

论著

日本血吸虫内皮分化相关因子-1基因的克隆、表达及鉴定

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摘要

【摘要】 目的 克隆、表达日本血吸虫内皮分化相关因子 (endothelial differentiation?-related factor) -1编码基因 (SjEDF-1), 并研究该基因在日本血吸虫不同发育阶段的特异性表达情况。方法 制备日本血吸虫卵、尾蚴、童虫和成虫等各发育阶段的总RNA, 采用RT-PCR方法, 以管家基因SjActin为内参, 根据SjEDF-1基因全长编码序列 (GenBank登录号为AY336498) 的开放阅读框设计引物进行RT-PCR扩增, 分析日本血吸虫各发育阶段SjEDF-1基因mRNA表达情况。将SjEDF-1基因亚克隆至载体pET28a, 转化至大肠埃希菌BL21 (DE3), 异丙基-β-D硫代半乳糖苷 (IPTG) 进行诱导表达。用组氨酸标签亲和层析法纯化表达产物, 以纯化的重组SjEDF-1蛋白免疫新西兰白兔制备免疫血清。蛋白质印迹 (Western blotting) 分析其免疫原性及该蛋白在日本血吸虫各发育阶段的表达差异。结果 RT-PCR结果显示, 除尾蚴外, 在虫卵、童虫、雄虫和雌虫期均扩增出约405 bp的片段。含重组质粒SjEDF-1/pET-28a的转化子细菌, 经IPTG诱导, 获得以不可溶的包涵体形式存在的重组蛋白, 其相对分子质量 (Mr) 为20 000, 与预测的融合蛋白大小相符。Western blotting分析结果显示, 纯化后的重组蛋白SjEDF-1可被日本血吸虫感染兔血清识别, 在血吸虫童虫和成虫阶段检测到目的蛋白的表达。结论 重组蛋白SjEDF-1具有较强的免疫原性, 在血吸虫童虫和成虫期检测到该蛋白特异表达。

关键词 [日本血吸虫; 内皮分化相关因子-; 虫期特异性; 免疫原性](#)

分类号

Cloning, Expression and Identification of Endothelial Differentiation-Related Factor-1 Gene of Schistosoma japonicum

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Abstract

【Abstract】 Objective To clone and express endothelial differentiation-related factor (SjEDF) - gene of Schistosoma japonicum, analyze its immunogenicity and the stage-specific expression at different developmental stages of S. japonicum. Methods Total RNA were extracted from eggs, cercariae, schistosomula and adult worms. The housekeeping gene SjActin was selected as the internal reference. According to the open reading frame for SjEDF- gene (GenBank accession number: AY336498), a pair of primers were designed to amplify the SjEDF- gene which was subcloned into pET-8a vector. The recombinant plasmid SjEDF-/pET-8a was transformed into E. coli BL21 and induced with IPTG for expression. The recombinant protein was purified with Ni-TA resin. The immune rabbit sera was prepared by immunizing New Zealand white rabbits with purified recombinant SjEDF- protein. Western blotting was used to analyze the immunogenicity and the expression level of SjEDF- at the different developmental stages. Results The SjEDF- gene was detected with a band of 405 bp in eggs, schistosomula, female and male worms. The recombinant protein (rSjEDF-) was expressed as inclusion bodies (Mr 20 000). Western blotting analysis showed that the purified rSjEDF- protein was recognized by pooled sera of infected rabbits. The target protein was detected only in schistosomulum and adult worms. Conclusion The recombinant protein (rSjEDF-) shows certain immunogenicity, and is detected only in schistosomula and adult worms.

Key words [Schistosoma japonicum; Endothelial differentiation-related factor-; Stage-specificity; Immunogenicity](#)

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