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TITLE PAGE

The Role of Sulfotransferases in Liver Diseases

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RUNNING TITLE PAGE

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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

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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 α - hepatocyte nuclear factor 4 α

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_2 - Estradiol

CFTR - cystic fibrosis transmembrane receptor

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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 α (HNF4 α). 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.

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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.

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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 (SO3-) 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.,

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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

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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

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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).

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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

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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*

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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

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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 diethynitrosamine (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*

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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 *IkbkbΔmye* mice with myeloid IKKβ deficiency in both genders but aggravated in male but not female *IkbkbΔhep* mice with hepatocyte IKKβ deficiency. Microarray analysis on liver tissues from male and female WT and *IkbkbΔhep* mice fed HCFD showed a significant upregulation of *Sult1e1* gene in *IkbkbΔhep* mice compared to WT mice. Among the four groups, the male *IkbkbΔhep* 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 LXRα to LXRE on the *Sult1e1* gene promoter of male *IkbkbΔhep* mice might be responsible for their heightened sensitivity to NASH. These results suggest hepatocyte IKKβ 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*

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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*$^{-/-}$ 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 HNF4α (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-kB. 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

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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

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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 (Radominska 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), LXRα (Uppal et al., 2007; Ou et al., 2014), HNF4α (Fang et al., 2007), and FXR (Song et

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al., 2001). The activation of SULT2A1 by PXR, CAR, and LXRα 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

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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 β-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 $Ldlr^{-/-}$ 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).

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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 HNF4α (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 HNF4α. In a subsequent study, we reported that SULT2B1b can be transcriptionally and positively regulated by HNF4α (Bi et al., 2018), an observation that led to our hypothesis that the transactivation of SULT2B1b by HNF4α represents a negative feedback to limit the gluconeogenic activity of HNF4α. Consistent with our hypothesis we showed that down-regulation of Sult2B1b promoted the gluconeogenic activity of HNF4α as a result of decreased expression of the HNF4α deacetylase Sirt1 and increased HNF4α acetylation. Sult2b1b expression was also induced by HNF4α 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 HNF4α 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 HNF4α-SULT2B1b-cholesterol sulfate axis represents a key endogenous mechanism to prevent uncontrolled gluconeogenesis.

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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 *HNF4α*, Hnf4α overexpression also sensitized mice to APAP-induced hepatotoxicity in a *Sult2b1b*-dependent manner, indicating that the *HNF4α-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

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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.

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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 β-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 HNF4α, a transcription factor that positively regulates *SULT2B1b* gene expression. The induction of SULT2B1b by HNF4α represents a negative feedback to limit the gluconeogenic activity of HNF4α. Overexpression of SULT2B1b sensitized mice to APAP-induced liver injury, and overexpression of Hnf4α increases the sensitivity to APAP-induced hepatotoxicity in a Sult2b1b-dependent manner.

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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

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

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

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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	17α-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)

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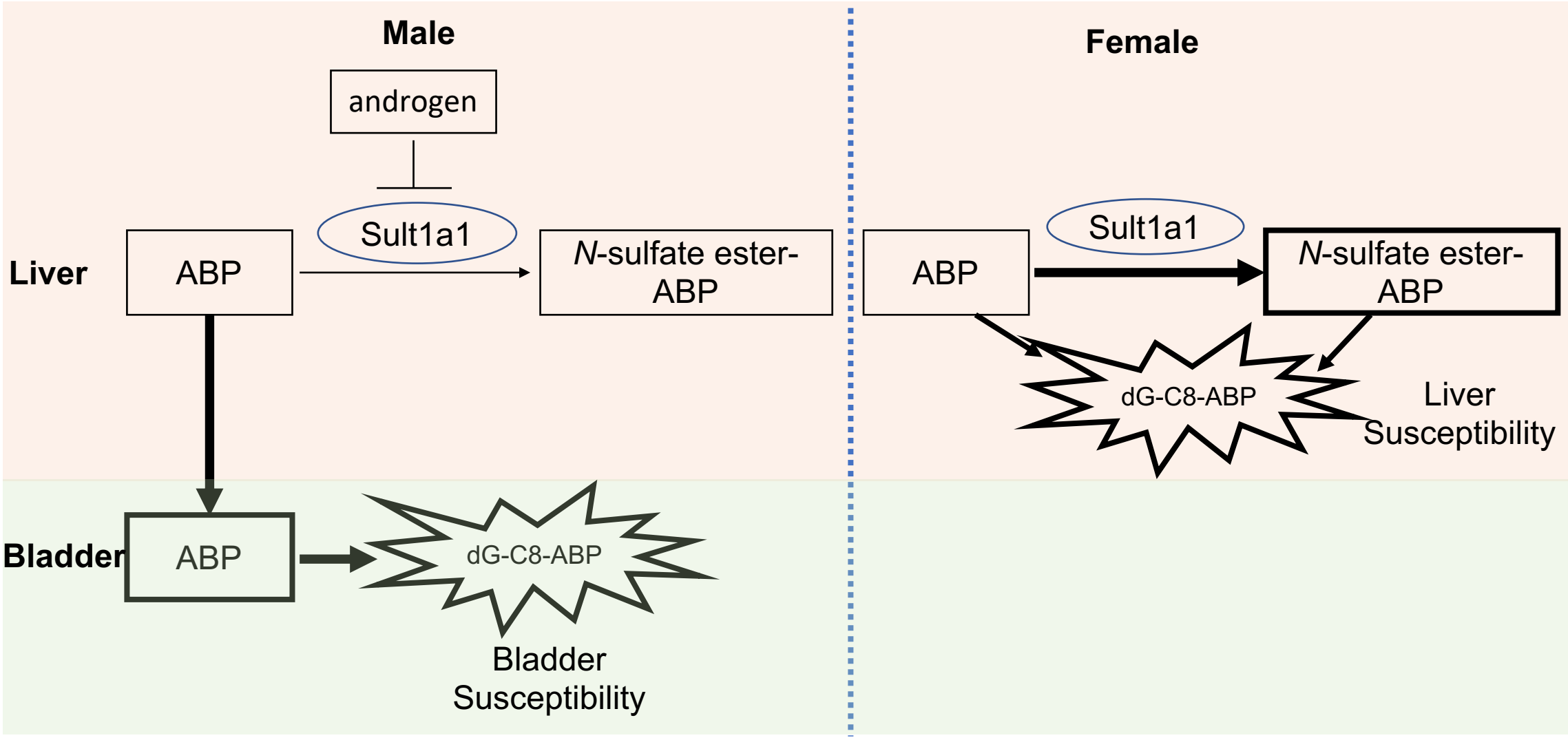
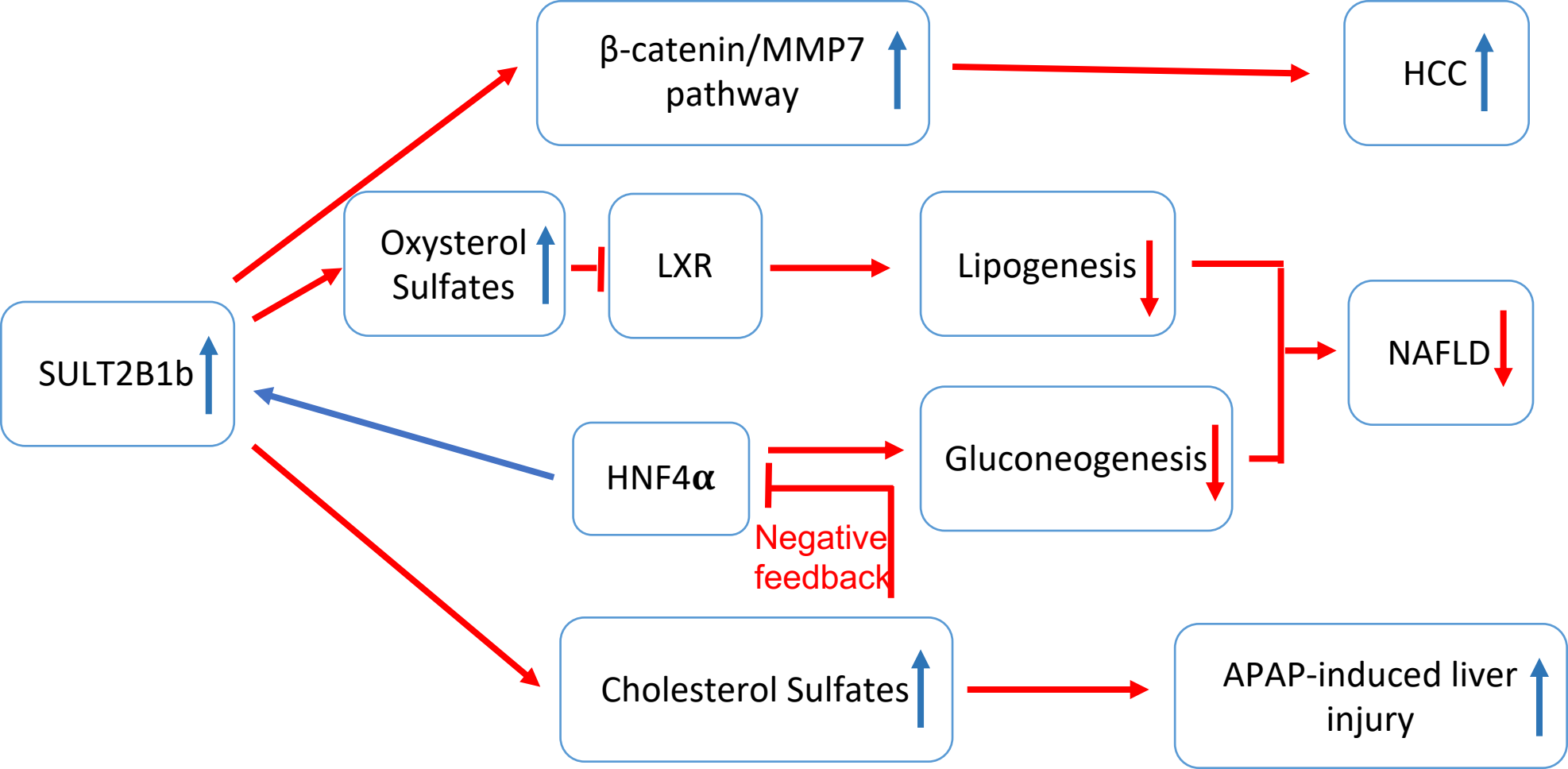
Alisol B 23-Acetate	ANIT-induced cholestasis	mouse	↑	FXR	attenuation	(Meng et al., 2015b)
Oleanolic acid	bile duct ligation-induced cholestasis	rat	↑	/	attenuation	(Chai et al., 2015a)
Oleanolic acid	lithocholic acid-induced cholestasis	mouse	↑	ERR2	attenuation	(Chen et al., 2014)

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


Male
androgen
Sult1a1
Liver
ABP
N-sulfate ester-ABP
Female
Sult1a1
ABP
N-sulfate ester-ABP
dG-C8-ABP
Liver Susceptibility
Bladder
ABP
dG-C8-ABP
Bladder Susceptibility


β-catenin/MMP7 pathway
HCC
Oxysterol Sulfates
LXR
Lipogenesis
NAFLD
SULT2B1b
HNF4α
Gluconeogenesis
Negative feedback
Cholesterol Sulfates
APAP-induced liver injury