GS02-3 **Application of next generation comprehensive quantitative proteomics to kinetic analysis of endogenous protein transport: Discovery of blood-brain barrier permeable liver soluble proteins**

○Kazuki SATO[1], Masanori TACHIKAWA[1], Michitoshi WATANABE[1], Yasuo UCHIDA[1], Tetsuya TERASAKI[1]
[1]Grad Sch of Pharmaceutical Sci, Tohoku

The blood-brain barrier (BBB) possesses the blood-to-brain transport systems for the specific proteins of insulin and transferrin via receptor-mediated transcytosis. The BBB-permeable proteins are considered to act as the transmitters of the central nervous system and peripheral interaction since some of the peripheral tissues-derived proteins play physiological functions in brain. However, only a limited number of proteins have been identified to date. The purpose of this study was to identify the potential candidates of BBB-permeable proteins by using comprehensive quantitative proteomics. Cytosolic proteins isolated from the mouse cerebrum and liver were labeled with biotin and intravenously injected in mice. The penetrated biotin-labeled proteins into the cerebrum were purified and analyzed by SWATH-based comprehensive proteomics. Four and nine proteins were identified from the cerebrum of mice injected with biotin-labeled cerebrum and liver cytosol proteins, respectively. Creatine kinase (CK) was commonly detected in both. The unique peptide of rabbit muscle CK (RCKM) was detected by quantitative targeted proteomics in the cerebrum cytosolic fraction of the mouse intravenously injected with RCKM and the brain penetration was validated. Moreover, RCKM was transported from apical to basolateral side across the monolayer of human brain endothelial cell line (hCMEC/D3) on the transwell and the internalized amount into the cells was decreased to less than 1.8% at 4°C. These results suggest that the transport system for CK is present at the BBB. In conclusion, we newly found the multiple candidates of BBB-permeable proteins.