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技术与方法

TAT-凋亡素基因重组质粒的构建、蛋白的表达纯化及体内活性实验

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摘 要 目的 克隆构建TAT-凋亡素质粒并提取融合蛋白，为进一步研究该蛋白功能奠定基础。方法 PCR合成TAT-凋亡素基因，与pTYB2质粒连接后转入Rosetta菌，经IPTG诱导表达，几丁质亲和层析一步纯化目的蛋白，用昆明小鼠H22动物模型检测活性。结果 克隆载体经过PCR筛选、测序鉴定，其大小和核苷酸序列正确，诱导后融合蛋白出现在上清，纯化出的TAT-凋亡素蛋白具有明显的抗肿瘤活性。结论 本实验所构建的重组质粒pTYB2/TAT-apoptin经诱导表达出了可溶性目的蛋白TAT-凋亡素，纯化后具有明显的生物活性，为研究TAT-凋亡素蛋白的进一步研究奠定了基础。

关 键 词 TAT-凋亡素 基因重组 纯化 几丁质亲和层析

分类号

Construction of recombinant plasmid of pTYB2/TAT-apoptin and Expression , Purification of TAT-apoptin protein

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Abstract Objective To construct pTYB2/TAT-apoptin expression system and producing TAT-apoptin protein. Methods The TAT-apoptin gene was cloned by PCR and was ligated to the expression plasmid pTYB2, and the recombinant plasmid was transformed into Rosetta. One-step affinity chromatography was used to purify the TAT-apoptin protein after inducing by IPTG,The protein activity was detected by using H22 animal model,Kunming mouse. Results The TAT-apoptin gene was screened by PCR ,and the result of sequencing was correct. The TAT-apoptin protein was obtained by affinity chromatography,it has conspicuous antineoplamic activity. Conclusion The recombinant plasmid ,pTYB2/TAT-apoptin,can express resoluble TAT-apoptin protein in the Rosetta， and it has obvious biological activity after purifying. This research build a base for the further study of the function for TAT-apoptin.

Key words TAT-apoptin recombination protein expression affinity chromatography

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