

## TITLE PAGE

### The Role of Sulfotransferases in Liver Diseases

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## **RUNNING TITLE PAGE**

**Running Title:** SULTs in liver diseases

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### **List of Nonstandard Abbreviations:**

SULTs – sulfotransferases

PAPS - phosphoadenosine-5'-phosphosulfate

NRs – nuclear receptors

PPARs - peroxisome proliferator-activated receptors

PXR - pregnane X receptor

CAR - constitutive androstane receptor

VDR - vitamin D receptor

LXR - liver X receptors

FXR - farnesoid X receptor

RORs - retinoid-related orphan receptors

ERRs - estrogen-related receptors

HNF4  $\alpha$  - hepatocyte nuclear factor 4  $\alpha$

FAS - fatty acid synthase

HCC – hepatocellular carcinoma

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

HCFD - high cholesterol and saturated fat diet

LPS - bacterial lipopolysaccharide

CLP - cecum ligation and puncture

I/R - ischemia-reperfusion injury

E<sub>2</sub> - Estradiol

CFTR - cystic fibrosis transmembrane receptor

## Abstract

The cytosolic sulfotransferases (SULTs) are phase II conjugating enzymes that catalyze the transfer of a sulfonate group from the universal sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to a nucleophilic group of their substrates to generate hydrophilic products. Sulfation has a major effect on the chemical and functional homeostasis of substrate chemicals. SULTs are widely expressed in metabolically active or hormonally responsive tissues, including the liver and many extrahepatic tissues. The expression of SULTs exhibits isoform-, tissue-, sex-, and development-specific regulations. SULTs display a broad range of substrates including xenobiotics and endobiotics. The expression of SULTs has been shown to be transcriptionally regulated by members of the nuclear receptor (NR) superfamily, such as the peroxisome proliferator-activated receptors (PPARs), pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR), liver X receptors (LXR), farnesoid X receptor (FXR), retinoid-related orphan receptors (RORs), estrogen-related receptors (ERRs), and hepatocyte nuclear factor 4  $\alpha$  (HNF4  $\alpha$ ). These NRs can be activated by numerous xenobiotics and endobiotics, such as fatty acids, bile acids, and oxysterols, many of which are substrates of SULTs. Due to their metabolism of xenobiotics and endobiotics, SULTs and their regulations are implicated in the pathogenesis of many diseases. This review is aimed to summarize the central role of major SULTs, including the SULT1 and SULT2 subfamilies, in the pathophysiology of liver and liver-related diseases.

### **Significant Statement**

SULTs are indispensable in the homeostasis of xenobiotics and endobiotics. Knowing SULTs and their regulations are implicated in human diseases, it is hoped that genetic or pharmacological manipulations of the expression and/or activity of SULTs can be used to affect the clinical outcome of diseases.

## 1. Introduction

Sulfate conjugation (sulfation or sulfonation) is a major conjugating pathway responsible for the deactivation, detoxification and excretion of xenobiotics and endogenous molecules (Falany, 1991). Sulfoconjugation was first recognized as an important metabolic pathway by Baumann in 1876 (Baumann, 1876). At the chemical level, the cytosolic sulfotransferases (SULTs) catalyze the transfer of a negatively charged sulfonate group ( $\text{SO}_3^-$ ) from the universal sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) onto a nucleophilic group of their substrates to generate hydrophilic products, which often promote the urinary excretion of the substrates. The high-energy sulfate donor PAPS can be generated by PAPS synthases, including PAPSS1 and PAPSS2, as well as by ATP sulfurylase and two forms of adenosine 5'-phosphosulfate kinase (APS kinases) (Mueller et al., 2018).

SULTs are widely expressed in the liver, as well as metabolically active or hormonally responsive extrahepatic tissues (Dooley et al., 2000; Gamage et al., 2006). This large family of enzymes are responsible for sulfating a variety of endogenous and exogenous molecules, including pharmaceuticals, procarcinogens, hormones, neurotransmitters, as well as intermediates of endogenous metabolism (Dooley et al., 2000; Glatt et al., 2001; Negishi et al., 2001; Jancova et al., 2010). Sulfation often results in the inactivation of the substrates or reduced potency of ligands (Strott, 2002; Bjerregaard-Olesen et al., 2015) but with some exceptions. Their abundance in the liver and wide range of substrates suggest SULTs may act as important mediators for the development of liver diseases, such as hepatocellular carcinoma (Xie et al., 2017; Zou et al., 2017), liver fibrosis (Hardwick et al., 2013; Krattinger et al., 2016; Yetti et al.,

2018), and drug-induced liver injury (Fang et al., 2016). Therefore, in this review we focus on the roles of the human and rodent SULTs in liver diseases.

## **2. The superfamily of sulfotransferases**

The SULTs superfamily contains 62 human *SULT* genes and 46 murine homologues as of 2016 (Mueller et al., 2015; Herrero et al., 2016). Among SULTs, the aryl-sulfotransferase (SULT1) and the hydroxysteroid sulfotransferase (SULT2) families are two principal sub-families of SULTs that are the major contributors to the sulfonation of many xenobiotics including pharmaceuticals and procarcinogens, and endobiotics including steroids, thyroid, and neurotransmitters (Kauffman, 2004; Reinen and Vermeulen, 2015).

SULT1 family comprises five isoforms: phenol-sulfotransferases (SULT1A1/2) (Raftogianis et al., 1999), catecholamine phenol sulfotransferase (SULT1A3/4) that is only present in humans (Zou et al., 2017), thyroid hormone sulfotransferase (SULT1B1) (Saeki et al., 1998), iodothyronine sulfotransferase (SULT1C2,1C4) (Dubaisi et al., 2019), and estrogen sulfotransferase (EST/SULT1E1) (Zhang et al., 1998; Guo et al., 2015). SULT2 family has two isoforms: alcohol/hydroxysteroid sulfotransferase (SULT2A1) that sulfonates hydroxysteroids (Mueller et al., 2018), such as androgens, estrogens at both the 3- and 17- positions (Ambadapadi et al., 2017), and bile acids (Huang et al., 2010), and SULT2B1b that has a greater selectivity for 3-hydroxysteroids, such as cholesterol (Bi et al., 2018), but not for bile acids.

In addition to the SULT1 and SULT2 families of enzymes, the human genome contains two more sulfotransferase gene families, *SULT4* and *SULT6*, encoding enzymes including

SULT4A1 that is a brain-specific sulfotransferase associated with antipsychotic treatment response (Wang et al., 2014) and SULT6B1 whose physiological function is largely unknown.

### **3. SULT1**

#### **3.1. SULT1A1/2**

##### **3.1.1. *SULT1A1/2 in liver cancers***

The human SULT1A1/2, also known as aryl/phenol or thermostable sulfotransferases (P-PSTs or TS-PSTs), exhibit a broad substrate range with specificity for phenolic compounds (Raftogianis et al., 1997; Raftogianis et al., 1999). SULT1A1 is widely distributed throughout the body, with high abundance in the liver, lung, brain, skin, platelets, gastrointestinal tissues, and kidney (Hempel et al., 2005). The *SULT1A2* gene is located on chromosome 16p11.2-12.1, in close proximity to its related isoform *SULT1A1* (Gamage et al., 2006). Besides their classical role in facilitating the detoxification and excretion of their substrates and metabolites, SULT1A1/2 is also known to play a major role in the bioactivation of environmental mutagens and carcinogens, such as hydroxymethyl polycyclic aromatic hydrocarbons, *N*-hydroxy derivatives of arylamines, allylic alcohols and heterocyclic amines, leading to mutagenicity and carcinogenesis through the binding of sulfonated metabolites to DNA (Falany, 1997; Weinshilboum et al., 1997; Hempel et al., 2005). Li and colleagues recently reported that the expression of liver SULT1A1/2 is highly associated with sex-dependent susceptibility of bladder and liver to the major human bladder carcinogen 4-aminobiphenyl (ABP) (Li et al., 2018). Both the parent ABP and its sulfonated metabolites are genotoxic (Chou et al., 1995). In this study, the authors observed that male bladders were more susceptible than female bladders to ABP. This was explained by the increased bladder exposure to ABP in male mice through androgen-dependent suppression of



ABP sulfation in the liver, leading to increased bladder delivery of carcinogenic ABP (Li et al., 2018). The male preference in the bladder's susceptibility to ABP was attenuated by knocking out the *Sult1a1* gene. In contrast, female livers were more susceptible than male livers to ABP in mice, which was believed to be due to their higher liver exposure to carcinogens, including APB and its toxic Sult1a1 metabolite, *N*-sulfate ester-ABP. Further studies revealed that Sult1a1 is positively associated with the increased formation of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP), a principal ABP-DNA adduct and the readout of tissue susceptibility to ABP, in hepatic cells. As summarized in Figure 1, androgen renders bladder more exposed to ABP in male mice by suppressing Sult-mediated ABP metabolism in liver; while the liver of female mice is more exposed to ABP and its carcinogenic metabolites because of the higher enzymatic activity of liver Sult1a1.

In humans, it was suggested that the most common polymorphism in SULT1A1 (Arg213His) may have accounted for variations in inter-individual susceptibility to hepatocellular carcinoma (HCC) (Boccia et al., 2015; Kim et al., 2015), because SULT1A1 activates environmental mutagens and carcinogens found in well-done meat (Bellamri et al., 2018), food processing contaminant (Hoie et al., 2016), and dietary flavonoids (Sak and Everaus, 2016). Interestingly, SULT1A1 deactivates carcinogens in cigarette smoke, and a significant interaction between SULT1A1 and smoking was found in a case-control study that included 221 HCC patients and 290 control subjects (Boccia et al., 2015). Specifically, up to 36% of HCC cases occurred in smokers who carried the Arg213His allele. The high incidence of HCC among Arg213His smokers was explained by the low catalytic activity of this SULT1A1 variant in detoxifying cigarette smoke carcinogens (Boccia et al., 2015).

### **3.1.2. *SULT1A1* in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)**

Non-alcoholic fatty liver disease encompasses a spectrum of diseases ranging from simple steatosis to NASH, which can progress to fibrosis, cirrhosis and HCC. The pathogenesis of NAFLD and NASH have been extensively studied. However, the roles of SULTs in the initiation and progression of these diseases are insufficiently studied. A hospital-based case control study in an Italian population including 294 NAFLD cases and 359 controls evaluated the effect of Arg213His *SULT1A1* gene polymorphism, which leads to a decreased enzymatic activity of SULT1A1, and the interaction of this polymorphism with lifestyle and dietary habits (Miele et al., 2014). Statistically significant interactions were reported for fruit intake and *SULT1A1* gene, as the carriers of Arg213His *SULT1A1* gene variant were riskier to develop NAFLD when taking high amounts of fruit. The presents of Arg213His *SULT1A1* gene variant was also positively associated with grilled meat or fish intake in the development of NAFLD. In addition, many studies found that flavonoids in vegetables and fruits were inhibitors of multiple SULTs (Pai et al., 2001; Huang et al., 2009), which may have affected sensitivity to the development of NAFLD. However, there was no direct evidence linking dietary flavonoids induced inhibition of SULT1A1 and NAFLD. On the other hand, NAFLD and its progression have a major effect on the expression and activity of multiple SULTs, such as SULT1A1, 1A3, 2A1, and 2B1 (Hardwick et al., 2013; Yalcin et al., 2013). Hardwick and colleagues reported an upregulation in protein and enzymatic activity of SULT1A1 in simple steatosis patients, but decreased expression of *SULT1A1* in NASH patients (Hardwick et al., 2013). However, these results are not without controversies. An independent study on normal subjects and patients with steatosis, diabetic cirrhosis, and alcoholic cirrhosis reported a significant decrease of SULT1A1 activity

with increasing severity of liver disease from simple steatosis to cirrhosis (Yalcin et al., 2013). A follow up study from the same group reported that in human liver tissues, sulfation of bisphenol A (BPA), an industrial chemical and endocrine disruptor, was substantially lower in livers from subjects with steatosis (23%), diabetic cirrhosis (16%), and cirrhosis (18%), relative to healthy livers (100%), resulting in a higher exposure of BPA in NAFLD patients (Yalcin et al., 2016). The discrepancies in reported *SULT1A1* expression in steatosis might result from the differences in the stages of steatosis, and the gender and age of patients when the liver tissues were collected.

### **3.1.3. *SULT1A1* in acetaminophen induced liver injury**

Acetaminophen (APAP), or Tylenol, is one of the most widely used drugs. Although the drug is safe at therapeutic doses, an overdose of APAP remains the leading cause of acute liver failure (ALF) in the United States. It has been known that human *SULT1A1* can catalyze APAP sulfation and facilitate its excretion from the urine. Consistent with their report that the expression and activity of *SULT1A1* was increased in steatotic livers and decreased in NASH, Hardwick and colleagues showed that the formation of sulfate-APAP was increased in cytosolic fractions of human steatotic liver tissue compared with normal liver, but reduced in NASH compared with steatotic liver tissue (Hardwick et al., 2013). A limitation of this study is that the results are largely associations. Future studies are necessary to determine whether *SULT1A1* is necessary and sufficient to affect APAP hepatotoxicity.

### **3.2. *SULT1A3/4* in liver cancers and NAFLD**

SULT1A3/4, also known as catecholamine phenol sulfotransferase, plays a key role in maintaining the homeostasis of monoamine neurotransmitters, such as dopamine, through sulfation. Different from SULT1A1/2 that is highly expressed in the hepatocytes, the SULT1A3/4 level is low in an adult human liver, but they are highly expressed in the fetal liver (Dubaisi et al., 2019) and tumor tissues (Yamamoto et al., 2016). A recent report confirmed that the expression of *SULT1A3/4* was increased in tumor tissues compared with adjacent normal tissues of HCC patients, and the highly expressed *SULT1A3/4* in liver tumor tissues was positively associated with its increased enzymatic activity in metabolizing dopamine and elevated metastatic capacity (Zou et al., 2017). Dopamine was a substrate for SULT1A3/4, as well as a regulator responsible for *SULT1A3/4* transcriptional activation (Sidharthan et al., 2013). Functional studies showed that the induced expression of *SULT1A3/4* in tumor tissues of HCC patients was negatively correlated with dopamine concentration, but positively associated with the epithelial-mesenchymal transition and cancer stem cells acquisition, which ultimately promotes tumor metastasis (Zou et al., 2017).

SULT1A3 activity was reported to be decreased in steatosis, diabetic cirrhosis, and alcoholic cirrhosis liver samples compared with non-fatty control livers (Yalcin et al., 2013), which may have led to reduced sulfation of acetaminophen and opioid drugs (Bairam et al., 2018).

### **3.3. *SULT1B1* in liver diseases**

SULT1B1 plays an important role in the metabolism of drugs, environmental toxins, and endogenous steroids, such as iodothyronines, thyroxine, 1-naphthol, and thyroid hormones (Fujita et al., 1997; Wang et al., 1998; Gamage et al., 2006). However, few studies have shown the

changes of *SULT1B1* expression or activity under different liver disease conditions. According to the Gene Expression Omnibus (GEO) database, conflicting observations in *SULT1B1* gene expression were reported among different species, the same disease models of the same species under different treatments, or even the same disease models of the same species with the same treatment. As shown in Table 1, there were no consistent changes of *SULT1B1* gene expression under HCC, or high fat diet induced NASH/NAFLD. Moreover, the lack of study on the roles of *SULT1B1* in liver diseases makes it even more challenging to interpret the pathophysiological significance of the altered expression of *SULT1B1* in liver diseases.

### **3.4. *SULT1E1* (EST)**

#### **3.4.1. *SULT1E1* in liver cancer**

Estrogen sulfotransferase (*SULT1E1*, also known as EST), widely expressed in human tissues such as the liver, lung, adipose tissue, and kidney (Barbosa et al., 2019), is best known for its function in the sulfoconjugation and deactivation of estrogens. This is because sulfonated estrogens fail to bind to the estrogen receptor and thus lose their hormonal activities (Song, 2001). As such, *SULT1E1* has long been implicated in female sex hormone related cancer, such as breast, endometrial, and ovarian cancers (Pasqualini, 2009; Mungenast et al., 2017; Sinreih et al., 2017; Xu et al., 2018). Interestingly, *SULT1E1* is also reported to be associated with the occurrence of HCC in rats. In a study on inflammatory liver disease, upregulation of *Sult1e1* in diethylnitrosamine (DEN)-treated mouse livers was observed compared to the vehicle-treated livers using real time RT-PCR (Lee et al., 2017). However, in the DEN-induced rat model of hepatoma, the expression of *SULT1E1* was decreased with the onset of hepatomas (Albrethsen et al., 2011). The causal relationship remains to be defined, because the expression of *SULT1E1*

was increased in regenerating liver (Albrethsen et al., 2011). Moreover, the species difference and the human relevance of *SULT1E1* in liver cancer need to be clarified.

### **3.4.2. *SULT1E1* in NAFLD and NASH**

Previous study in our lab found that loss of *Sult1e1/Est* in female mice improved metabolic function with improved insulin sensitivity and reduced hepatic steatosis in ob/ob mice lacking *Sult1e1* (also known as the obe mice), compared to the ob/ob (Gao et al., 2012). In an independent study, *Sult1e1* was suggested to play a role in sensitizing male mouse to NAFLD/NASH induced by a 20-wk feeding of a Western diet high in cholesterol and saturated fat diet (HCFD) (Matsushita et al., 2017). The authors found that NASH was attenuated in *Ikkkb $\Delta$ mye* mice with myeloid IKK $\beta$  deficiency in both genders but aggravated in male but not female *Ikkkb $\Delta$ hep* mice with hepatocyte IKK $\beta$  deficiency. Microarray analysis on liver tissues from male and female WT and *Ikkkb $\Delta$ hep* mice fed HCFD showed a significant upregulation of *Sult1e1* gene in *Ikkkb $\Delta$ hep* mice compared to WT mice. Among the four groups, the male *Ikkkb $\Delta$ hep* mice expressed the highest level of *Sult1e1* gene which was associated with decreased plasma estradiol levels. The authors' mechanism study indicated that the enrichment of LXR $\alpha$  to LXRE on the *Sult1e1* gene promoter of male *Ikkkb $\Delta$ hep* mice might be responsible for their heightened sensitivity to NASH. These results suggest hepatocyte IKK $\beta$  is protective in males due at least in part to its ability to repress LXR responsive induction of *Sult1e1* (Matsushita et al., 2017).

### **3.4.3. *SULT1E1* in cholestasis**

Few studies were focused on the role of SULT1E1 in cholestasis, but a recent study suggested that cholestasis induced FXR activation can lead to the suppression of SULT1E1, and hence impede hepatic deactivation of estrogens (Liu et al., 2018). In this study, the authors reported the positive correlation between elevated bile acid levels and estradiol levels in patients with obstructive cholestasis or primary biliary cirrhosis. By using female WT and *Fxr*<sup>-/-</sup> mice, the authors went on to show that cholestasis-induced accumulation of estradiol was associated with a down-regulation liver SULT1E1 expression in an FXR-dependent manner. Mechanistic studies using the human hepatoma Huh7 cells showed that bile acid activation of FXR repressed SULT1E1 by inhibiting the positive regulation of SULT1E1 by HNF4 $\alpha$  (Liu et al., 2018).

#### **3.4.4. *SULT1E1* in liver injury induced by sepsis and ischemia/reperfusion**

SULT1E1 is relatively thoroughly studied in inflammation-based conditions. Sepsis, resulting from the host's deleterious systemic inflammatory response to microbial infections, is a major cause of mortality in the intensive care unit. Although sepsis and its associated inflammation are known to decrease the expression and activity of many drug metabolizing enzymes, we observed a major induction of *Sult1e1* and compromised estrogen activity in the liver of mice subjected to the bacterial lipopolysaccharide (LPS) or cecum ligation and puncture (CLP) models of sepsis. The inflammatory induction of *Sult1e1* gene by sepsis was mediated by NF- $\kappa$ B. Reciprocally, the expression and activity of *Sult1e1* can impact the clinical outcome of sepsis. Specifically, ablation of the *Sult1e1* gene or pharmacological inhibition of the *Sult1e1* enzyme by Triclosan sensitized mice to sepsis-induced death in an estrogen dependent manner. The increased sepsis induced lethality in *Sult1e1* knockout mice was explained to be due to increased estrogen activity

and the resultant attenuated sepsis-induced pro-survival inflammatory response (Chai et al., 2015c).

Hepatic ischemia-reperfusion (I/R) injury is a major cause of liver damage. The pathogenesis of hepatic I/R injury is a dynamic process including the deprivation of blood and oxygen supply during the ischemic phase, followed by their restoration during the reperfusion phase, which is associated with oxidative stress and inflammation. An induction of *Sult1e1* in rats subjected to I/R was reported as early as 2006, but without a clearly defined mechanism or understanding of the biological significance (Svetlov et al., 2006). More recently, we reported a systemic analysis of the effect of liver I/R on the expression of *Sult1e1*. We showed that the expression of *Sult1e1* was markedly induced by I/R in the mouse liver. The ablation of *Sult1e1* protected female mice from the injury in an estrogen dependent manner, but heightened liver injury in male mice in an androgen-dependent manner. Further mechanism study established *Sult1e1* as a direct transcriptional target of nuclear factor erythroid 2-related factor (Nrf2), a key transcriptional factor responsible for the activation of an array of genes to adapt the cells to hypoxic or oxidative damages. Based on these results, we proposed that inhibition of SULT1E1, at least in females, may represent an effective approach to manage hepatic I/R injury (Guo et al., 2015).

### **3.4.5. SULT1E1 in cystic fibrosis**

Cystic fibrosis (CF), characterized by mutations of both copies of the cystic fibrosis transmembrane receptor (CFTR) gene, is an inherited disorder that causes progressive and eventually fatal damage to the lungs, digestive system, and other organs in the body. Liver is an organ that can be affected by CF. A series of papers published from the laboratory of Charles



Falany suggested that the hepatic *SULT1E1* may play a role in the progression of liver damage in CF patients (Li and Falany, 2007; He et al., 2008; Falany et al., 2009). Specifically, elevated hepatic *SULT1E1* activity was observed in mouse models of CF (Li and Falany, 2007) and HepG2 cells co-cultured with human MMNK-1 cholangiocytes with repressed CFTR (He et al., 2008). The induction of *SULT1E1* in CFTR-deficient MMNK-1 cells/HepG2 cells co-culture system was found to be dependent on the activation of LXR, and the *SULT1E1* induction led to alterations in the expression of estrogen responsive genes, including IGF-1, GST-P1 and carbonic anhydrase II, due to decreased  $E_2$  levels. These results suggest that the induction of hepatic *SULT1E1* in CF patients may have facilitated the development of CF liver disease (Falany et al., 2009).

## **4. SULT2**

### **4.1. SULT2A1**

#### **4.1.1. *SULT2A1* in cholestasis**

Cholestasis is an accumulation of bile acids in the liver as a result of increased bile acid production in the liver and/or insufficient detoxication and elimination of bile acids from the liver. *SULT2A1*, highly expressed in the liver and adrenal gland, mainly catalyzes the sulfation of hydroxysteroids including bile acids (Radomska et al., 1990), hydroxysteroid dehydroepiandrosterone (DHEA) (Otterness et al., 1992), and androgens. *SULT2A1* is reported to play an important role in the detoxification of bile acids and thereby preventing cholestasis via its enzymatic activity in bile acid sulfation. The expression of *SULT2A1* is transcriptionally regulated by several nuclear receptors, such as PXR (Sonoda et al., 2002), CAR (Saini et al., 2004),  $LXR\alpha$  (Uppal et al., 2007; Ou et al., 2014),  $HNF4\alpha$  (Fang et al., 2007), and FXR (Song et

al., 2001). The activation of *SULT2A1* by PXR, CAR, and LXR $\alpha$  was believed to play critical roles in the anti-cholestatic activity of these receptors in rodent models of cholestasis (Sonoda et al., 2002; Saini et al., 2004; Ou et al., 2014). In humans, it was reported that liver *SULT2A1* expression is decreased in human obstructive cholestasis due to gallstone biliary obstruction (Chai et al., 2015b). In a more recent clinical study, Wunsch and colleagues showed that liver *SULT2A1* expression is decreased in patients with primary sclerosing cholangitis, which was proposed to be due to *miR-378a-5p* mediated inhibition of the PXR/*SULT2A1* axis (Wunsch et al., 2015). Table 2 summarizes several studies suggesting that activation of *SULT2A1* expression through drug treatment protects against the development of cholestasis.

#### **4.1.2. *SULT2A1* in other liver diseases**

Down-regulations of *SULT2A1* gene were found in human HCC (Huang et al., 2005) and alcoholic liver disease (Yang et al., 2019b), but not in NASH (Suga et al., 2019). An increased liver *Sult2a1* gene expression was observed in choline-deficient, L-amino-acid-defined, high-fat diet-induced mouse model of NASH (Suga et al., 2019).

## **4.2. SULT2B1b**

### **4.2.1. *SULT2B1b* in hepatocellular carcinoma**

The cholesterol sulfotransferase *SULT2B1b* catalyzes the sulfation of cholesterol to synthesize cholesterol sulfate. Yang and colleagues found that *SULT2B1b* expression promotes the proliferation of HCC cells, which may have contributed to the progression of HCC. Specifically, the expression of *SULT2B1b* was found to be higher in HCC tumor tissues than their adjacent normal tissues. Moreover, overexpression of *SULT2B1b* promoted the growth of the mouse

HCC Hepa1-6 cells, while knockdown of *SULT2B1b* inhibited the cell growth with induced cell-cycle arrest and apoptosis via upregulating the expression of fatty acid synthase (*FAS*), downregulating the expression of *CyclinB1*, *BCL2* and *MYC* in vitro and in vivo (Yang et al., 2013). A follow-up mechanistic study from the same group reported that *SULT2B1b* promotes the proliferation of HCC cells via the activation of  $\beta$ -catenin/MMP7 pathway in hepatocytes and therefore enhances epithelial-mesenchymal transition (Yang et al., 2019a).

#### **4.2.2. *SULT2B1b* in NAFLD and metabolic liver disease**

*SULT2B1b* is also reported to play an important role in NAFLD. Overexpression of *SULT2B1b* decreased serum and hepatic lipids in mouse models of NAFLD via the suppression of liver X receptor (LXR)/sterol regulatory element binding protein-1c (SREBP1c) signaling pathway (Bai et al., 2012). In their study, the authors found *SULT2B1b* overexpression, combined with administration of the LXR agonist 25-hydroxycholesterol, significantly increased the formation of sulfated metabolite of 25-hydroxycholesterol in the liver tissue; decreased serum and hepatic lipid levels, including triglycerides, total cholesterol, free cholesterol, and free fatty acids, as compared to the control group both in WT and *Ldlr*<sup>-/-</sup> mice. *SULT2B1b* may have inhibited steatosis by sulfonation and deactivation of oxysterols, the endogenous agonists for the lipogenic nuclear receptor LXR. Indeed, gene expression analysis showed that overexpression of *SULT2B1b* was accompanied by the reduced expression of LXR target lipogenic genes, such as *SREBP-1*, *SREBP-2*, *acetyl-CoA carboxylase-1* and *FAS* (Bai et al., 2012). The inhibition of NAFLD was also observed in transgenic mice that overexpress *SULT2B1b* in the liver (Shi et al., 2014).

In addition to inhibiting lipogenesis, SULT2B1b and its metabolic byproduct cholesterol sulfate exhibit major activity in inhibiting hepatic gluconeogenesis and relieving metabolic liver disease (Shi et al., 2014; Bi et al., 2018). Initially, we reported that SULT2B1b can inhibit hepatic gluconeogenesis by suppressing the gluconeogenic activity of HNF4 $\alpha$  (Shi et al., 2014). In this study, we found that the treatment with cholesterol sulfate or transgenic overexpression of SULT2B1b in the liver inhibited hepatic gluconeogenesis and attenuated metabolic abnormalities in both the HFD-induced obesity and ob/ob mice. At the mechanistic level, treatment with cholesterol sulfate or overexpression of SULT2B1b inhibited gluconeogenesis via the suppression of acetyl-CoA synthetase (*Acss*) expression, leading to decreased acetylation and nuclear exclusion of HNF4 $\alpha$ . In a subsequent study, we reported that SULT2B1b can be transcriptionally and positively regulated by HNF4 $\alpha$  (Bi et al., 2018), an observation that led to our hypothesis that the transactivation of SULT2B1b by HNF4 $\alpha$  represents a negative feedback to limit the gluconeogenic activity of HNF4 $\alpha$ . Consistent with our hypothesis we showed that down-regulation of Sult2B1b promoted the gluconeogenic activity of HNF4 $\alpha$  as a result of decreased expression of the HNF4 $\alpha$  deacetylase Sirt1 and increased HNF4 $\alpha$  acetylation. Sult2b1b expression was also induced by HNF4 $\alpha$  upon fasting. Ablation of Sult2B1b in mice led to increased gluconeogenic gene expression and an elevated fasting glucose level, suggesting that SULT2B1b plays a restrictive role in HNF4 $\alpha$  mediated fasting responsive gluconeogenesis. In the same study, we also designed and synthesized thiocholesterol, a hydrolysis-resistant derivative of cholesterol sulfate, which showed more superior activity than the native cholesterol sulfate in inhibiting gluconeogenesis and improving insulin sensitivity in high-fat diet induced diabetic mice. Based on these results, we conclude that the HNF4 $\alpha$ -SULT2B1b-cholesterol sulfate axis represents a key endogenous mechanism to prevent uncontrolled gluconeogenesis.

#### **4.2.3. *SULT2B1b* in APAP-induced liver injury**

In a recent study, we uncovered an unexpected role for SULT2B1b in APAP-induced liver injury. Hepatic overexpression of *SULT2b1b* enhanced the sensitivity of mice to APAP-induced liver injury, whereas ablation of the *Sult2B1b* in mice conferred resistance to the APAP hepatotoxicity (An et al., 2019). This is a somewhat surprising result considering that sulfation is generally considered to be a metabolic pathway that detoxifies APAP. Consistent with our previous finding that *SULT2B1b* is a transcriptional target of *HNF4 $\alpha$* , *Hnf4 $\alpha$*  overexpression also sensitized mice to APAP-induced hepatotoxicity in a *Sult2b1b*-dependent manner, indicating that the *HNF4 $\alpha$* -*SULT2B1b* axis plays a unique role in APAP-induced hepatotoxicity, and SULT2B1b induction might be a risk factor for APAP toxicity (An et al., 2019).

As summarized in Figure 2, SULT2B1b plays important roles in the development of liver cancer, NAFLD, and sensitivity to APAP-induced liver injury.

### **5. Summary and Perspectives**

Over the past 20 years or so, significant advances have been achieved in our understanding of the roles of SULTs in multiple liver diseases, including liver cancers, non-alcoholic liver diseases, and drug-induced liver injury. Accumulating evidence shows altered expression and/or regulation of SULTs in liver diseases can reciprocally alter clinical consequences of liver diseases. Sex-, tissues-, and development-specific regulations of SULTs, such as SULT1A1/2 and SULT1E1, appeared to be important factors affecting the pathogenesis of liver diseases. In most cases, the effect of the SULTs on liver diseases can be explained by the sulfonation of

xenobiotics and endobiotics, which has a major impact on the chemical and functional homeostasis of these chemicals.

Despite the progress in our understanding of the role of SULTs in liver diseases, there are a number of remaining questions and challenges: 1) the identification of previously unknown pathophysiologic functions of SULT isoforms and understanding the molecular mechanisms underlying the roles of SULT beyond regulating the sulfation and deactivation of a variety of endogenous and exogenous molecules; 2) exploring SULTs and SULTs mediated signaling pathways as therapeutic targets for disease conditions such as NAFLD/NASH, sepsis- or ischemia reperfusion - induced liver injury, drug-induced liver injury, and even cancers; 3) investigating the species specificity of the SULTs' functions; the majority of the in vivo and mechanistic studies have been focused on rodents; human studies are necessary to determine whether the rodent results are translatable to humans; and 5) the humanizations of cytochrome P450 enzymes, such as CYP3A4 (Granvil et al., 2003; Cheung et al., 2006) and 2D6 (Corchero et al., 2001) have been reported, but the humanization of SULTs is a largely unexplored territory.

## **Authorship Contributions**

*Wrote or contributed to the writing of the manuscript: Y. Xie, W. Xie.*

## References

- Albrethsen J, Miller LM, Novikoff PM, and Angeletti RH (2011) Gel-based proteomics of liver cancer progression in rat. *Biochim Biophys Acta* **1814**:1367-1376.
- Ambadapadi S, Wang PL, Palii SP, and James MO (2017) Celecoxib affects estrogen sulfonation catalyzed by several human hepatic sulfotransferases, but does not stimulate 17-sulfonation in rat liver. *J Steroid Biochem Mol Biol* **172**:46-54.
- An Y, Wang P, Xu P, Tung HC, Xie Y, Kirisci L, Xu M, Ren S, Tian X, Ma X, and Xie W (2019) An Unexpected Role of Cholesterol Sulfotransferase and its Regulation in Sensitizing Mice to Acetaminophen-Induced Liver Injury. *Mol Pharmacol* **95**:597-605.
- Bai Q, Zhang X, Xu L, Kakiyama G, Heuman D, Sanyal A, Pandak WM, Yin L, Xie W, and Ren S (2012) Oxysterol sulfation by cytosolic sulfotransferase suppresses liver X receptor/sterol regulatory element binding protein-1c signaling pathway and reduces serum and hepatic lipids in mouse models of nonalcoholic fatty liver disease. *Metabolism* **61**:836-845.
- Bairam AF, Rasool MI, Alherz FA, Abunnaja MS, El Daibani AA, Kurogi K, and Liu MC (2018) Effects of human SULT1A3/SULT1A4 genetic polymorphisms on the sulfation of acetaminophen and opioid drugs by the cytosolic sulfotransferase SULT1A3. *Arch Biochem Biophys* **648**:44-52.
- Bansal S and Lau AJ (2019) Inhibition of Human Sulfotransferase 2A1-Catalyzed Sulfonation of Lithocholic Acid, Glycolithocholic Acid, and Taurolithocholic Acid by Selective Estrogen Receptor Modulators and Various Analogs and Metabolites. *J Pharmacol Exp Ther* **369**:389-405.



- Barbosa ACS, Feng Y, Yu C, Huang M, and Xie W (2019) Estrogen sulfotransferase in the metabolism of estrogenic drugs and in the pathogenesis of diseases. *Expert Opin Drug Metab Toxicol* **15**:329-339.
- Baumann E (1876) Ueber  $\alpha$ -Kresylschwefelsäure. *European Journal of Inorganic Chemistry* **9**:1389-1392.
- Bellamri M, Xiao S, Murugan P, Weight CJ, and Turesky RJ (2018) Metabolic Activation of the Cooked Meat Carcinogen 2-Amino-1-Methyl-6-Phenylimidazo[4,5-b]Pyridine in Human Prostate. *Toxicol Sci* **163**:543-556.
- Bi Y, Shi X, Zhu J, Guan X, Garbacz WG, Huang Y, Gao L, Yan J, Xu M, Ren S, Ren S, Liu Y, Ma X, Li S, and Xie W (2018) Regulation of Cholesterol Sulfotransferase SULT2B1b by Hepatocyte Nuclear Factor 4 $\alpha$  Constitutes a Negative Feedback Control of Hepatic Gluconeogenesis. *Mol Cell Biol* **38**.
- Bjerregaard-Olesen C, Bossi R, Bech BH, and Bonfeld-Jorgensen EC (2015) Extraction of perfluorinated alkyl acids from human serum for determination of the combined xenoestrogenic transactivity: a method development. *Chemosphere* **129**:232-238.
- Boccia S, Miele L, Panic N, Turati F, Arzani D, Cefalo C, Amore R, Bulajic M, Pompili M, Rapaccini G, Gasbarrini A, La Vecchia C, and Grieco A (2015) The effect of CYP, GST, and SULT polymorphisms and their interaction with smoking on the risk of hepatocellular carcinoma. *Biomed Res Int* **2015**:179867.
- Chai J, Du X, Chen S, Feng X, Cheng Y, Zhang L, Gao Y, Li S, He X, Wang R, Zhou X, Yang Y, Luo W, and Chen W (2015a) Oral administration of oleanolic acid, isolated from *Swertia mussotii* Franch, attenuates liver injury, inflammation, and cholestasis in bile duct-ligated rats. *Int J Clin Exp Med* **8**:1691-1702.

- Chai J, Feng X, Zhang L, Chen S, Cheng Y, He X, Yang Y, He Y, Wang H, Wang R, and Chen W (2015b) Hepatic expression of detoxification enzymes is decreased in human obstructive cholestasis due to gallstone biliary obstruction. *PLoS One* **10**:e0120055.
- Chai X, Guo Y, Jiang M, Hu B, Li Z, Fan J, Deng M, Billiar TR, Kucera HR, Gaikwad NW, Xu M, Lu P, Yan J, Fu H, Liu Y, Yu L, Huang M, Zeng S, and Xie W (2015c) Oestrogen sulfotransferase ablation sensitizes mice to sepsis. *Nat Commun* **6**:7979.
- Chen P, Li D, Chen Y, Sun J, Fu K, Guan L, Zhang H, Jiang Y, Li X, Zeng X, Chen X, Huang M, and Bi H (2017) p53-mediated regulation of bile acid disposition attenuates cholic acid-induced cholestasis in mice. *Br J Pharmacol* **174**:4345-4361.
- Chen P, Zeng H, Wang Y, Fan X, Xu C, Deng R, Zhou X, Bi H, and Huang M (2014) Low dose of oleanolic acid protects against lithocholic acid-induced cholestasis in mice: potential involvement of nuclear factor-E2-related factor 2-mediated upregulation of multidrug resistance-associated proteins. *Drug Metab Dispos* **42**:844-852.
- Cheung C, Yu AM, Chen CS, Krausz KW, Byrd LG, Feigenbaum L, Edwards RJ, Waxman DJ, and Gonzalez FJ (2006) Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. *J Pharmacol Exp Ther* **316**:1328-1334.
- Chou HC, Lang NP, and Kadlubar FF (1995) Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* **55**:525-529.
- Cieslak A, Trottier J, Verreault M, Milkiewicz P, Vohl MC, and Barbier O (2018) N-3 Polyunsaturated Fatty Acids Stimulate Bile Acid Detoxification in Human Cell Models. *Can J Gastroenterol Hepatol* **2018**:6031074.

- Corchero J, Granvil CP, Akiyama TE, Hayhurst GP, Pimprale S, Feigenbaum L, Idle JR, and Gonzalez FJ (2001) The CYP2D6 humanized mouse: effect of the human CYP2D6 transgene and HNF4alpha on the disposition of debrisoquine in the mouse. *Mol Pharmacol* **60**:1260-1267.
- Dong R, Wang J, Gao X, Wang C, Liu K, Wu J, Liu Z, Sun H, Ma X, and Meng Q (2019) Yangonin protects against estrogen-induced cholestasis in a farnesoid X receptor-dependent manner. *Eur J Pharmacol* **857**:172461.
- Dooley TP, Haldeman-Cahill R, Joiner J, and Wilborn TW (2000) Expression profiling of human sulfotransferase and sulfatase gene superfamilies in epithelial tissues and cultured cells. *Biochem Biophys Res Commun* **277**:236-245.
- Dubaisi S, Caruso JA, Gaedigk R, Vyhldal CA, Smith PC, Hines RN, Kocarek TA, and Runge-Morris M (2019) Developmental Expression of the Cytosolic Sulfotransferases in Human Liver. *Drug Metab Dispos* **47**:592-600.
- Falany CN (1991) Molecular enzymology of human liver cytosolic sulfotransferases. *Trends Pharmacol Sci* **12**:255-259.
- Falany CN (1997) Enzymology of human cytosolic sulfotransferases. *FASEB J* **11**:206-216.
- Falany CN, He D, Li L, Falany JL, Wilborn TW, Kocarek TA, and Runge-Morris M (2009) Regulation of hepatic sulfotransferase (SULT) 1E1 expression and effects on estrogenic activity in cystic fibrosis (CF). *J Steroid Biochem Mol Biol* **114**:113-119.
- Fang HL, Strom SC, Ellis E, Duanmu Z, Fu J, Duniec-Dmuchowski Z, Falany CN, Falany JL, Kocarek TA, and Runge-Morris M (2007) Positive and negative regulation of human hepatic hydroxysteroid sulfotransferase (SULT2A1) gene transcription by rifampicin:

- roles of hepatocyte nuclear factor 4alpha and pregnane X receptor. *J Pharmacol Exp Ther* **323**:586-598.
- Fang JL, Wu Y, Gamboa da Costa G, Chen S, Chitranshi P, and Beland FA (2016) Human Sulfotransferases Enhance the Cytotoxicity of Tolvaptan. *Toxicol Sci* **150**:27-39.
- Fujita K, Nagata K, Ozawa S, Sasano H, and Yamazoe Y (1997) Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J Biochem* **122**:1052-1061.
- Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, and McManus ME (2006) Human sulfotransferases and their role in chemical metabolism. *Toxicol Sci* **90**:5-22.
- Gao J, He J, Shi X, Stefanovic-Racic M, Xu M, O'Doherty RM, Garcia-Ocana A, and Xie W (2012) Sex-specific effect of estrogen sulfotransferase on mouse models of type 2 diabetes. *Diabetes* **61**:1543-1551.
- Gao X, Fu T, Wang C, Ning C, Liu K, Liu Z, Sun H, Ma X, Huo X, Yang X, Zou M, and Meng Q (2018) Yangonin protects against cholestasis and hepatotoxicity via activation of farnesoid X receptor in vivo and in vitro. *Toxicol Appl Pharmacol* **348**:105-116.
- Glatt H, Boeing H, Engelke CE, Ma L, Kuhlow A, Pabel U, Pomplun D, Teubner W, and Meinel W (2001) Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. *Mutat Res* **482**:27-40.
- Granvil CP, Yu AM, Elizondo G, Akiyama TE, Cheung C, Feigenbaum L, Krausz KW, and Gonzalez FJ (2003) Expression of the human CYP3A4 gene in the small intestine of transgenic mice: in vitro metabolism and pharmacokinetics of midazolam. *Drug Metab Dispos* **31**:548-558.

- Guo Y, Hu B, Huang H, Tsung A, Gaikwad NW, Xu M, Jiang M, Ren S, Fan J, Billiar TR, Huang M, and Xie W (2015) Estrogen Sulfotransferase Is an Oxidative Stress-responsive Gene That Gender-specifically Affects Liver Ischemia/Reperfusion Injury. *J Biol Chem* **290**:14754-14764.
- Hardwick RN, Ferreira DW, More VR, Lake AD, Lu Z, Manautou JE, Slitt AL, and Cherrington NJ (2013) Altered UDP-glucuronosyltransferase and sulfotransferase expression and function during progressive stages of human nonalcoholic fatty liver disease. *Drug Metab Dispos* **41**:554-561.
- He D, Wilborn TW, Falany JL, Li L, and Falany CN (2008) Repression of CFTR activity in human MMNK-1 cholangiocytes induces sulfotransferase 1E1 expression in co-cultured HepG2 hepatocytes. *Biochim Biophys Acta* **1783**:2391-2397.
- Hempel N, Negishi M, and McManus ME (2005) Human SULT1A genes: cloning and activity assays of the SULT1A promoters. *Methods Enzymol* **400**:147-165.
- Herrero J, Muffato M, Beal K, Fitzgerald S, Gordon L, Pignatelli M, Vilella AJ, Searle SM, Amode R, Brent S, Spooner W, Kulesha E, Yates A, and Flicek P (2016) Ensembl comparative genomics resources. *Database (Oxford)* **2016**.
- Hoie AH, Monien BH, Glatt H, Hjertholm H, and Husoy T (2016) DNA adducts induced by food mutagen PhIP in a mouse model expressing human sulfotransferases 1A1 and 1A2. *Toxicol Lett* **248**:34-38.
- Huang C, Chen Y, Zhou T, and Chen G (2009) Sulfation of dietary flavonoids by human sulfotransferases. *Xenobiotica* **39**:312-322.

- Huang J, Bathena SP, Tong J, Roth M, Hagenbuch B, and Alnouti Y (2010) Kinetic analysis of bile acid sulfation by stably expressed human sulfotransferase 2A1 (SULT2A1). *Xenobiotica* **40**:184-194.
- Huang LR, Coughtrie MW, and Hsu HC (2005) Down-regulation of dehydroepiandrosterone sulfotransferase gene in human hepatocellular carcinoma. *Mol Cell Endocrinol* **231**:87-94.
- Jancova P, Anzenbacher P, and Anzenbacherova E (2010) Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **154**:103-116.
- Kauffman FC (2004) Sulfonation in pharmacology and toxicology. *Drug Metab Rev* **36**:823-843.
- Kim IW, Han N, Kim MG, Kim T, and Oh JM (2015) Copy number variability analysis of pharmacogenes in patients with lymphoma, leukemia, hepatocellular, and lung carcinoma using The Cancer Genome Atlas data. *Pharmacogenet Genomics* **25**:1-7.
- Krattinger R, Bostrom A, Lee SML, Thasler WE, Schioth HB, Kullak-Ublick GA, and Mwinyi J (2016) Chenodeoxycholic acid significantly impacts the expression of miRNAs and genes involved in lipid, bile acid and drug metabolism in human hepatocytes. *Life Sci* **156**:47-56.
- Lee SR, Lee SY, Kim SY, Ryu SY, Park BK, and Hong EJ (2017) Hydroxylation and sulfation of sex steroid hormones in inflammatory liver. *J Biomed Res* **31**:437-444.
- Li L and Falany CN (2007) Elevated hepatic SULT1E1 activity in mouse models of cystic fibrosis alters the regulation of estrogen responsive proteins. *J Cyst Fibros* **6**:23-30.
- Li Y, Chen Z, Paonessa JD, Meinel W, Bhattacharya A, Glatt H, Vouros P, and Zhang Y (2018) Strong impact of sulfotransferases on DNA adduct formation by 4-aminobiphenyl in bladder and liver in mice. *Cancer Med* **7**:5604-5610.

Liu X, Xue R, Yang C, Gu J, Chen S, and Zhang S (2018) Cholestasis-induced bile acid elevates estrogen level via farnesoid X receptor-mediated suppression of the estrogen sulfotransferase SULT1E1. *J Biol Chem* **293**:12759-12769.

Matsushita N, Hassanein MT, Martinez-Clemente M, Lazaro R, French SW, Xie W, Lai K, Karin M, and Tsukamoto H (2017) Gender difference in NASH susceptibility: Roles of hepatocyte Ikkbeta and Sult1e1. *PLoS One* **12**:e0181052.

Meng Q, Chen X, Wang C, Liu Q, Sun H, Sun P, Huo X, Liu Z, Yao J, and Liu K (2015a) Protective Effects of Alisol B 23-Acetate Via Farnesoid X Receptor-Mediated Regulation of Transporters and Enzymes in Estrogen-Induced Cholestatic Liver Injury in Mice. *Pharm Res* **32**:3688-3698.

Meng Q, Chen XL, Wang CY, Liu Q, Sun HJ, Sun PY, Huo XK, Liu ZH, Yao JH, and Liu KX (2015b) Alisol B 23-acetate protects against ANIT-induced hepatotoxicity and cholestasis, due to FXR-mediated regulation of transporters and enzymes involved in bile acid homeostasis. *Toxicol Appl Pharmacol* **283**:178-186.

Miele L, Dall'armi V, Cefalo C, Nedovic B, Arzani D, Amore R, Rapaccini G, Gasbarrini A, Ricciardi W, Grieco A, and Boccia S (2014) A case-control study on the effect of metabolic gene polymorphisms, nutrition, and their interaction on the risk of non-alcoholic fatty liver disease. *Genes Nutr* **9**:383.

Mueller JW, Gilligan LC, Idkowiak J, Arlt W, and Foster PA (2015) The Regulation of Steroid Action by Sulfation and Desulfation. *Endocr Rev* **36**:526-563.

Mueller JW, Idkowiak J, Gesteira TF, Vallet C, Hardman R, van den Boom J, Dhir V, Knauer SK, Rosta E, and Arlt W (2018) Human DHEA sulfation requires direct interaction

- between PAPS synthase 2 and DHEA sulfotransferase SULT2A1. *J Biol Chem* **293**:9724-9735.
- Mungenast F, Aust S, Vergote I, Vanderstichele A, Sehouli J, Braicu E, Mahner S, Castillo-Tong DC, Zeillinger R, and Thalhammer T (2017) Clinical significance of the estrogen-modifying enzymes steroid sulfatase and estrogen sulfotransferase in epithelial ovarian cancer. *Oncol Lett* **13**:4047-4054.
- Negishi M, Pedersen LG, Petrotchenko E, Shevtsov S, Gorokhov A, Kakuta Y, and Pedersen LC (2001) Structure and function of sulfotransferases. *Arch Biochem Biophys* **390**:149-157.
- Otterness DM, Wieben ED, Wood TC, Watson WG, Madden BJ, McCormick DJ, and Weinshilboum RM (1992) Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA. *Mol Pharmacol* **41**:865-872.
- Ou Z, Jiang M, Hu B, Huang Y, Xu M, Ren S, Li S, Liu S, Xie W, and Huang M (2014) Transcriptional regulation of human hydroxysteroid sulfotransferase SULT2A1 by LXRalpha. *Drug Metab Dispos* **42**:1684-1689.
- Pai TG, Suiko M, Sakakibara Y, and Liu MC (2001) Sulfation of flavonoids and other phenolic dietary compounds by the human cytosolic sulfotransferases. *Biochem Biophys Res Commun* **285**:1175-1179.
- Pasqualini JR (2009) Estrogen sulfotransferases in breast and endometrial cancers. *Ann N Y Acad Sci* **1155**:88-98.
- Radomska A, Comer KA, Zimniak P, Falany J, Iscan M, and Falany CN (1990) Human liver steroid sulphotransferase sulphates bile acids. *Biochem J* **272**:597-604.



- Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, and Weinshilboum RM (1997) Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. *Biochem Biophys Res Commun* **239**:298-304.
- Raftogianis RB, Wood TC, and Weinshilboum RM (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. *Biochem Pharmacol* **58**:605-616.
- Reinen J and Vermeulen NP (2015) Biotransformation of endocrine disrupting compounds by selected phase I and phase II enzymes--formation of estrogenic and chemically reactive metabolites by cytochromes P450 and sulfotransferases. *Curr Med Chem* **22**:500-527.
- Saeki Y, Sakakibara Y, Araki Y, Yanagisawa K, Suiko M, Nakajima H, and Liu MC (1998) Molecular cloning, expression, and characterization of a novel mouse liver SULT1B1 sulfotransferase. *J Biochem* **124**:55-64.
- Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, Moore DD, Evans RM, and Xie W (2004) A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Mol Pharmacol* **65**:292-300.
- Sak K and Everaus H (2016) Sulfotransferase 1A1 as a Biomarker for Susceptibility to Carcinogenesis: From Molecular Genetics to the Role of Dietary Flavonoids. *Curr Drug Metab* **17**:528-541.
- Shi X, Cheng Q, Xu L, Yan J, Jiang M, He J, Xu M, Stefanovic-Racic M, Sipula I, O'Doherty RM, Ren S, and Xie W (2014) Cholesterol sulfate and cholesterol sulfotransferase inhibit gluconeogenesis by targeting hepatocyte nuclear factor 4alpha. *Mol Cell Biol* **34**:485-497.

- Sidharthan NP, Minchin RF, and Butcher NJ (2013) Cytosolic sulfotransferase 1A3 is induced by dopamine and protects neuronal cells from dopamine toxicity: role of D1 receptor-N-methyl-D-aspartate receptor coupling. *J Biol Chem* **288**:34364-34374.
- Sinreih M, Knific T, Anko M, Hevir N, Vouk K, Jerin A, Frkovic Grazio S, and Rizner TL (2017) The Significance of the Sulfatase Pathway for Local Estrogen Formation in Endometrial Cancer. *Front Pharmacol* **8**:368.
- Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, and Chatterjee B (2001) Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. *J Biol Chem* **276**:42549-42556.
- Song WC (2001) Biochemistry and reproductive endocrinology of estrogen sulfotransferase. *Ann N Y Acad Sci* **948**:43-50.
- Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, and Evans RM (2002) Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). *Proc Natl Acad Sci U S A* **99**:13801-13806.
- Strott CA (2002) Sulfonation and molecular action. *Endocr Rev* **23**:703-732.
- Suga T, Yamaguchi H, Ogura J, Shoji S, Maekawa M, and Mano N (2019) Altered bile acid composition and disposition in a mouse model of non-alcoholic steatohepatitis. *Toxicol Appl Pharmacol* **379**:114664.
- Svetlov SI, Xiang Y, Oli MW, Foley DP, Huang G, Hayes RL, Ottens AK, and Wang KK (2006) Identification and preliminary validation of novel biomarkers of acute hepatic ischaemia/reperfusion injury using dual-platform proteomic/degradomic approaches. *Biomarkers* **11**:355-369.

- Uppal H, Saini SP, Moschetta A, Mu Y, Zhou J, Gong H, Zhai Y, Ren S, Michalopoulos GK, Mangelsdorf DJ, and Xie W (2007) Activation of LXRs prevents bile acid toxicity and cholestasis in female mice. *Hepatology* **45**:422-432.
- Wang D, Li Q, Favis R, Jadwin A, Chung H, Fu DJ, Savitz A, Gopal S, and Cohen N (2014) SULT4A1 haplotype: conflicting results on its role as a biomarker of antipsychotic response. *Pharmacogenomics* **15**:1557-1564.
- Wang J, Falany JL, and Falany CN (1998) Expression and characterization of a novel thyroid hormone-sulfating form of cytosolic sulfotransferase from human liver. *Mol Pharmacol* **53**:274-282.
- Wang J, Fu T, Dong R, Wang C, Liu K, Sun H, Huo X, Ma X, Yang X, and Meng Q (2019) Hepatoprotection of auraptene from the peels of citrus fruits against 17 $\alpha$ -ethinylestradiol-induced cholestasis in mice by activating farnesoid X receptor. *Food Funct* **10**:3839-3850.
- Wang L, Wu G, Wu F, Jiang N, and Lin Y (2017) Geniposide attenuates ANIT-induced cholestasis through regulation of transporters and enzymes involved in bile acids homeostasis in rats. *J Ethnopharmacol* **196**:178-185.
- Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, and Raftogianis RB (1997) Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *FASEB J* **11**:3-14.
- Wunsch E, Klak M, Wasik U, Milkiewicz M, Blatkiewicz M, Urasinska E, Barbier O, Bielicki D, Bogdanos DP, Elias E, and Milkiewicz P (2015) Liver Expression of Sulphotransferase 2A1 Enzyme Is Impaired in Patients with Primary Sclerosing Cholangitis: Lack of the Response to Enhanced Expression of PXR. *J Immunol Res* **2015**:571353.

- Xiang D, Yang J, Liu Y, He W, Zhang S, Li X, Zhang C, and Liu D (2019) Calculus Bovis Sativus Improves Bile Acid Homeostasis via Farnesoid X Receptor-Mediated Signaling in Rats With Estrogen-Induced Cholestasis. *Front Pharmacol* **10**:48.
- Xie C, Yan TM, Chen JM, Li XY, Zou J, Zhu LJ, Lu LL, Wang Y, Zhou FY, Liu ZQ, and Hu M (2017) LC-MS/MS quantification of sulfotransferases is better than conventional immunogenic methods in determining human liver SULT activities: implication in precision medicine. *Sci Rep* **7**:3858.
- Xu Y, Lin X, Xu J, Jing H, Qin Y, and Li Y (2018) SULT1E1 inhibits cell proliferation and invasion by activating PPARgamma in breast cancer. *J Cancer* **9**:1078-1087.
- Yalcin EB, Kulkarni SR, Slitt AL, and King R (2016) Bisphenol A sulfonation is impaired in metabolic and liver disease. *Toxicol Appl Pharmacol* **292**:75-84.
- Yalcin EB, More V, Neira KL, Lu ZJ, Cherrington NJ, Slitt AL, and King RS (2013) Downregulation of sulfotransferase expression and activity in diseased human livers. *Drug Metab Dispos* **41**:1642-1650.
- Yamamoto A, Kurogi K, Schiefer IT, Liu MY, Sakakibara Y, Suiko M, and Liu MC (2016) Human Cytosolic Sulfotransferase SULT1A3 Mediates the Sulfation of Dextrorphan. *Biol Pharm Bull* **39**:1432-1436.
- Yang F, Wang Y, Li G, Xue J, Chen ZL, Jin F, Luo L, Zhou X, Ma Q, Cai X, Li HR, and Zhao L (2018) Effects of corilagin on alleviating cholestasis via farnesoid X receptor-associated pathways in vitro and in vivo. *Br J Pharmacol* **175**:810-829.
- Yang X, Du X, Sun L, Zhao X, Zhu J, Li G, Tian J, Li X, and Wang Z (2019a) SULT2B1b promotes epithelial-mesenchymal transition through activation of the beta-catenin/MMP7 pathway in hepatocytes. *Biochem Biophys Res Commun* **510**:495-500.

- Yang X, Xu Y, Guo F, Ning Y, Zhi X, Yin L, and Li X (2013) Hydroxysteroid sulfotransferase SULT2B1b promotes hepatocellular carcinoma cells proliferation in vitro and in vivo. *PLoS One* **8**:e60853.
- Yang Z, Kusumanchi P, Ross RA, Heathers L, Chandler K, Oshodi A, Thoudam T, Li F, Wang L, and Liangpunsakul S (2019b) Serum Metabolomic Profiling Identifies Key Metabolic Signatures Associated With Pathogenesis of Alcoholic Liver Disease in Humans. *Hepatology Commun* **3**:542-557.
- Yetti H, Naito H, Yuan Y, Jia X, Hayashi Y, Tamada H, Kitamori K, Ikeda K, Yamori Y, and Nakajima T (2018) Bile acid detoxifying enzymes limit susceptibility to liver fibrosis in female SHRSP5/Dmcr rats fed with a high-fat-cholesterol diet. *PLoS One* **13**:e0192863.
- Zhang H, Varlamova O, Vargas FM, Falany CN, Leyh TS, and Varmalova O (1998) Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. *J Biol Chem* **273**:10888-10892.
- Zou J, Li H, Huang Q, Liu X, Qi X, Wang Y, Lu L, and Liu Z (2017) Dopamine-induced SULT1A3/4 promotes EMT and cancer stemness in hepatocellular carcinoma. *Tumour Biol* **39**:1010428317719272.

## Footnotes

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## Legends for Figures

**Figure 1. Diagram showing the sex- and tissue-specific effect of Sult1a1 on the susceptibility to ABP.** In male mice, more ABP was transported into bladder from the liver because of the androgen-dependent suppression of SULT1A1, leading to an increased sensitivity of bladder to ABP induced carcinogenesis. In female mice, a relatively high expression of SULT1A1 leads to increased sensitivity of the liver to the exposure to ABP and ABP-sulfate.

**Figure 2. Summary of the role of SULT2B1B in liver diseases.** Overexpression of SULT2B1b promotes the progression of HCC via the activation of  $\beta$ -catenin/MMP7 pathway in hepatocytes. Overexpression of SULT2B1b increases the sulfonation and deactivation of oxysterols and thereby dampens the lipogenic function of LXR and decreases the serum and hepatic lipids. In addition to inhibiting lipogenesis, SULT2B1b and its metabolic byproduct cholesterol sulfate exhibit major activity in inhibiting hepatic gluconeogenesis and relieving metabolic liver disease by suppressing the gluconeogenic activity of HNF4 $\alpha$ , a transcription factor that positively regulates *SULT2B1b* gene expression. The induction of SULT2B1b by HNF4 $\alpha$  represents a negative feedback to limit the gluconeogenic activity of HNF4 $\alpha$ . Overexpression of SULT2B1b sensitized mice to APAP-induced liver injury, and overexpression of Hnf4 $\alpha$  increases the sensitivity to APAP-induced hepatotoxicity in a Sult2b1b-dependent manner.

**Table 1. Changes of liver SULT1B1 (Sult1b1) gene expression under different liver disease conditions**

Reporter	Species	Treatment/genotype	Liver disease	Sult1b1 expression
GDS3087	Mouse	Trim 24-/-	HCC	↓
GDS1385	Rat	choline-deficient L-amino acid defined diet	HCC	↑
GDS5320	Mouse	Pdgf-c transgenic	HCC	No difference
GDS2509	Mouse	ATP7B-/-	Wilson's disease	↑
GDS4387	Human		HBV-associated ALF	No difference
GDS4881	Human		NAFLD: steatosis	No difference
GDS6248	Mouse	High Fat Diet	liver steatosis	No difference
GDS2413	Mouse	High Fat Diet	liver steatosis	↑
GDS4166	Mouse	High Fat Diet	liver steatosis	↑
GDS4817	Mouse	High Fat Diet	liver steatosis	↑
GDS4013	Mouse	High Fat Diet	NASH	No difference
GDS4013	Mouse	High Fat Diet	pre-NASH	No difference
GDS4013	Mouse	High Fat Diet	liver steatosis	No difference
GDS4506	Mouse	ob/ob	liver steatosis	↑
GDS1354	Rat	inhalation of CCl4	liver cirrhosis	↑
GDS4271	Human		Infant biliary atresia: liver	No difference
GDS5526	Mouse	Bile Duct Ligation	liver fibrosis	No difference



GDS4389	Human		alcoholic hepatitis	
GDS3752	Mouse	Concanavalin A	immune- mediated hepatitis	↓

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HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; HBV - associated ALF: hepatitis B virus - associated acute liver failure.

**Table 2. SULT2A1 expression in cholestasis.**

Compounds	Disease Models	Species	Expression of Sult2a1	Mediators	Clinical Outcome	References
Yangonin	estrogen-induced cholestasis	mouse	↑	FXR	attenuation	(Gao et al., 2018; Dong et al., 2019)
Auraptene	17 $\alpha$ -ethinylestradiol-induced cholestasis	mouse	↑	FXR	attenuation	(Wang et al., 2019)
Tamoxifen	cholestasis	human	↓	/	enhancement	(Bansal and Lau, 2019)
Calculus Bovis Sativus	estrogen-induced cholestasis	rat	↑	FXR	attenuation	(Xiang et al., 2019)
N-3 Polyunsaturated Fatty Acids	/	human cell line	↑	/	Induce BAs detoxification	(Cieslak et al., 2018)
Corilagin	ANIT-induced cholestasis	rat	↑	FXR	attenuation	(Yang et al., 2018)
Doxorubicin	cholic acid-induced cholestasis	mouse	↑	p53	attenuation	(Chen et al., 2017)
Geniposide	ANIT-induced cholestasis	rat	↑	FXR	attenuation	(Wang et al., 2017)
Alisol B 23-Acetate	estrogen-induced cholestasis	mouse	↑	FXR	attenuation	(Meng et al., 2015a)

Alisol B 23- Acetate	ANIT- induced cholestasis	mouse	↑	FXR	attenuation	(Meng et al., 2015b)
Oleanolic acid	bile duct ligation- induced cholestasis	rat	↑	/	attenuation	(Chai et al., 2015a)
Oleanolic acid	lithocholic acid- induced cholestasis	mouse	↑	ERR2	attenuation	(Chen et al., 2014)

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FXR: farnesoid X receptor; ERR2: estrogen receptor related receptor 2



