105~109.重组抗HER2融合蛋白基因 ScFv/tBid 对骨肉瘤E10细胞的促凋亡作用[J].裘秀春,单乐群,纪振钢,等.中国肿瘤生物治疗杂志,2008,15(2)

重组抗HER2融合蛋白基因 ScFv/tBid 对骨肉瘤E10细胞的促凋亡作用 点此下载全文

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基金项目：国家杰出青年科学基金资助项目（No.39925036）;国家自然科学基金重点项目（No.30330610）;国家自然科学基金资助项目（No.30471988）;中国博士后基金资助项目（No.2005038259）

DOI：10.3872/j.issn.1007-385X.2008.2.20085635837485

摘要：

目的：构建抗HER2重组融合蛋白基因 ScFv/tBid ，并探讨其对骨肉瘤E10细胞的促调亡作用。方法：通过间接免疫荧光染色、流式细胞仪(FCM)检测E10细胞膜表面HER2的表达。将抗HER2单链抗体基因e23sFv与铜绿假单胞菌外毒素PE的转膜结构域基因（ PEⅡ）和tBid 基因连接，构建抗HER2重组融合蛋白基因 ScFv/tBid ，将其克隆入真核表达载体pCMV中构建重组pCMV ScFv/tBid载体，转染骨肉瘤E10细胞。间接免疫荧光法检测目的蛋白表达和细胞形态学变化， Annexin V 染色流式细胞术及TUNEL法检测E10细胞的凋亡情况。结果：流式细胞仪检测到E10细胞膜表面有HER2的表达。成功构建重组融合蛋白基因质粒 pCMV ScFv/tBid。重组质粒转染E10细胞后，间接免疫荧光双标记染色检测到E10细胞中 tBid 的过表达；细胞色素C在细胞质中出现；细胞出现明显的固缩、核浓缩等形态特征。Annexin V染色后流式细胞仪检测可见实验组细胞凋亡率较对照组明显升高(16.1% vs 4.5%)；TUNEL染色显示，E10细胞出现典型的凋亡特征。结论：重组抗HER2融合蛋白基因 ScFv/tBid 可以在转染的骨肉瘤E10细胞中表达，并诱导骨肉瘤细胞发生凋亡。

关键词：tBid 人表皮生长因子受体2 融合蛋白 骨肉瘤细胞 细胞凋亡

Pro apoptotic effect of recombinant anti HER2 fusion protein ScFv/tBid gene on osteosarcoma E10 cells Download Fulltext

Fund Project:Supported by the National Science Foundation for Distinguished Young Scholars of China (No.39925036); the Major National Natural Science Fondation of China (No.30330610); the National Natural Science Foundation of China (No.30471988); the National Postdoctoral Research Foundution of China (No.2005038259)

Abstract:

Abstract Objective: To construct a fusion gene ScFv/tBid against HER2 and investigate its pro popotic effect on osteosarcoma cell line E10. Methods: HER2 expression on the surface of E10 cells was detected by immunofluorescent staining and flow cytometry (FCM), then e23sFv fragment, a single chain HER2 antibody, was linked with a PE translocation domain (PE aa253-364) and tBid . The recombinant tBid gene was cloned into a pCMV plasmid to obtain pCMV ScFv/tBid, which was then transfected into E10 cells. Immunofluorescent staining was used to examine the expression of target protein and morphological changes of cells. Meanwhile, the pro apoptotic effect of ScFv/tBid gene was analyzed by Annexin V FITC staining and TUNEL staining. Results: Flow cytometry showed HER2 expression on cell surface, and the recombinant plasmid, pCMV ScFv/tBid, was successfully constructed and transfected into E10 cells. Overexpression of tBid protein was detected in E10 cells as revealed by immunofluorescent staining; and shrinkage and nuclear condensation were also noticed in E10 cells. Annexin V FITC staining and FCM revealed that the apoptosis rate of E10 cells was 16 1% after transfection with pCMV ScFv/tBid; the apoptosis rate in the control cells was 4.5%. TUNEL staining showed typical apoptosis characteristics of E10 cells after transfection. Conclusion: The recombinant anti HER2 fusion gene, ScFv/tBid, can be expressed in E10 cells transfected with pCMV ScFv/tBid, and subsequently induce apoptosis.

Keywords:BH3 interacting death agonist (tBid) human epidermal growth factor receptor 2(HER2) fusion protein osteosarcoma cell apoptosis

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