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日本血吸虫钙蛋白酶基因的原核表达及功能分析

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Prokaryotic Expression and Function Analysis of Schistosoma japonicum Calpain

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摘要 目的 克隆表达日本血吸虫钙蛋白酶（Sjcalpain），了解Sjcalpain在尾蚴内的分布及其在尾蚴侵染中的作用。 方法 根据SjCalpain基因全序列（GenBank登录号为AB016726）设计引物，PCR分别扩增Sjcalpain的催化位点区域和Ca2+ 结合位点区域的基因片段，克隆入pET-28a原核表达载体，并在大肠埃希菌（E. coli）BL21（DE3）中进行原核表达，组氨酸标签亲和层析法纯化表达产物。聚丙烯酰胺凝胶电泳（SDS-PAGE）分析蛋白表达和纯化情况。纯化的重组蛋白免疫新西兰白兔制备兔多克隆抗体，ELISA和蛋白质印迹（Western blotting）分别检测多克隆抗体的效价和特异性。利用免疫荧光定位技术观察Sjcalpain在尾蚴组织中的分布情况。将尾蚴和钙蛋白酶抑制剂孵育后感染小鼠，计算血吸虫减虫率。 结果 成功构建了Sjcalpain的催化位点/pET-28a和Ca2+结合位点/pET-28a原核表达重组质粒，在E. coli BL21（DE3）中成功表达目的蛋白，相对分子质量（Mr）约为43 000和39 000，与预期大小一致，且均为包涵体表达。组氨酸标签亲和层析成功纯化得到目的重组蛋白。间接ELISA结果显示，多克隆抗体的效价超过1 ： 80 000。免疫定位结果显示，Sjcalpain在日本血吸虫尾蚴头部分布较多。尾蚴侵染抑制实验中，钙蛋白酶抑制剂作用组平均获虫19条，对照组平均获虫23条，减虫率为17.4%。 结论 Sjcalpain蛋白产物主要分布在日本血吸虫尾蚴的头部；Sjcalpain被抑制后可以减少入侵宿主的尾蚴数量，说明Sjcalpain在尾蚴感染宿主皮肤过程中发挥一定的作用。

关键词： 日本血吸虫 尾蚴 钙蛋白酶 免疫定位 侵染

Abstract： Objective To clone and express recombinant calpain of Schistosoma japonicum（Sjcalpain）， observe the distribution of Sjcalpain in S. japonicum cercariae and analyze its role in skin invasion. Methods The primers were designed according to the full-length sequence of calpain（GenBank accession No. AB016726）. The genes encoding catalytic domain and Ca2+ binding domain of Sjcalpain were amplified by PCR， and the target fragments were subcloned into pET-28a. The recombinant proteins were expressed in E. coli BL21（DE3）and purified by Ni-NTA resin. The rabbit polyclonal antibodies were prepared with the two purified recombinant proteins by immunizing New Zealand white rabbits. ELISA was used to detect the titer of rabbit antiserum. Immunolocalization was used to investigate the distribution of Sjcalpain in S. japonicum cercariae. Cercariae were incubated with specific inhibitor before infection of mice and the worm reduction rate was calculated. Results The recombinant expression vector Sjcalpain catalytic domain/pET28a and Sjcalpain Ca2+ binding domain/pET28a were constructed and the recombinant proteins were successfully expressed in E. coli BL21（DE3）（about Mr 43 000 and Mr 39 000， respectively）. The two target proteins were expressed as inclusion bodies. The purified target proteins were obtained through Ni-NTA affinity purification. ELISA result showed that the titer of prepared rabbit polyclonal antibodies was higher than 1 ： 80 000. Immunolocalization study demonstrated that Sjcalpain protein was mainly expressed in the head of cercariae. Inhibition assays suggested that the average number of adult worms in calpain inhibitor-incubation group and control group was 19 and 23， respectively, with a worm reduction rate of 17.4%. Conclusion Sjcalpain is mainly expressed in the head of S. japonicum cercariae. Inhibition of Sjcalpain could reduce the number of invading cercariae in infected mice， which suggest that Sjcalpain may play a role in skin invasion by cercariae.

Keywords： Schistosoma japonicum Cercaria Calpain Immunolocalization Infection

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