论著·神经科学

SOCS3通过抑制NF-κB信号通路介导的小胶质细胞炎症反应改善帕金森病神经损伤

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[摘要] **目的** 探讨细胞因子信号抑制物3(suppressor of cytokine signaling 3,SOCS3)通过核因子-κB(nuclear factor-kappaB,NF-κB)通路调控小胶质细胞炎症反应的作用机制,为帕金森病(Parkinson's disease,PD)的发病机制提供新见解。**方法** ①以10~12周龄C57BL/6雄性小鼠(体质量20~25 g)为造模对象,腹腔注射15 mg/kg 1-甲基-4-苯基-1,2,3,6-四氢吡啶(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine,MPTP)以建立PD体内模型,采用旋转杆实验评估小鼠运动能力,Western blot法检测小鼠脑黑质区酪氨酸羟化酶(tyrosine hydroxylase,TH)及离子化钙结合衔接分子1(ionized calcium-binding adaptor molecule 1,IBA-1)表达,RT-qPCR检测脑黑质区SOCS3表达,免疫组化检测NF-κB p65亚基表达,Western blot检测SOCS3、NF-κB、p-NF-κB。②对小胶质细胞BV2给予1 000 ng/mL LPS刺激6 h以建立炎性小胶质细胞模型,在此基础上施以敲低SOCS3,并使用BAY 11-7082处理细胞以抑制NF-κB;采用RT-qPCR和Western blot检测BV2细胞SOCS3表达,采用Western blot检测NF-κB和p-NF-κB,ELISA检测细胞培养上清液中TNF-α和IL-1β,免疫荧光定位NF-κB核定位。③建立BV2-脑神经瘤细胞N2a细胞共培养体系探究小胶质细胞对神经细胞的调控作用,采用MTT法检测N2a细胞活性,TUNEL染色检测N2a细胞凋亡。**结果** ①相较于对照小鼠,PD小鼠模型表现为旋杆跌落时间缩短,TH和SOCS3下调($P<0.01$),IBA-1和p-NF-κB/NF-κB比值上调($P<0.01$)。②在BV2细胞中,LPS刺激导致TNF-α、IL-1β、p-NF-κB/NF-κB比值上调($P<0.01$),SOCS3下调($P<0.01$);在LPS刺激的BV2细胞中,敲低SOCS3导致p-NF-κB/NF-κB比值上调($P<0.01$),NF-κB多分布于细胞核,TNF-α和IL-1β上调($P<0.01$);在此基础上BAY 11-7082导致p-NF-κB/NF-κB比值、TNF-α、IL-1β下调($P<0.01$),NF-κB核分布减少。③LPS处理的小胶质细胞导致N2a细胞活性降低($P<0.01$),并引起细胞凋亡增加($P<0.01$);而敲低SOCS3在BV2细胞的表达后,N2a细胞活性降低($P<0.01$),细胞凋亡增加($P<0.01$);在此基础上BAY 11-7082处理小胶质细胞后,N2a细胞活性升高($P<0.01$),细胞凋亡减少($P<0.01$)。**结论** SOCS3通过下调NF-κB活性抑制小胶质细胞炎症反应,进而减少神经细胞死亡,改善PD神经损伤。

[关键词] 帕金森病;小胶质细胞;细胞因子信号抑制物3;核因子-κB;神经损伤

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SOCS3 ameliorates Parkinson's disease neuropathology by suppressing NF-κB signaling-mediated microglial inflammation

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[**Abstract**] Objective To investigate the mechanism by which suppressor of cytokine signaling 3 (SOCS3) regulates microglial inflammation through nuclear factor-kappaB (NF-κB), providing novel mechanistic insights into microglial involvement in Parkinson's disease (PD) pathogenesis. Methods ① Ten male C57BL/6 mice (12 weeks old, weighing 20~25 g) were subjected to intraperitoneal injection of 15 mg/kg MPTP to establish a PD model. Rotarod test was used to assess motor function. Western blotting was employed to detect the protein expression of tyrosine hydroxylase (TH) and ionized calcium-binding adapter molecule 1 (IBA-1) in the substantia nigra. RT-qPCR was utilized to measure the mRNA level of SOCS3 in the substantia nigra. Immunohistochemistry was performed to assess NF-κB p65 subunit expression. The expression of SOCS3, NF-κB and p-NF-κB was measured with Western blotting. ② Microglial cell line BV2 was stimulated with 1 000 ng/mL lipopolysaccharide (LPS) for 6 h to establish an inflammatory model. Subsequently, SOCS3 was knocked down. NF-κB inhibitor BAY 11-7082 was used to treat the cells. RT-qPCR and Western blotting were used to measure the expression of SOCS3 at mRNA and protein levels. Western blotting was also applied to detect the expression of NF-κB and p-NF-κB, and ELISA was conducted to measure TNF-α and IL-1β levels in the culture supernatant. Immunofluorescence assay was carried out to localize NF-κB (nuclear *vs* cytoplasmic). ③ A co-culture system of BV2 microglia and N2a neuroblastoma cells was established to investigate the regulatory effects of microglia on neuronal cells. MTT assay and TUNEL staining were used respectively to determine cell viability and apoptosis of N2a cells. Results ① Compared to the control mice, the PD mouse model exhibited reduced rotarod fall latency, down-regulation in TH and SOCS3($P<0.01$), up-regulation in IBA-1 and increased p-NF-κB/NF-κB ratio ($P<0.01$). ② In BV2 cells, LPS stimulation increased TNF-α, IL-1β, and p-NF-κB/NF-κB ratio($P<0.01$), while down-regulated SOCS3 expression($P<0.01$). SOCS3 knockdown in LPS-stimulated BV2 cells further increased the p-NF-κB/NF-κB ratio ($P<0.01$), increased nuclear localization of NF-κB, and elevated TNF-α and IL-1β levels ($P<0.01$). BAY 11-7082 treatment in these SOCS3-knockdown, LPS-stimulated cells resulted in reduced p-NF-κB/NF-κB ratio, TNF-α, and IL-1β ($P<0.01$), and decreased NF-κB nuclear distribution. ③ LPS-stimulated BV2 cells reduced cell viability and increased cell apoptosis in N2a cells($P<0.01$). SOCS3 knockdown in BV2 cells exacerbated the reduction in N2a cell viability ($P<0.01$) and the increase in cell apoptosis in N2a cells ($P<0.01$). BAY 11-7082 treatment of these SOCS3-knockdown BV2 microglia attenuated the reduction in N2a cell viability and decreased apoptosis in N2a cells ($P<0.01$). Conclusion SOCS3 inhibits microglia inflammatory response through down-regulation of NF-kB activity, and in turn attenuates neuronal cell death and ameliorates PD nerve injury.

[**Key words**] Parkinson's disease; microglia; suppressor of cytokine signaling 3; nuclear factor-kappa B; neuronal damage

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帕金森病(Parkinson's disease,PD)是常见的神经退行性疾病[1]。流行病学显示,PD的年发病率为5~35/10万,且发病率随年龄增长而增加[2]。PD导致患者出现运动迟缓、僵硬、震颤等运动症状,严重损害患者日常生活能力[3]。PD发病机制尚不明确,目前认为PD主要源于基底神经节功能障碍,其中黑质多巴胺能神经元丢失或损伤引起黑质纹状体功能不全,是PD早期运动症状的核心病理基础,同时神经元中路易小体的形成促进组织损伤[4-5]。PD的发病机制具有高度复杂性与异质性,深入揭示其分子与细胞机制,对于开发更有效的治疗策略具有重要意义。

近年来,小胶质细胞在PD发病机制中的作用日益受到关注。作为中枢神经系统内的常驻免疫细胞,小胶质细胞介导的神经炎症被认为是多种神经退行性疾病(包括PD)的共同特征[6-7]。在PD中,路易小体主要成分α-突触核蛋白激活小胶质细胞,引起小胶质细胞炎症反应,从而促进小胶质细胞向胞外释放促炎细胞因子,加剧神经炎症;α-突触核蛋白的异常聚集还会抑制小胶质细胞的吞噬功能,导致小胶质细胞对α-突触核蛋白的吞噬及清除活性受限[8-10]。目前对小胶质细胞调控PD发生的认识尚不完善,阐明其炎症反应的调控网络对于PD的干预治疗至关重要。

细胞因子信号抑制物3(suppressor of cytokine signaling 3,SOCS3)在神经退行性疾病中发挥重要的保护作用。最新证据显示,在PD中SOCS3上调可抑制神经炎症,并改善多巴胺耗竭及运动障碍,表明SOCS3表达在PD发病机制中具有重要意义[11]。同时该证据还指出核因子-κB(nuclear factor-kappaB,NF-κB)介导SOCS3在PD中的调控作用。NF-κB参与IL-6等促炎因子的转录过程,是炎症反应的关键介质[12-14]。α突触核蛋白激活小胶质细胞,并促进NF-κB易位至细胞核内以参与促炎因子转录[15],表明小胶质细胞的炎症活化依赖于NF-κB信号通路。另有研究表明,SOCS3参与调控小胶质细胞活化,在机制上SOCS3抑制小胶质细胞活化以下调促炎因子IL-6的胞外释放,从而抑制神经炎症并改善神经退行性症状[16]。这一研究表明,在神经退行性疾病中小胶质细胞活化受SOCS3调控,并提示SOCS3表达下调可能促进小胶质细胞活化及炎症,进而影响PD发生发展。鉴于NF-κB通路在小胶质细胞炎症反应中的重要意义,以及SOCS3对小胶质细胞的调控作用,本研究推测SOCS3可能通过NF-κB通路调控小胶质细胞的炎症反应,然而目前尚无直接实验证据支持该机制在PD中的作用。因此,本研究旨在证实NF-κB介导SOCS3对小胶质细胞炎症反应的调控机制,为小胶质细胞参与PD发病机制提供新的理论基础,并为PD治疗提供潜在的作用靶点。

1 材料与方法

1.1 PD小鼠模型构建

10~12周龄C57BL/6雄性小鼠(北京维通利华实验动物技术有限公司,体质量20~25 g)饲养于湿度60%±1%、(22±1)℃的环境下,并接受12 h光暗交替循环。小鼠接受适应性饲养1周后进行建模。取8只小鼠,按随机数字表法分为(n=4):Sham组和MPTP组。MPTP组和Sham组分别腹腔注射15 mg/kg 1-甲基-4-苯基-1,2,3,6-四氢吡啶(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine,MPTP)多巴胺能神经毒素[17-19](M0896,美国Sigma-Aldrich公司)、等体积的0.9%生理盐水,每天4次,每2小时注射1次,持续5 d。本研究已取得贵州省人民医院动物中心伦理委员会批准(伦预审字[2019]149号)。

1.2 旋转杆实验检测PD小鼠行为学

实验前准备:在正式测试前1~2 d,让所有小鼠进行适应性训练。例如,以较低转速让小鼠在杆上练习,使其学会停留在旋转杆上。正式测试阶段:轻柔地将小鼠面向远离实验者的方向放置于静止或缓慢旋转的杆上,启动仪器,使杆开始按预设模式加速旋转,记录小鼠从杆上掉落的潜伏期。同时观察并记录小鼠的抓附姿势和努力维持平衡的行为。每只小鼠在同一天内重复测试3~5次,多次测试间给予足够的休息时间。数据分析:计算每只小鼠多次测试的平均潜伏期。

1.3 免疫组化检测NF-κB p65

旋转杆实验完成后,采用CO_2麻醉法进行安乐死,收集小鼠脑组织,并行固定、脱水、石蜡包埋。将石蜡组织块切成3~5 μm厚的组织切片。对组织切片行脱蜡及水化处理后,浸入柠檬酸盐修复液(14746,美国CST公司),95 ℃加热10 min,冷却30 min。加入3%过氧化氢溶液孵育10 min。加入含5%正常羊血清(5425,美国CST公司)的封闭液,室温封闭1 h。移除封闭液,加入NF-κB p65抗体(8242,美国CST公司,1∶400),4 ℃孵育过夜。加入HRP交联的Boost免疫组化检测试剂(8114,美国CST公司),室温孵育30 min。加入DAB试剂复染1 min。对切片依次进行脱水和中性树胶封片。显微镜下观察。

1.4 细胞培养及分组

小鼠小胶质细胞BV2(武汉普诺赛生命科技有限公司)或小鼠脑神经瘤细胞N2a(武汉普诺赛生命科技有限公司)培养于含10%胎牛血清(美国Gibco公司)和100 U/mL青-链霉素混合液(北京索莱宝科技有限

公司)的DMEM培养基(美国Gibco公司)中,37 ℃、5%CO_2孵育细胞。

取BV2细胞,并分为:Control组,细胞正常培养,不进行额外处理;LPS组,细胞预先进行无血清培养基孵育2 h后,接受1 000 ng/mL LPS孵育6 h[18];LPS+sh-NC组,转染shRNA序列阴性对照(sh-NC),转染48 h后进行LPS处理;LPS+sh-SOCS3组,转染SOCS3 shRNA,转染48 h后进行LPS处理;LPS+sh-SOCS3+BAY组,转染SOCS3 shRNA,转染48 h后依次进行LPS处理和NF-κB抑制剂BAY 11-7082(10 μmol/L,美国MedChemExpress公司)处理。

shRNA转染方法如下:shRNA(sh-SOCS3-1和sh-SOCS3-2)及其阴性对照(sh-NC)设计、合成由上海生物生工公司完成。使用Lipofectamine™ 3000转染试剂盒(美国Invitrogen公司)进行细胞转染。转染48 h后,通过RT-qPCR及Western blot进行转染效率验证,选择转染效率较高者。

取BV2细胞及N2a细胞,并分为:N2a组,N2a细胞单独培养,不进行额外处理;N2a+BV2(Control)组,N2a细胞与Control组的BV2细胞共培养24 h;N2a+BV2(LPS)组,N2a细胞与LPS组的BV2细胞共培养24 h;N2a+BV2(LPS+sh-NC)组,N2a细胞与LPS+sh-NC组的BV2细胞共培养24 h;N2a+BV2(LPS+sh-SOCS3)组,N2a细胞与LPS+sh-SOCS3组的BV2细胞共培养24 h;N2a+BV2(LPS+sh-SOCS3+BAY)组,N2a细胞与LPS+sh-SOCS3+BAY组的BV2细胞共培养24 h。BV2及N2a共培养方法如下:将4×10^5个BV2细胞接种于Transwell上室;同时将8×10^5个N2a细胞接种于Transwell下室,共培养24 h。

1.5 ELISA检测IL-1β、TNF-α

吸取细胞培养基,在4 ℃下以1 000×*g*离心20 min,取上清液用于ELISA检测。使用小鼠IL-1β ELISA试剂盒(E-MSEL-M0003,武汉伊莱瑞特生物科技股份有限公司)及小鼠TNF-α ELISA试剂盒(E-EL-M3063,武汉伊莱瑞特生物科技股份有限公司)分别检测培养基中IL-1β、TNF-α含量,步骤严格遵循试剂盒说明书。

1.6 免疫荧光表征NF-κB p65

移除细胞培养基,加入4%多聚甲醛固定细胞15 min,移除固定液,洗涤3次后加入含5%正常羊血清的封闭液封闭细胞1 h。移除封闭液,洗涤3次后加入NF-κB p65抗体(美国CST公司,1∶400),4 ℃孵育过夜。移除NF-κB p65抗体,洗涤3次后加入荧光素偶联的山羊抗兔抗体(美国CST公司,1∶400),室温避光孵育1 h。加入DAPI试剂复染,进行中性树胶封片。荧光显微镜下观察NF-κB p65分布。

1.7 噻唑蓝(methyl thiazolyl tetrazolium, MTT)检测细胞活性

移除细胞培养基,加入10 μL MTT溶液(北京索莱宝科技有限公司)及90 μL新鲜培养基,37 ℃下孵育细胞4 h。移除细胞培养基,加入Formazan溶解液(北京索莱宝科技有限公司),摇床振荡10 min。采用酶标仪检测细胞样品在490 nm处的光密度[*D*(490)]值,计算细胞活性。

1.8 TUNEL检测细胞凋亡

移除细胞培养基,PBS溶液(北京索莱宝科技有限公司)洗涤1次后加入4%多聚甲醛(北京索莱宝科技有限公司)固定20 min,PBS溶液洗涤2次。采用TUNEL细胞凋亡检测试剂盒(北京索莱宝科技有限公司)进行TUNEL反应。加入DAPI染剂(北京索莱宝科技有限公司)复染5 min。荧光显微镜下观察细胞凋亡。

1.9 RNA提取和RT-qPCR

收集细胞样品或小鼠脑组织,采用TRIzol/氯仿法提取样品总RNA。采用TaqMan One Step RT-qPCR试剂盒(北京索莱宝科技有限公司)将RNA反转录为cDNA并进行荧光定量。PCR程序如下:反转录,50 ℃下反应20 min;预变性,95 ℃下反应3 min;变性,95 ℃下反应10~20 s,退火/延伸,60 ℃下反应20~60 s,循环35~40次。SOCS3引物序列如下:(上游引物)5'-GCTCCAAAAGCGAGTACCAG-3';(下游引物)5'-TGACGCTCAACGTGAAGAAG-3'。

1.10 Western blot检测蛋白相对水平

收集细胞或小鼠脑组织样品,加入裂解液(上海碧云天生物技术股份有限公司),待细胞裂解充分后以14 000×*g*离心5 min,取上清液。上清液中蛋白浓度经由BCA检测试剂盒(上海碧云天生物技术股份有限公司)检测。使用SDS-PAGE凝胶电泳系统(美国Bio-Rad公司)分离上清液蛋白,采用湿式转膜仪(美国Bio-Rad公司)进行冰转,将蛋白转移至PVDF膜(北京索莱宝科技有限公司)。加入封闭液(上海碧云天生物技术股份有限公司),室温摇床振荡1 h。移除封闭液,洗涤液洗涤3次,加入一抗酪氨酸羟化酶(tyrosine hydroxylase,TH)抗体(美国CST公司,1∶1 000),小胶质细胞标志物离子化钙结合衔接分子1(ionized calcium-binding adaptor molecule 1,IBA-1)抗体(美国CST公司,1∶1 000),路易小体标志物α-突触核蛋白(α-synuclein)抗体(美国CST公司,1∶1 000),SOCS3抗

体(美国CST公司,1∶1 000),NF-κB抗体(美国CST公司,1∶1 000),p-NF-κB抗体(美国CST公司,1∶1 000),β-actin抗体(美国CST公司,1∶1 000),4 ℃孵育过夜。移除一抗,洗涤液洗涤3次,加入二抗(美国CST公司,1∶10 000),室温孵育1 h。采用ECL显色试剂(上海碧云天生物技术股份有限公司)进行显色处理。上机,获取蛋白条带图像。以β-actin为内参,计算蛋白相对水平。

1.11 统计学分析

使用GraphPad Prism 10.0软件进行数据可视化处理,采用SPSS 26.0软件进行统计学分析。实验数据以$\bar{x}\pm s$表示。两组间比较采用独立样本t检验。多组间比较采用单因素方差分析,事后两两比较采用LSD-t检验。所有比较为双尾检验。P<0.05表示差异具有统计学意义。

2 结果

2.1 基于MPTP诱导的PD小鼠模型探究SOCS3和NF-κB的异常表现

采用MPTP刺激构建PD小鼠模型,通过旋转杆实验进行行为学评价,采用Western blot检测多巴胺能神经元标志物TH、α-synuclein、IBA-1。旋转杆实验结果显示,MPTP组跌落时间显著少于Sham组(P<0.01,图1A)。Western blot实验显示,与Sham组相比,MPTP组TH显著降低,IBA-1及α-synuclein显著升高(P<0.01,图1B、C)。可见PD小鼠建模成功,且PD小鼠表现为小胶质细胞激活。

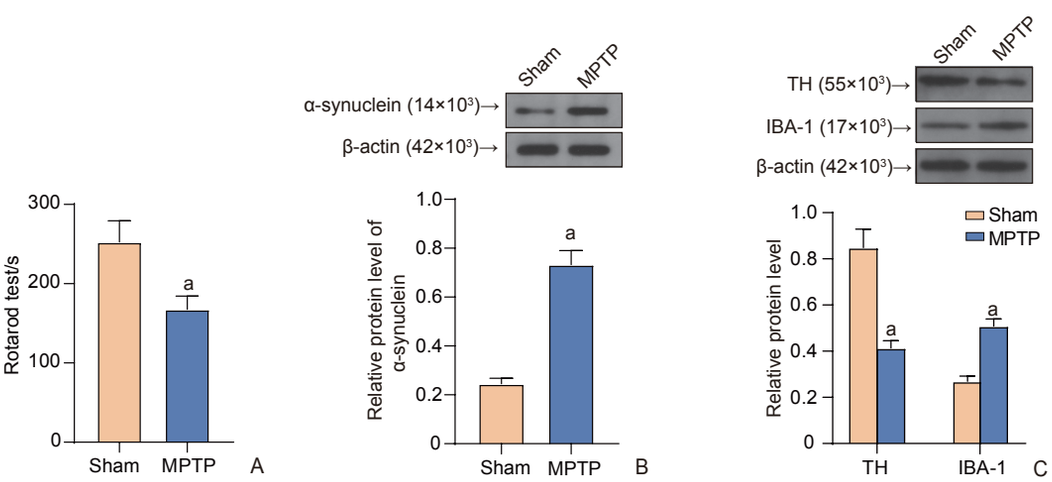
本研究检测SOCS3及NF-κB在小鼠脑组织中的表达。RT-qPCR结果显示,MPTP组SOCS3相对表达量显著低于Sham组(P<0.01,图2A)。Western blot结果显示,与Sham组相比,MPTP组SOCS3下调,p-NF-κB/NF-κB比值上调(P<0.01,图2B)。采用免疫组化检测小鼠脑组织NF-κB p65亚基蛋白水平,MPTP组NF-κB p65亚基阳性表达多于Sham组(P<0.01,图2C)。

2.2 基于LPS诱导的BV2细胞模型探究SOCS3和NF-κB的异常表现

鉴于小胶质细胞在PD小鼠脑组织中激活,本研究以LPS刺激的小胶质细胞模型为研究对象进行机制研究。ELISA检测结果显示,LPS组TNF-α和IL-1β含量显著高于Control组(P<0.01,图3A)。RT-qPCR结果显示LPS组SOCS3相对表达量显著低于Control(P<0.01,图3B)。Western blot结果表明,与Control相比,LPS组SOCS3显著降低,p-NF-κB/NF-κB比值显著升高(P<0.01,图3C)。

2.3 基于LPS诱导的BV2细胞模型观察SOCS3对NF-κB的影响

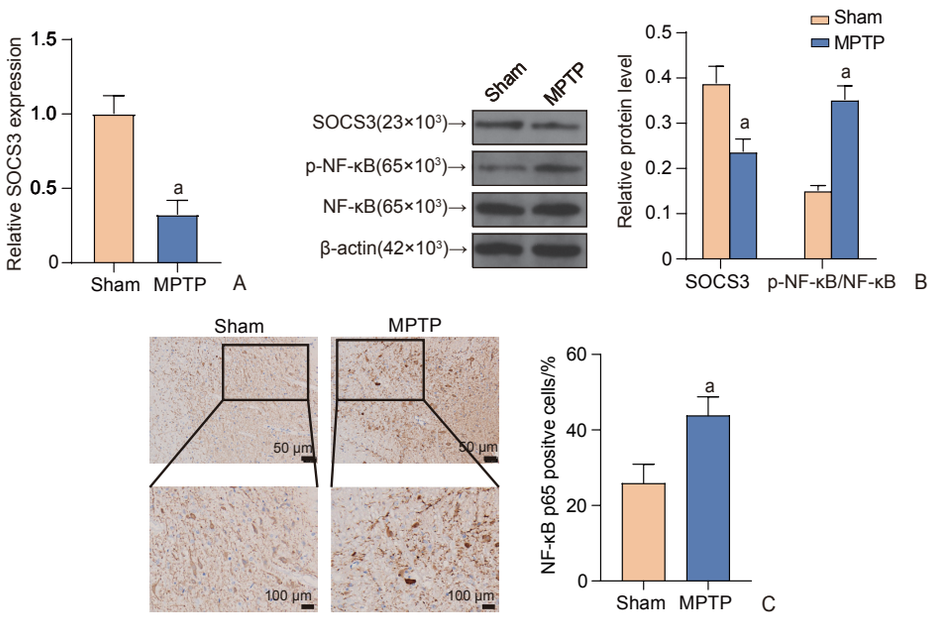
本研究构建两种SOCS3 shRNA序列以用于敲低SOCS3在BV2细胞中的表达。RT-qPCR及Western blot结果显示,sh-SOCS3-1组和sh-SOCS3-2组SOCS3表达均低于sh-NC组(P<0.01,图4A、B),sh-SOCS3-2组SOCS3表达显著低于sh-SOCS3-1组(P<0.01,图4A、B)。上述结果表明sh-SOCS3-2的敲低效果更佳。因此后续研究采用sh-SOCS3-2序列转染BV2细胞。相较于Control组,LPS+sh-NC组SOCS3表达降低,而p-NF-κB/NF-κB比值升高(P<0.01);相较于LPS+sh-NC组,LPS+sh-SOCS3组SOCS3降低,而p-NF-κB/NF-κB比值进一步升高(P<0.01,图4C、D)。



A: Rotarod test; B: Western blot analysis of protein level of α-synuclein; C: Western blot analysis of protein levels of TH and IBA-1. a: P<0.01, in comparison with the Sham

图1 基于MPTP构建PD小鼠模型

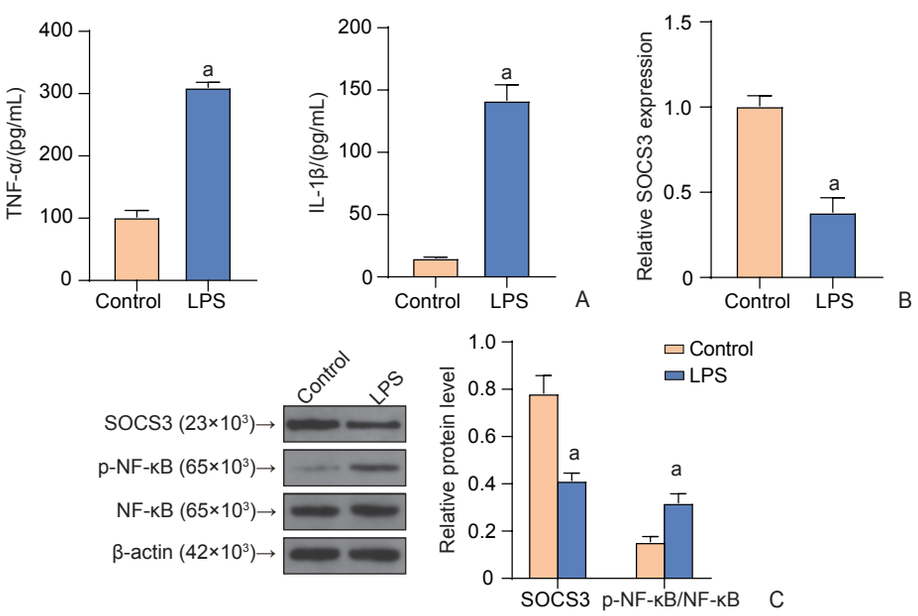
Figure 1 Establishment of MPTP-induced mouse model of Parkinson's disease (PD)



A: RT-qPCR analysis of relative expression of SOCS3; B: Western blot analysis of protein levels of SOCS3, NF-κB, and p-NF-κB; C: Immunohistochemical analysis of NF-κB p65. a: $P<0.01$, in comparison with the Sham

图2 SOCS3、NF-κB、p-NF-κB在Sham组和MPTP组脑组织中的表达水平

Figure 2 Expression of SOCS3, NF-κB and p-NF-κB in the striatum of Sham and MPTP-induced PD mice



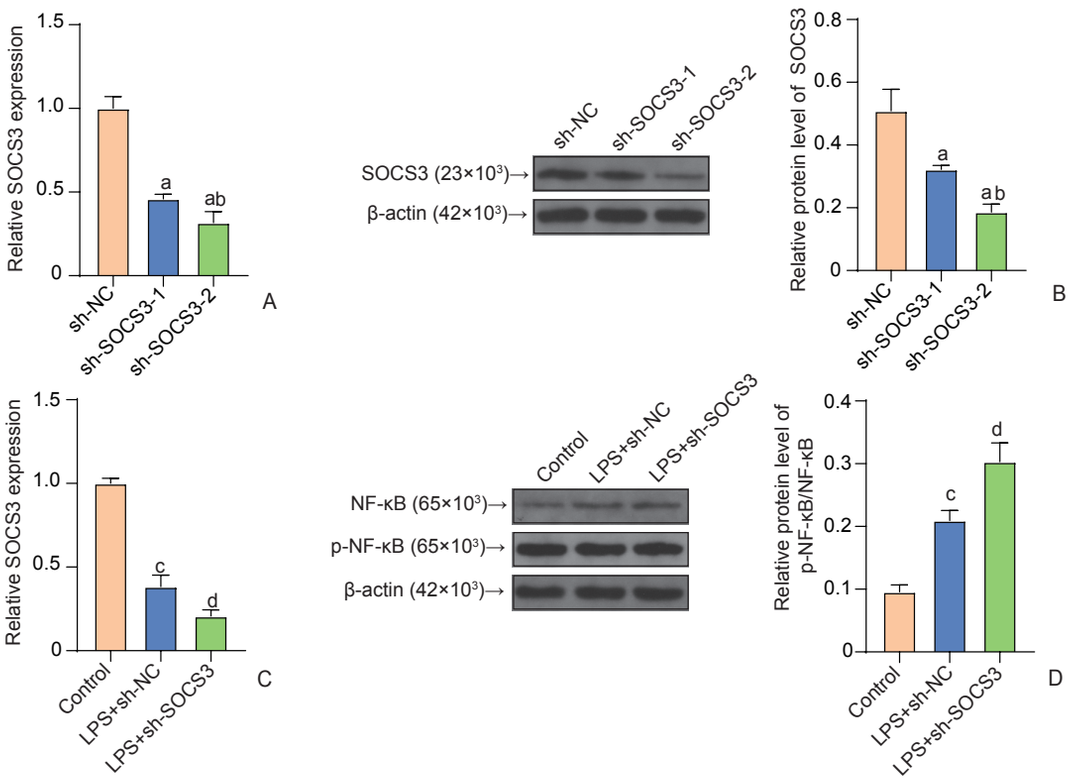
A: ELISA results of TNF-α and IL-1β; B: RT-qPCR analysis of relative expression of SOCS3; C: Western blot analysis of protein levels of SOCS3, NF-κB, and p-NF-κB. a: $P<0.01$, in comparison with the Control

图3 SOCS3和NF-κB在LPS诱导的BV2细胞模型中的蛋白水平

Figure 3 Protein levels of SOCS3 and NF-κB in an LPS-induced BV2 cell model

2.4 基于LPS诱导的BV2细胞模型证实NF-κB介导SOCS3的抗炎作用

Western blot结果显示,LPS+sh-NC组SOCS3低于Control组,而p-NF-κB/NF-κB比值高于Control组($P<0.01$)。LPS+sh-SOCS3组SOCS3低于LPS+sh-NC组($P<0.01$),而p-NF-κB/NF-κB比值高于LPS+sh-NC组($P<0.01$)。LPS+sh-SOCS3+BAY组与LPS+sh-SOCS3组的SOCS3无变化,而LPS+sh-SOCS3+BAY组p-NF-κB/NF-κB比值低于LPS+sh-SOCS3组($P<0.01$,图5A)。免疫荧光结果显示:LPS+sh-NC组与Control组相比,NF-κB p65亚基表达增强,部分细胞中呈现细胞核易位;LPS+sh-SOCS3组与LPS+sh-NC组相比,NF-κB p65



A: RT-qPCR analysis of relative expression of SOCS3; B: Western blot analysis of protein level of SOCS3; C: RT-qPCR analysis of relative expression of SOCS3; D: Western blot analysis of protein levels of NF-κB and p-NF-κB. a: $P<0.01$, in comparison with the sh-NC; b: $P<0.05$, in comparison with the sh-SOCS3-1; c: $P<0.01$, in comparison with the Control; d: $P<0.01$, in comparison with the LPS+sh-NC

图4 SOCS3对NF-κB激活的调控作用

Figure 4 Regulatory role of SOCS3 in NF-κB activation

亚基几乎发生核定位;LPS+sh-SOCS3+BAY组与LPS+sh-SOCS3组相比,NF-κB p65亚基核易位受抑制,胞质和胞核均有分布(图5B)。

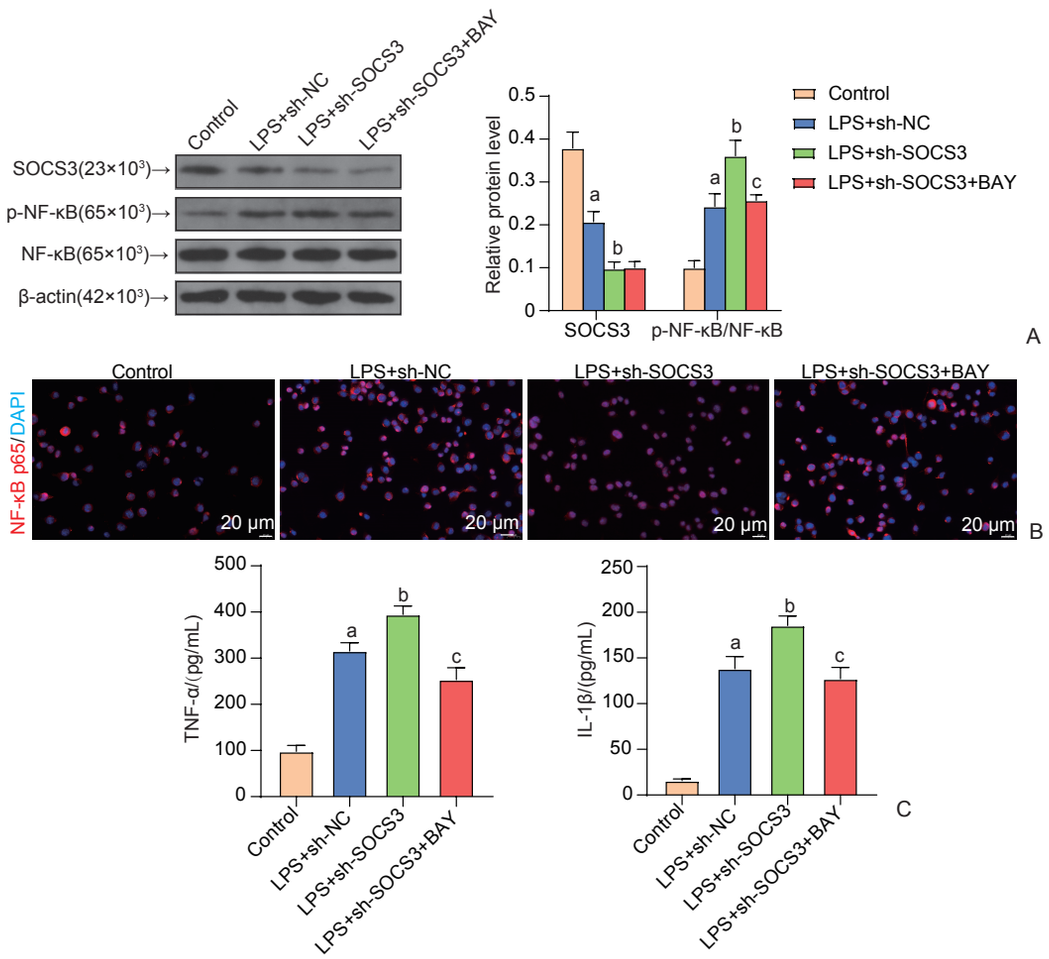
ELISA实验显示,LPS-sh-NC组与Control组相比,TNF-α及IL-1β含量升高($P<0.01$);LPS+sh-SOCS3组与LPS+sh-NC组相比,TNF-α及IL-1β含量进一步升高($P<0.01$);LPS+sh-SOCS3+BAY组与LPS+sh-SOCS3组相比,TNF-α及IL-1β含量降低($P<0.01$,图5C)。

2.5 基于细胞共培养模式证明SOCS3通过NF-κB调控小胶质细胞介导的神经元损伤

MTT结果显示,N2a+BV2(Control)组与N2a组的细胞活性差异无统计学意义,N2a+BV2(LPS)组细胞活性低于N2a+BV2(Control)组($P<0.01$),N2a+BV2(LPS+sh-SOCS3)组细胞活性小于N2a+BV2(LPS+sh-NC)组($P<0.01$),N2a+BV2(LPS+sh-SOCS3+BAY)组细胞活性高于N2a+BV2(LPS+sh-SOCS3)组($P<0.01$,图6A)。TUNEL染色结果显示,N2a+BV2(Control)组与N2a组相比细胞凋亡率差异无统计学意义,N2a+BV2(LPS)组与N2a+BV2(Control)组相比细胞凋亡率增加($P<0.01$),N2a+BV2(LPS+sh-SOCS3)组的细胞凋亡率高于N2a+BV2(LPS+sh-NC)组($P<0.01$),N2a+BV2(LPS+sh-SOCS3+BAY)组细胞凋亡率低于N2a+BV2(LPS+sh-SOCS3)组($P<0.01$,图6B)。

3 讨论

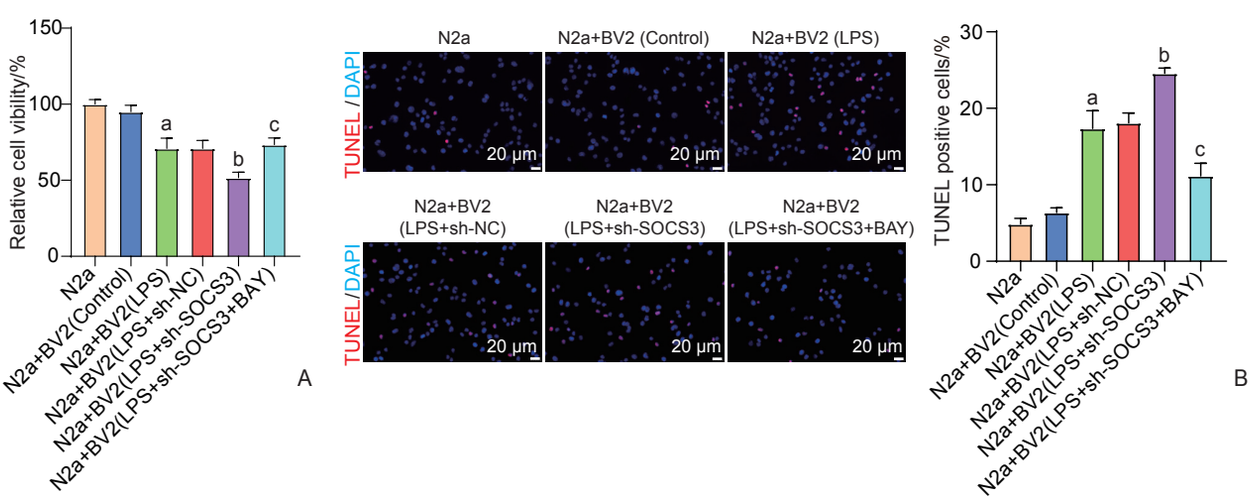
PD是一种常见的老年神经退行性疾病,可引起运动障碍、睡眠障碍等症状,严重降低患者日常生活质量。由于对PD发病机制的科学认知不足,因此尚缺失有效的临床治疗策略。本研究证实SOCS3在PD病理状况下表达显著下调,敲低SOCS3可进一步促进LPS诱导的小胶质细胞炎症。SOCS3具有神经保护作用,因而在PD等神经退行性疾病中发挥作用。新近研究发现,上调SOCS3通过抑制小胶质细胞活化及炎症反应干扰神经炎症诱导的脑组织损伤,提示SOCS3可能通过调控小胶质细胞发挥神经保护作用[20]。上述结果表明SOCS3表达下调可能是PD发病机制的必



A: Western blot analysis of protein levels of SOCS3, NF-κB, and p-NF-κB; B: Immunofluorescence analysis of protein level of NF-κB p65; C: ELISA results of TNF-α and IL-1β in each group. a: $P<0.01$, in comparison with the Control; b: $P<0.01$, in comparison with the LPS+sh-NC; c: $P<0.01$, in comparison with the LPS+sh-SOCS3

图5 NF-κB对SOCS3抗炎作用的影响

Figure 5 Effects of SOCS3 on NF-κB-mediated anti-inflammatory responses



A: CCK-8 assay of cell viability; B: TUNEL staining of apoptotic cells. a: $P<0.01$, in comparison with the N2a+BV2(Control); b: $P<0.01$, in comparison with the N2a+BV2(LPS+sh-NC); c: $P<0.01$, in comparison with the N2a+BV2(LPS+sh-SOCS3)

图6 SOCS3/NF-κB通路对小胶质细胞介导的神经元损伤的影响

Figure 6 Impact of the SOCS3/NF-κB signaling axis on microglia-mediated neuronal damage

要前提,其调控机制与小胶质细胞息息相关。

3.1 SOCS3下调通过促进小胶质细胞炎症加剧神经退行性病变

本研究发现NF-κB磷酸化水平在PD病理状况下升高,且SOCS3下调可进一步提高NF-κB磷酸化水平;进一步研究后证实SOCS3下调导致NF-κB p65亚基核定位增多。上述结果表明在炎性小胶质细胞中SOCS3抑制NF-κB活化和核转位。STAT3可能介导SOCS3对NF-κB的调控作用。STAT3既可与NF-κB结合,促进NF-κB活化,又可与NF-κB p65亚基相互作用,延长NF-κB核保留时间[21-22]。STAT3对NF-κB活化及核保留的影响最终增强NF-κB的转录活性。SOCS3是STAT3的负向调控因子,敲除SOCS3缺乏可增强STAT3活化,从而抑制下游炎症反应[23-25]。在机制上SOCS3通过抑制gp130下调STAT3磷酸化,从而干扰STAT3激活[26]。因此,在小胶质细胞中SOCS3可能通过抑制STAT3干扰NF-κB核保留和转录活性。

3.2 SOCS3通过STAT3抑制NF-κB在小胶质细胞中的活化与核转位

本研究证实敲低SOCS3诱导的NF-κB激活可促进小胶质细胞促炎因子的形成和释放,从而加剧神经炎症。NF-κB是关键的炎症介质。在正常生理状况下NF-κB在细胞质中保持非活化状态,外界刺激导致NF-κB激活,从而促进NF-κB易位至细胞核并作为转录因子调控促炎因子的转录表达[27]。因此,敲低SOCS3后小胶质细胞内NF-κB激活,从而促进促炎因子TNF-α及IL-6的转录。进一步研究后发现,当SOCS3敲低的小胶质细胞与神经元细胞共培养时,神经元细胞活性受抑制,神经元细胞凋亡显著增加。炎性小胶质细胞可向外大量释放促炎因子,进而导致神经炎症及神经元死亡,并最终引起多巴胺能神经变性及失调,加剧PD症状[28-30]。因此,SOCS3下调通过促进NF-κB激活,进而诱导小胶质细胞释放促炎细胞因子,由此加剧神经炎症及组织损伤,最终促进PD的病理进程。

本研究明确了SOCS3基于小胶质细胞抑制PD神经炎症的调控机制,并为“通过靶向调控SOCS3治疗PD症状”这一治疗思路提供了有力的理论基础。然而本研究仍存在不足之处:①本研究未能设置动物模型以验证SOCS3基于NF-κB抑制小胶质细胞炎症改善PD症状的作用机制;②本研究未能开展分子学实验验证SOCS3通过gp300/STAT3通路调控NF-κB转录活性的调控机制。后续研究将设置相应实验性模型,着重解决上述局限性。

综上所述,本研究证实SOCS3通过下调NF-κB活性抑制小胶质细胞炎症反应,进而减轻神经元细胞死亡,改善PD神经损伤。

利益冲突 本文所有作者声明不存在利益冲突

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