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论著

CYP3A4高表达肝细胞株建立及其对三氯乙烯毒性的影响

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摘要 目的 建立CYP3A4高表达肝细胞株并观察其对三氯乙烯毒性的影响。方法 PCR扩增CYP3A4基因并将其克隆到慢病毒高表达载体中,将已经构建的慢病毒载体进行转染后,收集病毒上清,感染正常L02肝细胞。用嘌罗霉素进行筛选得到CYP3A4高表达肝细胞株,通过荧光定量PCR和Western蛋白质印迹法鉴定细胞株。用三氯乙烯0, 0.25, 0.5, 1.0, 2.0和4.0 mmol·L^{-1}对正常肝肝细胞和CYP3A4高表达肝细胞进行染毒12 h,实时定量PCR检测凋亡基因*bcl*-2, 胱天蛋白酶3, 胱天蛋白酶8和胱天蛋白酶9以及癌基因*c-fos, c-myc, K-ras*和*p*53的表达。结果 测序证明重组慢病毒高表达载体CYP3A4基因序列正确,荧光定量PCR检测CYP3A4高表达肝细胞株比正常肝细胞CYP3A4基因表达提高94倍。Western蛋白质印迹结果显示,CYP3A4高表达肝细胞株CYP3A4蛋白表达水平是正常肝细胞的2.36倍。CYP3A4高表达肝细胞bcl-2 mRNA表达水平随三氯乙烯浓度增加呈下降趋势,与正常细胞相比,三氯乙烯0.25和0.5 mmol·L^{-1}使CYP3A4高表达肝细胞中的bcl-2 mRNA明显升高(P<0.01);三氯乙烯0.5,1.0,2.0和4.0 mmol·L^{-1}处理使CYP3A4高表达肝细胞组的凋亡基因胱天蛋白酶3、胱天蛋白酶8和胱天蛋白酶9的mRNA表达水平明显升高(P<0.05);三氯乙烯0.5,1.0,2.0和4.0 mmol·L^{-1}处理的CYP3A4高表达肝细胞*c-fos*、*c-myc*和*k-ras*基因的表达显著升高(P<0.01),*p*53表达水平明显下降(P<0.01)。结论 三氯乙烯对CYP3A4高表达肝细胞株中的凋亡基因和癌基因表达具有明显促进作用,说明CYP3A4是三氯乙烯在体内代谢的重要因素。

关键词 肝细胞 细胞色素P-450 CYP3A4 慢病毒感染 三氯乙烯

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Construction of CYP3A4 gene overexpressed hepatocytes and their effect on trichloroethylene toxicity

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Abstract

OBJECTIVE To construct hepatocytes L02 cells with cytochrome P-450(CYP)3A4 overexpression and to observe their effects on toxicity of trichloroethylene. METHODS CYP3A4 gene was amplified using PCR and ligated into the lentiviral vector pLVX-acGFP-C1. 293FT cells were transfected with the recombinant vector, viral supernatant was collected, and than L02 cells were transducted. After puromycin screening, CYP3A4-overexpressed hepatocytes strain was constructed, which was identified using quantitative PCR and Western blotting. Then, the constructed hepatocytes with CYP3A4 overexpression and normal L02 cells were treated with TCE 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mmol·L^{-1} for 12 h. The expression of apoptosis genes *bcl*-2, *caspase*3, *caspase*8, *caspase*9 and oncogenes *c-fos, c-myc, K-ras* and *p*53 was determined by real-time quantitative PCR. RESULTS Sequencing results showed that the recombinant vector contained the same sequence as CYP3A4 gene from GenBank. Quantitative PCR indicated that CYP3A4 gene expression level in CYP3A4 overexpressed hepatocytes was 94 times higher than in normal L02 cells. The Western blotting result revealed that CYP3A4 protein in CYP3A4-overexpressed hepatocytes was 2.36 times the level of normal L02 cells. After TCE 0.5, 1.0, 2.0 and 4.0 mmol·L^{-1} treatment,

compared with normal L02 cells, *p*53 expression levels in CYP3A4 overexpressed hepatocytes were significantly derceased($P<0.05$), while expression levels of other apoptosis genes caspase3, caspase8 and caspase9 expression levels and oncogenes (*c-fos*, *c-myc* and *k-ras*) significantly increased($P<0.05$). CONCLUSION Trichloroethylene significantly promotes the expression of apoptosis genes and oncogenes in CYP3A4 overexpressed hepatocytes, suggesting that CYP3A4 may play an important role in trichlorethylene metabolism *in vivo*.

Key words hepatocytes cytochrome P-450 CYP3A4 lentivirus infections trichloroethylene

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