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日本血吸虫核糖体蛋白SjRibosomal_L18a及其B细胞表位的筛选和评价

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Screening and Evaluation of Schistosoma japonicum SjRibosomal_L18a Protein and its B Cell Epitopes

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摘要 参考文献 相关文章

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摘要 目的 筛选获得日本血吸虫（Schistosoma japonicum）核糖体蛋白（SjRibosomal_L18a）编码基因，预测其B细胞表位。克隆、表达重组蛋白SjRibosomal_L18a，并比较分析其与合成的B细胞表位的潜在诊断价值。 方法 利用B细胞表位预测软件筛选日本血吸虫蛋白质序列，获取函数打分值较高且抗原表位片段多的免疫原性蛋白，合成B细胞表位片段。生物信息学方法分析免疫原性蛋白基因及其编码蛋白的相对分子质量（Mr）、等电点、亲水性、信号肽、跨膜区和功能结构域等理化性质。制备日本血吸虫各虫期阶段总RNA，逆转录PCR（RT-PCR）扩增目的基因，分析各虫期转录本丰度。将扩增产物克隆至原核表达载体pET-28a中，重组质粒转化至大肠埃希菌（E. coli）BL21（DE3）菌株，异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，组氨酸标记亲和层析法纯化重组蛋白。ELISA法比较分析重组蛋白和合成的B细胞表位的潜在诊断价值。 结果 预测软件筛选出免疫原性核糖体蛋白SjRibosomal_L18a以及2个B细胞表位（P1和P2）。SjRibosomal_L18a基因开放阅读框（ORF）由531个碱基组成，编码176个氨基酸，不含跨膜区和信号肽，为Mr 20 741，等电点为11.12。RT-PCR结果显示，各虫期均检测到SjRibosomal_L18a的转录本，且转录水平均较高。成功构建重组表达质粒SjRibosomal_L18a/pET-28a，诱导表达获得包涵体形式的重组蛋白，约为Mr 26 069。ELISA检测结果显示，重组蛋白、P1和P2检测15份慢性血吸虫病患者血清和15份健康人血清的敏感性和特异性分别为53.3%（8/15）和100%（15/15）、60%（9/15）和100%（15/15）、73.3%（11/15）和100%（15/15）。 结论 获得重组蛋白SjRibosomal_L18a及其免疫原性较高的表位片段P1、P2，P1和P2用于血清诊断的敏感性均高于重组蛋白。

关键词： 日本血吸虫；B细胞表位；核糖体蛋白；虫期特异性

Abstract： Objective To screen a ribosomal protein（SjRibosomal_L18a） of Schistosoma japonicum and predict its B cell epitopes, and evaluate the potential diagnostic value of the recombinant protein and the synthetic B cell epitopes. Method S. japonicum protein sequences were screened and analyzed by using B-cell epitope prediction softwares. The immunogenic protein was selected based on the predicted score and the quantity of epitopes. The epitopes with higher score（P1 and P2） were synthesized. The relative molecular mass（Mr）, isoelectric point, grand average of hydropathicity, signal peptide, and transmembrane domain were predicted by bioinformatics tools. RT-PCR was used to analyze the transcription level of the different development stages. The encoding sequence was amplified by PCR, and cloned into pET28a vector. The recombinant plasmid was transformed into in E. coli BL21（DE3） cells and induced with IPTG. The recombinant SjRibosomal_L18a protein was purified with Ni-NTA resin. ELISA was used to evaluate the potential diagnostic value of the recombined protein and the synthetic B cell epitopes. Results SjRibosomal_L18a protein was obtained, its B cell epitopes and physicochemical properties were predicted. The open reading frame of SjRibosomal_L18a was composed of 531 bp, and encoded a 176-amino-acid protein with Mr 20 741, pI 11.12. RT-PCR result showed that this gene was transcribed at high level in each developmental stage. The recombinant plasmid SjRibosomal_L18a/pET-28a was constructed and the protein was expressed as inclusion bodies （Mr 26 069）. The sensitivity and specificity of recombined protein, P1 and P2 were 53.3% （8/15） and 100% （15/15）, 60% （9/15） and 100% （15/15）, 73.3% （11/15） and 100% （15/15）, respectively. Conclusion The recombinant protein （SjRibosomal_L18a） and its epitopes with higher immunogenicity are obtained. The sensitivity of the two epitopes （P1 and P2） was higher than that of SjRibosomal_L18a protein.

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