



细粒棘球蚴原头节3种重组抗原的双向电泳定位分析

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Position Analysis of Three Recombinant Proteins of Echinococcus granulosus Protoscolex with Two-dimensional Electrophoresis

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摘要

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摘要 目的 利用已有的细粒棘球蚴(Echinococcus granulosus)原头节3种重组抗原rEgZW-5、rEg14-3-3和rEgP-29获得特异性抗体,以识别双向电泳图谱中相应的蛋白位点。方法 十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)初步分离细粒棘球蚴原头节蛋白,预判蛋白分布情况。应用双向电泳技术分离原头节蛋白,PDquest软件分析原头节的蛋白质数量、相对分子质量(Mr)和等电点(IP)。用原头节的3种重组蛋白rEgZW-5、rEg14-3-3和rEgP-29分别免疫新西兰家兔,制备血清,纯化抗体。蛋白质印迹(Western blotting)鉴定特异性抗体对原头节双向电泳图谱中相应蛋白的识别。结果 SDS-PAGE结果显示,细粒棘球蚴原头节蛋白主要分布于Mr 18 000~90 000区域。双向电泳分离出240个蛋白位点,为Mr 15 790~117 050,IP为4.0~9.5,其中85.8%(206/240)蛋白等电点为5~9。Western blotting结果显示,rEg14-3-3特异性抗体可识别细粒棘球蚴原头节双向电泳图谱中约为Mr 33 000、IP为4.86的14-3-3蛋白位点,rEgZW-5特异性抗体可识别约为Mr 23 000、IP为4.98的ZW-5蛋白位点,rEgP-29特异性抗体可识别约为Mr 29 000、IP为5.65的P-29蛋白位点。结论 细粒棘球蚴原头节的3种重组抗原rEgZW-5、rEg14-3-3和rEgP-29的抗体可识别双向电泳图谱中相应的蛋白位点。

关键词: 细粒棘球蚴 原头节 重组抗原 双向电泳

Abstract: Objective To obtain specific antibodies of the three recombinant antigens obtained previously, rEgZW-5, rEg14-3-3 and rEgP-29, for identifying the corresponding proteins in two-dimensional electrophoretogram of Echinococcus granulosus protoscolex. Methods The distribution of proteins from E. granulosus protoscoleces was judged by SDS-PAGE previously. Two-dimensional electrophoresis was used to separate proteins from E. granulosus protoscoleces, and the result was scanned and analyzed by the PDquest software to get the information about the quantity of proteins as well as their isoelectric point (IP) and relative molecular mass (Mr). Rabbits were immunized with the 3 recombinant antigens and antibodies were purified from antisera. Western blotting was used to identify the protein as marker in two-dimensional electrophoretogram of protoscolex. Results SDS-PAGE displayed that the proteins separated from Echinococcus granulosus protoscoleces mainly distributed in the Mr region of 18 000-90 000. 240 proteins were obtained by two-dimensional electrophoresis with Mr 15 790-117 050 and IP 4.0-9.5, and 85.8% (206/241) of the proteins showed the IP ranged from 5 to 9. Western blotting showed that the specific antibody of rEg14-3-3 identified the 14-3-3 protein in two-dimensional electrophoretogram of protoscolex with Mr 33 000 and IP 4.86, the specific antibody of rEgZW-5 identified the ZW-5 protein with Mr 23 000 and IP 4.98, and the specific antibody of rEg P-29 identified the P-29 protein with Mr 29 000 and IP 5.65. Conclusion The antibodies against the three recombinant proteins from Echinococcus granulosus protoscoleces can identify corresponding proteins in the two-dimensional electrophotoregrams.

Keywords: Echinococcus granulosus Protoscolex Recombinant protein Two-dimensional electrophoresis

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