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刚地弓形虫棒状体蛋白17（ROP17）真核表达载体的构建、表达及其激酶活性分析

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Construction，Expression and Kinase Function Analysis of an Eukaryocyte Vector of Rhoptry Protein 17 in Toxoplasma gondii

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摘要 【摘要】 目的 构建刚地弓形虫RH株棒状体蛋白17（ROP17）真核表达载体，对表达产物进行激酶活性的检测和功能分析。 方法 根据刚地弓形虫ROP17蛋白编码基因（GenBank登录号为AM075203.1）设计引物，以弓形虫RH株速殖子总RNA为模板，RT-PCR扩增目的基因片段，克隆至真核表达载体p3×Flag-CMV-14，构建重组质粒p3×Flag-CMV-14/TgROP17。经菌液PCR、双酶切和测序鉴定正确后，用脂质体法转染至人胚肾细胞（HEK 293T），RT-PCR和蛋白质印迹（Western blotting）分析表达产物；Western blotting检测其激酶活性；流式细胞术分析ROP17对转染宿主细胞凋亡的影响。 结果 RT-PCR结果显示，从RH株弓形虫速殖子中扩增出约1 850 bp的基因片段。菌落PCR、双酶切和测序结果显示，重组质粒p3×Flag-CMV-14/TgROP17构建成功且序列正确。RT-PCR和Western blotting结果显示，在转染p3×Flag-CMV-14/TgROP17的细胞中有TgROP17的表达，基因片段大小为1 850 bp，蛋白质相对分子质量（Mr）约为70 000，而转染空质粒组未见表达。Western blotting结果显示，ROP17可以磷酸化c-Jun蛋白；流式细胞术结果显示，ROP17抑制喜树碱（CPT）诱导的细胞凋亡，6 h和12 h的抑制率分别为20.6%和24.1%，两者差异有统计学意义（P＜0.05）。 结论 成功构建了真核表达载体p3×Flag-CMV-14/TgROP17，其表达产物具有激酶活性且能够抑制细胞凋亡。

关键词： 刚地弓形虫 棒状体蛋白17 真核表达 丝氨酸?鄄苏氨酸激酶 细胞凋亡

Abstract： 【Abstract】 Objective To construct and express the eukaryocytic expression vector of rhoptry protein 17 of Toxoplasma gondii RH strain(TgROP17) and analyze its kinase function. Methods The open reading frame of TgROP17 gene was amplified from total RNA in T. gondii RH strain by RT-PCR, and cloned into p3×Flag-CMV-14 vector to construct recombinant plasmid p3×Flag-CMV-14/TgROP17. After colony-PCR confirming, double restriction enzyme digestion and DNA sequencing, the eukaryotic expression vector p3×Flag-CMV-14/TgROP17 was transfected into HEK 293T cells. The target gene was examined by RT-PCR and the recombinant protein was detected by Western blotting. The kinase activity of TgROP17 was identified by Western blotting and its apoptotic function was assessed by flow cytometry. Results The size of RT-PCR product was 1 850 bp. The recombinant plasmid p3×Flag-CMV-14/TgROP17 was confirmed by colony-PCR, double restriction enzyme digestion and DNA sequencing. RT-PCR and Western blotting analysis showed that TgROP17 was expressed in the p3×Flag-CMV-14/TgROP17 transfected-HEK 293T cells rather than in mock cells. The amplified gene was with 1 850 bp and the target protein was about Mr 70 000. Western blotting analysis showed that c-Jun was phosphorylated by TgROP17. Flow cytometry analysis indicated that camptothecin-induced apoptosis was inhibited by TgROP17 with an inhibition rate of 20.6% and 24.1% at 6 h and 12 h after co-culture, respectively, which was higher than that of the control(P＜0.05). Conclusion The eukaryotic expression vector p3×Flag-CMV-14/TgROP17 is constructed. TgROP17 has kinase activity and playes an anti-apoptosis role.

Keywords： Toxoplasma gondii；Rhoptry protein 17；Eukaryotic expression；Serine-threonine kinase；Apoptosis

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