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RNA干扰沉默 PIN1 对肺癌A549细胞增殖、细胞周期和成瘤的影响 点此下载全文

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摘要：

目的： 应用RNA干扰技术沉默肺癌A549细胞中 PIN1 （protein interacting with N1MA1）基因的表达，探讨其对A549细胞增殖、细胞周期和裸鼠成瘤能力的影响。 方法: 构建靶向 PIN1 基因的shRNA真核表达质粒pGPU6-GFP-Neo-PIN1和无义对照质粒pGPU6-GFP-Neo，以脂质体法转染A549细胞，G418筛选稳定沉默 PIN1 基因的细胞株。Real-time PCR和Western blotting验证 PIN1 基因在mRNA和蛋白水平的表达，MTT法和流式细胞术检测A549细胞增殖和细胞周期分布。将稳定沉默 PIN1 的A549细胞与对照细胞皮下接种裸鼠，观察接种后肿瘤生长情况。 结果: 成功构建了pGPU6-GFP-Neo-PIN1载体，转染A549细胞并筛选获得稳定克隆。稳定转染pGPU6-GFP-Neo-PIN1的A549细胞中 PIN1 mRNA表达量较pGPU6-GFP-Neo转染组下降了89 3%；蛋白表达同时也显著抑制。 PIN 1 基因沉默组的A549细胞增殖速率明显下降（P<0.01），细胞出现G 1期阻滞。小鼠体内实验显示， PIN1 沉默的A549细胞在裸鼠体内成瘤能力降低（P<0.0 1)。结论: pGPU6-GFP-Neo-PIN1质粒稳定转染肺癌A549细胞能有效沉默 PIN1 基因的表达，从而抑制A549细胞的增殖、影响细胞周期和抑制成瘤能力。

关键词：RNA干扰 PIN1 基因 肺肿瘤 增殖

Effect of RNA interference-based silencing of PIN1 gene on proliferation, cell cycle and tumorigenicity of lung cancer A549 cells Download Fulltext

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Abstract:

Objective:To study the effect of PIN1 （protein interacting with N1MA1） gene on proliferation, cell cycle and tumorigenicity of lung cancer A549 cells by silencing PIN1 gene using RNA interference technique. Methods: The recombinant plasmid expressing short hairpin RNA (shRNA) targeting PIN1 gene was constructed and named pGPU6-GFP-Neo-PIN1. A549 cells were transfected with pGPU6-GFP-Neo-PIN1 and the negative control plasmid (pGPU6-GFP-Neo) by lipofectamine 2 000. Stable cell lines expressing PIN1 shRNA were obtained after G418 screening. Real-time PCR and Western blotting analysis were performed to determine the expressions of PIN1 at mRNA and protein levels, respectively. Proliferation and cell cycle distribution of A549 cells were detected by MTT assay and flow cytometry assay. Meanwhile, the growth of subcutaneously implanted tumors was observed in nude mice after inoculated with A549 cells with PIN1 stably silenced and the control A549 cells. Results: pGPU6-GFP-Neo-PIN1 plasmid vector was successfully constructed and transfected into A549 cells. PIN1 mRNA expression in A549 cells stably transfected with pGPU6-GFP-Neo-PIN1 decreased by 89.3% compared with that in pGPU6-GFP-Neo-transfected A549 cells, and PIN1 protein was also inhibited by pGPU6-GFP-Neo-PIN1 transfection. Proliferation of PIN1 -silenced A549 cells was significantly suppressed (P<0.01), and their cell cycle was arrested in G 1 phase. The tumorigenicity of A549 cells in nude mice was inhibited when PIN1 was silenced (P<0.01). Conclusion: pGPU6-GFP-Neo-PIN1 plasmid stably transfecting into lung cancer A549 cells can effectively silence PIN1 gene expression, inhibit cell proliferation, influence cell cycle and inhibit tumorigenicity of A549 cells.

Keywords:RNA interfere protein interacting with N1MA1 (PIN1) gene lung neoplasms proliferation

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