Published in final edited form as:

Adv Exp Med Biol. 2018 ; 1032: 203–221. doi:10.1007/978-3-319-98788-0_14.

Engineered Animal Models Designed for Investigating Ethanol Metabolism, Toxicity and Cancer

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Abstract

Excessive consumption of alcohol is a leading cause of lifestyle-induced morbidity and mortality worldwide. Although long-term alcohol abuse has been shown to be detrimental to the liver, brain

and many other organs, our understanding of the exact molecular mechanisms by which this occurs is still limited. In tissues, ethanol is metabolized to acetaldehyde (mainly by alcohol dehydrogenase and cytochrome p450 2E1) and subsequently to acetic acid by aldehyde dehydrogenases. Intracellular generation of free radicals and depletion of the antioxidant glutathione (GSH) are believed to be key steps involved in the cellular pathogenic events caused by ethanol. With continued excessive alcohol consumption, further tissue damage can result from the production of cellular protein and DNA adducts caused by accumulating ethanol-derived aldehydes. Much of our understanding about the pathophysiological consequences of ethanol metabolism comes from genetically-engineered mouse models of ethanol-induced tissue injury. In this review, we provide an update on the current understanding of important mouse models in which ethanol-metabolizing and GSH-synthesizing enzymes have been manipulated to investigate alcohol-induced disease.

Keywords

Transgenic; Alcohol; Aldehyde dehydrogenase; Glutathione; Cancer; Tumor; Acetaldehyde

14.1 Introduction

Alcoholic beverages have long been an integral part of our society, and serve many functions in medicine and social interactions. Not surprisingly, the effect of alcohol on human health and society has been subject to intensive research. Alcohol consumed in moderation (7 glasses/wk) is considered to have only a minor impact on health [1]. There is even some evidence that ethanol consumption (depending on the nature and speed at which it was consumed) may have health benefits [1]. However, excessive consumption of alcohol (or alcohol abuse) can lead to a plethora of adverse health outcomes, including injuries, violence, suicide, poisoning, cirrhosis, cancer, and possibly hemorrhagic stroke [2, 3]. Because of this, levels of alcohol consumption have been established by the NIAAA that represent abuse, i.e., typically 5 alcoholic drinks/day for males and 4 alcoholic drinks per day for females [4]. Alcohol abuse-related deaths total ≈ 3.3 million per year worldwide (equaling nearly 6% of total deaths), making it the fourth leading cause of preventable death [5]. Alcohol places a significant financial burden on the economy; its estimated healthcare costs surpassed \$223 billion in 2006 in the United States alone [1]. The development of interventions that prevent or treat diseases associated with alcohol consumption is dependent upon our understanding of the mechanisms by which ethanol induces pathophysiological changes. Consumed alcohol (ethanol) is metabolized to acetaldehyde, primarily by alcohol dehydrogenase (ADH) and cytochrome p450 2E1 (CYP2E1). Aldehyde dehydrogenases (ALDHs) eliminate acetaldehyde by oxidizing it to acetate (Fig. 14.1). Acetaldehyde is toxic in large quantities, and clinically causes a flushing syndrome manifesting as facial flushing, nausea, and tachycardia [6]. At the cellular level, it leads to increases in reactive oxygen species (ROS), depletion of antioxidants (e.g., glutathione), and formation of DNA and protein adducts. Acetaldehyde is classified as an International Agency for Research on Cancer (IARC) group I carcinogen [7] and has been shown to promote cancer [8]. In addition, byproducts of ethanol metabolism are hazardous to human health. For example, the accumulation of ROS, DNA adducts and lipid peroxidation products can cause significant

tissue damage by interfering with normal tissue functions. Genetically-modified mouse models have served as important tools for enhancing our understanding of the mechanisms by which alcohol induces tissue injury. While significant progress has been made, we still have much to discover regarding how specific mechanisms, such as stress, inflammation and cell signaling pathways interact and lead to alcohol-related pathologies, such as alcoholic liver disease, pancreatitis, cardiovascular disease, and diabetes mellitus, as well as various cancers, including oral, colorectal, liver, pancreatic, aerodigestive, breast, and colon [2–7]. We present here an update on the mouse models that hold the potential to lead to a deeper understanding of the pathways involved in the metabolism of ethanol and of the mechanisms that make cells vulnerable to alcohol-induced damage.

14.2 Animal Models for Alcohol-Induced Cancer

The pathological consequences of alcohol abuse occur over a spectrum. Aside from a few pathologies, such as acute steatohepatitis, prolonged alcohol use is usually associated with chronic disease. Specific lifestyle and genetic factors can increase the likelihood that alcohol consumption leads to disease [9]. Much research has been done on ethanol's contribution to esophageal, head and neck and oral cancers [10–12]. Heavy drinkers (i.e., those who have consumed 3 ethanol-containing beverages/day for women and 7 ethanol-containing beverages/day for men, for more for 5 days in the past month) appear to be at an increased risk for these cancers; they retain this increased risk even after 10 years of abstinence [13]. While a wealth of epidemiological data exists that relate alcohol consumption to cancer risk, a lack of appropriate experimental models has hindered our progress in understanding the molecular mechanisms by which ethanol promotes cancer formation [14]. Nevertheless, strong evidence suggests that acetaldehyde and free radical production from ethanol metabolism are significant contributors to alcohol-associated tumorigenesis [8]. The genetically-modified mouse models of ethanol, acetaldehyde and glutathione metabolism (presented herein, Table 14.1) hold the potential to facilitate the elucidation of the mechanisms by which ethanol promotes cancer development.

14.3 Mouse Models with Genetic Deficiencies in Ethanol-Metabolizing Enzymes

Ethanol is first oxidized to acetaldehyde through the enzymatic action of multiple isozymes of cytosolic alcohol dehydrogenase (ADH). This occurs primarily in the liver. Catalase and cytochrome p450 2E1 (CYP2E1) can also metabolize ethanol to acetaldehyde, albeit when ethanol is present in high concentrations, or in cells that only poorly express ADH [15]. The products of these reactions (e.g., acetaldehyde, ROS) are rapidly metabolized to less harmful products. Aldehyde dehydrogenase (ALDH) converts acetaldehyde to acetate (which enters into the citric acid cycle). Antioxidants, such as glutathione, are produced to detoxify the ROS generated from CYP2E1.

14.3.1 Adh1 Global Knockout

As noted, alcohol dehydrogenase (ADH) is primarily responsible for the metabolism of ethanol to acetaldehyde. ADH1 catalyzes unbranched alcohols to their corresponding

aldehydes. Duester and colleagues generated the *Adh1*^{-/-} mouse line [16]. Through evolution, humans have gained three genes for ADH1, viz. *ADH1A*, *ADH1B*, and *ADH1*, while mice retain just one ADH1 gene [11]. *Adh1*^{-/-} mice are phenotypically normal and fertile but have a limited capacity to oxidize ethanol and retinol. They have been reviewed in [17]. Upon ethanol feeding, *Adh1*^{-/-} mice exhibit reduced blood ethanol clearance, demonstrating that ADH1 has ethanol dehydrogenase activity [18]. These mice have been used successfully in mouse model studies of vitamin deficiencies and ethanol-induced tissue damage studies to elucidate the mechanism of action of retinoid dehydrogenases and aldehyde dehydrogenases.

14.3.2 Catalase *Global Knockout*

A small proportion of alcohol consumed is metabolized by catalase. The main biological role of catalase is the conversion of hydrogen peroxide (an important, harmful ROS metabolite) to water and oxygen. The catalase knockout (*Cat*^{-/-}) mouse strain was developed and characterized by Ho and colleagues [19]. These mice do not express catalase and develop normally, i.e., exhibit no gross abnormalities [17]. However, brain mitochondria of these animals show deficiencies in respiration. In spite of the absence of catalase, these animals do not show increased vulnerability to hyperoxia-induced lung injury [19]. Nevertheless, catalase appears to modulate ethanol sensitivity in the brain [20, 21]. Administration of 10% ethanol in drinking water to *Cat*^{-/-} mice (considered binge ethanol administration) increases serum alanine aminotransferase activity, plasma homocysteine levels, indicating liver damage, hepatic fat accumulation, and lipid peroxidation. Catalase has a role in protecting the liver against binge ethanol exposure [22]. Through the use of an anti-catalase shRNA gene-coding lentiviral vector, acetaldehyde was shown to be important in influencing preference for ethanol in rats [23]. Recently, it was revealed that in *Cat*^{-/-} mice fed a high-fat diet, mitochondrial-derived H₂O₂ is responsible for diminished insulin signaling in the heart [24]. Rindler and colleagues identified catalase as an important component of the immediate antioxidant response [25]. Thus, the *Cat*^{-/-} mice (alone or in combination with the other knockouts) are a valuable tool for examining determinants of ethanol drinking preference as well as ethanol-induced tissue damage.

14.3.3 Cyp2e1 *Global Knockout*

Cytochrome p450 2E1 (CYP2E1) is an ethanol-inducible enzyme with a role in hepatic ethanol oxidation. By genetically ablating exon 2 of the *Cyp2e1* gene, Gonzalez and colleagues developed *Cyp2e1*^{-/-} mice [26]. These mice do not express the CYP2E1 enzyme but develop normally [26]. Interestingly, they show a reduced sensitivity to the deleterious hepatic effects of the analgesic acetaminophen [26]. As one of the primary xenobiotic/endobiotic-metabolizing cytochrome P450 enzymes, CYP2E1 protects against a variety of endogenous or exogenous pathogens. Using the *Cyp2e1*^{-/-} mouse model, CYP2E1 has been shown to play a pivotal role in mediating ethanol-induced hepatotoxicity [27, 28]. *Cyp2e1*^{-/-} and *Cyp2e1* knock-in mice have been used to examine the potentiation of ethanol-induced hypoxia. *Cyp2e1* knock-in mice exhibited the lowest levels of hypoxia and HIF1- α protein expression compared to both WT mice and *Cyp2e1*^{-/-} mice [29]. In humans, up to 60% of ethanol metabolism can be attributed to CYP2E1 in excessive alcohol consumers; it has a higher K_m for alcohol than ADH and its activity is induced by chronic alcohol ingestion

[30–32]. It has been reported that the regulatory sequences in the promoter of CYP2E1 may play a role in the pre-absorptive metabolism of alcohol [33]. *Cyp2e1*^{−/−} mice are protected against apoptosis and steatohepatitis [34], and autophagy [35] in a chronic ethanol-fed mouse model. Similarly, ethanol-induced fatty liver and oxidant stress are blunted in these mice [36]; this study confirmed the important role CYP2E1 plays in ethanol-induced liver toxicities. *Cyp2e1*^{−/−} mice also display longer ethanol-induced sleep time [15], reinforcing the relevance of the *Cyp2e1*^{−/−} mouse line for the study of the CYP2E1 enzyme in ethanol toxicities and alcohol-related drinking preference.

14.3.4 Cat and Cyp2e1 Global Double-Knockout

We have recently generated the *Cat*^{−/−} and *Cyp2e1*^{−/−} double-knockout mouse line by crossing the two single knockout mouse lines (Matsumoto A, Chen Y, Vasiliou V et al., manuscript in preparation). No expression of catalase and CYP2E1 proteins were detectable in their liver or brain. These mice are viable, fertile and show no gross abnormalities. We are currently investigating alcohol metabolism, ethanol-induced sleep times and ethanol preference in these mice.

14.4 Mouse Models with Genetic Deficiencies in Acetaldehyde-Metabolizing Enzymes

After the first step of ethanol metabolism to acetaldehyde by ADH, aldehyde dehydrogenases (ALDHs) metabolize this toxic intermediate to a less active byproduct, acetate. This is normally a rapid step; however, under conditions of excessive ethanol consumption, acetaldehyde may accumulate, leading to increased ROS, lipid peroxidation, DNA adducts and physical manifestations, such as flushing and tachycardia. Multiple ALDH isozymes metabolize ethanol-derived acetaldehyde. The majority of acetaldehyde generated from ethanol is metabolized by ALDH2 in the mitochondria. Cytosolic ALDH1A1 also metabolizes acetaldehyde, but to a lesser extent than ALDH2. The ALDH1B1 isozyme shares 72% amino acid sequence homology with ALDH2 and has been shown to have an affinity for both acetaldehyde and retinaldehyde [1].

14.4.1 Aldh2 Global Knockout

The *Aldh2*^{−/−} strain was first developed and characterized by Isse et al. [37, 38]. These mice are fertile and display no overt phenotype. Following oral administration of ethanol, *Aldh2*^{−/−} mice exhibit higher ethanol and acetaldehyde levels and lower acetate levels in the blood, brain, and liver [39–41], and show a dose-dependent reduction in lifespan, body weight, and increased serum ALT activity [42]. *Aldh2*^{−/−} mice are also more sensitive to the toxic effects of inhaled acetaldehyde [43] and to the genotoxic effects of exposure to the biofuel ethyl *tert*-butyl ether (which generates acetaldehyde in the body) [44]. In addition, beneficial effects of low to moderate ethanol consumption, such as increased HDL-cholesterol levels and cardioprotection, are not observed in *Aldh2*^{−/−} mice [45, 46]. *Aldh2*^{−/−} mice show altered liver function under basal conditions [47] and after ethanol exposure [48]. Conversely, higher levels of hepatic antioxidant proteins have been found in naïve *Aldh2*^{−/−} mice fed standard chow [49]. Bone structure alterations have also been observed in naïve *Aldh2*^{−/−} mice [50] and in those treated with alcohol [51]. Cardiac function of *Aldh2*^{−/−}

mice is more adversely affected by exposure to an endoplasmic reticulum stress inducer [52] or alcohol [53]. On the other hand, *Aldh2*^{-/-} mice show diminished compensatory cardiac hypertrophy in an aortic ligation model [54]. Hence, there is no consensus on a positive or negative effect of ALDH2 absence on cardiac function. A diabetes phenotype induced in *Aldh2*^{-/-} mice exhibit more severe energy metabolism impairments and diastolic dysfunction [55]. *Aldh2*^{-/-} mice show higher acetaldehyde-DNA adducts in the esophagus [56, 57], stomach [58, 59] and liver [60, 61] after ethanol administration. In addition, mice with a polymorphism in ALDH2 (i.e., ALDH2*2) are more prone to liver tumor formation [62]. This suggests that acetaldehyde generation is likely involved in the pathogenesis of upper gastrointestinal, gastric and liver cancer. All of these findings demonstrate that the value of *Aldh2*^{-/-} mice as a strain that can be used to identify the contribution of ALDH2 to ethanol metabolism and toxicity.

14.4.2 *Aldh1b1* Global Knockout

Our laboratory generated the *Aldh1b1*^{-/-} strain [63]. These mice develop and breed normally without any overt phenotype. In agreement with the alcohol hypersensitivity and aversion associated with the ALDH1B1 polymorphism in Caucasians and with the catalytic properties of ALDH1B1 (i.e., the second lowest K_m for acetaldehyde oxidation) [64–66], *Aldh1b1*^{-/-} mice exhibit slower acetaldehyde clearance. Microarray analysis of a murine whole embryo culture model revealed ALDH1B1 to be expressed only in the ethanol-treated embryos with open neural tubes [67]. These findings suggest a crucial role of ALDH1B1 in ethanol toxicity during embryonic development. The *Aldh1b1*^{-/-} mouse strain represents the first animal model that allows the study of ALDH1B1 in ethanol-induced tissue injury. We found that ALDH1B1 may promote tumor formation by downregulating the Wntβ-catenin, Notch and PI3K/Akt signaling pathways [63]. ALDH1B1 is expressed in embryonic and adult pancreatic progenitor murine cells and has been proposed as a marker for these cells. Anastasiou and colleagues [68] showed alterations in beta cell functionality in the pancreas of *Aldh1b1*^{-/-} mice with decreased glucose sensing, stimulus-secretion coupling and secretory granule biogenesis, as well as decreased expression of *Nrf2* and other genes related to oxidative stress protection. Such changes may underlie the glucose intolerance and hyperglycemia that manifests in *Aldh1b1*^{-/-} mice over the long term [69, 70]. Centroacinar-like cells that express ALDH1B1 are present in the adult mouse pancreas, and their number increases dramatically following experimental pancreatic injury, such as that caused by caerulein or streptozotocin treatment [69]. These cells are also believed to be involved in pancreatic cancer initiation [69]. Recently, we showed that ALDH1B1 is strongly expressed in human pancreatic adenocarcinoma cells and is crucial for their proliferation [71]. As such, ALDH1B1 may play a central role in pancreatic development, regeneration and cancer [69–71]. The *Aldh1b1*^{-/-} mouse model provides an opportunity to examine the link between alcohol consumption and diabetes.

14.4.3 *Apc*^{F/F}*Cdx2*^{ERT2-Cre}/*Aldh1b1*^{-/-} Mutant

ALDH1B1 expression is confined to the base of crypts in the normal colon near (or in) the stem cells. In addition, it is highly expressed in human colon adenocarcinomas [72]. Our laboratory has shown that shRNA-mediated suppression of ALDH1B1 in a human colon cancer cell line (SW480) resulted in a decreased number and size of spheroids in 3D-

matrigel culture, and in reduced sizes of xenograft tumors in nude mice [63]. We are currently breeding the *Aldh1b1*^{-/-} and the *Apc*^{F/F}.*Cdx2*^{ERT2-Cre} mouse lines to generate an *Apc*^{F/F}.*Cdx2*^{ERT2-cre}/*Aldh1b1*^{-/-} mouse line. *Apc*^{F/F}.*Cdx2*^{ERT2-cre} mice carry a CDX2P-NLS Cre recombinase and a loxP-targeted *Apc* allele. These mice develop tumors predominantly in the large intestine (especially in distal colon and rectum). Tumors in *Apc*^{F/F}.*Cdx2*^{ERT2-cre} mice resemble human colorectal cancers in distribution, molecular changes and gender predisposition, i.e., are more common in males than in females [73]. Due to the finding that ALDH1B1 may be involved in tumor formation, crossing *Aldh1b1*^{-/-} mice with *Apc*^{F/F}.*Cdx2*^{ERT2-cre} mice will allow us to more quickly study the role of ALDH1B1 in the process of tumor formation induced by ethanol feeding. Accordingly, *Apc*^{F/F}.*Cdx2*^{ERT2-Cre}/*Aldh1b1*^{-/-} mutant mice will make an ideal model to study alcohol-induced colorectal cancer.

14.4.4 *Aldh1a1* Global Knockout

The *Aldh1a1*^{-/-} strain was developed and originally characterized by Fan and co-workers [74]. *Aldh1a1*^{-/-} mice are viable and develop normally. Following retinol treatment, livers from these mice display reduced retinoic acid synthesis and increased serum retinal levels [74], supporting a key role for hepatic *Aldh1a1* in oxidizing retinaldehyde. Interestingly, *Aldh1a1*^{-/-} mice are protected against diet-induced obesity and its comorbidity, and showed insulin resistance, suggesting that ALDH1A1 may regulate the metabolic response to a high-fat diet [75]. ALDH1A1 is highly expressed in the cornea, and its protective role in the eye is illustrated by the observation that *Aldh1a1*^{-/-} mice develop cataracts [76]. Gene expression profiling has shown *Aldh1a1* to be highly expressed in mouse hematopoietic stem cells [77, 78]. Interestingly, hematopoietic stem cells from *Aldh1a1*^{-/-} mice exhibit increased sensitivity to cyclophosphamide (a chemotherapy agent) and its decreased metabolism in the liver [79]. Based upon these results, it has been suggested that ALDH1A1 may be an important regulator of stem cell function. ALDH1A1 is also associated with metabolism of catecholamines and is expressed in the neurons of the mesencephalon where it converts potentially toxic 3,4-dihydroxyphenylacetaldehyde (DOPAL) metabolites to a non-toxic metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). Hence, by altering the metabolism of catecholamines, loss of ALDH1A1 expression can potentially increase neurotoxicity [80]. In humans, genetic variants of ALDH1A1 that exhibit low enzyme activity have been associated with increased alcohol sensitivity [81]. In addition, ALDH1 promoter polymorphisms have been proposed to contribute to the observed protection against alcohol disorders in Southwest California Indians [82]. In the Finnish population, alcohol consumption behavior and alcohol dependence risk are influenced by genetic variations in ALDH1A1 [83]. Therefore, the *Aldh1a1*^{-/-} mouse line represents a useful animal model for investigation of the ALDH1A1 enzyme in ethanol and retinol toxicities.

14.4.5 *Aldh1b1* and *Aldh1a1* Double Knockout

ALDH1A1 is highly expressed in some commonly-occurring carcinomas and is a marker for poor prognosis in humans (see Tomita et al. for review [84]). ALDH1B1 is expressed in several carcinomas that also express ALDH1A1; in some of these, the level of ALDH1B1 is higher than ALDH1A1, especially in colon adenocarcinoma [72]. An *Aldh1b1* and *Aldh1a1* double knockout (*Aldh1b1*^{-/-}/*Aldh1a1*^{-/-}) is being developed to identify the role of

retinaldehyde- and acetaldehyde- metabolizing enzymes in carcinogenesis. This strain will be the product of the crossbreeding of *Aldh1b1*^{-/-} and *Aldh1a1*^{-/-} mice.

14.4.6 *Aldh1b1 and Aldh2 Global Double-Knockout*

Beyond the pancreatic impairment that manifests over the long term, the normality of the phenotype of *Aldh1b1*^{-/-} mice could be attributable to compensatory changes in the activity of redundant enzymes, such as ALDH2. The *Aldh1b1* and *Aldh2* double knockout strain (*Aldh1b1*^{-/-}/*Aldh2*^{-/-}) is being developed to allow examination of the physiological and pathophysiological consequences of complete removal of mitochondrial acetaldehyde oxidation activity. This animal model will be the product of the breeding of *Aldh2*^{-/-} and *Aldh1b1*^{-/-} mice.

14.4.7 *Global Aldh3a1 Single and Aldh1a1/Aldh3a1 Double Knockouts*

Aldh3a1 is a member of the mouse aromatic hydrocarbon receptor (AHR) gene battery, which encode a highly regulated and coordinated group of drug-metabolizing enzymes [85]. The highest expression of *Aldh3a1* occurs in the cornea, stomach, colon, urinary bladder, and skin of the mouse [86]. Numerous studies suggest a role for ALDH3A1 in a variety of homeostatic mechanisms, including cell proliferation, differentiation and apoptosis, and in resistance to chemotherapeutics used to treat human breast, hepatocellular or prostate adenocarcinoma [87–89]. ALDH3A1 appears to mediate these effects through both enzymatic and non-enzymatic properties. Enzymatically, ALDH3A1 is involved in the detoxification of lipid peroxidation by-products and aromatic aldehydes [90]. Acetaldehyde is not a substrate for ALDH3A1, which is seemingly in line with the observation that ALDH3A1 protein is undetectable in the liver [91]. However, a recent report showed that pharmacological activation of ALDH3A1 facilitated acetaldehyde metabolism *in vivo*, suggesting that ALDH3A1 may contribute to cellular mechanisms that detoxify acetaldehyde [92]. A global *Aldh3a1* knockout (*Aldh3a1*^{-/-}) strain was originally generated by Nees and co-workers [93] and has been crossbred into B6 background [93]. *Aldh3a1*^{-/-} mice in the mixed background develop cataracts at one month of age [76] and B6 *Aldh3a1*^{-/-} mice display a phenotype of corneal haze [94]. Much like the *Aldh3a1*^{-/-} mice, the *Aldh1a1/Aldh3a1* double knockouts (*Aldh1a1*^{-/-}/*Aldh3a1*^{-/-}) mice develop cataracts, but to a more severe extent [76]. As such, the majority of the research using these mouse strains has focused on elucidating the enzymatic and non-enzymatic functions of ALDH3A1 and ALDH1A1 in the cornea [95]. Given the recent experimental evidence, *Aldh3a1*^{-/-} and *Aldh1a1*^{-/-}/*Aldh3a1*^{-/-} strains may provide new insights into the interactive actions of these ALDH isozymes in alcohol metabolism and toxicities.

14.4.8 *Aldh3a1C244A and Aldh1a1C303A Knock-in Mice*

A large body of evidence supports the notion that the accumulation of high concentrations of ALDH3A1 and ALDH1A1 proteins in the cornea exert multifaceted functions involving both enzymatic and non-enzymatic properties of these proteins [91], the latter of which has been proposed to involve direct protein-protein interactions [96]. To elucidate the specific enzymatic and/or non-enzymatic functions of ALDH3A1 and ALDH1A1, our group has recently generated knock-in (KI) mouse lines for *Aldh3a1* and *Aldh1a1*, respectively. These strains were developed by introducing a Cys→Ala mutation at the catalytically-essential site

of ALDH3A1 (codon 244) and ALDH1A1 (codon 303) proteins, respectively (unpublished work) and this results in the mice expressing enzymatically-inactive forms of these ALDH isozymes. These KI mouse strains may serve as additional models for alcohol research and are of particular utility for exploring non-enzymatic (or structural) functions of ALDH3A1 and ALDH1A1 in alcohol metabolism and toxicities.

14.5 Mouse Models with Glutathione Deficiency

It is well accepted that the generation of free radicals, such as ROS, during excessive consumption of ethanol depletes glutathione (GSH). It is believed that reductions in hepatic GSH levels are a key pathogenic event mediating ethanol-induced liver injury [97–99]. Glutathione is a ubiquitous antioxidant and is essential for maintaining cellular redox homeostasis by detoxifying reactive xenobiotics and scavenging ROS derived from cellular metabolism. Due to its abundance in the liver, GSH plays a crucial role in defending against oxidative liver injuries. It is synthesized by two sequential reactions, the first and rate-limiting step of which is catalyzed by the enzyme glutamate-cysteine ligase (GCL). In higher eukaryotes, GCL comprises a catalytic (GCLC) and a modifier (GCLM) subunit. GCLC possesses all of the catalytic activity of GCL, and GCLM serves to optimize the kinetic properties of GCLC. Due to its essential role in GSH biosynthesis, GCL has been the principal target for generating animal models with GSH deficiency. Comprehensive reviews on GSH metabolism and functions and the GCL enzyme can be found elsewhere [100, 101].

14.5.1 *Gclc* Conditional (*Gclc^{ff}*) Knockout

The global gene knockout of *Gclc* results in embryonic lethality, indicating that GSH is indispensable for early mouse development [102]. The *Gclc* floxed (*Gclc^{ff}*) strain was developed and originally characterized by Chen and colleagues [103]. The pathophysiological role of GSH deficiency in hepatocytes was investigated using the hepatocyte-specific *Gclc* knockout (*Gclc^{h/h}*) mice created by intercrossing *Gclc^{ff}* and *Alb-Cre* mice [103]. *Gclc^{h/h}* mice experience almost complete loss of hepatic GSH (~5% of normal) and die from acute liver failure when mitochondrial failure occurs [103]. Chronic administration of N-acetylcysteine, a treatment that promotes only a small increase in liver GSH levels (to 8% of normal), partially preserves the mitochondrial GSH pool and function, and allows *Gclc^{h/h}* mice to survive to adulthood, albeit with the serious liver pathologies fibrosis and cirrhosis [104]. The *Gclc^{ff}* mice represent a unique model that can be used to elucidate cell-specific functions of GSH in ethanol metabolism and toxicity.

14.5.2 *Global Gclm* (*Gclm^{-/-}*) knockout

The *Gclm^{-/-}* strain was developed and originally characterized by Yang and coworkers [79]. These mice are viable and fertile, despite having only 9–16% of the normal GSH levels in liver, lung, pancreas, erythrocytes, and plasma [105]. Except when challenged with oxidant stress [106, 107], *Gclm^{-/-}* mice exhibit no overt phenotype, making them a useful model for studying the pathophysiological roles of chronic GSH depletion. Interestingly, these mice are protected against liver injuries induced by a variety of hepatic insults, including chronic ethanol treatment [101]. In addition, these mice are completely protected from ethanol -

induced steatosis [108]. Thus, *Gclm*^{-/-} mice represent an animal model wherein the effects of significant depletion of GSH on interventions, such as alcohol, can be examined.

14.5.3 *Gclm* and *Nrf2* Global Double-Knockout

In examining the effects of GSH effects on alcohol metabolism, *Nrf2* was shown to be upregulated upon ethanol treatment of *Gclm*^{-/-} mice [108]. In a state of chronic GSH depletion, the Keap1-Nrf2-ARE signaling pathway appears necessary for the paradoxical resilience to ethanol-induced liver toxicity [109]. *Nrf2*^{-/-} mice are highly sensitive to ethanol toxicity and show increased morbidity and mortality upon ethanol treatment [110]. Our group has cross-bred *Nrf2*^{-/-} mice with the *Gclm*^{-/-} mice and have preliminary data to suggest that the resultant *Nrf2*^{-/-}/*Gclm*^{-/-} mice develop more serious hepatitis as they age than *Gclm*^{-/-} or WT mice. *Nrf2*^{-/-}/*Gclm*^{-/-} mice are viable and reach maturity; however, they do not produce progeny. This strain is an excellent model for exploring spontaneous development of hepatitis.

14.6 Concluding Remarks

Alcohol's deleterious effects have been implicated in various diseases, such as steatohepatitis, chronic liver disease, and cancers, such as those involving the mouth, liver, pancreas, colorectal region, and breast. Because alcohol metabolism is multifaceted, the genetically-engineered mouse models (discussed in this review) represent valuable tools that allow the further exploration of the mechanisms by which ethanol elicits its deleterious pathophysiological effects. Our diverse mouse model inventory contains a genetic variation for each step of the ethanol metabolic pathway, facilitating the way for the creation of novel therapeutic interventions to treat diseases associated with alcohol consumption.

Acknowledgements

This work was supported in part by the National Institutes of Health Grants. AA022057 (VV), AA017754 (VV), EY017963 (VV), EY11490 (VV), AA025093 (YC), and AA023699 (SMM).

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Fig. 14.1.

Description of enzymes that participate in ethanol metabolism. Ethanol is metabolized to acetaldehyde by catalase (CAT), alcohol dehydrogenase (ADH) and/or cytochrome P450 2E1 (CYP2E1). A lack of any of these enzymes will diminish the rate of synthesis of acetaldehyde and delay the elimination of ethanol. Acetaldehyde is primarily metabolized to acetic acid by aldehyde dehydrogenase 2 (ALDH2), although ALDH1B1 and ALDH1A1 may also contribute. Reductions in the activity of these enzymes result in elevated levels of acetaldehyde after ethanol consumption. ALDH1B1 and ALDH1A1 also participate in the metabolism of retinaldehyde to retinoic acid. Reactive oxygen species (ROS), by-products of ethanol metabolism, are scavenged by glutathione (GSH)-dependent mechanisms. Enzymes involved in GSH synthesis, such as glutamate-cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits are integral to neutralizing and maintaining homeostatic ROS levels that are generated after ethanol consumption preventing the deleterious effects of ethanol (adapted from [17])

Table 14.1

Overview of the phenotypes of the differing genetic strains of mice in the Vasiliou Lab

Strain	Genetic background	Phenotype	Reference
<i>Catalase</i> global knockout	C57BL6	Do not express catalase.	Ho 2004, Heit 2017
		Develop normally, i.e., exhibit no gross abnormalities. Brain mitochondria show deficiencies in respiration.	
		Have age-related weight gain when fed regular chow.	
<i>Cyp2e1</i> global knockout	C57BL6	Do not express CYP2E1.	Rindler 2016, Abdelmegeed 2013, Lu 2015
		Viable and develop normally.	
		Exhibit a lower sensitivity to the deleterious hepatic effects of acetaminophen.	
		Are protected against apoptosis and steatohepatitis and autophagy in a chronic ethanol-fed model.	
<i>Cat</i> and <i>Cyp2e1</i> global double-knockout	C57BL6/129 mixed	Do not express catalase or CYP2E1.	Unpublished
		Are viable, fertile and show no gross abnormalities. We are currently investigating alcohol metabolism, ethanol-induced sleep times and preference in these mice.	
<i>Aldh2</i> global knockout	C57BL6	Do not express ALDH2.	Isse 2005, Kiyoshi 2009, Jamal 2016, Matsumoto 2014, Oyama 2007
		Are viable and develop normally.	
		Exhibit lower ethanol and acetaldehyde clearance than wild-type mice.	
		Ethanol administration causes dose-dependent reduction in lifespan, body weight, and increased serum ALT activity.	
		More sensitive to the toxic effects of inhaled acetaldehyde.	
<i>Aldh1b1</i> global knockout	C57BL6J	Do not express ALDH1B1.	Singh 2015, Anastasiou 2016
		Are viable and develop normally.	
		Exhibit lower acetaldehyde clearance.	
		Show glucose intolerance and hyperglycemia.	
<i>Aldh1a1</i> global knockout	C57BL6	Do not express ALDH1A1.	Fan 2003
		Are viable and develop normally.	
		Following retinol treatment, livers display reduced retinoic acid synthesis and increased serum retinal levels.	
<i>Aldh3a1</i> global knockout	C57BL6	Do not express ALDH3A1.	Koppaka 2016, Chen 2015
		Are viable and develop normally.	
		Develop cataracts or corneal hazing	
<i>Aldh1a1</i> and <i>Aldh3a1</i> double knockout	C57BL6	Do not express ALDH1A1 or ALDH3A1.	Koppaka 2016
		Are viable and develop normally.	
		Develop cataracts	
GCLC conditional (<i>Gclc^{fl/y}</i>) knockout	C57BL6	Do not express GCLC in cre-targeted tissues.	Chen 2010, Chen 2014
		Mice are produced at expected mendillian ratios.	

Strain	Genetic background	Phenotype	Reference
		<i>Gclc^{hh}</i> mice die from liver failure due to lack of proper mitochondrial function	
		<i>Gclc^{de/de}</i> mice have defects in ocular development	
GCLM global(<i>Gclm</i> ^{-/-}) knockout	C57BL6	Do not express GCLM.	Yang 2002
		Viable, fertile and develop normally.	
		Protected against liver injuries induced by a variety of hepatic insults	
<i>Gclm</i> and <i>Nrf2</i> knockout mice	C57BL6	Do not express GCLM or NRF2.	Unpublished
		Are viable and reach maturity; however, have yet to produce progeny.	
		May develop hepatitis by 6 months of age.	
Mice still under investigation		<i>Apc^{F/F}Cdx2^{ERT2-Cre}/Aldh1b1^{-/-}</i> mutant line, <i>Aldh1b1</i> and <i>Aldh1a1</i> double knockout, <i>Aldh1b1</i> and <i>Aldh2</i> double knockout, <i>Aldh3a1</i> C244A knock-in, <i>Aldh1a1C303A</i> knock-in mouse strain	