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·基础研究·

lncRNA NEAT1促进脓毒症诱导肝细胞损伤和炎症反应的机制研究

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[摘 要] **目的** 基于lncRNA NEAT1调控的miR-129-5p/TLR4信号轴探讨脓毒症诱导的肝细胞损伤和炎症反应机制。**方法** 收集15例脓毒症患者外周血(脓毒症组)和15例健康者外周血(健康组)用于检测基因表达。体外实验使用小鼠正常肝细胞株NCTC1469,并用脂多糖(LPS)建立体外脓毒症模型(LPS组),将LPS刺激下转染mimic NC、mimic、siNC或siNEAT1质粒载体的细胞分别命名为LPS+mimic NC组、LPS+mimic组、LPS+siNC组或LPS+siNEAT1组,将LPS、siNEAT1、TLR4重组蛋白或LPS、mimic、TLR4重组蛋白联合处理的细胞命名为LPS+siNEAT1+TLR4组或LPS+mimic+TLR4组,而对照组不作处理。分别用qPCR和ELISA检测TNF-α、IL-6、IL-1β的mRNA和蛋白表达水平。TUNEL实验检测细胞的凋亡率。双荧光素酶报告基因分析法检测lncRNA NEAT1/miR-129-5p和miR-129-5p/TLR4的结合。Western blot检测细胞中TLR4的表达。**结果** 在体内实验中,与健康组比较,脓毒症组外周血中TNF-α、IL-6、IL-1β、lncRNA NEAT1和TLR4的表达明显增加($P<0.05$),miR-129-5p表达减少($P<0.05$)。在体外实验中,与对照组比较,LPS组lncRNA NEAT1表达增加($P<0.05$)。与LPS组、LPS+siNC组比较,LPS+siNEAT1组的lncRNA NEAT1表达降低($P<0.05$),miR-129-5p表达增加($P<0.05$)。与对照组比较,LPS+siNC组细胞凋亡率及TLR4、TNF-α、IL-6、IL-1β的表达均上调($P<0.05$)。与LPS+siNC组比较,LPS+siNEAT1组细胞凋亡率及TLR4、TNF-α、IL-6、IL-1β的表达均下调($P<0.05$)。与LPS+siNEAT1组比较,LPS+siNEAT1+TLR4组细胞凋亡率及TLR4、TNF-α、IL-6、IL-1β的表达均上调($P<0.05$)。双荧光素酶报告基因实验表明lncRNA NEAT1与miR-129-5p存在相互作用,TLR4是miR-129-5p的靶基因。与对照组比较,LPS+mimic NC组TNF-α、IL-6、IL-1β的表达和细胞凋亡率均增加($P<0.05$);与LPS+mimic NC组比较,LPS+mimic组TNF-α、IL-6、IL-1β的表达和细胞凋亡率均下调($P<0.05$);与LPS+mimic组比较,LPS+mimic+TLR4组TNF-α、IL-6、IL-1β的表达和细胞凋亡率上调($P<0.05$)。**结论** lncRNA NEAT1与miR-129-5p存在相互作用,并直接靶向TLR4以促进LPS诱导的肝细胞损伤和炎症反应。本研究可为脓毒症引起的肝细胞损伤提供新的治疗靶点。

[关键词] lncRNA NEAT1;miR-129-5p;脓毒症;肝细胞损伤;炎症反应

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Mechanism of lncRNA NEAT1 promoting hepatocyte injury and inflammatory reaction induced by sepsis

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Abstract: Objective To explore the mechanism of hepatocyte injury and inflammatory reaction induced by sepsis based on miR-129-5p/TLR4 signaling axis regulated by lncRNA NEAT1. **Methods** Peripheral blood from 15 sepsis patients (the sepsis group) and 15 healthy individuals (the healthy group) were collected for gene expression detection. The mouse normal liver cell line NCTC1469 was used in in vitro experiments, and an in vitro sepsis model (the LPS group) was established by lipopolysaccharide (LPS). The cells stimulated with LPS and transfected with mimic NC, mimic, siNC or siNEAT1 plasmid vectors were named as the LPS+mimic NC group, the LPS+mimic group, the LPS+siNC group or the LPS+siNEAT1 group, respectively. The cells treated with a combination of LPS, siNEAT1, and TLR4 recombinant protein or LPS, mimic, TLR4 recombinant protein were named as the LPS+siNEAT1+TLR4 group or the LPS+mimic+TLR4 group, while the control group was not treated. The mRNA and protein expression levels of TNF-α, IL-6, and IL-1β were detected by qPCR and ELISA, respectively. The cell apoptosis rate was detected by TUNEL assay. The binding of lncRNA NEAT1/miR-129-5p and miR-129-5p/TLR4 was detected by dual-luciferase reporter gene analysis. The TLR4 expression in cells was detected by Western blot. **Results** In the in vivo experiment, compared with the healthy group, the expression of TNF-α, IL-6, IL-1β, lncRNA NEAT1, and TLR4 of peripheral blood in the sepsis group was significantly increased (P<0.05), while the expression of miR-129-5p was decreased (P<0.05). In the in vitro experiment, compared with the control group, the expression of lncRNA NEAT1 in the LPS group was increased (P<0.05). Compared with the LPS group and the LPS+siNC group, the expression of lncRNA NEAT1 in the LPS+siNEAT1 group was decreased (P<0.05), and the expression of miR-129-5p was increased (P<0.05). Compared with the control group, the cell apoptosis rate and the expression of TLR4, TNF-α, IL-6, and IL-1β in the LPS+siNC group were up-regulated (P<0.05). Compared with the LPS+siNC group, the cell apoptosis rate and the expression of TLR4, TNF-α, IL-6, and IL-1β in the LPS+siNEAT1 group were down-regulated (P<0.05). Compared with the LPS+siNEAT1 group, the cell apoptosis rate and the expression of TLR4, TNF-α, IL-6, and IL-1β in the LPS+siNEAT1+TLR4 group were up-regulated (P<0.05). Dual-luciferase reporter gene assay revealed an interaction between lncRNA NEAT1 and miR-129-5p, and TLR4 was the target gene of miR-129-5p. Compared with the control group, the expression of TNF-α, IL-6, and IL-1β as well as cell apoptosis rate in the LPS+mimic NC group were increased (P<0.05). Compared with the LPS+mimic NC group, the expression of TNF-α, IL-6, and IL-1β as well as cell apoptosis rate in the LPS+mimic group were down-regulated (P<0.05). Compared with the LPS+mimic group, the expression of TNF-α, IL-6, and IL-1β as well as cell apoptosis rate in the LPS+mimic+TLR4 group were up-regulated (P<0.05). **Conclusion** lncRNA NEAT1 interacts with miR-129-5p and directly targets TLR4 to promote LPS-induced hepatocyte injury and inflammatory reaction. This study can provide a new therapeutic target for hepatocyte injury caused by sepsis.

Keywords: lncRNA NEAT1; miR-129-5p; sepsis; hepatocyte injury; inflammatory reaction

脓毒症是由感染引起的致命性疾病,可诱发全身炎症反应综合征(systemic inflammatory reaction syndrome,SIRS)。目前,脓毒症患者的病死率为25%~30%[1-2],但尚无特异且有效的脓毒症相关肝损伤的治疗干预措施[3]。因此,深入研究脓毒症的发病机制至关重要。

miRNA是一大类短链非编码RNA分子,其靶向mRNA并抑制基因的翻译。miRNA参与许多生物学过程,包括细胞分化、增殖、凋亡等。脓毒症患者存在miRNA水平失调或差异性表达[4]。有研究表明,miR-129-5p可以减轻脓毒症引起的肺损伤[5]。然而,炎症条件下miR-129-5p是否在脓毒症引起的肝损伤中起关键作用目前尚不清楚。lncRNA是长度超过200 nt的非编码RNA,参与多个生物过程。lncRNA和miRNA可以相互结合,进一步促进转录后调控。lncRNA核旁斑组装转录本1(nuclear paraspeckle assembly transcript 1,NEAT1)在肿瘤发生中发挥关键作用,其过表达与癌症患者的不良预后有关[6]。此外,lncRNA NEAT1在脓毒症诱导的急性肾损伤患者中明显上调,其上调与脓毒症患者急性肾损伤的严重程度相关[7]。然而,lncRNA NEAT1在脓毒症引起的肝损伤中的作用仍不清楚。

本研究通过分析lncRNA NEAT1对miR-129-5p及其预测的靶基因Toll样受体4(Toll-like receptor 4 gene,TLR4)的调控作用,探讨脓毒症诱导的肝细胞损伤和炎症反应机制,现报道如下。

1 材料与方法

1.1 临床标本

收集我院重症监护室15例脓毒症致肝损伤患者

的外周血和体检的15例健康者的外周血，分别设为脓毒症组和健康组。根据《赫尔辛基宣言》，本研究经我院医学伦理委员会审批通过（2021046），每个受试者均签署对本研究的知情同意书。

1.2 NCTC1469细胞培养

小鼠正常肝细胞NCTC1469购于武汉普诺赛生命科学有限公司。将NCTC1469细胞置于含2 mmol/L谷氨酰胺的无血清RPMI 1640培养基（美国Gibco公司）中，并在37 ℃、5%CO_2的湿润培养箱中培养。

1.3 细胞转染与分组

NCTC1469细胞用100 mmol/L脂多糖（lipopolysaccharide,LPS）单独刺激24 h，建立体外脓毒症模型，待细胞中炎症因子表达显著上调，表明LPS诱导的体外模型成功建立，将其设为LPS组。将未处理的NCTC1469细胞设为对照组。

转染前将NCTC1469细胞接种于12孔板中培养24 h。当细胞融合达70%~90%时，使用Lipofectamine 2000试剂（美国Invitrogen公司）转染6 h后，更换培养基培养24 h，收集细胞和细胞上清，用于后续细胞实验。单独转染mimic NC、mimic、inhibitor NC、inhibitor质粒载体（上海吉玛公司）的NCTC1469细胞分别设为mimic NC组、mimic组、inhibitor NC组、inhibitor组。

另外，当细胞融合达70%~90%时，使用Lipofectamine 2000试剂转染6 h后更换培养基，用100 mmol/L LPS刺激细胞24 h，或者LPS联合45 mg/L TLR4重组蛋白（英国Abcam公司）刺激细胞24 h，收集细胞和细胞上清，用于后续细胞实验。将LPS刺激下转染mimic NC、mimic、siNC、siNEAT1质粒载体的细胞分别设为LPS+mimic NC组、LPS+mimic组、LPS+siNC组和LPS+siNEAT1组，将LPS、siNEAT1、TLR4重组蛋白联合处理的细胞设为LPS+siNEAT1+TLR4组，将LPS、mimic、TLR4重组蛋白联合处理的细胞设为LPS+mimic+TLR4组。

1.4 RNA提取和qPCR分析

将健康组、脓毒症组的外周血及处理好的NCTC1469细胞置于冰上，加入TRIzol试剂（美国ThermoFisher公司）裂解5~10 min，将液体排入EP离心管，加入1/5体积的氯仿；液体混合后在4 ℃下放置10~15 min，随后离心15 min；取出400~500 μL上清液并放入新的EP管中。加入等体积的异丙醇，在4 ℃下孵育10 min，然后以12 000 r/min离心10 min。所得颗粒用75%乙醇洗涤，干燥后加入50 μL DEPC水完全溶解颗粒，然后于-80 ℃下储存。根据说明书，用TRIzol试剂提取血液或细胞总RNA。在溴化乙锭染色的1%琼脂糖凝胶上电泳确认RNA的完整性。使用PrimeScriptT-MRT试剂盒（日本Takara公司）逆转录1 μg总RNA。用于扩增的引物序列见表1。GAPDH用于内参基因。

表1 引物序列

基因		引物
NEAT1	正向	5′-GTAATTTTCGCTCGGCCTGG-3′
	反向	5′-TACCCGAGACTACTTCCCCA-3′
miR-129-5p	正向	5′-CAACCTACTACCTCATCCCA-3′
	反向	5′-TAGGAAAGACAGTAGATTGT-3′
TLR4	正向	5′-GGAGACTTGGCCCTAAACCA-3′
	反向	5′-GACATGGAAACACACCCAGG-3′
IL-6	正向	5′-GCAAGGGTCTGGTTTCAGCC-3′
	反向	5′-TGAGGTAAGCCTACACTTTCCAA-3′
IL-1β	正向	5′-ATTGCTCAAGTGTCTGAAGCAG-3′
	反向	5′-AGAGAGCACACCAGTCCAA-3′
TNF-α	正向	5′-CCCTCTCTCCCCTGGAAAGG-3′
	反向	5′-GCCACTGAATAGGGCGAT-3′

1.5 Western blot

使用RIPA裂解缓冲液制备用于裂解的全细胞裂解物，然后在SDS-PAGE凝胶上分离并转移到PVDF膜上。使用TLR4、GAPDH一抗和适当的辣根过氧化物酶偶联二抗进行蛋白免疫印迹分析，然后用增强的化学发光试剂检测蛋白表达，并使用光密度测定法进行量化。

1.6 ELISA法

NCTC1469细胞在96孔板中用含10%FBS和1%青霉素-链霉素的DMEM培养，以5 000个/孔的密度在37 ℃、5%CO_2加湿培养箱中培养。收集对数生长期的NCTC1469细胞及细胞培养上清液，按照ELISA试剂盒说明书分别检测TNF-α、IL-6、IL-1β水平。分离外周血血清后，按照说明书检测外周血中TNF-α、IL-6、IL-1β水平。

1.7 5′-脱氧肌啶核苷酸末端标记（TdT-mediated dUTP Nick-End Labeling,TUNEL）检测细胞凋亡

各组NCTC1469细胞用PBS洗涤，然后用4%多聚甲醛固定。实验根据TUNEL试剂盒（上海碧云天生物科技有限公司）说明书进行。TUNEL阳性细胞视为凋亡。使用荧光显微镜（德国蔡司Axio Vert A1）获得荧光图像。显微镜下以TUNEL阳性细胞占总细胞的比例计算细胞凋亡率。

1.8 双荧光素酶报告基因检测

将含预测的miR-129-5p结合位点或相应突变片段的lncRNA NEAT1/TLR4基因片段克隆到pGL3基本载体中。mimic的寡核苷酸用于miR-129-5p过表达。在转染过程中，pGL3、miRNAoligos和pRL质粒与Lipo-

fectamine3000(美国ThermoFisherScientific公司)以1∶3(DNA∶Lipofectamine)的比例共转染到NCTC1469细胞中。转染48 h后,使用测定试剂盒说明书(美国普洛麦格公司)检测海肾荧光素酶活性和萤火虫荧光素酶活性,计算萤火虫荧光素酶活性/海肾荧光素酶活性的比值。

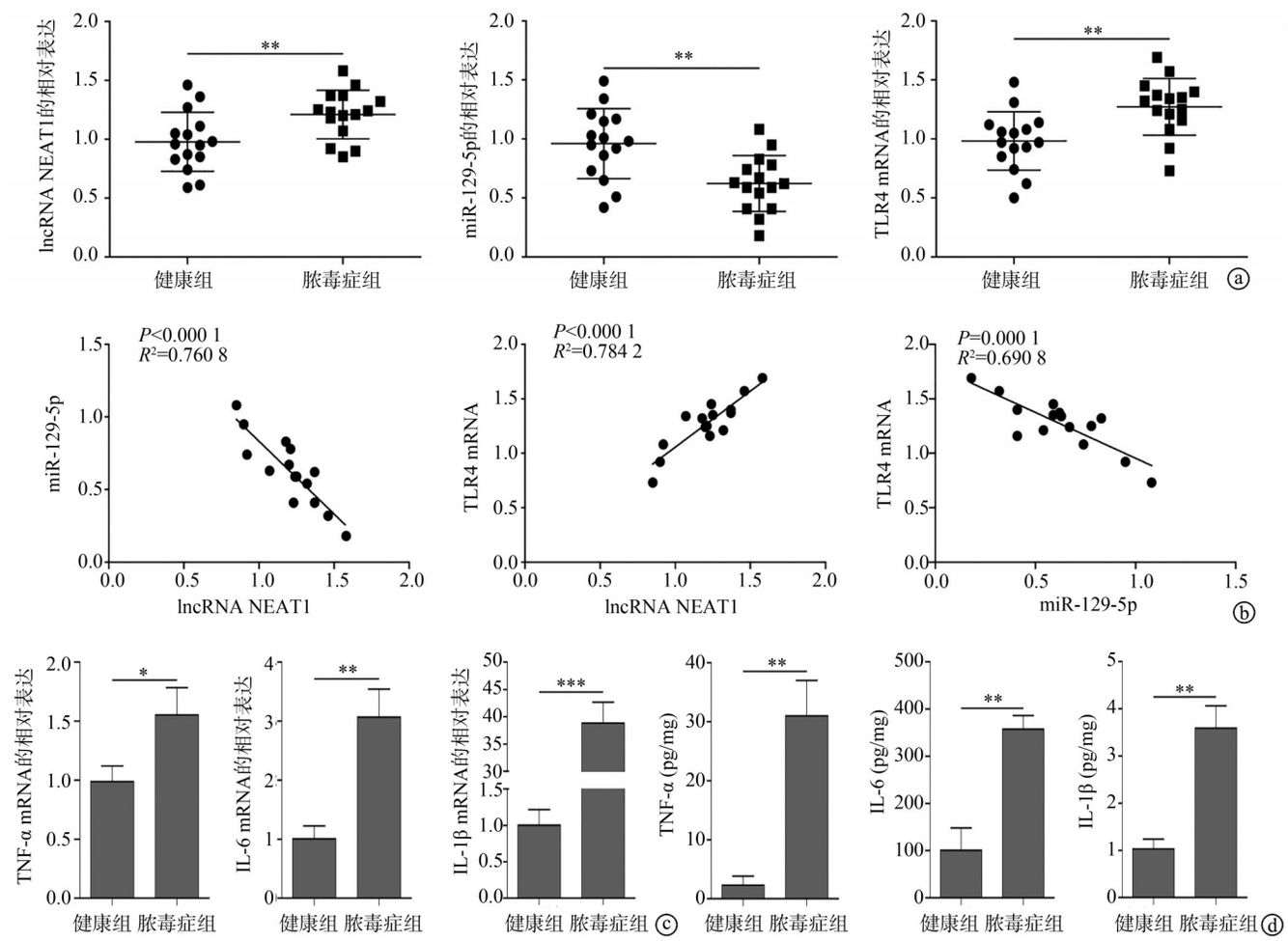
1.9 统计学分析

使用Graph Pad Prism 83.0进行统计分析。正态分布的数据以均数±标准差($\bar{x}\pm s$)表示。组间比较采用单因素方差分析,*LSD-t*法分析两两比较的差异。$P<0.05$为差异具有统计学意义。

2 结果

2.1 lncRNA NEAT1、miR-129-5p、TLR4和炎症因子在脓毒症诱导的肝损伤患者体内的表达

qPCR结果显示,与健康组相比,脓毒症组外周血中lncRNA NEAT1和TLR4的表达明显升高($P<0.05$),而miR-129-5p的表达明显降低($P<0.05$),见图1a。miR-129-5p表达与lncRNA NEAT1、TLR4表达均呈负相关($P<0.05$),lncRNA NEAT1表达与TLR4表达呈正相关($P<0.05$),见图1b。脓毒症组外周血中TNF-α、IL-6和IL-1β的表达比健康组分别高1.5、3.0、38.0倍($P<0.05$),且ELISA获得了类似的结果($P<0.05$),见图1c、d。



a:qPCR分析外周血中lncRNA NEAT1、miR-129-5p和TLR4的表达;b:lncRNA NEAT1、miR-129-5p和TLR4的相关性分析;c:qPCR检测脓毒症组和健康组外周血中TNF-α、IL-6和IL-1β的表达;d:ELISA检测脓毒症组和健康组外周血中TNF-α、IL-6和IL-1β的表达
*:$P<0.05$;**:$P<0.01$;***:$P<0.001$

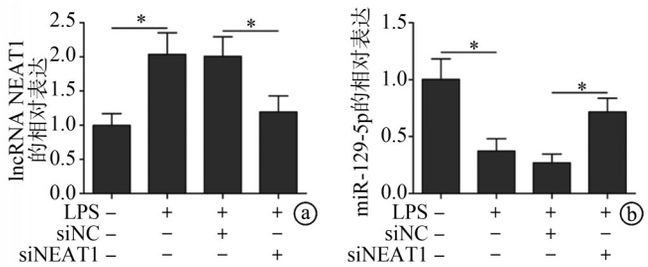
图1 脓毒症组和健康组外周血中lncRNA NEAT1、miR-129-5p、TLR4及炎症因子的表达

2.2 LPS刺激下沉默lncRNA NEAT1对miR-129-5p表达的影响

与对照组比较,LPS组lncRNA NEAT1的表达增加,miR-129-5p的表达减少($P<0.01$);与LPS组、LPS+siNC组比较,LPS+siNEAT1组中lncRNA NEAT1的表达降低,miR-129-5p的表达增加($P<0.01$),见图2。

2.3 LPS刺激下lncRNA NEAT1通过调控TLR4对肝细胞炎症和凋亡的作用

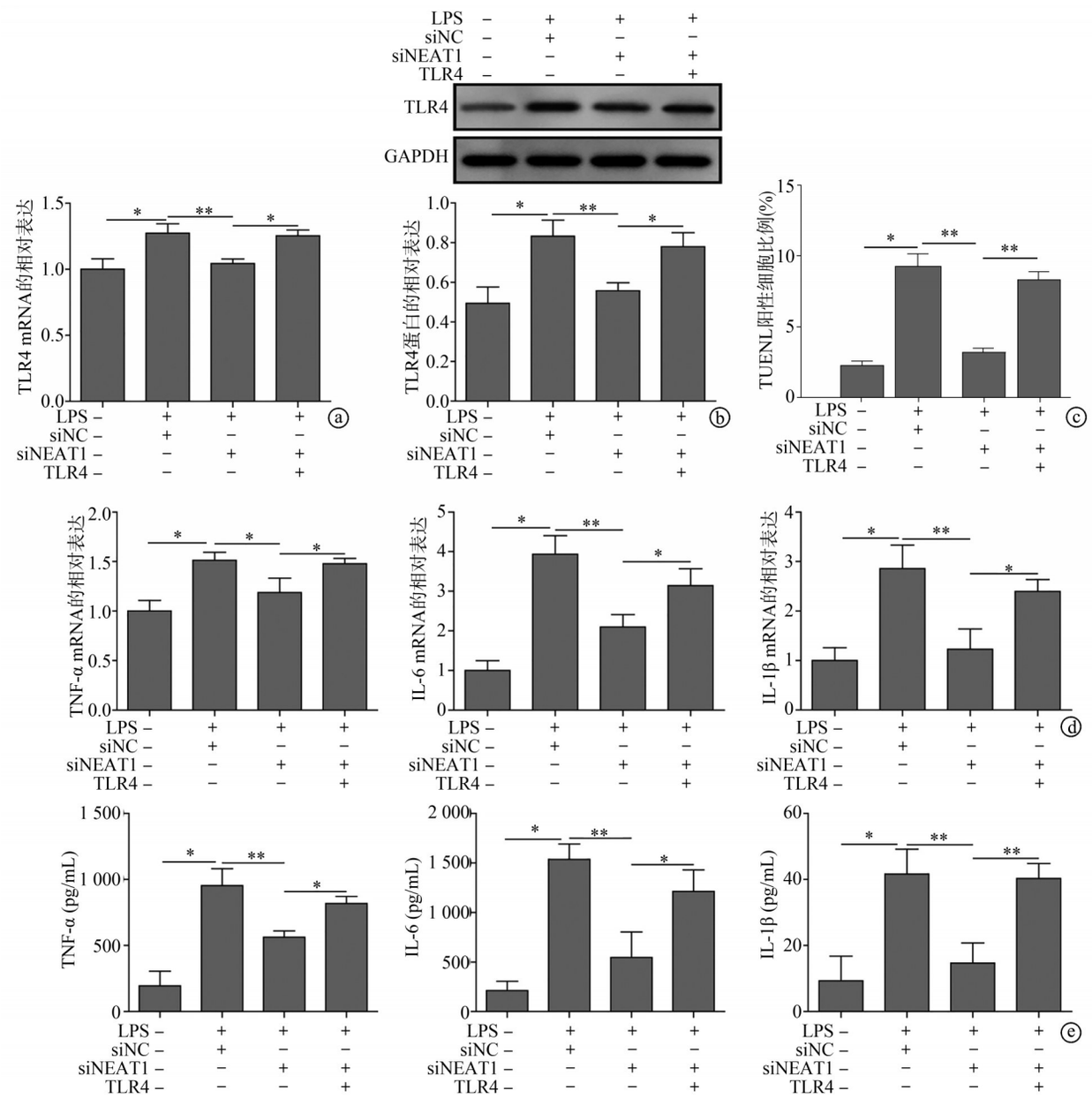
与对照组比较,LPS+siNC组中TLR4 mRNA和蛋白表达水平均上调($P<0.05$);与LPS+siNC组比较,



a:细胞中 lncRNA NEAT1 的表达;b:细胞中 miR-129-5p 的表达 *:P<0.05

图2 LPS刺激下沉默lncRNA NEAT1对miR-129-5p的影响

LPS+siNEAT1 组中 TLR4 mRNA 和蛋白表达水平均下调(P<0.05);与 LPS+siNEAT1 组比较,LPS+siNEAT1+TLR4 组中 TLR4 mRNA 和蛋白表达水平均上调(P<0.05),见图3a、b。与对照组比较,LPS+siNC 组中细胞凋亡率上调(P<0.05);与 LPS+siNC 组比较,与 LPS+siNEAT1 组中细胞凋亡率下调(P<0.05);LPS+siNEAT1 组比较,LPS+siNEAT1+TLR4 组中细胞凋亡率上调(P<0.05),见图3c。与对照组比较,LPS+siNC 组中 TNF-α、IL-6 和 IL-1β 的表达均上调(P<0.05);与 LPS+siNC 组比较,LPS+siNEAT1 组中 TNF-α、IL-6 和 IL-1β 的表达均下调(P<0.05);与 LPS+siNEAT1 组比较,LPS+siNEAT1+TLR4 组中 TNF-α、IL-6 和 IL-1β 的表达均上调(P<0.05),见图3d、e。



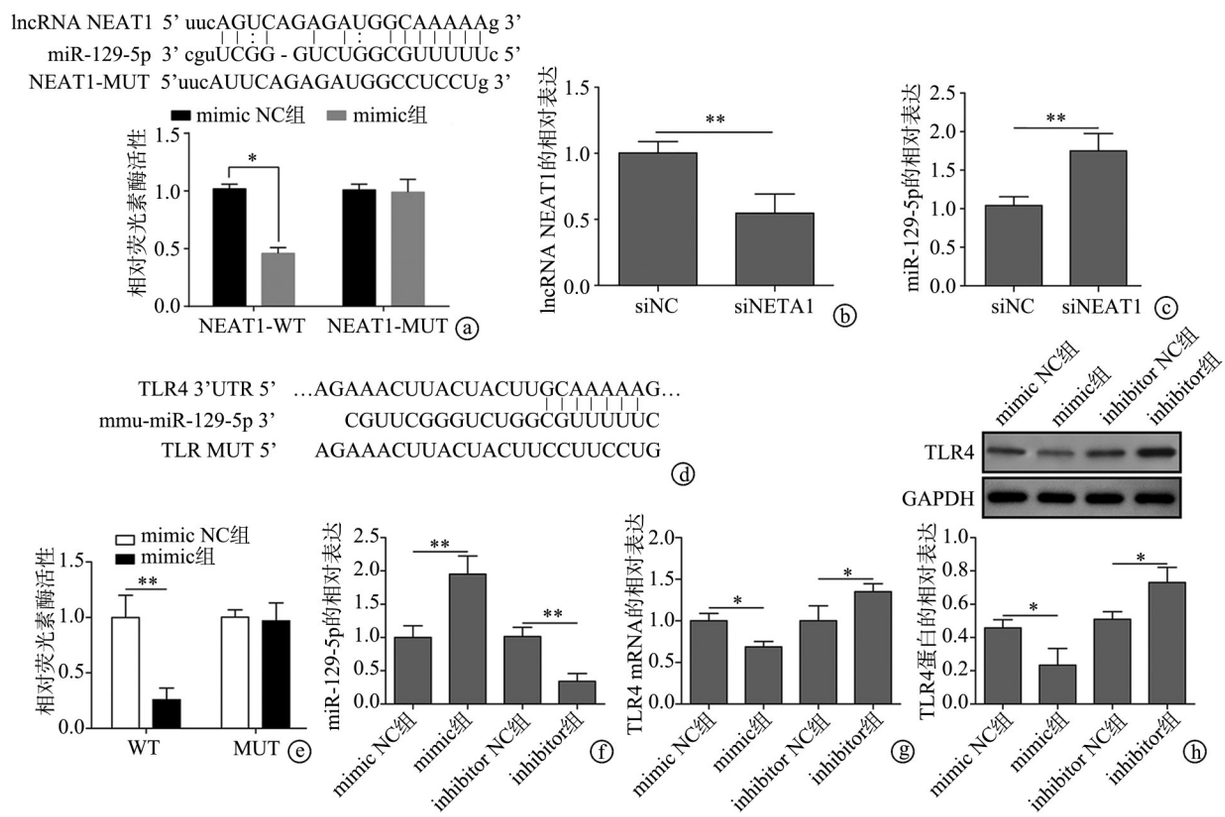
a、b:TLR4 在 LPS 诱导下和 NEAT1 沉默后的表达;c:TUNEL 阳性细胞比例;d:qPCR 检测细胞中 TNF-α、IL-6 和 IL-1β 的表达;e:ELISA 检测细胞中 TNF-α、IL-6 和 IL-1β 的表达 *:P<0.05;**:P<0.01

图3 LPS通过上调lncRNA NEAT1促进TLR4的表达诱导炎症反应和细胞损伤

2.4 lncRNA NEAT1/miR-129-5p及miR-129-5p/TLR4的相互作用

经生物信息学软件TargetScan预测发现，lncRNA NEAT1和miR-129-5p之间存在结合碱基区(图4a)。双荧光素酶基因报告实验测定结果显示，在野生型(NEAT1-WT)中，与mimic NC组比较，mimic组的荧光素酶活性降低($P<0.05$)；在突变型(NEAT1-MUT)中，与mimic NC组比较，mimic组的荧光素酶活性变化无统计学意义($P>0.05$)，见图4a。miR-129-5p与lncRNA NEAT1存在相互作用。qPCR分析显示，siNEAT1沉默了lncRNA NEAT1并增加了miR-129-5p的表达($P<0.01$)，见图4b、c。

进一步分析miR-129-5p与TLR4的相互作用。生物信息学软件TargetScan预测miR-129-5p和TLR4有多个结合位点(图4d)。双荧光素酶报告基因实验结果显示，miR-129-5p与TLR4之间存在靶向关系。mimic明显降低了TLR4野生型(WT)细胞的荧光素酶活性($P<0.01$)，而对TLR4突变型(MUT)细胞的荧光素酶活性无影响($P>0.05$)，见图4e。与mimic NC组比较，mimic组中miR-129-5p的表达增加，TLR4的mRNA和蛋白表达显著下调($P<0.05$)；与inhibitor NC组比较，inhibitor组中miR-129-5p的表达下调，TLR4的mRNA和蛋白表达上调($P<0.05$)，见图4f~h。

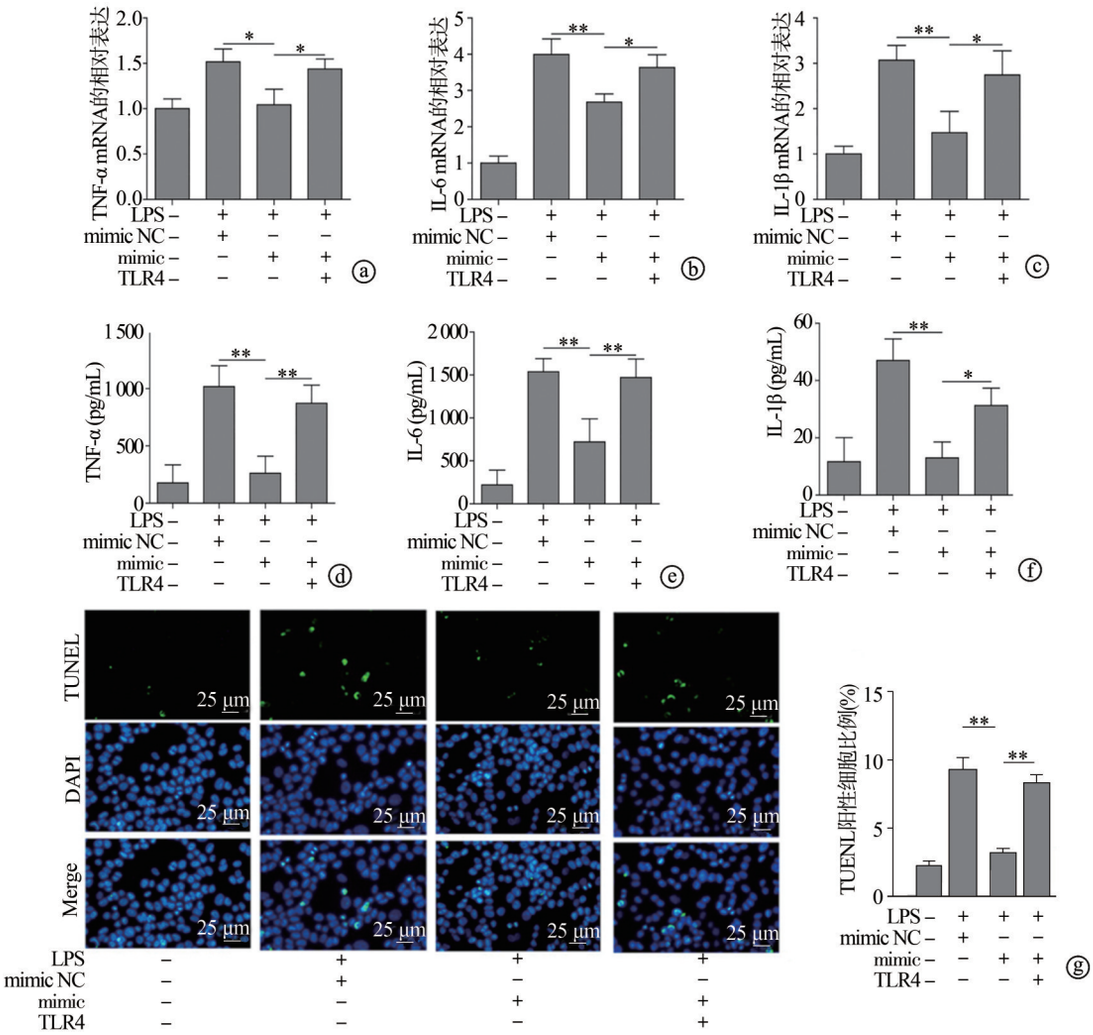


a：生物信息学预测lncRNA NEAT1/miR-129-5p相互作用的结合位点以及双荧光素酶报告基因分析lncRNA NEAT1和miR-129-5p之间的关系；b、c：qPCR检测lncRNA NEAT1和miR-129-5p的表达；d：miR-129-5p/TLR4相互作用的结合位点的生物信息学预测；e：双荧光素酶报告基因分析TLR4和miR-129-5p的关系；f~h：qPCR和Western blot检测miR-129-5p和TLR4的表达 *：$P<0.05$；**：$P<0.01$

图4 lncRNA NEAT1/miR-129-5p及miR-129-5p/TLR4的相互作用

2.5 lncRNA NEAT1通过miR-129-5p靶向TLR4调节LPS诱导的炎症反应

qPCR结果显示，与对照组比较，LPS+mimic NC组TNF-α、IL-6和IL-1β的mRNA表达增加($P<0.05$)；与LPS+mimic NC组比较，LPS+mimic组TNF-α、IL-6和IL-1β的mRNA表达降低($P<0.05$)；与LPS+mimic组比较，LPS+mimic+TLR4组TNF-α、IL-6和IL-1β的mRNA表达增加($P<0.05$)，见图5a~c。ELISA实验所观察到的TNF-α、IL-6和IL-1β表达结果与qPCR的结果相似($P<0.05$)，见图5d~f。因此，TLR4作为miR-129-5p的下游效应分子调节LPS诱导的炎症反应。TUNEL实验结果显示，与对照组比较，LPS+mimic NC组的细胞凋亡率增加($P<0.05$)；与LPS+mimic NC组比较，LPS+mimic组的细胞凋亡率降低($P<0.05$)；与LPS+mimic组比较，LPS+mimic+TLR4组的细胞凋亡率增加($P<0.05$)，见图5g。



a~c:qPCR检测细胞中TNF-α、IL-6和IL-1β的表达;d~f:ELISA检测细胞中TNF-α、IL-6和IL-1β的表达;g:TUNEL、DAPI、Merge染色检测细胞凋亡率 *:$P<0.05$;**:$P<0.01$

图5 miR-129-5p表达可诱导炎症反应和细胞损伤

3 讨论

脓毒症是一种全身性炎症反应,严重时可能导致器官功能障碍和/或循环障碍[7]。失调的炎症反应在脓毒症引起的肝损伤中起关键作用[8]。之前的研究证实了多种microRNA和lncRNA与炎症反应都有关[9-11],然而,其在调节脓毒症引起的肝损伤中的具体作用尚未明确。本研究根据TargetScan软件预测发现,lncRNA NEAT1与miR-129-5p之间存在相互作用,这可能与脓毒症患者的严重程度和预后不良有关。此外,本研究观察到脓毒症诱导的肝损伤患者体内的TNF-α、IL-6、IL-1β、lncRNA NEAT1和TLR4的表达均增加,但miR-129-5p的表达水平降低。进一步证明了lncRNA NEAT1可通过调节miR-129-5p的表达,靶向TLR4来调节LPS诱导的炎症反应。这些研究结果为脓毒症引起肝损伤的治疗提供了一个潜在治疗靶点。

研究表明,lncRNA NEAT1在脓毒症患者体内表达上调,其在脓毒症诱导的急性肾损伤中也扮演着重要角色[12-13]。本研究结果显示,在脓毒症诱导的肝损伤患者中,lncRNA NEAT1表达也升高,这与之前的研究一致。另外,lncRNA NEAT1的升高还伴随着miR-129-5p的抑制、TLR4的激活以及炎症反应的显著增加。本研究结果显示,miR-129-5p与lncRNA NEAT1、TLR4均呈负相关,而lncRNA NEAT1与TLR4呈正相关。之前研究也指出,lncRNA NEAT1可通过与miR-129-5p的结合,抑制miR-129-5p的表达从而促进酒精性脂肪肝的肝纤维化[14]。因此,本研究在脓毒症中证实了lncRNA NEAT1、miR-129-5p和TLR4在介导脓毒症诱导的肝损伤中具有显著的相关性。

在实验性脓毒症模型中,LPS通常用于研究脓毒症期间的炎症反应[15-17]。本研究发现,与脓毒症引起肝损伤患者的lncRNA NEAT1表达上调结果一致,LPS

刺激后肝细胞中lncRNA NEAT1的表达上调,而沉默lncRNA NEAT1则降低了LPS诱导的炎症细胞因子水平。此外,用TLR4重组蛋白上调TLR4的表达可以逆转沉默lncRNA NEAT1对细胞凋亡和炎症细胞因子表达的抑制作用,这表明miR-129-5p和TLR4在lncRNA NEAT1的下游扮演重要角色。

本研究结果还显示,lncRNA NEAT1/miR-129-5p和miR-129-5p/TLR4直接结合,这可能是lncRNA的内源竞争性RNA机制发挥的作用[18-19]。本研究通过双荧光素酶报告基因实验证实lncRNA NEAT1与miR-129-5p可直接结合,并且证实TLR4是miR-129-5p的靶基因,TLR4可以被miR-129-5p靶向抑制。因此,本研究认为在LPS刺激下,lncRNA NEAT1的表达显著上调,随后与miR-129-5p竞争性结合,使细胞中miR-129-5p的表达显著降低,提示lncRNA NEAT1扮演了内源竞争性RNA,从而激活被miR-129-5p抑制的靶基因TLR4,TLR4被激活后刺激下游信号传导,导致严重的炎症反应并诱导肝损伤。

综上,lncRNA NEAT1在脓毒症引起的肝损伤中具有重要的生物学功能,其在脓毒症诱导的肝损伤患者体内表达上调,且与miR-129-5p竞争并调节TLR4,以促进肝损伤。揭示了lncRNA NEAT1/miR-129-5p/TLR4轴在脓毒症诱导的肝损伤中的重要功能,为脓毒症诱导的肝损伤提供了潜在的治疗靶点。

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