吉林农业大学学报 2011, 33(3) 327-331 DOI: CNKI:22-1100/S.201104 ISSN: 1000-5684 CN: 22-1100/S

论文

生长抑素shRNA的慢病毒包装及对生长抑素的抑制作用

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摘要:

采用慢病毒(LV)载体介导siRNA感染宿主细胞,研究其对内源性生长抑素(Somatostatin,SS)的抑制作用。首先将筛选出的psh2与辅助质粒共转染293T细胞包装获得假病毒颗粒LV-sh2,同时,包装产生LV-sh0和LV-GFP作为阴性对照。超速离心,梯度稀释法测定病毒滴度,结果提示所构建的重组病毒滴度约为 6× 10 7 ifu / mL。病毒感染BHK-21细胞,通过荧光显微镜观察到了GFP的高效表达,在感染细胞的基因组中PCR检测到了病毒基因,表明病毒感染获得了高效的基因转移和表达效率。荧光定量PCR和RIA检测表明,LV-sh2对内源性SS 的mRNA和蛋白质水平均有显著的抑制作用(86.49%和78.80%, P <0.05)。

关键词: 生长抑素 shRNA 慢病毒载体 基因沉默

Packing of the Lentivirus Contained shRNA Targeting Somatostatin and Its Inhibitory Effect

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Abstract:

Somatostatin(SS) acts to inhibit secretion on a diverse array. In ourresearch, BHK-21 cells were infected by a Lentiviral-shRNA(Lv-shRNA) to examinewhether the endogenous SS would be down regulated by shRNA. First, the Lv—sh2was packed by the co transfection of the screened psh2 and the other three packing plasmids into 293T cells. Meanwhile, the Lv-sh0 and LvGFP were packed as thenegative controls. Next, high titer virus stocks (6×10 7 ifu / mL) obtained byulatracentrifugation were achieved routinely measuring by infecting BHK-21 cellsin tenfold serial dilutions. Finally, BHK-21 cells were infected with theconcentrated viral stocks, and the high infection and expression efficiency were observed under the fluorescence. The shRNA gene was detected to integrate into the genomic DNA of infected cells by PCR analysis. Significant downregulation of SS mRNA and protein level were detected by Real time PCR (reduced by 86 5% relative to the control, P <0 05) and RIA technique (lower 75.4% than the control, P <0.05). The results may lay a basis on the studies of SS functions in the cell.

Keywords: somatostatin shRNA Lentiviral vector gene silencing

收稿日期 2010-07-19 **修回日期** **网络版发布日期**

DOI: CNKI:22-1100/S.201104

基金项目:

吉林大学农学部人才引进建设项目（4305050102J4）

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