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Development and Application of a UPLC–MS/MS Method for P-glycoprotein Quantification in Human Tumor Cells

Zhaohui Qiu[1], Jie Peng[1], Lingli Mou[2], Xiao Li[3], Fanqi Meng [1], Peng Yu [1] *

1.School of Xiangya Pharmaceutical Sciences, Central South University, 172 Tong Zi Po Road, Changsha,410013, China

2.Medical College, Hunan Normal University, 371 Tong Zi Po Road, Changsha,410006, China

3.Changsha Duxact Biotech Co.,Ltd. C10 Building, Lugu S&T Park, 28 Lutian Rd, Changsha,411000, China

Abstract: Multidrug resistance (MDR) of tumors occurs when tumor cells exhibit reduced sensitivity to a large number of unrelated drugs. The molecular mechanism of MDR commonly involves overexpression of the plasma membrane drug efflux pump P-glycoprotein (P-gp). Overexpression of P-gp may be induced by the selection and/or adaptation of cells during exposure to chemotherapeutic drugs, referred to as acquired P-gp-mediated MDR. This study aimed to establish a P-gp quantification method by Ultra Performance Liquid Chromatography and Tandem Mass Spectrometry (UPLC-MS/MS) to better understand the regulation of P-gp expression and its relationship with the level of drug resistance. Absolute P-gp expression was determined in the human tumor cells MCF-7, HepG-2, and SMMC-7721 and their corresponding drug-resistant subclones MCF-7/ADMs, MCF-7/MXs, MCF-7/MTXs, HepG-2/ADMs, HepG-2/MXs, HepG-2/MTXs, SMMC-7721/ADMs, SMMC-7721/MXs and SMMC-7721/MTXs. A unique 10-mer tryptic peptide (IATEAIENFR) of P-gp was synthesized for developing the quantitative UPLC-MS/MS method with the stable isotope labeled signature peptide IATEAI ($^{13}C_{6,}$ $^{15}N_1$) ENFR as the internal standard (IS). The detection signal was linear in the range of 0.1-100 ng mL^{-1}. Quality control (QC) data showed that the within-run and between-run precision (%RSD) and accuracy (%RE) conformed to acceptable criteria of ±15% for the calibration standards and QCs (±20% at the LLOQ). The UPLC-MS/MS method was first applied to quantify P-gp in HepG-2 and SMMC-7721 cells and their drug-resistant subclones. The results confirmed that P-gp expression in most drug-resistant subclones increase significantly compared to parental tumor cells but varied among different types of drugs or tumor cells. This outcome was then compared with published reports and discrepancy was observed in HepG2 cell lines mainly due to different sample types and samples sources. Additionally, P-gp mRNA results ascertained that overexpression of P-gp in subclones was not only regulated by MDR1. The linear correlation between RI and logarithm-transformed P-gp expression was moderate or high and statistically significantly different in subclones, except for SMMC-7721/ADMs. The present study is the first to demonstrate the quantitative relationship between RI and P-gp expression by linear regression modeling and expanded the number of efflux transporters related to MDR quantifiable by LC-MS/MS to better understand the biological significance of effluent transporter expression.[1]

[1] * Corresponding author at: School of Xiangya Pharmaceutical Sciences, Central South University, 172 Tong Zi Po Road, Changsha 410013, China. Tel.:+86 13348716587;Fox:+86 89786965.Emial addresses: peng.yu@csu.edu.cn.

Abbreviations:

multidrug resistance (MDR), multiple reaction monitor(MRM), reverse-transcription-polymerase chain reaction(RT-PCR), relative standard deviation(RSD), relative error(RE), dithiothreitol (DTT), iodoacetamide(IAA), stable isotope-labeled internal standard (SIL-IS), trifluoroacetic acid(TFA), RI(resistance index)

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