Contributing Roles of CYP2E1 and Other Cytochrome P450 Isoforms in Alcohol-Related Tissue Injury and Carcinogenesis

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Abstract

The purpose of this review is to briefly summarize the roles of alcohol (ethanol) and related compounds in promoting cancer and inflammatory injury in many tissues. Long-term chronic heavy alcohol exposure is known to increase the chances of inflammation, oxidative DNA damage, and cancer development in many organs. The rates of alcohol-mediated organ damage and cancer risks are significantly elevated in the presence of co-morbidity factors such as poor nutrition, unhealthy diets, smoking, infection with bacteria or viruses, and exposure to pro-carcinogens. Chronic ingestion of alcohol and its metabolite acetaldehyde may initiate and/or promote the development of cancer in the liver, oral cavity, esophagus, stomach, gastrointestinal tract, pancreas, prostate, and female breast. In this chapter, we summarize the important roles of ethanol/acetaldehyde in promoting inflammatory injury and carcinogenesis in several tissues. We also review the updated roles of the ethanol-inducible cytochrome P450-2E1 (CYP2E1) and other cytochrome P450 isozymes in the metabolism of various potentially toxic substrates, and consequent toxicities, including carcinogenesis in different tissues. We also briefly describe the potential

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implications of endogenous ethanol produced by gut bacteria, as frequently observed in the experimental models and patients of nonalcoholic fatty liver disease, in promoting DNA mutation and cancer development in the liver and other tissues, including the gastrointestinal tract.

Keywords
Alcohol · Acetaldehyde · CYP2E1 · Oxidative stress · Inflammation · DNA mutation · Cancer

Introduction

Long-term chronic heavy alcohol (ethanol) intake is known to increase the incidences of cancer in many tissues, including the liver, mouth, esophagus, gastrointestinal tract, pancreas, prostate, and female breast [1–6]. The alcohol-mediated cancer rates are significantly increased in the presence of co-morbidity factors such as smoking, viral and bacterial infections, carcinogens, and potentially harmful diets, such as poor nutrition, western-style high fat diets, and soft drinks containing high fructose corn syrup. We have previously reviewed that the rates of alcohol-mediated cancer in experimental rodent models and alcoholic people are increased by the one or combinations of the following risk factors: (1) formation of etheno-(or acetaldehyde)-DNA adducts; (2) elevated production of reactive oxygen species (ROS), reactive nitrogen species (RNS), lipid peroxides, and metabolic conversion of procarcinogens to carcinogens via ethanol-inducible cytochrome P450-2E1 (CYP2E1); (3) accumulation of iron leading to increased ROS generation, lipid peroxidation, mutation of p53 gene or its covalent modifications of its protein; (4) decreased cellular levels of antioxidant glutathione (GSH) and S-adenosylmethionine (SAMe), resulting in oxidative stress and DNA hypomethylation of oncogenes and epigenetics changes; (5) depletion of retinoic acid with consequent cell proliferation through activation of activator protein-1 (AP-1); (6) activation of an inflammatory cascade via increased intestinal barrier dysfunction, resulting in endotoxemia, activation of tissue macrophages, including hepatic Kupffer cells via Toll-like receptor-4 (TLR-4), oxidative stress, the nuclear factor-KappaB (NF-kB) or early growth response-1 (Egr-1) activation, and production of inflammatory cytokines and chemokines; (7) reduced number and/or function of Natural Killer cells; (8) decreased activities of antioxidant enzymes and DNA repair enzymes. Since these areas have been covered in our previous review [1] and others [2–6], we specifically focus on the updated roles of CYP2E1-related oxidative stress, leaky gut, and metabolic activation of potentially toxic substrates in DNA adduct formation and cancer development in this chapter. The important roles of the ethanol-induced CYP2E1 and other cytochrome P450 isozymes in the metabolisms of various substrates and consequent cytotoxicities, including acetaldehyde and other carcinogenic substances in many tissues, are briefly summarized. Additive and/or synergistic interactions between ethanol and other risk factors toward increased levels of DNA adducts and carcinogenesis are also described. In addition, the potential roles of the endogenous ethanol and acetaldehyde produced by bacteria in certain tissues, as frequently observed in rodent models and patients with nonalcoholic fatty liver disease (NAFLD) and/or steatohepatitis (NASH), in promoting inflammatory tissue injury and cancer development are briefly discussed. Finally, based on the mechanistic study results, we have described the translational opportunities against alcohol-associated injury and carcinogenesis in many tissues.

Updated Mechanisms of Ethanol-Mediated Carcinogenesis

The International Agency for Research on Cancer (IARC) has concluded that both ethanol and its oxidative metabolite acetaldehyde are human

carcinogens [7]. One of the major factors for the increased cancer development in alcoholic individuals and ethanol-exposed animal models could be increased oxidative and nitrative stress, through activation of many pro-oxidant enzymes with decreased contents of small molecule antioxidants and suppressed antioxidant enzymes. In fact, chronic excessive alcohol intake is known to increase oxidative and nitrative stress which can be produced through impaired mitochondrial electron transport chain (i.e., mitochondrial dysfunction), elevated levels of the CYP2E1, NADPH oxidases, the inducible form of nitric oxide synthase (iNOS), xanthine oxidase, etc. [8–17]. In addition, chronic alcohol ingestion is known to decrease the levels of many small molecule antioxidants glutathione (GSH), *S*-adenosylmethionine (SAMe), folic acid, many vitamins, including retinol (vitamin A), thiamine (vitamin B1), ascorbic acid (vitamin C), vitamin D (ergocalciferol and cholecalciferol), α-tocopherol (vitamin E), menadione (vitamin K3) through insufficient absorption in the GI tract, suppression of biosynthesis, and increased metabolic degradation [1, 8, 18–21]. The depletion of these enzyme cofactors and co-enzymes can exert dramatic influences on major metabolic pathways and genetic/epigenetics changes. Moreover, the activities of antioxidant enzymes such as mitochondrial low-Km aldehyde dehydrogenase-2 (ALDH2), glutathione peroxidase (Gpx), superoxide dismutase (SOD), methionine adenosyltransferase-1 (MAT1), and catalase can be significantly suppressed through oxidative modifications in alcohol-exposed tissues [22–25]. It is likely that the decreased levels of antioxidants and suppressed antioxidant enzymes or proteins render the host more susceptible to inflammatory tissue injury and carcinogenesis [1–4].

Alcohol (ethanol) is not a strong carcinogen compared to its oxidative metabolite acetaldehyde. However, alcohol-induced carcinogenesis is significantly increased in the presence of a risk factor such as smoking, western-style high fat fast food, and viral infection. Elevated levels of highly reactive acetaldehyde and lipid aldehydes, through suppressed ALDH2 activity or genetic mutation in the *ALDH2* gene, as observed in many people in East Asian countries [26–28] or in various tissues of *Aldh2*-null mice exposed to alcohol gavages [29] can increase the amounts of DNA adducts and cancer. Alternatively, increased amounts of acetaldehyde produced from activated ADH through mutation of its gene [30] can interact with the amine groups of deoxyguanosine (dG), deoxyadenosine (dA), and deoxycytosine (dC) to generate N^2-ethylidene-DNA and more stable N^2-etheno-DNA adducts [31–33], contributing to increased mutagenesis and cancer development, as recently reviewed [34]. In the presence of reducing agents, such as polyamines, acetaldehyde dimer crotonaldehyde can interact with DNA and produce a stronger mutagenic propano-DNA adduct [35]. Furthermore, under increased oxidative stress following alcohol exposure, the amounts of lipid peroxides are significantly elevated and some of the highly reactive lipid aldehydes such as acrolein (ACR), malonaldehyde (MDA), MDA-Acetaldehyde (MDA-AA), and 4-hydroxynonenal (4-HNE) can interact with DNA, producing mutagenic DNA adducts, leading to increased carcinogenesis [36–38]. Analyses of human specimens revealed that the levels of the lipid-aldehyde DNA adducts in the liver and mucosa of the esophagus and colon in alcoholic people appear to depend on the levels of CYP2E1. In contrast, these adducts in some patients with NASH do not correlate with the CYP2E1 levels and are likely derived from inflammation-driven oxidative stress, as reviewed [39]. Furthermore, the rates of carcinogenesis could be markedly increased when p53 and DNA repair enzymes, such as oxoguanine DNA glycosylase (Ogg1), are inactivated in alcohol-exposed rodents [40, 41]. Although the molecular mechanisms for the inactivation of DNA repair enzymes in alcohol-exposed rodents have not been studied in detail, it is likely that these enzymes could be oxidatively modified and thus inactivated under increased oxidative and nitrative stress.

Contributing Roles of CYP2E1 and Other P450 Isoforms in Tissue Injury and Cancer Development

Multiple Regulations of CYP2E1 and Alcohol-Related Tissue Injury and Carcinogenesis

It is well-established that ingested ethanol is primarily metabolized by alcohol dehydrogenase (ADH, Km for ethanol 0.8–1 mM) expressed in the liver, esophagus, stomach, and intestine. However, after chronic alcohol exposure or intake of large amounts of ethanol, a significant amount of alcohol is also metabolized by another enzyme system so-called the microsomal ethanol oxidizing system (MEOS), consisting of CYP2E1, CYP1A2, and CYP3A with CYP2E1 being a major component [8, 9, 42, 43]. In fact, under higher blood alcohol concentration (BAC) up to ~100 mM, as observed in some alcoholics [44], ethanol-induced CYP2E1 (Km for ethanol 8–10 mM) becomes important in the oxidative metabolism of ethanol, producing acetaldehyde, which can impair intestinal barrier function and produce DNA adducts, contributing to inflammatory tissue injury [45] and carcinogenesis [34], respectively. Unlike other P450 enzymes, CYP2E1, a loosely bound enzyme to the ER membrane, exhibits NADPH oxidase activity, thus producing ROS during its catalytic cycle or even in the absence of its substrate, as reviewed earlier [8, 25, 42, 43]. The ROS include superoxide anion, hydrogen peroxide, and hydroxyethyl radical, depending on the local environment, including the presence of iron, which is known to be accumulated by alcohol exposure [46], and other preexisting conditions, contributing to DNA damage and carcinogenesis. In addition, CYP2E1 is known to produce RNS in certain conditions despite little induction of iNOS [47]. CYP2E1, present in both endoplasmic reticulum (ER) and mitochondria [48, 49] in the liver and extra-hepatic tissues such as kidney, colon, and brain [50], is induced and activated by acute or chronic exposure to alcohol and other small molecules such as acetone and isoniazid or pathophysiological conditions such as fasting and diabetes through different regulatory mechanisms [8, 18, 51–53]. Moreover, its level and activity are increased in obese and/or hyperglycemic diabetic rodents and in humans [8, 18, 52–55]. Because of different induction mechanisms of CYP2E1 (e.g., protein stabilization by ethanol or acetone [50, 56–58]), increased mRNA translation by isoniazid and pyridine, and mRNA increase by fasting, western-style high fat diet, over-feeding obesity, or diabetes [52–55], the overall levels and activities of CYP2E1 are expected to be increased in an additive or synergistic manner [8, 18, 59, 60]. For example, alcohol exposure in diabetic rodents and people would markedly elevate CYP2E1 activity, thus producing greater levels of oxidative stress and tissue injury [15, 18, 59]. Another example of additive or synergistic effect is interactions between alcohol drinking and other risk factors such as western-style high fat diet [52], nicotine [61], infection with hepatitis viruses [62], and certain chemicals or carcinogens, which are CYP2E1 substrates, such as dimethylnitrosamine (DMN) [56], diethylnitrosamine (DEN) [63], urethane, and benzene [64, 65], promoting acute toxicity or inflammatory tissue injury, as reviewed [15, 18, 59, 66]. Consequently, the degree of these interactions with cellular macromolecules, including DNA, is significantly increased in fasting or other pathological conditions with lower GSH levels [67], making the host more susceptible to oxidative DNA damage and mutations, contributing to inflammatory tissue injury and carcinogenesis.

In addition to the oxidative ethanol metabolism, CYP2E1 is known to metabolize many small molecule environmental toxicants and potential carcinogens, some of which are the inducers of CYP2E1 [51, 65]. The exogenous CYP2E1 substrate compounds are thioacetamide (TAA), acetaminophen (APAP), isoniazid, cisplatin, halothane, isoflurane, salicylic acid, solvents (e.g., ethylene, carbon tetrachloride, chloroform, dichloromethane, benzene, pyridine, and toluene), various long-chain fatty acids, DMN, DEN, bromodichloromethane, Vitamin A derivatives (retinoic acid), and others [51, 63–65]. Endogenous substrates of CYP2E1 can be acetone, long-chain

fatty acids, glycerol, 4-HNE, and others, including ethanol and acetaldehyde produced by oral or gut bacteria [18, 68–72]. Metabolism of these substrates by CYP2E1 and consequent organ damage appear to positively correlate with the levels of CYP2E1 activity, with a few exceptions of APAP- or carbon tetrachloride (CCL4)-exposed models, as reviewed [14, 18, 24, 66]. For instance, clinically relevant doses of APAP, halothane, thioacetamide, or CCL4 can cause acute drug-induced liver injury (DILI) or toxicity via alcohol and drug interactions, especially in alcohol-exposed or fasted individuals or rodents with increased CYP2E1. The APAP- or CCL4-mediated hepatic (and/or kidney) injury is initiated through their metabolism by CYP2E1, since pretreatment with CYP2E1 inhibitors or *Cyp2e1*-null mice was fully protected from these types of DILI or acute toxicity [24, 47, 73]. The decreased levels of retinoic acid by CYP2E1-mediated metabolism [74] and substrate competition with ethanol may also contribute to elevated hepatocyte proliferation and liver tumor progression in alcohol-exposed rodents and alcoholic individuals [74, 75]. By using knockout mice deficient of a specific pro-oxidant enzyme, Bradford and colleagues demonstrated that CYP2E1 but not NADPH oxidase is important in promoting alcohol-mediated DNA damage [76]. In this model, the levels of etheno-DNA adduct were significantly decreased in ethanol-exposed *Cyp2e1*-null mice compared to those of the wild-type mice. In contrast, the elevated levels of ethanol-related DNA adducts were unchanged and still observed in the corresponding *NADPH-oxidase*-null mice. In addition, the levels of exocyclic ethanol-DNA adduct were significantly increased in CYP2E1-overexpressing HepG2 cells upon ethanol exposure [77] and some patients with alcoholic fatty liver and fibrosis [78]. The levels of these DNA adducts can be significantly decreased in the presence of chlormethiazole (CMZ), a specific CYP2E1 inhibitor [75, 77]. Furthermore, the elevated levels of exocyclic ethanol-DNA adduct observed in experimental rodents were also observed in the biopsied esophagus specimens of human alcoholic patients with esophagus cancer [79]. The levels of etheno-DNA adduct significantly correlated with cell proliferation, which was markedly increased in people who both drank and smoked [80]. All these results strongly indicate an important role of CYP2E1 in producing carcinogenic etheno-DNA lesions in the experimental model and alcoholic individuals [75–77, 81].

Chronic inflammation plays an important role in cancer development and progression of malignant states [82–84]. A recent long-term epidemiological study with more than 121,000 health professional men and women revealed that consumption of pro-inflammatory diet is associated with colon cancer, underscoring the important role of inflammation in carcinogenesis [85]. Cancer-associated inflammation and inflammation-derived DNA lesions and malignancies seem to be genetically stable [36] and can be affected by the extrinsic and intrinsic factors. For instance, extrinsic factors, such as alcohol intake, smoking, viral and bacterial infections, exposure to environmental toxicants and pro-carcinogens, and pathophysiological conditions, can increase inflammation and cancer risk. Additionally, cancer-causing mutations can stimulate inflammatory reactions by activating and recruiting inflammatory cells through various pro-inflammatory cytokines and chemokines or complementary factors [82–84]. Additionally, it is well-established that excessive chronic alcohol intake can cause chronic inflammation through increased intestinal barrier dysfunction and endotoxemia [86–89]. Elevated plasma levels of bacterial endotoxin lipopolysaccharide (LPS) can interact with TLR-4 in the Kupffer cells, leading to inflammatory liver injury and carcinogenesis [82, 90]. It is possible that plasma LPS, a potent inducer of iNOS and nitration of many cellular proteins [89, 91, 92], can further stimulate inflammation and injury to the GI tract and other tissues. Recent data suggest that binge alcohol can stimulate gut leakiness and inflammatory liver injury in a CYP2E1-dependent manner [89, 93, 94]. The elevated CYP2E1 was responsible for increased oxidative and nitrative stress, causing nitration of several intestinal tight and adherent junction proteins [93]. Nitrated junctional complex proteins were degraded by

proteolytic degradation following ubiquitin conjugation. The markedly decreased amounts of the intestinal junctional complex proteins in binge ethanol-exposed rats compared to control counterparts were confirmed by quantitative mass-spectral analysis [93]. Subsequently, the levels of the gut junctional complex proteins were significantly decreased and contributed to leaky gut and endotoxemia in ethanol-exposed rodents and people who died suddenly due to heavy alcohol intoxication compared to people who died from nonalcoholic causes [93]. These events of ethanol-mediated leaky gut and inflammatory liver injury were not observed in the corresponding *Cyp2e1*-null mice or were significantly attenuated in the ethanol-exposed wild-type mice co-treated with CMZ, a specific inhibitor of CYP2E1 [93] or an antioxidant *N*-acetylcysteine (NAC) [89]. Consequently, elevated levels of serum LPS can upregulate TLR4 in the liver, stimulating inflammation and hepatic injury, including fibrosis, potentially leading to carcinogenesis [82, 95, 96]. Furthermore, both extrinsic and intrinsic inflammation are known to modulate or suppress immune responses, which can provide a suitable environment for alcohol-induced carcinogenesis and tumor progression, as reviewed [83, 84].

Distribution of CYP2E1 and Carcinogenesis in Extra-Hepatic Tissues

The majority of CYP2E1 is expressed in the liver. However, it is also expressed in many extrahepatic tissues such as kidney [50, 97], brain [50, 98, 99], lymphocytes [100], lung [101, 102], pancreas [103], nasal mucosa [104], esophagus [105], stomach [105], intestine [50, 93, 94], and female breast [106]. Induction of CYP2E1 (and other P450 isozymes) following exposure to ethanol, potentially environmental toxicants, or under pathophysiological conditions such as fasting and diabetes, is likely to result in production of ROS and RNS which can lead to increased levels of DNA adducts, inflammatory tissue injury, and carcinogenesis [75–81, 93, 94]. Furthermore, CYP2E1 was shown to stimulate post-translational modifications followed by inactivation of various proteins in different subcellular organelles, resulting in ER stress, mitochondrial dysfunction, and inflammatory cell death of many tissues, as reviewed [14, 18, 66, 92]. In many cases, the levels of tissue injury, DNA adducts, or cancer positively correlated with those of CYP2E1 [30–32, 61, 73–79, 93, 94, 107–109]. In contrast, no or little correlation between the severity of tissue injury and the CYP2E1 level was observed in some other cases [95, 97, 110]. However, the lack of correlation between the levels of CYP2E1 and DNA adduct or tissue injury does not necessarily rule out the important role of CYP2E1 because of its permissive role to allow other proteins or genes to exhibit their damaging effects, as described in some experimental models [95, 97, 110, 111].

Contribution of Other P450 Isoforms in Alcohol-Related Tissue Injury and Carcinogenesis

Most of the studies on alcohol-related DNA mutations and carcinogenesis appear to focus on the correlative roles of ADH, CYP2E1, and ALDH2 involved in the oxidative metabolism of ethanol and acetaldehyde [75–81, 96, 112]. However, it has been demonstrated that chronic ethanol exposure can induce CYP2E1 as well as other cytochrome P-450 isoforms, such as CYP1A1 [104], CYP2A5 [111, 113], and CYP3A [114, 115]. The levels of these P450 isoforms induced by ethanol exposure may be small compared to those of CYP2E1 induction. However, we also need to pay attention to their contributing roles in promoting DNA mutation, tissue injury, and caner in both liver and extra-hepatic tissues such as esophagus, gastrointestinal tract, nasal cavity, and lung in alcoholic individuals and/or alcohol-exposed rodents especially in the presence of another co-risk factors such as tobacco smoking and potentially harmful drugs, toxicants, or solvents. For instance, CYP1A1 is known to metabolize carcinogens aryl hydrocarbons [AHs] or polyaromatic hydrocarbons (PAHs) [116], such as benzo[a]pyrene and 3-methyl-cholanthrene, contained in charred foods and cigarette tars or smokes. Consequently,

ethanol-mediated induction of CYP1A would lead to increased levels of DNA adducts and carcinogenesis in the liver and other extra-hepatic tissues such as nose, esophagus, and lung. Similarly, ethanol-mediated inductions of CYP2A5 can increase DNA adducts in the lung since it is known to metabolize a tobacco carcinogen, the tobacco-related nitrosamine-related carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [117]. Likewise, alcohol-mediated induction of CYP3A4 [114, 115] and CYP2A5 [118] may render the host with increased levels of DNA adducts and carcinogenesis caused by a mycotoxin aflatoxin B1 (AFB1) [119]. In addition, elevated CYP3A isozymes are likely to metabolize many drugs, including tamoxifen, leading to their activation and production of DNA adducts and carcinogenesis in certain endometrial tissues such as uterus [120]. Furthermore, elevated CYP3A and other P450 isoforms may accelerate metabolic clearance of many drugs, including anti-retroviral agents, leading to oxidative stress and cellular injury [121, 122] in HIV-1-infected people, who exhibit higher rates of hepatic cirrhosis and cancer [123]. In fact, alcohol-mediated elevation/activation of other P450 isoforms in the esophagus, GI tract, nasal cavity, and lung is likely involved in increased DNA mutation, inflammatory injury, and cancer in these tissues in the presence of another risk factor, like smoking, and/or exposure to other potentially toxic substances, such as benzene and toluene [63–65], or western-style high fat fast foods [95].

Increased DNA Adducts, Inflammatory Tissue Injury, and Carcinogenesis in NAFLD/NASH Through the Production of Endogenous Ethanol and Acetaldehyde

In the previous section, we have focused on DNA mutations, inflammatory tissue injury, and carcinogenesis in alcoholic individuals and alcohol-exposed rodents. However, it is now known that people with NAFLD/NASH are more susceptible to DNA damage and cancer in the liver [124] and many extra-hepatic tissues, including the GI tract [125]. Some main reasons for increased DNA damage and cancer could be overgrowth of gut bacteria, increased alteration of gut microflora (dysbiosis), mucosal inflammation, oxidative/nitrative stress, and leaky gut after exposure to western-style high fat fast foods, fructose-rich soft drinks, and metabolic syndromes [124–128]. In addition, it was shown that ethanol and acetaldehyde can be endogenously produced in obese rodents with NAFLD [70, 129] and some people, including children with NAFLD/NASH [71, 129–131] without exogenous ethanol intake. Moreover, production of acetaldehyde was demonstrated in various bacteria present in the mouth [68, 69, 132], lung [132, 133], and GI tract, including colon [70, 129, 134]. Consequently, the levels of etheno-DNA adduct, inflammatory injury, and carcinogenesis could be increased in these tissues in rodents and people with NAFLD/NASH, as reviewed [124, 125]. For instance, gut dysbiosis with the increased population of the ethanol-producing bacterial family *Enterobacteriaceae*, including *Escherichia coli*, can lead to increased production of ethanol, local inflammation, and leaky gut, contributing to endotoxemia and inflammatory tissue injury, as demonstrated with pediatric patients with NASH [129, 131]. Although not studied, it would be of interest to know whether gut CYP2E1 is induced by the endogenously produced ethanol, albeit small amounts compared to those of alcohol intake. If gut CYP2E1 is induced in people or rodents with NAFLD/NASH, it may cause oxidative stress and oxidatively modify the intestinal junctional complex proteins, resulting in decreased amounts of the intestinal junctional complex proteins in alcohol-exposed rats and mice, as recently demonstrated [93, 94]. These events caused by the endogenous ethanol in NAFLD/NASH [135] may contribute to leaky gut, endotoxemia, and inflammatory tissue injury accompanied with DNA damage. Although the mechanisms for increased DNA mutation and carcinogenesis remain to be further studied, it is likely that CYP2E1 and other P450 isoforms, which can be induced by the endogenously produced ethanol, may be involved in the oxidative metabolisms of ethanol and acetaldehyde. In addition, these P450 isoforms can metabolize

pro-inflammatory substances n-6 long-chain fatty acids and/or other environmental toxicants or potential pro-carcinogens such as PAHs contained in charred western-style fast foods, contributing to elevated DNA adducts and carcinogenesis in the GI tract and other organs. Based on the damaging roles of the endogenously produced ethanol and acetaldehyde with potentially elevated CYP2E1 and other P450 isoforms, it is expected that people with diabetes or NAFLD/NASH could be more susceptible to DNA damage, inflammatory tissue injury, and cancer especially when they drink even small amounts of alcohol through additive or synergistic interactions [136, 137].

Translational Research Opportunities

Alcohol and acetaldehyde are human carcinogens [7]. The incidences of alcohol-related inflammatory tissue injury, DNA mutation, and carcinogenesis are significantly increased in the presence of another risk factor(s). As mentioned earlier, these risk factors are smoking, viral and bacterial infections, pro-inflammatory western-style high fat fast foods with fructose-containing soft drinks, poor nutrition, and preexisting pathophysiological conditions such as fasting and diabetes [18, 59, 66, 92]. Simultaneous exposure to these risk factors is likely to decrease cellular antioxidants, such as GSH and SAMe, and inactivate many antioxidant enzymes. Consequently, the rates of oxidative stress, lipid peroxidation, gut leakiness, endotoxemia, inflammatory tissue injury, DNA damage, and carcinogenesis would be increased [8, 18, 66, 92]. Based on these mechanisms, prevention or moderation of alcohol drinking would be the best remedy for alcohol-related tissue injury and carcinogenesis. Unfortunately, it would be difficult to decrease alcohol intake in many addicted alcoholic individuals. If alcohol drinking is not prevented, we may consider using adequately balanced diets with antioxidants (such as NAC) [89] or dietary supplements such as n-3 docosahexaenoic acid (DHA) [23], garlic compounds, including diallyl sulfide [37], resveratrol [138], walnut [139], indole-3-carbinol [140], ellagic acid [141], and pomegranate [94], many of which were shown to reduce or suppress the amount or activity of CYP2E1. As reported earlier [89, 93, 94, 140, 142], decreased CYP2E1 would lead to prevention of oxidative stress, leaky gut, and inflammatory tissue injury. Administration of soy protein isolate was also shown to protect from alcohol-mediated tumor promotion in DEN-exposed mice [143]. In addition, eubiosis by administering probiotics *Lactobacillus* [144] and *Bifidobacterium* strains [145] may be considered. In fact, a recent study showed that supplementation with *Akkermansia muciniphila* prevented alcohol-mediated intestinal barrier dysfunction and inflammatory liver injury through the gut–liver axis [146]. Furthermore, treatment with synthetic chemical inhibitors of CYP2E1, such as CMZ [75, 77, 89] and YH439 [147], can be considered to mitigate alcohol- and acetaldehyde-mediated inflammatory tissue injury, DNA mutation, and carcinogenesis.

Conclusion

As reviewed previously, both chronic and acute alcohol intake can change many different metabolic pathways and immunological dysregulations along with genetic and epigenetic changes. In the liver, alcohol drinking stimulates fatty liver, inflammation, fibrosis, cirrhosis, and cancer [148]. The development and progression of chronic liver disease usually depend on the amounts and duration of alcohol intake as well as the presence of another co-morbidity risk factor(s). Alcohol or acetaldehyde-mediated cancer in extra-hepatic tissues may also depend on the amounts of DNA adducts of the pro-carcinogens by CYP2E1 and other P450 isoforms-mediated metabolisms that can be increased by exposure to alcohol and/or another environmental toxicant(s). In this chapter, we have briefly summarized the biochemical properties of CYP2E1 and its roles in ethanol and acetaldehyde metabolism. We have also described its multiple regulations, tissue distribution, and causal roles in alcohol-mediated gut leakiness, inflammation, apoptosis, tissue injury, DNA

mutation, and carcinogenesis. In addition, we have mentioned the potential roles of other P450 isoforms, which are also induced or activated by alcohol or another environmental toxicant, in metabolizing potentially harmful substances, contributing to increased carcinogenesis in the liver and extra-hepatic tissues. The causal roles of CYP2E1 and other P450 isoforms in stimulating inflammatory tissue injury, DNA adducts, and carcinogenesis would be significantly increased in the presence of another risk factor such as smoking and/or western-style high fat fast foods. We have also briefly described the newly emerging roles of the gut microbiome changes and the endogenously produced ethanol in promoting DNA adduct formation and disease progression despite the absence of exogenous alcohol intake. Based on the understanding of the mechanisms of increased carcinogenesis, we have described potential methods of preclinical translational opportunities by preventing alcohol drinking or using dietary supplements, including various naturally occurring antioxidants and probiotics, and chemical inhibitors of CYP2E1. In fact, many drug candidates are being evaluated for preventing or treating liver disease through targeting the gut–liver axis [144–146, 149, 150]. Based on the important roles of gut microbiome changes in promoting leaky gut, endotoxemia, inflammatory tissue injury, some of these drug candidates may become a good candidate as an anti-cancer agent.

References

1. Purohit, V., Rapaka, R., Kwon, O. S., & Song, B. J. (2013). Roles of alcohol and tobacco exposure in the development of hepatocellular carcinoma. *Life Sciences, 92*, 3–9.
2. Neuman, M. G., French, S. W., Zakhari, S., Malnick, S., Seitz, H. K., Cohen, L. B., Salaspuro, M., Voinea-Griffin, A., Barasch, A., Kirpich, I. A., Thomes, P. G., Schrum, L. W., Donohue, T. M., Jr., Kharbanda, K. K., Cruz, M., & Opris, M. (2017). Alcohol, microbiome, life style influence alcohol and non-alcoholic organ damage. *Experimental and Molecular Pathology, 102*(1), 162–180.
3. Ratna, A., & Mandrekar, P. (2017). Alcohol and cancer: Mechanisms and therapies. Alcohol, microbiome, life style influence alcohol and non-alcoholic organ damage. *Biomolecules, 7*, E61.
4. Na, H. K., & Lee, J. Y. (2017). Molecular basis of alcohol-related gastric and colon cancer. *International Journal of Molecular Sciences, 18*(6), E1116.
5. Fernandes, G. M., Russo, A., Proença, M. A., Gazola, N. F., Rodrigues, G. H., Biselli-Chicote, P. M., Silva, A. E., Netinho, J. G., Pavarino, É. C., & Goloni-Bertollo, E. M. (2016). *CYP1A1*, *CYP2E1* and *EPHX1* polymorphisms in sporadic colorectal neoplasms. *World Journal of Gastroenterology, 22*(45), 9974–9983.
6. Lu, Y., Zhu, X., Zhang, C., Jiang, K., Huang, C., & Qin, X. (2017). Role of CYP2E1 polymorphisms in breast cancer: A systematic review and meta-analysis. *Cancer Cell International, 17*, 11.
7. International Agency for Research on Cancer. (2012). *IARC monographs on the evaluation of carcinogenic risks to humans* (Vol. 100E). Lyon: IARC.
8. Song, B. J., Koop, D. R., Ingelman-Sundberg, M., Nanji, A. A., & Cederbaum, A. I. (1996). Ethanol-inducible cytochrome P450 (CYP2E1): Biochemistry, molecular biology and clinical relevance: 1996 update. *Alcoholism, Clinical and Experimental Research, 20*(8 Suppl), 138A–146A.
9. Lieber, C. S. (1997). Cytochrome P-450 2E1: Its physiological and pathological role. *Physiological Reviews, 77*, 517–544.
10. Bailey, S. M., Pietsch, E. C., & Cunningham, C. C. (1999). Ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III. *Free Radical Biology and Medicine, 27*, 891–900.
11. Kono, H., Rusyn, I., Yin, M., Gäbele, E., Yamashina, S., Dikalova, A., Kadiiska, M. B., Connor, H. D., Mason, R. P., Segal, B. H., Bradford, B. U., Holland, S. M., & Thurman, R. G. (2000). NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. *The Journal of Clinical Investigation, 106*(7), 867–872.
12. McKim, S. E., Gabele, E., Isayama, F., Lambert, J. C., Tucker, L. M., Wheeler, M. D., Connor, H. D., Mason, R. P., Doll, M. A., Hein, D. W., & Arteel, G. E. (2003). Inducible nitric oxide synthase is required in alcohol-induced liver injury: Studies with knockout mice. *Gastroenterology, 125*, 1834–1844.
13. Venkatraman, S. S., Wigley, A., Ulasova, E., Chhieng, D., Bailey, S. D., & Darley-Usmar, V. M. (2004). The role of iNOS in alcohol-dependent hepatotoxicity and mitochondrial dysfunction in mice. *Hepatology, 40*, 565–573.
14. Song, B. J., Abdelmegeed, M. A., Henderson, L. E., Yoo, S. H., Wan, J., Purohit, V., Hardwick, J. P., & Moon, K. H. (2013). Increased nitroxidative stress promotes mitochondrial dysfunction in alcoholic and nonalcoholic fatty liver disease. *Oxidative Medicine and Cellular Longevity, 2013*, 781050.
15. Leung, T. M., & Nieto, N. (2013). CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. *Journal of Hepatology, 58*(2), 395–398.

16. Yun, J. W., Son, M. J., Abdelmegeed, M. A., Banerjee, A., Morgan, T. R., Yoo, S. H., & Song, B. J. (2014). Binge alcohol promotes hypoxic liver injury through CYP2E1-HIF1a-dependent apoptosis pathway in mice and humans. *Free Radical Biology and Medicine, 77*, 183–194.
17. Abbondanza, A., Battelli, M. G., Soffritti, M., & Cessi, C. (1989). Xanthine oxidase status in ethanol-intoxicated rat liver. *Alcoholism, Clinical and Experimental Research, 13*(6), 841–844.
18. Song, B. J., Akbar, M., Jo, I., Hardwick, J. P., & Abdelmegeed, M. A. (2015). Translational implications of the alcohol-metabolizing enzymes, including cytochrome P450-2E1, in alcoholic and nonalcoholic liver disease. *Advances in Pharmacology, 74*, 303–372.
19. Nanji, A. A., Jokelainen, K., Tipoe, G. L., Rahemtulla, A., Thomas, P., & Dannenberg, A. J. (2003). Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 284*(2), G321–G327.
20. Xiao, J., Ching, Y. P., Liong, E. C., Nanji, A. A., Fung, M. L., & Tipoe, G. L. (2013). Garlic-derived S-allylmercaptocysteine is a hepato-protective agent in non-alcoholic fatty liver disease in vivo animal model. *European Journal of Nutrition, 52*(1), 179–191.
21. Marí, M., Morales, A., Colell, A., García-Ruiz, C., & Fernández-Checa, J. C. (2009). Mitochondrial glutathione, a key survival antioxidant. *Antioxidants and Redox Signaling, 11*(11), 2685–2627.
22. Moon, K. H., Hood, B. L., Kim, B. J., Hardwick, J. P., Conrads, T. P., Veenstra, T. D., & Song, B. J. (2006). Inactivation of oxidized and S-nitrosylated mitochondrial proteins in alcoholic fatty liver of rats. *Hepatology, 44*(5), 1218–1230.
23. Song, B. J., Moon, K. H., Olsson, N. U., & Salem, N., Jr. (2008). Prevention of alcoholic fatty liver and mitochondrial dysfunction in the rat by long-chain polyunsaturated fatty acids. *Journal of Hepatology, 49*(2), 262–273.
24. Abdelmegeed, M. A., & Song, B. J. (2014). Functional roles of protein nitration in acute and chronic liver diseases. *Oxidative Medicine and Cellular Longevity, 2014*, e149627.
25. Song, B. J., Abdelmegeed, M. A., Yoo, S. H., Kim, B. J., Jo, S. A., Jo, I., & Moon, K. H. (2011). Post-translational modifications of mitochondrial aldehyde dehydrogenase and biomedical implications. *Journal of Proteomics, 74*(12), 2691–2702.
26. Yokoyama, A., Muramatsu, T., Ohmori, T., Yokoyama, T., Okuyama, K., Takahashi, H., Hasegawa, Y., Higuchi, S., Maruyama, K., Shirakura, K., & Ishii, H. (1998). Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis, 19*(8), 1383–1387.
27. Yokoyama, T., Muramatsu, T., Omori, T., Yokoyama, S., Matsushita, S., Higuchi, K., Maruyama, H., & Ishii, H. (2001). Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. *Carcinogenesis, 22*(3), 433–439.
28. Yokoyama, A., Mizukami, T., & Yokoyama, T. (2015). Genetic polymorphisms of alcohol dehydrogense-1B and aldehyde dehydrogenase-2, alcohol flushing, mean corpuscular volume, and aerodigestive tract neoplasia in Japanese drinkers. *Advances in Experimental Medicine and Biology, 815*, 265–279.
29. Isse, T., Matsuno, K., Oyama, T., Kitagawa, K., & Kawamoto, T. (2005). Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity show high acetaldehyde level in blood, brain, and liver after ethanol gavages. *Alcoholism, Clinical and Experimental Research, 29*, 1959–1964.
30. Bosron, W. F., Ehrig, T., & Li, T. K. (1993). Genetic factors in alcohol metabolism and alcoholism. *Seminars in Liver Disease, 13*, 126–135.
31. Seitz, H. K., & Stickel, F. (2010). Acetaldehyde as an underestimated risk factor for cancer development: Role of genetics in ethanol metabolism. *Genes and Nutrition, 5*(2), 121–128.
32. Seitz, H. K., & Mueller, S. (2015). Alcohol and cancer: An overview with special emphasis on the role of acetaldehyde and cytochrome P450 2E1. *Adv Exp Biol Med, 815*, 59–70.
33. Mueller, S., Peccerella, T., Qin, H., Glassen, K., Waldherr, R., Flechtenmacher, C., Straub, B. K., Millonig, G., Stickel, F., Bruckner, T., Bartsch, H., & Seitz, H. K. (2018). Carcinogenic Etheno DNA adducts in alcoholic liver disease: Correlation with cytochrome P-4502E1 and fibrosis. *Alcoholism, Clinical and Experimental Research, 42*(2), 252–259.
34. Balbo, S., & Brooks, P. J. (2015). Implications of acetaldehyde-derived DNA adducts for understanding alcohol-related carcinogenesis. *Advances in Experimental Medicine and Biology, 815*, 71–88.
35. Brooks, P. J., & Theruvathu, J. A. (2005). DNA adducts from acetaldehyde: Implications for alcohol-related carcinogenesis. *Alcohol, 35*, 187–193.
36. Bartsch, H., & Nair, J. (2005). Accumulation of lipid peroxidation-derived DNA lesions: Potential lead markers for chemoprevention of inflammation-driven malignancies. *Mutation Research, 591*(1–2), 34–44.
37. Sapkota, M., Hottor, T. K., DeVasure, J. M., Wyatt, T. A., & McCaskill, M. L. (2014). Protective role of CYP2E1 inhibitor diallyl disulfide (DADS) on alcohol-induced malondialdehyde-deoxyguanosine (M1dG) adduct formation. *Alcoholism, Clinical and Experimental Research, 38*(6), 1550–1558.
38. Sapkota, M., & Wyatt, T. A. (2015). Alcohol, aldehydes, adducts and airways. *Biomolecules, 5*(4), 2987–3008. (MAA-DNA adducts).
39. Linhart, K., Bartsch, H., & Seitz, H. K. (2014). The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts. *Redox Biology, 3*, 56–62.
40. Mufti, S. I., Salvagnini, M., Lieber, C. S., & Garro, A. J. (1988). Chronic ethanol consumption inhib-

its repair of dimethylnitrosamine-induced DNA alkylation. *Biochemical and Biophysical Research Communications, 152*(1), 423–431.
41. Espina, N., Lima, V., Lieber, C. S., & Garro, A. J. (1988). In vitro and in vivo inhibitory effect of ethanol and acetaldehyde on O6-methylguanine transferase. *Carcinogenesis, 9*, 761–766.
42. Caro, A. A., & Cederbaum, A. I. (2004). Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annual Review of Pharmacology and Toxicology, 44*, 27–42.
43. Cederbaum, A. I. (2012). Alcohol metabolism. *Clinics in Liver Disease, 16*(4), 667–685.
44. Lindblad, B., & Olsson, R. (1976). Unusually high levels of blood alcohol? *JAMA, 236*(14), 1600–1602.
45. Chaudhry, K. K., Shukla, P. K., Mir, H., Manda, B., Gangwar, R., Yadav, N., McMullen, M., Nagy, L., & Rao, R. (2016). Glutamine supplementation attenuates ethanol-induced disruption of apical junctional complexes in colonic epithelium and ameliorates gut barrier dysfunction and fatty liver in mice. *The Journal of Nutritional Biochemistry, 27*, 16–26.
46. Mueller, S., & Rausch, V. (2015). The role of iron in alcohol-mediated hepatocarcinogenesis. *Advances in Experimental Medicine and Biology, 815*, 89–112.
47. Abdelmegeed, M. A., Moon, K. H., Chen, C., Gonzalez, F. J., & Song, B. J. (2010). Role of cytochrome P450 2E1 in protein nitration and ubiquitin-mediated degradation during acetaminophen toxicity. *Biochemical Pharmacology, 79*(1), 57–66.
48. Robin, M. A., Sauvage, I., Grandperret, T., Descatoire, V., Pessayre, D., & Fromenty, B. (2005). Ethanol increases mitochondrial cytochrome P450 2E1 in mouse liver and rat hepatocytes. *FEBS Letters, 579*(30), 6895–6902.
49. Bansal, S., Liu, C. P., Sepuri, N. B., Anandatheerthavarada, H. K., Selvaraj, V., Hoek, J., Milne, G. L., Guengerich, F. P., & Avadhani, N. G. (2010). Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. *The Journal of Biological Chemistry, 285*(32), 24609–24619.
50. Roberts, B. J., Shoaf, S. E., Jeong, K. S., & Song, B. J. (1994). Induction of CYP2E1 in liver, kidney, brain and intestine during chronic ethanol administration and withdrawal: Evidence that CYP2E1 possesses a rapid phase half-life of 6 hours or less. *Biochemical and Biophysical Research Communications, 205*(2), 1064–1071.
51. Koop, D. R. (1992). Oxidative and reductive metabolism by cytochrome P4502E1. *The FASEB Journal, 6*(2), 724–730.
52. Yun, Y. P., Casazza, J. P., Sohn, D. H., Veech, R. L., & Song, B. J. (1992). Pretranslational activation of cytochrome P450IIE during ketosis induced by a high fat diet. *Molecular Pharmacology, 41*(3), 474–479.
53. Raucy, J. L., Lasker, J. M., Kraner, J. C., Salazar, D. E., Lieber, C. S., & Corcoran, G. B. (1991). Induction of cytochrome P450IIE1 in the obese overfed rat. *Molecular Pharmacology, 39*, 275–280.
54. Weltman, M. D., Farrell, G. C., Hall, P., Ingelman-Sundberg, M., & Liddle, C. (1998). Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology, 27*(1), 128–133.
55. Wang, Z., Hall, S. D., Maya, J. F., Li, L., Asghar, A., & Gorski, J. C. (2003). Diabetes mellitus increases the in vivo activity of cytochrome P450 2E1 in humans. *British Journal of Clinical Pharmacology, 55*, 77–85.
56. Song, B. J., Veech, R. L., Park, S. S., Gelboin, H. V., & Gonzalez, F. J. (1989). Induction of rat hepatic N-nitrosodimethylamine demethylase by acetone is due to protein stabilization. *The Journal of Biological Chemistry, 254*, 3568–3572.
57. Roberts, B. J., Song, B. J., Soh, Y., Park, S. S., & Shoaf, S. E. (1995). Ethanol induces CYP2E1 by protein stabilization. Role of ubiquitin conjugation in the rapid degradation of CYP2E1. *The Journal of Biological Chemistry, 270*(50), 29632–29635.
58. Oneta, C. M., Lieber, C. S., Li, J., Rüttimann, S., Schmid, B., Lattmann, J., Rosman, A. S., & Seitz, H. K. (2002). Dynamics of cytochrome P4502E1 activity in man: Induction by ethanol and disappearance during withdrawal phase. *Journal of Hepatology, 36*(1), 47–52.
59. Cederbaum, A. I. (2012). CYP2E1 potentiates toxicity in obesity and after chronic ethanol treatment. *Drug Metabolism and Drug Interactions, 27*(3), 125–144.
60. Gäbele, E., Dostert, K., Dorn, C., Patsenker, E., Stickel, F., & Hellerbrand, C. (2011). A new model of interactive effects of alcohol and high-fat diet on hepatic fibrosis. *Alcoholism, Clinical and Experimental Research, 35*(7), 1361–1367.
61. Micu, A. L., Miksys, S., Sellers, E. M., Koop, D. R., & Tyndale, R. F. (2003). Rat hepatic CYP2E1 is induced by very low nicotine doses: An investigation of induction, time course, dose response, and mechanism. *The Journal of Pharmacology and Experimental Therapeutics, 306*, 941–947.
62. Szabo, G. (2018). Alcoholic liver disease accelerates early hepatocellular cancer in a mouse model. *Advances in Experimental Medicine and Biology, 1032*, 71–80.
63. Kang, J. S., Wanibuchi, H., Morimura, K., Gonzalez, F. J., & Fukushima, S. (2007). Role of CYP2E1 in diethylnitrosamine-induced hepatocarcinogenesis in vivo. *Cancer Research, 67*(23), 11141–11146.
64. Kanagal-Shamanna, R., Zhao, W., Vadhan-Raj, S., Nguyen, M. H., Fernandez, M. H., Medeiros, L. J., & Bueso-Ramos, C. E. (2012). Over-expression of CYP2E1 mRNA and protein: Implications of xenobiotic induced damage in patients with de novo acute myeloid leukemia with inv(16)(p13.1q22); CBFβ-MYH11. *International Journal of Environmental Research and Public Health, 9*(8), 2788–2800.

65. Guengerich, F. P., & Avadhani, N. G. (2018). Roles of cytochrome P450 in metabolism of ethanol and carcinogens. *Advances in Experimental Medicine and Biology, 1032*, 15–35.
66. Abdelmegeed, M. A., Ha, S. K., Choi, Y. S., Akbar, M., & Song, B. J. (2017). Role of CYP2E1 in mitochondrial dysfunction and hepatic injury by alcohol and non-alcoholic substances. *Current Molecular Pharmacology, 10*(3), 207–225.
67. Chen, Y., Han, M., Matsumoto, A., Wang, Y., Thompson, D. C., & Vasiliou, V. (2018). Glutathione and transsulfuration in alcohol-associated tissue injury and carcinogenesis. *Advances in Experimental Medicine and Biology, 1032*, 37–53.
68. Kurkivuori, J., Salaspuro, V., Kaihovaara, P., Kari, K., Rautemaa, R., Grönroos, L., Meurman, J. H., & Salaspuro, M. (2007). Acetaldehyde production from ethanol by oral streptococci. *Oral Oncology, 43*(2), 181–186.
69. Yokoyama, S., Takeuchi, K., Shibata, Y., Kageyama, S., Matsumi, R., Takeshita, T., & Yamashita, Y. (2018). Characterization of oral microbiota and acetaldehyde production. *Journal of Oral Microbiology, 10*(1), 1492316.
70. Cope, K., Risby, T. H., & Diehl, A. M. (2000). Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. *Gastroenterology, 119*, 1340–1347.
71. Nair, S., Cope, K., Risby, T. H., & Diehl, A. M. (2001). Obesity and female gender increase breath ethanol concentration: Potential implications for the pathogenesis of nonalcoholic steatohepatitis. *The American Journal of Gastroenterology, 96*, 1200–1204.
72. de Faria Ghetti, F., Oliveira, D. G., de Oliveira, J. M., de Castro Ferreira, L. E. V. V., Cesar, D. E., & Moreira, A. P. B. (2018). Influence of gut microbiota on the development and progression of nonalcoholic steatohepatitis. *European Journal of Nutrition, 57*(3), 861–876.
73. Jang, S., Yu, L. R., Abdelmegeed, M. A., Gao, Y., Banerjee, A., & Song, B. J. (2015). Critical role of c-jun N-terminal protein kinase in promoting mitochondrial dysfunction and acute liver injury. *Redox Biology, 6*, 552–564.
74. Liu, C., Russell, R. M., Seitz, H. K., & Wang, X. D. (2001). Ethanol enhances retinoic acid metabolism into polar metabolites in rat liver via induction of cytochrome P450 2E1. *Gastroenterology, 120*(1), 179–189.
75. Peccerella, T., Arslic-Schmitt, T., Mueller, S., Linhart, K. B., Seth, D., Bartsch, H., & Seitz, H. K. (2018). Chronic ethanol consumption and generation of etheno-DNA adducts in cancer-prone tissues. *Advances in Experimental Medicine and Biology, 1032*, 81–92.
76. Bradford, B. U., Kono, H., Isayama, F., Kosyk, O., Wheeler, M. D., Akiyama, T. E., Bleye, L., Krausz, K. W., Gonzalez, F. J., Koop, D. R., & Rusyn, I. (2005). Cytochrome P450 CYP2E1, but not nicotinamide adenine dinucleotide phosphate oxidase, is required for ethanol-induced oxidative DNA damage in rodent liver. *Hepatology, 41*(2), 336–344.
77. Wang, Y., Millonig, G., Nair, J., Patsenker, E., Stickel, F., Mueller, S., Bartsch, H., & Seitz, H. K. (2009). Ethanol-induced cytochrome P4502E1 causes carcinogenic etheno-DNA lesions in alcoholic liver disease. *Hepatology, 50*, 453–461.
78. Frank, A., Seitz, H. K., Bartsch, H., Frank, N., & Nair, J. (2004). Immunohistochemical detection of 1,N6-ethenodeoxyadenosine in nuclei of human liver affected by diseases predisposing to hepatocarcinogenesis. *Carcinogenesis, 25*, 1027–1031.
79. Millonig, G., Wang, Y., Homann, N., Bernhardt, F., Qin, H., Mueller, S., Bartsch, H., & Seitz, H. K. (2011). Ethanol-mediated carcinogenesis in the human esophagus implicates CYP2E1 induction and the generation of carcinogenic DNA-lesions. *International Journal of Cancer, 128*(3), 533–540.
80. Seitz, H. K., & Cho, C. H. (2009). Contribution of alcohol and tobacco use in gastrointestinal cancer development. *Methods in Molecular Biology, 472*, 217–241.
81. Koehler, B. C., Arslic-Schmitt, T., Peccerella, T., Scherr, A. L., Schulze-Bergkamen, H., Bruckner, T., Gdynia, G., Jäger, D., Mueller, S., Bartsch, H., & Seitz, H. K. (2016). Possible mechanisms of ethanol-mediated colorectal carcinogenesis: The role of cytochrome P4502E1, etheno-DNA adducts, and the anti-apoptotic protein Mcl-1. *Alcoholism, Clinical and Experimental Research, 40*(10), 2094–2101.
82. Dapito, D. H., Mencin, A., Gwak, G. Y., Pradere, J. P., Jang, M. K., Mederacke, I., Caviglia, J. M., Khiabanian, H., Adeyemi, A., Bataller, R., Lefkowitch, J. H., Bower, M., Friedman, R., Sartor, R. B., Rabadan, R., & Schwabe, R. F. (2012). Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell, 21*(4), 504–516.
83. Todoric, J., Antonucci, L., & Karin, M. (2016). Targeting inflammation in cancer prevention and therapy. *Cancer Prevention Research, 9*(12), 895–905.
84. Meadows, Z. H. (2015). Effects of alcohol on tumor growth, metastasis, immune response and host survival. *Alcohol Research: Current Reviews, 37*(2), 311–322.
85. Tabung, F. K., Liu, L., Wang, W., Fung, T. T., Wu, K., Smith-Warner, S. A., Cao, Y., Hu, F. B., Ogino, S., Fuchs, C. S., & Giovannucci, E. L. (2018). Association of dietary inflammatory potential with colorectal cancer risk in men and women. *JAMA Oncology, 4*(3), 366–373.
86. Bode, C., Kugler, V., & Bode, J. C. (1987). Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. *Journal of Hepatology, 4*, 8–14.

87. Lambert, J. C., Zhou, Z., Wang, L., Song, Z., McClain, C. J., & Kang, Y. J. (2003). Prevention of alterations in intestinal permeability is involved in zinc inhibition of acute ethanol-induced liver damage in mice. *The Journal of Pharmacology and Experimental Therapeutics, 305*(3), 880–886.
88. Keshavarzian, A., Farhadi, A., Forsyth, C. B., Rangan, J., Jakate, S., Shaikh, M., Banan, A., & Fields, J. Z. (2009). Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. *Journal of Hepatology, 50*, 538–547.
89. Abdelmegeed, M. A., Banerjee, A., Jang, S., Yoo, S. H., Yun, J. W., Gonzalez, F. J., Keshavarzian, A., & Song, B. J. (2013). CYP2E1 potentiates binge alcohol-induced gut leakiness, steatohepatitis, and apoptosis. *Free Radical Biology and Medicine, 65*, 1238–1245.
90. Seki, E., De Minicis, S., Osterreicher, C. H., Kluwe, J., Osawa, Y., Brenner, D. A., & Schwabe, R. F. (2007). TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nature Medicine, 13*(11), 1324–1332.
91. Yoo, S. H., Park, O., Henderson, L. E., Abdelmegeed, M. A., Moon, K. H., & Song, B. J. (2011). Lack of PPARα exacerbates lipopolysaccharide-induced liver toxicity through STAT1 inflammatory signaling and increased oxidative/nitrosative stress. *Toxicology Letters, 202*(1), 23–29.
92. Song, B. J., Akbar, M., Abdelmegeed, M. A., Byun, K., Lee, B., Yoon, S. K., & Hardwick, J. P. (2014). Mitochondrial dysfunction and tissue injury by alcohol, high fat, nonalcoholic substances and pathological conditions through post-translational protein modifications. *Redox Biology, 3*, 109–123.
93. Cho, Y. E., Yu, L. R., Abdelmegeed, M. A., Yoo, S. H., & Song, B. J. (2018). Apoptosis of enterocytes and nitration of junctional complex proteins promote alcohol-induced gut leakiness and liver injury. *Journal of Hepatology, 69*(1), 142–153.
94. Cho, Y. E., & Song, B. J. (2018). Pomegranate prevents binge alcohol-induced gut leakiness and hepatic inflammation by suppressing oxidative and nitrative stress. *Redox Biology, 18*, 266–278.
95. Abdelmegeed, M. A., Choi, Y., Godlewski, G., Ha, S. K., Banerjee, A., Jang, S., & Song, B. J. (2017). Cytochrome P450-2E1 promotes fast food-mediated hepatic fibrosis. *Scientific Reports, 7*, 42566.
96. Gao, J., Wang, Z., Wang, G. J., Gao, N., Li, J., Zhang, Y. F., Zhou, J., Zhang, H. X., Wen, Q., Jin, H., & Qiao, H. L. (2018). From hepatofibrosis to hepatocarcinogenesis: Higher cytochrome P450 2E1 activity is a potential risk factor. *Molecular Carcinogenesis, 57*(10), 1371–1382.
97. Abdelmegeed, M. A., Choi, Y., Ha, S. K., & Song, B. J. (2017). Cytochrome P450-2E1 is involved in aging-related kidney damage in mice through increased nitroxidative stress. *Food and Chemical Toxicology, 109*(Pt 1), 48–59.
98. Heit, C., Dong, H., Chen, Y., Thompson, D. C., Deitrich, R. A., & Vasiliou, V. K. (2013). The role of CYP2E1 in alcohol metabolism and sensitivity in the central nervous system. *Sub-Cellular Biochemistry, 67*, 235–247.
99. Cho, Y. E., Lee, M. H., & Song, B. J. (2017). Neuronal cell death and degeneration through increased nitroxidative stress and tau phosphorylation in HIV-1 transgenic rats. *PLoS One, 12*(1), e0169945.
100. Song, B. J., Veech, R. L., & Saenger, P. (1990). Cytochrome P450IIE1 is elevated in lymphocytes from poorly controlled insulin-dependent diabetics. *The Journal of Clinical Endocrinology and Metabolism, 71*(4), 1036–1040.
101. Eom, S. Y., Zhang, Y. W., Kim, S. H., Choe, K. H., Lee, K. Y., Park, J. D., Hong, Y. C., Kim, Y. D., Kang, J. W., & Kim, H. (2009). Influence of NQO1, ALDH2, and CYP2E1 genetic polymorphisms, smoking, and alcohol drinking on the risk of lung cancer in Koreans. *Cancer Causes and Control, 20*(2), 137–145.
102. Saghir, S. A., Zhang, F., Rick, D. L., Kan, L., Bus, J. S., & Bartels, M. J. (2010). In vitro metabolism and covalent binding of ethylbenzene to microsomal protein as a possible mechanism of ethylbenzene-induced mouse lung tumorigenesis. *Regulatory Toxicology and Pharmacology, 57*(2–3), 129–135.
103. Kessova, I. G., DeCarli, L. M., & Lieber, C. S. (1998). Inducibility of cytochromes P-4502E1 and P-4501A1 in the rat pancreas. *Alcoholism, Clinical and Experimental Research, 22*(2), 501–504.
104. Longo, V., Ingelman-Sundberg, M., Amato, G., Salvetti, A., & Gervasi, P. G. (2000). Effect of starvation and chlormethiazole on cytochrome P450s of rat nasal mucosa. *Biochemical Pharmacology, 59*(11), 1425–1432.
105. Shimizu, M., Lasker, J. M., Tsutsumi, M., & Lieber, C. S. (1990). Immunohistochemical localization of ethanol-inducible P450IIE1 in the rat alimentary tract. *Gastroenterology, 99*(4), 1044–1053.
106. Kapucuoglu, N., Coban, T., Raunio, H., Pelkonen, O., Edwards, R. J., Boobis, A. R., & Iscan, M. (2003). Immunohistochemical demonstration of the expression of CYP2E1 in human breast tumour and non-tumour tissues. *Cancer Letters, 196*(2), 153–159.
107. Kang, J. S., Wanibuchi, H., Morimura, K., Wongpoomchai, R., Chusiri, Y., Gonzalez, F. J., & Fukushima, S. (2008). Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. *Toxicology and Applied Pharmacology, 228*(3), 295–300.
108. Malik, D. E., David, R. M., & Gooderham, N. J. (2018). Ethanol potentiates the genotoxicity of the food-derived mammary carcinogen PhIP in human estrogen receptor-positive mammary cells:

Mechanistic support for lifestyle factors (cooked red meat and ethanol) associated with mammary cancer. *Archives of Toxicology, 92*(4), 1639–1655.
109. Abdelmegeed, M. A., Banerjee, A., Yoo, S. H., Jang, S., Gonzalez, F. J., & Song, B. J. (2012). Critical role of cytochrome P450 2E1 (CYP2E1) in the development of high fat-induced non-alcoholic steatohepatitis. *Journal of Hepatology, 57*(4), 860–866.
110. Abdelmegeed, M. A., Choi, Y., Ha, S. K., & Song, B. J. (2016). Cytochrome P450-2E1 promotes aging-related hepatic steatosis, apoptosis and fibrosis through increased nitroxidative stress. *Free Radical Biology and Medicine, 91*, 188–202.
111. Lu, Y., Zhuge, J., Wu, D., & Cederbaum, A. I. (2011). Ethanol induction of CYP2A5: Permissive role for CYP2E1. *Drug Metabolism and Disposition, 39*(2), 330–336.
112. Marshall, S., Chen, Y., Singh, S., Berrios-Carcamo, P., Heit, C., Apostolopoulos, N., Golla, J. P., Thompson, D. C., & Vasiliou, V. (2018). Engineered animal models designed for investigating ethanol metabolism, toxicity and cancer. *Advances in Experimental Medicine and Biology, 1032*, 203–221.
113. Lu, Y., Zhang, X. H., & Cederbaum, A. I. (2012). Ethanol induction of CYP2A5: Role of CYP2E1-ROS-Nrf2 pathway. *Toxicological Sciences, 128*(2), 427–438.
114. Feierman, D. E., Melinkov, Z., & Nanji, A. A. (2013). Induction of CYP3A by ethanol in multiple in vitro and in vivo models. *Alcoholism, Clinical and Experimental Research, 27*(6), 981–988.
115. Je, Y. T., Sim, W. C., Kim, D. G., Jung, B. H., Shin, H. S., & Lee, B. H. (2015). Expression of CYP3A in chronic ethanol-fed mice is mediated by endogenous pregnane X receptor ligands formed by enhanced cholesterol metabolism. *Archives of Toxicology, 89*(4), 579–589.
116. Arif, J., & Gupta, R. (1996). Effect of inducer and inhibitor probes on DNA adduction of benzo[a] pyrene and 2-acetylaminofluorene and their roles in defining bioactivation mechanism(s). *International Journal of Oncology, 8*(4), 681–685.
117. Li, L., Megaraj, V., Wei, Y., & Ding, X. (2014). Identification of cytochrome P450 enzymes critical for lung tumorigenesis by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): Insights from a novel Cyp2abfgs-null mouse. *Carcinogenesis, 35*(11), 2584–2591.
118. Pelkonen, P., Lang, M. A., Negishi, M., Wild, C. P., & Juvonen, R. O. (1997). Interaction of aflatoxin B1 with cytochrome P450 2A5 and its mutants: Correlation with metabolic activation and toxicity. *Chemical Research in Toxicology, 10*(1), 85–90.
119. Jiang, H., Wu, J., Zhang, F., Wen, J., Jiang, J., & Deng, Y. (2018). The critical role of porcine cytochrome P450 3A46 in the bioactivation of aflatoxin B_1. *Biochemical Pharmacology, 156*, 177–185.
120. Kim, S. Y., Suzuki, N., Laxmi, Y. R., & Shibutani, S. (2006). Inefficient repair of tamoxifen-DNA adducts in rats and mice. *Drug Metabolism and Disposition, 34*, 311–317.
121. Rao, P. S., & Kumar, S. (2016). Chronic effects of ethanol and/or darunavir/ritonavir on U937 monocytic cells: Regulation of cytochrome P450 and antioxidant enzymes, oxidative stress, and cytotoxicity. *Alcoholism, Clinical and Experimental Research, 40*(1), 73–82.
122. Banerjee, A., Abdelmegeed, M. A., & Song, B. J. (2013). Zidovudine (AZT) and hepatic lipid accumulation: Implication of inflammation, oxidative and endoplasmic reticulum stress mediators. *PLoS One, 8*(10), e76850.
123. Ioannou, G. N., Bryson, C. L., Weiss, N. S., Miller, R., Scott, J. D., & Boyko, E. J. (2013). The prevalence of cirrhosis and hepatocellular carcinoma in patients with human immunodeficiency virus infection. *Hepatology, 57*(1), 249–257.
124. Michelotti, G. A., Machado, M. V., & Diehl, A. M. (2013). NAFLD, NASH and liver cancer. *Nature Reviews. Gastroenterology and Hepatology, 10*(11), 656–665.
125. Sanna, C., Rosso, C., Marietti, M., & Bugianesi, E. (2016). Non-alcoholic fatty liver disease and extrahepatic cancers. *International Journal of Molecular Sciences, 17*(5), E717.
126. Ahmad, R., Sorrell, M. F., Batra, S. K., Dhawan, P., & Singh, A. B. (2017). Gut permeability and mucosal inflammation: Bad, good or context dependent. *Mucosal Immunology, 10*(2), 307–317.
127. Spruss, A., Kanuri, G., Uebel, K., Bischoff, S. C., & Bergheim, I. (2011). Role of the inducible nitric oxide synthase in the onset of fructose-induced steatosis in mice. *Antioxidants and Redox Signaling, 14*(11), 2121–2135.
128. Yu, L. X., & Schwabe, R. F. (2017). The gut microbiome and liver cancer: Mechanisms and clinical translation. *Nature Reviews. Gastroenterology and Hepatology, 14*(9), 527–539.
129. Engstler, A. J., Aumiller, T., Degen, C., Durr, M., Weiss, E., Maier, I. B., Schattenberg, J. M., Jin, C. J., Sellmann, C., & Bergheim, I. (2016). Insulin resistance alters hepatic ethanol metabolism: Studies in mice and children with non-alcoholic fatty liver disease. *Gut, 65*, 1564–1571.
130. Volynets, V., Kuper, M. A., Strahl, S., Maier, I. B., Spruss, A., Wagnerberger, S., Königsrainer, A., Bischoff, S. C., & Bergheim, I. (2012). Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Digestive Diseases and Sciences, 57*, 1932–1941.
131. Zhu, L., Baker, S. S., Gill, C., Liu, W., Alkhouri, R., Baker, R. D., & Gill, S. R. (2013). Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology, 57*, 601–609.
132. Pikkarainen, P., Baraona, E., Seitz, H., & Lieber, C. S. (1980). Breath acetaldehyde: Evidence of acetaldehyde production by oropharynx microflora

and by lung microsomes. *Advances in Experimental Medicine and Biology, 132*, 469–474.
133. Lawal, O., Knobel, H., Weda, H., Bos, L. D., Nijsen, T. M. E., Goodacre, R., & Fowler, S. J. (2018). Volatile organic compound signature from co-culture of lung epithelial cell line with *Pseudomonas aeruginosa*. *Analyst, 143*(13), 3148–3155.
134. Tsuruya, A., Kuwahara, A., Saito, Y., Yamaguchi, H., Tenma, N., Inai, M., Takahashi, S., Tsutsumi, E., Suwa, Y., Totsuka, Y., Suda, W., Oshima, K., Hattori, M., Mizukami, T., Yokoyama, A., Shimoyama, T., & Nakayama, T. (2016). Major anaerobic bacteria responsible for the production of carcinogenic acetaldehyde from ethanol in the colon and rectum. *Alcohol and Alcoholism, 51*(4), 395–401.
135. Cho, Y. E., Kim, D. K., Seo, W., Gao, B., Yoo, S. H., & Song, B. J. (2019). Fructose promotes leaky gut, endotoxemia, and liver fibrosis through ethanol-inducible cytochrome P450-2E1-mediated oxidative and nitrative stress. Apr 8. doi:10.1002/hep. 30652. [Epub ahead of print]
136. Hassan, M. M., Hwang, L. Y., Hatten, C. J., Swaim, M., Li, D., Abbruzzese, J. L., Beasley, P., & Patt, Y. Z. (2002). Risk factors for hepatocellular carcinoma: Synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology, 36*, 1206–1213.
137. Xu, J., Lai, K. K. Y., Verlinsky, A., Lugea, A., French, S. W., Cooper, M. P., Ji, C., & Tsukamoto, H. (2011). Synergistic steatohepatitis by moderate obesity and alcohol in mice despite increased adiponectin and p-AMPK. *Journal of Hepatology, 55*(3), 673–682.
138. Wu, X., Li, C., Xing, G., Qi, X., & Ren, J. (2013). Resveratrol downregulates Cyp2e1 and attenuates chemically induced hepatocarcinogenesis in SD rats. *Journal of Toxicologic Pathology, 26*(4), 385–392.
139. Choi, Y. S., Abdelmegeed, M. A., & Song, B. J. (2016). Preventive effects of dietary walnuts on high-fat-induced hepatic fat accumulation, oxidative stress and apoptosis in mice. *The Journal of Nutritional Biochemistry, 38*, 70–80.
140. Choi, Y. S., Abdelmegeed, M. A., & Song, B. J. (2018). Preventive effects of indole-3-carbinol against alcohol-induced liver injury in mice via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms: Role of gut-liver-adipose tissue axis. *The Journal of Nutritional Biochemistry, 55*, 12–25.
141. Celik, G., Semiz, A., Karakurt, S., Arslan, S., Adali, O., & Sen, A. (2013). A comparative study for the evaluation of two doses of ellagic acid on hepatic drug metabolizing and antioxidant enzymes in the rat. *BioMed Research International, 2013*, 358945.
142. Forsyth, C. B., Voigt, R. M., Shaikh, M., Tang, Y., Cederbaum, A. I., Turek, F. W., & Keshavarzian, A. (2013). Role for intestinal CYP2E1 in alcohol-induced circadian gene-mediated intestinal hyperpermeability. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 305*, G185–G195.
143. Mercer, K. E., Pulliam, C. F., Hennings, L., Cleves, M. A., Jones, E. E., Drake, R. R., & Ronis, M. J. J. (2018). Diet supplementation with soy protein isolate, but not the isoflavone genistein, protects against alcohol-induced tumor progression in DEN-treated male mice. *Advances in Experimental Medicine and Biology, 1032*, 115–126.
144. Wang, Y., Kirpich, I., Liu, Y., Ma, Z., Barve, S., McClain, C. J., & Feng, W. (2011). Lactobacillus rhamnosus GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *The American Journal of Pathology, 179*(6), 2866–2875.
145. Elzouki, A. N. (2016). Probiotics and liver disease: Where are we now and where are we going? *Journal of Clinical Gastroenterology, 50*, S188–S190.
146. Grander, C., Adolph, T. E., Wieser, V., Lowe, P., Wrzosek, L., Gyongyosi, B., Ward, D. V., Grabherr, F., Gerner, R. R., Pfister, A., Enrich, B., Ciocan, D., Macheiner, S., Mayr, L., Drach, M., Moser, P., Moschen, A. R., Perlemuter, G., Szabo, G., Cassard, A. M., & Tilg, H. (2018). Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. *Gut, 67*(5), 891–901.
147. Jeong, K. S., Jeong, K. S., Lee, I. J., Roberts, B. J., Soh, Y., Yoo, J. K., Lee, J. W., & Song, B. J. (1996). Transcriptional inhibition of cytochrome P4502E1 by a synthetic compound, YH439. *Archives of Biochemistry and Biophysics, 326*(1), 137–144.
148. Fuster, D., & Samet, J. H. (2018). Alcohol use in patients with chronic liver disease. *New England Journal of Medicine, 379*, 1251–1261.
149. Brandl, K., & Schnabl, B. (2017). Intestinal microbiota and nonalcoholic steatohepatitis. *Current Opinion in Gastroenterology, 33*(3), 128–133.
150. Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S., & Jalan, R. (2017). Targeting the gut-liver axis in liver disease. *Journal of Hepatology, 67*(5), 1084–1103.