336~341.融合蛋白Kininogen D560-148 TRAIL114-281的表达及其抑制血管新生和诱导细胞凋亡的作用[J].宗 英,王梁华,孙铭娟,董晓毅,王 燕,陆国才,焦炳华.中国肿瘤生物治疗杂志,2009,16(4)

融合蛋白Kininogen D560-148 TRAIL114-281的表达及其抑制血管新生和诱导细胞凋亡的作用

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基金项目：上海市现代生物与新药产业发展基金（No. 024319115）

DOI：10.3872/j.issn.1007-385X.2009.4.004

摘要：

目的：采用原核表达系统表达人Kininogen D5 60 148 TRAIL 114 281 融合蛋白，并对其生物学活性进行研究。方法：PCR技术扩增Kininogen D5 60 148 和TRAIL 114 281 的编码序列，分别构建原核表达载体pMAL Kininogen D5 60 148 （pMAL KD5）、pMAL TRAIL 114 281 （pMAL TRAIL）和pMAL Kininogen D5 60 148 TRAIL 114 281 （pMAL KT），重组质粒分别转化大肠杆菌BL21，IPTG诱导表达融合蛋白MBP KD5、MBP TRAIL和MBP KT，并经亲和层析纯化。MTT法检测细胞的增殖，管状形成实验检测内皮细胞血管形成，流式细胞仪和电镜检测细胞凋亡。结果：成功构建原核表达载体pMAL KD5、pMAL TRAIL和pMAL KT，并获得纯化的融合蛋白MBP KD5、MBP TRAIL和MBP KT。融合蛋白MBP KT与MBP KD5、MBP TRAIL相比可显著抑制内皮细胞ECV304和胰腺癌细胞SW1990的增殖、明显抑制ECV304细胞体外管腔的形成，同时，MBP KT剂量依赖性地诱导SW1990细胞凋亡。结论：融合蛋白Kininogen D5 60 148 TRAIL 114 281 既能诱导肿瘤细胞凋亡又能抑制血管生成，为进一步开发靶向性抗肿瘤药物奠定了基础

关键词：高分子量激肽原 肿瘤坏死因子相关凋亡诱导配体 血管生成抑制因子 胰腺肿瘤细胞

Expression of Kininogen D560-148 TRAIL114-281 fusion protein and its angiogenesis inhibiting and apoptosis inducing effect

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Fund Project:Supported by the Modern Industrial Development Foundation for Biology and New Drugs of Shanghai (No. 024319115)

Abstract:

Objective:To express Kininogen D5 60 148 TRAIL 114 281 fusion protein using prokaryotic system and observe its biological functions.Methods: The Kininogen D5 60 148 gene and TNF related apoptosis inducing ligand (TRAIL 114 281) gene were amplified by PCR and were cloned into pMAL expression vector to construct recombinant pMAL Kininogen D5 60 148 (pMAL KD5), pMAL TRAIL 114 281 (pMAL TRAIL) and pMAL Kininogen D5 60 148 TRAIL 114 281 (pMAL KT) plasmids, respectively . The plasmids were transformed into E. coli BL21 and were efficiently expressed after IPTG induction. The purified MBP KD5, MBP TRAIL and MBP KT proteins were obtained by amylose resin affinity purification column. The proliferation of cells was measured by MTT; tube formation of endothelial cell was detected by tube formation assay; and the apoptosis of cells were observed by electron microscopic and FCM.Results: Prokaryotic expression vectors pMAL KD5, pMAL TRAIL and pMAL KT and their purified fusion proteins MBP KD5, MBP TRAIL and MBP KT were successfully obtained. MBP KT significantly inhibited the proliferation of ECV304 endothelial cells, SW1990 pancreatic cancer cells and the tube formation of ECV304 cells compared with those of MBP KD5 and MBP TRAIL. Meanwhile, MBP KT dose dependently induced the apoptosis of SW1990 cells.Conclusion: Kininogen D5 60 148 TRAIL 114 281 fusion protein can inhibit the proliferation of tumor cells and angiogenesis of endothelial cells, which lays a foundation for further research on tumor targeting drugs.

Keywords:high molecular weight kininogen tumor necrosis factor related apoptosis inducing ligand(TRAIL) antiangiogenesis pancreatic neoplasms cell

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