中国病理生理杂志 2009 25 (4): 656-660 ISSN: 1000-4718 CN: 44-1187/R

论著

雌激素受体2对前列腺癌细胞系PC-3M增殖的影响

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收稿日期 2008-4-25 修回日期 2008-9-19 网络版发布日期 2009-8-2 接受日期 2008-9-19

摘要 目的： 构建带有人雌激素受体2（ESR2）全长cDNA的重组质粒pcDNA3.1-hERβ，转染人前列腺癌PC-3M细胞株，观察ESR2对细胞增殖能力的影响。 方法：RT-PCR方法从人正常卵巢组织中获取ESR2全长cDNA，利用基因重组技术与真核表达载体pcDNA3.1连接，构建重组质粒pcDNA3.1-hERβ；瞬时转染前列腺癌PC-3M细胞株，应用细胞计数、MTT法及流式细胞术检测细胞增殖能力的改变；半定量RT-PCR法及Western blotting法分别检测增殖相关基因cyclinD1和 P21Cip1 mRNA及蛋白表达。 结果：DNA测序结果显示扩增的ESR2序列与GenBank（NM_001437）所公布的序列完全一致。重组质粒pcDNA3.1-hERβ瞬时转染人PC-3M细胞48 h后，与质粒对照组相比：RT-PCR法及Western blotting法显示，ESR2基因在mRNA水平和蛋白水平的表达均明显增加；细胞计数及MTT结果发现，细胞数目减少，细胞增殖活性下降（P<0.01）；流式细胞实验显示，G0/G1期细胞比例增加（P<0.05），S期及G2/M期细胞比例减少（P<0.05）；RT-PCR及Western blotting结果还发现cyclinD1的表达减弱，而P21Cip1的表达增强。 结论：成功构建pcDNA3.1-hERβ重组质粒；带有ESR2全长基因的重组质粒转染PC-3M细胞后，细胞的增殖活性受到抑制；RSR2可能通过影响细胞增殖相关基因cyclinD1和 P21Cip1的表达抑制细胞的增殖。

关键词 受体,雌激素； PC-3M细胞； 真核表达

分类号 R363

Effect of estrogen receptor 2 on the cell proliferation in prostate cancer cell line PC-3M

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Abstract

<FONT face=Verdana>AIM: To construct the recombination plasmid pcDNA3.1-hERβ with the human estrogen receptor 2 (ESR2) full length cDNA and transfect it into hormone-independent prostate cancer PC-3M cell line, and to study the effects of ESR2 on proliferation in transfected cells. METHODS: The complete cDNA of ESR2 was obtained from human ovary tissue by RT-PCR technique and cloned into eukaryotic expression vector pcDNA3.1 by using gene recombination technique to construct the pcDNA3.1-hERβ recombination plasmid. The plasmid was detected by endonuclease digestion and DNA sequencing and was transfected into PC-3M cells. MTT and FCAS assay were used to test the effects of ESR2 on the ability of proliferation in PC-3M cells. RT-PCR and Western blotting were used to detect the expressions of cyclinD1 and P21Cip1. RESULTS: The results of sequencing and endonuclease digestion demonstrated that the construction of pcDNA3.1-hERβ recombination plasmid was successful. The sequence analysis suggested that the ESR2 sequence detected by PCR was identical to that published in GenBank, and the product of endonuclease was as long as the complete human ESR2 gene. 48 h after transfected the pcDNA3.1-hERβ into PC-3M cells, the expression of ESR2 mRNA and protein levels increased significantly detected by RT-PCR and Western blotting. Compared to the cells transfected with vector as control, the PC-3M cells transfected with pcDNA3.1-hERβ showed that cell population decreased and proliferation activity degraded. FCAS showed that the cells in G0/G1 stage increased and in S stage or G2/M stage decreased. RT-PCR and Western blotting showed that the expression of cyclinD1 gene reduced and expression of P21Cip1 increased. CONCLUSION: The recombination of plasmid pcDNA3.1-hERβ is

constructed and transfected into the PC-3M cells successfully. The activity of cell proliferation is inhibited after pcDNA3 transfection.1-hERβ. It is possible that ESR2 inhibits cell proliferation by the expression of proliferation related genes cyclinD1 and P21Cip1. </FONT>

Key words Receptors estrogen PC-3M cells Eukaryotic expression

DOI: 1000-4718

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