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·基础研究·

TRAF6 对抑郁症大鼠海马区神经元细胞损伤和免疫紊乱的调节

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[摘 要] **目的** 探讨肿瘤坏死因子受体相关因子6(TRAF6)对慢性不可预知性轻度应激(CUMS)大鼠的抗抑郁作用。**方法** 将40只健康SPF级SD大鼠随机分为对照组、模型组(CUMS组)、CUMS+shRNA-NC组和CUMS+TRAF6-shRNA组,每组10只。除对照组外,所有大鼠每天随机接受CUMS刺激,5周后,分别向CUMS+shRNA-NC组和CUMS+TRAF6-shRNA组大鼠海马区注射慢病毒包被的shRNA-NC和TRAF6-shRNA序列。RT-PCR和Western blot检测TRAF6 mRNA和蛋白的表达;ELISA检测大鼠血清中5-羟色胺(5-HT)、5-羟吲哚乙酸(5-HIAA)、TNF-α、iNOS、IL-6和IL-10的含量;酶法测定大鼠血清血脂;HE染色观察海马区病理状况;TUNEL染色检测海马区细胞凋亡情况;Western blot检测海马谷氨酸转运体(GLT-1)和脑源性神经营养因子(BDNF)及凋亡蛋白的表达。**结果** 与对照组相比,CUMS组大鼠海马区TRAF6 mRNA和蛋白表达量明显上调($P<0.05$),GLT-1和BDNF蛋白表达显著下调($P<0.05$),血清中5-HT和5-HIAA水平显著较低($P<0.05$),TC、TG和LDL-c水平较高($P<0.05$),HDL-c水平较低($P<0.05$);大鼠血脂代谢紊乱,海马区神经元数量减少,核固缩;凋亡细胞数目明显较多($P<0.05$),Bax/Bcl-2及cleaved Caspase3/Caspase3比值显著上调($P<0.05$);TNF-α、iNOS、IL-6和IL-10含量明显较高($P<0.05$)。与CUMS组相比,CUMS+TRAF6-shRNA组大鼠海马区TRAF6 mRNA和蛋白表达量明显下调($P<0.05$),GLT-1和BDNF蛋白表达显著上调,血清5-HT和5-HIAA水平较高($P<0.05$),TC、TG和LDL-c水平较低($P<0.05$),HDL-c水平较高($P<0.05$);海马区神经元完整,很少有核固缩;凋亡细胞数目明显较少($P<0.05$),Bax/Bcl-2及cleaved Caspase3/Caspase3比值显著下调($P<0.05$),TNF-α、iNOS、IL-6含量显著较低($P<0.05$),IL-10含量较高($P<0.05$)。**结论** 敲低TRAF6水平治疗抑郁症大鼠可能是通过调节大鼠神经递质、抑制海马神经元凋亡并抑制炎症反应来实现。

[关键词] 抑郁症;TRAF6;慢性不可预知性轻度应激;BDNF;凋亡;炎症

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Regulation of TRAF6 on neuron damage and immune disorder in hippocampus of depressed rats

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Abstract: Objective To explore the antidepressant effect of tumor necrosis factor receptor-associated factor 6(TRAF6) on chronic unpredictable mild stress (CUMS) rats. **Methods** Forty healthy SPF Sprague-Dawley (SD) rats were randomly divided into the control group, the model group(CUMS group), the CUMS+shRNA-NC group and the CUMS+TRAF6 -shRNA group, with 10 rats in each group. Except for the control group, all rats were randomly stimulated by CUMS every day. After 5 weeks, the CUMS+shRNA-NC group and the CUMS+TRAF6-shRNA group were respectively injected with lentiviral-coated shRNA-NC and TRAF6-shRNA sequences into the hippocampus of rats. RT-PCR and Western blot were used to detect the expression of TRAF6 mRNA and protein. ELISA was used to assay the content of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid(5-HIAA), TNF-α, iNOS, IL-6 and IL-10 in rat serum. Serum lipids of rats were determined by enzymatic method. HE staining was performed to observe the pathological status of hippocampus. TUNEL staining was commended to determine cell apoptosis of hippocampus. Western blot was employed to measure the expression of hippocampal glutamate transporter 1 (GLT-1), brain-derived neurotrophic factor(BDNF), and apoptosis proteins. **Results** Compared with the control group, the expression levels of TRAF6 mRNA and protein were significantly up-regulated ($P<0.05$); the expression of GLT-1 and BDNF protein were significantly down-regulated ($P<0.05$); the levels of 5-HT and 5-HIAA in rat sreum were significantly lower ($P<0.05$); the levels of TC, TG and LDL-c were significantly higher ($P<0.05$); the level of HDL-c was significantly lower in the CUMS group ($P<0.05$). In the CUMS group, rat blood lipid metabolism was disordered, the number of neurons in the hippocampus was reduced, and the nucleus was constricted; the number of apoptotic cells increased significantly($P<0.05$); the ratio of Bax/Bcl-2 and cleaved Caspase3/Caspase3 were significantly up-regulated ($P<0.05$);

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the levels of TNF-α, iNOS, IL-6 and IL-10 were significantly higher ($P<0.05$). Compared with the CUMS group, the expression levels of TRAF6 mRNA and protein were significantly down-regulated ($P<0.05$); the expression of GLT-1 and BDNF protein were significantly up-regulated ($P<0.05$); the levels of 5-HT and 5-HIAA were significantly higher; the levels of TC, TG and LDL-c were significantly lower ($P<0.05$); the level of HDL-c was significantly higher in the CUMS + TRAF6-shRNA group ($P<0.05$). In the CUMS + TRAF6-shRNA group, rat blood lipid metabolism indexes returned to normal levels, neurons in the hippocampus were intact, and there was few nuclear constriction; the number of apoptotic cells was significantly reduced ($P<0.05$), the ratio of Bax/Bcl-2 and cleaved Caspase3/Caspase3 were significantly down-regulated ($P<0.05$), and the levels of TNF-α, iNOS and IL-6 were significantly lower, and the level of IL-10 was significantly higher ($P<0.05$). **Conclusion** The knockdown of TRAF6 can restore the normal level of neurotransmitters in CUMS rats, and reduce the neuronal damage in the hippocampus of depressed rats through anti-apoptosis and anti-inflammatory effects.

Keywords: depression; TRAF6; chronic unpredictable mild stress; BDNF; apoptosis; inflammation

抑郁症是一类严重的精神疾病,主要表现为患者长时间持续的情绪低落,已经成为世界范围内的一种常见病,影响了大约3.5亿人[1-2]。根据世界卫生组织的预测,抑郁症的发病率每年约增加113%,将成为世界第二大疾病[3]。研究表明,先天性和适应性免疫系统与神经递质和神经回路相互作用,可影响抑郁症的发生[4-5]。许多临床报告表明,与正常受试者相比,抑郁症受试者存在较高浓度的促炎细胞因子,如肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)和白介素-1β(interleukin-1β, IL-1β)[6-7]。因此,控制神经炎症,恢复神经营养蛋白和神经递质的功能可成为抗抑郁治疗的新方向。肿瘤坏死因子受体相关因子6(tumor necrosis factor receptor-associated factor 6, TRAF6)在多种免疫细胞中广泛表达,并作为重要的信号分子参与NF-κB和MAPK等炎症途径的激活[8]。研究表明,miRNA-194可通过靶向TRAF6抑制脂多糖诱导的椎间盘髓核细胞中的炎症反应[9]。本研究通过检测慢性不可预知性轻度应激(chronic unpredictable mild stress, CUMS)海马中神经递质、神经营养因子和炎性因子的变化,研究敲低TRAF6对抑郁大鼠的作用。

1 材料与方法

1.1 材料

健康SPF级雄性SD大鼠40只,5~6周龄,体质量为(180±20)g,购自海军军医大学实验动物中心,动物生产许可证号为SCXK(沪)2021-0003。实验动物在温度(24±2)℃、湿度50%左右的12 h/12 h光—暗环境下适应性喂养1周。TRIzol试剂购自中国赛默飞世尔公司,反转录试剂盒购自大连宝生物工程有限公司,白细胞介素-6(interleukin-6, IL-6)、白细胞介素-10(interleukin-6, IL-10)、TNF-α、诱导型一氧化氮合酶(inducible nitric oxide synthase, iNOS)酶联免疫吸附试验(enzyme linked immunosorbent assay, ELISA)试剂盒购自美国R&D公司,5-羟色胺(5-hydroxytryptamine, 5-HT)、5-羟吲哚乙酸(5-hydroxyindoleacetic acid, 5-HIAA)ELISA试剂盒购自北京北方生物技术研究所有限公司,RIPA裂解缓冲液、苏木精—伊红(hematoxylin-eosin, HE)染色试剂盒购自北京索莱宝公司,BCA试剂盒购自上海碧云天公司,GAPDH、Bax、Bcl-2、Caspase3一抗购自英国Abcam公司,TRAF6、谷氨酸转运体-1(glutamate transporter-1, GLT-1)、脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)抗体及辣根酶标记二抗购自北京博奥森公司,ECL发光液购自上海碧云天生物技术有限公司,聚偏二氟乙烯(polyvinylidene fluoride, PVDF)购自上海密理博公司,血脂生化检测试剂盒购自上海透景诊断科技有限公司,TUNEL试剂盒购自美国Sigma公司,Nanodrop 2000购自美国赛默飞世尔公司。

1.2 CUMS抑郁大鼠模型构建

将40只SD大鼠随机分为对照组、模型组(CUMS组)、CUMS + shRNA-NC组、CUMS + TRAF6-shRNA组,每组10只。对照组每笼2只饲养,正常饮食饮水;其他3组每笼1只饲养,35 d内随机接受以下刺激:倾斜45° 24 h;4 ℃冰水游泳5 min;禁食禁水24 h;夹尾2 min;潮湿垫料24 h;拥挤24 h(每笼10只);束缚应激6 h。每天接受其中1种应激源,连续2 d不重复相同刺激。通过大鼠精神状态、活动时间及生化指标(神经递质水平)判断模型是否成功建立。

1.3 立体定向注射

CUMS刺激5周后,CUMS + shRNA-NC组、CUMS + TRAF6-shRNA组大鼠分别注射慢病毒包被的shRNA-NC和TRAF6-shRNA序列(上海吉玛基因)。用10%水合氯醛(250 mg/kg)腹腔麻醉大鼠,然后置于立体定向框架内。根据Shen等[10]的研究方法,将纯化的高滴度shRNA-NC和TRAF6-shRNA慢病毒包装溶液以1 μL/min的注射速率在囟前后4.8 mm,中外侧±2.5 mm,背腹侧-3.5 mm处钻孔注射慢病毒包装溶液,注射完毕后针在注射部位保持5 min防止返流,所有大鼠术后继续观察饲养5 d。

1.4 血浆和海马组织样本收集

1.3项下实验完成后,大鼠禁食12 h,随后用10%

水合氯醛(250 mg/kg)对各组大鼠实施安乐死。从腹主动脉采集血液,分离血清,于 -80 ℃储存,用于检测生化指标和炎性因子含量(每组保存 8 份样品)。将大脑组织迅速取出,置于冰上,分离海马区组织,每组分别取 5 只大鼠的海马组织保存于 4% 多聚甲醛溶液,用于病理研究,另 5 只大鼠的海马组织用液氮快速冷冻后于 -80 ℃保存,用于检测基因的表达。

1.5 RT-PCR 实验检测 mRNA 表达

使用 TRIzol 试剂提取海马组织总 RNA,用 Nanodrop 2000 测定 RNA 的纯度和浓度。取等质量的总 RNA 作为模板,根据说明书反转录成 cDNA。以 cDNA 为模板,PCR 反应条件为:95 ℃ 30 s;95 ℃ 3 s,60 ℃ 30 s,40 个循环。通过 $2^{-\Delta\Delta ct}$ 法计算基因表达水平,以 β-肌动蛋白(β-actin)为内参。引物序列如下:TRAF6 上游引物 5'-TCATAATGTTAACCTCTCTT-3',下游引物 5'-TGTCTTACTAGGCGCCCCTC-3';β-actin 上游引物 5'-GCCTCCCGCGCGCGCACTAG-3',下游引物 5'-AAAGGCAAACGCTGGCCCGG-3'。

1.6 Western blot 检测蛋白表达

使用 RIPA 裂解缓冲液提取海马组织总蛋白,用 Nanodrop 2000 测量蛋白浓度并调平,用十二烷基硫酸钠—聚丙烯酰胺凝胶电泳分离蛋白,然后将蛋白转移到 PVDF 膜上。将膜用含有 5% 脱脂牛奶的 TBST 缓冲液封闭 1 h,然后与一抗(TRAF6 1∶1 000,GLT-1 1∶1 000,BDNF 1∶1 000,Bax 1∶1 000,Bcl-2 1∶1 000,Caspase3 1∶1 000 和 GAPDH 1∶1 000)于 4 ℃孵育过夜。TBST 洗涤 3 次,并与 HRP 偶联的二抗 IgG(1∶3 000)于 37 °C 孵育 2 h。使用 ECL 曝光成像并用 Image J 软件分析蛋白条带的灰度值。

1.7 ELISA 实验

按照 ELISA 试剂盒说明书检测大鼠血清炎性因子 TNF-α、IL-6、IL-10、iNOS 和血清神经递质 5-HT 和 5-HIAA 的含量。

1.8 血脂生化检测

按血脂生化试剂盒说明书酶法测定大鼠血清中总胆固醇(total cholesterol,TC)、三酰甘油(triglyceride,TG)、高密度脂蛋白胆固醇(high density lipoprotein cholesterin,HDL-c)和低密度脂蛋白胆固醇(low density lipoprotein cholesterin,LDL-c)水平。

1.9 HE 染色观察海马神经元病理变化

将大脑海马区组织用 4% 多聚甲醛溶液固定后,常规脱水制成石蜡切片,然后脱蜡、HE 染色、1% 盐酸酒精分化、0.6% 氨水返蓝、0.5% 伊红液染色,接着常规脱水,二甲苯透明,中性树胶封片,在 400 倍显微镜下观察神经细胞损伤情况。

1.10 TUNEL 染色海马区细胞凋亡情况

将石蜡切片脱蜡,按照 TUNEL 细胞凋亡试剂盒的操作进行染色,封片后使用光学显微镜观察凋亡细胞。

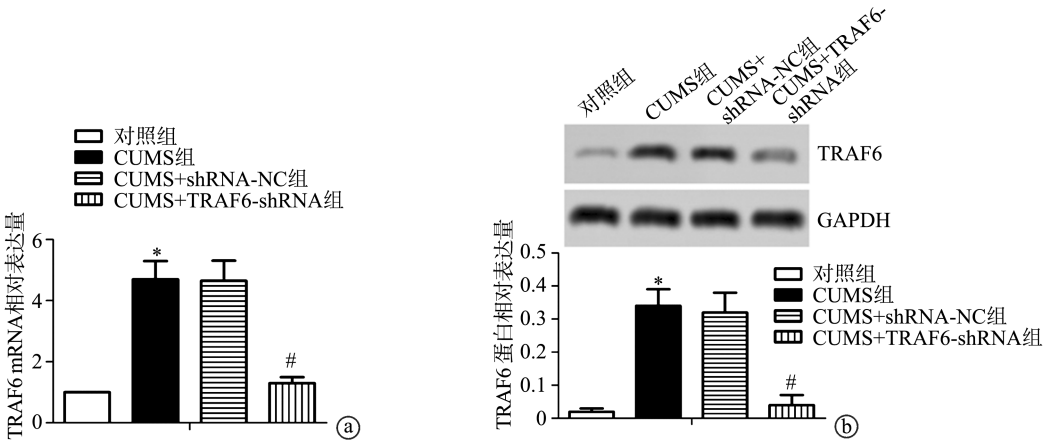
1.11 统计学分析

使用 SPSS 17.0 软件对数据进行统计分析,所有数据均以均数 ± 标准差($\bar{x} \pm s$)表示,采用单因素方差分析法比较多组间差异,两组间比较采用独立样本 t 检验。$P<0.05$ 为差异具有统计学意义。

2 结果

2.1 shRNA 敲低 TRAF6 效率验证

与对照组比较,CUMS 组大鼠海马区 TRAF6 mRNA和蛋白表达水平较高($P<0.05$);与 CUMS 组比较,CUMS + TRAF6-shRNA 组大鼠海马区 TRAF6 mRNA 和蛋白表达水平较低($P<0.05$);CUMS + shRNA-NC 组与 CUMS 组比较则差异无统计学意义($P>0.05$),提示 TRAF6-shRNA 干扰成功(图 1)。

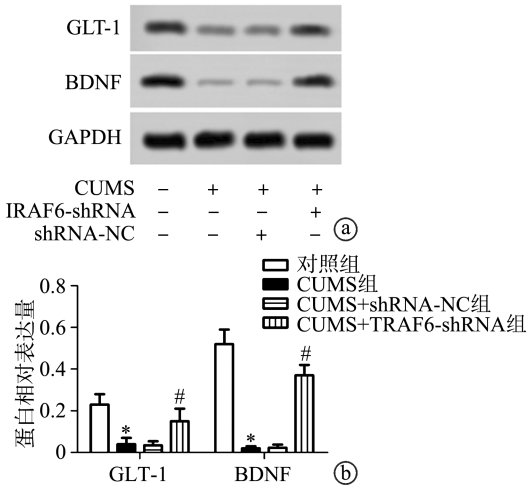


a:RT-PCR 检测 TRAF6 mRNA 相对表达水平;b:Western blot 检测 TRAF6 蛋白表达水平 *:与对照组比较,$P<0.05$;#:与 CUMS 组比较,$P<0.05$

图 1 大鼠海马区 TRAF6 mRNA 和蛋白的表达

2.2 敲低 TRAF6 对 CUMS 大鼠海马区神经递质表达水平的影响

与对照组比较,CUMS 组大鼠海马区 GLT-1 和 BDNF 表达显著下调($P<0.05$),血清中 5-HT 和 5-HIAA水平明显较低($P<0.05$);与 CUMS 组比较,CUMS + TRAF6-shRNA 组大鼠海马区 GLT-1 和 BDNF 表达显著上调($P<0.05$),血清中 5-HT 和 5-HIAA 水平明显较高($P<0.05$);CUMS + shRNA-NC 组与 CUMS 组各指标比较差异无统计学意义($P>0.05$),见图 2、表 1。



a:Western blot 检测 GLT-1 和 BDNF 蛋白的表达;b:GLT-1 和 BDNF 蛋白相对表达量 *:与对照组比较,$P<0.05$;#:与 CUMS 组比较,$P<0.05$

图 2 敲低 TRAF6 对 CUMS 大鼠海马区 GLT-1 和 BDNF 表达水平的影响

表 1 ELISA 检测大鼠血清中 5-HT 和 5-HIAA 的水平($\bar{x}\pm s$,$n=8$,ng/mg)

组别	5-HT	5-HIAA
对照组	21.33 ± 5.26	31.32 ± 6.06
CUMS 组	7.84 ± 2.52 *	10.61 ± 4.38 *
CUMS + shRNA-NC 组	7.77 ± 1.63	11.03 ± 3.24
CUMS + TRAF6-shRNA 组	16.91 ± 6.48#	24.84 ± 4.13#

*:与对照组比较,$P<0.05$;#:与 CUMS 组比较,$P<0.05$

2.3 敲低 TRAF6 对 CUMS 大鼠血清脂代谢的影响

与对照组比较,CUMS 组大鼠海马区 TC、TG 和 LDL-c 水平明显较高($P<0.05$),HDL-c 水平明显较低($P<0.05$);与 CUMS 组比较,CUMS + TRAF6-shRNA 组 TC、TG 和 LDL-c 水平明显较低($P<0.05$),HDL-c 水平明显较高($P<0.05$);CUMS + shRNA-NC 组与 CUMS 组各指标比较差异无统计学意义($P>0.05$),见表 2。

2.4 敲低 TRAF6 对 CUMS 大鼠海马神经元损伤的影响

对照组正常海马神经元呈圆形,结构清晰完整,排列良好;CUMS 组海马神经元核固缩,染色加深,呈不规则、多边形和梭形,且神经元数量减少,排列松散。与 CUMS 组比较,CUMS + TRAF6-shRNA 组大部分神经元完整,很少有核固缩;CUMS + shRNA-NC 组神经元形态与数量与 CUMS 组相似(图 3)。

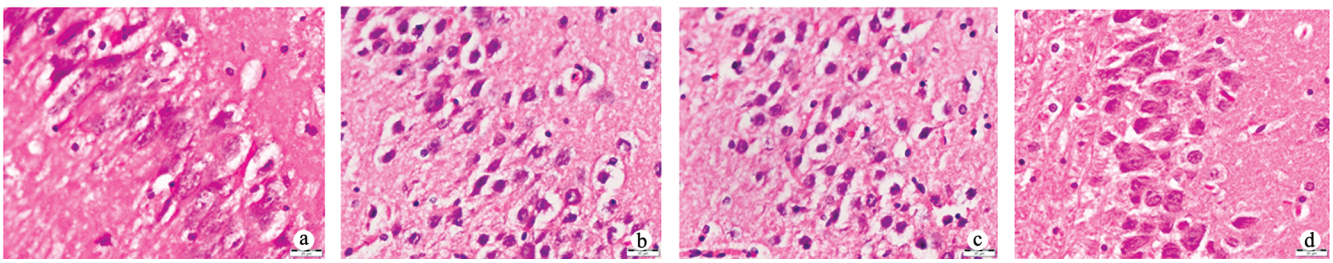
2.5 敲低 TRAF6 对 CUMS 大鼠海马区细胞凋亡的影响

与对照组比较,CUMS 组细胞凋亡数目显著较多($P<0.05$),Bax/Bcl-2 及 cleaved Caspase3/Caspase3 比值明显上调($P<0.05$);与 CUMS 组比较,CUMS + TRAF6-shRNA 组细胞凋亡数目明显较少($P<0.05$),Bax/Bcl-2 及 cleaved Caspase3/Caspase3 比值显著下调($P<0.05$);CUMS + shRNA-NC 组与 CUMS 组细胞凋亡数目、Bax/Bcl-2、cleaved Caspase3/Caspase3 比较差异无统计学意义($P>0.05$),见图 4。

表 2 大鼠血清 TC、TG、LDL-c 和 HDL-c 的水平($\bar{x}\pm s$,$n=8$,mmol/L)

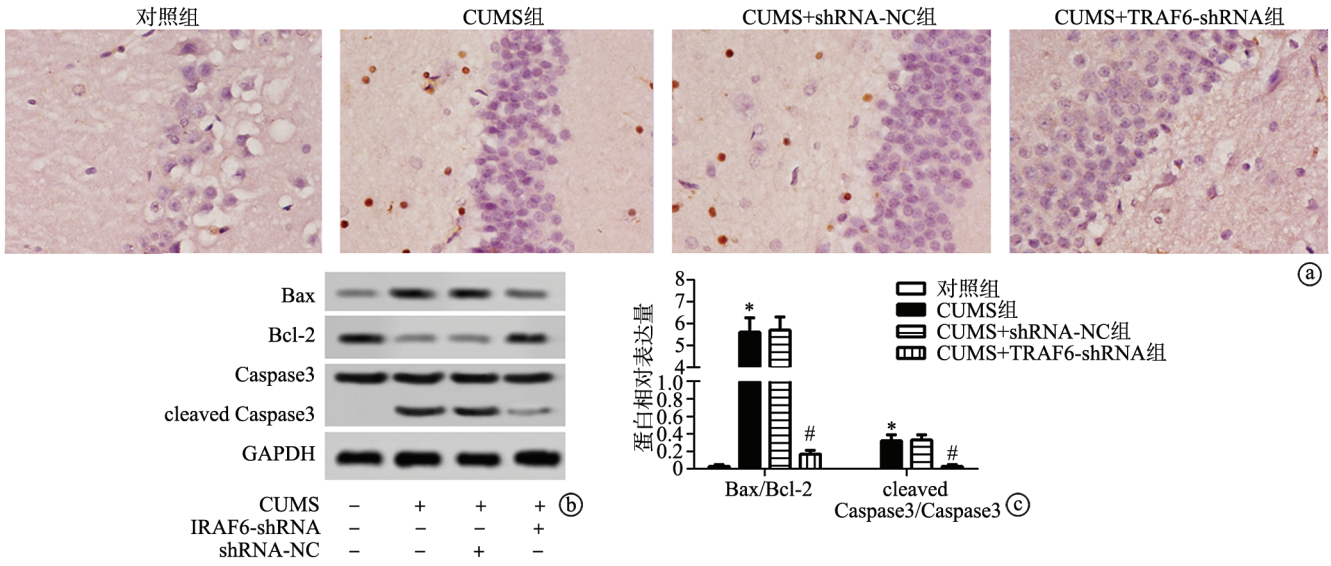
组别	TC	TG	LDL-c	HDL-c
对照组	1.03 ± 0.46	0.32 ± 0.09	0.13 ± 0.05	1.67 ± 0.49
CUMS 组	4.94 ± 0.57 *	1.71 ± 0.67 *	0.82 ± 0.07 *	0.61 ± 0.07 *
CUMS + shRNA-NC 组	4.71 ± 0.93	1.66 ± 0.24	0.85 ± 0.11	0.56 ± 0.09
CUMS + TRAF6-shRNA 组	1.97 ± 0.88#	0.84 ± 0.18#	0.31 ± 0.09#	1.28 ± 0.52#

*:与对照组相比,$P<0.05$;#:与 CUMS 组相比,$P<0.05$



a:对照组;b:CUMS 组;c:CUMS + shRNA-NC 组;d:CUMS + TRAF6-shRNA 组

图 3 HE 染色观察海马神经元病理变化(×400)



a:TUNEL 染色检测细胞凋亡(×400);b:Western blot 检测凋亡蛋白的表达;c:Bax/Bcl-2 和 cleaved Caspase3/Caspase3 蛋白比值

*:与对照组相比,$P<0.05$;#:与 CUMS 组相比,$P<0.05$

图 4 敲低 TRAF6 对 CUMS 大鼠海马区细胞凋亡的影响

2.6 敲低 TRAF6 对 CUMS 大鼠血清中炎性因子表达的影响

与对照组比较,CUMS 组血清中 TNF-α、iNOS、IL-6 和 IL-10 的水平均明显上调($P<0.05$);与 CUMS 组比较,CUMS + TRAF6-shRNA 组中 TNF-α、iNOS 和 IL-6 水平下调($P<0.05$),IL-10 水平上调($P<0.05$);CUMS + shRNA-NC 组与 CUMS 组炎性因子水平比较差异无统计学意义($P>0.05$),见表 3。

表 3 大鼠血清中炎性因子表达水平($\bar{x}\pm s$,$n=8$,pg/mL)

组别	TNF-α	iNOS	IL-6	IL-10
对照组	12.03 ± 4.32	15.32 ± 3.66	13.14 ± 4.25	4.57 ± 1.41
CUMS 组	124.84 ± 24.52*	97.61 ± 15.38*	78.82 ± 16.07*	8.51 ± 3.17*
CUMS + shRNA-NC 组	131.07 ± 31.63	98.06 ± 17.24	82.19 ± 14.06	8.46 ± 2.06
CUMS + TRAF6-shRNA 组	65.91 ± 16.08#	38.14 ± 6.13#	30.41 ± 8.14#	17.18 ± 4.32#

*:与对照组相比,$P<0.05$;#:与 CUMS 组相比,$P<0.05$

3 讨论

CUMS 诱导的大鼠抑郁模型模拟了多种慢性生活压力引发的抑郁症,已被广泛应用于评价药物的抗抑郁作用[11]。单胺类氧化酶抑制剂可通过抑制突触间隙中单胺类神经递质的降低,防止单胺类缺乏导致抑郁发生[12]。其中,5-HT 活性降低被认为与重度抑郁症的发展有关[13]。但有证据表明缺乏神经营养因子是抑郁症的另一个发病机制,BDNF 是介导中枢和外周神经系统神经元可塑性、迁移和存活的关键调节因子,抑郁患者的 BDNF 水平异常低[14-16]。此外,谷氨酸过量也与重度抑郁症的发生有关,GLT-1 可降低突触间隙谷氨酸水平,避免过高浓度的谷氨酸对神经元和神经胶质细胞的兴奋毒性作用[17]。本研究通过 CUMS 构建大鼠抑郁模型,结果显示,CUMS 组大鼠血清中 GLT-1、BDNF、5-HT 及其代谢产物 5-HIAA 的表达水平均显著下调,表明大鼠抑郁模型构建成功;敲低 TRAF6 可显著上调 CUMS 大鼠海马区 BDNF、GLT1 蛋白及血清中 5-HT 和 5-HIAA 的表达水平,提示敲低 TRAF6 可促进抑郁大鼠血清中低水平的神经递质恢复。

临床研究表明,抑郁症患者更易出现血脂代谢紊乱的状况,即 TG、TC、LDL-c 含量偏高和 HDL-c 含量偏低[18-19]。本研究结果显示,CUMS 组大鼠血清中 TG、TC 和 LDL-c 含量显著高于对照组,HDL-c 含量显著低于对照组;CUMS + TRAF6-shRNA 组大鼠血清中 TG、TC 和 LDL-c 含量明显低于 CUMS 组,HDL-c 含量明显高于 CUMS 组,提示敲低 TRAF6 的表达可使 CUMS 引起的大鼠血清参数紊乱恢复正常。

海马体是大脑对压力反应最脆弱的区域之一,事实上,慢性应激诱导的海马结构重塑和功能损伤(包括突触可塑性改变、神经发生减少、树突状萎缩增加和神经元凋亡增加)都与抑郁症和认知缺陷有关[20]。本研究观察到 CUMS 组大鼠海马神经元核固缩,染色加深,呈不规则、多边形和梭形,且神经元数量减少,排列松散;海马区细胞凋亡数目显著增加,Bax/Bcl-2 及 cleaved Caspase3/Caspase3 比值明显上调;与 CUMS 组相比,CUMS + TRAF6-shRNA 组大鼠海马神经元结构清晰完整,排列良好,很少有核固缩,而且细胞凋亡数

目显著减少,Bax/Bcl-2 和 cleaved Caspase3/Caspase3 比值表达明显下调。由此推测,敲低 TRAF6 可逆转抑郁引起的海马损伤及细胞凋亡。

研究已证实炎性因子的过度分泌与下丘脑—垂体—肾上腺轴功能障碍有关,并直接诱导糖皮质激素分泌,这种反应与抑郁症的发生有关[21]。炎症因子分为促炎症因子和抑炎症因子,促炎因子 TNF-α、IL-6 和 IL-1β在抑郁患者和抑郁动物模型的血清、脑脊液和海马中表达增加,会负反馈调节抑炎症因子 IL-10,使其表达降低,导致中枢神经系统的神经炎症,进而导致神经元损伤[22-23]。本研究结果表明 CUMS 可诱导促炎症因子 TNF-α、iNOS、IL-6 的表达升高。敲低 TRAF6 能下调 CUMS 大鼠促炎性因子 TNF-α、iNOS 和 IL-6 的表达,同时促进大鼠机体的自我免疫调节抑制炎症反应,从而使得 IL-10 的表达也升高。由于本研究检测时间与大鼠敲低 TRAF6 发生炎症较为接近,大鼠机体免疫尚未完全控制炎症,因此大鼠体内的抗炎因子表达也显著升高。

综上所述,在 CUMS 大鼠中,敲减 TRAF6 的表达具有较好的抗抑郁作用,可减轻海马神经元损伤,恢复神经递质和血脂代谢的正常水平,抑制神经元凋亡和神经炎症反应。

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