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人BNIP3基因真核表达载体的构建及其对HT-29细胞化疗敏感性的影响(PDF)

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Title: Construction of human BNIP3 eukaryotic expression vector and its effect on chemosensitivity of HT-29 cells

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关键词: 结肠肿瘤; HT-29细胞; BNIP3基因; 化疗; 凋亡; 表达载体

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摘要: 目的 构建人BNIP3真核表达载体，观察BNIP3高表达对人结肠癌细胞HT-29化疗敏感性的影响。 方法 PCR法扩增BNIP3基因，酶切后插入质粒pEGFP-C3，构建重组真核表达载体pEGFP-C3/BNIP3。脂质体转染人结肠癌细胞HT-29，Western blot检测BNIP3蛋白表达。MTT法检测5-氟尿嘧啶（5-Fu）的化疗敏感性和细胞增殖，Annexin V-APC/PI 双染流式细胞术检测细胞凋亡。 结果 酶切电泳分析和DNA序列测定证实，重组质粒pEGFP-C3/BNIP3构建成功；转染重组质粒的HT-29细胞BNIP3蛋白明显高表达。与未转染组和转染空质粒pEGFP-C3组比较，转染pEGFP-C3/BNIP3组5-Fu的IC_{50}值显著降低[(120.11±5.45)、(113.40±4.72) μg/mL *vs* (19.08±2.62) μg/mL，*P*<0.05]，细胞凋亡率显著增加[(5.51±0.32)%、(7.19±0.47)% *vs* (41.72±1.48)%，*P*<0.05]，细胞克隆形成显著减少[(52±6)、(49±5) *vs* (11±3)，*P*<0.05]，细胞增殖速度减慢。 结论 成功构建了人BNIP3真核表达载体，BNIP3高表达可增加HT-29细胞对5-Fu的化疗敏感性。

Abstract: Objective To construct an eukaryotic expression vector encoding human Bcl-2 and adenovirus E1B 19 kDa interacting protein (BNIP3) gene, and to investigate the effect of BNIP3 over-expression on the chemosensitivity of human colon cancer cell line HT-29. Methods The full-length cDNA of BNIP3 was

amplified by PCR and cloned into pEGFP-C3 vector using genetic engineering technology. The recombinant expression vector pEGFP-C3/BNIP3 was confirmed by enzyme digestion and sequencing, and then was transferred into HT-29 cells by liposome. The expression of BNIP3 was detected by Western blotting. The chemosensitivity of transfected HT-29 cells to 5-Fu and cell proliferation were evaluated by MTT assay. Cell apoptosis was measured by flow cytometry. Results An eukaryotic expression vector of BNIP3 was constructed successfully, and the BNIP3 protein was highly expressed in HT-29 cells transfected with pEGFP-C3/BNIP3. The IC_{50} of HT-29 cells transfected with pEGFP-C3/BNIP3 incubated with 5-Fu was significantly lower than those of HT-29 cells untransfected or transfected with pEGFP-C3 (120.11±5.45, 113.40±4.72 *vs* 19.08 ±2.62 μg/mL, *P*<0.05). The apoptotic rate of HT-29 cells transfected with pEGFP-C3/BNIP3 was significantly decreased compared with HT-29 cells untransfected or transfected with pEGFP-C3 [(5.51±0.32)%, (7.19±0.47)% *vs* (41.72±1.48)% , *P*<0.05]. Compared with the control cells, HT-29 cells transfected with pEGFP-C3/BNIP3 had significantly reduced cell colony formation (52±6, 49±5 *vs* 11±3, *P*<0.05) and decreased cell proliferation rate. Conclusion The recombinant expression vector pEGFP-C3/BNIP3 is successfully constructed. Over-expression of BNIP3 can enhance the chemosensitivity of HT-29 cells to 5-Fu.

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