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日本血吸虫脱尾童虫表膜结合短肽的筛选与鉴定

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Screening and Analysis of Peptides Specifically Binding to the Schistosomulum Tegument of Schistosoma japonicum

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摘要 目的 筛选噬菌体十二肽库中与日本血吸虫（Schistosoma japonicum）脱尾童虫表膜特异性结合而不与尾蚴表膜结合的短肽并鉴定。方法 利用M13噬菌体十二肽库，经体外逆向差异筛选日本血吸虫尾蚴和脱尾活童虫，从第3轮回收的结合噬菌体中随机挑取15个克隆进行测序。获取目标噬菌体后，采用ELISA法、洗脱回收率实验（以M13KE为阴性对照）和免疫组织化学法检测日本血吸虫脱尾童虫、尾蚴与噬菌斑克隆的特异性结合。荧光显微镜观察人工合成的阳性噬菌体短肽与日本血吸虫脱尾童虫体外的特异性结合。构建目的片段pEGFP-C2质粒体外转染日本血吸虫脱尾童虫。 结果 经3轮逆向差异筛选后，噬菌体回收率从第1轮的3.50×10-5%到第3 轮的3.20×10-2%，富集度明显提高。DNA测序结果表明，15个噬菌体克隆分别含有ZL6、ZL4 和ZL1 等3个不同的短肽序列。ELISA结果显示，M13噬菌体短肽ZL4（MppZL4）、MppZL6和MppZL1分别与脱尾童虫膜蛋白结合后的P/N值为6.72，3.65和2.22， 分别与尾蚴膜蛋白结合后的P/N值为1.58，5.15和1.20。洗脱回收率实验结果显示，MppZL4与脱尾童虫洗脱回收率［（4.60±0.27）×10-2%］远高于MppZL6［（2.10±0.23）×10-3%］、MppZL1［（1.20±0.28）×10-3%］和M13KE［（1.30±0.60）×10-7%）（P<0.01）。免疫组化结果显示，日本血吸虫脱尾童虫与MppZL4特异性结合，阳性率为83.0%（83/100）。荧光显微镜检测结果显示，人工合成的RhB?鄄ZL4可与日本血吸虫脱尾童虫体外特异性结合。构建的ZL4/pEGFP?鄄C2质粒体外可成功转染日本血吸虫脱尾童虫。 结论 筛选获得的短肽ZL4能与日本血吸虫童虫表膜特异性结合，不与尾蚴表膜结合。

关键词： 日本血吸虫 表膜 噬菌体展示技术

Abstract： Objective To screen and analyze the peptides in 12 phage-display peptide library specifically binding to the schistosomulum, not cercaria, tegument of Schistosoma japonicum. Methods A 12 phage-display peptide library was screened with the S. japonicum schistosomula and cercariae as the target cells for biopanning by degrees, 15 positive clones were picked randomly and deduced by DNA sequencing. According the sequencing result, ELISA test, elution recovery test and immunohistochemical staining were performed to determine the specificity of the phages to the tegument. To further examine its binding properties, the positive peptide conjugated to RhB and recombinant pEGFP?-C2 plasmid were similarly synthesized. Results After 3 rounds of biopanning, the phage recovery rate increased from 3.50 ×10-5% to 3.20×10-2%, indicating that the phage library was successfully enriched in the tegument of schistosomula. The analyzed sequences were identical with 3 peptide sequence of ZL6, ZL4 and ZL1. ELISA showed that the P/N value of MppZL4, MppZL6 and MppZL binding the schistosomulum membrane protein was 6.72，3.65 and 2.22, while 1.58，5.15 and 1.20 of binding the membrane protein of cercariae, respectively. Elution recovery test showed that the elution recovery rate of MppZL4 ［（4.60±0.27）×10-2%］ was much higher than that of MppZL6 ［（2.10±0.23）×10-3%］, MppZL1 ［（1.20±0.28）×10-3%］ and M13KE［（1.30±0.60）×10-7%］（P<0.01）. Immunohistochemical staining showed that MppZL4 specifically bound to the tegument of schistosomula with a positive rate of 83.0%（83/100）. Fluorescent microscopy revealed that the synthesized RhB-ZL4 bound to the tegument of schistosomula. The ZL4/pEGFP-C2 plasmid was introduced into juvenile S. japonicum and expressed in the parasite. Conclusion The peptide of ZL4 specifically binds to the schistosomulum tegument but not to that of cercaria.

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