



日本血吸虫卵SjE16、SjPPIase和SjRobl基因的真核表达及其在诊断中的应用

陈清, 吴琛耘, 冯艳, 吴健桦, 姚馨紫, 徐大刚, 王兆军*

上海交通大学医学院病原生物学教研室, 上海 200025

Eukaryotic Expression of SjE16, SjPPIase and SjRobl Genes from Schistosoma japonicum Egg and Evaluation of Their Role in Immunodiagnosis

CHEN Qing, WU Chen-yun, FENG Yan, WU Jian-hua, YAO Xin-ying, XU Da-gang, WANG Zhao-jun*

Department of Microbiology and Parasitology, Shanghai JiaoTong University School of Medicine, Shanghai 200025, China

摘要

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摘要 目的 真核表达日本血吸虫卵蛋白,并评价其在血吸虫病免疫学诊断中的作用。方法 提取日本血吸虫卵RNA,逆转录后获得cDNA。PCR扩增日本血吸虫卵特异性或高表达基因SJCHGC01695 (SjE16)、SJCHGC00856 (SjIMA8)、SJCHGC06249 (SjTOR)、SJCHGC06324 (SjP40)、SJEFTD02 (SjSLP)、SJCHGC06679 (SjPPIase)和SJCHGC06529 (SjRobl),亚克隆至真核表达载体pPIC9K。重组质粒电转至毕赤酵母GS115菌株后,甲醇诱导目的蛋白表达。镍-次氨基三乙酸(Ni-NTA)亲和层析纯化获取重组蛋白后,用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)和蛋白质印迹(Western blotting)对其进行分析鉴定。以血吸虫可溶性虫卵抗原(SEA)、重组蛋白SjE16、SjPPIase和SjRobl单用,3个重组蛋白两两伍用,及全部伍用作为包被抗原,ELISA法检测血吸虫感染小鼠血清、急性血吸虫病和慢性血吸虫病患者血清中相应抗体的反应性。结果 PCR扩增获得7个日本血吸虫卵高表达基因,经酵母重组表达和Ni-NTA亲和层析纯化后成功获得3个重组蛋白SjE16、SjPPIase和SjRobl。SDS-PAGE和Western blotting鉴定结果显示,所获得的重组蛋白为目的蛋白。ELISA检测结果显示,血吸虫感染小鼠血清和血吸虫病患者血清中均可检测出被SjE16、SjPPIase和SjRobl识别的特异性IgM和IgG抗体。在急性血吸虫病患者中上述3种抗原IgM的检出率分别为80%、60%和80%,IgG检出率分别为40%、80%和70%。伍用组中,SjE16和SjRobl伍用抗原,检测急性血吸虫病患者血清中IgM抗体的敏感性为100%,高于其单独为抗原的敏感性。结论 获得的3个日本血吸虫卵蛋白(SjE16、SjPPIase和SjRobl)均具有血吸虫病诊断的潜能,其中SjE16和SjRobl伍用抗原可提高急性血吸虫病诊断敏感性。

关键词: 日本血吸虫 虫卵高表达基因 真核表达 免疫学诊断

Abstract: Objective To express *Schistosoma japonicum* egg proteins by eukaryotic system and evaluate their role in schistosomiasis immunodiagnosis. Methods *S. japonicum* egg RNA was extracted and reversed to cDNA. Egg specific or highly expressed genes: SJCHGC01695 (SjE16), SJCHGC00856 (SjIMA8), SJCHGC06249 (SjTOR), SJCHGC06324 (SjP40), SJEFTD02 (SjSLP), SJCHGC06679 (SjPPIase) and SJCHGC06529 (SjRobl), were amplified and sub-cloned to eukaryotic expression vector pPIC9K. Recombinant vectors were transformed to yeast GS115 and the recombinant yeast was induced by methanol. Proteins were purified with Ni-NTA affinity chromatography and analyzed by SDS-PAGE and Western blotting. For the detection of specific antibodies, the wells of microtiter plate were coated with soluble egg antigen (SEA), SjE16, SjPPIase and SjRobl, respectively, or combination of recombinant proteins. The specific antibody reactivity in sera from schistosome-infected mice and patients were examined by ELISA. Results The highly expressed genes from *S. japonicum* eggs were cloned by PCR. The recombinant proteins of SjE16, SjPPIase and SjRobl were expressed and identified by SDS-PAGE and Western blotting. Those recombinant SjE16, SjPPIase and SjRobl were recognized by IgM and IgG in schistosome-infected mouse and patient sera. The sensitivity of the three antigens in detecting IgM and IgG in acute patients were 80%, 60%, 80% and 40%, 80%, 70%, respectively, while that of the combination of SjE16 and SjRobl in detecting IgM was 100%. Conclusion The above three *S. japonicum* egg enriched proteins were expressed using eukaryotic expression system and can be used in acute schistosomiasis diagnosis.

Keywords: *Schistosoma japonicum* Egg enriched gene Eukaryotic expression Immunodiagnosis

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