

截短型LEF-1 对 HeLa细胞系生物学行为的影响及其相关机制

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Effects and Related Mechanisms of Truncated LEF-1 Isoforms on Biological Behavior of HeLa Cell Lines

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摘要 目的 探讨淋巴细胞增强因子(LEF-1)截短亚型对宫颈癌HeLa细胞迁移的影响及其分子机制。方法 利用PCR方法从人淋巴结cDNA文库中克隆LEF-1截短型的编码基因,将其插入pCDNA3.1/V5-His载体中构建截短型LEF-1的真核表达质粒,脂质体法将重组质粒pcDNA3.1-Δ-LEF-1转染HeLa细胞, G418筛选稳定表达目的基因的细胞株, Western blot鉴定目的基因的表达,流式细胞术分析转染后细胞的增殖,凋亡和胞周期变化,以及CXCR4的表达情况;细胞外基质实验检测细胞的黏附能力;Transwell检测细胞的迁移能力。结果成功构建截短型LEF-1真核表达质粒,获得稳定表达LEF-1截短亚型的HeLa细胞株,目的基因稳定表达,转染后的细胞增殖速度减慢,凋亡增加,细胞阻滞在G0/G1期,迁移能力降低, CXCR4的表达降低。结论LEF-1截短亚型可以抑制HeLa细胞增殖和迁移,而这种调控作用可能和CXCR4的表达有关。

关键词: LEF-1 增殖 凋亡 迁移 宫颈癌 CXCR4

Abstract:

Abstract: Objective To study the effects of truncated LEF-1 isoforms on the biological behavior of HeLa cell lines and the related mechanisms. Methods Truncated LEF-1 gene was obtained by PCR from human lymphoid node cDNA library and inserted into pCDNA3.1/V5 His vector to construct the eukaryotic expression plasmid pcDNA3.1-Δ-LEF-1. Using LipofectamineTM 2000, the plasmid pcDNA3.1-Δ-LEF-1 or empty vector was transfected into Hela cells. Then the stable cell lines which expressed the truncated LEF-1 isoforms were screened by G418 and expression of target protein was identified by Western blot. Subsequently, the proliferation, apoptosis, cell cycle and expression of CXCR4 of transgenic cell lines were analyzed by flow cytometry. In addition, the capability of adhesion and migration of transgenic cell lines were investigated by extracellular matrix adhesion assay and transwell assay respectively. Results The truncated LEF-1 eukaryotic expression plasmid and the stable HeLa cell lines expressing the truncated LEF1 isoforms were constructed successfully. The proliferation of the transgenic cell lines was inhibited, but their apoptosis was increased, meanwhile the cells were blocked at G0/G1 stage. The ability of adhesion and migration of the transgene cell lines were reduced and the expression of CXCR4 was upregulated. Conclusion The truncated LEF-1 isoforms can inhibit the proliferation and migration of HeLa cells, and promote their apoptosis, which maybe depend on the expression CXCR4 partly.

Key words: LEF-1 Proliferation Apoptosis Migration Uterine cervix cancer CXCR4

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