论著

黏附素5胞外区克隆、转染及抗人乳腺癌细胞生长的研究

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摘要 目的: 克隆和转染黏附素5胞外区,并研究其对人乳腺癌MDA-MB435细胞株生长的影响。方法: RT-PCR技术克隆黏附素5胞外区(称为CED1-4),将其插入pMSCV质粒载体,在大肠杆菌XL-blue扩增,提取和纯化pMSCV-CED1-4,酶切、电泳和测序检测CED1-4序列。CED1-4基因转染MDA-MB435细胞株,RT-PCR和Western blotting检测MDA-MB435细胞表达CED1-4。细胞增殖实验和乳腺癌裸小鼠致瘤实验检测CED1-4对MDA-MB435细胞体内外生长的影响。结果: 构建了重组体pMSCV-CED1-4,电泳显示CED1-4条带在 1 636 bp-1 018 bp区间,测序显示CED1-4基因长1 452 bp,编码484氨基酸。经PCR和Western blotting证实,CED1-4基因转染的MDA-MB435细胞在mRNA和蛋白质水平表达CED1-4。细胞增殖实验结果表明,实验组MDA-MB435细胞增殖低于实验对照组和空白对照组(P<0.05)。乳腺癌裸小鼠致瘤实验显示,实验组移植瘤平均体积和重量低于实验对照组和空白对照组(P<0.05)。结论: 黏附素5胞外区CED1-4能在体内外抑制人乳腺癌MDA-MB435细胞株的生长。

关键词 钙粘着糖蛋白类; 克隆; 乳腺肿瘤; 基因转染

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Cloning, transfer and anti-human breast cancer cell growth of the extracellular domain of cadherin 5

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Abstract

AIM: In order to study the effect of the extracellular domain of cadherin 5 on the growth of a human breast cancer cell line MDA-MB435.
METHODS: Cadherin extracellular domain repeats 1 to 4(CED1-4) was cloned by using RT-PCR technique, and inserted into the plasmid vector pMSCV.pMSCV-CED1-4 was propagated in XL-blue strain of Escherichia coli, extracted and purified. CED1-4 was cut by restriction endonuclease, examined by using agar gel electrophoresis, and finally sequenced. CED1-4 gene was transferred into MDA-MB435 cell line. The expression of CED1-4 gene in MDA-MB435 cell was analyzed by methods of RT-PCR and Western blotting. The effect of CED1-4 on the growth of MDA-MB435 cell was observed by the methods of proliferation experiments in vitro and the experiments in nude mice in vivo. < BR > RESULTS: The recombinant vector pMSCV-CED1-4 was successfully constructed.CED1-4 band appeared between the 1 636 bp and 1 018 bp in agar gel electrophoresis. The sequence result showed that CED1-4 had 1 452 bp and codes 484 amino acids. PCR and Western blotting identified that CED1-4 mRNA and protein were expressed in the transfected MDA-MB435 cells Cell proliferation experiments showed that the proliferation rate of MDA-MB435 cells was lower in the experimental group than that in the experimental control group and the blank control group. The mean volume and weight of tumors in nude mice were lower in the experimental group than those in the experimental control group and the blank control group. < BR > CONCLUSION: The growth of a human breast cancer cell line MDA-MB435 is inhibited in vitro and in vivo by cadherin 5 extracellular domain CED1-4.

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Key words Cadherins Clone Breast neoplasms Gene transfection

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