复旦学报（医学版） 2008 35 (4): ISSN: 1672-8467 CN: 31-1885/R

实验研究报道

RNAi抑制水通道蛋白5表达及其对细胞株粘蛋白合成、分泌的影响

陈智鸿 高磊 祝蓉 白莉 白春学

复旦大学附属中山医院肺科,上海 200032

收稿日期 2008-2-21 修回日期 网络版发布日期 2008-10-14 接受日期

摘要
目的：观察瞬时及稳定转染的RNA干扰法对水通道蛋白5（AQP5）的抑制效果，并观察AQP5抑制后细胞株粘蛋白合成、分泌的变化。方法：用化学合成siRNA和质粒介导的shRNA分别转染SPC-A1细胞，并在转染后7d内的不同时点收集细胞，检测AQP5 mRNA和蛋白的变化。用G418筛选稳定转染细胞株，用Western blot法检测稳转株AQP5蛋白的抑制情况。用ELISA法检测AQP5抑制后各时点及稳定转染细胞株MUC5AC表达量的变化。结果： siAQP5及shAQP5转染24h对AQP5 mRNA的抑制率分别为65%、79%。对蛋白的抑制在转染后第7d最明显，分别为93%，98%。稳转株（5株）对AQP5蛋白的抑制率为45-64%。ELISA显示瞬转第5d，MUC5AC的合成和分泌分别增加 57.9% 、85.3%。在5株稳定转染细胞中 (shAQP5-G1,G2,G3,A1,A5) MUC5AC的合成和分泌分别增加 59-156% 及33-166% 。结论：化学合成及质粒介导的RNA干扰法均能有效抑制SPC-A1细胞AQP5的表达，后者较前者更经济，抑制效应更优。与瞬时转染比较，稳定转染可长时间抑制特定基因的表达。AQP5抑制后，细胞株粘蛋白的合成、分泌增高。

关键词 RNAi,siRNA,shRNA,AQP5,粘蛋白,稳定转染

分类号

Inhibition of AQP5 expression by RNAi and its effect on mucin synthesis and secretion

CHEN Zhi-hong, GAO Lei, ZHU Rong, BAI Li, BAI Chun-xue

Pulmonary Department, Zhongshan Hospital, Fudan University, Shanghai, China, 200032

Abstract
Objective In this experiment, we attempted to investigate whether the expression of AQP5 could be inhibited by RNAi transiently and stably, compared effects among the 3 methods, and the effect of AQP5 gene silencing on mucin synthesis and secretion. Methods SPC-A1 cells were firstly transfected with synthetic siRNA or vector driven hairpin RNA. Then, cells were collected at different time within 7 days and mRNA and protein level of AQP5 were detected using quantitative real-time PCR and Western blot. Stably transfected cell lines were selected by G418, AQP5 protein were detected by Western blot. MUC5AC was measured by ELISA. Results : AQP5 mRNA were suppressed by 65% and 79% using siAQP5 and shAQP5 respectively, and AQP5 protein were decreased 93%, 98% on day 7 after transfection. The inhibitory rate for stable transfection cell lines were 45-64%. In transiently tranfected cells, the results of ELISA showed MUC5AC synthesis and secretion were increased by 57.9% and 85.3% respectively on day 5 after transfection. In 5 stable transfection clones, the elevated levels of MUC5AC synthesis and secretion varied from 59-156% and 33-166% respectively. Conclusion The results indicate both chemically synthesized siRNA and vector-based hairpin RNA could dramatically inhibit targeting gene AQP5, and the later is a more efficient and economical strategy than the former one. Stable transfection could inhibit gene expression longer than the transient one. After AQP5 silencing, mucin synthesis and secretion increased.

Key words RNAi,siRNA,shRNA,AQP5,mucin stable transfection

DOI:

通讯作者 白春学 bai-chunxue@zshospital.sh.cn

作者个人主页 陈智鸿 高磊 祝蓉 白莉 白春学