Metabonomics in translational research – Current status

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The move towards precision medicine requires an in-depth understanding of how both internal (e.g. genetics) and external (e.g. environment) factors impact the risk of disease development and treatment outcomes. Genetic information can provide rich information on disease classification and susceptibility, while environmental factors, such as drug exposure, lifestyle and gut microbiota can contribute to disease development and diverse responses to drug treatments. Disease phenotypes resulting from the interplay between genetic and environmental factors manifest through different perturbations of metabolic compositions that can be measured by various metabolic profiling techniques. In this editorial, advances in metabolic profiling techniques and their applications in translational medicines are summarised.

Advances in translational research in the field of precision medicine are expected to be facilitated by the development of new metabolic profiling platforms. Quantitatively detecting as many metabolites as possible with the highest sensitivity and throughput is the ultimate goal of metabolic profiling techniques. Currently, metabolite detection in biofluids is achieved by nuclear magnetic resonance spectroscopy and mass spectrometry, and the combination of these two techniques enable the detection of a broad range of metabolites with high sensitivity. To meet the demands of broad coverage with high sensitivity, derivatisation techniques are often employed. Recently, simultaneous quantification of over 100 metabolites containing the amide group was accomplished in 14 minutes with a detection limit of sub-femtomole using ultra-performance liquid chromatography (UPLC)-Triple Quad mass spectrometry.[1] These metabolites covered 20 important metabolic pathways. Applications of chemical-derivatisation assisted methodology in the clinic is therefore likely to be beneficial for molecular diagnosis in precision medicine, particularly when it comes to surgeons making decisions in real-time instead of waiting for results of histological examinations. The coupling of rapid evaporative ionisation mass spectrometry with electrosurgery, known as intelligent knife (iKnife), allows near-real-time profiling from the aerosol released during electrosurgical dissection.[2] The aerosol carries biological information that differentiates whether the tissue is cancerous or benign, with the aid of multivariate data analysis. This tissue identification technique can be translated into routine operational procedures, allowing surgeons to make precise and fast decisions to remove cancerous sites while preventing the removal of healthy tissue. In recent years, important progress has been made in measuring metabolites from single cells, which is attributed to the development of a microfluidic platform.[3] Translational medical research using single cell techniques is expected to advance the areas of cancer biology, stem cell research, and monitoring drug response. These newly developed metabolic profiling techniques provide innovative tools for translational clinical studies.

For biomarker discovery, metabolic profiling techniques in clinical medicine have generated a wide range of biomarkers for disease prediction and diagnosis. Increased levels of branched chain amino acids, sugars (glucose, mannose, fructose and hexose) and α–hydroxybutyrate, and decreased levels of Lyso-phosphatidylcholine (C18:2), which have been studied in numerous cohorts of patients, are predictive for early onset of diabetes.[4] These metabolites also change in the same fashion for diabetes population.

Lipoprotein and lipids have also been found to be highly relevant to cardiovascular disease (CVD). The combination of NMR metabolic profiling and accurate targeted lipidomics assays are likely to generate biomarkers that could predict the onset of CVD. This approach has been applied to a population study, in which biomarkers were generated from 7256 individuals in the CVD free FINRISK population, and 800 CVD cases during a 15-year follow-up. The candidate biomarkers were validated in two population studies with a total of 6185 healthy individuals and 941 CVD cases during a period of 12-23 years. This type of large population metabolic

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profiling study identified that high levels of phenylalanine and increased proportion of monounsaturated fatty acids are predictive of CVD.[5]

Recent studies found that trimethylamine *N*-oxide (TMAO), generated from choline metabolism through action of gut microbiota, is also associated with cardiovascular disease in the clinic.[6] This biomarker was identified when investigating the relationship between fasting plasma levels of TMAO and major cardiovascular incidents in 4007 patients during a 3-year follow-up study, which highlighted the important role of gut microbes in cardiovascular disease. Modulation of enzymatic activities for trimethylamine (TMA) formation by inhibiting trimethylamine lyases through low levels of 3,3-dimethyl-1-butanol exposure showed great potential in preventing cardiovascular disease.[7]

Niemann-Pick disease type C (NPC) is a rare progressive neuro-degenerative and cholesterol storage disorder. By using metabolic profiling of plasma, an unusual bile acid, 3β,5α,6β-trihydroxycholanic acid and its glycine conjugate, were identified and validated to be specific bile acids for distinguishing NPC disease from other causes of cholestasis. A high-throughput mass spectrometry method for detecting the glycine conjugate was further developed and validated with 100% sensitivity and specificity from dried blood spots of 4992 controls, 134 NPC carriers and 44 NPC patients. This newly developed diagnostic method provides a fast and robust NPC screening tool for newborns, allowing the possibility of drug interventions to be implemented during the early stages of life.[8]

Drug resistance is common in anti-microbial and cancer treatment patients, and new drug development is slow compared to the speed of development of drug resistance. Finding the cause of drug resistance and making drugs with resistance become sensitive again appear to be a cost effective strategy in combatting drug resistance. Metabolic profiling technique has been used to identify metabolites associated with epidermal growth factor receptor (EGFR) inhibitor-resistance in the treatment of non-small cell lung cancer. Reduction in the levels of glutathione was discovered to be associated with EGFR inhibitor-resistance, which was found in two independent pairs of erlotinib-sensitive/resistant cells. Down-regulation of glutathione anabolic enzymes and up-regulation of glutathione catabolic enzymes in resistant cells contributed to the reduced levels of glutathione. Ethacrynic acid, a diuretic agent, is known to have the ability to increase the levels of glutathione in cells, and by using ethacrynic acid, the resistant cells became re-sensitised to erlotinib treatment. *In vivo* studies have also proved the effectiveness of ethacrynic acid and erlotinib combination treatment.[9] This investigation highlighted an innovative approach to drug-resistance issues and benefits of combinational drug therapies.

In conclusion, enhancing diagnosis, prognosis and therapeutics is the overarching goal of translational research. Innovative analytical methods that enable robust, quick and cost-effective measurements are mandatory in ensuring biomarker discovery. A range of biomarkers have been discovered to predict the onset of diabetes, CVD, inborn errors of metabolism, and to tackle drug resistance issues; however, implementing these discoveries in the clinic requires approval from regulatory bodies. Therefore, biomarker discoveries and translation of these biomarkers into clinical medicine still require concerted efforts from clinical doctors, biomedical research scientists and regulatory bodies.

Key words: Translational research, biomarkers, metabolic diseases, drug resistant.

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