中国寄生虫学与寄生虫病杂志 2007 25 (4): 320-324 ISSN: 1000-7423 CN: 31-1248/R

实验研究

细粒棘球绦虫Eg95重组分泌型卡介苗的构建

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收稿日期 修回日期 网络版发布日期 接受日期

摘要

【摘要】 目的 构建细粒棘球绦虫Eg95重组分泌型卡介苗(rsBCG-Eg95)。 方法 分别以卡介苗BCG基因组DNA和pGEX-4T-Eg95重组质粒为模板, PCR扩增获117 bp 的BCG抗原85B(BCG-Ag85B)信号肽序列和 471 bp 的Eg95基因序列。将这两个序列定向克隆至大肠埃希菌-BCG穿梭质粒pMV261, 经酶切、 PCR扩增及测序鉴定得到重组质粒pSMEg95。电穿孔法将重组质粒导入BCG菌构建rsBCG-Eg95疫苗, 卡那霉素抗性基因筛选并经PCR扩增鉴定。 结果质粒pSMEg95经双酶切、 PCR扩增及测序鉴定, 证实克隆基因Ag85B信号肽和Eg95基因序列正确插入载体pMV261, 并将此重组质粒导入BCG菌, 经PCR扩增鉴定证实细粒棘球绦虫Eg95重组分泌型卡介苗 (rsBCG-Eg95)构建成功。 结论 构建了含有BCG信号肽Ag85B和保护性抗原Eg95基因序列的细粒棘球绦虫Eg95重组分泌型卡介苗rsBCG-Eg95。

关键词 细粒棘球绦虫 分泌型 Eg95 BCG疫苗

分类号

Construction of the Recombinant Secretion Type BCG-Eg95 Vaccine of *Echinococcus granulosus*

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Abstract

【Abstract】 Objective To construct the recombinant secretion type BCG-Eg95 vaccine of *Echinococcus granulosus* (rsBCG-Eg95). Methods BCG-Ag85B signal sequence with 117 bp and Eg95 gene with 471 bp were amplified from the genome of BCG and pGEX-4T-Eg95 by PCR, respectively. BCG-Ag85B signal coding gene and Eg95 gene were cloned into *E. coli*-BCG shuttle-vector pMV261 to get the recombinant plasmid pSMEg95, which was confirmed by restriction endonuclease digestion, PCR amplification and gene sequencing. These recombinant plasmids were introduced into BCG by electroporation for the construction of rsBCG-Eg95 vaccine. The rsBCG-Eg95 positive clones were screened by Kan^+ and identified by PCR amplification. Results BCG-Ag85B signal sequence coding gene and Eg95 coding gene were successfully cloned into pMV261, which was confirmed by restriction endonuclease digestion, PCR amplification and sequencing of the plasmid pSMEg95. The plasmids were introduced into BCG and confirmed as the recombinant secreting BCG-Eg95 vaccine of *E. granulosus* (rsBCG-Eg95). Conclusion The recombinant secretion type BCG?鄄Eg95 vaccine (rsBCG-Eg95) of *E. granulosus* with BCG-Ag85B signal sequence and Eg95 gene has been constructed.

Key words *Echinococcus granulosus* Secretion type Eg95 BCG vaccine

DOI:

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