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慢病毒介导shRNA特异性沉默livin基因促进SPC-A1细胞凋亡 点此下载全文

陈愉生 李鸿茹 林 明 陈 刚 谢宝松 许能銮 林立芳

福建医科大学省立临床医学院 呼吸科 福州 350001;福建医科大学省立临床医学院 呼吸科 福州 350001

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摘要:

目的:建立慢病毒介导的livin基因沉默系统,探讨其对肺癌细胞凋亡的影响。方法:Livin shRNA慢病毒感染肺腺癌细胞株SPC A1沉默livin基因表达。应用PI染色经荧光镜下观察SPC A1细胞凋亡形态,流式细胞术检测SPC A1细胞凋亡率及亚二倍体峰形成,Real time PCR及Western blotting方法检测livin和caspase 3 表达的改变。结果:livin基因在肺腺癌细胞株SPC A1中持续高表达。经慢病毒介导shRNA使livin基因表达沉默后,镜下可见肺腺癌细胞出现典型凋亡形态特征,流式细胞术检测出现亚二倍体峰,细胞凋亡率较空白对照及阴性病毒对照细胞明显增加(8.21% vs 0.08%,0.13%; P <0.05),RT PCR及Western blottin g 检测结果显示,caspase 3 mRNA表达无改变,但cleaved caspase 3蛋白表达上调。结论:慢病毒载体介导的shRNA能抑制肺腺癌细胞株SPC A1中livin基因的表达,从而促进SPC A1细胞凋亡。

关键词: 肺肿瘤 livin基因 RNA干扰 慢病毒载体 细胞凋亡

Lentivirus mediated shRNA silencing of livin gene promotes apoptosis of SPC-A1 cells
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CHEN Yu sheng LI Hong ru LIN Ming CHEN Gang XIE Bao song XU Neng luan LIN Li fang

Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical College, Fujian Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 350001, Fujian, China; Department of Respiratory Medicine, Fujian Provincial Medical University, Fuzhou 35000

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Abstract:

Objective: To construct a lentiviral livin shRNA vector to silence livin gene expression, and to study its effect on apoptosis of lung carcinoma cells. Methods: Livin expression in human lung adenocarcina SPC A1 cells was silenced by lentiviral livin shRNA infection. The morphology of apoptotic cells was observed by propidine iodide staining and fluoroscope; apoptosis rate and sub lipliod apoptotic peak of SPC A1 cells were assessed by flow cytometry; expression of livin and caspase 3 in SPC A1 cells was examined by real time PCR and Western blotting analysis. Results: Livin was constitutively expressed in SPC A1 cells. After livin expression was silenced by lentiviral livin shRNA infection, SPC A1 cells showed the characteristic morphology of apoptosis under fluoroscope, and the sub lipliod apoptotic peak was identified by flow cytometry. Apoptosis rate in livin shRNA infected SPC A1 cells was significantly higher than that in blank and negative control groups (8.3% vs 0.08% and 0.13%, P <0.05). caspase 3 mRNA expression in SPC A1 cells had no change but the expression of cleaved caspase 3 was greatly upregulated after lentiviral livin shRNA infection as showed by RT PCR and Western blotting analysis. Conclusion: Lentiviral livin shRNA can inhibit livin expression in human lung adenocarcina SPC A1 cells and induce cell apopotosis.

Keywords: <u>lung neoplasms</u> <u>livin gene</u> <u>RNA interference</u> <u>lentiviral vector</u> <u>cell apoptosis</u>

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