取的髓核组织覆盖于左侧L5 DRG。假手术组仅行L5 DRG的暴露,取尾部髓核组织但不将其置于L5 DRG。逐层缝合组织关闭切口。

### 1.6 PWT的测定

将大鼠放置在箱底为金属网的透明有机玻璃箱中至少15 min,使其充分适应测试环境并处于安静状态。采用Up-Down方法<sup>[14]</sup>,用Von Frey纤维丝(0.41、0.70、1.20、2.04、3.63、5.50、8.51、15.14 g)对大鼠后肢左脚足心部进行机械性刺激,每次刺激持续时间为6~8 s。大鼠在刺激时间内或撤离刺激时出现撤足或舔足现象,则为阳性反应。以2.041g刺激强度为初始刺激强度,若撤足反应为阳性,则选择相邻递减的刺激强度给予刺激;若撤足反应为阴性,则选择相邻递增的刺激强度给予刺激。以第一个转折点的前一点为起点连续6次的刺激结果。使用计算机程序根据测试计算出PWT。

### 1.7 Western Blot实验

实验一术后第3、7、14天,每组3只大鼠用于检测蛋白表达。实验二中术后第7天每组3只大鼠检测pERK、pAKT表达变化。戊巴比妥钠(70 mg/kg, ip.)深麻醉大鼠后断头处死,在冰面上迅速取出大鼠L4~L6节段脊髓。加入蛋白裂解液,匀浆和裂解30 min,裂解后的样品4  $^{\circ}$ C 14 000 g离心10 min,取上清液,行BCA法蛋白定量。配平并变性蛋白。SDS-PAGE胶电泳分离蛋白后转至PVDF膜。5% BSA中封闭1 h,随后加入一抗孵育,4  $^{\circ}$ C过夜。漂洗后加入相应的二抗室温孵育1h。洗膜后加入ECL发光液显色曝光。使用Biorad ChemiDoc MP凝胶成像系统进行显色拍照,最后采用Image J进行条带的灰度分析。

### 1.8 免疫荧光实验

用2%戊巴比妥(60~80 mg/kg)深度麻醉大鼠后,经主动脉快速灌注200 mL生理盐水后,继而40 g/L多聚甲醛灌注固定30 min。将L4~6脊髓节段取出,40 g/L多聚甲醛后固定24 h,然后依次用10%、20%和30%蔗糖溶液梯度脱水3 d。做连续冠状冰冻切片(20  $\mu$ m/片),切片漂洗、封闭后加入一抗,4  $^{\circ}$ C孵育12 h,漂洗后加入荧光二抗,室温孵育2 h。漂洗、裱片、封片,加入抗荧光淬灭剂后在倒置荧光显微镜下观察摄片。

### 1.9 统计学分析

统计学分析通过SPSS 20.0(SPSS Inc., USA)进行,所有数据表示为均数 $\pm$ 标准差。PWT数据应用重复测量的方差分析检验差异。对Western Blot数据采用单因素方差分析进行检验。以 $P < 0.05$ 为差异有统计学意义。

## 2 结果

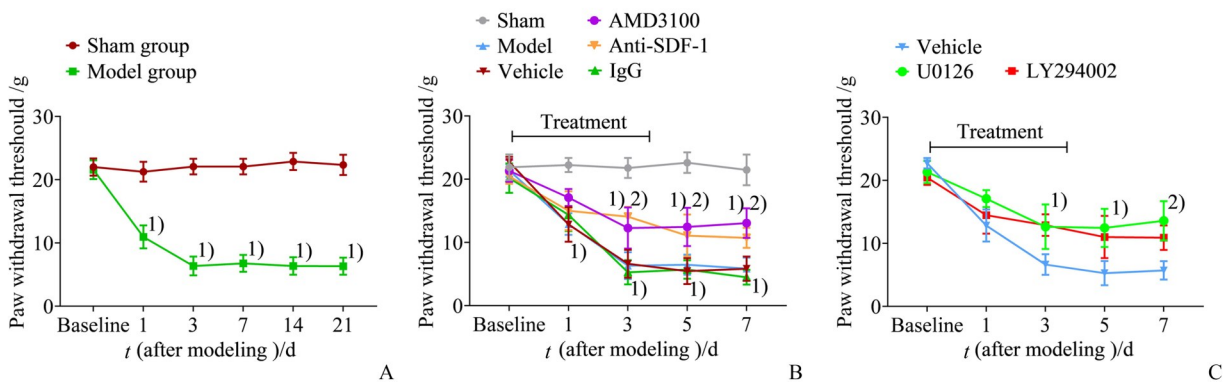
### 2.1 髓核自体移植导致大鼠机械痛敏, 脊髓中 SDF-1、CXCR4、pERK、pAKT 表达上调

PWT测试结果显示(图1A):经重复测量方差分析,模型组与假手术组间差异有统计学意义( $F=54.55, P<0.001; F=703.9, P<0.001$ );进一步采用Sidak法作两两比较发现,模型组术后第1、3、7、14、21天PWT较术前降低( $P$ 均 $<0.001$ ),且与假手术组比较有统计学差异( $P$ 均 $<0.001$ )。Western blot结果显示(图2A-D):经单因素方差分析,模型组SDF-1、CXCR4、pERK、pAKT蛋白表达与假手术组比较有统计学差异( $F=15.98, P=0.045; F=26.37, P=0.035; F=82.87, P=0.008; F=65.04, P=0.010$ ),进一步两两比较发现模型组SDF-1、CXCR4、pERK、pAKT蛋白术后比假手术组表达上调(术后第3天 $P=0.029, P=0.016, P=0.006, P<0.001$ ;术后第7天 $P=0.035, P=0.022, P=0.003, P=0.015$ ;术后第14天 $P=0.031, P=0.021, P=0.011, P=0.001$ )。免疫荧光双染结果显示,SDF-1主要在神经元分布(图3),而CXCR4主要在神经元和星形胶质细胞分布(图4)。

### 2.2 鞘内注射 SDF-1 中和性抗体或 CXCR4 抑制剂 AMD3100 减轻髓核自体移植引起的痛觉过敏, 并下调脊髓中 pERK、pAKT 表达水平

PWT测试结果显示(图1B):经重复测量方差分析,各组间差异有统计学意义( $F=188.9, P<$

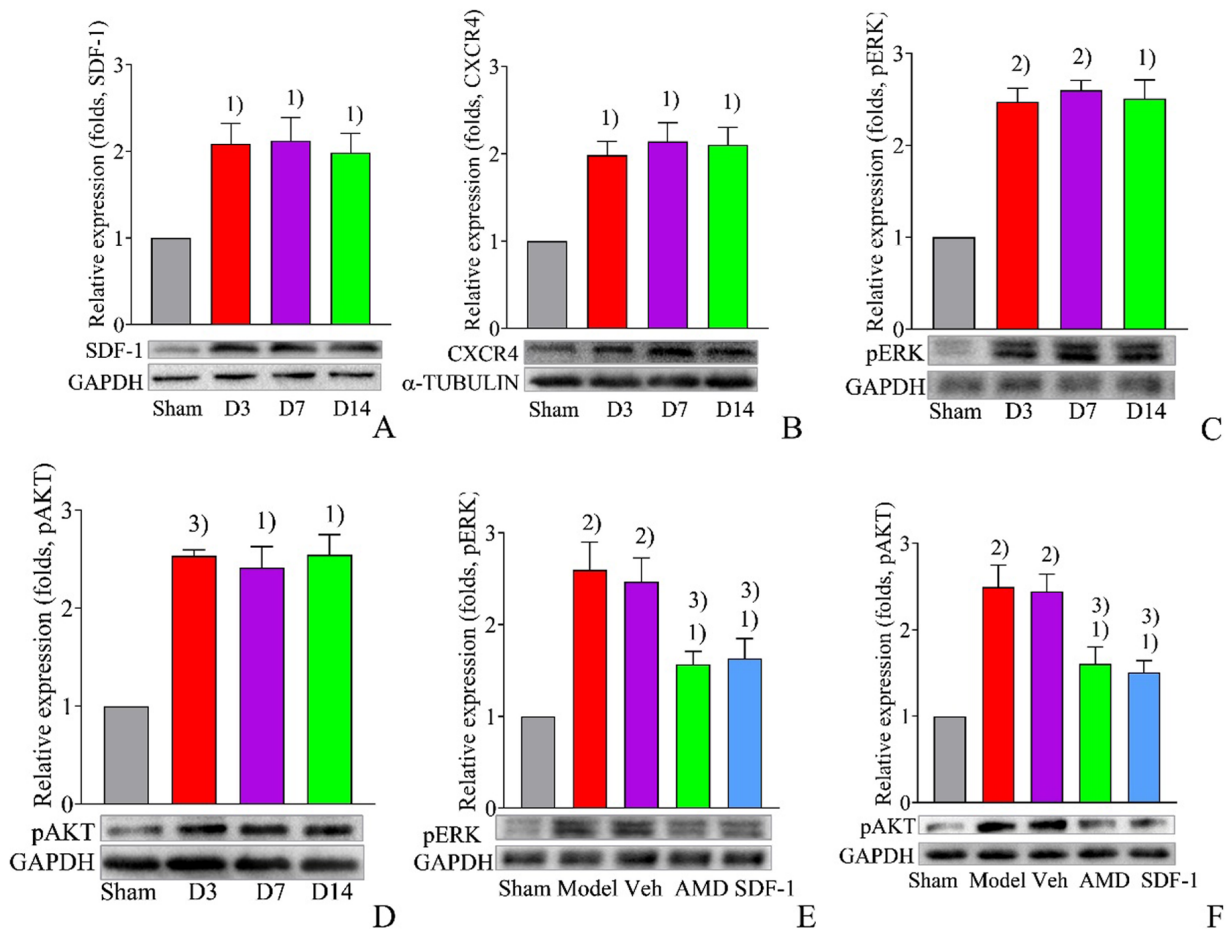
$0.001; F=76.21, P<0.001$ );进一步采用Sidak法作两两比较发现,模型组、溶剂组和对照IgG组PWT在术后第1、3、5、7天比假手术组降低( $P$ 均 $<0.001$ );AMD3100组和SDF-1中和抗体组的PWT比模型组(第3天: $P<0.001$  vs. AMD3100组或SDF-1中和抗体组;第5天: $P<0.001$  vs. AMD3100组,  $P=0.008$  vs. SDF-1中和抗体组;第7天: $P<0.001$  vs. AMD3100组,  $P=0.004$  vs. SDF-1中和抗体组),溶剂组(第3、5天: $P$ 均 $<0.001$  vs. AMD3100组或SDF-1中和抗体组;第7天: $P<0.001$  vs. AMD3100组,  $P=0.004$  vs. SDF-1中和抗体组)或对照IgG组(第3、5、7天: $P$ 均 $<0.001$  vs. AMD3100组或SDF-1中和抗体组)提高;与假手术组比较,AMD3100组和SDF-1中和抗体组的PWT仍下降( $P$ 均 $<0.001$ )。Western blot结果显示(图2E、F):经单因素方差分析,各组间pERK( $F=29.91, P<0.001$ )和pAKT( $F=38.37, P<0.001$ )差异有统计学意义;进一步两两比较发现,模型组和溶剂组中pERK和pAKT比假手术组表达上调( $P$ 均 $<0.001$ );与模型组或溶剂组比较,AMD3100组pERK( $P=0.001$  vs. 模型组,  $P=0.003$  vs. 溶剂组)和pAKT( $P<0.001$  vs. 模型组,  $P=0.001$  vs. 溶剂组)表达下调;与模型组或溶剂组比较,SDF-1中和抗体组的pERK( $P=0.002$  vs. 模型组,  $P=0.005$  vs. 溶剂组)和pAKT( $P$ 均 $<0.001$ )表达下调;与假手术组比较,AMD3100组和SDF-1中和抗体组的pERK( $P=0.048, P=0.029$ )和pAKT( $P=0.014, P=0.040$ )表达仍上调。



PWT for rats was tested in different groups. A: 1)  $P<0.001$  compared with sham group or baseline by Sidak- $t$  after two-way ANOVA;  $n=6$  per group. B: 1)  $P<0.001$  compared with sham group; 2)  $P<0.01$  compared with model group or vehicle group, or IgG group by Sidak- $t$  after two-way ANOVA;  $n=6$  per group. C: 1)  $P<0.05$ , 2)  $P<0.01$  compared with sham group by Sidak- $t$  after two-way ANOVA;  $n=6$  per group.

图1 各组大鼠PWT变化情况

Fig.1 PWT for rats in different groups



Western blot shows that the expression of SDF-1 (A), CXCR4 (B), pERK (C), pAKT (D) in the spinal cord of model group was upregulated, 1)  $P < 0.05$ , 2)  $P < 0.01$ , 3)  $P < 0.001$  compared with sham group by Tukey- $t$  after ANOVA;  $n = 3$  per group. SDF-1 neutralizing antibody and AMD3100 reduced upregulation of pERK (E) and pAKT (F) in the spinal cord of nucleus pulposus autograft rats, 1)  $P < 0.05$ , 2)  $P < 0.001$  compared with sham group, 3)  $P < 0.01$  compared with model group or vehicle group by Tukey- $t$  after ANOVA;  $n = 3$  per group.

图2 各组大鼠脊髓蛋白表达情况

Fig.2 Protein expression in spinal cord of rats in different groups

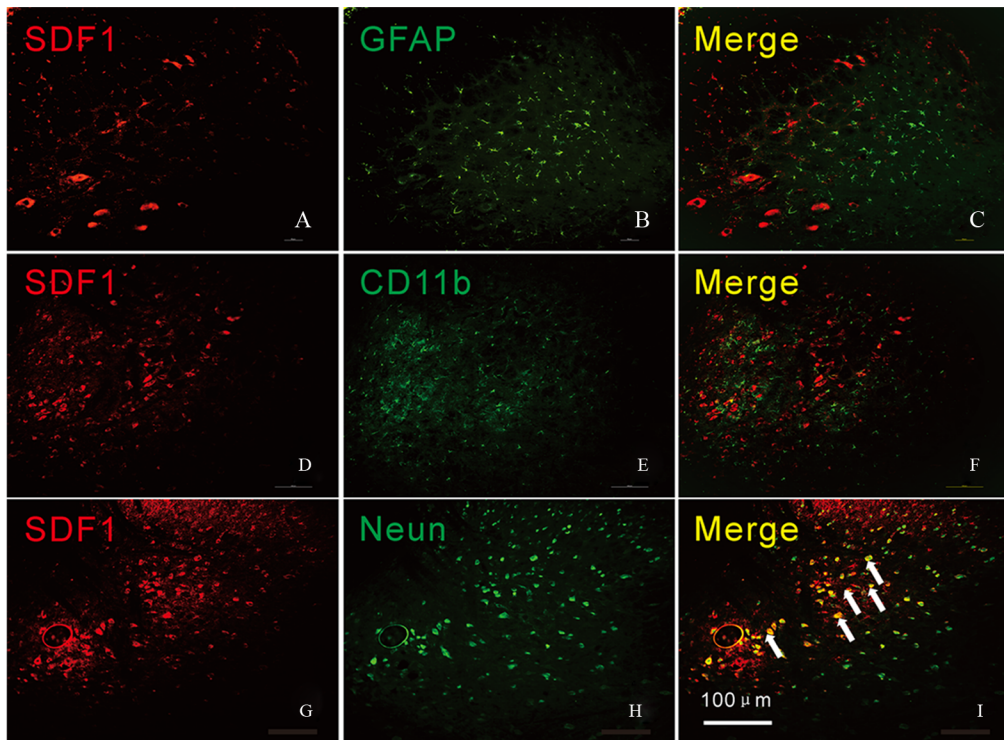
### 2.3 鞘内注射MEK的抑制剂U0126或PI3K抑制剂LY294002减轻髓核自体移植引起的痛觉过敏

PWT测试结果显示(图1C):经重复测量方差分析,各组间差异有统计学意义( $F = 88.58$ ,  $P < 0.001$ ;  $F = 15.7$ ,  $P < 0.001$ );进一步采用Sidak法作两两比较发现,与溶剂组相比U0126组和LY294002组PWT在术后升高(术后第3天: $P = 0.04$ ,  $P = 0.001$ ; 术后第5天 $P = 0.009$ ,  $P = 0.04$ ; 术后第7天: $P = 0.008$ ,  $P = 0.005$ )。

## 3 讨论

SDF-1/CXCR4通路被证明参与多种类型疼痛的形成和发展。有研究结果显示,大鼠单次足底或

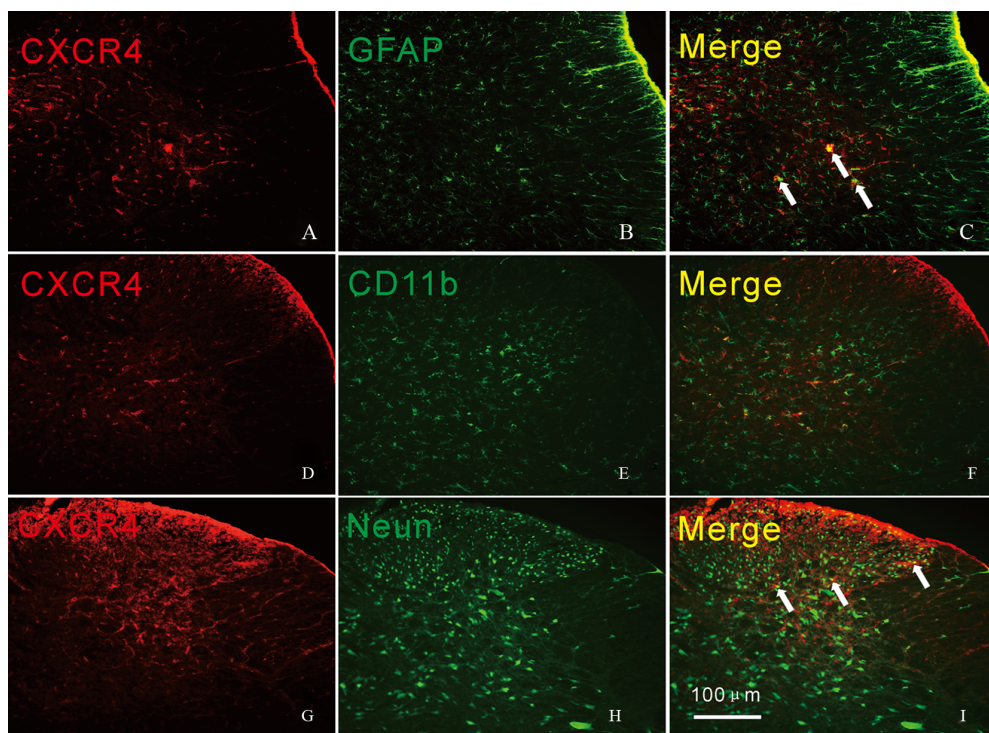
鞘内注射SDF-1后可出现持续的痛觉过敏,给予CXCR4的siRNA或AMD3100可减轻痛敏<sup>[15]</sup>;在神经病理性疼痛的模型如坐骨神经分支选择切断大鼠模型或坐骨神经慢性压迫性损伤大鼠模型中,其DRG及脊髓中SDF-1和CXCR4的表达均发生上调,在鞘内注射SDF-1中和抗体或AMD3100可减轻模型的痛敏<sup>[16-17]</sup>;另外,在完全弗式佐剂诱导炎性痛模型<sup>[4]</sup>、癌痛模型<sup>[5-6]</sup>、术后急性切口痛模型<sup>[7]</sup>、再缺血再灌注疼痛模型<sup>[18]</sup>,化疗药引起的疼痛<sup>[10-19]</sup>等模型中,均有报道显示SDF-1/CXCR4参与了痛敏的形成和发展。但目前文献中尚无SDF-1/CXCR4在髓核致炎根性疼痛模型中作用的相关报道。在本研究中,鞘内给予SDF-1中和抗体或CXCR4抑制剂AMD3100阻断SDF-1/CXCR4信号通路可减



Double immunofluorescence shows that SDF-1 is mainly co-localized with Neun (I) in the spinal cord. The arrows indicate double-labeled cells. Scale bar: 100  $\mu\text{m}$ .

图3 SDF-1与星形胶质细胞、小胶质细胞和神经元双染结果

Fig.3 Co-localization of SDF-1 and astrocyte, microglia or neuron in the spinal cord



Double immunofluorescence shows that CXCR4 is mainly co-localized with GFAP (C) and Neun (I). The arrows indicate double-labeled cells. Scale bar: 100  $\mu\text{m}$ .

图4 CXCR4与星形胶质细胞、小胶质细胞和神经元双染结果

Fig.4 Co-localization of CXCR4 and astrocyte, microglia or neuron in the spinal cord

轻髓核自体移植引起的痛觉过敏,说明在髓核致炎根性疼痛的发生发展过程中,SDF-1/CXCR4也参与其中。

神经元和胶质细胞之间的相互作用参与了疼痛的中枢敏化机制<sup>[20-21]</sup>。在本研究中,免疫荧光双染显示SDF-1主要分布在神经元,而CXCR4主要分布在神经元和星形胶质细胞。这与Liu等<sup>[15]</sup>报道的在脊神经结扎神经病理性疼痛模型中的研究结果一致。这提示,在髓核致炎根性痛大鼠模型中,神经元分泌的SDF-1可能作用于自身或胶质细胞的CXCR4,从而参与疼痛的形成和发展。

有文献报道,SDF-1/CXCR4通过其下游的ERT和PI3K/AKT通路发挥其生物学作用。在不同的疼痛模型中,SDF-1/CXCR4激活的下游通路有所差异。大鼠足趾切口急性疼痛模型中,预先鞘内注射CXCR4拮抗剂AMD3100可以抑制ERK1/2的激活,却不能抑制AKT的激活<sup>[7]</sup>;脊神经结扎神经病理性疼痛模型和BV注射痛敏预点燃模型中,ERK和PI3K/AKT通路则均被激活<sup>[8]</sup>。本研究证明,在髓核自体移植根性疼痛模型中,SDF-1/CX-

CR4同时激活了ERK和PI3K/AKT通路而介导痛觉过敏的产生。

细胞信号通路中,存在着广泛的环形反馈环路和交互作用。体外实验中,给予LY294002或GDC0941(PI3K抑制剂)可以抑制ERK的激活;而给予PD98059(MEK抑制剂),可以抑制AKT激活<sup>[22-23]</sup>;AKT也可以在B-Raf水平上调节Raf/MEK/ERK通路<sup>[24]</sup>。即抑制ERK或PI3K/AKT其中一条通路,另外一条通路也可被抑制。这说明ERK和PI3K/AKT通路间存在交互作用。然而,在本研究的实验二中,并未检测U0126组和LY294002组中脊髓pERK和pAKT的表达情况,因此并不能显示在髓核致炎根性疼痛大鼠模型中ERK和PI3K/AKT通路之间相互作用的情况,它们之间的关系有待进一步的研究探讨。

综上所述,本研究证明髓核致炎根性疼痛的大鼠模型中,SDF-1/CXCR4通过激活ERK和PI3K/AKT通路介导疼痛的发生与发展。这为阐明LDH导致根性疼痛的发生机制提供了新的证据。

#### 参考文献

- [1] Deyo RA, Mirza SK. CLINICAL PRACTICE. Herniated Lumbar Intervertebral Disk [J]. *N Engl J Med*, 2016, 374(18): 1763-1772.
- [2] Altun I. Cytokine profile in degenerated painful intervertebral disc: variability with respect to duration of symptoms and type of disease [J]. *Spine J*, 2016, 16(7): 857-861.
- [3] Reaux-Le Goazigo A, Rivat C, Kitabgi P, et al. Cellular and subcellular localization of CXCL12 and CXCR4 in rat nociceptive structures: physiological relevance [J]. *Eur J Neurosci*, 2012, 36(5): 2619-2631.
- [4] Li F, Xue ZY, Yuan Y, et al. Upregulation of CXCR4 through promoter demethylation contributes to inflammatory hyperalgesia in rats [J]. *CNS Neurosci Ther*, 2018, 24(10): 947-956.
- [5] Shen W, Hu XM, Liu YN, et al. CXCL12 in astrocytes contributes to bone cancer pain through CXCR4-mediated neuronal sensitization and glial activation in rat spinal cord [J]. *J Neuroinflammation*, 2014, 11: 75.
- [6] Hang LH, Li SN, Luo H, et al. Connexin 43 Mediates CXCL12 production from spinal dorsal horn to maintain bone cancer pain in rats [J]. *Neurochem Res*, 2016, 41(5): 1200-1208.
- [7] Xing F, Kong C, Bai L, et al. CXCL12/CXCR4 signaling mediated ERK1/2 activation in spinal cord contributes to the pathogenesis of postsurgical pain in rats [J]. *Mol Pain*, 2017, 13: 1-12.
- [8] Yang F, Sun W, Luo WJ, et al. SDF1-CXCR4 signaling contributes to the transition from acute to chronic pain state [J]. *Mol Neurobiol*, 2017, 54(4): 2763-2775.
- [9] Zhu HY, Liu X, Miao X, et al. Up-regulation of CXCR4 expression contributes to persistent abdominal pain in rats with chronic pancreatitis [J]. *Mol Pain*, 2017, 13: 1-11.
- [10] Xu T, Zhang XL, Ou-Yang HD, et al. Epigenetic up-regulation of CXCL12 expression mediates antitubulin chemotherapeutics-induced neuropathic pain [J]. *Pain*, 2017, 158(4): 637-648.
- [11] Bareiss SK, Dugan E, Brewer KL. PI3K mediated activation of GSK-3 $\beta$  reduces at-level primary afferent growth responses associated with excitotoxic spinal

- cord injury dysesthesias [J]. *Mol Pain*, 2015, 11: 35.
- [12] Liu C, Luan S, OuYang H, et al. Upregulation of CCL2 via ATF3/c-Jun interaction mediated the Bortezomib-induced peripheral neuropathy [J]. *Brain Behav Immun*, 2016, 53: 96-104.
- [13] Wei M, Mo SL, Nabar NR, et al. Modification of rat model of sciatica induced by lumbar disc herniation and the anti-inflammatory effect of osthole given by epidural catheterization [J]. *Pharmacology*, 2012, 90 (5-6): 251-263.
- [14] Chaplan SR, Bach FW, Pogrel JW, et al. Quantitative assessment of tactile allodynia in the rat paw [J]. *J Neurosci Methods*, 1994, 53(1): 55-63.
- [15] Liu ZY, Song ZW, Guo SW, et al. CXCL12/CXCR4 signaling contributes to neuropathic pain via central sensitization mechanisms in a rat spinal nerve ligation model [J]. *CNS Neurosci Ther*, 2019, 25 (9): 922-936.
- [16] Bai L, Wang X, Li Z, et al. Upregulation of chemokine CXCL12 in the dorsal root ganglia and spinal cord contributes to the development and maintenance of neuropathic pain following spared nerve injury in rats [J]. *Neurosci Bull*, 2016, 32(1): 27-40.
- [17] Yu Y, Huang X, Di Y, et al. Effect of CXCL12/CXCR4 signaling on neuropathic pain after chronic compression of dorsal root ganglion [J]. *Sci Rep*, 2017, 7 (1): 5707.
- [18] Li XQ, Zhang ZL, Tan WF, et al. Down-Regulation of CXCL12/CXCR4 expression alleviates ischemia-reperfusion-induced inflammatory pain via inhibiting glial TLR4 activation in the spinal cord [J]. *PLoS One*, 2016, 11(10): e0163807.
- [19] Li YY, Li H, Liu ZL, et al. Activation of STAT3-mediated CXCL12 up-regulation in the dorsal root ganglion contributes to oxaliplatin-induced chronic pain [J]. *Mol Pain*, 2017, 13: 1-11.
- [20] Hossain MZ, Unno S, Ando H, et al. Neuron-glia crosstalk and neuropathic pain: involvement in the modulation of motor activity in the orofacial region [J]. *Int J Mol Sci*, 2017, 18(10): 2051.
- [21] 魏绪红, 杨涛, 宫庆娟, 等. 大鼠脊髓小胶质细胞 p38 MAPK 介导 rIL-1 $\beta$  诱导的机械痛敏 [J]. *中山大学学报(医学科学版)*, 2010, 31(3): 327-331. Wei XH, Yang T, Gong QJ, et al. p38 MAPK in spinal microglia mediates mechanical allodynia induced by rIL-1 $\beta$  [J]. *J Sun Yat-sen Univ (Med Sci)*, 2010, 31(3): 327-331.
- [22] Huang W, Zhao Y, Zhu X, et al. Fluoxetine upregulates phosphorylated-AKT and phosphorylated-ERK1/2 proteins in neural stem cells: evidence for a crosstalk between AKT and ERK1/2 pathways [J]. *J Mol Neurosci*, 2013, 49(2): 244-249.
- [23] Ebi H, Costa C, Faber AC, et al. PI3K regulates MEK/ERK signaling in breast cancer via the Rac-GEF, P-Rex1 [J]. *Proc Natl Acad Sci USA*, 2013, 110(52): 21124-21129.
- [24] Hong SK, Jeong JH, Chan AM, et al. AKT upregulates B-Raf Ser445 phosphorylation and ERK1/2 activation in prostate cancer cells in response to androgen depletion [J]. *Exp Cell Res*, 2013, 319(12): 1732-1743.

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