Estrogen Sulfotransferase Is an Oxidative Stress-responsive Gene That Gender-specifically Affects Liver Ischemia/Reperfusion Injury*

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Background: EST regulates estrogen homeostasis by sulfonating and deactivating estrogens. Liver ischemia and reperfusion (I/R) involves both hypoxia during the ischemic phase and oxidative damage during the reperfusion phase. In this report, we showed that the expression of EST was markedly induced by I/R. Mechanistically, oxidative stress-induced activation of Nrf2 was responsible for the EST induction, which was abolished in Nrf2−/− mice. EST is a direct transcriptional target of Nrf2. In female mice, the I/R-responsive induction of EST compromised estrogen activity. EST ablation attenuated I/R injury as a result of decreased estrogen deprivation, whereas this benefit was abolished upon ovariectomy. The effect of EST ablation was sex-specific because the EST−/− males showed heightened I/R injury. Reciprocally, both estrogens and EST regulate the expression and activity of Nrf2. Estrogen deprivation by ovariectomy abolished the I/R-responsive Nrf2 accumulation, whereas the compromised estrogen deprivation in EST−/− mice was associated with increased Nrf2 accumulation. Our results suggested a novel I/R-responsive feedback mechanism to limit the activity of Nrf2 in which Nrf2 induces the expression of EST, which subsequently increases estrogen deactivation and limits the estrogen-responsive activation of Nrf2. Inhibition of EST, at least in females, may represent an effective approach to manage hepatic I/R injury.

Results: Liver ischemia and reperfusion (I/R) induced the expression of EST and comprised estrogen activity in an Nrf2-dependent manner. EST ablation gender-specifically affected I/R sensitivity.

Conclusion: EST is an oxidative stress responsive gene that affects liver I/R injury.

Significance: Inhibition of EST, at least in females, represents an effective approach to manage hepatic I/R injury.

Estrogen sulfotransferase (EST) regulates estrogen homeostasis by sulfonating and deactivating estrogens. Liver ischemia and reperfusion (I/R) involves both hypoxia during the ischemic phase and oxidative damage during the reperfusion phase. In this report, we showed that the expression of EST was markedly induced by I/R. Mechanistically, oxidative stress-induced activation of Nrf2 was responsible for the EST induction, which was abolished in Nrf2−/− mice. EST is a direct transcriptional target of Nrf2. In female mice, the I/R-responsive induction of EST compromised estrogen activity. EST ablation attenuated I/R injury as a result of decreased estrogen deprivation, whereas this benefit was abolished upon ovariectomy. The effect of EST ablation was sex-specific because the EST−/− males showed heightened I/R injury. Reciprocally, both estrogens and EST regulate the expression and activity of Nrf2. Estrogen deprivation by ovariectomy abolished the I/R-responsive Nrf2 accumulation, whereas the compromised estrogen deprivation in EST−/− mice was associated with increased Nrf2 accumulation. Our results suggested a novel I/R-responsive feedback mechanism to limit the activity of Nrf2 in which Nrf2 induces the expression of EST, which subsequently increases estrogen deactivation and limits the estrogen-responsive activation of Nrf2. Inhibition of EST, at least in females, may represent an effective approach to manage hepatic I/R injury.

Hepatic ischemia/reperfusion (I/R) injury occurs in various clinical situations when the blood flow to the liver is blocked or the liver is in a low-flow state, such as during liver resection, solid organ transplantation, cardiac and vascular surgery, massive trauma, hemorrhagic shock, and cardiogenic shock. I/R injury can cause a series of clinical manifestations ranging from asymptomatic elevation of liver enzymes to acute liver failure or even death (1).

The pathogenesis of hepatic I/R injury is a dynamic process, including the deprivation of blood and oxygen supply, followed by their restoration. The pathological events associated with I/R include direct hypoxic damage as the result of ischemia as well as a delayed and more severe oxidative damage that eventually leads to the activation of inflammatory pathways (2). During the hypoxic phase, sublethal cellular damage leads to the release of reactive oxygen species and damage-associated molecular pattern molecules from macrophages and hepatocytes. Reperfusion augments the injury by triggering sterile inflammatory responses, causing irreversible liver damage (3).

Meanwhile, a series of protective pathways are also activated, and, as such, the extent of organ damage is determined by the balance between these two systems. During I/R, there are two transcriptional factors, hypoxia inducible factor 1 (HIF-1) and nuclear factor erythroid 2-related factor 2 (Nrf2), that play important roles in protecting the liver from I/R injury. Stabilization and accumulation of HIF-1 or Nrf2 lead to the activation of an array of genes to adapt the cells to hypoxic or oxidative damages and, therefore, affect numerous cellular functions, such as cell apoptosis, proliferation, survival, metabolism, and angiogenesis (2, 4).

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The abbreviations used are: I/R, ischemia/reperfusion; SULT, sulfotransferase; EST, estrogen sulfotransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; H/R, hypoxia and reoxygenation; NACA, N-acetylcysteine amide; ARE, antioxidant response element.
Both clinical and animal studies have shown a sexual dimorphic response of the liver to various stresses, including liver I/R, hemorrhagic shock-resuscitation, liver cirrhosis, and endotoxemia. Many studies have suggested that female livers were more tolerant than male livers under stress conditions, suggesting estrogen as a responsible factor for this sexual dimorphism (5–8). The proposed mechanisms for the protective effect of estrogens on liver I/R include inhibition of apoptosis (9), an increased serum level of nitric oxide, a decreased serum level of tumor necrosis factor α (10), regulation of heat shock protein expression (11), and selective modulation of MAPK kinase activities (12). However, most of these experiments were performed by challenging male mice or ovariectomized female animals with pharmacological doses of estrogens, and the dynamics of the endogenous estrogen metabolism during I/R remains unknown. Considering the potential side effects associated with pharmacological estrogen therapies (13), it is necessary to develop novel therapeutic strategies to attenuate hepatic I/R injury through the regulation of endogenous estrogen metabolism.

An important metabolic pathway to deactivate estrogens is sulfotransferase (SULT)-mediated sulfation. Sulfonated estrogens cannot bind to and activate estrogen receptors, therefore losing their hormonal activities (14). Estrogen sulfotransferase (EST or SULT1E1) is the major SULT isoform responsible for estrogen sulfation because of its high affinity for estrogens (15). EST is known to express in multiple human tissues, and decreased EST expression may have led to increased availability of estrogens and contributed to the pathogenesis of hormone-dependent breast cancers (16). The basal expression of EST in the liver is low (17), but its expression is highly inducible in response to ligands for nuclear receptors, such as the liver X receptor (17), the glucocorticoid receptor (18), and the constitutive androstane receptor (19, 20). A major induction of EST has also been reported in the liver of mouse models of obesity and type 2 diabetes, and EST ablation improved the energy metabolism in female mice by increasing estrogenic activity in the liver (21).

Here we report that hepatic I/R injury induced the expression of EST, which was accounted for by the oxidative stress-responsive activation of Nrf2 and subsequent transactivation of EST, a novel Nrf2 target gene. Reciprocally, the Nrf2-responsive induction of EST facilitated the deactivation of estrogen, which is a positive regulator of Nrf2. Our results have uncovered a novel I/R-responsive and EST/estrogen-mediated feedback mechanism to prevent the sustained overactivation of Nrf2.

### Experimental Procedures

#### Chemicals

The HIF-1 inhibitor was purchased from EMD Millipore Chemicals (San Diego, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

#### Animals

EST−/− mice have been described previously (22). Nrf2−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were in the C57BL/6 background. The use of mice in this study complied with the relevant federal guidelines and institutional policies.

### Liver Ischemia and Reperfusion

Liver warm ischemia was performed by placing a microvascular clamp across the portal vein, hepatic artery, and bile duct just above the branching to the right lateral lobe to occlude the blood supply to the left lateral and median lobes (~70% ischemia of the whole liver). After 60 min of partial hepatic ischemia, the clamp was removed to initiate hepatic reperfusion. Sham control mice underwent the same protocol without vascular occlusion. Ischemic liver samples and blood were collected for analysis.

#### Primary Hepatocyte Hypoxia and Reoxygenation

Primary hepatocytes were isolated from 6- to 8-week-old female C57BL/6 mice and seeded at a density of 4 × 10^5 cells/well in 6-well plates. The initial culture medium was Williams medium E containing 10% FBS. Hepatocytes were allowed to attach to plates for 2 h and then changed to RPMI 1640 medium containing 10% FBS and incubated overnight. To initiate hypoxia, the medium was replaced with hypoxic medium (equilibrated with 1% O₂, 5% CO₂, and 94% N₂) and placed into a modular incubator chamber (Billups-Rothenburg) that was flushed with the same hypoxic gas mixture. After hypoxia, plates were returned to the normal incubator to start the reoxygenation.

#### Histology and Immunohistochemistry

Liver paraffin sections were stained with hematoxylin-eosin. Necrosis was estimated by dividing the necrotic area by the entire histological section using ImageJ Software. For EST immunostaining, antigen retrieval was performed by conventional steam heating for 10 min in 0.01 M sodium citrate retrieval buffer. The sections were incubated with 0.3% H₂O₂ for 30 min to quench endogenous peroxidase activity. Then they were incubated with blocking serum for 10 min, followed by incubation with the primary monoclonal rabbit antibody against EST/SULT1E1 (1:100) from Proteintech (Chicago, IL) overnight at 4°C. The staining was detected with the Vectastain ABC kit from Vector (Burlingame, CA). The slides were counterstained with hematoxylin.

#### Serum Aminotransferase Quantification

Blood samples were collected by cardiac puncture. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using assay kits from Stanbio (Boerne, TX).

#### Estrogen Sulfotransferase Activity Assay

Liver cytosols were prepared by homogenization, and the cytosols were then used for sulfotransferase assay by using [35S]phosphoadenosine phosphosulfate from PerkinElmer Life Sciences (Waltham, MA) as the sulfate donor, as described previously (17).

#### Northern Blotting, Real-time PCR, and Western Blotting

Total RNA was extracted from tissues using TRIzol reagent. Northern hybridization and real-time PCR were carried out as described previously (18). For Western blotting, 30 μg of total proteins for each sample were separated on 10% SDS-polyacrylamide gel. The primary antibody was a polyclonal rabbit antibody against SULT1E1 (1:1000) from Proteintech or Nrf2 (1:250) from Santa Cruz Biotechnology (Dallas, TX).

#### Cell Culture, Plasmid Constructs, and Reporter Gene Assay

HepG2 cells were maintained in DMEM supplemented with 10% FBS. The 2.5- and 3.0-kb 5’-regulatory sequences of the mouse EST gene were cloned by PCR. HepG2 cells were transiently transfected with the reporter constructs and the Nrf2 and MafG expression vectors in 48-well plates and measured for luciferase and β-gal activities 24 h after transfection. Trans-
Ablation of EST in female mice—ATTENUATED I/R LIVER INJURY IN FEMALE MICE—known to deactivate estrogens. Consistent with their induction compared with the sham control.

Portion of the central veins but a reduced level in the portal region (Fig. 1).

Expression pattern, with the highest expression in hepatocytes that I/R-induced EST expression exhibited a partially zonal consistent with each other. Immunohistochemistry showed fold inductions at the mRNA and protein levels were not always compared with the sham control.

Results

Liver I/R Induced the Expression of EST—EST is known to have a low basal expression in the liver (18). Interestingly, we found a dramatic induction of hepatic EST mRNA expression upon I/R in both male and female mice in a time-dependent manner, as shown by real-time PCR (Fig. 1A) and Northern blot analysis (Fig. 1B). The induction of EST was also confirmed at the protein level by Western blotting (Fig. 1C), although the fold inductions at the mRNA and protein levels were not always consistent with each other. Immunohistochemistry showed that I/R-induced EST expression exhibited a partially zonal expression pattern, with the highest expression in hepatocytes surrounding the central veins but a reduced level in the periportal region (Fig. 1D).

I/R Compromised EST Activity by Inducing EST, and EST Ablation Attenuated I/R Liver Injury in Female Mice—EST is known to deactivate estrogens. Consistent with their induction of EST, female mice that were subjected to I/R showed increased estrogen sulfotransferase activity (Fig. 2A, left panel) and a higher level of estrone sulfate (Fig. 2A, right panel) in their livers. Moreover, the expression of hepatic estrogen-responsive genes Igfbp4 and Sulf2 was decreased in female mice upon I/R, presumably because of increased estrogen deactivation. The I/R-responsive suppression of estrogen-responsive genes was EST-dependent because it was abolished in EST−/− mice (Fig. 2B).

To determine the functional relevance of EST and its regulation in I/R injury, we subjected WT and EST−/− mice to liver I/R. Female EST−/− mice were significantly protected from I/R injury compared with their WT counterparts after 6- or 12-h reperfusion, as evidenced by their decreased necrotic areas (Fig. 2C, top panel) and lower serum levels of ALT and AST (Fig. 2C, bottom panel). The protective effect of EST ablation was estrogen-dependent because ovariectomy abolished the differences in liver injury between WT and EST−/− mice (Fig. 2D). Ovariectomy seemed to weaken the ALT increase after I/R, the mechanism of which remains to be understood. Nevertheless, these results suggested that the I/R responsive EST induction may have led to the deactivation of estrogens and, therefore, contributed to the pathogenesis of I/R injury.

To our surprise, the effect of EST ablation on I/R injury was gender-specific because male EST−/− mice were more sensitive to I/R injury than WT males, which was confirmed by histology (Fig. 2E, top panel) and measurements of serum ALT and AST levels (Fig. 2E, bottom panels). The differences in the ALT and AST levels between WT and EST−/− males were abolished upon castration (Fig. 2F).

![Figure 1](image1.png)
FIGURE 2. I/R compromised estrogen activity by inducing EST, and EST ablation attenuated I/R liver injury in female mice. A, female WT mice were subjected to 60-min/12-h liver I/R. Left panel, liver cytosolic extracts were measured for estrogen sulfotransferase activity using estrone as the substrate. Right panel, the liver tissue level of estrone sulfate. B, female WT and EST⁻/⁻ mice were subjected to I/R. The hepatic expression of estrogen-responsive genes was measured by real-time PCR. C, mice were the same as in B. The I/R injuries were measured by histology (top panel, with the necrotic areas (N) circled) with the quantification of the necrotic area (center panel), as well as by serum ALT and AST levels. D, female mice were subjected to ovariectomy (OVX) at 4 weeks of age before being subjected to I/R at 9 weeks of age. Shown are the serum ALT and AST levels. E, experiments were the same as in C except that male WT and EST⁻/⁻ mice were used. F, male mice were subjected to castration (CST) at 4 weeks of age before being subjected to I/R at 9 weeks of age. Shown are the serum ALT and AST levels. n = 5–6; *, p < 0.05; **, p < 0.01; NS, statistically not significant.
We next wanted to determine the mechanism by which I/R induced EST gene expression. Liver I/R is characterized by initial hypoxic tissue damage, followed by the return of blood flow and oxygen delivery, which accelerates the damage. To determine which stage of I/R is important for EST induction, we exposed primary mouse hepatocytes isolated from female mice to hypoxia and reoxygenation (H/R) to simulate the microenvironment of I/R. We found that 1–12 h of hypoxia alone was not sufficient to induce EST gene expression. In contrast, hypoxia followed by 12-h reoxygenation significantly induced EST expression (Fig. 3A, top panel). These results suggested that it was the reperfusion/oxidative phase, but not the ischemia/hypoxic phase, that played a dominating role in inducing EST. The expression of EST was also induced in primary human hepatocytes isolated from a female patient fol-

**I/R-responsive Induction of EST Was Independent of HIF-1 but Nrf2-dependent**—We next wanted to determine the mechanism by which I/R induced EST gene expression. Liver I/R is characterized by initial hypoxic tissue damage, followed by the return of blood flow and oxygen delivery, which accelerates the damage. To determine which stage of I/R is important for EST induction, we exposed primary mouse hepatocytes isolated from female mice to hypoxia and reoxygenation (H/R) to simulate the microenvironment of I/R. We found that 1–12 h of hypoxia alone was not sufficient to induce EST gene expression. In contrast, hypoxia followed by 12-h reoxygenation significantly induced EST expression (Fig. 3A, top panel). These results suggested that it was the reperfusion/oxidative phase, but not the ischemia/hypoxic phase, that played a dominating role in inducing EST. The expression of EST was also induced in primary human hepatocytes isolated from a female patient fol-

**FIGURE 3.** I/R-responsive induction of EST was independent of HIF-1, whereas oxidative stress was sufficient to induce EST. A, primary mouse hepatocytes were exposed to normoxia or hypoxia (H), followed by a different duration of reoxygenation (R). The mRNA expression of EST (top panel) and VEGF (bottom panel) and the protein expression of EST (top panel, inset) were measured by real-time PCR analysis and Western blotting, respectively. *, p < 0.05; **, p < 0.01; all compared with the normoxia group. B, primary mouse hepatocytes were pretreated with increasing doses of HIF-1 inhibitor before undergoing H/R. The mRNA expression of VEGF and EST (top panel) and the protein expression of EST (bottom panel) were measured by real-time PCR and Western blotting, respectively. **, p < 0.01; NS, statistically not significant; comparisons are labeled. The quantifications of the Western blotting bands are labeled. Veh, vehicle. C, primary mouse hepatocytes were treated with the indicated concentrations of H2O2 for the indicated amounts of time. The mRNA and protein expression of EST was measured by real-time PCR and Western blotting, respectively. *, p < 0.05; **, p < 0.01; all compared with the vehicle group. D, primary mouse hepatocytes were pretreated with increasing doses of NACA before undergoing H6/R12. The mRNA (top panel) and protein (bottom panel) expression of EST was measured by real-time PCR and Western blotting, respectively. **, p < 0.01 compared with the normoxia group. The quantifications of the Western blotting are labeled. E and F, female mice were pretreated with NACA (200 mg/kg) 1 h before undergoing 60-min/12-h I/R. Shown are the EST mRNA and protein expression (E) and serum ALT level (F). n = 3–5; *, p < 0.05; **, p < 0.01.
following 6-h hypoxia and 12-h reoxygenation (data not shown), suggesting that the effect is conserved in human hepatocytes. It is known that hypoxia increases the HIF-1 protein level by stabilizing this protein, whereas the HIF-1 protein is rapidly degraded when the oxygen is present (4). VEGF is a typical HIF-1 target gene (4). Consistent with these notions, in the same hepatocyte cultures, we found that hypoxia alone induced the expression of VEGF, whereas the expression of VEGF decreased upon reoxygenation (Fig. 3A, bottom panel).

In vivo, the hepatic expression of VEGF in WT mice subjected to I/R was not induced following 6 h of reperfusion (data not shown), consistent with the hepatocyte results. Moreover, treatment of hepatocytes with HIF-1 inhibitor abolished the hypoxia-responsive induction of VEGF mRNA (Fig. 3B, top panel) but had little effect on the H/R-responsive induction of EST at the mRNA (Fig. 3B, top panel) and protein (Fig. 3B, bottom panel) levels. The results suggested that the H/R-responsive induction of EST was independent of HIF-1 activation.

To directly test whether oxidative stress played a role in EST induction, we treated mouse primary hepatocytes isolated from female mice with a typical oxidant, H$_2$O$_2$, and found a time-dependent EST induction at the mRNA and protein levels (Fig. 3C). In contrast, pretreatment of hepatocytes with N-acetylcysteine amide (NACA), a membrane-penetrating antioxidant (23), abolished the H/R-responsive induction of EST mRNA (Fig. 3D, top panel) and protein (Fig. 3D, bottom panel) expres-
Nrf2 is a transcriptional target of Nrf2—

**EST Is a Transcriptional Target of Nrf2**—Nrf2 is a transcriptional factor that regulates gene expression by binding to the antioxidant response element (ARE) in its target gene promoters (26). In determining whether EST is a transcriptional target of Nrf2, we found two putative AREs, ARE1 and ARE2, in the −5.0 kb and −2.7 kb positions of the mouse EST gene promoter, respectively (Fig. 5A). The binding of these two AREs to Nrf2 was evaluated by EMSA. Nrf2 can bind to DNA as homodimers or as heterodimers with its partner protein MafG (27). As shown in Fig. 5B, Nrf2 alone bound to both ARE1 and ARE2, and the bindings were enhanced when the MafG protein was added to the binding reactions. The binding was specific because efficient competition was achieved by adding an excess unlabeled wild-type competitor, whereas the unlabeled mutant competitor failed to compete (Fig. 5B). A ChIP assay was used to determine whether Nrf2 could be recruited onto the EST gene promoter. As shown in Fig. 5C, treatment of primary hepatocytes with H2O2 increased the recruitment of Nrf2 to a known ARE in the Nqo1 gene promoter. The recruitment of Nrf2 to the EST gene promoter was also increased in vitro synthesized Nrf2 and MafG proteins. In contrast, the ARE-less 2.5-kb EST promoter that contained ARE2 was activated by the cotransfection of Nrf2 and MafG. In contrast, the ARE-less 2.5-kb promoter was not activated but modestly suppressed by Nrf2-MafG. These results suggest that the mouse EST gene is a transcriptional target of Nrf2.

**Nrf2 Ablation Protected Female Mice from I/R Liver Injury**—Having shown that Nrf2 is a positive regulator of EST and that EST plays a role in I/R-responsive liver injury, we went on to determine whether Nrf2 ablation can affect the I/R injury. We first showed that male Nrf2−/− mice had heightened liver I/R injury compared with their WT counterparts, as evidenced by increased liver necrosis (Fig. 6A) and serum levels of ALT and...
The sensitizing effect of Nrf2 ablation was associated with the loss of I/R-responsive induction of Nrf2 target genes (Fig. 6C), which was consistent with previous reports (25, 29). Surprisingly and unexpectedly, we found that female Nrf2−/− mice were protected from I/R injury compared with WT females, as confirmed by histology (Fig. 6D) and measurements of serum ALT and AST activities (Fig. 6E). I/R-responsive Nrf2 target gene expression was similarly abolished in female Nrf2−/− mice (Fig. 6F), suggesting that the reduced antioxidant gene expression might not account for this sexual dimorphic effect of Nrf2 ablation.

**Discussion**

Hepatic I/R injury contributes to morbidity and mortality in a wide range of clinical events, such as liver transplantation (24). In this study, we showed that the estrogen-deactivating enzyme EST was markedly induced in the mouse liver by I/R. Our results suggest that the up-regulation of EST in the liver may have played a pathogenic role in I/R injury because EST ablation in female mice attenuated I/R-responsive liver injury in an estrogen-dependent manner.

We were surprised to find that EST ablation had an opposite effect in males, because the male EST−/− mice showed heightened sensitivity to I/R injury. Previous reports suggested that estrogens protected male mice from I/R injury (10, 12). However, most of the reported protection was observed in male animals that were treated with pharmacological doses of estrogen.

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Our results suggest differential effects between the administration of pharmacological doses of estrogens and alteration in endogenous estrogen metabolism. The increased sensitivity to I/R in male EST/H11002/H11002 mice was abolished upon castration, suggesting that the sensitization was androgen-dependent. Indeed, androgens have been reported to play a role in liver I/R injury (31). The detailed mechanism for the sex-specific effect of EST ablation on I/R injury remains to be understood.

One of our interesting findings was the Nrf2-dependent but HIF-1 independent regulation of EST during I/R. HIF-1 and Nrf2 are activated during the ischemia and reperfusion stages, respectively. On the basis of the dynamics of EST regulation and the use of an HIF-1 inhibitor, our results suggest that the I/R-responsive induction of EST was HIF-1-independent. Instead, the activation of Nrf2 was found to be essential for EST induction because I/R failed to induce EST in Nrf2-/- mice. Nrf2 is a master regulator of the oxidative response (32). Most of the Nrf2 target genes encode antioxidant proteins and detoxifying enzymes (33). Consistent with the notion that Nrf2 is an oxidative responsive gene, we showed that treatment of primary hepatocytes with H2O2 induced EST, whereas the antioxidant NACA attenuated the H/R- and I/R-responsive induction of EST, further suggesting that Nrf2 activation triggered by oxidative stress is mechanistically involved in the I/R-responsive EST induction. We went on to show that EST is a direct transcriptional target of Nrf2 (Fig. 7). Equally intriguing is the biological significance of this feedback mechanism. Although Nrf2 has been reported to have various protective effects, activation of Nrf2 does not always lead to a favorable physiological outcome. It has been reported that enhanced Nrf2 activity worsened insulin resistance, impaired lipid accumulation in adipose tissue, and increased hepatic steatosis in leptin-deficient mice (35). In another report, oxidative stress-
induced Nrf2 activation contributed to the pathogenesis of alcoholic liver disease by up-regulating the hepatic expression of very low density lipoprotein receptor (36). In this work, female Nrf2−/− mice showed attenuated sensitivity to I/R liver injury, which was reasoned to be due to the loss of Nrf2-dependent EST induction and estrogen deprivation. Our results suggest a novel estrogen/EST-mediated mechanism to prevent the overactivation or sustained activation of Nrf2, which can be potentially harmful.

Interestingly, like EST ablation, the loss of the Nrf2 effect was also gender-specific because male Nrf2−/− mice showed heightened sensitivity to I/R liver injury, as shown by others (25, 29) and by us. The sensitization in male Nrf2−/− mice was believed to be due to the loss of I/R-responsive Nrf2 target gene expression (25, 29). I/R-responsive Nrf2 target gene expression was similarly abolished in female Nrf2−/− mice, suggesting that the reduced antioxidant gene expression might not account for the sexual dimorphic effect of Nrf2 ablation on I/R injury. Instead, Nrf2−/− female mice showed a loss of Nrf2-dependent EST induction. Our results suggest that, at least in female Nrf2−/− mice, the gain of the estrogenic benefit might have outweighed the harm of loss of the induction of antioxidant genes, leading to the net outcome of attenuated I/R injury.

In summary, our results suggest that the induction of hepatic EST, which was mediated by oxidative stress-induced Nrf2 activation, may have played a pathogenic role in I/R liver injury. As such, both EST ablation and Nrf2 ablation conferred protection in female mice. Pharmacological inhibition of EST, at least in females, might represent a novel therapeutic approach to manage hepatic I/R injury. It is encouraging that major progress has been made in the identification and characterization of chemical EST inhibitors (37).

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