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摘要：

目的：探讨RNAi（RNA interference）技术抑制乳腺癌MCF 7细胞中AKT1和PI3K P85亚基的表达对MCF 7细胞增殖和侵袭等的影响。方法：将包含AKT1、PI 3K P85两种siRNA开放阅读框的短发夹RNA（shRNA）重组腺病毒质粒表达载体rAd5 siAKT1 siPI3K转染至乳腺癌MCF 7细胞。应用real time PCR和Western bl otting检测转染后目的基因mRNA和蛋白的表达水平，并用Western blotting检测目的基因被沉默后PCNA、cyclin D1和P53的表达情况。应用MTT法、流式细胞术、2 D和3 D Matrigel实验检测MCF 7细胞转染前后的细胞增殖周期和侵袭能力。结果：重组腺病毒质粒表达载体rAd5 siAKT1 siPI3K 介导的靶向 〖STBX〗AKT1, PI3K P85 shRNA可以有效抑制目的基因AKT1和PI3 Kp85 的mRNA和蛋白表达；下游相关因子 PCNA 、cyclin D1的表达亦下调，P53表达则上调。MTT法结果显示rAd5 siAKT1 siPI3K组细胞生长抑制率>50%，与未转染组和rAd5 siCtrl转染组比较，出现明显的G 1/G 0细胞周期阻滞；2 D和3 D Matrigel实验显示，未转染组和rAd5 siCtrl转染组细胞呈正常形态，而rAd5 siAKT1 siPI3K 转染组细胞贴壁生长能力明显减低，细胞团块明显缩小。结论：靶向AKT1、PI3K P 85亚基的shRNA技术可以抑制MCF 7细胞中AKT1、PI3K P85亚基的表达，抑制MCF 7细胞的体外增殖。

关键词：RNA干扰 乳腺肿瘤 AKT1 PI3K P85 增殖

RNAi targeting AKT1 and PI3K P85 suppresses proliferation of breast carcinoma MCF-7 cells Download Fulltext

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Abstract:

Objective:To investigate the effect of RNA interference (RNAi) targeting AKT1 and PI3K P85 on the proliferation and invasion of breast carcinoma MCF 7 cells. Methods:The recombinant adenovirus expression vector, which contained short hairpin RNA (shRNA) targeting open reading frames of AKT1 and PI3K P85 (rAd5 siAKT1 siPI3K), was transfected into human breast carcinoma MCF 7 cells. AKT1 and PI3K P85 mRNA and protein expressions were detected by real time PCR and Western blotting analysis. The expressions of PCNA, cyclinD1, and P53 were also detected by Western blotting analysis. The proliferation and apoptosis of MCF 7 cells were measured by MTT, flow cytometry and 2 dementinal and 3 dementional matrigel assay. Results:Recombinant adenovirus vector rAd5 siAKT1 siPI3K dramatically down regulated AKT1 and PI3K P85 mRNA and protein expressions in MCF 7 cells; the downstream factors PCNA and cyclin D1 were also down regulated, while P53 was up regulated. Growth of MCF 7 cells was inhibited by over 50% in rAd5 siAKT1 siPI3K group as measured by MTT assay, and cell cycle was arrested in G 1/G 0 phase compared with untransfected and rAd5 siCtrl transfected groups. Cell growth on matrigel matrix showed normal cell shapes, while the cells in rAd5 siAKT1 siPI3K transfected group were detached from the matrix or grew in scattered clustering patterns, forming only small aggregates. Conclusion:shRNA targeting AKT1 and PI3K P85 can significantly down regulate the expression of AKT1 and PI3K P85 in breast carcinoma MCF 7 cells, and inhibit the growth of MCF 7 cells in vitro.

Keywords:RNA interference breast neoplasms AKT1 PI3K P85 proliferation

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