简报

日本血吸虫副肌球蛋白全基因克隆、测序及体内表达

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收稿日期 修回日期 网络版发布日期 接受日期

目的: 将日本血吸虫大陆株副肌球蛋白(S j c 9 7) 编码区全基因克隆及测序, 并研究 S j c 9 7 全基因核酸疫苗在小鼠体内的表达。方法: 抽提日本血吸虫成虫总 R N A, 逆转录 聚合酶 链反应 (R T P C R) 扩增编码 S j c 9 7 的全基因 c D N A, 双脱氧链末端终止法测 D N A 序列。构建含 S j c 9 7 全基因的质粒表达载体 (p C M V S j c 9 7) , 并用免 疫荧光染色法研究 p C M V S j c 9 7 在小鼠体内的表达特性。结果与结论: 用 R T P C R 扩增获得了 S j c 9 7 的编码区全基因 c D N A, 并测定了该基因编码区的全序列。与日本血吸虫菲律宾株、日本株及曼氏血吸虫副肌球蛋白编码区全基因比较, 核苷酸序列同源性分别为: 9 9 4 % 、9 9 2 % 和 9 1 0 % ; 推导的氨基酸序列同源性分别为: 9 9 7 % 、9 9 8 % 和 9 6 0 % 。 p C M V S j c 9 7 核酸疫苗能在注射的小鼠局部肌肉组织表达 S j c 9 7

关键词 <u>日本血吸虫</u> <u>副肌球蛋白</u> <u>基因克隆</u> <u>DNA序列</u> <u>核酸疫苗</u> 分类号

CLONING, SEQUENCING AND EXPRESSION OF THE FULL-LENGTH GENE ENCODING PARAMYOSIN OF SCHISTOSOMA JAPONICUM IN VIVO *

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Abstract

AIM: To clone and sequence the gene encoding paramyosin of S. japonicum (Chinese strain) and to study the expression of the DNA-based vaccine encoding the full-length paramyosin of S. japonicum in vivo . METHODS: Total RNA was isolated from adult S. japonicum using TRIzol reagent. The full-length cDNA encoding paramyosin of S. japonicum was amplified by RT-PCR and cloned into pGEM-T vector and sequenced by the method of dideoxy-mediated chain-termination. The cDNA encoding paramyosin of S. japonicum was subcloned into the expressive plasmid vector pCDNA/AMP(pCMV-Sjc97), and the recombinants were identified by restriction enzyme digestion and sequencing. The immunofluorescence assay was used to study the expression of Sjc97 in vivo in mice. RESULTS AND CONCLUSION: The 2.6 kb cDNA encoding the full-length paramyosin of Chinese S. japonicum has been successfully cloned and sequenced for the first time. The full-length sequence of paramyosin of S. japonicum was determined. Comparison of the nucleotide sequence and the deduced amino acid sequence of Sjc97 with that of S.japonicum paramyosin(Philippine strain)(Sjp97), S.japonicum paramyosin (Japanese strain) (Sjj97), S.mansoni (Sm97), B6 and Y6 clone (the partial cDNA encoding paramyosin of Chinese strain) showed that Sjc97 differed from Sjp97 by 16/2 601 nucleotide and 3/866 amino acid substitutions (99.4% on nt-levlel and 99.7% on aa-level in homology); from Sjj97 by 20/2 601 nucleotide and 2/866 amino acid substitutions (99.2% on nt-levlel and 99.8% on aa-level in homology); and from B6 by 11/1 329 nucleotide and 1/443 amino acid substitutions (99.0% on nt-level and 99.8% on aa-level in homology); from Y6 by 13/1 329 nucleotide and 1/443 amino acid substitutions (98.9% on nt-levlel and 99.8% on aa-level in homology); Sjc97 differed from the Sm97 by 2 235/2 601 nucleotide and 34/866 amino acid (91.0% on nt-levlel and 96.0% on aa-level in homology). The plasmid expression vector encoding the fulllength paramyosin of Chinese S. japonicum has been successfully constructed. The pCMV-Sjc97 vaccine could express Sjc97 protein in vivo in mice after intramuscular immunization.

Key words Schistosoma japonicum (Chinese strain) paramyosin gene cloning DNA sequence DNA-based vaccine

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