Progress of drug transport study based on absolute quantitative method for membrane transporter proteins

○Junichi Kamiie, Yuki Katsukura, Sumio Ohtsuki, Tetsuya Terasaki
(Graduate School of Pharmaceutical Sciences, Tohoku University, SORST・JST)

Knowing the amount of membrane transporter expression in human tissue is one of key issues for the rational and reliable prediction of pharmacokinetic profile in human. Recently, we have developed a simultaneous and highly sensitive method for the absolute quantification of multiple membrane transporter proteins in the mammalian tissues. To develop the quantitative analysis for high molecular membrane protein, we have solved problems by proteomics technology as follows; 1) The target proteins are detected via tryptic peptides which can be dissolved and analyzed by LC-MS/MS, while membrane protein is hard to dissolve. 2) LC-MS/MS in Multiple Reaction Monitoring (MRM) mode produce the highly sensitive and selective response for the transporter protein of low expression by separation from high abundance molecules. 3) Analyte specificity for each peptide was demonstrated in amino acid sequence by multiple MRM detection. Selection of peptide probe was very important for highly sensitive analysis with LC-MS/MS. We set criteria for peptide selection with informatics approach. The peptides without unstable residue, double basic residues and integral membrane domain were selected as useful probe.
We clarified the amount of 10 ABC transporters (Mdr1a, Bcrp, Mrp1-7 and 9) in mouse tissues using developed method. In the brain capillaries, Mdr1a, Bcrp and Mrp4 were determined significantly to be 15 fmol/μg protein, 4.6 fmol/μg protein and 2.1 fmol/μg protein, respectively, while Mrp3, Mrp5 and Mrp6 were detected at lower than 1.0fmol/μg protein. These results demonstrate that presently developed method has enough sensitivity to determine the protein amount of functionary important drug transporters. Numerous membrane transporter proteins could be measured by the established quantitative method. The developed method will provide an inclusive assay platform for all transporter proteins expressed both in animal/human tissues and would contribute progress of drug discovery and development.