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GLUTATHIONE DEFENSE MECHANISM IN LIVER INJURY: INSIGHTS FROM ANIMAL MODELS

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Abstract

Glutathione (GSH) is the most abundant cellular thiol antioxidant and it exhibits numerous and versatile functions. Disturbances in GSH homeostasis have been associated with liver diseases induced by drugs, alcohol, diet and environmental pollutants. Until recently, our laboratories and others have developed mouse models with genetic deficiencies in glutamate-cysteine ligase (GCL), the rate-limiting enzyme in the GSH biosynthetic pathway. This review focuses on regulation of GSH homeostasis and, specifically, recent studies that have utilized such GSH-deficient mouse models to investigate the role of GSH in liver disease processes. These studies have revealed a differential hepatic response to distinct profiles of hepatic cellular GSH concentration. In particular, mice engineered to not express the catalytic subunit of GCL in hepatocytes [*Gclc(h/h)* mice] experience almost complete loss of hepatic GSH (to 5% of normal) and develop spontaneous liver pathologies characteristic of various clinical stages of liver injury. In contrast, mice globally engineered to not express the modifier subunit of GCL [*Gclm*(*-/-*) mice] show a less severe hepatic GSH deficit (to $\approx$ 15% of normal) and exhibit overall protection against liver injuries induced by a variety of hepatic insults. Collectively, these transgenic mouse models provide interesting new insights regarding pathophysiological functions of GSH in the liver.

Keywords

Glutathione; glutamate-cysteine ligase; oxidative stress; steatosis; steatohepatitis; liver injury

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Conflict of interest

The authors declare there are no conflicts of interest.

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I. Introduction

Oxidative stress occurs when reactive oxygen species (ROS) are produced at levels exceeding those capable of being sequestered by normal cellular antioxidant defenses. Glutathione (GSH) is the most abundant cellular non-protein thiol, attaining concentrations in the high millimolar range in liver (Kretzschmar 1996). GSH is a ubiquitous tripeptide composed of glutamate, cysteine, and glycine. The oxidized form of GSH is glutathione disulfide (GSSG). As an important cellular redox buffer, the relative levels of GSH and GSSG maintain cellular redox homeostasis (Dalton et al. 2004). Because of its abundance, GSH serves to protect cells against toxicity arising from exposure to excessive amounts of endogenous and exogenous electrophiles (Meister 1991). GSH scavenges hydroxyl radical and superoxide directly, and serves as a cofactor for the enzyme glutathione peroxidase (GPx) in metabolizing hydrogen peroxide (H₂O₂) and lipid peroxides (Arthur 2000). Through action of the glutathione *S*-transferase (GST) family of enzymes, GSH may conjugate to a variety of electrophilic endogenous compounds and xenobiotics, and this results in their safe and efficient elimination from the body (Rinaldi et al. 2002). Furthermore, GSH is able to regenerate other important antioxidants, such as vitamins C and E, back to their active forms. Aside from its important role as a major antioxidant, GSH has been shown to participate in other physiological processes including nucleotide metabolism, formation of lipid second messengers (Luthman et al. 1979; Meister 1991), regulation of nitric oxide homeostasis (Hogg 2002; Lipton et al. 2001), and modulation of protein function by redox modification (Pompella et al. 2003).

The liver plays a critical role in metabolism and detoxication of ingested and blood-borne substances. Many drugs, environmental toxicants and selected dietary components have the potential to cause liver damage by inducing oxidative stress. Pathophysiological consequences of hepatic oxidative damage include dysregulation of lipid metabolism (steatosis), impaired liver function (hepatocyte degeneration and death), and activation of the immune response (inflammation and fibrosis/cirrhosis) (Pessayre et al. 2004). These conditions become manifest in liver diseases of various etiologies—such as non-alcoholic fatty liver disease (NAFLD) (Pessayre et al. 2002), alcoholic liver disease (ALD) (Albano 2008), and drug-induced liver injury (DILI) (Pessayre et al. 2012).

Under physiological conditions, the liver is protected from oxidative stress by the capacity of its hepatocytes to synthesize GSH. Studies in human subjects and in animal models have implicated an important role for disrupted GSH homeostasis in pathogenesis of the above hepatopathies (Kretzschmar 1996). This review provides a current brief discussion about: (i) the GSH biosynthetic system and mouse models with genetic deficiencies of the proteins involved in GSH biosynthesis, and (ii) studies using these models to address the role of GSH in NAFLD, ALD and liver injuries resulting from drug or environmental toxicant exposure.

II. GSH metabolism

2.1 GSH biosynthesis

GSH is synthesized by two successive enzymatic reactions (Fig. 1). The first reaction, catalyzed by glutamate cysteine ligase (GCL), couples L-glutamate to L-cysteine to form γ -glutamylcysteine (γ -GC) (Meister 1988). The second reaction couples glycine to γ -GC and is catalyzed by GSH synthase (GSS). Both enzymes consume one molecule of ATP per catalytic cycle. Since formation of γ -GC by GCL is the rate-limiting enzymatic step in GSH biosynthesis, GCL has been the principal target of drugs designed to inhibit GSH biosynthesis (Meister 1991) and of genetic manipulations to generate GSH-deficient mice.

The eukaryotic GCL holoenzyme, in its most catalytically efficient form, is a heterodimer composed of a catalytic (GCLC) and a modifier (GCLM) subunit, each of which is encoded by separate genes. GCLC possesses all the catalytic activity of GCL, whereas GCLM serves to optimize the catalytic properties of GCL holoenzyme (Chen et al. 2005). The overall rate of GSH synthesis is controlled by several factors—including (i) amount of GCL, (ii) relative ratio between its two subunits (Chen et al. 2005), (iii) availability of the rate-limiting substrate *L*-cysteine (Bannai 1986; Dall'Asta et al. 1983; Meister and Anderson 1983), and (iv) extent of feedback inhibition of GCL by GSH (Richman and Meister 1975; Taylor et al. 1996). Complementation studies using recombinant GCL proteins and tissue lysate indicate that the GCLC subunit exists in excess in most mouse tissues, making GCLM subunit a limiting factor in determining the level of GSH biosynthesis in these tissues (Chen et al. 2005).

2.2 Cellular compartmentalization and degradation of GSH

The available evidence indicates that most, if not all, of the GSH biosynthetic activity resides in cytoplasm (Meister 1982); cytosolic GSH is distributed into intracellular organelles including mitochondria, endoplasmic reticulum (ER), and nucleus (Lu 1999) (Fig. 1). GSH is the major water-soluble antioxidant that maintains redox homeostasis in these subcellular compartments. GSH exists predominantly in its reduced form except in the ER. In liver, about 10% of total cytosolic GSH is actively transported into mitochondria, such that GSH concentrations in mitochondria are comparable to cytosolic concentrations, *viz.* 5–10 mM (Garcia-Ruiz et al. 1994; Griffith and Meister 1985). GSH plays a pivotal role in normal functioning of this vital organelle, where oxygen consumption and generation of ROS occur. GSH in the nucleus maintains the redox status of critical protein sulfhydryl groups that are necessary for expression, transcription activity, and DNA repair (Green et al. 2006). In contrast to other organelles, GSH in ER exists more in the oxidized state, which is believed to be necessary for providing the appropriate environment for assembly and secretory pathways for proteins (Hwang et al. 1992).

GSH contains an unusual γ -glutamyl amide bond between the γ -carbon of the glutamate side chain and amino group of cysteine. As a result, GSH is not a substrate for peptidases inside the cell. Instead, GSH is metabolized extracellularly by membrane-bound γ -glutamyl transferases (GGTs) when GSH is transported through the plasma membrane (Meister 1988) (Fig. 1).

GGT catalyzes the ATP-dependent cleavage of the γ -glutamyl amide bond and transfers the glutamyl residue to another amino acid. This reaction also generates cysteinylglycine, the standard α -amide bond which is cleaved by an extracellular dipeptidase (DP), producing free cysteine and glycine that can then be used by the cell. The highest-affinity acceptor amino acid for the glutamyl residue generated by GGT is cysteine disulfide, cystine.

After reduction of γ -glutamylcystine, γ -GC can be further metabolized to GSH. As such, this salvage pathway represents a means by which GSH can be produced independently of GCL. However, it is important to recognize that this pathway can help maintain GSH levels over the short term because its synthesis of GSH relies on consumption of GSH. Under conditions of decreased new GSH synthesis, such as GCL deficiency, or GSH loss through other pathways, however, the salvage pathway would be incapable of resurrecting and sustaining GSH levels over the long term. The reactions described above for synthesis and degradation of GSH form a metabolic pathway known as the γ -glutamyl cycle (Njalsson and Norgren 2005) (Fig. 1). By way of this cycle, GSH participates in amino acid transport for cellular resynthesis of GSH and other proteins.

2.3 Genetic mouse models with deficiencies in GSH biosynthesis

Polymorphisms in human GCLC and GCLM genes have been reported in several ethnic groups. Several single nucleotide Polymorphisms (SNPs) in the coding region of GCLC have been identified (Beutler et al. 1999; Ristoff et al. 2000; Ristoff and Larsson 1998). These SNPs appear to be rare in the human population and are often associated with low GCL activity and severe GSH depletion (Beutler et al. 1999; Hamilton et al. 2003; Ristoff et al. 2000). Individuals carrying these nonsynonymous SNPs clinically suffer hemolytic anemia, jaundice and some also present with a central nervous system disorder (Ristoff et al. 2000). On the other hand, some common synonymous polymorphisms of *GCL* genes have been reported to be associated with increased risks for certain diseases, including cardiovascular disease (Koide et al. 2003; Nakamura et al. 2002; Nakamura et al. 2003), neurological disorder (Berk et al. 2011; Tosic et al. 2006), pulmonary disease (Bentley et al. 2008; McConnachie et al. 2013) and cancer (Nichenametla et al. 2012). Functional studies indicate that these SNPs generally impair the transcriptional induction of *GCL* genes following oxidant challenge, resulting in moderate changes in GSH homeostasis and consequently increased susceptibility to disease (Butticaz et al. 2011; Gysin et al. 2007; Nakamura et al. 2003; Nichenametla et al. 2011). To our best knowledge, to date there is no reported study yet on the association between *GCL* polymorphisms and drugs- or alcohol-induced tissue injuries.

Historically, cellular and pathophysiological effects of GSH deficiency have been studied through the use of agents that inhibit GCL. Of these, *L*-buthionine-(S,R)-sulfoximine (BSO) has been the one most commonly used (Meister 1991). Such pharmacological approaches are considered limited by the lack of specificity of these inhibitors and by toxic effects of these inhibitors during chronic administration. However, a recent study indicates that BSO is rather a specific inhibitor of GCLC in cell culture system (Mandal et al. 2010).

Several mouse models of GSH depletion have been developed based on genetic manipulation, specifically by disruption of the two *Gcl* genes. Given that the GCLC subunit is required for all GCL-catalyzed γ -GC synthesis, cells lacking *Gclc* do not have the capacity for *de novo* synthesis of GSH. Two independent mouse lines carrying a global *Gclc* deletion have been developed. Studies using these mouse lines demonstrate that *Gclc* and, by extension, GSH, is essential for mouse embryonic development (Dalton et al. 2000; Shi et al. 2000). *Gclc*($-/-$) embryos fail to differentiate beyond embryonic day 7.5 (E7.5), at which time *Gclc*($-/-$) embryos reveal massive distal apoptosis and die. Surprisingly, *Gclc*($-/-$) blastocysts can be grown indefinitely in culture when either GSH or *N*-acetylcysteine (NAC) is added to the medium. Considering that cellular GSH levels in *Gclc*($-/-$) blastocysts maintained in NAC are below detectable limits, it is possible that GSH is dispensable for cell growth in culture or that a minimal amount of GSH can be generated by scavenging pathways in the γ -glutamyl cycle to support cell growth. Embryonic lethality resulting from the *Gclc* deletion greatly limits the utility of these mouse models to study the effects of GSH depletion *in vivo*. This limitation can be avoided through use of a conditional *Gclc* floxed mouse line (Chen et al. 2007) (*vide infra*).

Two mouse lines lacking *Gclm* have been independently generated. In contrast to the lethality resulting from *Gclc* deletion, *Gclm*-deleted mice show no overt phenotype despite having very low tissue GSH levels. For the remainder of this review, the lines will be empirically referred to, according to their laboratory of creation as *Gclm*($-/-$) mice (Yang et al. 2002) and GCLM-null mice (McConnachie et al. 2007).

Gclm($-/-$) (KO) mice exhibit GSH levels in liver, kidney, pancreas, erythrocytes, and plasma that are respectively 15, 14, 11, 9 and 16% of control (wild-type, WT) mice (Yang et al. 2002). Whereas GSH was diminished in these tissues, cysteine levels were found to be

decreased only in kidney and pancreas (by 91% and 65%, respectively), *i.e.* tissues with high GGT levels. Mouse fetal fibroblasts (MFFs) derived from KO fetuses are extremely sensitive to oxidant-induced cell death and exhibit premature cellular senescence (Chen et al. 2009). In liver of KO mice, low (15% control) levels of GSH result in a decreased GSH redox potential (E_{GSH}) (Table 1). Mitochondrial GSH is maintained at 40% of WT levels, and accompanied by increased H_2O_2 release. Nevertheless, mitochondrial function of KO (as reflected by respiration control ratio and hepatic ATP) was not different from that of WT mice. The generally good health of *Gclm*($-/-$) mice makes them a valuable model for studying the impact of chronic GSH deficiency. Indeed, several studies have used *Gclm*-deleted mouse models to elucidate the role of GSH in hepatic responses to numerous insults (Table 2) (& *vide infra*).

III. GSH deficiency in non-alcoholic fatty liver disease (NAFLD)

3.1 Spontaneous steatohepatitis occurring in hepatocyte-specific GSH-depleted mice

The specific role of hepatic GSH *in vivo* has been evaluated in hepatocyte-specific *Gclc*(*h/h*) knockout mice (Chen et al. 2007). Loss of the hepatocyte GCLC subunit through targeted deletion of *Gclc* results in progressive depletion of total hepatic GSH from 90% at post-natal day 21 (PND21) to 95% at PND28, relative to the *Gclc*(*f/f*) floxed control (Chen et al. 2007). The extent of this reduction should not be surprising—given that hepatocytes account for $\approx 85\%$ of the total cell volume in liver. Coincident with severe depletion of hepatic GSH, livers from *Gclc*(*h/h*) mice display massive steatosis, hepatocyte death and inflammatory infiltration (Chen et al. 2007). Ultrastructural examination of *Gclc*(*h/h*) hepatocytes reveals aberrant mitochondria, manifesting as vesicular and ballooning morphologies; these alterations are accompanied by dramatic decreases in mitochondrial GSH pool [to $\approx 15\%$ of *Gclc*(*f/f*) mice], *in vitro* mitochondrial function (reflected in respiration control ratio), and cellular ATP production (Chen et al. 2007).

By PND30, without any intervention, all *Gclc*(*h/h*) mice die of acute liver failure. However, supplementation of NAC (1 g/L in drinking water), an antioxidant that increases tissue GSH levels by supplying cysteine for GSH synthesis (Sjodin et al. 1989), is able to rescue *Gclc*(*h/h*) mice into adulthood (Chen et al. 2010). Whereas total hepatic GSH remains depleted by $>90\%$, NAC treatment restores the mitochondrial GSH pool ($>50\%$) in *Gclc*(*h/h*) mice and partially preserves mitochondrial morphology and function. However, abnormal liver biochemical tests and hepatic oxidative stress persist in these rescued mice. Under histological examination, livers from NAC-rescued *Gclc*(*h/h*) mice show persistent steatohepatitis, which progresses to hepatic fibrosis and cirrhosis by PND120 (Chen et al. 2010). The partial compensation for GSH deficiency afforded by NAC in *Gclc*(*h/h*) hepatocytes is likely mediated by: (i) GSH synthesis by the salvage pathway, in which GGT utilizes cystine to produce γ -glutamylcystine thereby bypassing the action of GCL (Njalsson and Norgren 2005); (ii) enhanced active transport of cytoplasmic GSH into mitochondrial pool by SLC25A10 and SLC25A11 transporters (Chen et al. 2010); and (iii) compensation from actions of other antioxidant enzymes, such as glutathione peroxidase 4 (GPX4) which does not rely on GSH as the sole reducing power but also readily uses cysteine for enzyme catalysis (Toppo et al. 2009; Yoo et al. 2012).

3.2 High-fat-diet-induced metabolic disorder

Dietary intake of high fat is associated with increased risk of metabolic diseases—such as obesity, fatty liver disease and diabetes mellitus. The mechanism by which this occurs is thought to involve oxidative stress (Matsuzawa-Nagata et al. 2008). The role of GSH in high-fat-diet (HFD)-induced liver damage has been evaluated using *Gclm* KO mice (Table 2) (Kendig et al. 2011).

KO and WT mice received for 11 weeks a normal diet (ND) or a HFD that contains 6-fold higher energy from fat. When compared to HFD-fed WT mice, HFD-fed KO mice do not gain excessive body weight nor do they accumulate more storage fat. This lean KO phenotype is associated with a higher basal metabolic rate, a result of enhanced activity of liver mitochondrial respiration complex I. Despite having an elevated systemic and hepatic oxidative stress response, KO mice are protected from HFD-induced glucose intolerance, insulin resistance and steatosis, all of which are observed in HFD-fed WT mice. The observed protection against HFD-induced insulin resistance in KO mice is in agreement with another study reporting increased insulin sensitivity in mice lacking the glutathione peroxidase 1 (GPX1), one of the key enzymes involved in eliminating physiological ROS (Loh et al. 2009). In this study, it was found that increased production of ROS enhanced insulin-induced phosphatidylinositol-3-kinase/Akt signaling and glucose uptake in muscle, providing evidence for a causal role of ROS in upregulating insulin signaling *in vivo* (Loh et al. 2009).

In addition, liver microarray analysis reveals dramatic suppression of genes involved in metabolism of cholesterol, sterol and fatty acids (Kendig et al. 2011), suggesting a possible causal link between dysregulated lipid metabolism and lower susceptibility to HFD-induced metabolic disorders.

3.3 Methionine- and choline-deficient-diet-induced steatohepatitis

Although feeding mice a high fat diet results in development of steatosis as the primary hepatic lesion, a high-sucrose diet deficient in methionine and choline (MCD) leads not only to steatosis but also to hepatocellular injury, inflammation, and fibrosis. These reflect pathological changes characteristic of human NAFLD (Vetelainen et al. 2007). It should be noted that, despite causing highly similar liver pathology, the MCD diet fails to reproduce other systemic metabolic disorders, such as weight gain and insulin resistance, that are commonly seen in patients with NAFLD. The MCD feeding regimen has been used as an experimental model to study the pathogenesis of non-alcoholic steatohepatitis (NASH) (Rinella et al. 2008). The *GCLM*-null mouse line has been tested for development of MCD diet-induced steatohepatitis (Table 2) (Haque et al. 2010). Similar to changes seen in *Gclm*($-/-$) and HFD-fed mice, *GCLM*-null mice were, in general, protected from MCD-induced hepatic damage. This was accompanied by inhibition of hepatic lipogenesis, low levels of oxidative stress, and enhanced metabolic capacity and antioxidant activity.

IV. GSH deficiency in environmental toxicant- and drug-induced liver injury

4.1 TCDD-mediated liver toxicity

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a by-product of various industrial processes and is a persistent environmental contaminant. Diseases associated with exposure to TCDD include metabolic disorders, cancer, cardiovascular disease and birth defects (Nebert et al. 1993). TCDD is hepatotoxic, causing hepatocellular injury and steatosis. TCDD activates the heterodimeric transcription factor aromatic hydrocarbon receptor (AHR), leading to activation of downstream pathways that promote oxidative stress (Shen et al. 2005; Shertzer et al. 2004).

The role of GSH in TCDD-induced hepatotoxicity has been explored using *Gclm*($-/-$) KO mice (Chen et al. 2012) (Table 2). TCDD treatment (15 μ g/kg/day i.p. $\times$ 3 consecutive days) leads to decreased GSH redox potential, elevated plasma liver enzymes, and diminished hepatic ATP production in KO mice relative to WT mice. These results are consistent with KO mice being more vulnerable to TCDD-induced liver toxicity. Consistent with HFD and MCD treatments, TCDD-induced fatty liver was absent from KO mice.

4.2 Acetaminophen-induced liver injury

Acetaminophen (APAP), one of the best-selling analgesics in the US, is also one of the leading drug-associated causes of severe acute liver injury. At therapeutic doses, APAP is readily detoxified by conjugation with GSH. However, at toxic doses, APAP induces acute hepatotoxicity through multifactorial mechanisms including, among others, depletion of GSH, increased oxidative/nitrative stress, activation of the c-Jun N-terminal kinase (JNK)-associated cell death signaling pathway and mitochondrial dysfunction (Jaeschke et al. 2011). A recent study further demonstrates a new mechanism by which nitration of mitochondrial proteins and consequent suppression of their activities promotes APAP-induced mitochondrial dysfunction and hepatotoxicity (Abdelmegeed et al. 2013). Studies in a variety of species including humans indicate that toxic effects of APAP are intimately associated with depletion of hepatocyte GSH (Ferret et al. 2001; Villeneuve and Pichette 2004). Supporting this notion is the observation that APAP (500 mg/kg, i.p.) induces more histologically-evident liver injury in *GCLM*-null mice than in WT mice (Table 2) (McConnachie et al. 2007). Notably, this effect was gender-specific in that the increased toxicity was evident only in female *GCLM*-null mice.

V. GSH deficiency in alcohol-associated liver damage

Alcoholic liver disease (ALD) is a major cause of chronic liver disease worldwide. Alcohol abuse leads to early liver pathology manifesting as simple steatosis, which can progress to steatohepatitis, fibrosis and cirrhosis. Oxidative stress appears to play a central role in many pathways involving initiation and progression of ALD. Ethanol metabolism, CYP2E1 induction, compromised antioxidant defenses, mitochondrial injury, activation of Kupffer and stellate cells, hypoxia, and iron overload have all been implicated as contributing to the alcohol-induced oxidative environment (Albano 2008). The role of GSH in ethanol metabolism and alcohol-induced hepatotoxicity has been examined using the *Gclm*($-/-$) KO mouse model (Table 2) (Chen & Vasiliou et al, manuscript in preparation). Following single acute administration of ethanol (5 g/kg i.p.), total blood levels of ethanol and acetaldehyde are respectively 30% and 50% less in KO mice. When ethanol is administered chronically (2–6% v/v in a modified Lieber-DeCarli liquid diet for six weeks), KO mice are less susceptible to ethanol-induced body weight loss and liver weight gain, and are resistant to ethanol-induced steatosis. As expected, higher levels of oxidative stress are evident in KO livers. Quite unexpectedly, KO mice exhibit differential beneficial changes in the profiles of lipid composition, expression of genes involved in ethanol and lipid metabolism, and induction of antioxidant genes in the liver (Chen & Vasiliou et al, manuscript in preparation).

VI. Concluding remarks

Oxidative stress is considered to be a critical contributor to liver injury caused by various etiologies. Although GSH depletion has been associated with numerous liver diseases, the precise mechanism(s) by which such depletion promotes pathophysiological changes in liver have not been clearly defined. The transgenic rodent models of GSH deficiency, described herein, represent valuable experimental tools for delineating the role of GSH in liver disease. From relatively few studies that have been conducted to date in these models, some overall observations might be made and are summarized in Fig. 2.

Studies in hepatocyte-specific *Gclc* KO mice indicate that: (i) minimal levels of hepatic GSH (5% normal) are required for normal liver function, primarily by preserving mitochondrial functional integrity; (ii) mitochondria are the most affected hepatocyte organelle by GSH deficiency; the extent of disruption of mitochondrial GSH homeostasis appears to determine severity and progression of liver pathologies. On the other hand, in

Gclm($-/-$) KO mice in which the hepatic GSH deficit is chronic and less severe (15% normal) and, most importantly, mitochondrial function remains intact, metabolic and stress response adaptations are activated that serve to protect these animals from developing hepatic steatosis. By contrast, GSH plays a pivotal role in conferring protection against APAP- and TCDD-mediated hepatocellular toxicity.

Taken together, these studies show a differential hepatic response to GSH depletion—depending on the extent of depletion and nature of the insult applied to liver. It is conceivable that the hepatic GSH pool functions to fine-tune metabolic and stress responses to liver injuries. Through redox regulation of protein functions (Dalle-Donne et al. 2007; Janssen-Heininger et al. 2008; O'Brian and Chu 2005), GSH may also function as a vital modulator of key proteins involved in hepatocellular responses to toxic compounds. Whatever the precise mechanism, it is evident that GSH depletion is not always associated with negative cellular consequences in liver. Clearly, much remains to be learned about this remarkable tripeptide. The transgenic models described herein have provided some tantalizing and unexpected new insights regarding GSH in liver. We anticipate further studies will provide additional exciting mechanistic information that can be exploited clinically to prevent or treat liver diseases.

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Highlights

GSH-deficient mouse models have been developed by targeting GCL, the rate-limiting enzyme of GSH biosynthesis

Liver-specific *Gclc* knockout mice have 95% depletion of hepatic GSH and develop spontaneous steatohepatitis

Universal *Gclm* deleted mice have 15% of normal hepatic GSH and are protected from liver injuries induced by hepatic insults

Animal studies indicate a potential role of hepatic GSH in fine-tuning metabolic and stress responses to liver injuries

Figure 1. Pathways for glutathione (GSH) biosynthesis, transport and metabolism

Details of these pathways are discussed in the text. Cys, *L*-cysteine; Glu, *L*-glutamate; Gly, *L*-glycine; DP, dipeptidase; GCL, glutamate-cysteine ligase; GGT, γ -glutamyltransferase; GSS, glutathione synthase; GSH, glutathione; GSSG, glutathione disulfide; ER, endoplasmic reticulum; MT, mitochondrion; NU, nucleus. *Pink boxes* represent catalytic enzymes; *grey boxes* represent transporters.

Figure 2. Schematic summary of observations from GSH-deficient mouse models

Studies in hepatocyte-specific *Gclc(h/h)* mice indicate that: (i) minimal levels of hepatic GSH (5% normal) are required for normal liver function, primarily by preserving mitochondrial functional integrity; and (ii) partial restoration of mitochondrial function by *N*-acetylcysteine (NAC) signals the induction of hepatic regeneration. In *Gclm*($-/-$) KO mice in which the hepatic GSH deficit is less severe (15% normal) and chronic, mitochondrial function remains intact; metabolic and stress response adaptations protect these animals from steatosis and hepatic injuries induced by a variety of insults. Collectively, these studies show a differential hepatic response to GSH depletion, depending on the extent of depletion and nature of the insults. Molecular mechanism(s) underlying such GSH-mediated effects remain(s) to be elucidated.

Table 1

Biochemical measurements in *Gclm*($-/-$) (KO) and wild-type (WT) liver

Biochemical parameters	WT	KO
GSH (mM)	7.4 $\pm$ 0.5	1.1 $\pm$ 0.2 *
E _{GSH} (mV)	-235 $\pm$ 5	-159 $\pm$ 12 *
Mitochondrial GSH (nmol/mg)	7.7 $\pm$ 0.6	3.1 $\pm$ 0.3 *
Mitochondrial H ₂ O ₂ released (pmol/min * mg)	195 $\pm$ 10	370 $\pm$ 25 *
Respiration control ratio	3.9 $\pm$ 0.1	4.4 $\pm$ 0.4
ATP (μ mol/g)	5.7 $\pm$ 0.4	5.6 $\pm$ 0.7

Data are reported as means $\pm$ SEM of 4–5 mice.

* $p < 0.05$, when compared with WT mice. (Adapted from (Kendig et al., 2011)).

Table 2Hepatic response to challenges in *Gclm*-deleted mice

Challenge	Phenotype	Ref
High-fat diet (11 weeks)	Increased oxidative stress Increased basal metabolic rate Protection against metabolic disorder and steatosis Suppression of lipid metabolism genes	(Kendig et al., 2011)
Methionine- and choline-deficient diet (3 weeks)	Decreased oxidative stress Attenuated hepatic damage (steatohepatitis) Suppression of lipogenesis genes Increased metabolic capacity and antioxidant activity	(Rinella et al., 2008)
TCDD (i.p. $3 \times 15 \mu\text{g/kg/day}$)	Increased oxidative stress Enhanced hepatocellular damage Protection against steatosis	(Chen et al., 2012)
APAP (i.p. 500 mg/kg)	Increased oxidative stress Enhanced hepatocellular damage Gender difference	(McConnachie et al., 2007)
Lieber-DeCarli ethanol liquid diet (2–6% v/v, 6 weeks)	Increased oxidative stress Increased ethanol and acetaldehyde metabolism Protection against hepatotoxicity and steatosis Differential change in lipid profile, lipid metabolism genes, and activation of antioxidant genes	(Chen et al., manuscript in preparation)