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MDR 逆转因子筛选技术P-gp 细胞系的建立及其生物学特性评价

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The Establishment of P-gp Expression on Cell Line and its Biological Characteristics

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摘要

目的 通过基因转导和药物诱导，建立稳定表达P—gp的细胞系，用于筛选特异性逆转P-gp的药物。方法 经RT-PCR反应得到小鼠肿瘤细胞P-gp DNA，转化大肠杆茵，构建并将质粒DNA转导逆转录病毒载体中，进而感染B-MD-C1细胞。采用Northern印迹法和流式细胞技术检测细胞P-gp mRNA和P-gp分子的表达；以MTT法分析细胞的增殖活性；应用药物聚集和排放实验检测其生物学活性。结果 成功构建了稳定表达特异性P-gp基因和P-gP分子的B-MD-C1(ADR+ / +)细胞系。经阿霉素诱导，B-MD-C1(ADR+ / +)细胞P-gp的表达明显增高，在阿霉素增加至12000ng / mL时，仍有80%的细胞保持良好增殖状态，而B-MD-C1(wt)细胞在1000ng / mL时100%细胞死亡。B-MD-C1(ADR+ / +)较(wt)细胞对MI>123有较低的聚集量和较高的排放量，加入P—gP逆转剂Verapamil后，B-MD-C1(ADR+ / +)细胞MD-123聚集量增加，药物外排作用消失。结论 B-MD-C1(ADR+ / +)细胞系具有较强的药物耐受性，可用于筛选特异性逆转P-gp的药物，Verapamil可逆转P—gP耐药性，具有Verapamil特性者可视为具有逆转P—gp耐药性。

关键词： 多药耐药 P-糖蛋白 基因转导 P-gp 细胞系

Abstract: Objective To establish a cell line expressing P-glycoprotein (P-gp) stably by gene transduction and drug inducement , to screen drugs used to specifically reverse P-gp. Methods P-gp DNA of tumor cells in mice that was obtained by RT-PCR was transfered into colibacillus , constructed plasmids DNA and transduced it into ret roviral vector , then to t ransfect B-MD-C1 cell. Northern blot analysis was used to investigate P-gp mRNA and flow cytometry was performed to assess the expression of P-gp. Proliferation assay was measured by MTT method. The biological characteristics of P-gp positive cell line were evaluated by drug accumulation assays and drug efflux assays. Results B-MD-C1 (ADR + / +) cell expressing P-gp stably was established. Significant overexpression of P-gp on B-MD-C1 (ADR + / +) cell was induced by Adriamycin , the cell survior of B-MD-C1 (ADR + / +) was 80 % when the concentration of Adriamycin was 12 000ng/ mL , while the cells of B-MD-C1 (wt) were all died when the concent ration of Adriamycin was 1 000ng/ mL. The accumulation of MD-123 in B-MD-C1 (ADR + / +) was lower than in B-MD-C1 (wt) , the efflux of MD-123 in B-MD-C1 (ADR + / +) was higher. But the accumulation of MD-123 was significantly increased and the cells efflux was vanished in B-MD-C1 (ADR + / +) with Verapami. Conclusion B-MD-C1 (ADR + / +) is multidrug resistant , Verapami can reverse its resistance of P-gp. B-MD-C1 (ADR + / +) cell line can be used to screen drugs , those having the same character with Verapami may specifically reverse P-gp resistance.

Key words: Multidrug resistance P-glycoprotein Gene transduction P-gp expression cell line

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