癌变.畸变.突变 ISSN: 1004-616x CN:44-1063/R 2009; 21(1) :036-041

论著

TrKA特异小干扰 RNA表达载体构建及对乳腺癌细胞增殖的影响

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收稿日期 2008-7-8 修回日期 2008-10-6 网络版发布日期:

摘要 背景与目的： 构建TrKA特异小干扰 RNA(siRNA)表达载体，并观察其对神经生长因子(NGF)促细胞增殖作用和对细胞周期的影响。 材料与方法： 通过Genbank 提供的 TrKA 基因mRNA全序列，应用设计软件选择设计能转录短发夹状 RNA(short hairpin RNAs, shRNA)的 DNA 序列，并与 PsilencerTM4.1-CMV neo 质粒载体连接，经 DNA测序鉴定重组体DNA序列正确后转染乳腺癌 MCF-7 细胞。利用G418 筛选稳定表达TrKA-siRNA 的 MCF-7 细胞株，通过Real-time PCR、Western blot和免疫组化在mRNA和蛋白水平检测TrKA 的表达水平。MTT法检测TrKA干扰后NGF对细胞增殖作用的影响。流式细胞术观察细胞周期的变化。 结果： 序列测定表明成功构建 TrKA-siRNA 表达载体， Real-time PCR 显示TrKA mRNA下调74.7％(P<0.05)，Western blot 显示蛋白表达下降80.5％，免疫组化结果也显示TrKA 蛋白表达下降。试剂盒提供阳性对照 GAPDH 基因其表达水平下降85.0％(P<0.05)。MTT检测显示，NGF 组细胞增殖反应均较NGF＋siRNA 组、siRNA 组和对照组明显升高(P<0.05)，NGF＋siRNA 组的细胞增殖反应较其余各组下降(P<0.05)，表明TrKA干扰能有效抑制NGF诱导的乳腺癌细胞MCF-7的增殖。流式细胞术检测细胞周期结果显示：与对照组相比，NGF＋siRNA组 和 siRNA 组细胞G0/G1期增加，S 期减少(P<0.05)，细胞周期阻滞于G0/G1期。 结论： TrKA 特异 siRNA 表达载体有效下调TrKA基因的表达，并抑制NGF引起的细胞增殖效应。

关键词 小干扰 RNA； TrKA； 乳腺癌MCF-7细胞

Construction of TrKA-specific Small Interfering RNA Expression Vector and Its Anti-proliferative Effect on Breast Cancer Cells

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Abstract BACKGROUND AND AIM: To construct the expression vector of TrKA small interfering RNA,and to observe its effect on cell proliferation induced by nerve growth factor (NGF) and cell cycle. MATERIALS AND METHODS: Using the mRNA complete sequence of TrKA gene provided by Genbank, DNA sequence which could transcribe short hairpin RNAs was selected and designed by software,and was connected with the vector of PsilencerTM 4.1-CMV neo .Then it was transfected into MCF-7 cells after confirmed by sequencing. The stable cell line expressing TrKA small interfering RNA were selected by G418. The mRNA and protein levels of TrKA were tested by real-time PCR, Western blot, and immunohistochemistry. The alteration of cell proliferation induced by NGF was assessed by MTT assay after TrKA interference and cell cycle was measured by flow cytometry. RESULTS: The expression vector of TrKA siRNA was successfully constructed . The level of TrKA mRNA and protein was decreased by 74.7％ (P<0.01) and 80.5％,respectively. Immunohistochemistry also showed that TrKA was down-regulated.The expression of GAPDH gene was decreased by 85.0％ (P<0.05). The density value of NGF group was higher than the group of NGF＋siRNA,siRNA and control each time(P<0.05),but the density value of NGF＋siRNA group was lower than other group(P<0.05) . The result indicated that TrKA could effectively inhibit the proliferation of breast cancer cells MCF-7 induced by NGF.The cell cycle showed that compared with control, G0/ G1 period of NGF＋siRNA was higher and the S cell of siRNA was lower (P<0.05), cell

cycle was arrested by G0/G1. CONCLUSION: The expression vector of TrKA siRNA could decrease the expression of TrKA in MCF-7 cells effectively,and inhibit the proliferation of MCF-7 induced by NGF.

Keywords small interfering RNA TrKA breast cancer cell MCF-7

DOI

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