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慢病毒介导bcr/abl基因RNAi对白血病K562细胞生物学特性的影响 [点此下载全文](#)

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摘要:

目的: 研究慢病毒介导RNAi致 bcr/abl 基因长期沉默对K562白血病细胞各种生物学特性的影响。方法: 构建含 bcr/abl 重组质粒载体并包装病毒, 感染K562细胞, 挑取稳定转化的克隆(B/A K562)。Real time PCR及Western blotting检测细胞增殖能力变化; 联苯胺染色观察细胞分化; ELISA法检测酪氨酸激酶活性; AO EB染色观察细胞凋亡; 比色法检测Ca²⁺以K562细胞和转染空质粒EGFP K562作对照。结果: 成功构建 bcr/abl 基因RNAi稳定转染的B/A K562细胞, Real time PCR检测bcr/abl mRNA及P210 bcr/abl 蛋白含量明显下调。bcr/abl 基因稳定下调后, K562细胞倍增时间(h)、集落形成能力减弱 (P <0.01), K562细胞向红系分化, 酪氨酸激酶活性下降 (P <0.01), 自发凋亡率显著增加 (P <0.05) 及Caspase 9 (P <0.01) 活性明显提高。结论: 慢病毒介导的RNAi能够实现 bcr/abl 基因长期沉默分化及凋亡。

关键词: [RNA干扰](#) [白血病细胞](#) [bcr/abl基因](#) [慢病毒](#) [稳定转染](#)

Effects of lentivirus mediated long term bcr/abl RNAi on biologic characteristics of human leukemia
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Abstract:

Objective: To study the effects of lentivirus mediated bcr/abl RNAi on the biological characteristics of K562 cells. Methods: Bcr/abl RNAi lentivirus vector pNL B/A EGFP was constructed and was used to transfect K562 cells. RNAi efficiency was assessed by Real time PCR and Western blotting. Cell proliferation was measured by cell counting kit-8 (CCK-8) staining and colony formation assay, cell differentiation was investigated by benzidine staining, PTK activation was observed by AO EB staining, and caspase 3 and caspase 9 activation were measured by fluorescence-activated cell sorting (FACS) analysis. Results: pNL B/A EGFP stably transfected K562 cells (B/A K562) were selected. Real time PCR and Western blotting analysis confirmed that lentivirus mediated bcr/abl RNAi significantly reduced bcr/abl mRNA and P210 bcr/abl protein expression in K562 cells. The doubling time of B/A K562 cells was significantly longer in B/A K562 cells and EGFP K562 cells (37.1 vs 20.4, 23.3 h). Furthermore, B/A K562 cells showed decreased colony formation, decreased differentiation toward erythrocytes, decreased activation of PTK, increased apoptosis and enhanced caspase 3 and caspase 9 activation (P <0.05 or P <0.01). Conclusion: Lentivirus mediated bcr/abl RNAi can result in long time silencing of bcr/abl gene and induce differentiation and apoptosis in K562 cells.

Keywords: [RNA interfere](#) [leukemia](#) [bcr-abl gene](#) [lentivirus](#) [stably transfection](#)

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