中国药理学与毒理学杂志 2008 22 (6): 463-469 ISSN: 1000-3002 CN: 11-1115/R

实验方法

携带人生存素基因短发夹RNA表达载体重组腺病毒的构建及其生物学作用

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收稿日期 2008-1-16 修回日期 网络版发布日期 2008-11-28 接受日期 2008-8-1

摘要 目的 构建携带人生存素(survivin)基因短发夹RNA(shRNA)表达载体的重组腺病毒。方法 构建并筛选重组质粒pShuttle-*survivin*，将其与腺病毒骨架质粒共转染HEK-293细胞，经同源重组产生腺病毒；PCR鉴定并测定病毒滴度；β-半乳糖染色检测病毒对人乳腺癌细胞MCF-7的感染效率；流式细胞术、RT-PCR和Western免疫印迹法检测其生物学功能。结果 经PCR鉴定重组腺病毒构建成功；48 h后感染率达75%以上；腺病毒感染MCF-7细胞后48 h，生存素mRNA和蛋白的表达均受到抑制；腺病毒感染MCF-7细胞后，细胞分裂受阻于G_2/M期，在48和72 h有凋亡峰出现。结论 成功构建人生存素基因shRNA表达载体的重组腺病毒。

关键词 生存素 基因表达调控, 病毒 DNA, 重组

分类号 R965.2

Construction of recombinant adenovirus with short hairpin RNA expression vector of survivin and its biological effect

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Abstract

AIM To construct recombinant adenovirus with short hairpin RNA (shRNA) expression vector of survivin. **METHODS** After pShuttle-*survivin* was constructed and screened, cotransfected it with bone plasmid of adenovirus into HEK-293 cells to get recombinant adenovirus. Virus titer was determined after verification by PCR. Efficiency of adenovirus infecting MCF-7 cells was detected by β-galactose staining. Its biological effects were observed by means of flow cytometric analysis, RT-PCR and Western blot. **RESULTS** Adenovirus was constructed successfully. Total of 75% of MCF-7 cells were infected after treated with adenovirus. After infected with adenovirus, RT-PCR and Western blot analysis showed that survivin was suppressed at 48 h on the level of mRNA and protein. Flow cytometric analysis indicated that survivin shRNA induced the cell cycle arrest at G_2/M phase, and induced apoptosis at 48 and 72 h. **CONCLUSION** Recombinant adenovirus with shRNA targeting survivin may be an effective way of gene therapy on human breast cancer.

Key words survivin gene expression regulation viral DNA recombinant

DOI: 10.3867/j.issn.1000-3002.2008.06.012

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