459~463.p70/p85 核糖体蛋白S6激酶1重组真核表达载体的构建及其在人乳腺癌MCF-7细胞内功能的初步鉴定[J].赵莉,侯敬申,白晓春,贾春宏.中国肿瘤生物治疗杂志,2014,21(4)

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摘要：

目的：构建p70 核糖体蛋白S6激酶1（p70 ribosomal protein S6 kinase 1，p70 S6K1）及p85 S6K1基因的真核表达载体pcDNA3.1(-)-flag-p70 S6K1和pcDNA3.1(-)-flag-p85 S6K1，并鉴定其在人乳腺癌MCF-7细胞内的表达及功能。 方法： 以pRK7-HA-S6K1为模板，采用PCR扩增出目的基因片段p70 S6K1、p85 S6K1，克隆入真核表达载体pcDNA3.1(-)-flag构建重组表达载体pcDNA3.1(-)-flag-p70 S6K1和pcDNA3.1(-)-flag-p85 S6K1，采用PCR、双酶切和DNA测序鉴定。将重组载体转染MCF-7细胞，24 h后采用Western blotting方法检测细胞内p70 S6K1、p85 S6K1蛋白的表达；同时向转染细胞内加入1 mmol/L H 2O 2处理36 h，观察p70 S6K1、p85 S6K1蛋白对H 2O 2诱导的细胞死亡的影响。 结果： 成功扩增得到p70 S6K1、p85 S6K1基因片段并构建重组真核表达载体pcDNA3.1(-)-flag-p70 S6K1和pcDNA3.1(-)-flag-p85 S6K1，重组载体经PCR、双酶切鉴定均出现p70 S6K1和p85 S6K1预期条带，DNA测序结果显示其全长基因阅读框完整、正确。重组载体在MCF-7细胞中高效表达flag-p70 S6K1和flag-p85 S6K1，且p85 S6K1能增强H 2O 2诱导的细胞死亡。 结论：成功构建重组真核表达载体pcDNA3.1(-)-flag-p70 S6K1和pcDNA3.1(-)-flag-p85 S6K1，均能在MCF-7细胞中高效表达，且p85 S6K1能够增强H 2O 2诱导的细胞死亡。

关键词：核糖体蛋白S6激酶1 基因重组 载体构建 真核表达 细胞死亡 乳腺癌 MCF-7细胞

Construction of p70 /p85 ribosomal protein S6 kinase 1 expression vectors and functional assessment in human breast cancer MCF-7 cells

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Abstract:

Objective: To optimize the construction of eukaryotic expression vectors encoding p70/p85 ribosomal protein S6 kinase 1 (S6K1)and to evaluate the function of the constructed vectors in human breast cancer MCF-7 cells. Methods: Fragments of p70 S6K1 and p85 S6K1 cDNAs with restriction endonucleases sites were amplified by PCR with pRK7-HA-S6K1 as a template and cloned into an eukaryotic expression vector with a flag tag, pcDNA3.1 (-)-flag. MCF-7 cells were transfected with the constructed vectors, pcDNA3.1(-)-flag-p70 S6K1 and pcDNA3.1(-)-flag-p85 S6K1. At 24 h after transfection, protein contents of p70 S6K1 and p85 S6K1 were assessed by Western blotting using anti-flag and anti-p70/85 S6K1 antibodies and cell death following induction with 1 mmol/L hydrogen peroxide for another 36 h was analyzed by microscopy. Results: Eukaryotic expression vectors pcDNA3.1(-)-flag-p70 S6K1 and pcDNA3.1(-)-flag-p85 S6K1 were successfully constructed; the full-length open reading frames were confirmed by DNA sequencing. Overexpression of S6K1 and S6K1 was detected in MCF-7 cells transfected with pcDNA3.1(-)-flag-p70 S6K1 and pcDNA3.1(-)-flag-p85 S6K1 respectively. Overexpression of p85 S6K1 but not p70 S6K1 enhanced MCF-7 cell death induced by 1 mmol/L hydrogen peroxide. Conclusion: Expression vectors pcDNA3.1(-)-flag-p70 S6K1 and pcDNA3.1(-)-flag-p85 S6K1 were constructed successfully. Overexpression of p85 S6K1 may enhance H 2O 2-induced breast cancer cell death.

Keywords:ribosomal protein S6 kinase 1(S6K1) gene recombination vector construction eukaryotic expression cell death breast cancer MCF-7 cell

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