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RNA干扰下调 Slug 表达对肺癌细胞A549的细胞周期、增殖和侵袭的影响 点此下载全文

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摘要：

目的：应用RNA干扰技术下调肺癌A549细胞中 Slug基因 的表达，探讨其对A549细胞增殖、细胞周期和细胞侵袭能力的影响。方法：构建靶向 Slug基因的shRNA真核表达质粒pGPU6-GFP-Neo-Slug，与阴性对照质粒pNeg-shRNA分别用脂质体法转染A549细胞。Real-time PCR和Western blotting验证转染后 Slug mRNA和蛋白的表达。CCK-8法、流式细胞术和Transwell实验分别检测下调Slug表达对A549细胞增殖、细胞周期和侵袭能力的影响。结果：成功构建pGPU6-GFP-Neo-Slug载体并转染A549细胞，转染率达90%。与pNeg-shRNA组和空白对照组相比，pSlug-shRNA组A549细胞中 Slug mRNA\[（0.23±0.01） vs （0.97±0.08）、（1.0±0.09）， P <0.05\]和蛋白\[（0.20±0.09） vs （1.0±0.32）、（1.13±0.26）， P <0.05\]表达量显著降低；细胞增殖抑制率明显上升\[（35.3±5.4）% vs （1.5±0.2）%、（3.3±0.7）%，均 P < 0.01\]；细胞增殖指数显著降低 \[（32.92±0.69）% vs （48.19±0.71）%、（42.88±0.75）%，均 P <0.05\]，并且处于G 1期细胞数明显增多\[（67.08±0 92）% vs （52.81±0.78）%、（56.12±0.73）%，均 P <0.05\]；细胞侵袭能力显著降低\[穿透基底膜细胞数：（55±9） vs （169±12）、（173±15），均 P <0.01\]。结论：pGPU6-GFP-Neo-Slug转染肺癌A549细胞能有效下调 Slug基因 的表达，从而抑制A549的增殖侵袭能力、阻滞细胞周期于G 1期。

关键词：RNA干扰 Slug基因 肺癌 A549细胞 增殖 细胞周期 侵袭

Effect of down-regulation of the Slug gene expression by RNAi on cell cycle, proliferation and invasion of lung cancer A549 cells Download Fulltext

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Abstract:

Objective:To study the effect of silencing Slug gene on proliferation, cell cycle and invasion ability of lung cancer A549 cells by RNA interference technique. Methods: The recombinant plasmid expressing short hairpin RNA (shRNA) targeting Slug gene was constructed and named as pGPU6-GFP-Neo-Slug. A549 cells were transfected with pGPU6-GFP-Neo-Slug and the negative control plasmid pNeg-shRNA with liposome method. Real-time PCR and Western blotting analysis was performed to determine the expression of Slug mRNA and protein after transfection, respectively. The proliferation, cell cycle distribution and cell invasion of A549 cells were detected by CCK-8 assay, flow cytometry and transwell assay, respectively. Results: pGPU6-GFP-Neo-Slug vector was successfully constructed and transfected into A549 cells with transfection rate of nearly 90%. Compared to the control and pNeg-shRNA group, Slug mRNA (\[0.23±0.01\] vs \[0.97±0.08\], \[1.0±0.09\]; all P <0.05) and protein (\[0.20±0.09\] vs \[1.0±0.32\], \[1.13±0.26\]; all P <0.05) expression level was significantly reduced in pSlug-shRNA group. Compared to the control and pNeg-shRNA group, the inhibition rate of proliferation in Slug-silencing A549 cells was significantly increased (\[35.3±5.4\]% vs \[1 5±0 2%\], \[3.3±0.7%\]; all P < 0.05); the cell multiplication index was significantly decreased(\[32.92±0 69\] vs \[48.19±0.71\], \[42.88±0.75\]; all P <0.05); the cell number in G 1 phase was significantly increased(\[67.08±0 92\] vs \[52.81±0.78\], \[56.12±0.73\]; all P <0.05); and the cell invasion ability of A549 was significantly reduced (number of alive A549 cells through the matrigel chamber: \[55±9\] vs \[169±12\], \[173±15\]; all P <0.01). Conclusion: pGPU6-GFP-Neo-Slug vector transfected into lung cancer A549 cell can effectively silence Slug gene expression, inhibit cell proliferation, influence cell cycle and inhibit cell invasion of A549 cells.

Keywords:RNA interference Slug gene lung cancer A549 cell proliferation cell cycle invasion

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