[1]刘洋博,何刚,章波,等.慢病毒介导shRNA沉默HDAC1表达对EC109细胞生长特性的影响[J].第三军医大学学报,2013,35(10):961-964.

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Title: Effects of lentivirus-mediated HDAC1 silencing on cell growth in esophageal cancer cell line EC109

作者: 刘洋博; 何刚; 章波; 庞学利
第三军医大学：西南医院肿瘤科；基础医学部医学遗传学教研室

Author(s): Liu Yangbo; He Gang; Zhang Bo; Pang Xueli
Department of Oncology, Southwest Hospital,Department of Medical Genetics, College of Basic Medical Sciences, Third Military Medical University, Chongqing, 400038, China

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Keywords: HDAC1; lentivirus; esophageal cancer cell; cell growth

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摘要: 目的 应用慢病毒介导的RNA干扰技术，有效沉默食管癌细胞株EC109的HDAC1表达并观察其对细胞生长的影响。 方法 以HDAC1为靶基因，合成寡核苷酸，退火形成双链DNA，与经*Age* Ⅰ和*Eco*R Ⅰ酶切后的pGCSIL-PUR载体连接获得重组慢病毒载体；将其与病毒复制包装质粒共感染293T细胞，收集并浓缩上清液获得重组病毒，测定病毒滴度；病毒颗粒转染EC109细胞，通过荧光显微镜等观察转染效率并经有限稀释法获得单克隆来源的细胞系；定量PCR及Western blot检测HDAC1的表达；并观察EC109细胞的生长特性。 结果 成功构建干扰HDAC1表达的慢病毒载体pGCSIL-SiHDAC1，并在293T细胞中包装获得病毒。重组病毒感染EC109细胞后，HDAC1mRNA和蛋白水平检测显示干扰组细胞的HDAC1的表达水平较对照组显著降低（$P<0.05$）。HDAC1下调的EC109细胞的增殖能力明显下降（$P<0.05$）。 结论 慢病毒介导的shRNA能有效的沉默食管癌细胞EC109中的HDAC1的表达，并且能抑制其细胞的增殖。

Abstract: Objective To suppress the gene expression of HDAC1 in human esophageal cancer cell line EC109 and to observe its impact on cell growth. Methods Synthesized oligonucleotide was cloned into a lentiviral vector pGCSIL-PUR digested with *Age* Ⅰ and *Eco*R Ⅰ. The recombinant vector was further screened by PCR and verified by sequencing, and was transfected into package cells 293T with helper vectors. Recombinant lentivirus and control were extracted from cell culture and were used to infect EC109 cells. Individual cell clone was picked up and gene expression of HDAC1 was determined by real-time PCR and Western blotting. Cell growth and cell cycle distribution were detected by CCK-8 and FACS, respectively. Results Recombinant vector was successfully constructed, and sufficient lentivirus was obtained from package cells. The recombinant lentivirus can efficiently infect EC109 cells, and two clones were picked up from the infected EC109 cells. The results of real-time PCR and Western blotting showed HDAC1 gene expression was efficiently suppressed in EC109 cells, of which cell growth and cell cycle distribution were significantly altered. Conclusion The RNAi technology based on lentivirus can efficiently suppress the gene expression of HDAC1 in EC109 cells, and lead to cell growth inhibition.

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