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论文

金葡菌肠毒素C_2的克隆表达及其生物学活性

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摘要：

目的克隆金葡菌肠毒素C_2全长基因，构建SEC_2的表达载体，实现其可溶性表达，并对纯化的$rSEC_2$蛋白的生物学活性进行研究。方法通过聚合酶链式反应(polymerase chain reaction，PCR)从金葡菌FRI1230菌株基因中得到肠毒素SEC_2的基因，将其克隆至融合表达载体pGEX-4T-1，转化大肠杆菌进行表达并对融合蛋白进行亲和色谱纯化。通过考察重组SEC_2对淋巴细胞的增殖作用及其对肿瘤细胞杀伤活性的影响，对其超抗原活性和免疫学活性进行研究。结果得到正确的肠毒素SEC_2基因序列并得到高效表达的融合蛋白，MTT法结果表明，重组SEC_2表现出良好的促淋巴细胞增殖活性，且能够增强淋巴细胞对肿瘤细胞的杀伤活性。结论本研究成功克隆了SEC_2基因，表达并纯化出具有抗肿瘤生物学活性的重组SEC_2蛋白，为进一步对其分子抗肿瘤作用机制进行研究以及构建靶向抗肿瘤融合蛋白奠定了基础。

关键词： $GST\text{-}SEC_2$ 超抗原 融合表达 大肠杆菌 MTT法

Expression and bioactivity analysis of staphylococcal enterotoxin C_2

XUE Qiao; YING Yue-bin; PAN Ying-qiu; LI Dan-xi; SUN Hong-ying; CHEN Shu-qing

Abstract:

AimTo clone the gene of staphylococcal enterotoxin C_2 and express it in the form of a soluble fusion protein in *E.coli*. Then the activation of SEC_2 on mice lymphocyte and its lethal effects on tumor cells were studied. MethodsStaphylococcus aureus SEC_2 gene was cloned into GST gene fusion vector pGEX-4T-1. The resultant plasmid pGEX-4T-SEC_2 was used to transform *E.coli* BL21, where the $GST\text{-}SEC_2$ fusion protein was expressed efficiently. The $rSEC_2$ protein was purified with Glutathione Sepharose 4B affinity column and digested with thrombin. The *in vitro* culture system was utilized to observe the activation of the SEC_2 on mice lymphocyte and the lethal effects on tumor cells of the activated mice lymphocyte. ResultsThe proper gene of SEC_2 was cloned and purified $rSEC_2$ was obtained. The MTT results indicated that $rSEC_2$ have strong ability to stimulate mice lymphocyte to proliferate with a dose-dependent manner. With the proliferation of mice splenic lymphocyte, $rSEC_2$ has a strong lethal effect on tumor cells B16, K562 and K562-AD. ConclusionIn this study, the gene of SEC_2 was cloned and the $rSEC_2$ protein was obtained, which had strong lethal effect on tumor cells B16, K562 and K562-AD.

Keywords: superantigen fusion expression *E.coli* MTT $GST\text{-}SEC_2$

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