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基础研究

WTX 慢病毒载体及其稳转结肠癌细胞株Lovo/WTX-EGFP 的建立

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摘要：

目的：构建抑癌基因WTX慢病毒载体，建立稳定转导WTX的结肠癌Lovo/WTX-EGFP细胞株，为研究WTX在结肠癌中的作用机制提供有效工具。方法：通过Gateway技术构建WTX慢病毒载体pLV.Ex3d.null-EF1A>WTX>IRES/EGFP并通过菌落PCR筛选鉴定，将其与辅助质粒pLV/helper-SL3，PLV/helper-SL4，PLV/helper-SL5共转染293FT细胞包装慢病毒并在荧光显微镜下行滴度值测定。用WTX慢病毒载体转导结肠癌Lovo细胞株，并通过多次挑克隆培养建立稳定表达WTX的Lovo/WTX-EGFP细胞株。结果：Gateway技术构建的慢病毒载体pLV.Ex3d.null-EF1A>WTX>IRES/EGFP经鉴定完全正确；慢病毒包装48 h后视野下可见清晰绿色荧光表达，病毒滴度为5×107 TU/mL。慢病毒载体成功转导Lovo细胞，经qPCR及Western blot检测WTX表达水平明显升高；通过多次挑克隆培养成功建立了稳定转导WTX的Lovo/WTX-EGFP细胞株。结论：通过Gateway技术可成功构建WTX慢病毒载体并获稳定转导WTX的Lovo/WTX-EGFP细胞株，为研究WTX在结肠癌中的作用机制提供了实验基础。

关键词： 结肠肿瘤；基因 肿瘤抑制；WTX；慢病毒载体

Establishment of WTX-containing lentiviral vector and its stably transduced colon cancer cell line Lovo/WTX-EGFP

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Abstract:

Objective: To construct the lentiviral vector containing tumor suppressor gene WTX (Wilms tumor gene on the X chromosome) and the colon cancer cell line stably transduced with this WTX-containing vector (Lovo/WTX-EGFP), so as to provide a useful tool for studying the role of WTX in colon cancer. Methods: The lentiviral vector pLV.Ex3d.null-EF1A>WTX>IRES/EGFP was constructed by Gateway technology, which was screened and identified by colony PCR. After that, it was co-transfected into the 293FT cells with three helper plasmids that were PLV/helper-SL3, PLV/helper-SL4 and PLV/helper-SL5 to package lentivirus and then the viral titer was determined under fluorescence microscope. Finally, the human colon cancer Lovo cells were transduced with the WTX-containing lentiviral vector to obtain the Lovo/WTX-EGFP cell line with stable expression of WTX gene through several subcultures by repeated colony picking. Results: The lentiviral vector pLV.Ex3d.null-EF1A>WTX>IRES/EGFP constructed by Gateway technology was completely and correctly identified. The distinct green fluorescence was seen under fluorescence microscope 48 h after virus packaging and the virus titer was 5×107 TU/mL. The vector was successfully transduced into Lovo cells as evidenced by the significantly increased WTX expression level determined by both qPCR and Western blot was obviously higher than cells without transducting. The Lovo/WTX-EGFP colon cancer cell line with stable transduction of WTX containing vector was established by repeated colony picking and subcultures. Conclusion: Through Gateway technology, the WTX-containing lentiviral vector can be successfully constructed and colon cancer Lovo/WTX-EGFP cell line can be stably transduced by this WTX-containing lentiviral vector. It may provide an experimental basis for studying the role of WTX in colon cancer.

Keywords: Colonic Neoplasms Genes, Tumor Suppressor WTX Lentiviral Vector

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