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LC–MS–based metabolomics: an update

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Abstract

Liquid chromatography–mass spectrometry (LC–MS)-based metabolomics can have a major impact in multiple research fields, especially when combined with other technologies, such as stable isotope tracers and genetically modified mice. This review highlights recent applications of metabolomic technology in the study of xenobiotic metabolism and toxicity, and the understanding of disease pathogenesis and therapeutics. Metabolomics has been employed to study metabolism of noscapine, an aryl hydro-carbon receptor antagonist, and to determine the mechanisms of liver toxicities of rifampicin and isoniazid, trichloroethylene, and gemfibrozil. Metabolomics-based insights into the pathogenesis of inflammatory bowel disease, alcohol-induced liver diseases, non-alcoholic steatohepatitis, and farnesoid X receptor signaling pathway-based therapeutic target discovery will also be discussed. Limitations in metabolomics technology such as sample preparation and lack of LC–MS databases and metabolite standards, need to be resolved in order to improve and broaden the application of metabolomic studies.

Keywords

Metabolomics; Xenobiotic metabolism; Toxicology; Pathogenesis

Introduction

Metabolomics is an area that studies low Mr chemical fingerprints through an unbiased analysis of all the metabolites in biofluids (serum, urine), and extracts of cells, tissues, and organs (Johnson and Gonzalez 2012; Johnson et al. 2012a). In combination with other technologies in systems biology (e.g., genomics, transcriptomics, proteomics), the full cellular pathways can be dissected and understood. Metabolomics can give a comprehensive view of the combinatorial results of genetic factors, and xenobiotic exposure through the diet, drug administration and environment (Johnson and Gonzalez 2012). Metabolomics has found broad application in studies of xenobiotic metabolism (Chen et al. 2007) and toxicity (Robertson 2005), and in disease diagnostic biomarker discovery (Dumas et al. 2014).

The most common platforms used for metabolomics are gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and proton nuclear magnetic resonance (^1H NMR). Each platform has advantages and disadvantages

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that have been detailed in previous reviews (Patterson et al. 2010). Compared with the limited sensitivity of ^1H NMR and extensive sample preparation (e.g., derivatization) and low throughput for GC–MS, high sensitivity and ease of sample preparation make LC–MS among the most widely used platforms in metabolomics.

The combination of metabolomics with some other technologies such as stable isotope tracers and genetically modified mouse models can further broaden the scope of application. Metabolomics using steady-state concentration-based metabolite profiling sometimes does not reflect the contribution of each biochemical pathway. However, the introduction of isotopes into metabolomics can be successfully used to both monitor metabolite flux and study the metabolism of xenobiotics. Stable isotope tracers can reveal the metabolic networks. For example, LC–MS-based metabolomics with D_4 -cholic acid (CA) as a metabolic tracer, was used to successfully determine the metabolism and re-conjugation of bile acids and demonstrated that silencing of 90 % of *Slc27a5* expression significantly decreases the re-conjugation of $\text{D}(4)$ -CA to $\text{D}(4)$ -taurocholic acid ($\text{D}(4)$ -TCA) (Castro-Perez et al. 2011). Stable isotope resolved metabolomics (SIRM) was also successfully applied for pathway tracing to obtain metabolic parameters in human cancer subjects in situ (Lane et al. 2011). Stable isotope tracers have also been used to profile the metabolic fate of therapeutic drugs. After introduction of labeled drugs into the system, LC–MS was used to probe their metabolites in biological fluids. For example, three new metabolites of acetaminophen (APAP) were found through principle components analysis (PCA) of urine obtained from the mice dosed with unlabeled APAP and [acetyl- $^2\text{H}_3$] APAP (Chen et al. 2008a). A more detailed review of the application of isotope-labeled compounds in metabolism was published earlier (Mutlib 2008). Genetically modified mice are also valuable tools to understand the role of specific genes in the xenobiotic metabolism, toxicology mechanisms, or diseases pathogenesis. A metabolomics study was performed to compare the in vivo metabolism of procainamide in wild-type mice, CYP2D6-humanized mice, and human liver microsomes to demonstrate that the major metabolite *N*-acetylprocainamide in human urine was approximately 25-fold higher in humans than wild-type mice (Li et al. 2012), providing a potential clue to understanding the species difference in procainamide-induced lupus. LC–MS-based metabolomics studies have been applied in multiple research fields (Table 1), a few of which are detailed below.

LC–MS–based metabolomics study in the study of xenobiotic metabolism

Humans are frequently exposed to various xenobiotics (e.g., drugs, herbs, food, environmental pollutants), and different metabolic fates exist for xenobiotics from all these sources. Elucidation of their metabolic pathways will help to understand the beneficial (therapeutic roles) and deleterious (toxic) effects of chemicals. LC–MS-based metabolomics was first used to study drug metabolism (Plumb et al. 2003), and this method has been successfully applied to the metabolism of numerous xenobiotics.

The metabolic profile and bioactivation of nescapine

Nescapine, a clinically effective cough suppressant drug, has drawn much attention due to its anti-tumor activities toward multiple tumor types, including lymphoma, breast cancer,

melanoma, ovarian carcinoma, glioblastoma, colon cancer and non-small cell lung cancer (Mahmoudian and Rahimi-Moghaddam 2009). However, safety concerns exist for noscapine, including its potential carcinogenic properties (Schuler et al. 1999), decreased glutathione and enhanced lipid peroxidation (Aneja et al. 2004), and drug–drug interaction with warfarin (Fang et al. 2010; Zhang et al. 2013a). The metabolism of noscapine was investigated and some first-pass metabolic pathways detected, including C–C cleavage, O-demethylation, and cleavage of methylenedioxy group (Tsunoda and Yoshimura 1981). Yet, limitation exists on the methodology to obtain a more complete metabolic profile of this agent. Recently, the complete metabolic profile of noscapine was elucidated using ultra-performance chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOFMS)-based metabolomics (Fang et al. 2012). Besides the metabolites previously observed, novel metabolites were found after oral gavage of noscapine (300 mg/kg), including an *N*-demethylated metabolite, two hydroxylated metabolites, one metabolite undergoing both demethylation and cleavage of the methylenedioxy group, and a bis-dem-ethylated metabolite. Additionally, new glucuronide conjugates were detected (Fig. 1). One reactive ortho-quinone metabolite was identified that might be involved in the clinical noscapine–warfarin interaction and the potential toxicity risk of noscapine.

In vivo application of the pure aryl hydrocarbon receptor antagonist GNF-351 is limited to the gastrointestinal track

Development of aryl hydrocarbon receptor (AHR) antagonist is an area of great interest given the role of this receptor in toxicology and physiology. GNF-351 is a recently developed AHR antagonist with the capacity to inhibit dioxin response element (DRE)-dependent and independent activity (Smith et al. 2011). Given the important role of pharmacokinetic properties toward the development of the therapeutic agents, UPLC-ESI-QTOFMS-based metabolomics study was employed to evaluate the absorption and metabolic behavior of GNF-351. GNF-351 was poorly absorbed into the blood after oral administration with nearly all of the parent compound and its metabolites appearing in the feces and not in blood and urine (Fig. 2). Several phase I metabolites were produced by human and mouse microsomes, including two oxidized (VI and VII) and one tri-demethylated (VIII) metabolite of GNF-351. UGT1A4-catalyzed glucuronidation of GNF-351 also exhibited species differences. Additionally, profiling the metabolic enzymes indicated that the major enzymes involved in metabolism of GNF-351 were in the CYP1A family that are encoded by target genes regulated by AHR, which serve to accelerate metabolism of GNF-351 and further weaken the antagonism activity of GNF-351. In this study, metabolomics was successfully applied to demonstrate the limitation of a potential drug candidate by its poor metabolism and absorption behavior (Fang et al. 2014).

LC–MS–based metabolomics study mechanisms of xenobiotic toxicity

Metabolomic approaches have been increasingly employed as an important tool in toxicology. Through a comprehensive analysis of metabolomes, the alterations in response to toxicity stressors can be detected, and clues to the mechanism of toxicity can be determined

through correlating the relationship between the chemical perturbations and the influenced biochemical pathways. Compared with the application of other omics technologies, genomics, transcriptomics, and proteomics, in toxicity studies, metabolomics-based study results reflect the final results induced by a cascade of toxic events, which are closer with the toxic phenotype.

Mechanism of liver toxicity induced by co-therapy with rifampicin and isoniazid

Both rifampicin (RIF) and isoniazid (INH) are first-line anti-tuberculosis drugs, and the co-therapy with these two drugs in humans can result in the liver toxicity. This toxicity is difficult to reproduce in rats and mice, and the mechanism of hepatotoxicity has remained elusive. In a recent study, pregnane X receptor (PXR) humanized (hPXR) were engaged to successfully reproduce the liver toxicity induced by co-treatment with rifampicin (RIF) and isoniazid (INH); toxicity was not observed in wild-type mice and *Pxr*-null mice. This is due to the fact that mouse PXR is not activated by pharmacological concentrations of rifampicin in mice, while this drug readily activates human PXR. Additionally, liver toxicity was not observed in hPXR mice treated with either RIF or INH; both drugs need to be administered simultaneously. To provide mechanistic clues on the RIF, INH-co-therapy-induced liver toxicity, bile metabolomics was adopted revealing extremely high concentrations of an important intermediate in porphyrin biosynthesis protoporphyrin IX (PPIX) in hPXR mice treated with RIF and INH, which might be caused by PXR-regulated over-expression of aminolevulinic acid synthase (ALAS).

The potential mechanism of toxicity is RIF activation of PXR and induction of the PXR target gene *Alas* which leads to an increase in PPIX levels. In a mechanism that has yet to be determined, INH could inhibit the downstream enzyme leading to heme production that causes a backup in the pathway and a massive toxic increase in PPIX (Fig. 3). This study is an example of the use of metabolomics and genetically modified mice to provide a new paradigm to understand hepatotoxicity mechanisms associated with drug therapy in humans (Li et al. 2013b).

Major contribution of trichloroacetate (TCA) toward trichloroethylene (TCE)-induced biomarkers alteration

Trichloroethylene (TCE), a chlorinated solvent, has been widely used for degreasing metals and dry cleaning (Bakke et al. 2007). Exposure to TCE can induce severe health problems, due to multiple organs damage, including liver, kidney and the immune system toxicities, and carcinogenesis toward multiple types of cancers (e.g., renal cell carcinoma, non-Hodgkin's lymphoma, liver or biliary cancer). Toxicity has been attributed to the metabolism of TCE to its major metabolites dichloroacetate (DCA) and trichloroacetate (TCA). However, the mechanism of toxicity and the contribution of DCA and TCA toward TCE toxicity remain unclear. Metabolomics revealed that TCE treatment resulted in a decrease in urine of endogenous metabolites related to fatty acid metabolism, possibly a result of TCE-induced expression of fatty acid metabolism-associated peroxisome proliferator-activated

receptor α (PPAR α) target genes (Fig. 4). TCE treatment also disrupted the homeostasis of serum phospholipids as revealed by increased serum lysophosphatidylcholine (LPC) 18:0, LPC 18:1 (9Z), and phosphatidylcholine (PC) metabolites. Additionally, through comparison of metabolomics alteration induced by TCA and DCA, the more comparable metabolites profile alteration was observed for TCA than DCA, indicating more contribution of TCA toward TCE-induced toxicity (Fang et al. 2013).

PPAR α -dependent disruption of homeostasis of lysophosphatidylcholine and bile acid induced by gemfibrozil

Gemfibrozil, one of the most widely prescribed anti-dyslipidemia fibrate drugs, was reported to induce many adverse effects, including alterations of liver function, cholestatic jaundice, and cholelithiasis in some patients. However, the mechanisms of these toxicities remain unclear. A combination of UPLC-MS-based metabolomics and *Ppara*-null mice was used to elucidate the mechanism of gemfibrozil-induced hepatotoxicity. Disrupted homeostasis of bile acids and phospholipids was detected, and this alteration was PPAR α -dependent (Liu et al. 2014), which is closely correlated with the alteration of the genes involved in the metabolism and transport of LPC and bile acids compounds (Fig. 5). This study provides a new insight into the mechanism of gemfibrozil-induced hepatotoxicity using the combination of LC-MS-based metabolomics and traditional toxicological strategies.

Mechanism identification of cadmium (Cd) toxicity through analysis of integrated redox proteomics and metabolomics of mitochondria

Cd exposure leads to hepatotoxicity, nephrotoxicity, pulmonotoxicity, neurotoxicity, bone toxicity, and even carcinogenesis. Metabolomics showed that Cd exposure can result in alteration of levels of branched-chain amino acid (BCAA) and carnitine metabolites, indicating disruption of amino acid and fatty acid metabolism. Furthermore, redox proteomics using isotope-coded affinity tag (ICAT) combined mass spectrometry was adopted to analyze the redox states of liver mitochondrial proteins, revealing alterations in 24 Cys in proteins functioning in branched-chain amino acid (BCAA) metabolism and 14 Cys in proteins functioning in fatty acid (acylcarnitine/carnitine) metabolism, which closely correlated with the metabolomics results. Based on the above results, the conclusion was drawn that Cd-induced hepatotoxicity was due in part to alterations in mitochondrial protein redox state and metabolites (Go et al. 2014). This study is a good example of elucidating toxicity mechanism using a combination of proteomics and metabolomics.

Metabolomics to understand the pathogenesis and related therapeutic targets of diseases

Metabolomics can give an accurate description of the bio-chemical profiles of blood and tissues, which can facilitate an understanding of the alterations in complex biological networks involved in diseases.

Metabolomics to elucidate the disease mechanism of inflammatory bowel disease (IBD) and colon cancer

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract and includes ulcerative colitis and Crohn's disease. To date, although multiple factors such as environmental stress, microbial insults, and auto-immunity, have been reported to contribute to the etiology of IBD (Schreiber and Hampe 2000; Xavier and Podolsky 2007), the mechanism of IBD is considered diverse and inadequately described. LC-MS-based metabolomic analysis of deproteinized serum from control and dextran sulfate sodium (DSS)-treated mice was carried out revealing that experimental IBD significantly shifted the balance between saturated LPC (LPC 18:0) and unsaturated LPCs (LPC 18:1, LPC 18:2), which results from the inhibition of stearoyl-CoA desaturase 1 (SCD1) activity in liver. This alteration exacerbates pro-inflammatory responses, which is considered to be a key event in the DSS-induced IBD model; this was supported by the evidence that *Scd1*-null mice are more susceptible to DSS treatment than wild-type mice (Chen et al. 2008b). A combination of metabolomics and a corresponding mouse model was further employed to explore the role of nuclear receptors in IBD. Treatment with rifaximin, a human intestine-specific PXR activator, significantly protects against DSS-induced and trinitrobenzene sulfonic acid (TNBS)-induced IBD in hPXR mice but not in wild-type and *Pxr*-null mice. Serum metabolomics revealed a similar metabolomics profile among rifaximin-treated wild-type, *Pxr*-null and hPXR mice, indicating that rifaximin may be acting locally in the colon and not systemically to decrease the severity of DSS-induced colitis (Cheng et al. 2010). An intestine-specific vitamin D receptor (VDR) knockout mouse and showed that disruption of the intestinal *Vdr* gene exacerbated the IBD, as indicated by high score of IBD symptoms, severe damage of the intestine mucosal layer and increased expression of genes encoding pro-inflammatory cytokines (Kim et al. 2013). Furthermore, feces metabolomics showed decreased concentrations of taurine and taurine-conjugated bile acids in intestine-specific vitamin D receptor knockout mice, indicating disruption of taurine metabolism by IBD. Given that taurine plays an important role in anti-inflammation and taurine treatment can ameliorate the symptoms of IBD, the metabolomics comparison between wild-type mice and intestine-specific vitamin D receptor knockout mice demonstrated the mechanism by which loss of VDR expression in the intestine exacerbated IBD.

Colon cancer remains a leading cause of cancer mortality worldwide. Metabolomics was used to analyze the serum metabolic profiles between healthy volunteers and colon cancer patients, revealing a significant alteration of amino acid profiles (Leichtle et al. 2012). Urine metabolomics analysis of patients with colon cancer indicated increased tryptophan metabolism and altered tricarboxylic acid cycle and gut microflora metabolism in patients (Qiu et al. 2010). In another study, metabolomics revealed differences in urine metabolites between wild-type and *Apc*^{min/+} mice (mice bearing heterozygous mutation in adenomatous polyposis coli gene that develops multiple intestinal neoplasia) and found alteration of metabolites related to polyamine metabolism, nucleic acid metabolism, and methylation. The changes in these metabolites were also found in mice with azoxymethane-induced tumors and in tumor tissues of colon cancer patients, indicating the involvement of these metabolic pathways in the pathogenesis of colon cancer (Manna et al. 2014).

Metabolomics to reveal the pathogenesis of liver diseases

Since various liver diseases have become a leading reason for deaths in the USA and the world, metabolomics was applied to explore the pathogenesis of experimentally induced liver disorders. Metabolomics profiling was carried out on urine obtained from alcohol-treated wild-type and *Ppara*-null mice. Besides some common metabolites (e.g., ethyl sulfate, ethyl- β -D-glucuronide, 4-hydroxy-phenylacetic acid, 4-hydroxyphenylacetic acid sulfate, 2-hydroxyphenylacetic acid, adipic acid, pimelic acid) identified in alcohol-treated wild-type and *Ppara*-null mice, one specific metabolite, indole-3-lactic acid, was observed to only increase in alcohol-treated *Ppara*-null mice (Manna et al. 2010). The proposed mechanism is that the loss of PPAR α impairs conversion of tryptophan to NAD⁺ through quinolinic acid and fatty acid β -oxidation. The oxidation of alcohol to acetaldehyde and acetic acid resulted in a reduction in the ratio of NAD⁺/NADH, followed by accumulation of free fatty acid in the liver. The increased indole-3-lactic acid in urine was due to increased reduction of indole-3-pyruvic acid to indole-3-lactic acid driven by the buildup of NADH.

Non-alcoholic steatohepatitis (NASH), a progressive form of non-alcoholic fatty liver disease, can develop into cirrhosis, hepatic failure, and hepatocellular carcinoma. Serum metabolomics showed significant decreases in palmitoyl-, stearoyl-, and oleoyl-lysophosphatidylcholine (LPC), and increase in tauro- β -muricholate, taurocholate, and 12-hydroxyeicosatetraenoic acid (12-HETE) in mice treated with a methionine and choline-deficient (MCD) diet that produces a liver pathology mimicking NASH. The relevant mechanism is the pro-inflammation cytokine regulation of genes involved in LPC degradation and the synthesis and transport of bile acids (Tanaka et al. 2012). Others also demonstrated an increase in azelaic acid-mono ester in serum that resulted from attenuation of hepatic carboxy-lesterase 3 (CES3) expression in the MCD-treated NASH model (Matsubara et al. 2012). Monitoring metabolites related to energy production in both wild-type mice and estrogen-related receptor α (ERR α) knockout mice showed that loss of orphan nuclear receptor estrogen-related receptor α (ERR α) promoted the necrosis of hepatocytes in response to carcinogen due to a deficiency of energy production, which also helps the development of agents (e.g., rapamycin) for chemoprevention of hepatocarcinogenesis through decreasing the level of ERR α (Hong et al. 2013).

Microbiome remodeling results in the inhibition of intestinal farnesoid X receptor (FXR) signaling and decreased obesity

Metabolomics was applied to find a new therapeutic target of obesity (Li et al. 2013a). The influence of antioxidant tempol on the genus *Lactobacillus*-mediated bile salt hydrolase (BSH) activity resulted in the accumulation of bile acids including tauro- β -muricholic acid (T β MCA) in intestine. T β MCA was found to be an antagonist of FXR, and inhibition of FXR signaling was thought to mediate the anti-obesity effects of tempol (Fig. 6). The role of FXR signaling in obesity was demonstrated by use of mice lacking this receptor in the intestine; tempol treatment does not have an anti-obesity effect in intestine-specific *Fxr*-null mice and suggest that targeting intestinal FXR with an FXR antagonist could be a promising anti-obesity therapy.

Challenges for the utilization of metabolomics in xenobiotic metabolism, toxicology mechanism, and diseases pathogenesis

Sample preparation in metabolomics studies

Proper sample preparation is vital for a successful metabolomics study. Inadequate sample handling and storage can compromise the reliability of metabolomics data. Different extraction protocols were reported to result in different metabolite profiles and the corresponding interpretation of metabolic pathways (Duportet et al. 2012). Other studies evaluated the pre-analytical aspects and sample quality assessment in metabolomics studies of human blood, and showed that many factors were important for the final results, including even placement of samples in ice water, the utilization of EDTA plasma, and the preferred use of non-refrozen biobank samples (Yin et al. 2013). Sample preparation is particularly important for the ability to compare data between laboratories. Therefore, development of unselective, reproducible, simple, and fast sample preparation methods should be an area of immediate concern.

Impact of the lack of metabolite standards and LC-MS databases

In order to accurately identify and quantify ions discovered by metabolomics, standards should be available. Metabolomics-based xenobiotic metabolism study often identifies metabolites with low abundance, and the MS/MS data are further employed to aid in the prediction of metabolite structures through the analysis of MS/MS fragmentation patterns (Chen et al. 2007). However, this method can-not be used for some compounds in which it is difficult to obtain many MS/MS fragments. Additionally, some metabolites can produce the same MS/MS fragmentation pattern, which can be used in the prediction of metabolite structures that are difficult to decipher. Therefore, obtaining metabolite standards is vital for proper structural identification. In the absence of a commercially available standard, chemical synthesis and purification of the predicted structure based on the accurate mass and MS/MS fragmentation pattern of an ion must be carried out where feasible. However, in xenobiotic metabolism studies, this is not practical nor is it necessary. For example, as shown above, 22 metabolites of nescapine were found in the urine of treated mice, many of which are of low abundance. However, knowing the structure of the parent compound and the conjugates makes prediction possible, but precise stereochemistry cannot be determined. Thus, it is not necessary to synthesize all possible metabolites in order to produce a metabolic map of the drug. Indeed, synthesis of all the metabolites of a particular xenobiotic is nearly impossible and costs considerable time and money. In addition, for some metabolites, the synthesis is especially difficult, such as glucuronides, glutathiones, and oxidates, and the introduction of chirality (Walker et al. 2011). Another method to obtain the metabolite standards is to isolate the compound from an in vitro metabolic incubation system or from in vivo biological fluids (e.g., urine, bile). However, this method cannot be used to prepare metabolite standards present at low abundance (Espina et al. 2009).

It is vitally important to determine the structures of in vivo biomarkers associated with diseases. Although the structures can be predicted with the combination of accurate masses, MS/MS data, and several databases (e.g., Human Metabolome Database (HMDB), METLIN, ChemDB), standards are ultimately needed for comparison of the retention times

and MS/MS fragmentation patterns with the ion in question and for accurate quantitation. Additionally, endogenous metabolites are more complex than xenobiotic metabolites, with over tenfold lower magnitude concentrations found in vivo. Therefore, it is a major challenge to cover a wide concentration range and measure metabolites present at trace levels.

Conclusions

The compilation of recent metabolomic studies in xenobiotic metabolism and toxicity, and biomarkers for diseases pathogenesis and mechanism, demonstrate the power and importance of LC–MS-based metabolomics studies. Metabolomics can give a wealth of information, especially when combined with isotopes tracers and genetically modified mice and integrated with a systems biology analysis. Some limitations such as sample preparation, limited metabolite standards, and more comprehensive development of LC–MS databases are areas that required attention.

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Abbreviations

APAP	Acetaminophen
ALAS	Aminolevulinic acid synthase
AHR	Aryl hydrocarbon receptor
BSH	Bile salt hydrolase
CES3	Carboxylesterase 3
CYP	Cytochrome P450
CVD	Cardiovascular diseases
DSS	Dextran sulfate sodium
DCA	Dichloroacetate
DRE	Dioxin response elements
FXR	Farnesoid X receptor
GC–MS	Gas chromatography–mass spectrometry
GVHD	Graft-versus-host disease
IBD	Inflammatory bowel disease
INH	Isoniazid

LC-MS	Liquid chromatography–mass spectrometry
LPC	Lysophosphatidylcholine
GNF351	<i>N</i> -(2-(1H-Indol-3-yl)ethyl)-9-isopropyl-2-(5-methylpyridin-3-yl)-9H-purin-6-amine
NASH	Non-alcoholic steatohepatitis
PPARα	Peroxisome proliferator-activated receptor α
PC	Phosphatidylcholine
PCA	Principle components analysis
PCOS	Polycystic ovary syndrome
PXR	Pregnane X receptor
^1H NMR	Proton nuclear magnetic resonance
PPIX	Protoporphyrin IX
RIF	Rifampicin
SCD1	Stearoyl-CoA desaturase 1
TβMCA	Tauro- β -muricholic acid
TNBS	Trinitrobenzene sulfonic acid
TCA	Trichloroacetate
TCE	Trichloroethylene
VDR	Vitamin D receptor

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Fig.1.

Metabolic mapping of noscapine by use of metabolomics. Novel metabolites discovered by UPLC-ESI-QTOFMS-based metabolomics (e.g., metabolomics based on ultra-performance liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry) are shown by *red arrows*

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Fig.3.

Metabolomics reveals the mechanism of liver toxicity induced by co-therapy with rifampicin and isoniazid. RIF activates human PXR and induction of the PXR target gene *Alas* leading to increase in protoporphyrin IX (PP-IX) levels. In a mechanism that has yet to be determined, INH could inhibit the downstream enzyme ferrochelatase leading to less heme production and a massive toxic increase in PP-IX

Fig.4.

Major contribution of trichloroacetate (TCA) in trichloroethylene (TCE)-induced biomarkers alteration. TCE can be metabolized to form two PPAR α agonists dichloroacetate (DCA) and TCA. The up-regulated expression genes by TCA activation of PPAR α results in increased metabolism of fatty acids that results in lower levels of urinary metabolites that reflect fatty acid. TCE-induced hepatomegaly induces altered phospholipid homeostasis in serum

Fig.5. Metabolomics reveals that PPAR α mediated gemfibrozil-induced hepatotoxicity. The therapeutic role of gemfibrozil can be explained by PPAR α -induced expression of target genes involved in fatty acid β -oxidation. PPAR α -dependent regulation of the metabolic genes involved in the LPC and bile acid metabolism and/or transport result in alteration of downstream metabolic pathways

Fig.6.

Metabolomics reveals the importance of intestinal farnesoid X receptor (FXR) signaling pathway in obesity. Tempol alters the gut microbiome by preferentially killing *Lactobacillus* and which decreased bile salt hydrolase activity (BSH), which hydrolyzes tauro- β -muricholic acid (T β MCA) that is formed in liver as a result of cholesterol (Chol) metabolism. *Lactobacillus* BSH efficiently deconjugates T β MCA to MCA. Lower intestinal *Lactobacillus* BSH results in increased T β MCA that inhibits intestinal FXR signaling resulting in decreased obesity in high-fat diet-fed mice

Table 1

List of metabolomics application in the study of xenobiotic metabolism, toxicology mechanisms, and the pathogenesis and of diseases

Xenobiotic/diseases	Model	Key finding	References
Isoniazid	Humans	7 Novel metabolites	(Li et al. 2011)
Ethanol	Wild-type and <i>Cyp2e1</i> -null mice	A novel metabolite <i>N</i> -acetyltaurine	(Shi et al. 2012)
α -Tocopherol	Wild-type mice	3 Novel metabolites	(Johnson et al. 2012b)
Cocaine	Wild-type mice and rats	Species-dependent metabolism of cocaine	(Yao et al. 2013)
Tempol	Wild-type mice	8 Novel metabolites	(Li et al. 2013c)
Saquinavir	<i>In vitro</i> system, wild-type and <i>Cyp3a4</i> -null mice	20 Novel metabolites and one reactive metabolite α -hydroxyaldehyde	(Li et al. 2014a)
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	Wild-type, <i>Ahr</i> ^{-/-} , and <i>Ahr</i> ^{Liv} mice	The role of CES3, TGF β in TCDD-induced steato-hepatitis	(Matsubara et al. 2012)
Rifaximin	Wild-type, Pxr-null, and hPXR mice	Long-term exposure of rifaximin-induced hepatic steatosis in hPXR mice	(Cheng et al. 2012)
Acetaminophen	Wild-type, <i>Ppara</i> -null, <i>Ucp2</i> -null Mice	The protection of APAP-induced hepatotoxicity by PPAR α -induced expression of UCP2	(Patterson et al. 2012)
CCl ₄	Rats	Oxidative stresses to multiple rat organs	(Jiang et al. 2012)
Acetaminophen	Wild-type, and <i>Txnrd1</i> ^{Liv} mice	Important role of Txnrd1 in APAP-induced hepatotoxicity	(Patterson et al. 2013)
Buprenorphine	Wild-type mice	Key role of GABA receptor signaling pathway in cerebral lesion	(Zhang et al. 2013b)
Isoniazid	Wild-type and <i>Cyp2e1</i> -null mice	The involvement of bile acids accumulation and mitochondria β -oxidation in isoniazid toxicity	(Cheng et al. 2014)
Lithocholic acid	Wild-type, Tg-3A4, <i>Vdr</i> ^{IEpC} and <i>Vdr</i> ^{IEpC/3A4} mice	Protection role of intestinal CYP3A4 in LCA-induced hepatotoxicity	(Cheng et al. 2013)
3-Chloropropane-1,2-diol	Rats	The alteration of indoxyl sulfate, xanthurenic acid, phenylacetylglutamine, non-anedioic acid, and taurine	(Li et al. 2013e)
Aflatoxin B1	Rats	Gluconeogenesis and lipid metabolism disorder in aflatoxin B1-induced acute hepatotoxicity	(Lu et al. 2013)
Bisphenol a	Rats	DNA methylation damage, disrupted choline pathway	(Chen et al. 2014)
Type 2 diabetes mellitus	Monkey, mice	The important role of SLC6A20 kidney transporter in type 2 diabetes mellitus	(Patterson et al. 2011a)
Hepatocellular carcinoma	Human	Aberrant lipid metabolism in hepatocellular carcinoma	(Patterson et al. 2011b)
Autosomal dominant polycystic kidney disease	Wild-type and <i>Pkd1</i> ^{cko} mice	The important role of Pkd1 in autosomal dominant polycystic kidney disease	(Menezes et al. 2012)
Cholestatic liver disease	Wild-type and <i>Acb1</i> ^{Liv} -null mice	Impaired mitochondrial fatty acid β -oxidation in <i>Acb1</i> ^{Liv} -null mice precedes cholestasis	(Zhang et al. 2012)

Xenobiotic/diseases	Model	Key finding	References
Squamous cell carcinoma	SCCVII-tumor-bearing mice	Liver dysfunction induced by tumor growth imposed inflammatory response	(Li et al. 2013d)
Breast cancer	Human	Altered FA β -oxidation in patients with breast cancer	(Shen et al. 2013)
Pseudoxanthoma elasticum	Wild-type and ABCC6-null mice	The important role of circulated pyrophosphate via ABCC6-dependent mechanism	(Jansen et al. 2013)
Type 2 diabetes mellitus	Human	The key role of 2-aminoadipic acid in type 2 diabetes mellitus	(Wang et al. 2013)
Prostate cancer	Human	Biochemical alterations associated with cell growth, energetics, stress	(McDunn et al. 2013)
Pancreatic tumor	Pdx-Cre <i>Kras^{LSL-G12D}</i> mice, <i>p53^{fl/fl}</i> , <i>Age⁷ fl/fl</i> , <i>Age⁵ fl/fl</i>	p53 status determines the role of autophagy in pancreatic tumor development	(Rosenfeldt et al. 2013)
Alzheimer's disease	Human	Disturbance of phospholipids metabolism	(Gonzalez-Dominguez et al. 2014)
Acute respiratory distress syndrome (ARDS)	Human	The alteration of amino acid metabolism, glycolysis and gluconeogenesis, fatty acid biosynthesis, phospholipids, and purine metabolism	(Evans et al. 2013)
Gastric ulcer	Rats	The disruption of sphingophospholipid and fatty acid metabolism pathway	(Tianjiao et al. 2014)
Graft-versus-host disease (GVHD)	Mice	Early dysregulation of host hepatic GSH metabolism and oxidative stress	(Suh et al. 2014)
Rhegmatogenous retinal detachment	Human	The disruption of histidine metabolism and citrate cycle	(Li et al. 2014b)
Cardiovascular diseases (CVD)	Human	The important role of mitochondrial damage and dysfunction in CVD	(Rizza et al. 2014)
Polycystic ovary syndrome (PCOS)	Human	Abnormalities of lipid- and androgen-metabolism in PCOS patients	(Zhao et al. 2014)
Pulmonary embolism	Pigs	Involvement of many metabolic pathways (e.g., glycolysis, TCA cycle)	(Bujak et al. 2014)