

论著

日本血吸虫特异性IgE相关抗原编码基因的克隆和鉴定

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摘要

目的 从日本血吸虫成虫cDNA文库中筛选并克隆日本血吸虫特异性IgE相关抗原编码基因。方法 用ABC ELISA法从日本血吸虫病流行区筛选出高水平抗血吸虫成虫抗原IgE抗体的个体 15人,采集血清并混合。混合血清经Protein G柱吸收后,用于日本血吸虫成虫cDNA文库的免疫学筛选。PCR扩增阳性克隆插入cDNA片段。序列分析后,于该序列第一开读框两端设计引物并分别引入EcoR I 和Not I 位点,PCR扩增并纯化目的基因片段后克隆入质粒载体pGEM T,再亚克隆入表达载体pGEX 6p 1。经IPTG诱导表达,对表达产物进行SDS PAGE和Westernblotting鉴定。结果 阳性克隆插入片段约 12 0 0bp,第一开读框长 5 0 7bp,编码 16 9个氨基酸,理论分子量为 19 3kDa。DNA序列分析显示,与已知序列同源性小于 4 0 %。重组质粒pGEX 6p 1/Sj4 3B能高效表达融合蛋白,且能被日本血吸虫特异性IgE抗体识别。结论 成功构建的重组质粒pGEX 6p 1/Sj4 3B,Sj4 3B可编码日本血吸虫特异性IgE抗体相关抗原

关键词 [日本血吸虫](#) [IgE](#) [基因克隆](#) [重组抗原](#) [融合蛋白](#)

分类号

Cloning and Identification of the Gene Encoding Specific IgE Antibody-Related Antigen of *Schistosoma japonicum*

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Abstract

Objective To obtain the gene encoding specific IgE-related antigen of *Schistosoma japonicum* from the cDNA library of adult *Schistosoma japonicum*. Methods The pooled sera from 15 individuals with high levels of specific IgE antibody against SWAP were absorbed with Protein-G and used for screening of IgE-related antigen from the adult worm cDNA library of *Schistosoma japonicum*. The inserted cDNA was amplified by PCR and sequenced. According to the first reading frame of the sequence, a pair of new probes, in which Eco R I and Not I sites were incorporated respectively, were designed and used to amplify the target gene. Then, the gene was cloned into vector pGEM-T and subcloned into expression vector pGEX-6p-1. The fusion protein was expressed, analysed by SDS-PAGE and identified by Western blotting with the specific IgE antibody, respectively. Results The inserted cDNA fragment from the positive clone was about 1 200 bp, with the ORF of 507 bp which encoded 169 amino acids. The deduced molecular weight of the recombinant protein was 19.3 kDa. The homology between the target gene(Sj43B) and other known DNA sequences was less than 40%. The fusion protein expressed by the recombinant vector pGEX-6p-1/Sj43B could be recognized by schistosome specific IgE antibody. Conclusion Sj43B may encode the specific IgE-related antigen of *Schistosoma japonicum*. The successful construction of recombinant plasmid pGEX-6p-1/Sj43B lay the groundwork for further studies on immunological characteristics and protection immunity of the recombinant protein.

Key words [Schistosoma japonicum](#) [IgE](#) [gene cloning](#) [recombinant antigen](#) [fusion protein](#)

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