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论文

真核表达载体pEGFP-N1-ZIP10的构建及其对人乳腺癌细胞中其他锌转运体基因表达的影响

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摘要：

目的 构建pEGFP-N1-ZIP10表达载体，观察其在乳腺癌MCF-7和MDA-MB-231细胞中的表达，并检测ZIP10过表达对其他锌转运体的影响。方法 从外周血经 RT-PCR扩增ZIP10 cDNA序列，并将其定向克隆至真核表达质粒pEGFP-N1中，pEGFP-N1-ZIP10经酶切及测序鉴定后，转染乳腺癌MCF-7和MDA-MB-231细胞，RT-PCR检测ZIP10的表达及其他锌转运体ZnT1、ZIP1、ZIP6等表达的变化。结果 酶切和测序证实目的基因片段大小、方向均正确，转染MCF-7和MDA-MB-231细胞，RT-PCR检测到ZIP10在mRNA水平过表达，并发现使ZIP1的表达显著降低。结论 成功构建真核表达载体pEGFP-N1-ZIP10，并在乳腺癌MCF-7和MDA-MB-231细胞瞬时表达成功，转染后使细胞中ZIP1的表达明显降低，为进一步研究ZIP10在乳腺癌发生发展中的作用奠定基础。

关键词： 乳腺癌细胞；ZIP10；ZIP1；表达载体

Construction of the recombinant plasmid pEGFP-N1-ZIP10 and its effect on expressions of other zinc transporters in breast cancer cells

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Abstract:

Objective To construct the pEGFP-N1-ZIP10 expression vector and observe its expression in human breast cancer MCF-7 and MDA-MB-231 cell lines, and also to detect expressions of other zinc transporters when ZIP10 is over-expressed. Methods The target sequence of ZIP10 was obtained and amplified from human blood by RT-PCR. Then, the cDNA segment was cloned into eukaryote plasmid pEGFP-N1. The pEGFP-N1-ZIP10 was identified by restriction enzyme digestion and checked by DNA sequence analysis. MCF-7 and MDA-MB-231 cells were transiently transfected, and expressions of ZIP10 and other zinc transporters were detected by RT-PCR. Results Identification of pEGFP-N1-ZIP10 by enzyme digestion and PCR showed that the length, location of insertion and direction of the target gene inserted into the recombinant were correct. After the transfection, over-expression of ZIP10 was found in human breast cancer MCF-7 and MDA-MB-231 cells, while expression of ZIP1 was significantly reduced in the transfected cells. Conclusion The eukaryotic expression plasmid pEGFP-N1-ZIP10 has been successfully constructed and it can be expressed transiently in MCF-7 and MDA-MB-231 cells. The decreased expression of ZIP1 in the transfected cells may indicate the role of ZIP10 in breast carcinogenesis and development.

Keywords: Breast cancer cells; ZIP10; ZIP1; Expression vector

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