



肿瘤坏死因子 α 诱导滋养细胞NOD1-RICK-NF- κ B信号轴 激活与炎症因子释放*

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【摘要】目的 解析核苷酸结合寡聚化结构域1(nucleotide-binding oligomerization domain 1, NOD1)在妊娠早期和晚期的母胎界面细胞表达差异及其介导炎症反应的分子机制。**方法** 通过免疫组化(IHC)和逆转录PCR(RT-PCR)检测30例正常早孕绒毛、30例正常早孕蜕膜及30例正常足月胎盘组织中NOD1的mRNA和蛋白的表达;用不同质量浓度(0、0.01、0.1、1、10和100 ng/mL)和不同刺激时间(24、48和72 h)的肿瘤坏死因子(tumor necrosis factor- α , TNF α)刺激人绒毛膜滋养细胞HTR8/SVneo细胞,通过实时荧光定量PCR(qRT-PCR)、Western blot检测NOD1、受体结合丝氨酸苏氨酸激酶2[receptor-interacting protein (RIP)-like interacting CLARP kinase, RICK]以及p65的表达差异,ELISA分析炎症因子IL-6、IL-8分泌水平,并用EMSA检测核因子 κ B(nuclear factor-kappa B, NF- κ B)的DNA结合活性,从而探讨滋养细胞内NOD1信号转导通路的调控机制。**结果** NOD1在正常早孕绒毛、蜕膜以及足月胎盘组织中均有表达,主要表达于滋养细胞、血管内皮细胞以及蜕膜细胞的胞浆,NOD1在足月胎盘和早孕绒毛、蜕膜组织中的表达差异具有统计学意义($P < 0.05$),足月胎盘NOD1表达高于早孕绒毛和蜕膜组织。TNF α 上调了HTR8/SVneo细胞中NOD1的表达,且呈现剂量依赖性和时间依赖性。同时,IL-6的分泌水平与TNF α 质量浓度呈正相关,而IL-6和IL-8的释放呈现TNF α 刺激时间依赖性。此外,TNF α 刺激增强了RICK和p65的表达,并提高了NF- κ B的DNA结合活性。**结论** NOD1持续表达于正常妊娠母胎界面的滋养细胞及蜕膜细胞内,参与了正常妊娠过程的调控。NOD1表达变化与TNF α 诱导的炎症反应存在关联,TNF α 可能通过NOD1-RICK-NF- κ B信号轴触发炎症因子释放,介导滋养细胞的炎症级联反应,为深入理解妊娠期免疫调控机制提供了新的见解。

【关键词】 NOD1 固有免疫 妊娠 滋养层

Tumor Necrosis Factor- α Induces Activation of the NOD1-RICK-NF- κ B Signaling Axis and Inflammatory Cytokine Release in Trophoblasts

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[Abstract] Objective To analyze the differential expression of nucleotide-binding oligomerization domain 1 (NOD1) in maternal-fetal interface cells during early and late pregnancy, and to investigate its molecular mechanisms in mediating inflammatory responses. **Methods** Immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR) were used to detect NOD1 mRNA and protein expression in normal first-trimester villi tissues ($n = 30$), normal early pregnancy decidua tissues ($n = 30$), and normal full-term placental tissues ($n = 30$). Human chorionic trophoblast HTR8/SVneo cells were stimulated with tumor necrosis factor- α (TNF α) at various concentrations (0,

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0.01, 0.1, 1, 10, and 100 ng/mL) and exposure times (24, 48, and 72 h). Real-time quantitative PCR (qRT-PCR) and western blot analysis were performed to detect differential expression of NOD1, receptor-interacting protein (RIP)-like interacting CLARP kinase (RICK), and p65. ELISA was used to analyze the secretion levels of inflammatory cytokines IL-6 and IL-8. Electrophoretic mobility shift assay (EMSA) was conducted to assess the DNA-binding activity of nuclear factor-kappa B (NF- κ B) and to investigate the regulatory mechanisms of NOD1 signaling pathways in trophoblasts.

Results NOD1 was expressed in normal first-trimester villi, decidua, and full-term placental tissues, primarily localized in the cytoplasm of trophoblasts, vascular endothelial cells, and decidual cells. The difference in NOD1 expression between full-term placental tissues and first-trimester villi and decidua was statistically significant ($P < 0.05$), with higher NOD1 expression observed in full-term placental tissues than in first-trimester villi and decidua. TNF α upregulated NOD1 expression in HTR8/SVneo cells in a dose- and time-dependent manner. IL-6 secretion levels positively correlated with TNF α concentration, while IL-6 and IL-8 release exhibited time-dependent responses to TNF α stimulation. Furthermore, TNF α stimulation enhanced RICK and p65 expression and increased NF- κ B DNA-binding activity.

Conclusion NOD1 is constitutively expressed in trophoblasts and decidual cells at the maternal-fetal interface during normal pregnancy, participating in the regulation of the gestational process. Changes in NOD1 expression correlate with TNF α -induced inflammatory responses. TNF α may trigger inflammatory cytokine release via the NOD1-RICK-NF- κ B signaling axis, mediating inflammatory cascades in trophoblasts. These findings provide novel insights into immune regulatory mechanisms during pregnancy.

[Key words] NOD1 Innate immunity Pregnancy Trophoblast

固有免疫在正常滋养细胞植入子宫及胎盘抗感染防御中发挥着关键作用。胎盘作为一个特殊的免疫器官,在介导母胎免疫耐受和抵御病原体侵袭方面具有重要功能^[1],这一过程依赖于固有免疫细胞的模式识别受体对病原体相关分子模式的识别^[2-3]。核苷酸结合寡聚肽结构域(nucleotide-binding oligomerization domains, NOD)是一类在结构上都含有NOD蛋白受体的总称,是重要的胞浆内模式识别受体,其中NOD1作为NOD家族中最具代表性的成员在启动固有免疫应答中扮演核心角色^[4-7]。

以往研究表明,NOD1识别特异性配体后可与受体结合丝氨酸/苏氨酸-蛋白激酶2[receptor-interacting protein (RIP)-like interacting CLARP kinase, RICK]形成复合物,进而可能激活核因子 κ B(nuclear factor-kappa B, NF- κ B)信号通路,从而促进IL-8、IL-6、IL-1 β 、IL-12和肿瘤坏死因子(tumor necrosis factor- α , TNF α)等多种促炎因子的释放^[8]。这种信号级联反应不仅参与固有免疫应答,还影响适应性免疫的建立,并通过诱导细胞凋亡从而清除病原体 and 受损细胞^[9]。NOD1可以特异性识别革兰氏阴性菌和某些革兰氏阳性菌中特有的内消旋二氨基庚二酸(γ -D-glutamyl-meso-diaminopimelic acid, iE-DAP)肽聚糖组分^[10],已有研究表明iE-DAP的刺激可以诱导上皮细胞等产生TNF α ^[11-12]。TNF α 主要由活化的巨噬细胞或单核细胞分泌,可活化各种炎性细胞、促进肿瘤细胞的凋亡,在免疫应答和细胞凋亡中发挥核心作用。

母胎界面及生殖道免疫微环境变化一直是产科研究的热点^[13-15],也有研究表明滋养层细胞功能异常是导致复发性自然流产的关键因素之一,其免疫调控机制复杂^[16]。

研究表明,NOD1可能参与妊娠期炎症调控,其异常表达可能与复发性自然流产、早产、子痫前期等妊娠并发症相关^[7, 9, 17-18]。尽管NOD1在肠道疾病和感染相关疾病中的功能已较明确^[19-20],但其在母胎界面的表达特征和作用机制尚未完全阐明。本研究旨在通过母胎界面样本检测和细胞实验探讨正常妊娠过程中NOD1的动态表达以及NOD1在TNF α 介导的滋养细胞炎症反应中的作用及分子调控机制,从而为深入理解NOD1在维持正常妊娠及妊娠免疫调控中的作用奠定理论基础。

1 材料与方法

1.1 试剂与细胞株

RPMI 1640培养基(Gibco, 美国), Opti-MEMTM I无血清培养基(Gibco, 美国), 新生牛血清(Gibco, 美国), 胎牛血清(Hyclone, 美国), 兔抗人NOD1抗体(Santa Cruz, 美国), 过氧化物酶标记的链霉亲和素(SP)检测试剂盒(北京中杉金桥生物技术有限公司, 中国), GAPDH(上海康成生物工程有限公司, 中国), 兔抗人RICK抗体(eBioscience, 美国), 兔抗NF- κ B p65抗体(SAB, 美国), 酶标山羊抗兔IgG二抗(北京中杉金桥生物技术有限公司, 中国), SV Total RNA Isolation System(Promega, 美国), cDNA试剂盒(TOYOBO, 日本), PCR反应试剂盒(TaKaRa Ex-Taq, 中国), Trizol(Invitrogen, 美国), SYBR Premix Ex Taq试剂盒(TaKaRa, 日本), TNF α (PeproTech, 美国), NE-PER试剂盒(Pierce, 美国), EMSA试剂盒(Millipore, 美国)。人绒毛膜滋养细胞株HTR8/SVneo由加拿大CHARLES GRAHAM博士于2010年惠赠^[21]。

1.2 临床样本收集

本研究于2021年9月-2022年12月在四川大学华西第二医院共采集90例临床样本。样本组成包括: 30例妊娠早期(孕8~12周)自愿接受子宫刮除术孕妇的正常绒毛($n=30$)及蜕膜组织($n=30$), 以及30例住院终止妊娠孕妇的足月($\geq$ 孕39周)正常胎盘组织($n=30$)。所有纳入研究的孕妇均符合以下标准: 术前体温正常, 血常规及白带常规检查结果均在正常范围内, 无血源性传染病史, 且术前未接受任何抗炎治疗。入组的60例妊娠早期与足月妊娠孕妇在年龄、产次、白细胞总数及中性粒细胞计数等方面均无差异($P>0.05$)。采集的组织经甲醛溶液固定或液氮冻存。本研究经四川大学华西第二医院医学伦理委员会审批通过, 批准号: 医学科研2020年(122)号, 并严格遵循《赫尔辛基宣言》的伦理原则, 所有参与研究的患者均签署了知情同意书。

1.3 逆转录PCR(RT-PCR)检测绒毛和蜕膜组织中NOD1 mRNA的表达

采用SV Total RNA Isolation System提取RNA, cDNA试剂盒进行反转录反应。使用TaKaRa Ex-Taq酶体系进行PCR扩增。使用的扩增引物包括: NOD1-F: 5'-CCAGCCTCACCTCATTC-3', NOD1-R: 5'-CCCGTTTAGTCACCCTTCA-3'; GAPDH-F: 5'-AAAGGTGGA GGAGTGGGT-3', GAPDH-R: 5'-GGGAAACTGTG GCGTGAT-3'。PCR反应程序为: 50℃ 30 min, 95℃ 5 min; 36个循环(95℃ 1 min, 55℃ 1 min, 72℃ 1 min); 72℃ 6 min。PCR产物经2%琼脂糖凝胶电泳分离后, 采用Image Mastervos System测定条带平均光密度, Typhoon成像系统和Total Lab软件(5.0版本)进行图像分析。

1.4 免疫组化(IHC)检测绒毛及蜕膜组织中NOD1蛋白表达

采用EnVision二步法、兔抗人NOD1蛋白(1:1500)、SP试剂盒进行NOD1蛋白IHC检测。通过Image-Pro Plus软件(Version 5.0)对棕黄色阳性染色区域的光密度进行定量测定, 切片在光镜下按统一放大倍数($\times 400$ 倍)进行分析。IHC检测过程中的组织制备、切片厚度、抗体浓度、孵育时间、温度及显色反应的时间、温度均进行标准化处理; 在图像分析过程中, 由三名操作者独立将每张切片按等距抽样原则随机摄取5个视野, 输入计算机作为测定视场, 所有摄像参数保持统一。

1.5 体外实验

1.5.1 细胞培养及TNF α 处理

HTR8/SVneo细胞培养于含10%胎牛血清的RPMI 1640培养基, 培养基另添加10 mmol/L Hepes、0.1 mmol/L

MEM非必需氨基酸、1 mmol/L丙酮酸钠及100 nmol/L青霉素/链霉素。当细胞生长至70%时, 弃上清, PBS清洗两次, 加入无血清OptiMEM(Gibco, 美国)培养基培养4 h后, 使用不同质量浓度(0、0.01、0.1、1、10和100 ng/mL)TNF α 进行刺激^[22-23], 24 h后收集细胞及培养上清。确定最佳TNF α 刺激浓度后, 采用该质量浓度分别处理HTR8/SVneo细胞不同时间(24、48和72 h), 并于各时间点收集细胞及上清。

1.5.2 实时荧光定量PCR(qRT-PCR)检测HTR8/SVneo细胞NOD1及RICK mRNA表达

采用Trizol法提取细胞总RNA, 使用SYBR Premix Ex Taq试剂盒进行扩增, 引物包括: NOD1-F: 5'-AAGCGAAGAGCTGACCAAAT-3', NOD1-R: 5'-TCC CAGTTTAAGATGCGTGA-3'; RICK-F: 5'-TTCAAG TGCCATTACCTAT-3', RICK-R: 5'-TTTCAGGA GAACCACTATTT-3'; GAPDH-F: 5'-CGCTCTCTGC TCCTCCTGTT-3', GAPDH-R: 5'-CCATGGTGTCTG AGCGATGT-3'。反应程序为: 95℃ 10 min; 39个循环(95℃ 15 s, 55℃ 1 min, 72℃ 13 s); 72℃ 5 min。通过 $2^{-\Delta\Delta Ct}$ 法计算各基因相对表达量。

1.5.3 Western blot检测细胞中NOD1、RICK和p65蛋白表达水平

提取细胞总蛋白, 采用Western blot检测NOD1、RICK和p65蛋白的表达水平, 一抗浓度分别为: 山羊抗人NOD1抗体(1:500), 兔抗人RICK抗体(1:250)及p65抗体(1:300)。使用Image-Pro Plus图像处理软件测量各目的条带灰度值, 与相应GAPDH条带灰度比值作为目的条带相对灰度值。

1.5.4 检测HTR8/SVneo细胞IL-8和IL-6分泌水平和NF- κ B转录活性

收集细胞培养上清, 400 $\times$ g离心10 min后, 使用ELISA试剂盒和Upstate Luminex 100 IS系统(Upstate, 美国)检测IL-8和IL-6浓度。

采用核蛋白提取试剂盒分离核蛋白, 通过EMSA试剂盒检测NF- κ B的DNA结合活性。

1.6 统计学方法

数据以 $\bar{x} \pm s$ 表示, 组间比较采用单因素方差分析或 t 检验。使用SPSS 20.0软件(美国SPSS Inc.)进行统计分析, $P<0.05$ 为差异有统计学意义。所有实验均独立重复3次。

2 结果

2.1 NOD1 mRNA及蛋白在绒毛与蜕膜组织中的表达

对采集的30例妊娠早期正常绒毛、30例妊娠早

期正常蜕膜组织和30例足月正常胎盘组织进行*NOD1* mRNA及*NOD1*蛋白检测。RT-PCR检测结果显示,*NOD1* mRNA在早孕绒毛组织、蜕膜组织及足月胎盘组织中均有表达(图1),早孕绒毛(0.22 ± 0.06)、蜕膜组织*NOD1* mRNA表达水平(0.22 ± 0.05)低于足月胎盘组织(0.30 ± 0.05),差异有统计学意义($P < 0.05$)。

免疫组化结果显示,*NOD1*蛋白广泛表达于正常妊娠的绒毛细胞滋养细胞、合体滋养细胞、绒毛外细胞滋养细胞、血管内皮细胞以及蜕膜细胞的胞浆内(图2)。早孕绒毛(129.62 ± 1.37)、蜕膜组织*NOD1*蛋白表达强度(129.83 ± 1.16)低于足月胎盘组织(134.44 ± 2.03),差异有统计学意义($P < 0.05$)。

2.2 TNF α 对HTR8/SVneo细胞中*NOD1*表达的影响

见图3、图4。与空白对照组(0 ng/mL TNF α)相比,0.01 ~ 10 ng/mL质量浓度组TNF α 可改变*NOD1* mRNA及蛋白表达水平($P < 0.05$)。其中,1 ng/mL TNF α 刺激时表达量达到峰值,而随着质量浓度继续增加,表达水平反而

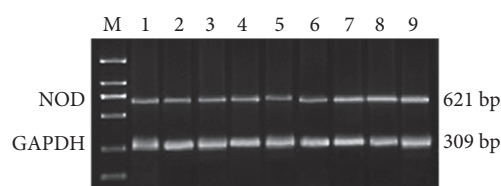


图 1 RT-PCR检测组织中*NOD1* mRNA的表达

Fig 1 RT-PCR detection of *NOD1* mRNA expression levels in tissue

Lane 1-3: The villi of the first trimester; lane 4-6: the decidua of the first trimester; lane 7-9: the term placental tissue; M: Marker.

下降($P < 0.05$)。当采用1 ng/mL TNF α 处理HTR8/SVneo细胞时,各时间点*NOD1* mRNA和蛋白表达量均高于0 h对照组($P < 0.05$),且在24 h达到最大值,随后逐渐降低($P < 0.05$)。

2.3 TNF α 调控HTR8/SVneo细胞中IL-8和IL-6的分泌

采用不同质量浓度TNF α 刺激HTR8/SVneo细胞24 h后,结果显示,当TNF α 为1 ng/mL时,IL-8和IL-6的分泌水平随TNF α 质量浓度增加而升高($P < 0.05$)(图5A、5B)。

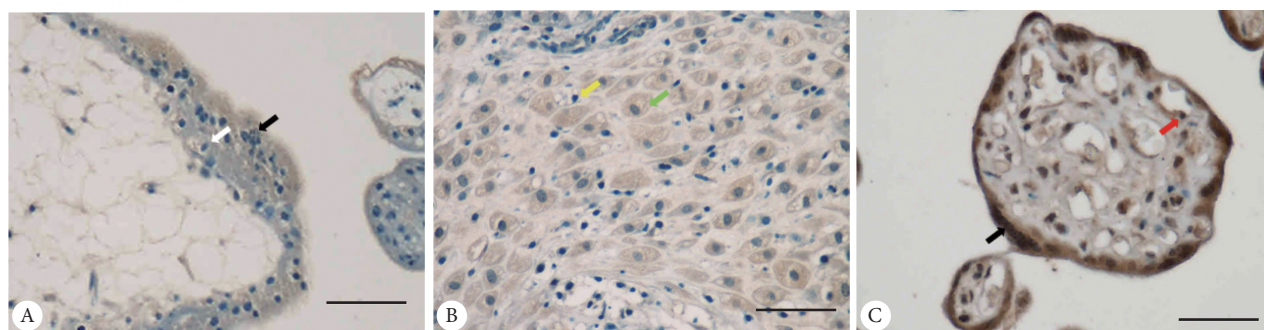


图 2 *NOD1*蛋白的免疫组化检测结果

Fig 2 Immunochemical detection of *NOD1* protein

Immunohistochemistry (IHC) was performed to detect *NOD1* expression in tissues, with brown staining indicating positive signals. Black arrows indicate villous syncytiotrophoblasts, the white arrow indicates villous cytotrophoblasts, the green arrow indicates extravillous cytotrophoblasts, the red arrow indicates vascular endothelial cells, and the yellow arrow indicates decidual cells: villi of the first trimester (A); decidua of the first trimester (B); term placental tissue (C). Scale bar = 200 μ m.

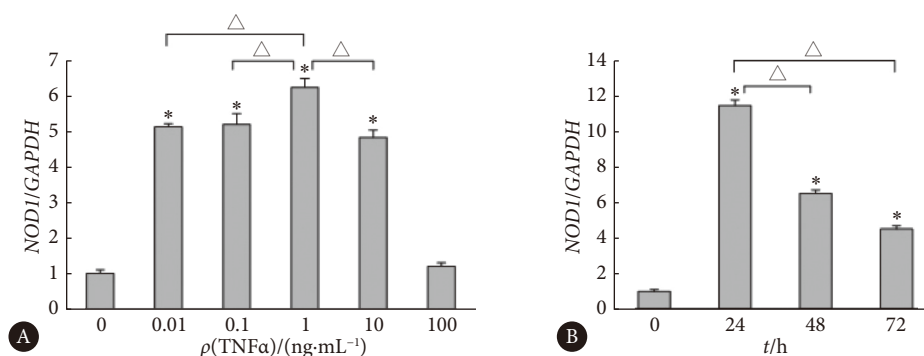


图 3 qRT-PCR检测HTR8/SVneo细胞经不同质量浓度TNF α 处理24 h (A) 及1 ng/mL TNF α 处理不同时间 (B) 后的*NOD1* mRNA表达水平

Fig 3 qRT-PCR analysis of *NOD1* mRNA expression levels in HTR8/SVneo cells treated with different concentrations of TNF α for 24 h (A) and with 1 ng/mL TNF α for varying durations (B)

* $P < 0.05$, vs. the control group (0 ng·mL $^{-1}$ TNF α or 0 h); Δ $P < 0.05$. $n = 3$.

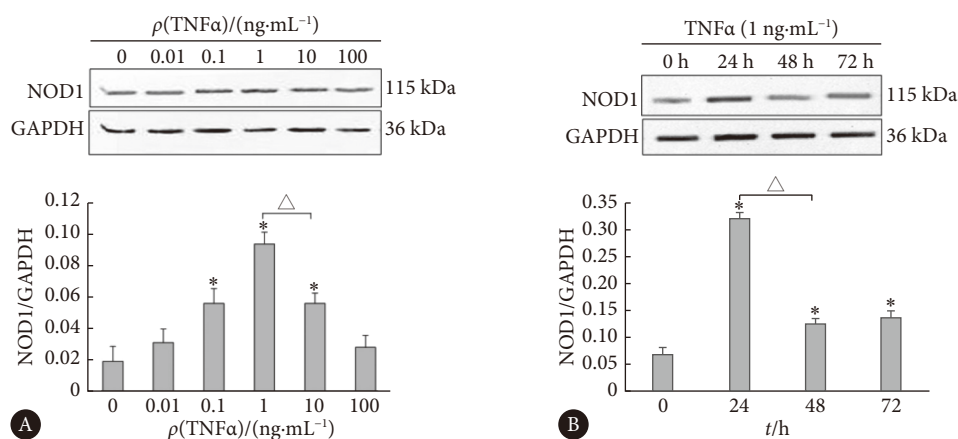


图4 Western blot检测HTR8/SVneo细胞经不同质量浓度TNF α 处理24 h (A) 及1 ng/mL TNF α 处理不同时间 (B) 后的NOD1蛋白表达水平

Fig 4 Analysis of NOD1 protein expression levels in HTR8/SVneo cells treated with different mass concentrations of TNF α for 24 h (A) and with 1 ng/mL TNF α for varying durations (B) by Western blot

* $P < 0.05$, vs. the control group (0 ng/mL⁻¹ TNF α or 0 h); $\Delta P < 0.05$, $n = 3$.

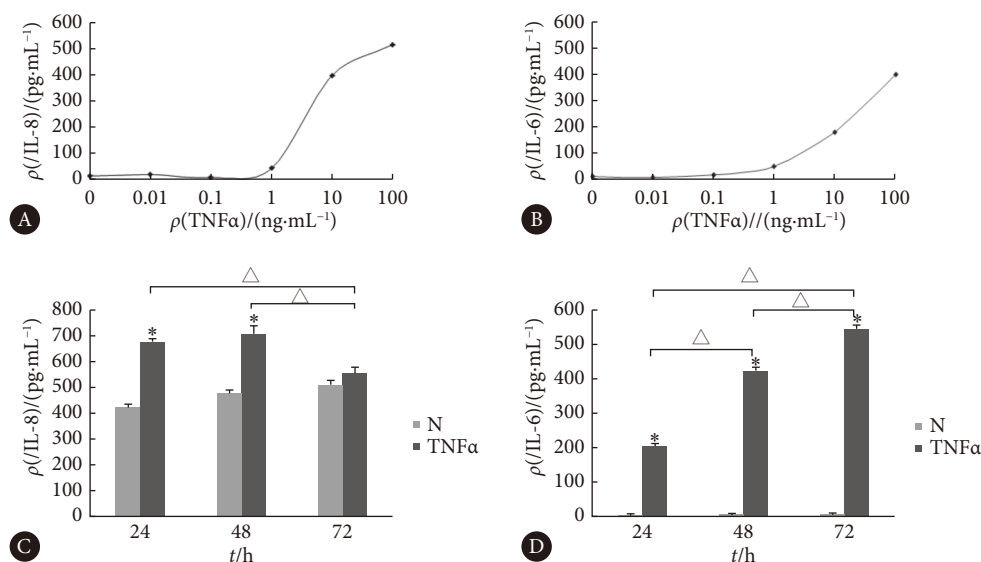


图5 ELISA检测HTR8/SVneo细胞经不同质量浓度TNF α 处理24 h (A、B) 及1 ng/mL TNF α 处理不同时间 (C、D) 后的IL-8和IL-6分泌水平

Fig 5 ELISA analysis of IL-8 and IL-6 secretion levels in HTR8/SVneo cells after 24 h of treatment with different concentrations of TNF α (A, B) and after varying durations of treatment with 1 ng/mL TNF α (C, D)

* $P < 0.05$, vs. the N group; $\Delta P < 0.05$, $n = 3$.

用1 ng/mL TNF α 处理不同时间后发现, 与对照组相比, IL-8表达在24 h和48 h增加($P < 0.05$), 72 h下降至与对照组相当的水平; 而IL-6表达则随时间延长持续升高, 并于72 h达到峰值($P < 0.05$, 与对照组相比)(图5C、5D)。

2.4 TNF α 对RICK、p65表达及NF- κ B活化的调控作用

与对照组(0 ng/mL)相比, 0.01 ~ 10 ng/mL TNF α 处理后RICK表达升高($P < 0.05$), 当TNF α 为1 ng/mL时, RICK表达达到峰值, 随后开始下降($P < 0.05$)(图6A、6B)。0.1 ~ 10 ng/mL TNF α 处理后p65表达水平与对照组相比均有差异($P < 0.05$), 且TNF α 为1 ng/mL时p65表达水平达到峰值, 10 ng/mL时开始下降(图6A、6C)。此外, 不同质量浓度

TNF α 处理组(0.01 ~ 10 ng/mL)NF- κ B活性均高于对照组, 其中0.1 ng/mL TNF α 处理时NF- κ B活性最高, 随后逐渐降低(图6D)。

3 讨论

机体遭遇病原体入侵时首先通过防御细胞膜模式识别受体(如TLRs)对病原体识别, 继而发生一系列的炎症效应以清除病原体, 但当病原体逃过胞膜模式识别受体的识别而进入细胞内时, 需要胞浆内模式识别受体发挥作用, 进一步清除病原菌, 维持机体内环境的平衡。NOD1作为近年来新发现的一类胞浆内病原模式识别受

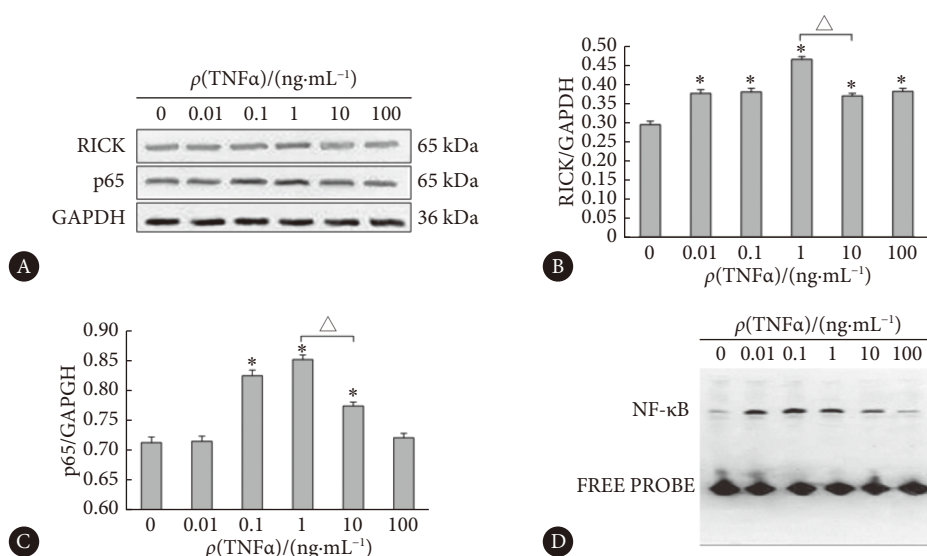


图 6 Western blot检测HTR8/SVneo细胞经不同质量浓度TNFα处理24 h后的RICK和p65蛋白表达水平 (A ~ C) 和NF-κB转录活性 (D)

Fig 6 Western blot analysis of RICK and p65 protein expression levels (A-C) and NF-κB transcriptional activity (D) in HTR8/SVneo cells treated with different concentrations of TNFα for 24 h

* $P < 0.05$, vs. the control group (0 ng·mL⁻¹ TNFα); Δ $P < 0.05$, $n = 3$.

体受到广泛关注。以往研究发现, NOD1蛋白在口腔、食管、肠黏膜上皮细胞、宫颈上皮细胞、肺上皮细胞、间皮细胞广泛表达^[24-26], 且已证实NOD1蛋白在HTR8/SVneo细胞的胞浆内表达^[27]。本研究发现NOD1在早孕绒毛、蜕膜和足月胎盘组织的滋养细胞、母体蜕膜细胞和血管内皮细胞中呈现弥漫性表达, 表明NOD1在正常妊娠母胎界面广泛存在, 可能对正常妊娠的维持有重要作用, 并参与正常妊娠母胎界面的炎症调控, 与既往研究的结果一致^[28-29]。足月胎盘组织中NOD1的表达明显高于早孕绒毛和蜕膜组织, 提示NOD1在正常妊娠进程中可能呈现上调趋势。已有动物实验证实, 细菌成分iE-DAP激活孕鼠NOD1可导致早产、胎鼠体质量减轻和胎鼠炎症^[18], 提示妊娠期滋养细胞表达的NOD1可能在介导感染相关炎症和早产中发挥重要作用^[30-31]。RAKNER等^[7]研究表明, 在正常妊娠和子痫前期患者中, 蜕膜及胎盘组织中NOD1主要表达于母体蜕膜细胞和胎儿滋养细胞, 其中滋养细胞表达水平最高; 与正常妊娠相比, 先兆子痫在蜕膜和胎盘中显示出更高的NOD1表达水平, iE-DAP体外刺激后IL-6和IL-8的增加证实了NOD1的功能。WANG等^[9]研究发现, 与正常妊娠孕妇相比, 经历反复自然流产的孕妇绒毛中观察到较高的NOD1和NOD2表达; ZHANG等^[17]也发现蜕膜基质细胞中NOD1的表达失调可能导致复发性流产。以上研究均表明绒毛中异常升高的NOD1与妊娠结局相关, NOD1参与的免疫调控对妊娠结局具有重要影响。

TNFα作为炎症和免疫应答的关键调节因子, 能够激

活多种炎症细胞并诱导肿瘤细胞凋亡, 研究发现高龄孕妇妊娠早期外周血的TNFα水平高于非高龄孕妇^[32]。本研究用不同质量浓度TNFα刺激HTR8/SVneo细胞, 在显微镜下HTR8/SVneo细胞呈多边形, 未随着TNFα质量浓度的增加而发生改变。HUBER等^[33]报道10 ng/mL TNFα不影响HTR8/SVneo细胞增殖及细胞周期的分布, 从而排除了TNFα对细胞增殖能力的影响。本研究发现, 随着TNFα质量浓度升高, NOD1表达呈剂量依赖性增强, 但在持续刺激条件下, 其表达水平随时间延长反而逐渐下调。这可能是由于TNFα作为细胞表达NOD1蛋白后产生的前炎症因子^[34], 可通过IL-1、IL-6等介质放大炎症级联反应。因此, 低剂量TNFα能快速诱导HTR8/SVneo细胞的NOD1表达, 表明滋养细胞对炎症启动具有高度敏感性。而长时间或高剂量TNFα刺激导致的NOD1下调, 提示细胞通过负反馈调节避免过度炎症反应和病原体应答通路的耗竭。以上结果表明, TNFα介导的滋养细胞NOD1调控既能增强感染过程中的炎症放大效应, 又可能在反馈机制失调时导致炎症状态异常, 该免疫调控机制具有重要的病理生理意义。另外, 本研究还证实TNFα刺激可剂量依赖性地促进滋养细胞IL-8和IL-6的分泌, IL-8水平在24 h达峰值后随刺激时间延长而下降, IL-6则表现为持续累积效应, 表明TNFα在滋养细胞中可能触发双相细胞因子应答, 其中IL-6较IL-8具有更持久的效应。IL-6的持续分泌提示其在TNFα介导的HTR8/SVneo细胞免疫调控机制中具有重要作用, 可能通过促进T/B淋巴细胞活化,

以及借助NOD1阳性滋养细胞桥接固有免疫与适应性免疫^[35-36]。IL-6与IL-8的差异调控可能反映二者在炎症应答中的不同功能分工, IL-8主导早期炎症反应, 而IL-6维持持续性免疫活化。

RICK是含丝氨酸-苏氨酸激酶的胞浆蛋白分子, 其结构包含了同NOD分子效应相同的caspase募集结构域(Caspase recruitment domain, CARD), 可以与含有CARD的蛋白分子相结合发挥转接分子的作用。本研究发现, 滋养细胞中RICK表达呈现TNF α 剂量依赖性调控, 且与NOD1表达模式高度一致, 提示RICK可能是滋养细胞NOD1信号通路中的下游效应分子, 其活性与病原微生物入侵量密切相关, 这与RICK在其他细胞中的研究结果一致^[26]。NF- κ B作为一种重要的核转录因子, 当受到刺激时活化并转位入核, 进而调控多种生理和病理基因表达程序^[37], 其活化与表达可间接受其重要亚基p65是否转入核内的影响^[38]。STROBER等^[39]研究发现, 小鼠胚胎成纤维细胞中RICK基因敲除可完全阻断NOD1/NOD2介导的NF- κ B活化, 而RICK回补能完全恢复NF- κ B应答, 与本研究的结果一致。研究表明10 ng/mL和20 ng/mL TNF α 刺激HTR8/SVneo细胞后会引发NF- κ B活性的降低^[23], 10 ng/mL TNF α 刺激HTR8/SVneo细胞导致p65的核积累^[33]。本研究发现TNF α 质量浓度为0.1 ~ 1 ng/mL时, RICK、NF- κ B和p65的表达及转录活性最高, 随后开始下降。这揭示了NOD1介导的病原识别可能存在明确的耐受阈值, 以及过度刺激下潜在的负反馈调节和固有免疫信号的剂量依赖性脱敏现象。NOD1、RICK和p65在1 ng/mL TNF α 时达峰值, 而NF- κ B结合活性在0.1 ng/mL时最高, 存在峰值错位, 而且IL-6与IL-8的分泌在他们表达降低时继续升高, 提示TNF α 作为滋养细胞发挥作用的效应因子, 刺激滋养细胞NF- κ B活化可能还存在其他信号通路的参与, NOD1-RICK轴仅是这一调控网络的一个组成部分。WANG等^[9]研究表明, 在早孕期绒毛组织中, NOD1/NOD2表达异常升高会通过激活丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)/p38信号通路, 抑制滋养细胞侵袭能力, 提示滋养细胞中多种固有免疫通路间可能存在复杂的交互作用, 因此系统阐明这些替代性NF- κ B调控机制, 包括但不限于MAPK通路的参与, 可能是未来研究的重要方向。

综上, 本研究证实NOD1持续表达于正常妊娠母胎界面的滋养细胞及蜕膜细胞内, 参与了正常妊娠过程的调控, 在正常妊娠的发展过程中可能呈升高趋势, 适度NOD1表达有利于妊娠维持。TNF α 刺激可同时引起NOD1表达上调以及IL-6、IL-8等炎症因子的释放, 并伴

随RICK-NF- κ B信号通路的激活, 提示TNF α 可能通过NOD1-RICK-NF- κ B信号轴触发炎症因子释放, 介导滋养细胞的炎症级联反应, 其确切分子机制仍需进一步通过NOD1功能学实验及相应细胞行为学实验进行验证。本研究为深入理解正常妊娠维持及妊娠期免疫调控机制提供了新的实验依据和研究方向。

* * *

作者贡献声明 邓测川负责论文构思、调查研究、可视化、初稿写作和审读与编辑写作, 康红、李娜娜和薛志伟负责调查研究和研究方法, 邓莹和王美先负责数据审编和正式分析, 李露负责验证, 李玉婷负责研究项目管理, 周淑负责论文构思、经费获取、监督指导和审读与编辑写作, 于萍负责论文构思、监督指导和审读与编辑写作。所有作者已经同意将文章提交给本刊, 且对将要发表版本进行最终定稿, 并同意对工作的所有方面负责。

Author Contribution DENG Cechuan is responsible for conceptualization, investigation, visualization, writing--original draft, and writing--review and editing. KANG Hong, LI Nana, and XUE Zhiwei are responsible for investigation and methodology. DENG Ying and WANG Meixian are responsible for data curation and formal analysis. LI Lu is responsible for validation. LI Yuting is responsible for project administration. ZHOU Shu is responsible for conceptualization, funding acquisition, supervision, and writing--review and editing. YU Ping is responsible for conceptualization, supervision, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突 人绒毛膜滋养细胞株HTR8/SVneo由加拿大CHARLES GRAHAM博士于2010年惠赠。除此之外, 所有作者均声明不存在利益冲突。

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