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· 基础研究 ·

## 慢病毒介导 shRNA 特异性沉默 livin 基因促进 SPC-A1 细胞凋亡

陈榆生\*, 李鸿茹, 林明, 陈刚, 谢宝松, 许能奎, 林立芳(福建医科大学省立临床医学院呼吸科福州 350001)

[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 blotting 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

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## Lentivirus-mediated shRNA silencing of livin gene promotes apoptosis of SPC-A1 cells

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)

[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 adenocarcinoma SPC-A1 cells was silenced by lentiviral livin shRNA infection. The morphology of apoptotic cells was observed by propidium iodide staining and fluorescence microscope; apoptosis rate and sub-diploid 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 fluorescence microscope, and the sub-diploid 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 adenocarcinoma SPC-A1 cells and induce cell apoptosis.

[Key words] lung neoplasms; livin gene; RNA interference; lentiviral vector; cell apoptosis

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凋亡抑制蛋白家族(inhibitor of apoptosis proteins, IAPs)成员在调节细胞凋亡和增殖中起到至关重要的作用。研究<sup>[1]</sup>表明, IAP 通过有效抑制细胞凋亡导致肿瘤发生, 干扰 IAP 表达可抑制肿瘤增殖, 故 IAP 成员成为肿瘤治疗的热门靶点。Livin 是新发现的 IAP 成员, 实验<sup>[2-4]</sup>表明 livin 在各种肿瘤中高表达, 参与肿瘤发生、发展, 并与某些恶性肿瘤如骨肉瘤、膀胱癌、小儿急性淋巴瘤的预后密切相关;

一些抑制 livin 基因表达以促细胞凋亡的研究<sup>[5-6]</sup>也提示 livin 基因可能是肿瘤治疗新靶点之一。但目

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[作者简介] 陈榆生(1957-), 女, 福建省福州市人, 主任医师, 教授, 主要从事肺癌靶向治疗方面的研究

\* 通信作者( Corresponding author)。E-mail: slyyywb@yahoo.com.cn