中国寄生虫学与寄生虫病杂志 2009 28 (6): 463-466 ISSN: 1000-7423 CN: 31-1248/R

论著

约氏疟原虫环子孢子蛋白对TNF-α刺激人肝癌细胞株核转录因子活化的抑制作用

丁艳,陈继德,周桃莉,付雍,彭小红,徐文岳*

第三军医大学病原生物学教研室，重庆 400038

收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的 观察约氏疟原虫环子孢子蛋白（CSP）对肿瘤坏死因子α（TNF-α）刺激人肝癌细胞株HepG2核转录因子-κB （NF-κB）活化的影响。 方法 以约氏疟原虫BY265株子孢子总RNA为模板，用RT-PCR扩增CSP基因的编码区序列并克隆至pFLAG-CMV8载体，构建重组质粒pFLAG-CMV8-CSP。以兔抗CSP多克隆抗体间接免疫荧光法观察pFLAG-CMV8-CSP能否在HepG2细胞中正确表达，及其在细胞中的分布。实验分为3组，A组（阴性对照组）为转染质粒pFLAG-CMV8的HepG2细胞，B组以100 ng/ml TNF-α刺激转染质粒pFLAG-CMV8的HepG2细胞，C组以100 ng/ml TNF-α刺激转染质粒pFLAG-CMV8-CSP的HepG2细胞。采用双荧光素酶试验和凝胶迁移试验（EMSA）检测NF-κB 的核转位及其活化，观察pFLAG-CMV8?鄄CSP对于TNF-α刺激HepG2细胞活化NF-κB是否具有抑制作用。 结果 质粒pFLAG-CMV8-CSP主要在HepG2细胞胞浆中表达。 检测HepG2细胞浆中NF-κB活性，C组萤火虫荧光素酶活性与海肾荧光素酶活性比值为0.228±0.029，明显低于B组（0.571±0.030）和A组（0.438±0.085）（P＜0.05）。EMSA结果显示，C组的条带明显弱于B组。 结论 位于细胞浆中的疟原虫CSP蛋白通过抑制NF-κB核转位, 从而抑制TNF-α刺激HepG2细胞活化NF-κB。

关键词 约氏疟原虫 环子孢子蛋白 核转录因子-κB 人肝癌细胞株

分类号

Inhibition of Plasmodium yoelii Circumsporozoite Protein on the Activation of Nuclear Transcription Factor in Hepatoma Cells Stimulated by TNF-α

DING Yan,CHEN Ji-de,ZHOU Tao-li,FU Yong,PENG Xiao-hong,XU Wen-yue*

Department of Pathogenic Biology，The Third Military Medical University，Chongqing 400038，China

Abstract

Objective To investigate on the effect of *Plasmodium yoelii*（BY265 strain） circumsporozoite protein （CSP）on the activation of nuclear transcription factor κB （NF-κB）in hepatoma cells（HepG2）stimulated by TNF-α. Methods Entire coding sequence of CSP was reverse transcribed and amplified by RT-PCR with sporozoite total RNA as template，then cloned into pFLAG-CMV8 for construction of the recombinant plasmid pFLAG-CMV8-CSP. Indirect immunofluorenscence staining with rabbit anti-csp was applied to verify the expression and distribution of FLAG-CSP fusion protein in HepG2. Three groups were established for the experiment: group A with HepG2 transfected by pFLAG-CMV8 as negative control, group B with HepG2 transfected by pFLAG-CMV8 and stimulated by 100 ng/ml TNF-α, and group C with HepG2 transfected by pFLAG-CMV8-CSP and stimulated by 100 ng/ml TNF-α. Dual-luciferase assay and EMSA were performed to detect the nuclear translocation and activation of NF-κB, to observe if pFLAG-CMV8-CSP suppressed the activation of NF-κB in HepG2 stimulated by TNF-α. Result The expression of pFLAG-CMV8-CSP was localized on cytoplasm of HepG2. The activity ratio of firefly luciferase to *Renilla* luciferase in group C（0.228±0.029） was significantly lower than both groups A（0.438±0.085）and B（0.571±0.030）（P<0.05）. EMSA showed that the band in group C was significantly weaker than group B. Conclusion *Plasmodium yoelii* CSP localizes in the cytoplasm of HepG2 and inhibits the activation and nuclear translocation of NF-κB in HepG2 stimulated by TNF-α.

Key words *Plasmodium yoelii* Circumsporozoite protein Nuclear transcription factor κB Hepatoma cell

DOI:

通讯作者

作者个人主页 丁艳;陈继德;周桃莉;付雍;彭小红;徐文岳*