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Luffin-β-KDEL-uPAcs融合毒素的构建及其抗胃癌SG性研究(PDF)

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Title: Construction, expression and purification of prokaryotic fusion protein luffin-β-KDEL-uPAcs and its cytotoxic effect on gastric carcinoma cell line SGC-7901

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关键词: Luffin-β; 尿激酶纤溶酶原激活剂裂解位点; KDEL驻留信号序列; 融合毒素; 杀瘤活性

Keywords: luffin-β; urokinase plasminogen activator; KDEL (Lys-Asp-Glu-Leu); fusion protein; cytotoxic effect

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摘要: 目的 构建含尿激酶型纤溶酶原激活剂（urokinase plasminogen activator, uPA）裂解位点（uPAcs）和KDEL（Lys-Asp-Glu-Leu）驻留信号序列的丝瓜毒素(Luffin-β)基因的原核载体，表达并纯化其融合毒素蛋白Luffin-β-KDEL-uPAcs（LKP），并探讨融合毒素蛋白LKP抗胃癌SGC-7901细胞的活性。 方法 RT-PCR两步法克隆Luffin-β基因，引物延伸法构建Luffin-β-KDEL-uPAcs融合基因并亚克隆至原核表达载体pET-32a(+)中，诱导其表达融合蛋白Trx-EK-Luffin-β-KDEL-uPAcs（TELKP）并纯化TELKP，肠激酶（enterokinase，EK）切割TELKP后，纯化与回收目的毒素蛋白LKP，SDS-PAGE对LKP蛋白予以检测鉴定，高效液相色谱法（HPLC）对其进行纯度检测。采用cell counting kit-8 (CCK-8)、RT-PCR、Western blot等方法，体外检测毒素蛋白LKP经uPA酶裂解后释放Luffin-β的抗胃癌SGC-7901细胞的活性。 结果 成功诱导重组载体pET-32a (+)/Luffin-β-KDEL-uPAcs表达相对分子质量约48.8×10^3含载体表达标签（Trx）的融合免疫毒素TELKP，EK酶切该蛋白获含290个氨基酸，相对分子质量约31.8×10^3的目的蛋白LKP。SDS-PAGE检测鉴定表明，LKP蛋白与预期大小一致，其纯度

达98.8%。CCK-8、RT-PCR、Western blot等法检测显示，LKP经uPA酶体外裂解可释放具杀瘤活性的Luffin-β小分子毒素。　　结论　　成功克隆到Luffin-β-KDEL-uPAcs融合基因，并将其构建于原核表达载体pET-32a(+)中，且诱导该载体表达了相对分子质量约31.8×10^3的融合毒素LKP。LKP毒素经uPA酶体外裂解能释放具杀瘤活性的Luffin-β小分子毒素。

Abstract: Objective To construct a prokaryotic expression vector carrying a fusion gene containing tandemly ligated luffin-β, KDEL (Lys-Asp-Glu-Leu) and urokinase plasminogen activator cleavage site (uPAcs), to express and purify the fusion protein, and to investigate the cytotoxic effect of the fusion protein on gastric carcinoma cell line SGC-7901. Methods Luffin-β gene was cloned by two-step reverse transcriptase-polymerase chain reaction (RT-PCR). The fusion gene of luffin-β-KDEL-uPAcs was obtained by primer extension, and was inserted into pET-32a(+) vector to construct a recombinant vector pET-32a(+)/luffin-β-KDEL-uPAcs. The fusion protein Trx-EK-luffin-β-KDEL-uPAcs (TELKP) was expressed under induction, purified, and digested by enterokinase (EK) to obtain luffin-β-KDEL-uPAcs (LKP). The LKP protein was purified and its purity was determined by high performance liquid chromatography (HPLC). Cell counting kit-8 (CCK-8), RT-PCR and Western blotting were employed to detect the cytotoxic effect of LKP on gastric carcinoma cell line SGC-7901 after uPA cleavage. Results The recombinant vector pET-32a(+)/luffin-β-KDEL-uPAcs could successfully express the fusion protein TELKP (48.8×10^3), and the LKP protein (31.8×10^3) could be obtained by digesting the fusion protein TELKP with EK. The purity of the LKP protein was about 98.8%. The results of CCK-8, RT-PCR and Western blotting revealed that immunotoxin luffin-β, which had cytotoxic effect on tumor cells, could be released from the LKP protein cleaved by uPA *in vitro*. Conclusion The fusion gene luffin-β-KDEL-uPAcs and its prokaryotic expression vector are successfully constructed. Recombinant fusion protein LKP (31.8×10^3) is successfully prepared. The immunotoxin luffin-β, which possesses cytotoxic effect on tumor cells, could be released from the LKP protein cleaved by uPA *in vitro*.

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