# Nuclear Factor (Erythroid-Derived 2)-Like 2/Antioxidant Response Element Pathway in Liver Fibrosis

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**ABSTRACT |** The liver is the major site of first-pass metabolism and, accordingly, is highly exposed to oxidative injury caused by reactive intermediates, resulting in the stimulation of different biological targets. Diseases such as nonalcoholic steatohepatitis, which is characterized histologically by hepatic steatosis, necroinflammation, and progressive substitution of the functioning hepatic parenchyma by fibrotic tissue, are widely related to oxidative stress, although the mechanisms are not completely understood. A rational attempt to comprehend the pathways underlying redox-mediated fibrogenic signaling may be investigating the adaptive responses to oxidative stress by interacting with the antioxidant response. The expression of a variety of downstream targets aimed at cytoprotection, primarily mediated through antioxidant response elements, are largely under the control of nuclear factor E2-related factor 2 (Nrf2). In this study, the regulation of the cellular response to oxidative stress was determined in the presence of Nrf2 activators or Nrf2-null mice influencing lipid metabolism and targeted cytoprotection of hepatocytes during inflammation/fibrosis. These interactions participate in a multi-tiered, integrated reaction to chemical stress, in which Nrf2 signaling pathway can be considered as a key factor in orchestrating adaptive responses in liver disease.

**ABBREVIATIONS |** ARE, antioxidant response element; BDL, bile duct ligation; CDDO, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid; CDDO-Im, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole; CDAA, choline-deficient, l-amino acid-defined; ECM, extracellular matrix; GSH, reduced glutathione; GSK-3β, glycogen synthase kinase 3; HFD, high fatty diet; HSC, hepatic stellate cell; Keap1, kelch-like ECH-associated protein-1; MAF, musculo-aponeurotic fibrosarcoma; NQO1, NADPH:quinone oxidoreductase 1; NASH, nonalcoholic steatohepatitis; Nrf2, nuclear factor erythroid-2-related factor 2; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; α-SMA, alpha-smooth muscle actin; TGF-β, transforming growth factor-beta; TIMP-1, tissue inhibitor of metalloproteinase

## CONTENTS

## 1. INTRODUCTION

Oxidative stress has been implicated in the pathogenesis of chronic liver diseases [1–3]. Numerous recent studies have focused on steatosis, and increased concentrations of free fatty acids provide insight into the mechanism of perpetuation/propagation of oxidative stress involving lipid peroxidation [4]. In particular, reactive oxygen species (ROS) can enhance the peroxidation of fatty acids in mitochondrial membranes, where fatty acids are inadequately oxidized and undergo excessive esterification into triglycerides, accumulating in the liver [5, 6]. Furthermore, while ultrastructural mitochondrial irregularities due to impaired adenosine triphosphate (ATP) synthesis have been observed in steatohepatitis [7], we and others have shown that cells recruited in response to injury emit pro-inflammatory responses including cytokines, chemokines, and ROS promoting profibrotic signaling by differentiation of fibroblasts, epithelial, or hepatic stellate cells (HSCs) to myofibroblasts, which synthesize increased levels of extracellular matrix (ECM) [8–11]. However, the ensuing anti-inflammatory immune response in the liver protects against the damage actions using a sophisticated antioxidant defense system [12–14], in which nuclear factor (erythroid-derived 2)-like 2, also known as NF-E2 or Nrf2, plays a major role in defending against oxidative stress [15, 16]. Cytoprotective enzymes are encoded by genes containing antioxidant response elements (AREs), and Nrf2 has been recognized as an important regulator of antioxidants and phase II detoxification enzymes based on the transcriptional upregulation of ARE-containing genes [17, 18]. As such, Nrf2 signaling exerts essential functions in the cellular defense system by optimizing hepatoprotection [19] and is a target of new approaches for treating liver disease as part of the characteristic activation response. Overall, this area of Nrf2 biology surely requires further exploration; once Nrf2 deficiency is established, the capacity to combat oxidative insults of electrophilic compounds in liver fibrosis is reduced with impaired up-regulation of various antioxidant and detoxification enzyme genes [20, 21].

## 2. NRF2 PLAYS A ROLE IN MEDIATING ACTIVATION OF OXIDATIVE STRESS RESPONSE

Nrf2 was firstly identified in a screen for proteins of the β-globin gene, and elevated sequence homology was observed between Nrf2 and the p45 subunit of the previously recognized transcription factor nuclear factor erythroid 2 (p45-NF-E2) [22]. While p45-NF-E2 is expressed exclusively in megakaryocytes, erythroid cells, and mast cells, Nrf2 appears to be expressed nearly ubiquitously, although at different levels in various tissues [22]. The basic-region leucine-zipper combined with a small musculo-aponeurotic fibrosarcoma (Maf) protein forms a transactivation complex that binds to AREs [23]. Of particular interest is the transcriptional activation of several Nrf2 target genes including heme oxygenase-1 and those involved in drug metabolism, transport of xenobiotics, and various other stress responses (**Table 1**). By inducing the expression of this array of genes, Nrf2 can increase a variety of cell defense processes, enhancing the capacity of detoxification and protecting cells from damage.

Nrf2, characterized in 1999 by Itoh and co-workers [24], is principally localized in the cytoplasm with Kelch-like ECH associating protein 1 (Keap1) and the actin cytoskeleton, where polyubiquitination and degradation result in a short protein half-life [25]. Hepatocyte-specific Keap1-null mice exhibit elevated levels of expression of Nrf2-regulated genes in the liver, supporting that the activation and subsequent cytoprotective gene induction maintain the balance between oxidants and antioxidants. The dissociation of Keap1

**TABLE 1. Examples of genes regulated by Nrf2** [23, 87, 88]

| Function | Name |
|---|---|
| Detoxification: phase I | Alcohol dehydrogenase class 4 mu/sigma chain (ADH7) |
| | Aldo-keto reductase family 1 (AKR1) |
| | Aldehyde dehydrogenase (ALDH) |
| | Carbonyl reductase 1 (CBR1) |
| | Cytochrome P450 family 1, subfamily B, polypeptide 1 (CYP1B1) |
| | Cytochrome P450 family 2, subfamily B, polypeptide 9 (CYP2B9) |
| | Microsomal epoxide hydrolase 1 (mEPH) |
| | NADPH:quinine oxidoreductase (NQO1) |
| Detoxification: phase II | Glutathione *S*-transferase (GST) |
| | Sulfotransferase family (SULT) |
| | UDP Glucuronosyltransferase (UGT) |
| Detoxification: phase III | ATP-binding cassette, subfamily B, member 6 (ABCB6) |
| | ATP-binding cassette, subfamily C, member 1 (ABCC1) |
| | ATP-binding cassette, subfamily C, member 2 (ABCC2) |
| | ATP-binding cassette, subfamily C, member 3 (ABCC3) |
| | ATP-binding cassette, subfamily C, member 4 (ABCC4) |
| | ATP-binding cassette, subfamily C, member 5 (ABCC5) |
| Glutathione-based system | Glutamate-cysteine ligase, catalytic subunit (GCLC) |
| | Glutamate-cysteine ligase, modifier subunit (GCLM) |
| | Gamma-glutamyltransferase 1 (GGT1) |
| | Glutaredoxin 1 (GLRX) |
| | Glutaminase (GLS) |
| | Glutathione peroxidase (GPx) |
| | Glutathione reductase (GSR) |
| Heme and iron metabolism | Biliverdin reductase (BLVR) |
| | Ferochelatase (FECH) |
| | Ferritin (FTH) |
| | Heme oxygenase (decycling) 1 (HMOX1) |
| Carbohydrate metabolism | Glucose-6-phosphate 1-dehydrogenase (G6PD) |
| | Isocitrate dehydrogenase 1 (IDH1) |
| | 6-Phosphogluconate dehydrogenase (PGD) |
| | UDP-glucose dehydrogenase (UGDH) |
| Lipid metabolism | Acetyl-CoA thioesterase (ACOT) |
| | Acetyl-CoA oxidase (ACOX) |
| | Carboxylesterase (CES) |
| | Stearoyl-CoA desaturase-2 (SCD2) |
| | Lipase, member H (LIPH) |
| | Phospholipase A2 (PLA2G7) |
| | Patatin-like phospholipase domain-containing protein 2 (PNPLA2) |
| Ubiquitin ligase substrate adaptor | Kelch-like ECH-associated protein 1 (KEAP 1) |
| Transcription factors | CCAAT/enhancer-binding protein (C/EBP) |
| | MafG protein (MAFG) |
| | Nuclear factor-erythroid 2-like 2 (NFE2L2) |
| | Peroxisome proliferator-activated receptor gamma (PPARγ) |
| | Retinoid X receptor alpha (RXRα) |

has been proposed as a mechanism by which some chemical inducers stabilize Nrf2 [26, 27]. However, exposure to oxidative stress results in disruption of the Keap-1 and Nrf2 interaction, and Nrf-2 translocates into the nucleus (**Figure 1**). Once in the nucleus, Nrf2 can heterodimerize with diverse transcriptional regulatory proteins, while attaching to motifs identified as antioxidant or electrophile response elements (ARE/EpRE) located in the promoters or upstream promoter regions of detoxification genes [28]. Several signals are likely necessary for the dissociation of this complex, which then allows Nrf2 translocation to the nucleus. Notably, the Keap1-Nrf2 complex is conserved in rodents and humans, supporting its essential role in controlling antioxidant defense.

Recent studies have demonstrated that Nrf2 activity is not solely controlled by Keap1-mediated proteasomal degradation. In addition to Keap1, Nrf2 protein stability is regulated by another E3 ubiquitin ligase adaptor, $\beta$-transducin, via the Neh6 domain. Nrf2 degradation independent of Keap1 is promoted by glycogen synthase kinase 3 (GSK-3$\beta$), which phosphorylates specific serine residues, and GSK-3$\beta$ inhibition by the PI3K-pathway leads to increased levels of Nrf2 [29]. Moreover, Bach1 (BTB and CNC homology 1, basic leucine zipper transcription factor 1) is considered as a transcription factor that is ubiquitously expressed in tissues and indistinctly Nrf2-associated. Under normal physiological conditions, Bach1 forms heterodimers with the small Maf proteins and binds to the ARE [30] to repress gene expression. Under oxidative stress conditions, Bach1 dissociates from the ARE and is replaced by Nrf2. Thus, Bach 1 competes with Nrf2 for ARE binding [31].

## 3. ACTIVATION OF NRF2 IN HEPATIC INJURY AND DISEASE

In humans, Nrf2-regulated redox genes were found to be reduced in end-stage liver disease of different etiologies, indicating that Nrf2 pathway impairment may compromise the hepatic detoxification and the antioxidative stress system [32]. The first method used for Nrf2 activation was pharmacological molecules, such as butylated hydroxyanisole, the natural triterpenoid oleanolic acid, and the synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) as well as its derivative 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) [33].

Triterpenoids protect rodents from chemical- and drug-mediated oxidative stress, including methylazoxymethanol acetate-induced liver necrosis [34], acetaminophen hepatotoxicity [35, 36], concanavalin A-mediated inflammatory liver injury [37], and aflatoxin-induced carcinogenesis [38]. Moreover, other studies suggested administration of selective Nrf2-activating compounds under conditions of liver injury (**Table 2**). Chemical activators of Nrf2 protected mice against obesity [39, 40]. A high-fat diet (HFD), for example, stimulates oxidative stress in Nrf2$^{-/-}$-mice which cannot adapt to insults, indicating a failure to preserve homeostatic levels of the reduced form of glutathione (GSH) and thioredoxin-based antioxidant systems [41]. Notably, GSH defends cells against oxidative stress by detoxifying damaging chemicals through direct binding or enzymatic conjugation of GSH to the toxicant [42], with Nrf2 playing an important role in maintaining the cellular redox status by regulating GSH synthesis [43]. Nevertheless, prolonged exposure to a toxicant typically results in depletion of GSH and, eventually, oxidative stress. This consequence appears to result from reduced levels of the GSH-producing enzyme. In fact, livers from Nrf2-null mice showed decreased expression of GSH-synthesizing enzyme [44]. One of the main notable effects of abolishing Nrf2 function involves NADPH:quinone oxidoreductase 1 (NQO1), whose gene is known as a prototypical Nrf2-target gene [45]. NQO1 is critical for cytoprotection against many highly reactive and potentially damaging quinones [46]. Moreover, in humans with nonalcoholic fatty liver disease, increasing NQO1 expression and activity levels may improve the capacity of protecting patients from nonalcoholic steatohepatitis (NASH) progression caused by oxidative stress [47].

### 3.1. Nrf2 Signaling Inhibits Stellate Cell Activation

Upon hepatic fibrosis, quiescent stellate cells transdifferentiate into myofibroblasts that express alpha-smooth muscle actin (α-SMA) and excrete ECM proteins [48]. Nrf2 overexpression reduces the basal expression of α-SMA and transforming growth factor-beta (TGF-β) [49], a key fibrosis mediator produced in activated stellate cells that regulate cell growth, differentiation, and synthesis of ECM proteins in liver fibrosis [50]. Notably, a recent study showed that Nrf2
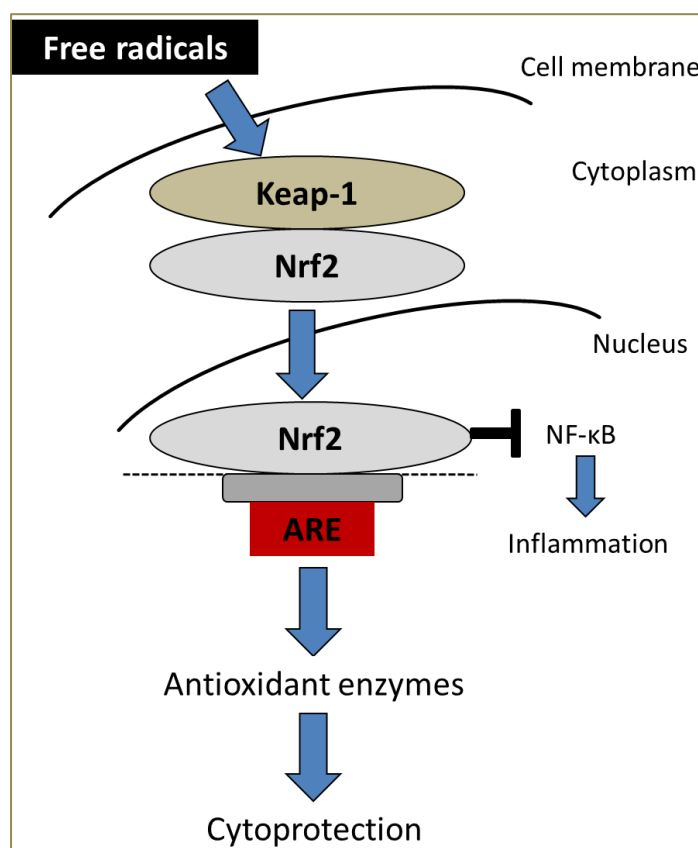
**FIGURE 1. Representative image of Nrf2-mediated cytoprotection.** Nrf2 positively regulates antioxidant genes through antioxidant responses in the regulatory regions of target genes, and negatively modulates nuclear factor-κB (NF-κB)-mediated pro-inflammatory reactions.

limits HSCs activation by inhibiting the TGF-β1/Smad pathway in HSCs [51]. Nrf2 protects stellate cells in the SMAD family of transcription factors that enhance expression of fibrogenic genes such as collagen 1α1 and tissue inhibitor of metalloproteinases (TIMP-1), which prevents ECM degradation. TGF-β provokes ROS accumulation, particularly hydrogen peroxide ($H_2O_2$), which acts as an intracellular signal mediator of the profibrogenic action of TGF-β1 [8]. This suggests that ROS stimulate induction of fibrogenic gene expression. A study by Oh et al. [52] indicated that sulforaphane, a dietary isothiocyanate, exerts chemopreventive actions through Nrf2-mediated induction of antioxidant/phase II enzymes and prevents bile-duct ligation (BDL)-induce liver fibrosis by suppressing HSC activation and subsequently

downregulating hepatic ECM proteins via inhibiting TGF-β-stimulated expression.

### 3.2. Induction of Nrf2 Reduces Accumulation of Hepatic Lipids

Treatment with the potent Nrf2 activator acetylenic tricyclic *bis*-TBE-31 in C57BL/6 mice, fed long-term with a high-fat plus fructose diet, resulted in suppression of hepatic steatosis, a decreased abundance of triglycerides and cholesterol in the livers with decreased expression of lipid-synthesis enzymes, and reduced fibrosis in an Nrf2-dependent manner [53]. The severity of liver fibrosis has been attributed to classic Nrf2-target genes that cannot be induced by *bis*-TBE-31 in the livers of Nrf2$^{-/-}$ mice fed a high-fat plus fructose diet.

In agreement with these observations, Nrf2$^{-/-}$ mice fed a methionine-and choline-deficient (MCD) diet accumulated total hepatic lipids and polyunsaturated fatty acids [63] and the HFD altered gene expression patterns favoring the synthesis and retention of lipids [64]. Nrf2 influenced hepatic lipid metabolism in HFD-fed mice, but the period of HFD feeding widely varied in previous studies [41]. Genetic activation of Nrf2 in mice by knockdown of its repressor Keap1 has been shown to reduce steatosis and NASH induced by an MCD diet [65], while a deficiency in Nrf2 increases the susceptibility of mice to MCD diet-induced hepatosteatosis and liver injury. Shin et al. [39] also found that CDDO-Im regulates hepatic lipid homeostasis in an Nrf2-dependent manner in mice on a HFD, where expression of lipogenic genes was significantly suppressed in Nrf2-null mice. However, Nrf2 activation in Keap1-knockdown mice under HFD caused NASH [66], suggesting that persistent Nrf2 activation in the liver can promote lipid accumulation. It has generally been established that the livers of Nrf2$^{-/-}$ mice accumulate lipids to a greater extent than those of wild-type mice, and lipid catabolism genes are downregulated in Nrf2$^{-/-}$ livers, but persistent Nrf2 activation may be related to increased metabolic syndrome risk of HFD feeding.

## 3.3. Amplification of Nrf2 Activation Decreases Inflammation-Mediated Hepatotoxicity

Another manner by which Nrf2 activation may protect against nonalcoholic fatty liver disease and NASH is by preventing inflammation. Steatosis commonly causes inflammation leading to NASH. It has been shown that NASH can develop in individuals with metabolic syndrome because of excessive delivery of fatty acids/triglycerides (increased lipid metabolism), leading to oxidative stress by overproduction of ROS and consequent inflammation [67]. Previous studies indicated that endotoxins and pro-oxidants cause greater damage and lethality [68], and lipopolysaccharide, for example, has been shown to be associated with liver fibrosis [69]. Moreover, context-dependent pro-inflammatory interactions with the stimuli bacteria, gut microbiota, and their products can induce Nrf2 activation, where activated Nrf2 translocates into the nucleus (**Figure 2**). Another approach demonstrated in a variety of cell culture and rodent systems to induce Nrf2 is the nuclear factor kappa B (NF-κB) [70]. Consistently, the mRNA levels of the inflammatory cytokine genes, innate immunity genes, and fibrogenesis-related genes were significantly higher in the livers of Nrf2-null mice than in control mice after 6 and

**TABLE 2. Some compounds that have been shown to activate Nrf2/ARE signaling system against oxidative stress in liver fibrosis development**

| | Effect | Ref. |
|---|---|---|
| Sulforaphane | Attenuated hepatic fibrosis induced by BDL | [52] |
| Ganodermanondiol | Increased cellular GSH levels and the expression of the glutamine-cysteine ligase gene | [54] |
| Ursolic acid | Prevented CCl$_4$-induced hepatotoxicity and fibrosis | [55] |
| Lignan Sauchinone | Ameliorated liver injury caused by a high dose of acetaminophen | [56] |
| CDDO-Im | Prevented HFD-induced increases in body weight, adipose mass, and steatosis | [57] |
| Oltipraz, NK-252 | Attenuated hepatic fibrosis induced by CDAA diet | [58] |
| Gastrodin | Increased expression of antioxidants enzymes in HFD | [59] |
| Ginsenoside Rg1 | Protects against alcohol- and CCl$_4$-induced hepatic-fibrosis | [60] |
| Lycopene | Reduced hepatocyte injury on the development of NASH-promoted early hepatocarcinogenesis | [61] |
| Baicalein | Decreased inflammation and oxidative hepatocyte injury | [62] |
| Acetylenic tricyclic bis(cyano enone) TBE-31 | Antagonize lipogenesis, endoplasmic reticulum stress, inflammation, oxidative stress, and fibrosis | [53] |

24 weeks of starting an atherogenic diet plus a HFD [71]. The impaired expression of antioxidative genes potentially leads to higher NF-κB activation, highlighting the activation of antioxidant enzymes as a potential treatment for NASH. The NF-κB pathway, which regulates pro-inflammatory biomarkers, such as IL-1β, IL-6, and tumor necrosis factor-alpha, was found to be highly induced in Nrf2-deficient mice, indicating that Nrf2 suppression accelerates NF-κB-mediated pro-inflammatory reactions [72]. Moreover, carbon tetrachloride ($CCl_4$) treatment that increases aminotransferase levels in the serum and causes histopathologic modifications, such as leukocyte infiltration, hepatic cell necrosis, pseudo-lobe, and collagen fiber formation, also inhibits nuclear translocation of Nrf2 [55]. Markedly, Nrf2-null mice showed aggravated liver injury following treatment with $CCl_4$, with an amplified inflammatory response and reduced detoxification which may contribute to more severe liver injury in Nrf2 knockout mice [73]. While the MCD diet caused NASH more rapidly in Nrf2-deficient than in wild-type mice [19], a transgenic strategy resulting in knockout of Keap1 decreased inflammatory gene expression in T cell-mediated acute inflammatory liver injury [37]. In this study, hepatocytes were targeted by activated inflammatory cells to prevent the amplification of inflammatory responses on late-phase hepatic pro-inflammatory gene expression, although genetic magnification of Nrf2 signaling in hepatocytes had no effect on the initial immune reaction generated by concanavalin A injection. This was supported by the results of Collins et al. [74] who examined low-density lipoprotein receptor-deficient mice (a model that mimics human atherosclerosis) transplanted with either wild-type or Nrf2-deficient bone marrow cells and fed on a HFD for 7 months. Decreased antioxidant gene expression and an increased number of inflammatory cell foci associated with more fibrosis than in control mice were observed. This is related to increased macrophage migration, inflammation, and oxidative stress contributing to atherosclerosis and enhanced hepatic fibrosis.

### 3.4. Nrf2 Activators Can Regulate Liver Fibrotic Response

Consistent with data showing the onset of tissue remodeling, Xu et al. [73] demonstrated that the expression of genes such as collagen type 1 alpha 1 and TIMP-1 are inhibited by an Nrf2 activator in toxin-induced liver injury and fibrosis. A causal relationship was revealed between reduced expression of cytoprotective proteins and the initiation and development of liver fibrosis within increased mRNA levels of type I collagen and plasminogen activator inhibitor-1, contributing to excess accumulation of collagen and other ECM components [75]. Furthermore, BDL surgery in mice had a protective effect with pharmacological activation of Nrf2, as observed by histopathological evaluation of livers that included extensive proliferation of bile ducts in dilated portal spaces, and fibrosis characterized by excessive deposition of collagen on hepatic fibrosis [52]. Importantly, Nrf2 activators reduced fibrosis scores compared to controls in a fibrosis rat model fed a choline-deficient (CDAA) diet [58]. This result indicates that Nrf2 signaling delays liver fibrosis progression in NASH. The Nrf2 activators (oltipraz and NK-252) showed protective effects against $H_2O_2$-induced cytotoxicity in Huh-7cells. Nrf2-null mice fed a HFD and atherogenic diet exhibited more pronounced bridging and pericellular fibrosis in the livers [71]. Yet, Choi et al. [49] found that solubilized coenzyme Q10, an endogenous antioxidant, suppresses the expression of TGF-β1 induced by dimethylnitrosamine in liver fibrogenesis via Nrf2/ARE activation. Therefore, while hepatic injury accelerates profibrogenic cytokine secretion and stimulates the ECM, Nrf2 activation may initiate signal transduction pathways that regulate cell proliferation in response to injury [43].

## 4. NRF2 AND THERAPEUTIC APPROACH: DUAL FUNCTION

Remarkably, cells can adapt to the presence of increased ROS by up-regulating antioxidant genes. Specifically, individual heterogeneity in pathways may lead to adaptation to oxidative stress [76]; thus, antioxidative therapy with antioxidants is much more complex than simple free radical scavenging. Additionally, while routine clinical employment of antioxidant dietary supplementation perturbs the natural balance of endogenous antioxidants, Nrf2 has emerged as a transcription factor with extremely advantageous effects under oxidative stress conditions [77, 78]. The role of Nrf2 in detoxification and hepatoprotection has been employed with the use of new pharmacological Nrf2 activators, but only some of these activators have entered the clinical trial stage
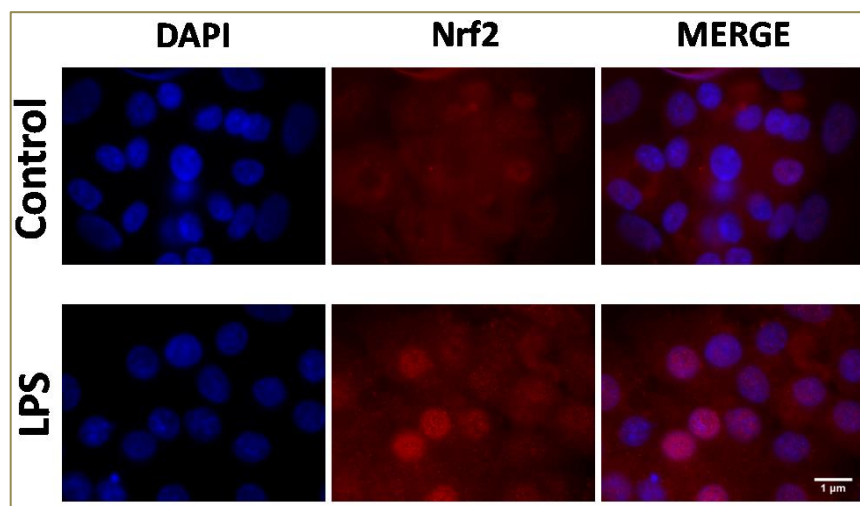
**FIGURE 2. Nuclear localization of Nrf2 in HepaRG cells treated with lipopolysaccharide (100 ng/ml for 6 h).** Localization of Nrf2 distribution was observed by immunofluorescence under a confocal microscope. HepaRG cells grown on coverslips were fixed in 4% paraformaldehyde for 15 min at 4°C and blocked with 10% normal goat serum in phosphate-buffered saline (PBS). Cells were incubated with anti-Nrf2 rabbit polyclonal antibody (1:250 dilution) in blocking solution overnight at 4°C and then for 1 h in rhodamine-conjugated goat anti-rabbit secondary antibody (1:100) for 1 h at room temperature in the dark. DAPI (Sigma-Aldrich, St. Louis, MO, USA) was added for nuclear staining. The cells were observed under a DMRXA epifluorescence microscope (Leica, Germany) using a 63/1.4 NA objective and a charge-coupled device camera (CoolSNAP HQ2; Roper Scientific, Trenton, NJ, USA). Images were acquired using the Metamorph software (Universal Imaging, Bedford Hills, NY, USA). Original magnification ×200; scale bar, 1 µm.

[33, 79]. Whether Nrf2 activators are clinically effective in NASH is unclear, but these agents may represent a promising therapy for liver injury and disease in humans. However, although Nrf2 signaling may defend against cancer cell initiation by chemical carcinogens, it also causes tumorigenesis when initiation occurs. The precise function of Nrf2 signaling in tumorigenesis remains undefined. Using Nrf2-deficient mice, Kitamura et al. [80] demonstrated the protective role of Nrf2 against xenobiotic-mediated hepatocarcinogenesis. Paradoxically, while Nrf2 deficiency led to increased local tumor growth in mice following subcutaneous injection of B16-F10 melanoma cells [81], increased Nrf2 activation was observed in some types of cancer tissue [82–84]. In fact, somatic mutations were found to disrupt the interaction between Nrf2 and Keap1, leading to stable Nrf2 and an increase in the constitutive transcription of Nrf2 target genes. Mutations in the Keap1 gene associated with Nrf2 stabilization have also been recognized in liver cancer associated with elevated levels and activity of Nrf2 in cancerous cells [83, 84]. Mutations in Keap1 gene affecting amino acids at the site of interaction with Nrf2 lead to its upregulation [85]. Whereas disruption of Nrf2 signaling seems to be associated with cancer cell cycle progression and proliferation of cancer cell lines in vitro, constitutive activation of Nrf2 was shown to enhance the resistance of cancerous cells to various forms of chemotherapy [84]. Nevertheless, inhibition of Nrf2 by RNAi rendered cancerous cells more sensitive to chemotherapeutic agents [86].

## 5. CONCLUDING REMARKS AND FUTURE DIRECTION

While Nrf2 signaling appears to play a pivotal role in protecting cells from oxidative stress and subsequent responses such as inflammation and fibrotic

mechanisms, studies of the function of Nrf2 in liver fibrosis protection have been performed mostly using Nrf2-deficient models. Significantly, disruption of Nrf2 is an extremely useful strategy both for assigning function and for mapping the interrelationship of different components in intracellular regulatory pathways, and further offers a framework for observing alterations operating at a higher stage of biological organization. This is necessary when there is a variation in the expression or activity of multiple members of the same pathway. For instance, somatic inactivation of the Keap1 gene in hepatocytes does not interfere with the development or morphological and physiological integrity of the liver, but rather leads to activation of the Nrf2-ARE pathway and acquisition of resistance against toxicity. Indeed, analyses of global transcriptional responses revealed that connections in Nrf2 signaling can arise from diverse modifications in the regulation of gene expression/function, and hence, a pre-clinical database and clinical studies of antifibrotic activity are needed using more precise targeting approaches in staging conditions to orchestrate wound healing responses.

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