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论著

pcDNA3.1-*UDPGT1A9*表达质粒的构建及其转基因细胞系的建立

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摘要 目的 为建立能稳定表达人葡糖醛酸转移酶UDPGT1A9蛋白的CHL-*UDPGT1A9*转基因细胞系，并鉴定其对药物的葡糖醛酸缀合活性。方法 利用基因亚克隆技术，将*UDPGT1A9* cDNA从pREP9-*UDPGT1A9*构建到哺乳动物表达载体pcDNA3.1中，形成真核细胞表达重组子pcDNA3.1-*UDPGT1A9*，再转染于中国仓鼠肺细胞（CHL细胞）中，通过G418筛选阳性克隆，建立稳定表达UDPGT1A9的CHL-*UDPGT1A9*转基因细胞系，并以山萘酚为底物，采用HPLC方法对其代谢活性进行鉴定。结果 建立了CHL-*UDPGT1A9*转基因细胞系，通过HPLC分析其对山萘酚代谢活性为（1.02±0.11）μmol•min^{-1}•g^{-1}蛋白，而对照CHL细胞对底物无明显代谢。结论 构建完成的转基因细胞系能稳定表达人体葡萄糖醛酸转移酶UDPGT1A9，并具有对山萘酚的代谢活性。

关键词 葡糖醛酸基转移酶 转染, 细胞系 色谱法, 高压液相 山萘酚

分类号 R966

Construction of pcDNA3.1 *UDPGT1A9* expression plasmid and establishment of its transgenic cell line

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Abstract

AIM To establish a cell line CHL-UDP-glucuronosyltransferase gene (*UDPGT1A9*) which will stably express human UDPGT1A9 protein and determine the activity of expressed UDPGT1A9 in drug glucuronidation. **METHODS** Complimentary DNA (cDNA) of human *UDPGT1A9* was subcloned from pREP9-*UDPGT1A9* into the mammalian expression vector pcDNA3.1 with DNA subclone techniques. Recombinant expression plasmid pcDNA3.1 *UDPGT1A9* was constructed and transfected into Chinese hamster lung (CHL) cells. G418 was used to select transfected clones. The activity and kinetic parameters of expressed UDPGT1A9 were measured using kaempferol as substrate by HPLC. **RESULTS** The CHL-*UDPGT1A9* transgenic cell line was established. With kaempferol as the substrate, the enzyme activity of UDPGT1A9 expressed in CHL-*UDPGT1A9* transgenic cell line was（1.02±0.11）μmol•min^{-1}•g^{-1} protein, whereas the native UDPGT1A9 activity was not detectable in control CHL cells. **CONCLUSION** The CHL-*UDPGT1A9* transgenic cell line established in this study expressed human UDPGT1A9 protein and had metabolic activity to kaempferol.

Key words glucuronosyltransferase transgenic cell line chromatography high pressure liquid kaempferol

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