

## DOCTORAL THESIS

### Mass spectrometry-based metabolomics to unravel alterations in hepatic cell lines and transgenic mouse model of Alzheimer's disease

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## ABSTRACT

This thesis research concentrates on the development and applications of mass spectrometry-based metabolomics to elucidate biochemical alterations involved in basic research models for two common human diseases: mammalian cell culture model of hepatocellular carcinoma (HCC) and transgenic mouse model of Alzheimer's disease (AD). Two major approaches were developed: (1) targeted quantitative metabolomics for elucidation of altered cancer metabolism in human liver cell lines caused by the overexpression of the oncogene eukaryotic translation initiation factor 5A2 (*EIF5A2*) and O-Linked  $\beta$ -*N*-acetylglucosamine (O-GlcNAc) modification; (2) non-targeted metabolite profiling for early discovery of potential non-invasive urinary metabolite markers in the transgenic mouse model TgCRND8 of AD.

For cancer metabolism research, much effort has been focused on development of ultrahigh performance liquid chromatography triple quadrupole mass spectrometry (UPLC-MS/MS)-based targeted metabolomics method and its emerging applications in exploiting oncogene-induced metabolic alterations. To achieve our goal, more than one hundred intermediate and/or metabolite were selected and broadly categorized into cationic species and anionic species. Tandem mass spectrometric conditions were extensively optimized for each analyte by using energy-resolved collision-induced dissociation. Two crucial operating parameters of tandem mass spectrometry, namely, cone voltage and collision energy were finely tuned to get the highest signal response of the

parent ion and fragment ions. Multiple reaction monitoring (MRM) transitions were created for each targeted compound, providing foundation for MRM-based assays. Meanwhile, to enhance the retention and separation of the water-soluble metabolites on reversed-phase C18 column, hydrophobic ion-pairing interactions separation (HIPS) strategies were proposed and established via complementary use of two ion-pairing reagents, heptafluorobutyric acid and tributylamine, for the cationic species and anionic species, respectively. The ‘HIPS’ strategies led to efficient retention and resolution of polar intermediates/metabolites, covering the majority of components involved in central carbon metabolism and amino acid metabolism. Even isomeric pairs, like citrate-isocitrate and leucine-isoleucine, were almost baseline resolved. The performance evaluation of the developed UPLC MRM-based assays showed that nanomolar levels of limit of quantification were achieved. The developed methods enabled quantitative analysis of central carbon metabolism in mammalian cells. The altered metabolism induced by the overexpression of the oncogene *EIF5A2* in human normal liver cell line LO2 was studied. We found that the altered aerobic glycolysis and pentose phosphate pathway dysregulated the tricarboxylic acid (TCA) cycle and amino acid imbalances presented as distinct metabolic features in *EIF5A2* overexpressed LO2 cells.

In chapter 3, we performed quantitative analysis of central carbon metabolism and amino acid metabolism via the established UPLC-MRM-based metabolomics, which was combined with pharmacological inhibition of the

catalytic enzymes, O-linked N-acetylglucosamine transferase (O-GlcNAc transferase, OGT) and  $\beta$ -N-acetylglucosaminidase (O-GlcNAcase, OGA) in order to uncover the contribution of protein (including the glycolytic enzymes) O-GlcNAc modification to metabolic alterations in cancer cells. We found that OGA inhibition led to decreased levels of intermediates in both glycolysis and TCA cycle, but increased level of pentose phosphate pathway. Interestingly, the opposite phenotypes were obtained in OGT inhibition, i.e., the increased levels of glycolysis and TCA cycle were observed. Our data suggested that O-GlcNAc modification could direct switches of glucose metabolism through coordinated glycolysis and TCA cycle pathways in HCC cell line.

In chapter 4, an improved UPLC-MS/MS method for accurate and rapid assessment of the content and redox state of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) and the crucial component of electron transport chain (ETC) was described. Non-aqueous reversed phase liquid chromatography on a C18 column was hyphenated with tandem mass spectrometry working in the electrospray ionization positive MRM mode, with methanol serving dual roles as sample preparation solvent and mobile phase. This rapid extractive and analytical method could avoid artificial auto-oxidation of reduced form of CoQ<sub>10</sub>, enabling the native redox state assessment. To demonstrate the utility of the developed method, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposed mice liver tissue were analyzed, revealing the down-regulated mitochondrial ETC in TCDD exposed mice group.

Chapter 5 reported the study to assess whether the urinary metabolic alterations linked to early pathophysiological changes in the TgCRND8 mouse model of AD. An unbiased metabolomics approach using high resolution Orbitrap mass spectrometry coupled with hydrophilic interaction liquid chromatography was conducted to uncover the metabolic alterations as a relevant readout of biochemical activity that implicated in the pathogenesis and progression of AD in the TgCRND8 mice. A total of 73 differential metabolites of urine sample sets was identified in 12-week and 18-week transgenic mice compared to wild-type littermates, covering perturbations of aromatic amino acids metabolism, TCA cycle and one-carbon metabolism. Of particular interest, divergent tryptophan metabolism, such as up-regulation of serotonin pathway while down-regulation of kynurenine pathway, was observed. The accumulation of both *N*-acetylvani­lalanine and 3-methoxytyrosine indicated the aromatic *L*-amino acid decarboxylase deficiency. The microbial metabolites derived from tryptophan metabolism and drug-like phase II metabolic response via the glycine conjugation reactions were also highlighted, indicating that genetic modification in mouse brain not only alters genotype but also disturbs gut microbiome. Together, our study demonstrated that the integrative approach employing mass spectrometry-based metabolomics and a transgenic mouse model for AD might provide new insights into the metabolic phenotypes of AD with a noninvasive approach.

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