

论著

## 人细胞色素P450 3A4突变体CYP3A4.3, CYP3A4.4, CYP3A4.5和CYP3A4.18重组酶的异源表达与活性

王晓雯, 曾 苏

(浙江大学药学院药物分析与药物代谢实验室, 浙江 杭州 310058)

收稿日期 2009-1-16 修回日期 网络版发布日期 2009-11-24 接受日期 2009-9-24

**摘要** 目的 重组表达人细胞色素P450(CYP)3A4突变体CYP3A4.3, CYP3A4.4, CYP3A4.5和CYP3A4.18蛋白, 为CYP3A4代谢活性的体外研究提供单一酶源。方法 应用杆状病毒表达系统构建含有各CYP3A4突变体基因序列的重组病毒, 将其连同含人源还原型烟酰胺腺嘌呤二核苷酸-P450氧化还原酶(POR)和细胞色素b5基因的重组病毒共同感染昆虫草地夜蛾细胞Sf9得到CYP3A4突变体与POR和细胞色素b5共表达的重组蛋白, 分别以高效液相色谱法和荧光分析法测定各重组酶对睾酮和7-甲氧基-4-三氟甲基香豆素的代谢活性。结果 在mRNA分子水平上验证了CYP3A4突变体CYP3A4\*3, CYP3A4\*4, CYP3A4\*5和CYP3A4\*18基因在Sf9细胞中的转录。感染了各重组病毒的Sf9细胞裂解液对睾酮和7-甲氧基-4-三氟甲基香豆素有明显代谢。结论 应用杆状病毒-昆虫细胞表达系统在体外成功表达了具有催化活性的人CYP3A4突变体CYP3A4.3, CYP3A4.4, CYP3A4.5和CYP3A4.18蛋白, 其中CYP3A4.5活性显著低于野生型蛋白, CYP3A4.18活性显著高于野生型蛋白, 而CYP3A4.3和CYP3A4.4与野生型蛋白活性近似。

**关键词** [细胞色素P450 CYP3A4](#) [突变](#) [杆状病毒科](#) [睾酮](#)

分类号 [R965.1](#)

## Heterogenous expression and activity analysis of human cytochrome P450 3A4 mutants CYP3A4.3, CYP3A4.4, CYP3A4.5 and CYP3A4.18

WANG Xiao-Wen, ZENG Su

(Department of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China)

### Abstract

**AIM** To express recombinant human cytochrome P450 3A4 mutants CYP3A4.3, CYP3A4.4, CYP3A4.5 and CYP3A4.18, and to employ them for *in vitro* metabolism studies of CYP3A4. **METHODS** Use Bac-to-Bac baculovirus expression system to recombinant baculovirus carrying cDNA of CYP3A4 mutants CYP3A4.3, CYP3A4.4, CYP3A4.5 and CYP3A4.18. *Spodoptera frugiperda* 9 (Sf9), cells were co-infected by recombinant viruses of CYP3A4 mutants, human NADPH-P450 oxidoreductase and cytochrome b5 to obtain recombinant proteins CYP3A4.3, CYP3A4.4, CYP3A4.5 and CYP3A4.18 with metabolic activity. **RESULTS** The mRNA transcription of CYP3A4 mutants in Sf9 cells were validated by RT-PCR. Testosterone and 7-benzyloxy-4-(trifluoromethyl) coumarin were metabolized by the lysates of Sf9 cells infected by the recombinant viruses. **CONCLUSION** CYP3A4 mutants CYP3A4.3, CYP3A4.4, CYP3A4.5 and CYP3A4.18 with metabolic activity were successfully expressed by baculovirus-insect cell expression system. The results indicated that recombinant CYP3A4.5 showed lower activity comparing to the wild type protein towards testosterone, while CYP3A4.18 with higher activity, and for CYP3A4.3 and CYP3A4.4 showing similar activity to the wild type protein.

**Key words** [cytochrome P-450 CYP3A4](#) [mutation](#) [Baculoviridae](#) [testosterone](#)

DOI: 10.3867/j.issn.1000-3002.2009.06.007

通讯作者 曾 苏 [zengsu@zju.edu.cn](mailto:zengsu@zju.edu.cn)

扩展功能

本文信息

▶ [Supporting info](#)

▶ [PDF\(1927KB\)](#)

▶ [\[HTML全文\]\(0KB\)](#)

▶ [参考文献](#)

服务与反馈

▶ [把本文推荐给朋友](#)

▶ [加入我的书架](#)

▶ [加入引用管理器](#)

▶ [复制索引](#)

▶ [Email Alert](#)

▶ [文章反馈](#)

▶ [浏览反馈信息](#)

相关信息

▶ [本刊中 包含“细胞色素P450 CYP3A4”的 相关文章](#)

▶ [本文作者相关文章](#)

· [王晓雯](#)

· [曾 苏](#)