Regulation of drug metabolism and toxicity by multiple factors of genetics, epigenetics, lncRNAs, gut microbiota, and diseases: a meeting report of the 21st International Symposium on Microsomes and Drug Oxidations (MDO)

Ai-Ming Yu, *University of California Davis*
Magnus Ingelman-Sundberg, *Karolinska Institutet*
Nathan J. Cherrington, *University of Arizona*
Lauren M. Aleksunes, *Rutgers State University*
Ulrich M. Zanger, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology*
Wen Xie, *University of Pittsburgh*
Hyunyoung Jeong, *University of Illinois*
Edward T Morgan, *Emory University*
Peter J. Turnbaugh, *University of California San Francisco*
Curtis D. Klaassen, *University of Washington*

Only first 10 authors above; see publication for full author list.

MEETING REPORT

Regulation of drug metabolism and toxicity by multiple factors of genetics, epigenetics, lncRNAs, gut microbiota, and diseases: a meeting report of the 21st International Symposium on Microsomes and Drug Oxidations (MDO)

Ai-Ming Yu[a,*], Magnus Ingelman-Sundberg[b], Nathan J. Cherrington[c], Lauren M. Aleksunes[d], Ulrich M. Zanger[e,f], Wen Xie[g], Hyunyoung Jeong[h], Edward T. Morgan[i], Peter J. Turnbaugh[j], Curtis D. Klaassen[k], Aadra P. Bhatt[l], Matthew R. Redinbo[l], Pengying Hao[m], David J. Waxman[m], Li Wang[n], Xiao-bo Zhong[o,*]

[a]Department of Biochemistry and Molecular Medicine, School of Medicine, University of California at Davis, Sacramento, CA 95817, USA
[b]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
[c]Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA
[d]Department of Pharmacology and Toxicology, School of Pharmacy, Rutgers University, Piscataway, NJ 08854, USA
[e]Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany
[f]Eberhard Karls University, Tuebingen, Germany
[g]Center of Pharmacogenomics and Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA
[h]Department of Pharmacy Practice, School of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA
[i]Department of Pharmacology, School of Medicine, Emory University, Atlanta, GA 30322, USA
[j]Department of Microbiology and Immunology and the George William Hooper Research Foundation, University of California at San Francisco, San Francisco, CA 94143, USA
[k]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA 98195, USA
[l]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[m]Department of Biology and Bioinformatics Program, Boston University, Boston, MA 02215, USA
[n]Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269, USA
[o]Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT 06269, USA

*Corresponding authors. Tel.: +1 916 734 1566 (Ai-Ming Yu), +1 860 486 3697 (Xiao-bo Zhong).
E-mail addresses: aimyu@ucdavis.edu (Ai-Ming Yu), xiaobo.zhong@uconn.edu (Xiao-bo Zhong).

KEY WORDS

Drug metabolism and toxicity;
Genetics;
Epigenetics;
Gut microbiota;
Long non-coding RNAs;
Disease;
Personalized medication

Abstract Variations in drug metabolism may alter drug efficacy and cause toxicity; better understanding of the mechanisms and risks shall help to practice precision medicine. At the 21st International Symposium on Microsomes and Drug Oxidations held in Davis, California, USA, in October 2–6, 2016, a number of speakers reported some new findings and ongoing studies on the regulation mechanisms behind variable drug metabolism and toxicity, and discussed potential implications to personalized medications. A considerably insightful overview was provided on genetic and epigenetic regulation of gene expression involved in drug absorption, distribution, metabolism, and excretion (ADME) and drug response. Altered drug metabolism and disposition as well as molecular mechanisms among diseased and special populations were presented. In addition, the roles of gut microbiota in drug metabolism and toxicology as well as long non-coding RNAs in liver functions and diseases were discussed. These findings may offer new insights into improved understanding of ADME regulatory mechanisms and advance drug metabolism research.

1. Introduction

Efficacy and safety are two elements that determine the utility of a therapeutic drug for the treatment of a particular disease. Drug efficacy and safety profiles are governed by how the drug interacts with on-target and off-target molecules. Furthermore, how the drug is processed in the body will inevitably affect the accessibility of drug to its targets, by which the former consists of a number of critical processes namely absorption, distribution, metabolism and excretion (ADME). Mechanistically, drug-metabolizing enzymes and transporters (DMET) are the molecular determinants of the ADME processes. Therefore, pharmaco-/toxico-kinetic properties of a drug, such as bioavailability, half-life and exposure, are dictated by the interactions of the drug with DMETs, which will ultimately determine drug efficacy and safety profiles following their actions on targets.

Variations in drug ADME or pharmacokinetics are common among a defined population as well as various groups, which may alter drug efficacy or cause toxicity[1–3]. Indeed, the inhibition or activation of DMET protein functions often leads to remarkable variations in pharmacokinetics and consequently drug efficacy or toxicity. Secondly, changes in transcriptional gene expression of DMETs, which are governed by xenobiotic receptors or transcription factors in cells, have been revealed as another major cause of variations in drug metabolism and toxicity. Thirdly, genetic variations of ADME genes could lead to significant changes in DMET expression or activity in the metabolism and transport of drugs. In addition, recent studies have demonstrated that many other factors such as epigenetics, noncoding RNAs (ncRNAs), and gut microbiota[4–9] may modulate ADME gene expression and cause variations in drug metabolism and toxicity.

The International Symposia on Microsomes and Drug Oxidations (MDO) are a well known series of conferences in the field of drug metabolism and related areas (https://mdo.ki.se/). The 21st MDO conference was held in Davis, California, USA, in October 2–6, 2016, to provide a unique opportunity for investigators and scientists to interact with each other. The programs consisted of 1 keynote lecture, 4 plenary lectures, 24 parallel sessions, and 120 posters. With a focus on the most recent advances in the fields of drug metabolism, the programs covered a wide range of important topics, including DMET structure and function, ADME gene regulation, drug development, and clinical pharmacology and toxicology. Some new merging fields, such as gene editing, gut microbiota, metabolomics, and lncRNAs, were also included. This report is to summarize some talks presented at the 21st MDO conference that are related to the regulation of drug metabolism and toxicity, particularly by multiple factors of genetics, epigenetics, lncRNAs, gut microbiota, and diseases.

2. Plenary lecture on "genetic and epigenetic regulation of ADME gene expression and drug response" by Dr. Magnus Ingelman-Sundberg

Dr. Ingelman-Sundberg started off by reviewing the current important pharmacogenomic biomarkers used in clinical medicine and in guidance by European Medicines Agency (EMA) and The US Food and Drug Administration (FDA). About 15% of medical products approved by EMA and 138 medicines approved by FDA contain pharmacogenomic labels. The most important pharmacogenomic biomarkers are related to genes encoding HLA molecules, enzymes, transporters, drug targets, specific markers and mutations in the somatic genome where mutations in the genes of *ABL*, *ALK*, *BRAF*, *EGRF*, *HER2*, *KRAS*, *KIT*, and *MET* are of importance for selection of anticancer therapy.

Dr. Ingelman-Sundberg then explained the developmental origin of human polymorphisms. He pointed out genetic drift and genetic selection as the most important bases for the occurrence of today's polymorphism. He gave examples of genetic selection from the animal world where tolerance to new environments has been developed through selection for *CYP* gene inducibility, expression and substrate specificities. They are exemplified, *e.g.*, by the microsatellite 100-fold amplification of *CYP6CY3* in *Myzus persicae* as adaptation for the tobacco plant as host, since the corresponding enzyme is active in nicotine metabolism. The genetic selection was also exemplified comparing the difference between mice and humans in alkaloid detoxification as well as the evolution of the *CYP2D6* duplication in North East Africa.

Dr. Ingelman-Sundberg further presented the €15 million Ubiquitous Pharmacogenomics project (http://upgx.eu/) that will

run until December 31, 2020 in 10 different EU countries. Pharmacotherapy of 8000 patients in 7 different clinical centers will either be based on pharmacogenetic parameters or conducted in the standard mode for 18 months each. In addition, outliers will be more thoroughly analyzed using next generation sequencing.

Dr. Ingelman-Sundberg then discussed at length the influence of rare mutations on drug pharmacokinetics and effects. He concluded that rare mutations might be absent in some populations but indeed common in specific geographical regions as exemplified by *CYP3A4*20* which is only expressed in some parts of Spain, and is important for, *e.g.*, paclitaxel treatment regimen in carriers of this allele. He presented data from several papers where they had presented the number and types of rare mutations in 57 *P450* genes and 146 genes encoding other phase I, phase II enzymes, transporters, and nuclear receptors mainly from human exome data bases. He concluded that as a whole the number of variants per kb gene was similar among transporters and phase II enzymes, but that severe differences exist in the number of rare mutations in different genes. Thus among the ABC transporter family, the *ABCA4* gene carried 370 variant alleles, whereas only 40 variant alleles were identified in the *ABCB7* gene. Similar differences occurred in the other classes of ADME genes analyzed. He presented data regarding the contribution of rare alleles to the overall fractional variability of different genes and concluded that in many genes the role of rare variants is very high. Among the P450 genes, *CYP1A2*, *CYP2A6*, *CYP2C19*, and *CYP3A4* genes have a high contribution of rare alleles to the genetic variations and overall it can be calculated that the rare variants contribute to 30%–40% of the inherited variability in drug pharmacokinetics.

He reviewed data from the ExAc project[10], analyzing the exomes from 60,706 humans where they conclude that in the exomes each 8th nucleotide is subject to polymorphism and that 55% of all 7.5 million mutations identified were present as singletons. He reviewed results by Kerb and Schwab[11] from twins studies showing that only 40% of the genetic variability of the pharmacokinetics of torsemide or metoprolol can be explained by known polymorphisms. He concluded that overall both the analyses of different rare mutations in the different ADME genes and the results from the twin studies indeed indicate that by using standard analyses for polymorphic genes only 50%–60% of the true genetic variation can be identified. He elaborated how this information will influence future pharmacogenetic testing of patients.

Dr. Ingelman-Sundberg then presented his laboratory's novel system for culturing viable liver cells for 5 weeks in a 3D spheroid system[12]. The proteome of the spheroid to a great extent mimic the proteome in the liver from where they were isolated, whereas cells cultivated in 2D had a very different proteome. In the spheroid system it was thus possible to study true inter-individual variation. He presented the use of this system in *in vitro* disease models including steatosis and cholestasis. It was also shown that non-parenchymal cells integrated well in the 3D spheroids for long periods of time and resulted in higher responsivity to pro-inflammatory stimuli, *e.g.* lipopolysaccharide (LPS). Dr. Ingelman-Sundberg laboratory's data indicate that the spheroid system is very suitable for analyzing chronic drug toxicity in many cases.

Dr. Ingelman-Sundberg ended his lecture by presenting data from single nucleotide resolution analyses of ADME genes in human liver[13] and could conclude that 5 hmC is very important in liver and that the bisulfite method does not provide a true epigenetic analyses. Furthermore, the data revealed that ADME gene expression is related to the overall presence of 5 mC and 5 hmC in the open reading frame of the gene, but not by specific elements with altered composition of 5 mC and 5 hmC.

3. Parallel session on "disease effect on drug metabolism and disposition", chaired by Drs. Wen Xie and Lauren M. Aleksunes

3.1. Regulation of drug transporters and drug disposition by fatty liver disease, Nathan J. Cherrington from University of Arizona, USA

Numerous drug-induced and environmental exposure–related toxicities are the result of inter-individual variations in the ADME processes of absorption, distribution, metabolism, and elimination that control the fate of these compounds from the body. Alterations in these processes provide the mechanistic basis for individual variability in response to drugs and environmental exposures. A common perception is that variability in response is due to genetic polymorphisms within the drug metabolizing enzyme and transporter genes. While there are numerous examples of these differences that play a major role in the susceptibility of genetic subpopulations for specific toxicities, the potential for transient phenotypic conversion due to temporary environmental changes, such as inflammation and diseases, are often overlooked. Due to the ensuing liver damage caused by the progressive stages of non-alcoholic fatty liver disease (NAFLD), gene expression patterns can change dramatically resulting in a phenoconversion resembling genetic polymorphisms. Because the liver plays such a key role in the metabolism and disposition of xenobiotics, it is well recognized that liver diseases can alter drug disposition and require dose adjustment to maintain drug concentrations within the therapeutic window. This temporary phenoconversion could lead to the inability of patients to properly metabolize and excrete drugs and environmental toxicants, increasing the risk of some adverse drug reactions and environmental toxicities. Dr. Cherrington's laboratory has made significant strides in identifying liver-specific disturbances in the expression and function of xenobiotic biotransformation enzymes and membrane drug transporters. Importantly, these molecular alterations in the expression and functions of drug transporters and biotransformation enzymes lead to *in vivo* perturbations in the disposition of numerous xenobiotics. Therefore, Dr. Cherrington suggests that patients with NAFLD present as a subpopulation of individuals that are at higher risk for developing adverse drug reactions, due to aberrant disposition of drugs and other xenobiotics. Specifically, Dr. Cherrington's laboratory has documented individual differences in ADME genes and proteins, such as metabolizing enzymes and transporters that cause a profound alteration in the pharmacokinetics, overall exposure, and toxicity of clinically relevant drugs and xenobiotics.

3.2. Endobiotic and xenobiotic disposition in pregnancy and maternal cholestasis, Lauren M. Aleksumes from Rutgers University, USA

Pregnancy is a critical period with high nutritional demands in order to support fetal growth and development. To accommodate these needs, the enterohepatic, renal, and cardiovascular systems undergo a number of adaptive molecular and physiological changes. Circulating hormones and growth factors modify global transcription factor signaling and drug disposition. Dr. Aleksunes

described adaptive changes in the expression of drug and bile acid metabolizing enzymes as well as transporters in pregnant mice[14–18]. To enhance the absorption of lipids during pregnancy, bile acid synthesis is increased and transport is reduced[16]. An enhanced supply of bile acids coupled with reduced enterohepatic circulation predisposes pregnant women to develop intrahepatic cholestasis. Interestingly, in pregnancy, Aleksunes demonstrated a down-regulation of the fibroblast growth factor 15 (mice)/19 (human), an ileal endocrine factor that represses hepatic bile acid synthesis[19]. *Ex vivo* studies using primary hepatocytes and serum from pregnant mice further supports the critical role of circulating hormones as modulators of the classic bile acid synthesis enzyme, CYP7A1. Treatment with recombinant FGF19 restored the expression of *Cyp7a1* in primary hepatocytes cultured with serum from pregnant mice[19]. Additional data pointed to a potential role for 17β-estradiol to down-regulate FGF19 in cultured human intestinal cells primed with the bile acid, chenodeoxycholic acid. Using pharmacological activators of the farnesoid X receptor GW4064 and the constitutive androstane receptor TCPOBOP, the Aleksunes laboratory has demonstrated restored expression of bile acid and drug metabolizing enzyme and transporter pathways in the livers and intestines of pregnant mice[19]. These data point to a novel pharmacological approach to regulate liver–intestine bile acid crosstalk during pregnancies complicated by maternal cholestasis.

3.3. Role of nuclear receptors and microRNAs in the regulation of drug metabolism by inflammation, Ulrich M. Zanger from Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Germany

Variability in drug response is caused by various genetic and nongenetic factors[20]. Data from the genome-wide expression studies in human liver ($n = 150$) from Dr. Zanger's laboratory[21] show that the clinical inflammation marker C-reactive protein (CRP) is associated with a broad "negative acute phase response" that comprises most drug metabolizing enzymes and transporters (DMET), supporting a well-documented negative influence of inflammation on drug metabolism[22]. To investigate mechanisms leading to coordinated DMET downregulation, Dr. Zanger's laboratory treated human primary hepatocytes (PHH) with IL-6 and selective signal transduction inhibitors. Reverse-phase phosphoproteomics analysis suggested a more important role of MAPK and PI3K over JAK/STAT signaling for DMET regulation. Mathematical modeling using fuzzy-logics and extensive time-resolved data from five liver donors suggested a central role of heterodimeric RXRα/nuclear receptor complexes to coordinately downregulate most DMET genes, which was confirmed by siRNA-mediated knock-down in PHH[23]. Zanger's laboratory also observed a significant downregulation of RXRα protein but not its transcript in response to IL-6 and therefore investigated a possible contribution of microRNAs. They identified numerous miRNAs strongly elevated in cholestatic liver and during inflammation, of which miR-130b was previously shown to downregulate various cytochromes P450 enzyme activities and to directly target CYP2C9[24]. Preliminary data indicate that miR-130b also directly targets RXRα. Taken together, the data indicate that coordinated downregulation of DMET genes in hepatocytes in inflammatory conditions involves IL-6 activated MAPK and PI3K signaling and inactivation or down-regulation of RXRα/nuclear receptor complexes. MiR-130b appears to play a role in downregulating RXRα and certain cytochrome P450s.

3.4. Regulation of sulfotransferase by local and systemic liver injuries, Wen Xie from University of Pittsburgh, USA

Sulfotransferases are phase II drug-metabolizing enzymes (DMEs) that play critical roles in maintaining the chemical and functional homeostasis of xenobiotics and endobiotics. In the case of estrogen homeostasis, estrogens can be sulfated and deactivated by the estrogen sulfotransferase (EST, or SULT1E1), because estrogen sulfates cannot bind to the estrogen receptor and thus are hormonally inactive[25]. Accumulating evidence suggests that many hepatic and systemic diseases can affect drug metabolism and disposition by regulating the hepatic expression and/or activity of DMEs and transporters, including the sulfotransferases[26]. This presentation focused on the recent progress in describing and understanding the hepatic injury and sepsis responsive regulation of sulfotransferases in animal models. Liver ischemia and reperfusion (I/R) were used as a typical model of hepatic injury, whereas LPS and cecal ligation and puncture (CLP) were used as the sepsis models. Dr. Xie's laboratory showed that the hepatic expression and activity of EST was markedly induced in a mouse model of I/R, which was associated with a higher level of estrone sulfate and decreased expression of estrogen responsive genes in the liver in a EST-dependent manner[27]. The up-regulation of EST in the liver may have played a pathogenic role in I/R injury, because EST ablation in female mice attenuated I/R responsive liver injury. Interestingly, the effect of EST ablation was sex specific, because the EST$^{-/-}$ male mice exhibited heightened I/R injury. The gender specific role of EST in I/R injury remains to be better understood. In an independent study, they showed that sepsis induced the expression of EST and compromised the activity of estrogen in the liver[28]. The sepsis responsive induction of EST in the liver may have played a protective role, because EST ablation sensitized mice to sepsis-induced death, which was recapitulated in wild-type mice pre-treated with triclosan, a pharmacological inhibitor of EST[29]. Mechanistically, Xie's laboratory showed that EST ablation attenuated sepsis-induced inflammatory responses due to compromised estrogen deactivation, leading to an increased sepsis lethality. It is hoped that understanding the disease effect on drug metabolism will facilitate the efficient and safe use of drugs in the clinic. In the meantime, DMEs such as sulfotransferases may be therapeutic targets that can affect the outcome of the diseases[26].

4. Parallel session on "role of gut microbiota in drug metabolism and toxicology", chaired by Drs. Hyunyoung Jeong and Edward T. Morgan

4.1. The microbial pharmacists within us, Peter J. Turnbaugh from University of California at San Francisco, USA

Large inter-individual variability in drug response has been a major limiting factor for achieving optimal drug therapy. The important role of the intestinal microbiota in drug metabolism has been known since the discovery by Domagk of the antibacterial activity of prontosil, and the subsequent determination of its activation to sulfanilamide[30]. However, the roles of the microbiome in modern pharmacology, drug metabolism and toxicology are underappreciated. Dr. Turnbaugh's laboratory is pursuing a more comprehensive view of pharmacology that includes the structure and activity of the resident microbial communities in the human gut and a deeper understanding of their interactions

with each other, with their host habitat, and with the nutritional milieu of the gastrointestinal (GI) tract. More than 50 drugs are known to be metabolized by the microbiome, mostly by reductions and hydrolyses[31]. Many of the hydrolytic reactions provide the bacteria with nutritional substrates. One important such drug is digoxin, which is reduced by the Actinobacteria *Eggerthalla lenta* to pharmacologically inactive dihydrodigoxin. About 10% of individuals taking digoxin excrete large amounts of dihydrodigoxin. The enzymes responsible have been identified as products of the bacterial cardiac glycoside reductase *Cgr* operon, *Cgr1* and *Cgr2*[32]. Only a subset of *E. lenta* strains encode the *Cgr* operon and are thus able to metabolize digoxin. The abundance of the *Cgr* operon in the human gut microbiota was shown to be predictive of the inter-individual differences in digoxin inactivation using an *ex vivo* assay. *Cgr* expression and function are inhibited by arginine. Studies in germ-free mice colonized with wild-type *E. lenta* suggested that dietary protein reduced the inactivation of digoxin, *via* increasing the luminal concentration of arginine, resulting in a significant increase in digoxin bioavailability. These results demonstrate the interplay between strain-level differences in the gut microbiota and dietary intake in modulating drug disposition and thus drug response. Dr. Turnbaugh's laboratory is continuing to study the molecular mechanisms responsible for digoxin reduction and using this proof-of-principle to inform the study of other drugs that are metabolized by gut microbes.

4.2. RNA-Seq quantification of hepatic drug-processing genes in germ-free mice, Curtis D. Klaassen from University of Washington, USA

Dr. Klaassen's presentation focused on the effect of intestinal bacteria on the expression of hepatic drug-processing genes in the host. RNA sequencing (RNA-Seq) was used to profile drug metabolizing enzymes and transporters in germ-free (GF) and conventional (CONV) mice, as well as in GF mice treated with probiotics (*i.e.*, *bifidobacterium* and *lactobacillus*, VSL3)[33,34]. In the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including *Cyp1a2*, *2c54*, *2e1* and *4a10*. Consistent with the increases in *Cyp1a2* and *Cyp4a10*, mRNA levels of the aryl hydrocarbon receptor and peroxisome proliferator–activated receptor *α* were also up-regulated in GF mice. *Cy3a11* mRNA was markedly reduced by 87%, and *Cyp2b10* was decreased by 57%. However, the expression of constitutive androstane receptor (CAR) or pregnane X receptor (PXR) did not decrease in GF mice. Non-P450 phase I enzymes in the livers of GF mice were only moderately affected. Glutathione *S*-transferase enzymes p1 and p2 were down-regulated whereas sulfotransferases *Sult1a1* and *1d7* were induced in GF mice. A number of solute carrier and ATP-binding cassette transporters were up-regulated. Speculatively, the observed differences in GF mice may be due to production of endogenous ligands of nuclear receptors (*e.g.*, secondary bile acids) by the microbiota, but this has yet to be determined. In general, smaller effects were seen in the intestines than in livers of GF mice, although *Cyp3a* subfamily transcripts were down-regulated in the intestine. Administration of probiotics to GF mice had little effect, whereas housing GF mice with CONV mice reversed the observed effects. Overall, it is clear that intestinal bacteria can affect drug metabolism and transport, and are likely to be responsible for some individual differences in drug responses.

4.3. Drugging the microbiome, Aadra P. Bhatt and Mathew R. Redinbo from University of North Carolina at Chapel Hill, USA

Glucuronide metabolites of drugs are hydrolyzed by intestinal *β*-glucuronidase (GUS) enzymes, with the glucuronide moiety feeding in to the microbial citric acid cycle. This can lead to long half-lives of drugs due to enterohepatic circulation, and in the case of the anticancer drug irinotecan and non-steroidal anti-inflammatory drug (NSAIDs), to gastrointestinal toxicity in the form of severe diarrhea or ulcerations, respectively. Irinotecan is converted to bioactive SN-38 by carboxylesterases in the body, which is subsequently glucuronidated and excreted to the small intestine *via* bile. Hydrolysis of the nontoxic SN-38 glucuronide metabolite by the microbiota GUS enzymes leads to the release of SN-38 in the gut and subsequent related toxicity. These bacterial enzymes thus present a therapeutic target to reduce irinotecan toxicity *via* inhibition of SN-38 glucuronide hydrolysis. Dr. Redinbo's laboratory used crystal structures of bacterial GUS enzymes to elucidate the enzymatic mechanism, and identified potent and bacteria-selective inhibitors of these enzymes by high-throughput screening[35]. In a mouse xenograft model, one such inhibitor did not alter the efficacy of irinotecan in reducing tumor growth, but did prevent irinotecan-induced toxicity (*e.g.*, weight loss) in the mice. GUS inhibition also reduced the formation of intestinal ulcers in mice treated with diclofenac, which is also due to hydrolysis of its glucuronide in the intestine[36]. The current inhibitors have been developed with a focus on the *Escherichia coli* enzyme, but Dr. Redinbo's laboratory is examining the diversity of GUS enzymes in the GI microbiota. While this illustrates the challenges of developing drugs to related microbial targets whose representation may differ greatly among individuals, the study underscores the enzymes in the GI microbiota as potential drug targets that can be manipulated to achieve optimal drug responses.

5. Parallel session on "roles of long non-coding RNAs in liver development, functions, and diseases", chaired by Drs. Xiao-bo Zhong and Li Wang

5.1. Non-coding RNAs and hepatic responses to drugs, environmental chemicals, and endogenous hormones, Pengying Hao, Tisha Melia, Nicholas J. Lodato, and David J. Waxman from Boston University, USA

The liver responds to both xenobiotic and hormonal stimulation with dynamic changes in gene expression and the epigenetic landscape. Some of these responses may involve the action of long non-coding RNAs (lncRNAs), which are increasingly recognized as potential chromatin regulators, as well as microRNAs, which are important post-transcriptional regulators. In work presented by David Waxman's laboratory, non-coding RNA dynamics in liver was examined under diverse conditions, including: (1) exposure to TCPOBOP, an agonist ligand of the nuclear receptor CAR, representing short-term liver responses to environmental chemical exposure; and (2) stimulation of the liver by plasma growth hormone, whose sex-differential pituitary secretion pattern imparts widespread hepatic sex-differences[37]. First, they used a stringent computational discovery pipeline[38] to identify 15,558 mouse liver-expressed lncRNAs, based on an analysis of a diverse set of 186 mouse liver RNA-seq datasets, representing 30 biological

conditions. Strikingly, they found that liver-expressed lncRNA gene promoters show greater species conservation and a higher frequency of proximal binding by liver transcription factors than corresponding protein-coding gene promoters. Further, activation of the nuclear receptor CAR in mouse liver (TCPOBOP treatment for 3–27 h) was found to significantly alter the expression of 166 lncRNAs. Many of these lncRNAs are intragenic or transcribed anti-sense to CAR-regulated CYP genes and genes that encode other drug-metabolizing enzyme RNAs, suggesting their co-regulation. Comparing the male and female liver transcriptome, they identified 247 lncRNAs showing strong sex bias and tight regulation by growth hormone[38], with significant enrichment for nearby growth hormone-regulated DNase hypersensitive sites[39] and sex-dependent binding sites for growth hormone-regulated transcription factors such as STAT5[40] and HNF6[41]. In other studies, the Waxman laboratory investigated the role of micro-RNAs in liver sex differences, and identified 13 sex-biased liver microRNAs by small RNA sequencing. Two of these microRNAs were found to be tightly regulated by the transcription factor STAT5 following its activation by growth hormone. To assess functionality, one of the male-specific miRNAs was over-expressed in female mouse liver using adenovirus, which led to widespread gene expression changes in liver. This work leverages deep sequencing and integrative data analyses to elucidate exogenous and endogenous stimuli-induced liver transcriptome dynamics.

5.2. LncRNAs in liver metabolic functions, Li Wang from University of Connecticut, USA

Dr. Li Wang reported a novel function of lncRNA MEG3 in bile acid homeostasis and cholestatic liver injury. Bile acids play critical physiological functions in cholesterol homeostasis and deregulation of bile acid metabolism causes cholestatic liver injury. Maternally expressed gene 3 (*MEG3*) was recently shown as a potential tumor suppressor; however, its basic hepatic function remains elusive. Using RNA pull-down with biotin-labeled sense or anti-sense *MEG3* RNA followed by mass spectrometry, Dr. Wang's laboratory identified RNA binding protein polypyrimidine tract-binding protein 1 (PTBP1) as a *MEG3* interaction protein and validated their interaction by RNA immunoprecipitation (RIP). Bioinformatics analysis revealed putative binding sites for PTBP1 within the coding region (CDS) of small heterodimer partner (SHP); a key repressor of bile acid biosynthesis. Forced expression of *MEG3* in hepatocellular carcinoma (HCC) cells guided and facilitated PTBP1 binding to *Shp* CDS, resulting in *Shp* mRNA decay. Transient overexpression of *MEG3* RNA *in vivo* in mouse liver caused rapid *Shp* mRNA degradation and cholestatic liver injury, which was accompanied by the disruption of bile acid homeostasis, elevation of liver enzymes, and dysregulation of bile acid synthetic enzymes and metabolic genes. Interestingly, RNA-seq coupled with qPCR revealed a drastic induction *Meg3* RNA in *Shp*$^{-/-}$ liver. SHP inhibited *MEG3* gene transcription by repressing cAMP response element-binding protein (CREB) transactivation of the *MEG3* promoter. In addition, the expression of *MEG3* and PTBP1 was activated in human fibrotic and NASH cirrhotic liver. At the end of the presentation, Dr. Wang concludes that *MEG3* causes cholestasis by destabilizing *Shp via* serving as a guide RNA scaffold to recruit PTBP1 to *Shp* mRNA. SHP in turn represses CREB-mediated activation of *MEG3* expression in a feedback regulatory fashion.

5.3. Role of lncRNAs in postnatal liver maturation, Chad Pope and Xiao-bo Zhong from University of Connecticut, USA

The adult liver conducts critical functions in metabolism of various endogenous and exogenous compounds, including drugs. How-ever, the functions are not mature yet in liver at neonatal and infant ages. There is a postnatal maturation process in liver to reflect a functional transition, in which the molecular mechanisms control-ling the process have not been fully established. LncRNAs have been implicated to play important roles in organ development and cell proliferation. Dr. Zhong presented an initial study to establish the role of lncRNAs in postnatal liver maturation in a mouse model. In a preliminary screening[42], Dr. Zhong's laboratory applied RNA-Seq to examine ontogeny of all annotated lncRNAs in mouse liver during postnatal maturation from perinatal (day −2) to adult (day 60). They found nearly 2000 lncRNAs were differentially expressed in liver during liver maturation. In general, lncRNAs were expressed at a lower level than the coding mRNAs. Both coding mRNAs and lncRNAs displayed three major onto-genic patterns with a similar proportion among the neonatal, adolescent, or adult enriched patterns. Closed neighboring pairs of coding mRNAs and lncRNAs showed the trend to exhibit highly correlated ontogenic expression patterns, indicating that lncRNAs may share similar regulatory mechanisms with their *cis*-coding genes in same chromatin segments. In a comparison with the previously deciphered developmental dynamics of the mouse liver transcriptome of all coding mRNAs[43], gene ontology (GO) analysis revealed that some lncRNAs enriched at neonatal ages had their neighbor protein-coding genes also enriched at neonatal ages and functions of those proteins were associated with liver growth, immune activation related processes, cell proliferation, tissue organization, and hematopoiesis. Some other lncRNAs enriched at adult ages had their neighboring protein-coding genes associated with different metabolic functions.

Dr. Zhong's laboratory further identified 433 pairs of such lncRNAs and coding mRNAs, in which lncRNAs were signifi-cantly differentially expressed during postnatal liver maturation and their neighboring protein-coding genes were also expressed in the same ontogenic patterns. Several top candidate lncRNAs in the list were selected for further validation. One of the selected lncRNAs is H19, a lncRNA located in a genomic imprinting region differentially expressed in liver regulated by a DNA methylated region on maternal and paternal chromosomes. At the end of the presentation, Dr. Zhong described an experimental design to use a genetic modified mouse strain with a deletion of H19 on the maternal chromosome to further investigate the role of H19 in liver postnatal maturation.

6. Summary

A number of talks presented at the 21st MDO conference have overviewed recent important findings on the effects on drug metabolism and toxicity by multiple factors of genetics, epige-netics, lncRNAs, gut microbiota, and diseases. The programs nicely covered both traditional and rising topics in drug metabo-lism and related areas. These exceptional presentations, following questions, and insightful discussions shall undoubtedly stimulate further studies on the regulatory mechanisms underlying variable drug metabolism and toxicity, which will ultimately advance the field of drug metabolism.

Acknowledgments

References

1. Tracy TS, Chaudhry AS, Prasad B, Thummel KE, Schuetz EG, Zhong XB, et al. Interindividual variability in cytochrome P450-mediated drug metabolism. *Drug Metab Dispos* 2016;**44**:343–51.
2. Tolson AH, Wang H. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv Drug Deliv Rev* 2010;**62**:1238–49.
3. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999;**286**:487–91.
4. Swanson HI. Drug metabolism by the host and gut microbiota: a partnership or rivalry?. *Drug Metab Dispos* 2015;**43**:1499–504.
5. Yip LY, Chan EC. Investigation of host-gut microbiota modulation of therapeutic outcome. *Drug Metab Dispos* 2015;**43**:1619–31.
6. Ingelman-Sundberg M, Zhong XB, Hankinson O, Beedanagari S, Yu AM, Peng L, et al. Potential role of epigenetic mechanisms in the regulation of drug metabolism and transport. *Drug Metab Dispos* 2013;**41**:1725–31.
7. Yu AM, Tian Y, Tu MJ, Ho PY, Jilek JL. MicroRNA pharmacoepigenetics: posttranscriptional regulation mechanisms behind variable drug disposition and strategy to develop more effective therapy. *Drug Metab Dispos* 2016;**44**:308–19.
8. Peng L, Zhong X. Epigenetic regulation of drug metabolism and transport. *Acta Pharm Sin B* 2015;**5**:106–12.
9. Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. *Trends Pharmacol Sci* 2014;**35**:384–96.
10. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;**536**:285–91.
11. Matthaei J, Brockmöller J, Tzvetkov MV, Sehrt D, Sachse-Seeboth C, Hjelmborg JB, et al. Heritability of metoprolol and torsemide pharmacokinetics. *Clin Pharmacol Ther* 2015;**98**:611–21.
12. Bell CC, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, et al. Characterization of primary human hepatocyte spheroids as a model

13. system for drug-induced liver injury, liver function and disease. *Sci Rep* 2016;**6**:25187.
14. Ivanov M, Kals M, Lauschke V, Barragan I, Ewels P, Käller M, et al. Single base resolution analysis of 5-hydroxymethylcytosine in 188 human genes: implications for hepatic gene expression. *Nucleic Acids Res* 2016;**44**:6756–69.
15. Aleksunes LM, Xu J, Lin E, Wen X, Goedken MJ, Slitt AL. Pregnancy represses induction of efflux transporters in livers of type I diabetic mice. *Pharm Res* 2013;**30**:2209–20.
16. Fortin MC, Aleksunes LM, Richardson JR. Alteration of the expression of pesticide-metabolizing enzymes in pregnant mice: potential role in the increased vulnerability of the developing brain. *Drug Metab Dispos* 2013;**41**:326–31.
17. Aleksunes LM, Yeager RL, Wen X, Cui JY, Klaassen CD. Repression of hepatobiliary transporters and differential regulation of classic and alternative bile acid pathways in mice during pregnancy. *Toxicol Sci* 2012;**130**:257–68.
18. Yacovino LL, Gibson CJ, Aleksunes LM. Down-regulation of brush border efflux transporter expression in the kidneys of pregnant mice. *Drug Metab Dispos* 2013;**41**:320–5.
19. Wen X, Donepudi AC, Thomas PE, Slitt AL, King RS, Aleksunes LM. Regulation of hepatic phase II metabolism in pregnant mice. *J Pharmacol Exp Ther* 2013;**344**:244–52.
20. Moscovitz JE, Kong B, Buckley K, Buckley B, Guo GL, Aleksunes LM. Restoration of enterohepatic bile acid pathways in pregnant mice following short term activation of Fxr by GW4064. *Toxicol Appl Pharmacol* 2016;**310**:60–7.
21. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013;**138**:103–41.
22. Schröder A, Klein K, Winter S, Schwab M, Bonin M, Zell A, et al. Genomics of ADME gene expression: mapping expression quantitative trait loci relevant for absorption, distribution, metabolism and excretion of drugs in human liver. *Pharmacogenomics J* 2013;**13**:12–20.
23. Harvey RD, Morgan ET. Cancer, inflammation, and therapy: effects on cytochrome p450-mediated drug metabolism and implications for novel immunotherapeutic agents. *Clin Pharmacol Ther* 2014;**96**:449–57.
24. Keller R, Klein M, Thomas M, Dräger A, Metzger U, Templin MF, et al. Coordinating Role of RXRα in downregulating hepatic detoxification during inflammation revealed by Fuzzy-Logic modeling. *PLoS Comput Biol* 2016;**12**:e1004431.
25. Rieger JK, Reutter S, Hofmann U, Schwab M, Zanger UM. Inflammation-associated microRNA-130b down-regulates cytochrome P450 activities and directly targets CYP2C9. *Drug Metab Dispos* 2015;**43**:884–8.
26. Song WC. Biochemistry and reproductive endocrinology of estrogen sulfotransferase. *Ann N Y Acad Sci* 2001;**948**:43–50.
27. Guo Y, Hu BF, Xie Y, Billiar TR, Sperry JL, Huang M, et al. Regulation of drug-metabolizing enzymes by local and systemic liver injuries. *Expert Opin Drug Metab Toxicol* 2016;**12**:245–51.
28. Guo Y, Hu BF, Huang H, Tsung A, Gaikwad NW, Xu MS, et al. Estrogen sulfotransferase is an oxidative stress-responsive gene that gender-specifically affects liver ischemia/reperfusion injury. *J Biol Chem* 2015;**290**:14754–64.
29. Chai XJ, Guo Y, Jiang MX, Hu BF, Li ZG, Fan J, et al. Oestrogen sulfotransferase ablation sensitizes mice to sepsis. *Nat Commun* 2015;**6**:7979.
30. Wang LQ, Falany CN, James MO. Triclosan as a substrate and inhibitor of 3′-phosphoadenosine 5′-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metab Dispos* 2004;**32**:1162–9.
31. Fuller AT, Lond FI. Is *p*-aminobenzenesulphonamide the active agent in prontosil therapy?. *Lancet* 1937;**229**:194–8.
32. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat Rev Microbiol* 2016;**14**:273–87.

32. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science* 2013;**341**:295–8.

33. Selwyn FP, Cheng SL, Klaassen CD, Cui JY. Regulation of hepatic drug-metabolizing enzymes in germ-free mice by conventionalization and probiotics. *Drug Metab Dispos* 2016;**44**:262–74.

34. Selwyn FP, Cheng SL, Bammler TK, Prasad B, Vrana M, Klaassen C, et al. Developmental regulation of drug-processing genes in livers of germ-free mice. *Toxicol Sci* 2015;**147**:84–103.

35. Wallace BD, Wang HW, Lane KT, Scott JE, Orans J, Koo JS, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* 2010;**330**:831–5.

36. Boelsterli UA, Redinbo MR, Saitta KS. Multiple NSAID-induced hits injure the small intestine: underlying mechanisms and novel strategies. *Toxicol Sci* 2013;**131**:654–67.

37. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol* 2009;**76**:215–28.

38. Melia T, Hao PY, Yilmaz F, Waxman DJ. Hepatic long intergenic noncoding RNAs: high promoter conservation and dynamic, sex-dependent transcriptional regulation by growth hormone. *Mol Cell Biol* 2016;**36**:50–69.

39. Ling GY, Sugathan A, Mazor T, Fraenkel E, Waxman DJ. Unbiased, genome-wide *in vivo* mapping of transcriptional regulatory elements reveals sex differences in chromatin structure associated with sex-specific liver gene expression. *Mol Cell Biol* 2010;**30**:5531–44.

40. Zhang YJ, Laz EV, Waxman DJ. Dynamic, sex-differential STAT5 and BCL6 binding to sex-biased, growth hormone-regulated genes in adult mouse liver. *Mol Cell Biol* 2012;**32**:880–96.

41. Conforto TL, Steinhardt GF, Waxman DJ. Cross talk between GH-regulated transcription factors HNF6 and CUX2 in adult mouse liver. *Mol Endocrinol* 2015;**29**:1286–302.

42. Peng L, Paulson A, Li H, Piekos S, He X, Li LH, et al. Developmental programming of long non-coding RNAs during postnatal liver maturation in mice. *PLoS One* 2014;**9**:e114917.

43. Gunewardena SS, Yoo B, Peng L, Lu H, Zhong XB, Klaassen CD, et al. Deciphering the developmental dynamics of the mouse liver transcriptome. *PLoS One* 2015;**10**:e0141220.