Journal of Cellular Physiology

Challenges and Opportunities of Metabolomics

CAROLINE H. JOHNSON AND FRANK J. GONZALEZ*

Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

The metabolome is a data-rich source of information concerning all the low-molecular-weight metabolites in a biofluid, which can indicate early biological changes to the host due to perturbations in metabolic pathways. Major changes can be seen after minor stimuli, which make it a valuable target for analysis. Due to the diverse and sensitive nature of the metabolome, studies must be designed in a manner to maintain consistency, reduce variation between subjects, and optimize information recovery. Technological advancements in experimental design, mouse models and instrumentation have aided in this effort. Metabolomics has the ultimate potential to be valuable in a clinical setting where it could be used for early diagnosis of a disease and as a predictor of treatment response and survival. During drug treatment, the metabolic status of an individual could be monitored and used to indicate possible toxic effects. Metabolomics therefore has great potential for improving diagnosis, treatment and aftercare of disease.

J. Cell. Physiol. 227: 2975–2981, 2012. Published 2011.

Metabolomics is the unbiased global survey of all the low-molecular-weight molecules or metabolites in a biofluid, cell, tissue, organ, or organism (Johnson et al., 2011a). These metabolites are the final downstream products of genomic, transcriptomic, and/or proteomic perturbations (see Fig. 1). Global metabolomics studies have effectively shown biological changes resulting from disease or environmental interactions, and revealed novel mechanisms not previously identified by other biochemical experiments. Metabolomics has also revealed possible routes for further investigation. Both nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) based metabolomics have been applied successfully to numerous in vitro and in vivo studies. These studies have revealed metabolic pathway disruption in many disease types including cardiovascular disease (Brindle et al., 2002; Kirschenlohr et al., 2006), diabetes (Fiehn et al., 2010; Patterson et al., 2011), inborn errors of metabolism (Arn, 2007; Wikoff et al., 2007), alcohol-induced liver injury (Manna et al., 2010, 2011), and various cancers (Cheng et al., 2005; Chan et al., 2009). Exposure to environmental influences such as ionizing radiation in cellular and rodent models (Patterson et al., 2008; Tyburski et al., 2008, 2009; Lanz et al., 2009; Johnson et al., 2011b), and xenobiotic use in rodents and non-human primates (Cheung et al., 2005b; Giri et al., 2006, 2007; Chen et al., 2006, 2007, 2008; Ma et al., 2008a; Cheng et al., 2009; Liu et al., 2009; Li et al., 2010, 2011; Pang et al., 2011) have been explored by metabolomics and revealed numerous novel metabolites and mechanisms of action. The influence of individual gene alterations on the metabolome has also been demonstrated by metabolomics (Zhen et al., 2007; Bonzo et al., 2010; Cheng et al., 2010). Although metabolomics can successfully identify metabolic signatures and mechanisms of disease, there are some challenges that need to be considered when implementing a metabolomic study. Here, we will discuss the issues faced in metabolomics analysis with possible solutions, along with a selection of innovative ideas for future applications.

Challenges of Metabolomics Analysis

The metabolome is sensitive to various genetic and environmental stimuli. Therefore the execution of a metabolomic study requires the consideration of a number of factors so that confounders can be limited and information recovery optimized. Augmenting experimental design is essential to reduce interindividual variation, and a decision has to be made as to which analytical platform is the most effective for the experiment or laboratory, both of which can affect the outcome of the experiment and data recovery. Another limitation to metabolomics, particularly with liquid chromatography (LC)-MS-based metabolomics, is assigning identity to the biomarkers.

Reducing Interindividual Variation

The metabolome is influenced by genetic, environmental and gut microbiota pressures, thus subtle variations between individuals can result in large perturbations to metabolite concentrations and flux. Environmental factors include diet, stress, xenobiotic use, lifestyle, and disease, while genetic variation can result from differences in gender, epigenetics, and gene polymorphisms (Johnson et al., 2011a). The gut microbiota can modify the host metabolome through co-metabolism of compounds such as dietary phenols and phenylalanine. It can also co-metabolize xenobiotics (Wilson and Nicholson, 2009; Wallace et al., 2010) including prontosil (Gingell et al., 1971), fostamatinib (Sweeny et al., 2010), and acetaminophen (Mikov et al., 1988). Furthermore, alterations in the gut microbe environment have been shown to be associated with diseases such as obesity (Ley, 2010). These gut microbiota, genetic, and environmental factors contribute to interindividual differences in the metabolome, but the interplay between them can further confound results. For example, a group of individuals exhibiting variation in genes encoding xenobiotic-metabolizing enzymes and transporters will metabolize xenobiotics differently; there will also be the added influence of co-metabolism from their gut microbiota. Dietary grapefruit ingestion inhibits xenobiotic-metabolizing enzymes such as cytochrome P450 (CYP)3A4 and some transporters (Hanley et al., 2011). Indeed, grapefruit-drug interactions have been seen with antihypertensives, antimicrobials, benzodiazepines, antihistamines, statins, and chemotherapeutics (Kiani and Imam, 2007). Therefore, this dietary environmental intervention could additionally confound

*Correspondence to: Frank J. Gonzalez, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
E-mail: gonzalef@mail.nih.gov

Manuscript Received: 14 October 2011
Manuscript Accepted: 17 October 2011

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 27 October 2011.
DOI: 10.1002/jcp.24002

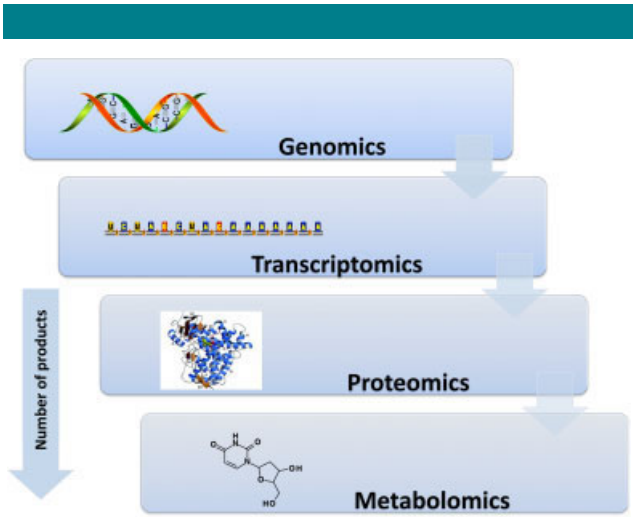


Fig. 1. Metabolomics contains the downstream products of genomic, transcriptomic, and proteomic processes.

the metabolism of xenobiotics administered to these individuals and their metabolomic profiles.

To overcome the confounding effects of variation, the concept of a metabolic fingerprint or the metabotype was conceived to encompass and identify all the possible variation in an individual. The metabotype was first defined as "a probabilistic multiparametric description of an organism in a given physiological state based on analysis of its cell types, biofluids, or tissues" (Gavaghan et al., 2000). It is an important consideration when studying the effect of a specific intervention on a system. It could aid in patient stratification for clinical trials where pharmacogenomics is primarily used to stratify populations by genotype. Trials may benefit from using metabotypes for population stratification, so that responders and non-responders can be defined. This could aid in greater success in drug development by considering both the environmental and genomic influences.

Metabolomics experiments' with in vitro and in vivo models have been utilized to control some of the factors that propagate interindividual variation. Single-cell metabolomics has recently been implemented to observe the metabolic phenotype of an individual cell. It is known that populations of cells are not always homogenous due to differences in microenvironment, cell-cycle stages or age, thus variation can exist between them (Heinemann and Zenobi, 2011). Single-cell metabolomics can reveal metabolic differences between these cells to provide more information about a cell type. Regulating environmental variation is more complex in in vivo models but can be accomplished through co-housing and breeding of animal models in the same conditions. A distinct urinary metabolome was seen in a set of Sprague–Dawley rats; these rats were housed in a different room to the other rats and excreted a higher concentration of aromatic metabolites, most likely due to metabolism by the gut microbiota (Robosky et al., 2005). This study shows the importance of environmental consistency in metabolomics experiments. The diet can also be regulated, but due to the hierarchy between cage-mates, some animals may eat more than others which could also cause variation to the metabolome. In clinical metabolomics studies, environmental variation can be reduced by obtaining samples from a large number of volunteers; this could eliminate some non-specific variation. Strict inclusion criteria such as age, body mass index and health status must also be included. The volunteers should ideally be admitted to a clinic so that environmental factors such as stress, pollutants, diet, pharmaceuticals, and others can be restricted. This has been demonstrated in the experimental design and implementation of a metabolomics study to look at acetaminophen-induced liver toxicity in humans (Winnike et al., 2010). Healthy volunteers were admitted into a clinic and placed on a standard whole-food diet for 14 days during dosing; another advantage to this study was that they were able to collect 24-h urine samples continuously over the 14 days.

The genetic differences between mice and humans are large. Orthologs for some genes such as those from CYP family have been identified and show similar function, but differences in activity have been seen (Donato et al., 2000; Pang et al., 2011). Transgenic humanized mouse models have been developed to understand human responses to environmental interventions such as the *CYP1A2*-humanized and *CYP2E1*-humanized mouse models. These have been used to study aminoflavone (Chen et al., 2006), melatonin (Ma et al., 2008a), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Cheung et al., 2005a; Chen et al., 2007), and acetaminophen metabolism (Cheung et al., 2005b). The double transgenic humanized-PXR/Tg3A4 mouse model was created to eliminate the effects of species-selective ligand activation of PXR and gene expression of CYP3A4 (Ma et al., 2008b). Acetaminophen (Cheng et al., 2009) and black cohosh (Pang et al., 2011) metabolism/toxicity have been studied using this double transgenic model and provided an effective model for evaluating the metabolism of these drugs in a human system.

In order to control the effects of the microbiome on the metabolome, gnotobiotic animal models have been developed that establish a human microbiome within the mouse. These mice are germ-free at birth and are colonized with human adult fecal microbiota (Turnbaugh et al., 2009) or human newborn fecal microbiota (Martin et al., 2007). The effects of human gut microbiome can thus be observed on the metabolome.

Choice of Instrumentation

The metabolome is chemically diverse and requires different methods to capture most of the compounds in a complex mixture. The two main analytical platforms used in metabolomics analysis are NMR spectroscopy and MS. The decision on which platform to use depends on the biofluid to be analyzed and the ultimate aims of the experiment. Both NMR spectroscopy and MS have numerous configurations for optimizing and improving metabolite analysis. Advances in NMR spectroscopy have involved higher magnetic field strengths and the invention of cryoprobes. These developments have improved spectral dispersion and sensitivity thus aiding in metabolite identification (Keun et al., 2002). NMR spectroscopy can detect different nuclides which is advantageous for sample analysis. The ^{19}F nuclide for example can identify and track the metabolism of fluorine-containing drugs, whereas the ^{1}H and ^{13}C nuclides can show endogenous metabolite changes; these nuclides can also be used in radiolabeled studies. Various methodologies and techniques have been adopted that optimize analysis of specific sample types such as magic-angle spinning for intact tissues (Moka et al., 1998). The identification of particular metabolite species have been improved through statistical recovery techniques that increase signal dispersion and information recovery (Cloarec et al., 2005; Johnson et al., 2008). Various pulse sequences have also been utilized such as the Carr–Purcell–Meiboom–Gill spin echo sequence, which attenuates broad protein and lipoprotein signals (Waldram et al., 2009). NMR spectroscopy can be useful for analysis of larger molecules (>500 Da), sugars, amines, or volatile liquids (Wishart, 2011) but has some disadvantages for analysis including its lack of sensitivity compared to MS.

For MS, the chromatography component depends on the expected outcomes of the experiment and expertise/budget of

the laboratory. For metabolite separation, gas chromatography (GC), and LC can be used, some metabolomics studies have also utilized capillary electrophoresis (CE) which can provide complementary information (Lee and Britz-McKibbin, 2010; D'Agostino et al., 2011). Ultra high performance liquid chromatography (UHPLC), a recent LC development (Wilson et al., 2005), is now commonly employed as the LC system of choice for MS-based metabolomics. The columns for UHPLC are composed of a small particle size (1.7–1.8 μm) and the mobile phase is applied at pressures of up to 15,000 psi, a combination that greatly improves sensitivity compared to traditional high performance LC (three to fivefold). To improve identification of polar metabolites, which are difficult to retain on reverse phase columns, hydrophobic interaction chromatography (HILIC) has been introduced to the UHPLC system (Gika et al., 2008; Spagou et al., 2011; Swann et al., 2011). Recent advances in GC-MS technology has led to development of the GCxGC system which uses two orthogonal separation phases. The advantage is enhanced resolution with a reduction in coeluting peaks; therefore many more peaks can be detected (Marriott and Shellie, 2002). An example of the separation that can be achieved by this system can be seen in Figure 2. The three-dimensional surface and contour plots effectively show how metabolites can elute at the same retention time from one column but can be separated using a secondary column/ dimension.

With so many different types of analysis tools available, the decision on which platform to use can be problematic as no single platform can reveal the whole metabolome. This is due to the heterogeneity of the chemical constitution of the metabolites and specific analytical limitations of each instrument. Table 1 displays some advantages and disadvantages of GC-MS, UHPLC-MS, and NMR spectroscopy. The optimal platform for identification of particular metabolite species is also shown; some species can be identified on more than one instrument. It should also be noted that GC-MS platforms are typically the cheapest to purchase compared to UHPLC-MS and NMR spectrometers, but the extensive sample preparation required for GC samples is more expensive than those for NMR spectroscopy and UHPLC-MS analysis.

Biomarker Identification

The most challenging part of a metabolomic study is confirmation of biomarker identity. This is an essential step toward understand the biological changes occurring within the system and remains a major bottleneck in metabolomics investigations. Technological advances with better databases could help solve this problem. The databases developed for GC-MS data [NIST/EPA/NIH (Babushok et al., 2007), Fiehnlib (Kind et al., 2009), and Golm (Kopka et al., 2005)] can provide effective identification of metabolites (Dunn, 2008). Identifying metabolites from LC-MS data however is more problematic as the databases are much less developed than those for GC-MS analysis; they include the Human Metabolome Database (HMDB) (http://www.hmdb.ca) and the METLIN Metabolite Database (http://metlin.scripps.edu). It is also difficult to compare data between laboratories due to differences in analytical platforms, columns, gradients, and collision energies for tandem MS. There are a number of chemical databases such as ChemSpider (http://www.chemspider.com) that contain millions of chemical structures to aid in structural elucidation after fragmentation of the metabolite, with a common sense component to decipher its origin (endogenous or xenobiotic). To aid in identification of metabolites through NMR spectroscopy databases can be used for comparison of chemical shifts including HMDB and METLIN, and the Biological Magnetic Resonance Databank (http://www.bmrb.wisc.edu/ metabolomics/). Two-dimensional experiments such as total correlation spectroscopy or heteronuclear multiple bond correlation can also be applied.

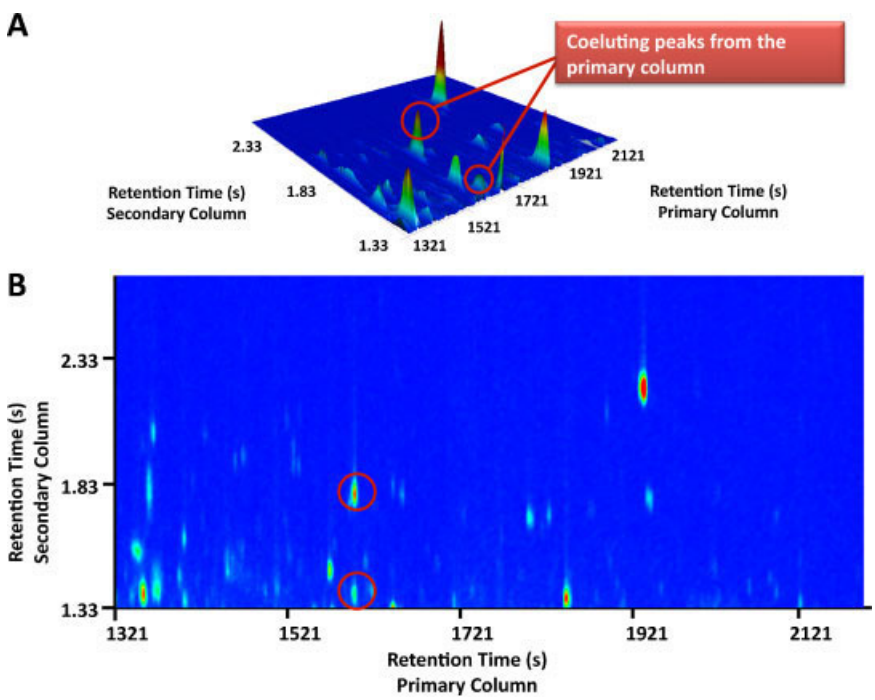
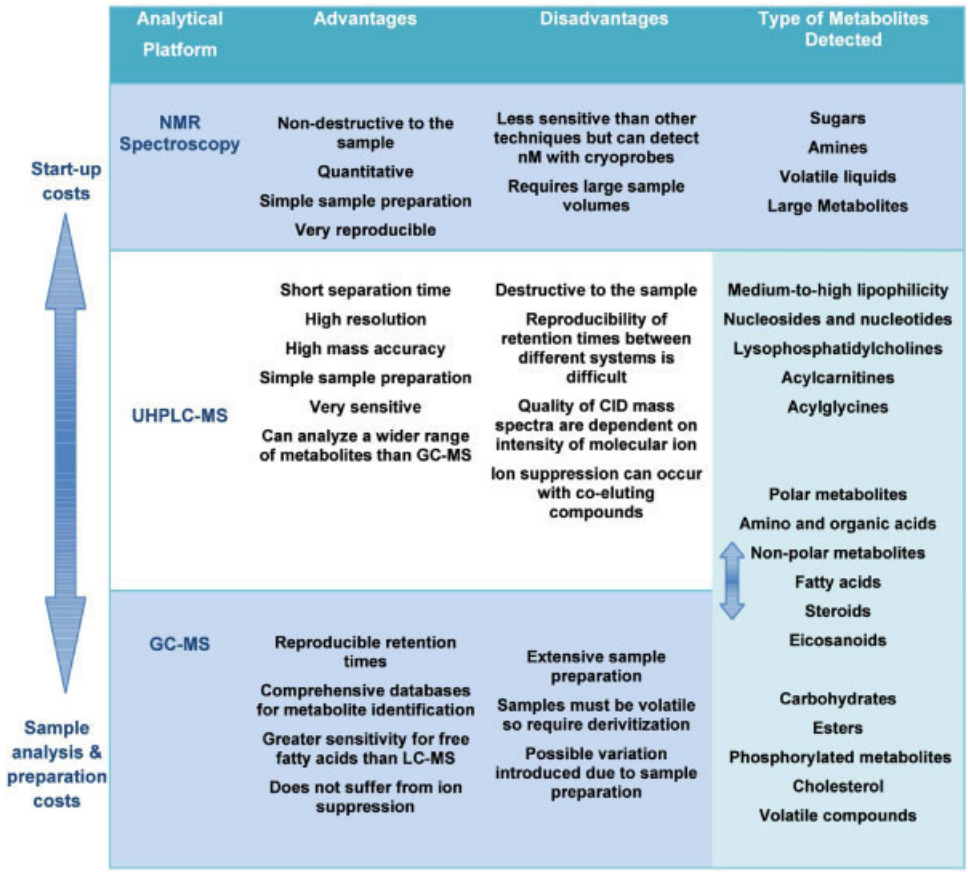


Fig. 2. GC × GC-TOFMS analysis of standard mixture showing (A) a three-dimensional surface plot of a selected region where metabolites elute at the same retention time on the primary column and can then be resolved by using a secondary column and (B) a contour plot of the same region showing separation of coeluting peaks.

TABLE 1. Selection guide for analytical platform



Variation in sampling methods, sample preparation, instrumentation, and data mining exists not only between laboratories but also between scientists in the same laboratory (Villas-Boas et al., 2010). It is therefore essential to ensure standardization between personnel, but in practice, the broad range of metabolomics applications prevents this somewhat. A central reporting database needs to be established so that investigators can share their methodologies and results. This would allow for standard operating procedures to be developed and comparisons of data between laboratories. There have been some initiatives toward producing these data collection sites: MIAMET (Bino et al., 2004), ArMet (Jenkins et al., 2004), SMRS (Lindon et al., 2005), and MSI (Hardy and Jenkins, 2010). Further development of databases is also necessary, but limited by cost. Unpublished data are also unlikely to make it onto these databases due to restrictions from funding bodies. Authentic standards would need to be synthesized or purchased for each reported metabolite; therefore a collaborative effort between metabolomics laboratories is needed.

Future Directions for Metabolomics

Metabolomics has the potential to be an effective tool for early diagnosis of disease, through identification of one, or a signature of prognostic biomarkers. It could also serve as a predictor of treatment response and survival. The metabolome is quick to respond to environmental stimuli including therapeutic or surgical intervention and thus could be used to monitor the metabolic status of the individual and indicate any possible toxic effects; it could also be used to detect any remaining disease or recurrence after therapy.

Personalized Medicine and Pharmacometabolomics

One of the major opportunities that can arise from metabolomics is its contribution to the development of personalized medicine. The metabolome contains all the biological endpoints of genomic, transcriptomic, and proteomic perturbations, as well as environmental and gut microbiota influences. Therefore, stratifying populations based on their metabolic phenotype or metabotype could be an effective way to tailor a dose/type of drug or other therapy to an individual. This could lead to greater success in drug development by decreasing adverse drug reactions and improving disease outcomes. Patient stratification has previously focused on genotype, but pharmacogenomics has not shown promising results. These studies tend to stratify based on CYP or other drug metabolizing enzymes and drug transporters (Nicholson and Holmes, 2006), but there are other influences that could affect disease or drug efficacy such as gut microflora and diet; the outcomes of these effects can be seen in the metabolome. Therefore, metabolomics could be developed toward personalized medicine approaches. Pharmacometabolomics, which can predict drug efficacy and drug-induced side effects through knowledge of the individual's metabotype, could inevitably aid in designing a patient stratification approach, improving drug development, and greater success in clinical trials (Nicholson et al., 2011).

Pharmacometabolomics was first defined by its pseudonym pharmacometabonomics as "the prediction of the outcome of a drug or xenobiotic intervention in an individual based on a mathematical model of preintervention metabolite signatures" (Clayton et al., 2006). The technique has been used to examine galactosamine and acetaminophen toxicity in rats

(Clayton et al., 2009). Clinical studies have also used this approach to analyze acetaminophen-induced hepatotoxicity in humans (Winnike et al., 2010) and has been further developed to lead a pharmacogenomic investigation (Ji et al., 2011).

Technological Advances in Metabolomics

There have been many recent technological advances in metabolomic research that show promise for improving metabolite identification and clinical applications. Nanostructure-Initiator Mass Spectrometry (NIMS) uses a matrix-free desorption/ionization approach which can analyze metabolites within tissues and biofluids. NIMS has many advantages for MS-based metabolomics; no sample preparation is required, it does not require a matrix, produces minimal fragmentation and can detect metabolites in the yoctomole (10^{-24}) range (Greving et al., 2011). It has many applications and has so far been used for analyzing blood and urine (Northen et al., 2007), imaging tissues to reveal the localization of a drug in mouse brain (Yanes et al., 2009) and for measuring enzyme activity in biological samples (Northen et al., 2008).

Stable isotope tracers have been successfully adapted for metabolomics studies to monitor metabolic flux. Metabolomics can reveal metabolites that have been perturbed due to an intervention but the concentration flux in a biological pathway is important to determine directional changes in metabolism. Stable isotope tracers can reveal which parts of a metabolic network are being utilized. Isotope tracing has been used successfully in cellular models to analyze fatty acid metabolism and in animal models to monitor bile acid metabolism (Castro-Perez et al., 2011). Cancer cells are also good model systems for the stable isotope tracer method. Increased aerobic glycolysis is one of the hallmarks of malignant transformation (Warburg, 1956) and proliferating cancer cells have a high demand for glucose which is depleted over time. The decrease of glucose can be measured using ^{13}C-glucose and concomitantly monitored by the formation of the ^{13}C-lactate isotopomer (Lane et al., 2011). In humans a non-small cell lung cancer study was carried out using [U-^{13}C]-glucose. The isotope was administered to patients 3 h prior to surgical resection of the tumor and surrounding non-cancerous tissues. NMR spectroscopy and MS were used to analyze the paired cancerous and non-cancerous tissues. The flow of ^{13}C atoms could be monitored from glucose, through glycolysis into the Krebs cycle and revealed distinct metabolic activities in the cancerous tissues (Fan et al., 2009b; Lane et al., 2011). Positron emission tomography (PET) has already been used to detect tumors using radiolabeled tracers such as ^{18}F-fluorodeoxyglucose (FDG) or ^{11}C-choline. FDG accumulates in cancerous cells that have increased aerobic glycolysis, and ^{11}C-choline is increased in cancerous cells due to the upregulation of choline kinase (Kassis et al., 2010). In vivo metabolomics studies have started to use this technology as a method to predict drug response: patients receiving sunitinib for gastrointestinal stromal tumors (Prior et al., 2009) were monitored by NMR spectroscopy and GC-MS-based metabolomics to look for metabolic distinctions and tumor response in (a) normal lung tissue, (b) in a bronchioalveolar erlotinib-sensitive carcinoma, and (c) in an erlotinib-insensitive lesion from a patient (Fan et al., 2009a). This type of metabolomics imaging could aid in patient stratification for therapy and optimization of clinical outcomes.

Isotopes have also been used in drug metabolism studies to aid in the identification of novel metabolites; three new urinary metabolites of acetaminophen were discovered by UPLC-MS-based metabolomics after administration of [acetyl-$^{2}H_3$] and 2,3,5,6-[$^{2}H_4$] acetaminophen (APAP) to mice (Chen et al., 2008). Figure 3A shows a PCA scores plot of urine collected from mice dosed with either unlabeled APAP or [acetyl-$^{2}H_3$] APAP, the ions contributing to the separation were due to APAP and its metabolites. The PCA loadings plot in Figure 3B displays these metabolites from APAP and [acetyl-$^{2}H_3$] APAP in a symmetrical pattern. This study illustrates an effective novel way to identify and validate xenobiotic metabolites.

Technological advances in surgical metabolomics studies have included the installation of a MAS NMR spectrometer at St. Mary's Hospital, London, UK (Imperial College Healthcare NHS Trust). The spectrometer is situated in the operating room and will allow surgeons to have diagnostic information while the patient is undergoing treatment. This will provide phenotypic information regarding the tissue status such as viability and morbidity assessment, and aims to improve the quality of surgical care of the patient. There are also further aims to develop a real-time MS-based metabolomics intelligent knife; electrocautery devices used during surgery release metabolites as they cut into the tissues, this could show valuable information such as the disease state of the tissue that could be relayed to the surgeon as they are performing the surgery (Kinross et al., 2011).

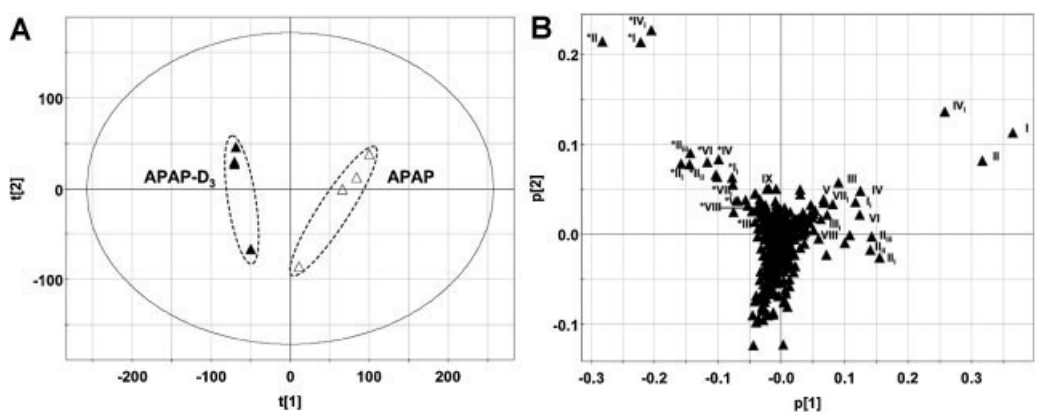


Fig. 3. Identification of urinary APAP metabolites by UPLC-MS-based metabolomics and stable-isotope tracing. A: PCA scores plot on 24 h urine samples from mice dosed with 400 mg/kg APAP▵ or 400 mg/kg [acetyl-$^{2}H_3$] APAP▲. B: PCA loadings plot revealing ions in the urine samples after APAP and [acetyl-$^{2}H_3$] APAP dosing.

Summary

Metabolomics has been shown to be an evolving analytical tool that has prospects in all areas of scientific research. The introduction of innovative technologies for metabolomics is exciting and holds great promise for clinical applications to improve patient diagnosis, treatment, and after-care. Due to the complexity of the metabolome there are a number of experimental considerations that need to be made before embarking on a study, such as identifying and limiting sources of variation and the choice of instrumentation for analysis. Novel methods for metabolite analysis and identification are being developed in all aspects to help overcome some of the hurdles associated with metabolomics analysis.

Literature Cited

Arn PH. 2007. Newborn screening: Current status. Health Aff (Millwood) 26:559–566.

Babushok VI, Linstrom PJ, Reed JJ, Zenkevich IG, Brown RL, Mallard WG, Stein SE. 2007. Development of a database of gas chromatographic retention properties of organic compounds. J Chromatogr A 1157:414–421.

Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K, Draper J, Nikolau BJ, Mendes P, Roessner-Tunali U, Beale MH, Trethewey RN, Lange BM, Wurtele ES, Sumner LW. 2004. Potential of metabolomics as a functional genomics tool. Trends Plant Sci 9:418–425.

Bonzo JA, Patterson AD, Krausz KW, Gonzalez FJ. 2010. Metabolomics identifies novel Hnf1{alpha}-dependent physiological pathways in vivo. Mol Endocrinol 24:2343–2355.

Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ. 2002. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nat Med 8:1439–1444.

Castro-Perez JM, Roddy TP, Shah V, Wang SP, Ouyang X, Ogawa A, McLaren DG, Tadin-Strapps M, Robinson MJ, Bartz SR, Ason B, Chen Y, Previs SF, Wong KK, Vreeken RJ, Johns DG, Hubbard BK, Hankemeier T, Mitnaul L. 2011. Attenuation of Slc27a5 gene expression followed by LC-MS measurement of bile acid reconjugation using metabolomics and a stable isotope tracer strategy. J Proteome Res 10:4683–4691.

Chan EC, Koh PK, Mal M, Cheah PY, Eu KW, Backshall A, Cavill R, Nicholson JK, Keun HC. 2009. Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). J Proteome Res 8:352–361.

Chen C, Meng L, Ma X, Krausz KW, Pommier Y, Idle JR, Gonzalez FJ. 2006. Urinary metabolite profiling reveals CYP1A2-mediated metabolism of NSC686288 (aminoflavone). J Pharmacol Exp Ther 318:1330–1342.

Chen C, Ma X, Malfatti MA, Krausz KW, Kimura S, Felton JS, Idle JR, Gonzalez FJ. 2007. A comprehensive investigation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) metabolism in the mouse using a multivariate data analysis approach. Chem Res Toxicol 20:531–542.

Chen C, Krausz KW, Idle JR, Gonzalez FJ. 2008. Identification of novel toxicity-associated metabolites by metabolomics and mass isotopomer analysis of acetaminophen metabolism in wild-type and Cyp2e1-null mice. J Biol Chem 283:4543–4559.

Cheng LL, Burns MA, Taylor JL, He W, Halpern EF, McDougal WS, Wu CL. 2005. Metabolic characterization of human prostate cancer with tissue magnetic resonance spectroscopy. Cancer Res 65:3030–3034.

Cheng J, Ma X, Krausz KW, Idle JR, Gonzalez FJ. 2009. Rifampicin-activated human pregnane X receptor and CYP3A4 induction enhance acetaminophen-induced toxicity. Drug Metab Dispos 37:1611–1621.

Cheng J, Shah YM, Ma X, Pang X, Tanaka T, Kodama T, Krausz KW, Gonzalez FJ. 2010. Therapeutic role of rifaximin in inflammatory bowel disease: Clinical implication of human pregnane X receptor activation. J Pharmacol Exp Ther 335:32–41.

Cheung C, Ma X, Krausz KW, Kimura S, Feigenbaum L, Dalton TP, Nebert DW, Idle JR, Gonzalez FJ. 2005a. Differential metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in mice humanized for CYP1A1 and CYP1A2. Chem Res Toxicol 18:1471–1478.

Cheung C, Yu AM, Ward JM, Krausz KW, Akiyama TE, Feigenbaum L, Gonzalez FJ. 2005b. The cyp2e1-humanized transgenic mouse: Role of cyp2e1 in acetaminophen hepatotoxicity. Drug Metab Dispos 33:449–457.

Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, Provost JP, Le Net JL, Baker D, Walley RJ, Everett JR, Nicholson JK. 2006. Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature 440:1073–1077.

Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. 2009. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc Natl Acad Sci USA 106:14728–14733.

Cloarec O, Dumas ME, Craig A, Barton RH, Trygg J, Hudson J, Blancher C, Gauguier D, Lindon JC, Holmes E, Nicholson J. 2005. Statistical total correlation spectroscopy: An exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. Anal Chem 77:1282–1289.

D'Agostino LA, Lam KP, Lee R, Britz-McKibbin P. 2011. Comprehensive plasma thiol redox status determination for metabolomics. J Proteome Res 10:592–603.

Donato MT, Viitala P, Rodriguez-Antona C, Lindfors A, Castell JV, Raunio H, Gomez-Lechon MJ, Pelkonen O. 2000. CYP2A5/CYP2A6 expression in mouse and human hepatocytes treated with various in vivo inducers. Drug Metab Dispos 28:1321–1326.

Dunn WB. 2008. Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes. Phys Biol 5:011001.

Fan TW, Lane AN, Higashi RM, Bousamra M, II, Kloecker G, Miller DM. 2009a. Metabolic profiling identifies lung tumor responsiveness to erlotinib. Exp Mol Pathol 87:83–86.

Fan TW, Lane AN, Higashi RM, Farag MA, Gao H, Bousamra M, Miller DM. 2009b. Altered regulation of metabolic pathways in human lung cancer discerned by (13)C stable isotope-resolved metabolomics (SIRM). Mol Cancer 8:41.

Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH. 2010. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS ONE 5:1–10.

Gavaghan CL, Holmes E, Lenz E, Wilson ID, Nicholson JK. 2000. An NMR-based metabonomic approach to investigate the biochemical consequences of genetic strain differences: Application to the C57BL10J and Alpk: ApfCD mouse. FEBS Lett 484:169–174.

Gika HG, Theodoridis GA, Wilson ID. 2008. Hydrophilic interaction and reversed-phase ultra-performance liquid chromatography TOF-MS for metabonomic analysis of Zucker rat urine. J Sep Sci 31:1598–1608.

Gingell R, Bridges JW, Williams RT. 1971. The role of the gut flora in the metabolism of prontosil and neoprontosil in the rat. Xenobiotica 1:143–156.

Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. 2006. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol 19:818–827.

Giri S, Krausz KW, Idle JR, Gonzalez FJ. 2007. The metabolomics of (+/−)-arecoline 1-oxide in the mouse and its formation by human flavin-containing monooxygenases. Biochem Pharmacol 73:561–573.

Greving MP, Patti GJ, Siuzdak G. 2011. Nanostructure-initiator mass spectrometry metabolite analysis and imaging. Anal Chem 83:2–7.

Hanley MJ, Cancalon P, Widmer WW, Greenblatt DJ. 2011. The effect of grapefruit juice on drug disposition. Expert Opin Drug Metab Toxicol 7:267–286.

Hardy N, Jenkins H. 2010. Reporting standards. In: Neilsen J, Jewett MC, editors. Metabolomics: A powerful tool in systems biology. Berlin: Springer. pp 53–73.

Heinemann M, Zenobi R. 2011. Single cell metabolomics. Curr Opin Biotechnol 22:26–31.

Jenkins H, Hardy N, Beckmann M, Draper J, Smith AR, Taylor J, Fiehn O, Goodacre R, Bino RJ, Hall R, Kopka J, Lane GA, Lange BM, Liu JR, Mendes P, Nikolau BJ, Oliver SG, Paton NW, Rhee S, Roessner-Tunali U, Saito K, Smedsgaard J, Sumner LW, Wang T, Walsh S, Wurtele ES, Kell DB. 2004. A proposed framework for the description of plant metabolomics experiments and their results. Nat Biotechnol 22:1601–1606.

Ji Y, Hebbring S, Zhu H, Jenkins GD, Biernacka J, Snyder K, Drews M, Fiehn O, Zeng Z, Schaid D, Mrazek DA, Kaddurah-Daouk R, Weinshilboum RM. 2011. Glycine and a glycine dehydrogenase (GLDC) SNP as citalopram/escitalopram response biomarkers in depression: Pharmacometabolomics-informed pharmacogenomics. Clin Pharmacol Ther 89:97–104.

Johnson CH, Athersuch TJ, Wilson ID, Iddon L, Meng X, Stachulski AV, Lindon JC, Nicholson JK. 2008. Kinetic and J-resolved statistical total correlation NMR spectroscopy approaches to structural information recovery in complex reacting mixtures: Application to acyl glucuronide intramolecular transacylation reactions. Anal Chem 80:4886–4895.

Johnson CH, Patterson AD, Idle JR, Gonzalez FJ. 2011a. Xenobiotic metabolomics: Major impact on the metabolome. Annu Rev Pharmacol Toxicol In press.

Johnson CH, Patterson AD, Krausz KW, Lanz C, Kang DW, Luecke H, Gonzalez FJ, Idle JR. 2011b. Radiation metabolomics. 4. UPLC-ESI-QTOFMS-based metabolomics for urinary biomarker discovery in gamma-irradiated rats. Radiat Res 175:473–484.

Kassis AI, Yongliang YS, Adelstein J. 2010. Putative molecular signatures for the imaging of prostate cancer. Expert Rev Mol Diagn 10:65–74.

Keun HC, Beckonert O, Griffin JL, Richter C, Moskau D, Lindon JC, Nicholson JK. 2002. Cryogenic probe ^{13}C NMR spectroscopy of urine for metabonomic studies. Anal Chem 74:4588–4593.

Kiani J, Imam SZ. 2007. Medicinal importance of grapefruit juice and its interaction with various drugs. Nutr J 6:33.

Kind T, Wohlgemuth G, Lee do Y, Lu Y, Palazoglu M, Shahbaz S, Fiehn O. 2009. FiehnLib: Mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Anal Chem 81:10038–10048.

Kinross JM, Holmes E, Darzi AW, Nicholson JK. 2011. Metabolic phenotyping for monitoring surgical patients. Lancet 377:1817–1819.

Kirschenlohr HL, Griffin JL, Clarke SC, Rhydwen R, Grace AA, Schofield PM, Brindle KM, Metcalfe JC. 2006. Proton NMR analysis of plasma is a weak predictor of coronary artery disease. Nat Med 12:705–710.

Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmuller E, Dormann P, Weckwerth W, Gibon Y, Stitt M, Willmitzer L, Fernie AR, Steinhauser D. 2005. GMD@CSB. DB: The Golm Metabolome Database. Bioinformatics 21:1635–1638.

Lane AN, Fan TW, Bousamra M, II, Higashi RM, Yan J, Miller DM. 2011. Stable isotope-resolved metabolomics (SIRM) in cancer research with clinical application to nonsmall cell lung cancer. OMICS 15:173–182.

Lanz C, Patterson AD, Slavik J, Krausz KW, Ledermann M, Gonzalez FJ, Idle JR. 2009. Radiation metabolomics. 3. Biomarker discovery in the urine of gamma-irradiated rats using a simplified metabolomics protocol of gas chromatography-mass spectrometry combined with random forests machine learning algorithm. Radiat Res 172:198–212.

Lee R, Britz-McKibbin P. 2010. Metabolomic studies of radiation-induced apoptosis of human leukocytes by capillary electrophoresis-mass spectrometry and flow cytometry: Adaptive cellular responses to ionizing radiation. Electrophoresis 31:2328–2337.

Ley RE. 2010. Obesity and the human microbiome. Curr Opin Gastroenterol 26:5–11.

Li F, Patterson AD, Hofer CC, Krausz KW, Gonzalez FJ, Idle JR. 2010. Comparative metabolism of cyclophosphamide and ifosfamide in the mouse using UPLC-ESI-QTOFMS-based metabolomics. Biochem Pharmacol 80:1063–1074.

Li F, Patterson AD, Hofer CC, Krausz KW, Gonzalez FJ, Idle JR. 2011. A comprehensive understanding of thioTEPA metabolism in the mouse using UPLC-ESI-QTOFMS-based metabolomics. Biochem Pharmacol 81:1043–1053.

Lindon JC, Nicholson JK, Holmes E, Keun HC, Craig A, Pearce JT, Bruce SJ, Hardy N, Sansone SA, Antti H, Jonsson P, Daykin C, Navarange M, Beger RD, Verheij ER, Amberg A, Baunsgaard D, Cantor GH, Lehman-McKeeman L, Earll M, Wold S, Johansson E, Haselden JN, Kramer K, Thomas C, Lindberg J, Schuppe-Koistinen I, Wilson ID, Reily MD, Robertson DG, Senn H, Krotzky A, Kochhar S, Powell J, van der Ouderaa F, Plumb R, Schaefer H, Spraul M. 2005. Summary recommendations for standardization and reporting of metabolic analyses. Nat Biotechnol 23:833–838.

Liu A, Patterson AD, Yang Z, Zhang X, Liu W, Qiu F, Sun H, Krausz KW, Idle JR, Gonzalez FJ, Dai R. 2009. Fenofibrate metabolism in the cynomolgus monkey using ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry-based metabolomics. Drug Metab Dispos 37:1157–1163.

Ma X, Chen C, Krausz KW, Idle JR, Gonzalez FJ. 2008a. A metabolomic perspective of melatonin metabolism in the mouse. Endocrinology 149:1869–1879.

Ma X, Cheung C, Krausz KW, Shah YM, Wang T, Idle JR, Gonzalez FJ. 2008b. A double transgenic mouse model expressing human pregnane X receptor and cytochrome P450 3A4. Drug Metab Dispos 36:2506–2512.

Manna SK, Patterson AD, Yang Q, Krausz KW, Li H, Idle JR, Fornace AJ, Jr., Gonzalez FJ. 2010. Identification of noninvasive biomarkers for alcohol-induced liver disease using urinary metabolomics and the Ppara-null mouse. J Proteome Res 9:4176–4188.

Manna SK, Patterson AD, Yang Q, Krausz KW, Idle JR, Fornace AJ, Gonzalez FJ. 2011. UPLC-MS-based urine metabolomics reveals indole-3-lactic acid and phenyllactic acid as conserved biomarkers for alcohol-induced liver disease in the Ppara-null mouse model. J Proteome Res 10:4120–4133.
Marriott P, Shellie R. 2002. Principles and applications of comprehensive two-dimensional gas chromatography. Trend Anal Chem 21:573–583.
Martin FP, Dumas ME, Wang Y, Legido-Quigley C, Yap IK, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC, Sprenger N, Fay LB, Kochhar S, van Bladeren P, Holmes E, Nicholson JK. 2007. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol Syst Biol 3:112.
Mikov M, Caldwell J, Dolphin CT, Smith RL. 1988. The role of intestinal microflora in the formation of the methylthio adduct metabolites of paracetamol. Studies in neomycin-pretreated and germ-free mice. Biochem Pharmacol 37:1445–1449.
Moka D, Vorreuther R, Schicha H, Spraul M, Humpfer E, Lipinski M, Foxall PJ, Nicholson JK, Lindon JC. 1998. Biochemical classification of kidney carcinoma biopsy samples using magic-angle-spinning 1H nuclear magnetic resonance spectroscopy. J Pharm Biomed Anal 17:125–132.
Nicholson JK, Holmes E. 2006. Global systems biology and personalized healthcare solutions. Discov Med 6:63–70.
Nicholson JK, Lindon JC, Wilson ID. 2011. Pharmacometabonomics as an effector for personalized medicine. Pharmacogenomics 12:103–111.
Northen TR, Yanes O, Northen MT, Marrinucci D, Uritboonthai W, Apon J, Golledge SL, Nordstrom A, Siuzdak G. 2007. Clathrate nanostructures for mass spectrometry. Nature 449:1033–1036.
Northen TR, Lee JC, Hoang L, Raymond J, Hwang DR, Yannone SM, Wong CH, Siuzdak G. 2008. A nanostructure-initiator mass spectrometry-based enzyme activity assay. Proc Natl Acad Sci USA 105:3678–3683.
Pang X, Cheng J, Krausz KW, Guo DA, Gonzalez FJ. 2011. Pregnane X receptor-mediated induction of Cyp3a by black cohosh. Xenobiotica 41:112–123.
Patterson A, Li H, Eichler G, Krausz K, Weinstein J, Fornace AJ, Gonzalez F, Idle J. 2008. UPLC-ESI-TOFMS-based metabolomics and gene expression dynamics inspector self-organizing metabolomic maps as tools for understanding the cellular response to ionizing radiation. Anal Chem 80:665–674.
Patterson AD, Bonzo JA, Li F, Krausz KW, Eichler GS, Aslam S, Tigno X, Weinstein JN, Hansen BC, Idle JR, Gonzalez FJ. 2011. Metabolomics reveals attenuation of the SLC6A20 kidney transporter in nonhuman primate and mouse models of type 2 diabetes mellitus. J Biol Chem 286:19511–19522.
Prior JO, Montemurro M, Orcurto MV, Michielin O, Luthi F, Benhattar J, Guillou L, Elsig V, Stupp R, Delaloye AB, Leyvraz S. 2009. Early prediction of response to sunitinib after imatinib failure by 18F-fluorodeoxyglucose positron emission tomography in patients with gastrointestinal stromal tumor. J Clin Oncol 27:439–445.
Robosky LC, Wells DF, Egnash LA, Manning ML, Reily MD, Robertson DG. 2005. Metabonomic identification of two distinct phenotypes in Sprague–Dawley (Crl:CD(SD)) rats. Toxicol Sci 87:277–284.
Spagou K, Wilson ID, Masson P, Theodoridis G, Raikos N, Coen M, Holmes E, Lindon JC, Plumb RS, Nicholson JK, Want EJ. 2011. HILIC-UPLC-MS for exploratory urinary metabolic profiling in toxicological studies. Anal Chem 83:382–390.
Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. 2011. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci USA 108:4523–4530.
Sweeny DJ, Li W, Clough J, Bhamidipati S, Singh R, Park G, Baluom M, Grossbard E, Lau DT. 2010. Metabolism of fostamatinib, the oral methylene phosphate prodrug of the spleen tyrosine kinase inhibitor R406 in humans: Contribution of hepatic and gut bacterial processes to the overall biotransformation. Drug Metab Dispos 38:1166–1176.
Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. 2009. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 1:6ra14.
Tyburski JB, Patterson AD, Krausz KW, Slavik J, Fornace AJ, Jr., Gonzalez FJ, Idle JR. 2008. Radiation metabolomics. 1. Identification of minimally invasive urine biomarkers for gamma-radiation exposure in mice. Radiat Res 170:1–14.
Tyburski JB, Patterson AD, Krausz KW, Slavik J, Fornace AJ, Jr., Gonzalez FJ, Idle JR. 2009. Radiation metabolomics. 2. Dose- and time-dependent urinary excretion of deaminated purines and pyrimidines after sublethal gamma-radiation exposure in mice. Radiat Res 172:42–57.
Villas-Boas SG, Koulman A, Lane GA. 2010. Analytical methods from the perspective of method standardization. In: Neilsen J, Jewett MC, editors. Metabolomics: A powerful tool in systems biology. Berlin: Springer. pp 11–52.
Waldram A, Holmes E, Wang Y, Rantalainen M, Wilson ID, Tuohy KM, McCartney AL, Gibson GR, Nicholson JK. 2009. Top-down systems biology modeling of host metabotype-microbiome associations in obese rodents. J Proteome Res 8:2361–2375.
Wallace BD, Wang H, Lane KT, Scott JE, Orans J, Koo JS, Venkatesh M, Jobin C, Yeh LA, Mani S, Redinbo MR. 2010. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 330:831–835.
Warburg O. 1956. On the origin of cancer cells. Science 123:309–314.
Wikoff WR, Gangoiti JA, Barshop BA, Siuzdak G. 2007. Metabolomics identifies perturbations in human disorders of propionate metabolism. Clin Chem 53:2169–2176.
Wilson ID, Nicholson JK. 2009. The role of gut microbiota in drug response. Curr Pharm Design 15:1519–1523.
Wilson ID, Nicholson JK, Castro-Perez J, Granger JH, Johnson KA, Smith BW, Plumb RS. 2005. High resolution "ultra performance" liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies. J Proteome Res 4:591–598.
Winnike JH, Li Z, Wright FA, Macdonald JM, O'Connell TM, Watkins PB. 2010. Use of pharmaco-metabonomics for early prediction of acetaminophen-induced hepatotoxicity in humans. Clin Pharmacol Ther 88:45–51.
Wishart DS. 2011. Advances in metabolite identification. Bioanalysis 3:1769–1782.
Yanes O, Woo HK, Northen TR, Oppenheimer SR, Shriver L, Apon J, Estrada MN, Potchoiba MJ, Steenwyk R, Manchester M, Siuzdak G. 2009. Nanostructure initiator mass spectrometry: Tissue imaging and direct biofluid analysis. Anal Chem 81:2969–2975.
Zhen Y, Krausz KW, Chen C, Idle JR, Gonzalez FJ. 2007. Metabolomic and genetic analysis of biomarkers for peroxisome proliferator-activated receptor alpha expression and activation. Mol Endocrinol 21:2136–2151.