四川大学学报（医学版） 2025，56（3）：722－729
J Sichuan Univ（Med Sci）

在线全文

YTHDF3调控巨噬细胞活化的机制研究*

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【摘要】 目的 探究m^{6}A识别蛋白YTHDF3对巨噬细胞活化功能的影响及其作用机制。 **方法** 利用shRNA敲低RAW264.7细胞中的*Ythdf3*，检测LPS刺激后RAW264.7细胞促炎因子表达水平、吞噬功能及肿瘤杀伤效应的变化；分析敲低*Ythdf3*对TLR4下游MAPK、NF-κB通路活化水平的影响；对*Ythdf3*敲低后TLR4通路关键的接头蛋白、信号转导分子的表达水平、mRNA稳定性进行分析，探究YTHDF3的靶基因及其调控机制。 **结果** LPS刺激野生型RAW264.7细胞后，其促炎因子水平表现为先上升后下降的趋势；但 YTHDF3的水平在此过程中表现出与促炎因子相反的变化趋势，提示YTHDF3可能发挥负调控巨噬细胞活化的功能。利用shRNA敲低*Ythdf3*可显著增强RAW264.7细胞促炎因子表达水平、NO的分泌和吞噬功能；且在与肿瘤细胞共培养实验中，敲低*Ythdf3* 的RAW264.7细胞肿瘤杀伤能力增强。证实YTHDF3缺失可促进LPS诱导的RAW264.7细胞活化，增强其促炎因子产生及肿瘤杀伤功能。进一步的机制研究发现，敲低*Ythdf3*可抑制TLR4通路关键接头蛋白、信号转导分子*Cd36*、*Irak1*、*Tab1/2*、*Tirap* mRNA的降解，进而增强下游关键激酶p38的磷酸化水平，促进巨噬细胞的活化。 **结论** YTHDF3通过靶向TLR4通路关键接头蛋白、信号转导分子的mRNA，促进其快速降解，抑制巨噬细胞的活化；敲低*Ythdf3*能显著促进巨噬细胞活化，增强其抗肿瘤活性。

【关键词】 YTHDF3 巨噬细胞活化 TLR4通路

YTHDF3 Regulates Macrophage Activation: Investigation of the Mechanisms Involved

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[Abstract] Objective To investigate the role and the underlying mechanisms of N^6-methyladenosine (m^{6}A) reader YTHDF3 in macrophages activation. **Methods** shRNA-mediated *Ythdf3* knockdown in RAW264.7 cells was performed and these RAW264.7 cells were stimulated with LPS. Then, changes in the pro-inflammatory and anti-tumor functions, including cytokine production, phagocytosis, and tumoricidal ability were evaluated. The effect of *Ythdf3* knockdown on the activation of the Toll-like receptor 4 (TLR4) downstream MAPK and NF-κB pathways was assessed by immunoblotting. After *Ythdf3* knockdown, the expression levels and mRNA stability of key junction proteins and signaling molecules of the TLR4 signaling pathway were analyzed to identify YTHDF3 target genes and investigate the underlying regulatory mechanism. **Results** After LPS stimulation of wild-type RAW264.7 cells, the level of pro-inflammatory factors increased and then decreased. However, the level of YTHDF3 showed the opposite trend to that of pro-inflammatory factors, suggesting that YTHDF3 might play a role in the negative regulation of macrophage activation. shRNA-mediated *Ythdf3* knockdown in RAW264.7 cells significantly increased the expression of pro-inflammatory factors, nitric oxide (NO) production, and phagocytosis. In addition, *Ythdf3* knocked-down RAW264.7 cells co-cultured with tumor cells exhibited enhanced tumor killing ability. The findings suggested that YTHDF3 deletion could promote LPS-induced activation of RAW264.7 cells and enhance their production of pro-inflammatory factors and tumor killing function. further investigation into the underlying mechanisms revealed that *Ythdf3* knockdown inhibited the degradation of *Cd36*, *Irak1*, *Tab1/2*, and *Tirap* mRNAs, which were key junction proteins and signaling molecules in the TLR4 pathway, which in turn, enhanced the phosphorylation of p38, a downstream key kinase and the activation of

* 国家自然科学基金（No. 32170905、No. 32470941）和四川省自然科学基金杰出青年科学基金（No. 25NSFJQ0228）资助

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出版日期：2025-05-20

macrophages. **Conclusion**　By targeting the mRNA of the key junction proteins and signaling molecules of the TLR4 pathway, YTHDF3 accelerates their rapid degradation and suppresses macrophage activation. *Ythdf3* knockdown significantly promotes macrophage activation and enhances the tumor killing activities of macrophages.

[Key words]　YTHDF3　Macrophage activation　TLR4 signaling pathway

巨噬细胞是固有免疫系统中重要的效应细胞，直接或间接地参与宿主对病原体的感知和防御[1]，具有较强的可塑性，可在不同微环境刺激下极化形成两种功能几乎完全相反的细胞亚群，即具有促炎作用的经典激活M1型和具有抑炎作用的交替激活M2型[2]。M1 型巨噬细胞极化依赖于 Toll样受体4(TLR4)-丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)/核因子κB(nuclear factor kappa-B, NF-κB)信号通路，抑制该信号通路可以抑制 M1 型巨噬细胞的极化，并进一步诱导 M2 型巨噬细胞极化[3]。巨噬细胞的可塑性使其在多种生理及病理状态下都能发挥相应的效应功能，对机体的新陈代谢及免疫系统的协调运转至关重要。

m^{6}A甲基化修饰是最早发现且最丰富的RNA转录后修饰之一[4]，该修饰是动态可逆的，由甲基化转移酶(m^{6}A writer)、去甲基化酶(m^{6}A eraser)、m^{6}A结合蛋白(m^{6}A reader)三者相互协调完成并进一步对RNA的代谢及功能进行调控[5-6]。m^{6}A修饰对免疫细胞具有重要的调控作用，在T细胞[7-8]、巨噬细胞[9]、树突状细胞[10]、自然杀伤细胞[11]等免疫细胞中的表达异常会引起免疫细胞功能失调并导致相关疾病[12]。m^{6}A识别蛋白，YT521-B 同源 (YTH) 域家族蛋白 (YTH domain family protein 2, YTHDF) 影响着RNA剪切、转录、迁移等多个代谢过程[13]。YTHDF家族1-3蛋白主要参与mRNA稳定性、RNA降解及翻译的调控，它们在功能上或存在一定程度的重复，但在不同组织内表达量不同，在RNA的生理代谢过程中也发挥着不同的调控作用[14-15]。YTHDF3作为YTH家族中发挥重要功能的成员，主要参与促进mRNA翻译、介导mRNA降解等过程[16-17]，但其对巨噬细胞的活化、促炎及抗肿瘤功能的调节及作用机制尚不清晰。本实验通过shRNA敲低RAW264.7细胞内的*Ythdf3*，探究YHTDF3对巨噬细胞的调控作用，为抗肿瘤免疫提供新的潜在靶点。

1　材料与方法

1.1　主要试剂

RAW264.7小鼠单核巨噬细胞白血病细胞系和B16小鼠黑色素瘤细胞系购于上海富衡生物；pLKO.1-puro质粒、脂多糖(lipopolysaccharide, LPS)、放线菌素D(actinomycin D, ACTD)、一氧化氮(NO)检测试剂盒(Greiss reagent)购于美国Sigma；RPMI1640基础培养基、DMEM基础培养基、胎牛血清、青霉素-链霉素(Penicillin-Streptomycin, P/S)溶液购于美国Gibco；嘌呤霉素(Puromycin；Puro)购于美国MCE；总RNA抽提试剂(Trizol)购于美国Thermo Fisher；Phospho-NF-κB/p65、NF-κB/p65、Phosph-p38/MAPK、p38/MAPK一抗购于美国CST；YTHDF3一抗购于武汉三鹰生物；GAPDH一抗，山羊抗小鼠、山羊抗兔二抗购于成都正能生物；荧光颗粒(pHrodo™ BioParticles™ Conjugates)、CellTrace™ CFSE细胞增殖试剂盒、细胞死活染料(Violet fluorescent reactive dye)购于美国Invitrogen；细胞增殖染料(Annexin V-FITC)购于美国Biolegend；磷酸盐缓冲溶液(PBS)、SDS-PAGE蛋白上样缓冲液购于上海碧云天生物；逆转录试剂盒、质粒提取试剂盒购于上海翌圣生物。

1.2　LPS刺激实验

将生长状态良好的RAW264.7细胞接种于6孔板(密度为2×10^6/孔)，100 ng/mL LPS分别刺激细胞0 h、3 h、6 h、12 h、24 h后，PBS清洗并收集蛋白及RNA用以检测YTHDF3和炎症因子白细胞介素-1β(interleukin 1β, IL-1β)、白细胞介素-6(interleukin 6, IL-6)、肿瘤坏死因子-α(tumor necrosis factor α, TNF-α)mRNA的表达。

1.3　Western blot

取蛋白样品进行SDS-PAGE凝胶电泳。待Marker条带清晰分离后进行转膜，后用5%脱脂奶粉室温封闭1 h。封闭完成后在4 ℃冰箱孵育一抗过夜。清洗后用加入辣根过氧化物酶(horseradish peroxidase, HRP)标记的二抗室温孵育1 h，再次清洗。在膜上滴加显色液进行蛋白曝光并采集图像。采用ImageJ软件对蛋白条带进行灰度分析，以内参蛋白作为标准，目的蛋白与内参蛋白灰度值的比值即为目的蛋白的相对表达量。

1.4　逆转录-实时荧光定量PCR（RT-qPCR）

对提纯后的RNA样品定量并进行逆转录。逆转录程序为25 ℃ 5 min；55 ℃ 15 min；85 ℃ 5 min。以逆转录产物为模板进行qPCR扩增。扩增程序为95 ℃ 5 min，一个循环；95 ℃ 10 s，60 ℃ 20 s，72 ℃ 20 s，40个循环。使用qPCR soft 4.0进行数据分析。引物由擎科生物公司合成，序列见表1。

1.5　构建*Ythdf3*敲低细胞系

本研究利用短发夹RNA(shRNA)敲低RAW264.7细胞中的*Ythdf3*[18]。在Sigma官方网站上检索已验证效果的

表1 qPCR引物序列

Table 1 The primer sequences used for qPCR

Gene	Primer sequence (5'-3')
TNF-α	Forward: CCCTCACACTCAGATCATCTTCT
	Reverse: GCTACGACGTGGGCTACAG
IL-1β	Forward: GCAACTGTTCCTGAACTCAACT
	Reverse: ATCTTTTGGGGTCCGTCAACT
IL-6	Forward: TAGTCCTTCCTACCCCAATTTCC
	Reverse: TTGGTCCTTAGCCACTCCTTC
Ythdf3	Forward: CATAGGGCAACAGAGGAAACAG
	Reverse: ATCTCCAGCCGTGGACCAT
CD36	Forward: ATGGGCTGTGATCGGAACTG
	Reverse: GTCTTCCCAATAAGCATGTCTCC
Irak1	Forward: CAGAACCACCACAGATCATCATC
	Reverse: AGGCTTCAATTCCAATAGCATCA
Tab1	Forward: TCCAACCGCAGCTACTCTG
	Reverse: CCCGTACAGGAAGCAGTTATTTT
Tab2	Forward: CATGACCTGCGACAAAAATTCC
	Reverse: TGATTGCGTAGACCAGAAATTCC
Tirap	Forward: CCTCCTCCACTCCGTCCAA
	Reverse: CTTTCCTGGGAGATCGGCAT
GAPDH	Forward: AGTGCCAGCCTCGTCTCATA
	Reverse: GAGAAGGCAGCCCTGGTAAC
β-actin	Forward: GGCACCACACCTTCTACAATG
	Reverse: GGGGTGTTGAAGGTCTCAAAC
18S-rRNA	Forward: GTAACCCGTTGAACCCCATT
	Reverse: CCATCCAATCGGTAGTAGCG

TNF-α: tumor necrosis factor; *IL-1β*: interleukin 1β; *IL-6*: interleukin 6; *Irak1*: IL-1 receptor associated kinase1; *Tab1*: MAP3K7 binding protein 1; *Tab2*: MAP3K7 binding protein 2; *Tirap*: TIR domain containing adaptor protein.

靶向*Ythdf3*的shRNA序列并进行BLAST比对，选择评分前3的序列构建sh-*Ythdf3* RAW264.7细胞系，无靶向功能的随机序列(sh-CTL)作为阴性对照。将候选序列构建成完整的shRNA引物序列或sh-CTL序列送至擎科生物合成。将合成的双链sh-*Ythdf3*或sh-CTL与pLKO.1-puro质粒连接构成重组质粒。将重组质粒转入感受态大肠杆菌，挑取氨苄西林(Ampicillin)抗性克隆扩大培养并进行质粒提取。提取的重组质粒包装成慢病毒后感染生长状态良好的RAW264.7，孵育2 d后，用浓度为4 μg/mL的puro进行筛选，收集细胞并扩大培养即获得*Ythdf3*敲低的RAW264.7细胞。以sh-CTL组细胞作为对照，检测3个sh-*Ythdf3*敲低RAW264.7细胞系的*Ythdf3* mRNA和蛋白质水平，选用敲除效果最好的RAW264.7细胞进行后续实验。本研究所用sh-*Ythdf3*或sh-CTL序列分别为5'-CCGGCCTATGGACAAATGAGTAACTCGAGTTACTCATTTGTCCATAGGTTTTTG-3'; 5'-CCGGTCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG-3'。

1.6 NO分泌实验

将生长状态良好的sh-CTL组和sh-*Ythdf3*组RAW264.7细胞接种于96孔板(密度为1.5×10^5/孔)。设置LPS未处理组和LPS处理组。处理组细胞用100 ng/mL LPS刺激24 h后向所有细胞上清液中加入等体积Greiss reagent，并用酶标仪在570 nm波长下测定吸光度值。

1.7 吞噬实验

将生长状态良好的sh-CTL组和sh-*Ythdf3*组RAW264.7细胞接种于96孔板(密度为1×10^5/孔)，100 ng/mL LPS刺激24 h后，用PBS清洗以去除LPS。实验设置为不加荧光颗粒组和加荧光颗粒组。按实验组别设置向相应细胞中加入100 μg/mL荧光颗粒，并于37 ℃ CO_2培养箱培养30 min。孵育完成后用PBS清洗多余的荧光颗粒，收集细胞进行流式细胞术验证。

1.8 杀伤实验

将生长状态良好的sh-CTL组和sh-*Ythdf3*组RAW264.7细胞用100 ng/mL LPS刺激24 h，PBS清洗细胞以去除LPS，收集效应细胞(effector cells, E)。CFSE标记B16肿瘤细胞(tumor cells, T)。按E: T分别为20∶1、10∶1和5∶1接种至24孔板中，其中B16肿瘤细胞数量固定，为5×10^4/孔。将上述细胞混匀，于细胞培养箱培养24 h后，收集细胞并进行流式细胞术验证。

1.9 流式细胞术

收集需要检测的细胞，流式抗体染色后用300 μL FACS(fluorescence activated cell sorting) buffer(PBS+0.5%FBS)重悬，用70 μm滤网过滤制成单细胞悬液，流式细胞仪检测对应的荧光强度。用Flowjo V10对数据进行处理和分析。

1.10 信号通路检测实验

将生长状态良好的sh-CTL组和sh-*Ythdf3*组RAW264.7细胞接种于6孔板(密度为2×10^6/孔)，100 ng/mL LPS分别刺激细胞0、5、15、30、60、120 min。刺激完成后，收集细胞蛋白检测信号通路关键蛋白Cd36、白细胞介素-1受体相关激酶1(IL-1 receptor associated kinase1, Irak1)、转化生长因子β激活激酶1(MAP3K7)结合蛋白1(MAP3K7 binding protein 1, Tab1)、MAP3K7结合蛋白2(MAP3K7 binding protein 2, Tab2)、含衔接蛋白的TIR结构域(TIR domain containing adaptor protein, Tirap)的mRNA的表达。

1.11 mRNA稳定性实验

将生长状态良好的sh-CTL组和sh-*Ythdf3*组RAW264.7细胞接种于6孔板(密度为2×10^6/孔)，100 ng/mL LPS刺激6 h后，去除原有培养基。5 μg/mL ACTD分别处理0 h、0.5 h、1 h、2 h后收集细胞并提取RNA，RT-qPCR检测RNA表达水平。

1.12 统计学方法

所有统计学分析均在GraphPad Prism9软件上进行，数据以平均值±标准差形式呈现，两组单因素样本数据比较采用未配对的*t*检验；多组单因素样本数据比较采用单因素多重ANOVA检验；两组双因素样本数据比较采用双

因素多重ANOVA检验。$P < 0.05$为差异有统计学意义。

2　结果

2.1　LPS激活后，RAW264.7细胞中YTHDF3与促炎因子水平的动态变化特征

LPS刺激后，RAW264.7细胞主要分泌的促炎因子IL-1β、IL-6、TNF-α的mRNA表达水平先上升后下降(图1A)，在刺激后3~6 h内达到峰值。与此相反的是，*Ythdf3* mRNA(图1B)与蛋白质(图1C)水平在刺激后先下降再上升。

2.2　RAW264.7细胞*Ythdf3*基因敲低验证

本实验构建了3种*Ythdf3*敲低的RAW264.7细胞系，经过对其*Ythdf3* mRNA和蛋白水平的检测(图2)，发现3号候补shRNA(sh-*Ythdf3*#3)敲低效果最佳，遂选用sh-*Ythdf3*#3敲低的RAW264.7细胞系进行后续实验。

2.3　敲低*Ythdf3*促进RAW264.7细胞的炎症因子表达、吞噬和肿瘤杀伤功能

sh-*Ythdf3*组RAW264.7细胞促炎因子*IL-1β*、*IL-6*、*TNF-α* mRNA表达水平显著高于sh-CTL组(图3A)；sh-*Ythdf3*组RAW264.7细胞NO产生水平显著高于sh-CTL组(图3B)。随后，我们通过检测巨噬细胞吞噬荧光颗粒的能力对其吞噬活性进行评估，发现sh-*Ythdf3*组荧光阳性群比例显著高于sh-CTL组(图3C)。我们又将sh-CTL组和sh-*Ythdf3*组RAW264.7分别与CFSE标记的B16肿瘤细胞共培养以评估其肿瘤杀伤能力，发现sh-*Ythdf3*组中肿瘤细胞凋亡的比例显著升高(图3D)，且E: T比值越低，凋亡比例越高。

2.4　敲低*Ythdf3*促进RAW264.7细胞MAPK信号通路活化

为进一步探究YTHDF3对巨噬细胞活化负向调控的机制，我们对MAPK和NF-κB通路上关键激酶和蛋白亚基p38和p65的磷酸化水平进行检测，发现敲低*Ythdf3* 不影响p38和p65的蛋白水平，但能显著增强p38蛋白磷酸化水平(图4)。

2.5　*Ythdf3*通过促进TLR4通路关键信号转导分子mRNA的降解抑制巨噬细胞活化

敲低*Ythdf3*可显著上调MAPK通路上游TLR4信号通

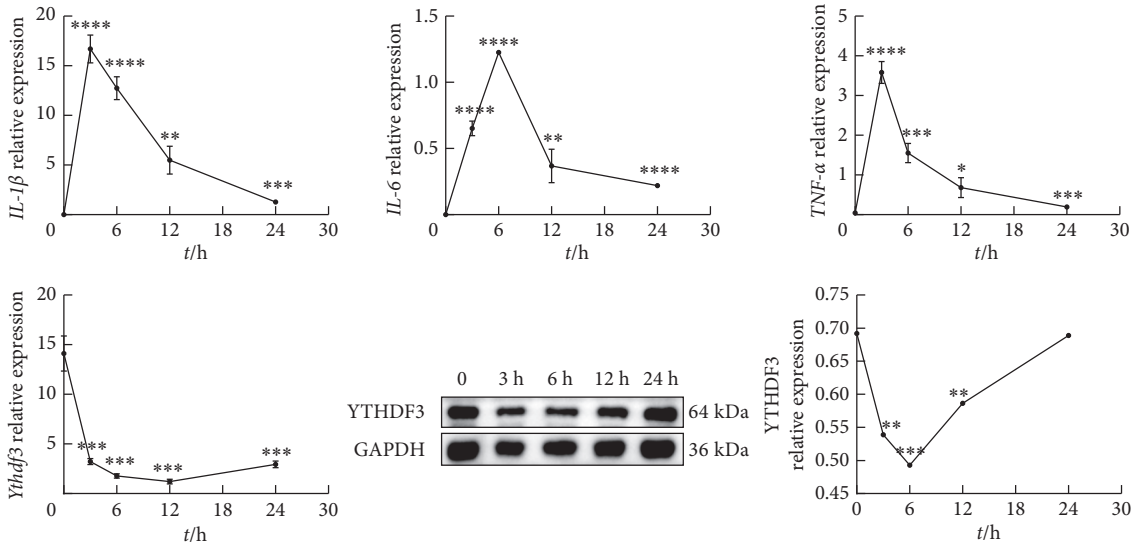

图 1　100 ng/mL LPS刺激对RAW264.7细胞促炎因子和YTHDF3水平的影响

Fig 1　Effects of 100 ng/mL LPS stimulation on pro-inflammatory factors and YTHDF3 in RAW264.7 cells

$n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.000\,1$, vs. 0 h.

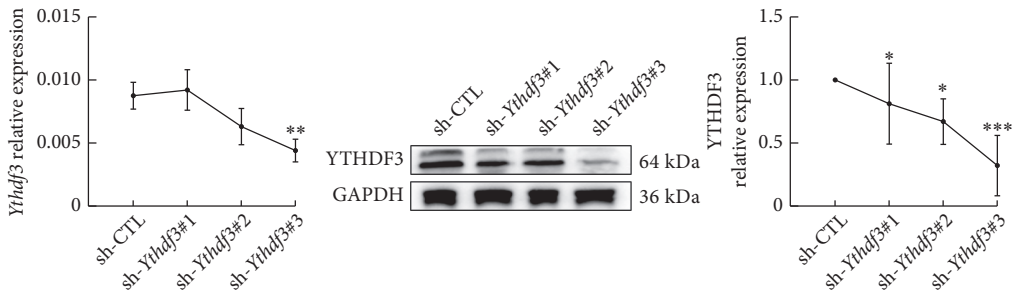

图 2　RAW264.7细胞*Ythdf3*基因敲低效果

Fig 2　Effect of *Ythdf3* knockdown on RAW264.7 cells

$n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, vs. sh-CTL.

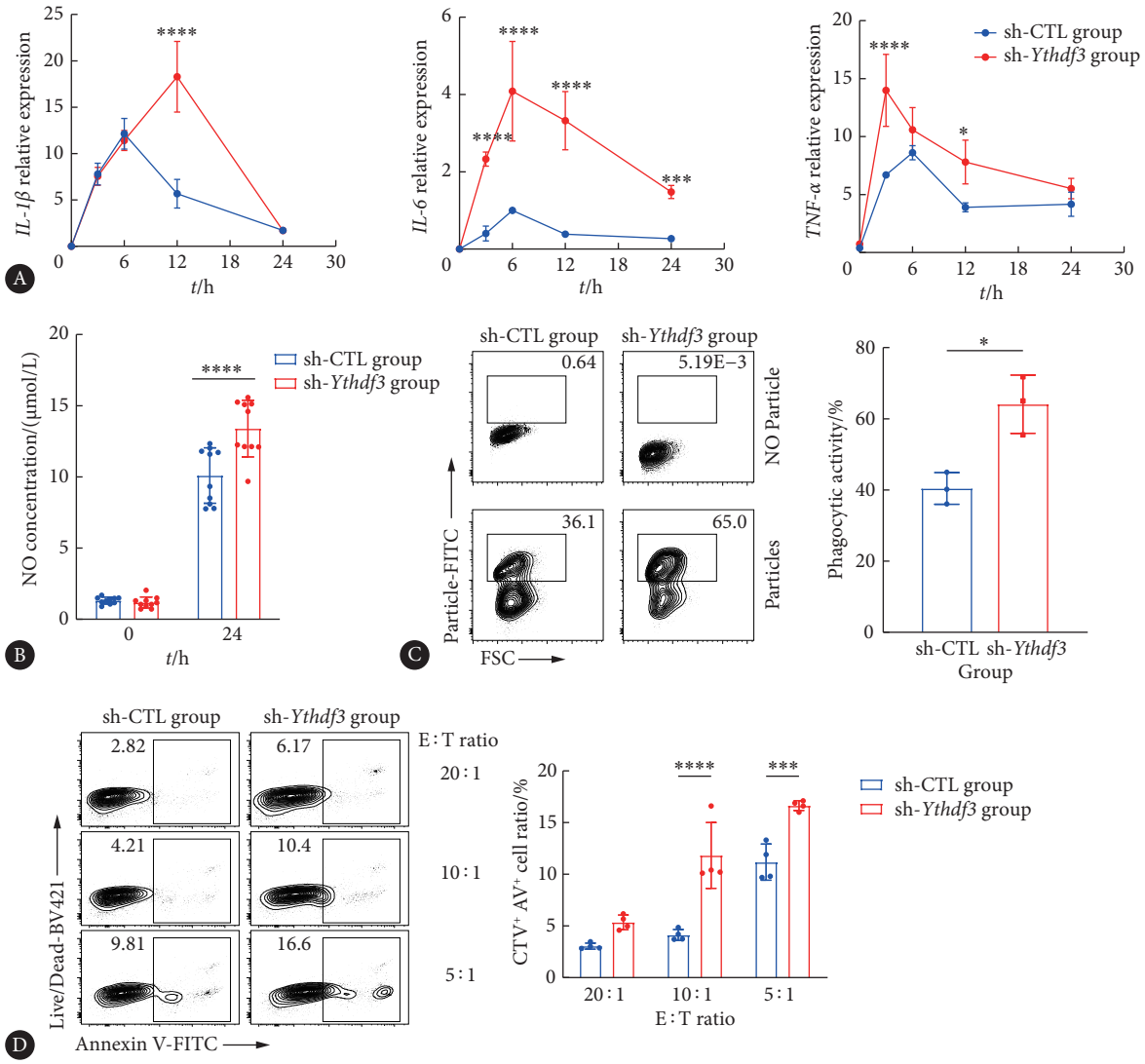

图 3 敲低*Ythdf3*对RAW264.7细胞促炎因子的产生及其吞噬、肿瘤杀伤能力的影响

Fig 3 Effect of *Ythdf3* knockdown on pro-inflammatory cytokine production and phagocytic and tumor killing ability of RAW264.7 cells

A, Relative mRNA levels of pro-inflammatory factors in sh-CTL and sh-*Ythdf3* groups of RAW264.7 cells stimulated with LPS ($n = 3$, $^{****}P < 0.000\,1$, $^{***}P < 0.001$, $^{*}P < 0.05$, vs. sh-CTL group). B, NO secretion in sh-CTL and sh-*Ythdf3* group RAW264.7 cells stimulated with LPS ($n = 10$, $^{****}P < 0.000\,1$). C, Representative dot plots (left) and the frequencies (right) of FITC-positive particles in sh-CTL and sh-*Ythdf3* group RAW264.7 cells stimulated with LPS ($n = 3$, $P = 0.011\,7$). D, Representative dot plots (left) and the frequencies (right) of apoptotic B16 cells after co-culture with LPS-stimulation ($n = 4$, $^{****}P < 0.000\,1$, $^{***}P < 0.001$).

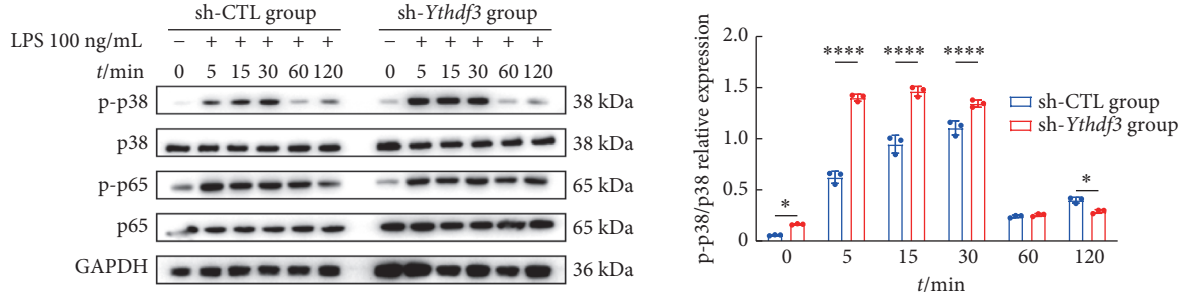

图 4 敲低*Ythdf3*对RAW264.7细胞 MAPK和NF-κB细胞信号通路的影响

Fig 4 Effect of *Ythdf3* knockdown on MAPK and NF-κB signaling pathways in RAW264.7 cell

$n = 3$. $^{*}P < 0.05$, $^{****}P < 0.000\,1$.

路关键的接头蛋白、信号转导分子*Cd36*、*Irak1*、*Tab1*、*Tab2*、*Tirap*的mRNA水平(图5A)。

我们利用ACTD阻断LPS刺激后上述基因的转录,通过检测ACTD处理后不同时间点各基因的mRNA水平,探

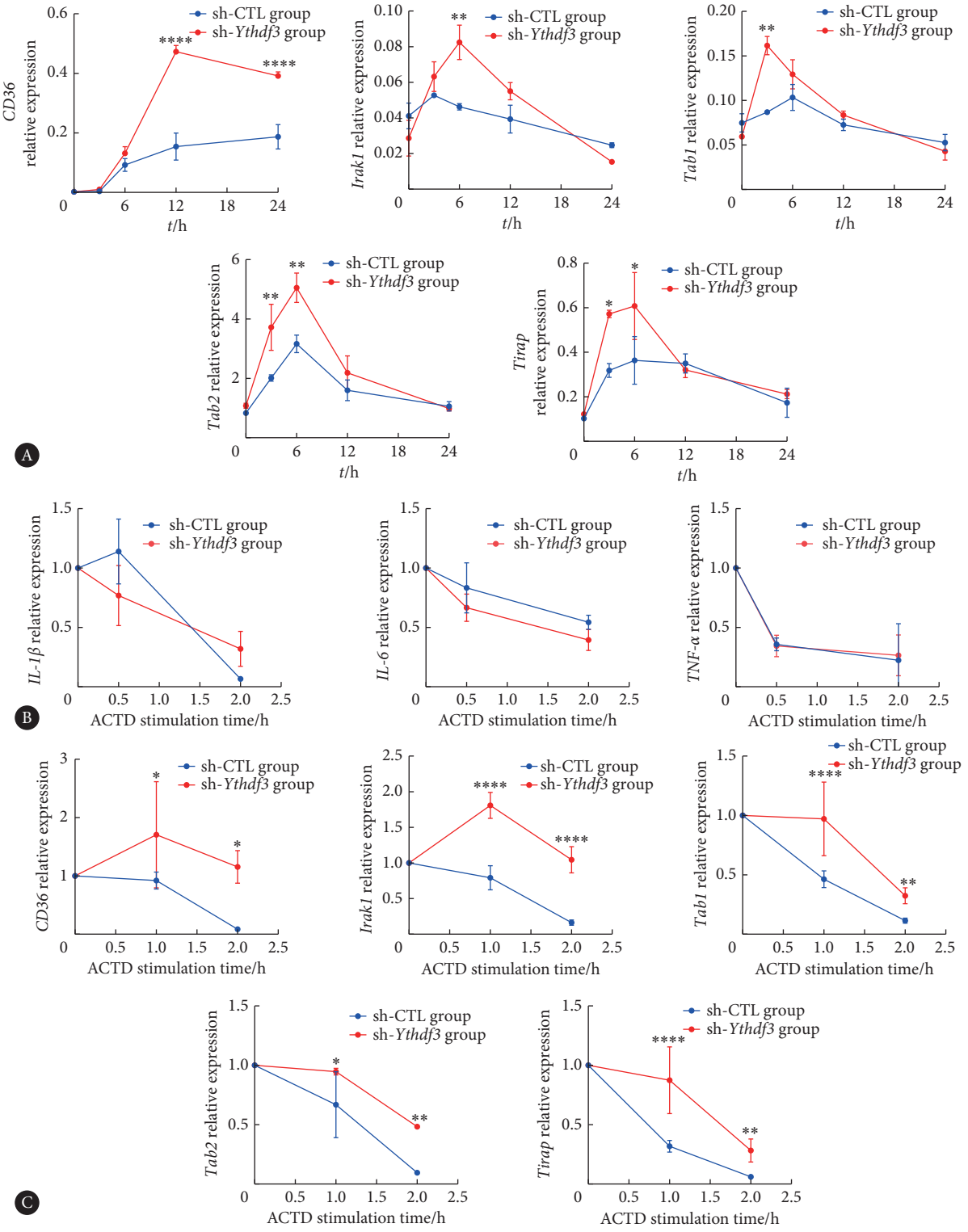

图 5　敲低 *Ythdf3*对RAW264.7细胞TLR4通路信号分子的影响

Fig 5　Effect of *Ythdf3* knockdown on TLR4 pathway signaling adaptors of RAW264.7 cells

A, Relative mRNA levels of TLR4 pathway signaling adaptors in sh-CTL and sh-*Ythdf3* group RAW264.7 cells stimulated with LPS. B and C, Relative mRNA levels of pro-inflammatory factors (B) and TLR4 pathway signaling adaptors (C) in sh-CTL and sh-*Ythdf3* group RAW264.7 cells treated with ACTD (n = 3, * P < 0.05, ** P < 0.01, **** P < 0.000 1, vs. sh-CTL group).

究YTHDF3对上述mRNA稳定性的影响。发现*Ythdf3*敲低并不会影响RAW264.7中促炎因子mRNA的稳定性(图5B)，但TLR4信号通路关键的接头蛋白*Cd36*、*Irak1*、*Tab1*、*Tab2*、*Tirap*的mRNA的稳定性显著增加(图5C)。

3 讨论

巨噬细胞的活化由病原体、组织损伤和免疫信号分子等引发,而后执行监视、识别、杀伤和清除外源性病原体及坏死细胞等一系列免疫功能[19]。RNA代谢包括RNA合成、修饰、转运、降解和翻译等多个环节,它们环环相扣,不仅维持着巨噬细胞的基本功能,还在巨噬细胞极化、炎症响应及抗病毒感染等过程中发挥重要作用[20-21]。RNA转录后修饰是RNA代谢的重要组成部分,m^{6}A修饰作为最常见的mRNA转录后修饰,在巨噬细胞中也发挥着重要作用。YTHDF1/2/3是目前研究最为广泛的m^{6}A阅读蛋白,它们既各司其职又相互协同,在基因表达调控及细胞命运决定方面发挥着重要作用[8]。研究发现,YTHDF1可通过介导SPRED2(sprouty related EVH1 domain containing 2)的翻译促进M1型巨噬细胞的极化[20];YTHDF2可通过控制 *Pyk2*(protein tyrosine kinase)mRNA 稳定性,增强MAPK 和 AKT(protein kinase B)信号,促进巨噬细胞活化[22]。以往研究认为,YTHDF3的角色更多的是作为辅助因子,参与YTHDF1和YTHDF2介导的mRNA代谢调控[16-17]。但在巨噬细胞中,YTHDF3是否参与调控巨噬细胞活化,是否也作为辅因子协同YTHDF1和YTHDF2起作用仍不清楚,因此,本研究聚焦于YTHDF3,探讨其在巨噬细胞活化过程中的调控机制。

LPS激活巨噬细胞的TLR4信号通路后,可迅速诱导下游炎症因子表达,并在LPS刺激后6-12小时表达水平达到高峰。TLR4的激活也会诱导一系列负调控因子的产生,如SARM(sterile alpha- and armadillo-motif-containing protein)[23], TIPE2(tumour necrosis factor α-induced protein 8-like 2)[24], Axl(AXL receptor tyrosine kinase)[25]等,这些负调控因子抑制TLR4持续活化和炎症因子的产生,促进巨噬细胞恢复至稳态。我们发现,*Ythdf3* mRNA与蛋白质水平与炎症因子的表达动态相反,提示YTHDF3可能对巨噬细胞的活化具有负向调控作用。通过敲低*Ythdf3*,我们首次对YTHDF3在巨噬细胞活化中的功能进行研究,发现敲低*Ythdf3*可显著增强RAW264.7细胞中*Il-1β*、*Il-6*、*Tnf-α*等炎症性细胞因子的表达,促进NO的分泌增加,增强其吞噬和肿瘤杀伤能力,表明YTHDF3确实对巨噬细胞促炎、吞噬、肿瘤杀伤功能具有重要的调控作用,且与YTHDF1和YTHDF2的促炎功能不同,YTHDF3主要发挥抑制巨噬细胞活化的功能。

TLR信号通过激活MAPK和NF-κB通路,启动下游效应分子的表达[26-29]。前期研究发现,m^{6}A修饰识别蛋白YTHDF1及YTHDF2可通过促进TLR通路关键负调控因子mRNA的降解,促进巨噬细胞活化[30]。通过对MAPK和NF-κB通路上关键激酶和蛋白亚基p38和p65的磷酸化水平进行检测,我们发现敲低*Ythdf3* 并不影响p38和p65的总蛋白水平,但能显著增强p38的磷酸化。表明YTHDF3主要通过抑制MAPK通路的活性,抑制RAW264.7细胞的活化,提示不同的m^{6}A识别蛋白可能在巨噬细胞激活过程中发挥不同的调控功能。

进一步机制研究发现,敲低*Ythdf3*可显著上调TLR4通路关键接头蛋白、信号转导分子*Cd36*、*Irak1*、*Tab1/2*、*Tirap* 的mRNA水平,提示YTHDF3可能通过下调TLR4通路上游关键调控因子的表达水平,影响TLR的活性和巨噬细胞活化。YTHDF3作为m^{6}A的识别蛋白之一,其主要通过结合m^{6}A参与调控靶mRNA的稳定性[17]。我们利用ACTD阻断RNA的转录后,对上述mRNA稳定性进行了分析,发现*Ythdf3*敲低可显著增强TLR4通路关键接头蛋白、信号转导分子的mRNA稳定性,但对促炎因子mRNA的稳定性并无影响。上述结果表明,YTHDF3可通过促进TLR4通路关键信号转导分子mRNA的降解,抑制TLR4-MAPK通路介导的巨噬细胞活化,并可能与YTHDF1、YTHDF2协同调控TLR4信号通路,共同影响巨噬细胞功能。

综上,本研究首次发现YTHDF3对巨噬细胞活化的负向调控功能,靶向YTHDF3可增强巨噬细胞炎症因子的产生和肿瘤杀伤能力,为靶向YTHDF3重编程巨噬细胞进而治疗肿瘤提供新思路。

* * *

作者贡献声明 彭可仁负责调查研究、验证、可视化和初稿写作,尹敏敏负责论文构思、数据审编、经费获取、研究方法、研究项目管理、提供资源、监督指导和审读与编辑写作,童吉宇负责论文构思、数据审编、经费获取、研究方法、研究项目管理、提供资源、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,且对将要发表的版本进行最终定稿,并同意对工作的所有方面负责。

Author Contribution PENG Keren is responsible for investigation, validation, visualization, and writing--original draft. YIN Qimin is responsible for conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, and writing--review and editing. TONG Jiyu is responsible for conceptualization, data curation, funding acquisition, methodology, project administration, resources, 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 所有作者均声明不存在利益冲突

Declaration of Conflicting Interests All authors declare no competing interests.

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（2024 – 12 – 19收稿, 2025 – 05 – 01修回）
编辑　汤　洁