[1]项颖,黄德鸿,唐显军,等.VEGF受体单抗与Luffin双靶融合毒素的构建及其对非小细胞肺癌细胞的抑制作用[J].第三军医大学学报,2013,35(18):1921-1927.

Xiang Ying,Huang Dehong,Tang Xianjun,et al.Construction of double-targeting fused immunotoxin KDRscFv-uPAcs-Luffin-β-KDEL and its cytotoxic effect on non-small-cell lung carcinoma cells[J].J Third Mil Med Univ,2013,35(18):1921-1927.

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分享到:

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第18期 页码: 1921-1927 栏目: 论著 出版日期: 2013-09-30

Title: Construction of double-targeting fused immunotoxin KDRscFv-uPAcs-Luffin-β-KDEL and its cytotoxic effect on non-small-cell lung carcinoma cells

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关键词: KDRscFv; Luffin-β; 尿激酶纤溶酶原激活剂; 重组融合毒素; 杀瘤活性

Keywords: KDRscFv; Luffin-β; uPAcs; Recombinant fusion immunotoxin; cytotoxic effect on tumor cells

分类号: R394-33;R73-362;R734.2

文献标志码: A

摘要: 目的　构建含血管内皮生长因子(vascular endothelial growth factor, VEGF)受体单链抗体（KDRscFv）、尿激酶纤溶酶原激活剂（urokinase plasminogen activator, uPA）裂解位点（uPAcs）、丝瓜毒素(luffin-β)与KDEL（Lys-Asp-Glu-Leu）内质网驻留信号序列的双靶向融合毒素，并探讨其对非小细胞肺癌（non-small-cell lung carcinoma, NSCLC）细胞的抑制作用。　方法　全基因合成KDRscFv基因，RT-PCR法克隆luffin-β，重叠PCR法将两基因融合，其连

接部位与C端分别引入uPA裂解位点uPAcs与KDEL，形成融合基因KDRscFv-uPAcs-Luffin-β-KDEL。将该融合基因克隆至原核表达载体pET-32a（+）中，转入大肠杆菌后，诱导其表达融合毒素蛋白Trx-EK-KDRscFv-uPAcs-Luffin-β-KDEL（TEKPLK）并纯化TEKPLK。用肠激酶（enterokinase，EK）切割TEKPLK后，纯化与回收靶向融合毒素KDRscFv-uPAcs-Luffin-β-KDEL（KPLK）。采用CCK-8（cell counting kit-8）、RT-PCR、Western blot等方法，体外检测靶向融合毒素KPLK经uPA酶裂解后释放Luffin-β对NSCLC细胞的抑制作用。 结果 成功诱导重组载体pET-32a（+）/KDRscFv-uPAcs-Luffin-β-KDEL表达相对分子质量约7.5×10^4含载体表达标签（Trx）的融合毒素蛋白TEKPLK，EK酶切该蛋白获得相对分子质量约5.8×10^4的靶向融合免疫毒素KPLK。CCK-8法检测表明，KPLK毒素的杀瘤活性成剂量-效应关系，其IC_{50}约为35 ng/mL；RT-PCR和Western blot检测结果显示，KPLK经uPA酶体外裂解后能释放细胞毒素小分子Luffin-β，上调瘤细胞促凋亡基因caspase-3及其蛋白的表达。 结论 成功构建了KDRscFv-uPAcs-Luffin-β-KDEL融合基因及其原核表达载体，并获得相对分子质量约5.8×10^4的靶向融合毒素KPLK，该毒素经uPA酶体外裂解后能释放具杀瘤活性的Luffin-β毒素小分子。

Abstract: Objective To construct a double-targeting fused immunotoxin KDRscFv-uPAcs-Luffin-β-KDEL that containing a single-chain variable fragment (scFv) against vascular endothelial growth factor (VEGF) receptor KDR, urokinase plasminogen activator（uPA）cleavage site (uPAcs), tandemly ligated luffin-β and KDEL（Lys-Asp-Glu-Leu）, which is a signal for retention of proteins in the endoplasmic reticulum, and to investigate its cytotoxic effect on non-small cell lung carcinoma (NSCLC) cell line. Methods The complete sequence of KDR scFv gene was synthesized. And luffin-β gene was cloned through reverse transcriptase-polymerase chain reaction (RT-PCR). KDR scFv gene was fused together with luffin-β gene by overlaying PCR. The uPAcs sequence was placed the linking position between KDRscFv and luffin-β gene, and the KDEL sequence was fused at the C-terminal of luffin-β gene. In doing so, the fused gene KDRscFv-uPAcs-Luffin-β-KDEL was constructed and cloned into pET-32a(+) vector to form recombinant vector pET-32a(+)/KDRscFv-uPAcs-Luffin-β-KDEL. Subsequently, the recombinant vector pET-32a(+)/KDRscFv-uPAcs-Luffin-β-KDEL was transfected into *E.coli* BL21, and the fusion protein Trx-EK- KDRscFv-uPAcs-Luffin-β-KDEL（TEKPLK） was expressed under induction, and then purified and digested through enterokinase (EK) to produce double-targeting fused immunotoxin KDRscFv-uPAcs-Luffin-β-KDEL（KPLK）. Cell count kit-8 (CCK-8), RT-PCR and Western blotting were employed to test the cytotoxic effect of KPLK cleavaged by uPA for setting free immunotoxin luffin-β on H460 cells. Results After induction, recombinant vector pET-32a(+)/KDRscFv-uPAcs-Luffin-β-KDEL

expressed the fusion protein TEKPLK, which contained the Trx tag