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论著

日本血吸虫四跨膜蛋白第二亲水基团（TSP_2HD）基因合成、表达与免疫原性研究

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摘要

目的 合成和表达日本血吸虫四跨膜蛋白第二亲水基团（TSP_2HD）基因，并研究其免疫原性。方法 采用重叠PCR人工合成日本血吸虫TSP2HD（aa107～aa182）完整基因片段，经测序正确后，将此片段插入表达载体pGEX-4T-3，构建重组表达质粒TSP_2HD-PG，转化大肠埃希菌BL21（DE3），获得含重组表达质粒的转化子，异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）观察TSP_2HD融合蛋白表达情况。用谷胱甘肽（GST）融合蛋白纯化胶（glutathione sepharose 4B）从表达产物裂解上清中纯化GST-TSP_2HD融合蛋白，用凝血酶切割融合蛋白，制备纯化的重组TSP_2HD蛋白。通过蛋白质印迹（Western blotting）分析重组TSP_2HD蛋白与血吸虫病患者血清及血吸虫重感染兔血清的免疫反应性；重组TSP_2HD蛋白刺激血吸虫感染小鼠淋巴细胞进行淋巴细胞增殖试验，通过比较实验组与对照组脉冲指数（cpm）值的差异研究重组TSP_2HD蛋白的免疫原性。 结果 经过3轮重叠PCR扩增，获得长228 bp的TSP_2HD基因，序列分析证实与天然基因序列完全一致。含重组质粒TSP_2HD-PG的转化子细菌，经IPTG诱导后表达相对分子质量（*Mr*）约为 34 000的可溶性GST-TSP_2HD融合蛋白。凝血酶切割GST-TSP_2HD融合蛋白获得纯化的重组TSP_2HD蛋白。Western blotting分析证明，重组表达蛋白可被血吸虫重感染兔血清和血吸虫病患者血清识别，有较好的免疫反应性；重组TSP_2HD蛋白能刺激血吸虫感染小鼠脾细胞增殖，实验组cpm值明显高于对照组，两者间差异有统计学意义（$P<0.01$）。 结论 日本血吸虫TSP_2HD基因合成及表达获得成功，重组TSP_2HD蛋白有天然免疫原性。

关键词 日本血吸虫；四跨膜蛋白第二亲水基团基因；重叠PCR；基因合成；表达；免疫原性

分类号

Gene Synthesis, Expression and Immunogenicity Analysis of TSP_2 Hydrophilic Domain（TSP_2HD） of *Schistosoma japonicum*

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Abstract

Objective To synthesize and express the gene of TSP_2 hydrophilic domain of *Schistosoma japonicum*, and investigate the immunogenicity of the recombinant TSP_2HD protein. Methods The whole DNA fragment encoding the TSP_2 hydrophilic domain was synthesized by overlapping PCR, and confirmed by DNA sequencing. The recombinant plasmid TSP_2HD-PG was constructed by inserting the purified TSP_2HD DNA fragment into expression vector pGEX-4T-3 and the GST-TSP_2HD fusion protein was expressed by transforming the recombinant plasmid TSP_2HD-PG into Escherichia coli BL21（DE3）and induced the recombinant with isopropyl β-D-1-thiogalactopyranoside（IPTG）. The expressing situation of fusion protein was analyzed by SDS-PAGE. The GST-TSP_2HD fusion protein was purified by affinity chromatography with glutathione sepharose 4B gel, and the purified recombinant TSP_2HD protein was prepared by digesting the GST-

TSP_2HD fusion protein with thrombin. The immuno-response of the recombinant TSP_2HD recognized by the pool sera of schistosomiasis patients and the pool sera of heavily infected rabbits was explored by Western blotting analysis. The immunogenicity of the recombinant TSP_2HD was investigated by comparing the difference of counts per minute （cpm） value of lymphocyte proliferation test between experiment group and control group. Results A 228 bp of TSP_2HD gene fragment was obtained after overlapping PCR of three times and its DNA sequence was confirmed by DNA sequencing，which was same to one of the native TSP_2HD. The recombinant containing recombinant plasmid TSP_2HD-PG expressed a soluble fusion protein of GST-TSP_2HD（*Mr*≈34 000） after being induced with IPTG. The purified recombinant TSP_2HD protein was obtained through digesting the GST-TSP_2HD fusion protein with thrombin. The recombinant TSP_2HD was recognized by pool sera of schistosomiasis patients and pool sera of infected rabbits, indicating that the recombinant TSP_2HD has a good response activity. The recombinant TSP_2HD also stimulated proliferation of lymphocytes in infected mouse，the cpm value of experiment group was higher than that of the control （P<0.01）. Conclusion The Sj TSP_2HD gene has been synthesized and expressed with immunogenicity which is similar to that of the native antigen.
Key words *Schistosoma japonicum*；TSP_2HD；Overlapping PCR；Gene synthesis；Expression；Immunogenicity

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