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Is Nrf2-ARE a potential target in NAFLD mitigation?

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is defined as a metabolic syndrome associated with obesity and type 2 diabetes mellitus (T2DM) and characterized as excessive triglyceride accumulation in 5% or more of the hepatocyte population without significant alcohol consumption. Although the worldwide prevalence of NAFLD is increasing, except dietary control, there remains no effective treatment to improve NAFLD clinically. Nuclear factor erythroid-derived 2-like 2 (NFE2L2, also known as Nrf2) is one of the most important transcription factors in regulating adaptive antioxidants and xenobiotic stress responses. Recently, a variety of studies have started to investigate the potential involvement of Nrf2 in the development of NAFLD and to utilize Nrf2 activators in an attempt to ameliorate hepatic steatosis and inflammation. However, the results are, so far, controversial and complex. This review attempts to highlight the hypothetical roles of Nrf2 in the incidence of NAFLD. In addition, we provide recommendations on therapeutic strategies of Nrf2 activators in NAFLD, including use in the proper phase of the disease for the greatest impact.

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Keywords

Liver, NAFLD, Nrf2, Oxidative stress.

Abbreviations

ACC, acetyl-CoA carboxylase; AcCoA, acetylcoenzyme A; ACOX1, acyl-coenzyme A oxidase 1; AMPK, adenosine monophosphate-activated protein kinase; ApoB-100, apolipoproteinB-100; ARE, antioxidant response element; ChREBP, carbohydrate response element–binding protein; Cox2, cyclooxygenase 2; CPT1/2, carnitine palmitoyltransferase 1/2; Elovl3, elongation of very-long-chain fatty acids protein 3; Elovl6, elongation of very-long-chain fatty acids 6; ECM, extracellular matrix; FABP1/4/5, fatty acid-binding protein 1/4/5; Fas, fatty acid synthetase; FFAs, free fatty acids; GSH, glutathione; GPx2, glutathione peroxidase 2; Ho-1, heme oxygenase-1; HSCs, hepatic stellate cells; IL-1β, Interleukin 1β; IL6, Interleukin 6; IP-10, Interferon gamma-induced protein 10; KCs, kupffer cells; LXRα, liver X receptor-α; MCD, methionine-choline deficient diet; MCP-1, monocyte chemoattractant protein 1; MPO, myeloperoxidase; MTTP, microsomal triglyceride transfer protein; NF-κB, IkappaB kinase (IKK)/nuclear factor-κB; Nos2, nitricoxidesynthase 2; Nrf2, nuclear factor erythroid 2 like 2; Nqo1, NAD(P)H, quinone oxidoreductase-1; NAD, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; PEPCK, phosphoenolpyruvate carboxykinase; PPARα, peroxisome proliferator-activating receptor α; ROS, reactive oxygen species; SHP, small heterodimer partner; sMaf, small musculoaponeurotic fibrosarcoma; SREBP1c, sterol regulatory element-binding protein 1c; Scd1, stearoyl-CoA desaturase 1; TCA, tricarboxylic acid cycle; TG, triglyceride; TNFα, tumor necrosis factor α; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoproteins; VLDLR, very low-density lipoprotein receptor.

Introduction

With continued worldwide economic development driven largely by globalization, the prevalence of nonalcoholic fatty liver disease (NAFLD) has been steadily increasing not only in developed countries [1,2] but also in developing countries [3–5]. The global NAFLD incidence has increased up to 25% by 2016 [6]. NAFLD is a metabolic syndrome associated with obesity and type 2 diabetes mellitus (T2DM), characterized as excessive triglyceride (TG) accumulation in the hepatocytes (5% or more) in the absence of alcohol intake [7]. In a broad sense, NAFLD consists of simple steatosis and nonalcoholic steatohepatitis (NASH), which involves the development of inflammation and fibrosis [8]. Indeed, about 10–30% of patients with simple steatosis develop NASH [9], and afterwards, 10–29% of patients with NASH progress to overt cirrhosis in about 10 years [10]. Ultimately, those with cirrhosis (up to 27%) can eventually end up developing hepatocellular carcinoma [11]. Since liver transplantation, which has various limiting factors, is the only therapeutic strategy for cirrhosis [12], an early treatment of NAFLD becomes extremely important.

This review summarizes the roles of nuclear factor erythroid-derived 2-like 2 (Nrf2) in the development of NAFLD according to the diverse research results published in recent years and highlights the potential

therapeutic application of Nrf2 activators in NAFLD treatment.

Risk factors and etiology of NAFLD

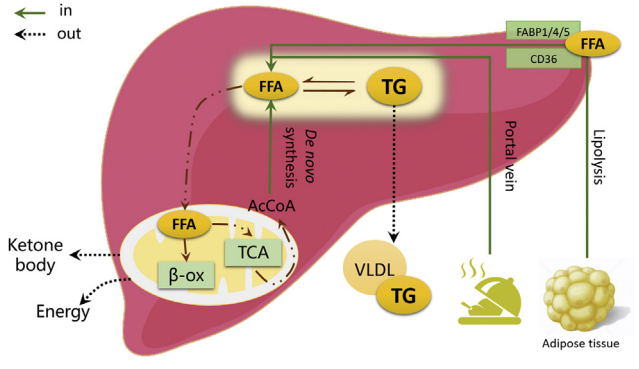
Risk factors

The development of NAFLD is associated with many metabolic disorders and factors related to genetics and lifestyle. Early in 1979, Adler and Schaffner [13] discovered the relationship between obesity and NAFLD. Since then, extensive studies in a variety of populations established that NAFLD is positively associated with obesity, insulin resistance and T2DM [9,14–19]. Importantly, T2DM and NAFLD combine to set up a vicious cycle to exaggerate the impact of one another. In 2004, Browning et al. [20] reported the difference in prevalence of NAFLD between distinct ethnic groups, which points toward genetic diversity in the disease etiology. More recently, Eslam et al. reviewed concisely the importance of various genetic and epigenetic factors that impact NAFLD susceptibility and progression [21]. With regard to lifestyle, a variety of dietary factors, including fat and fructose, have been clearly demonstrated as risk factors in prevalence of NAFLD [22–24].

Etiology

An imbalance between the acquisition and cleanup of TG is the essence of hepatic steatosis. Free fatty acids (FFAs) and glycerol are the raw materials by which hepatocytes synthesize TGs. As illustrated in Figure 1, there are three major sources for the FFAs in hepatocytes: ① dietary intake where the fat in food is digested into FFAs that are absorbed into blood and transported into the liver through the action of fatty acid transporters (FATP2 and FATP5) and fatty acid transposition enzymes (FAT/CD36); ② *de novo* synthesis, using other energy substances through hepatic acetylcoenzyme A carboxylase (ACC) and fatty acid synthase (FAS) to activate lipogenesis; and ③ lipolysis in adipose tissue and subsequent transport FFAs to the liver [25]. On the other hand, there are also three destinies for hepatic FFAs: ① β-oxidation, by which FFAs enter the mitochondria to be oxidized into ketone bodies producing energy; ② conjugated with lipoprotein as a very-low-density lipoprotein (VLDL) and secretion into the blood; and ③ esterification and storage as TGs in hepatocytes. The equilibrium of acquisition and elimination of lipids in hepatocytes, which is regulated by a variety of nuclear receptors, including peroxisome proliferator–activating receptors (PPARs) and liver X receptor (LXR), as well as some related transcription factors such as hepatic steroid response element–binding protein (SREBP-1c) and carbohydrate response element–binding protein (ChREBP), is the key in the pathogenesis of NAFLD.

Figure 1



Metabolic pathways of lipid in the liver. There are three ways for TG accumulation in hepatocytes: dietary intake daily, FFAs transported from peripheral adipose tissue and *de novo* lipogenesis. There are two routes for TG elimination. One way is β-oxidation in mitochondria, and the other way is binding to VLDL which helps TG export from hepatocytes. TG, triglyceride; FFAs, free fatty acids; TCA, tricarboxylic acid cycle; AcCoA, acetylcoenzyme A; VLDL, very-low-density lipoproteins; β-ox, β-oxidation.

Oxidative stress and inflammation in NAFLD

Oxidative stress

Last century, the famous 'two-hit' model to NAFLD was proposed [26]. When insulin resistance, hyperglycemia and hyperlipidemia occur, the hepatocytes initially develop steatosis, which was called the first hit. The second hit was considered as subsequent oxidative stress and resulting inflammation and fibrosis. Excess diet fat, smoking and other environmental factors may drive mitochondrial dysfunction, resulting in oxidative stress. Reactive oxygen species (ROS) can transmit signals for normal physiological activity and are at normal levels alone insufficient to cause significant liver injury, but ROS overproduction is involved in many diseases, including diabetes [27], obesity [28], cancer [29], cardiovascular disease [30], chronic respiratory diseases [31] and neurological diseases [32]. ROS may not only damage proteins, lipids and DNA, but also specifically impair lipid homeostasis in hepatocytes, which is directly associated with NAFLD [33].

Inflammation

Inflammation with fibrosis is the critical component of the second hit for NAFLD development, and significant as it is the point that renders the disease progress into NASH. The inflammation may come from the influx of FFAs to the liver and adaptive mechanisms concerning oxidative stress–induced liver injury. This likely activates endothelial cells of the liver, increasing the expression of cytokines and finally activating the hepatic stellate cells (HSCs) associated with the acquisition of proinflammatory and profibrogenic functions [34,35]. In addition, it was reported that tumor necrosis factor α (TNFα) produced by hepatic Kupffer cells (KCs) could induce lipid accumulation and monocyte infiltration into the liver, which accelerated the development of NASH [36]. Inflammatory cytokines including interferon

gamma—induced protein 10 (IP-10) and monocyte chemoattractant protein 1 (MCP-1) were downregulated by silencing TNFα in KCs and monocytes [36].

Nrf2 and NAFLD

Nature of Nrf2

Nrf2, a CNC-bZIP family member, is one of the most important transcription factors in regulating adaptive antioxidant response and xenobiotic stress response. Nrf2 may heterodimerize with small avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF) proteins to regulate the transcription of many antioxidant and detoxification genes via the antioxidant response element (ARE), a DNA motif located in the upstream promoter regions of target genes. Under normal circumstances, Nrf2 and its main inhibitory regulatory protein Kelch-like ECH-associated protein 1 (Keap1) with two specific coupling sites form a cyclic E3-ubiquitin ligase and Cullin-3/Rbx1 complex, which sends Nrf2 into the ubiquitin proteasome to be degraded [37,38]. Electrophilic agents and oxidants may change the specific cysteine residues of Keap1 chemically, modify the conformation, and then inhibit the ubiquitination of Nrf2, resulting in the accumulation and activation of Nrf2 [39]. In addition to the well-defined regulatory model for Nrf2-ARE activation, other proteins and signaling cascades, such as p62 and GSK3β, may also be involved in the response [40]. The downstream genes of Nrf2 include antioxidant enzymes, such as NADPH quinone oxidoreductase-1 (*NQO1*), heme oxygenase-1 (*HO-1*), gamma glutamyl cysteine ligase, and many II phase detoxification enzymes [41]. Interestingly, accumulating evidence indicates that Nrf2 may also regulate a variety of genes involved in cell proliferation and differentiation, lipid metabolism and inflammatory response [40].

The distinctive roles of Nrf2 in NAFLD

The pathogenesis of NAFLD is clearly associated with oxidative stress, and Nrf2 is adept at dealing with ROS produced under various stress conditions [42,43]. Thus, Nrf2 is believed to help ameliorate NAFLD. Accordingly, methionine-choline deficient diet (MCD) treatment induced extraordinarily higher malondialdehyde (MDA) levels and reduced GSH/GSSG ratio in *Nrf2*-knockout (KO) mice compared to wild-type mice [44,45]. In hepatocyte-specific *Nrf2*-overexpressing mice, antioxidant genes glutathione peroxidase 2 (*Gpx2*), thioredoxin-1 (*Txn1*) and *Nqo1* were all amplified in animals fed either normal chow diet or MCD, and major markers of oxidative stress, such as 4-hydroxynonenal (4-HNE) and nuclear dihydroethidium (DHE), were reduced [46]. These results are consistent with previous studies showing that specific overexpression of hepatic Nrf2 in mice protects against oxidative stress induced by prolonged MCD exposure [47]. To clarify the diverse results of various Nrf2 modulations on the development of NAFLD/NASH in a balanced manner, most of the published results related to the topic have been grouped into 'Yang' and 'Yin' effect and summarized in Tables 1 and 2, respectively.

Hepatic lipid metabolism concerning NAFLD mainly consists of FFAs uptake and transport, *de novo* synthesis, β-oxidation and TG exportation from hepatocytes. An early proteomic analysis using *Nrf2*-deficient mice found that Nrf2 not only was a positive regulator of phase 2 detoxification enzymes, but also played a negative role in regulating the factors related to lipid metabolism, such as ATP-citrate lyase that is responsible for the production of acetyl-CoA, an important substrate of lipid metabolism [48]. FABP1/4/5, which is responsible for FFAs uptake and transport from blood into hepatocytes, was negatively regulated by Nrf2 [46,49,50] and could increase the levels of FFAs in hepatocytes and interrupt VLDL assembly [51]. CD36 as an integral membrane protein plays a role of importing FFAs into cells, and has been identified as a downstream of Nrf2 [51—53]. SREBP1c is a classical transcription factor for lipogenesis, which upregulates expression of many downstream genes, including ELOVL5/6, ACC1/2, SCD1 and FAS [49,54,55]. In addition, liver X receptor-α (LXRα), which serves as an insulin-sensitive sensor to increase lipid synthesis, along with PPAR gamma coactivator 1-alpha (PGC1α) activates SREBP1c to regulate lipid homeostasis [49,56,57]. PGC1α with its related protein phosphoenolpyruvate carboxykinase (PEPCK) mediates gluconeogenesis without affecting mitochondrial biogenesis [58]. However, the relationship between Nrf2 and LXRα is controversial, as well as the relationship between Nrf2 and PGC1α.

Ludtmann et al. [59] reported that the efficiency of fatty acid oxidation is reduced when *Nrf2* is absent in isolated mouse embryonic fibroblasts (MEFs). In addition, *Nrf2* deletion increases the expression of peroxisome proliferator—activated receptor α (PPARα), which regulates β-oxidation in mitochondria, where PGC1α serves as a coactivator [49,54,60]. Similar results were showed in an Nrf2 overexpression model, in which PPARα decreased [46,61]. Carnitine palmitoyltransferase 1/2 (CPT1/2) consists of a complex for transporting FFA into mitochondria. Early research demonstrated that CPT1 was decreased in *Nrf2*-KO mice [49,60], and recent data have shown that CPT2 is also decreased [47]. CD36 is an extracellular lipid carrier and is upregulated in hepatocyte-specific *Keap1*-knockdown mice, which means CD36 is positively regulated by Nrf2 [46], in accord with early evidence reviewed in the study by Hayes and Dinkova-Kostova [62]. Peroxisomal acyl-coenzyme A oxidase 1 (ACOX1) is the first enzyme of the fatty acid β-oxidation pathway, which is upregulated by Nrf2 [60]. Nrf2 can influence TG export by upregulating microsomal triglyceride transfer protein (MTTP) in hepatocyte-specific Nrf2 constitutively

Table 1 The *Yang* effect of Nrf2 in HFD-induced NAFLD.

Model (mouse line)	Started wks	Diet	Expo time	Hepatic phenotype			Ref
				Steatosis	inflammation	Oxidative stress	
Nrf2 deletion (B6)	8 wks	HFD (68.5%)	4 wks	↑	↑		[68]
Nrf2 deletion (B6)	8–10 wks	HFD (45%) Test diets	24 wks	↑	↑	↑	[49]
Hepatocyte *Nrf2* overexpression (B6)	6–8 wks	MCD (MP Bio. 0296043910)	4 wks	↓	–	–	[47]
Nrf2 deletion (ICR)	8 wks	HFD (68.5%)	8 wks	↑		↑	[54]
Nrf2 deletion (B6)	11–12 wks	MCD (MP Bio. 0296043910)	2 wks	↑	↑	↑	[44]
Hepatocyte *Nrf2* overexpression (B6)	8–10 wks	MCD (Research Diets D09100301)	4 wks	↓	↓		[46]
Nrf2 deletion (B6J)	8 wks	HFD (60%) (Research Diets #D12492)	14 wks	↑			[89]
Nrf2 deletion (B6)	7 wks	HFD (16%)	12 wks	↑		↑	[60]
Nrf2 deletion (B6)	8 wks	HFD (35%) (Bio-Serv, Inc. F5194)	4 wks	↑		↑	[45]

B6, C57BL/6; ICR, Institute of Cancer Research; NAFLD, nonalcoholic fatty liver disease HFD, high-fat diet.
ICR mice were selected by Hauschka Swiss mouse population with the goal of fertility. Later, the Institute of Cancer Research of the United States sent the ICR mice to different countries for breeding experiments, which were called ICR mice in different countries.

Table 2 The *Yin* effect of Nrf2 HFD-induced NAFLD.

Model (mouse line)	Started wks	Diet	Expo time	Hepatic phenotype			Ref
				Steatosis	Inflammation	Oxidative stress	
Nrf2 overexpression (B6)	3 wks	HFD (60%) (Research Diets #D12492)	24 wks	↑	↑		[70]
Nrf2 deletion (B6)	9–10 wks	HFD (60%) (Research Diets)	26 wks	↓			[90]
Nrf2 deletion (B6JL)	8–10 wks	HFD (42%), (Harlan Teklad TD 88137)	12 wks	↓			[51]
Nrf2 deletion (B6)	12 wks	HFD (42%) (Test Diets #1813906)	12 wks	↓		–	[91]
Nrf2 overexpression (B6)				↑		–	
Nrf2 deletion (B6 and 129SV mixed)		HFD (Harlan Laboratories TD 88137)	16 wks	↓			[92]
Nrf2 deletion (B6J)	12–16 wks	HFD (60%) (Harlan Teklad TD 06414)	6 wks	↓			[93]

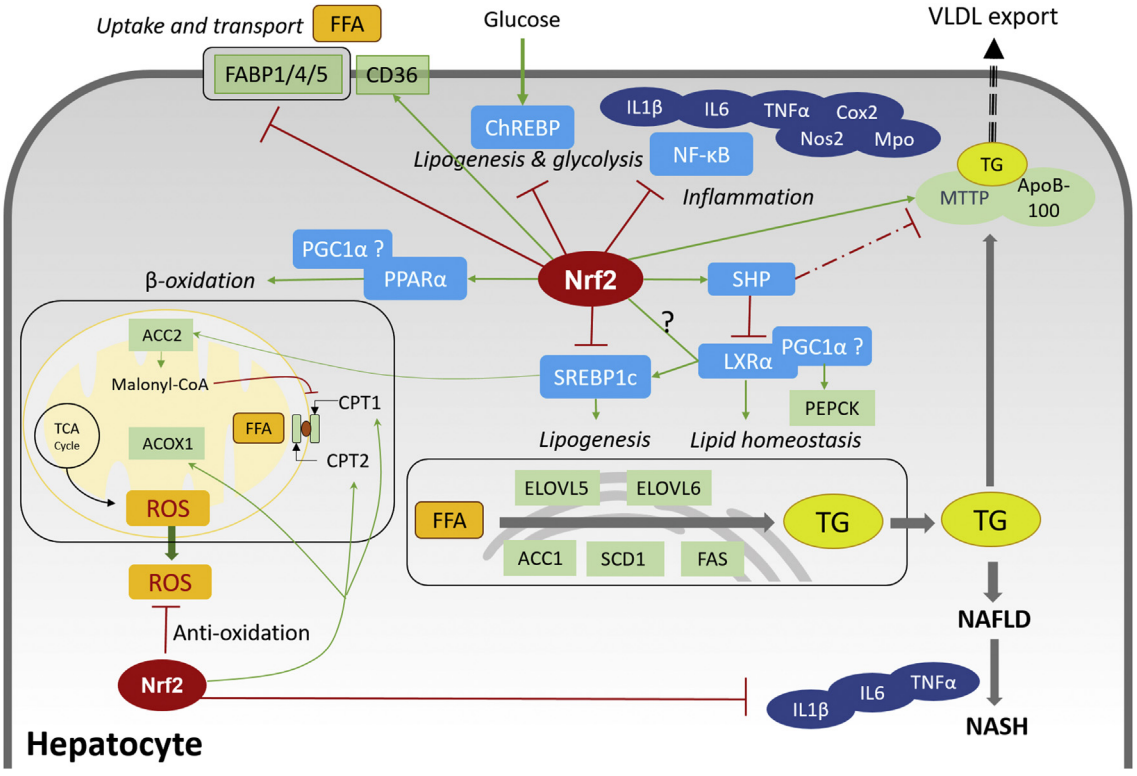
B6, C57BL/6; NAFLD, nonalcoholic fatty liver disease HFD, high-fat diet.

activation mice [47]. Park et al. and Jiang et al. [63,64] confirmed that p62, an adapter protein in autophagy, interacts with Nrf2 to prevent lipotoxicity. Adenosine monophosphate–activated protein kinase (AMPK) can phosphorylate Nrf2 at Ser40, which causes it to translocate into the nucleus where it activates downstream antioxidative enzymes [43]. Nrf2 can initiate the AMPK pathway to provoke β-oxidation via Cpt1 [49]. According to the distinct roles of Nrf2 in lipid metabolism, adaptive antioxidant response and anti-inflammatory response, the development of NAFLD/NASH and involvement of Nrf2 in the process are summarized in Figure 2.

Considering diverse types of cells in the liver that are involved in the pathogenesis of NAFLD, it is not surprising that Nrf2 plays roles in a cell- and stage-specific fashion. Lee et al. utilized hepatocyte-specific Nrf2 constitutive activation mice fed 4 weeks of MCD as a NASH model and found decreased hepatic steatosis and hepatocellular damage without inducing inflammation, oxidative stress or HSC activation [47]. Similarly, Ramadori et al. [46] reported that constitutive Nrf2 overactivation in hepatocytes enhanced lipid catabolism and repressed *de novo* lipogenesis but observed no differences in inflammatory F4/80- and CD11b-positive cells or profibrogenic genes when mice were exposed to MCD for 4 weeks. In contrast, *Nrf2* deficiency specifically in hepatocytes of mice resulted in lower insulin levels and improved insulin sensitivity without any changes in liver TG accumulation in mice fed 6 months high-fat diet (HFD) [65].

The involvement of inflammation in the development of NASH, and particularly the role of Nrf2 in this response, has been studied extensively. In addition, Nrf2 has been revealed to control inflammation by inhibiting the c-Jun N-terminal kinase (JNK) and nuclear factor-κB pathways [66]. In accord with diverse characteristics of various diet-induced NAFLD models in mice, major types are HFD- and MCD-induced fatty liver disease [67]. HFD induces simple steatosis with low inflammation, and MCD is used for arousing NASH with inflammation. As a result, animal models utilizing HFD do not show significant inflammation, whereas in the MCD-induced NASH model, there are often remarkable results associated with

Figure 2



Major roles of Nrf2 in the progression of NAFLD. In hepatocytes, TG synthesized from FFAs via synthetase finally exports by binding VLDL with ApoB-100 and MTTP complex. Alternatively, TGs accumulate in hepatocytes leading to NAFLD progression. The remaining FFAs are transported to mitochondrial entering β-oxidation cycle which may generate by-product — ROS. ROS, reactive oxygen species; ApoB-100, apolipoproteinB-100; SREBP1c, sterol regulatory element–binding protein 1c; ChREBP, carbohydrate-responsive element–binding protein; LXRα, liver X receptor-α; PGC1α, peroxisome proliferator–activated receptor gamma coactivator 1-alpha; PPARα, peroxisome proliferator–activated receptor α; CPT1/2, carnitine palmitoyltransferase 1/2; ACOX1, acyl-coenzyme A oxidase 1; MTTP, microsomal triglyceride transfer protein; PEPCK, phosphoenolpyruvate carboxykinase; FABP1/4/5, fatty acid–binding protein 1/4/5; NF-κB, IkappaB kinase (IKK)/nuclear factor-κB; IL6, interleukin 6; IL-1β, interleukin 1β; TNFα, tumor necrosis factor α; Nos2, nitricoxidesynthase 2; Cox2, cyclooxygenase 2; MPO, myeloperoxidase; SHP, small heterodimer partner; Elovl5/6, elongation of very-long-chain fatty acids 5/6; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase-1; ACC1/2, acetyl-CoA carboxylase 1/2.

inflammatory responses. Interestingly, deficiency of *Nrf2* in mice may substantially worsen the inflammation. For instance, in *Nrf2*-KO mice fed MCD for 14 days, the transcript levels of *Il-1β*, *Tnfα*, *Nos2* and *Cox2* and the protein levels of nuclear factor-κB were increased compared to wild-type mice [44]. Similar results were also reported by Meakin et al. [49] and Wang et al [68]. In contrast, there were no differences in inflammatory F4/80- and CD11b-positive cells between hepatocyte-specific Nrf2 overexpression mice and wild-type mice [46,47]. These findings suggested that hepatocytes alone cannot provoke inflammation. Alternatively, Nrf2 may regulate *Gpx2*, which in turn mediates glutathione biosynthesis and thioredoxin [69]. Thus, a loss of GPx2 in *Nrf2*-deficient mice could allow oxidative stress, thereby contributing to inflammation via inducing inflammatory cytokines. However, other research finds that *Keap1*-knockdown mice will develop more severe inflammation than wild-type mice and show increased mRNA levels of inflammatory cytokines *Mcp1* and *Tnfα* [70].

Fibrosis plays a critical role in the development of cirrhosis and NASH. HSC activation can proliferate and deposit collagen, resulting eventually in fibrosis. Although the specific role of Nrf2 in HSCs is still unclear, Nrf2 activation alone is sufficient to attenuate NASH-associated hepatic fibrosis in a rat model [71]. During hepatic fibrogenesis, apoptosis markers decreased which indicates selective perturbation of programmed cell death in the process of NAFLD. The antiapoptotic mitochondrial protein Bcl-2 is significantly increased in the hepatocyte-specific Nrf2-overexpressing mouse model [46], suggesting that Nrf2 may affect fibrosis in either direct and/or indirect ways.

Use of Nrf2-ARE modulators in the treatment of NAFLD

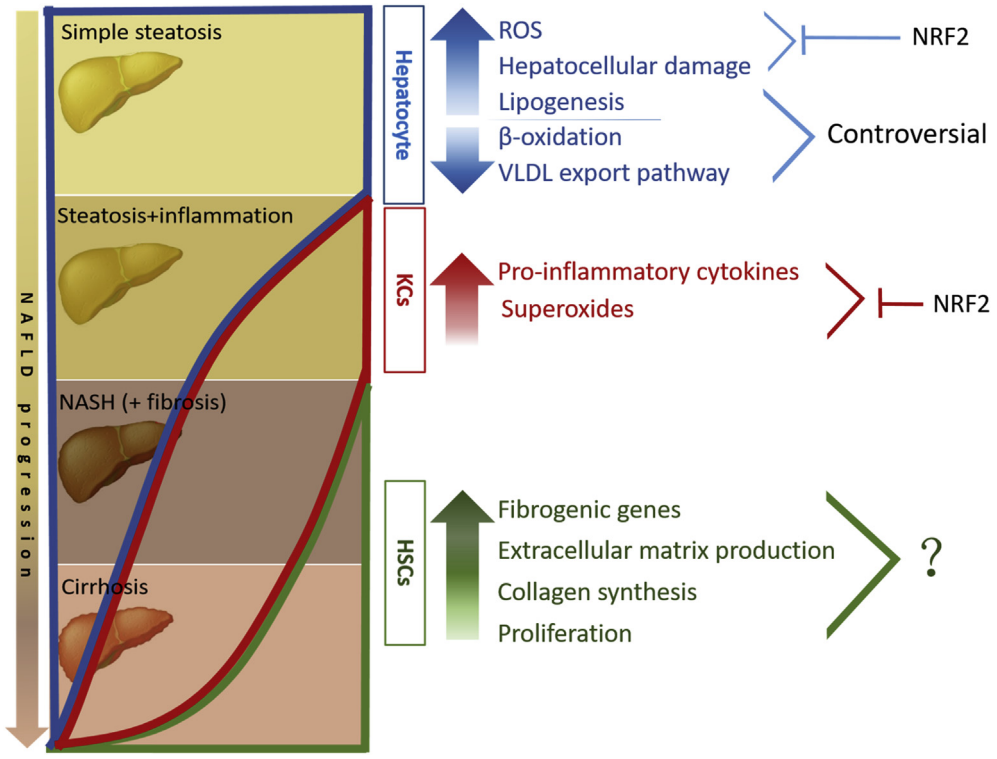
At present, lifestyle modification is the most effective and reliable intervention for NAFLD. Reducing excess body weight by 5–10% may improve liver histology, fibrosis, and serology indicative of liver damage in

Table 3 Applications of Nrf2-ARE modulators in preventing/treating NAFLD.

Subject/model	Chemical	Description	Steatosis	Inflammation	Oxidative stress	Other effects and results	Ref
In vivo							
C57BL/6J mice	Nigaichigoside F1 (NI)	A plant-derived bioactive substance	↓		↓		[75]
Sprague–Dawley rats	Scutellarin (SCU)	Drug for cerebral infarction, atherosclerosis, and coronary heart disease	↓		↓	↑PI3K/AKT with subsequent Nrf2 nuclear translocation; ↑HO-1 and NQO1	[76]
C57BL/6J mice	Apigenin (Api)	A natural flavonoid, antioxidant, and anticancer agent	↓		↓	↑Nrf2 and PPARα	[42]
C57BL/6J mice	Hydroxytyrosol (HT)	A polyphenol in extra-virgin olive oil, an Nrf2 activator	↓			↓PPARα; ↓NF-κB.	[77]
C57BL/6J mice	Docosahexaenoic acid and extra-virgin olive oil	Antioxidant properties enriched with tocopherols and polyphenols	↓	↓	↓	↑PPARα and Nrf2; ↓NF-κB and SREBP-1c.	[80]
Sprague–Dawley rats	Saponins	Antiinflammatory and antioxidative effects	↓	↓	↓	↑Nrf2-mediated antioxidant enzymes; ↑PPARα-regulated fatty acid oxidation	[85]
C57BL/6 mice	Myricetin	A bioactive flavonoid	↓			↑Nrf2 and the PPAR signaling pathway	[81]
C57BL/6J mice	Ezetimibe (Eze)	A potent cholesterol absorption inhibitor (clinical), an Nrf2 activator	↓	↓		↓fibrosis	[84]
Fisher 344 rats	NK-252	A biaryl urea compound, an Nrf2 activator	↓	↓		↓fibrosis	[71]
Sprague–Dawley rats	Baicalein	Huang qin in China, a traditional Chinese herbal medicine and an Nrf2 activator	↓	↓	↓		[82]
Wistar rats	Linoleic acid (LA)	A conjugated linoleic acid (CLA) mixture, an Nrf2 activator	↓			Modulate mitochondrial uncoupling	[83]
Nrf2(–/–) mice	6-Methylsulfinylhexyl isothiocyanate	A bioactive ingredient present in Japanese horseradish (wasabi); an Nrf2 activator.	↓				[94]
C57BL/6 mice	S-Propargyl-cysteine	The substrate for endogenous H_2S synthesis via CSE catalysis	↓		↓	↑PI3K/Akt/Nrf2/HO-1 signaling pathway	[78]
C57BL/6 mice	Osteocalcin	A noncollagenous small protein secreted by osteoblasts	↓			↑Nrf2; ↓JNK activation.	[79]
C57BL/6J mice	Gastrodin	A Chinese herbal medicine	↓	↓	↓	↑AMPK and Nrf2 pathway	[43]
In vitro							
HepG2 cells	Nigaichigoside F1 (NI)	A plant-derived bioactive substance	↓			↑Nrf2 nuclear translocation	[75]
Hepa1c1c7 cells	Ezetimibe (Eze)	A potent cholesterol absorption inhibitor (clinical); an Nrf2 activator				Nrf2 ↑ is dependent on p62 and ↑AMPK	[84]
HepG2 cells	S-Propargyl-cysteine	The substrate for endogenous H_2S synthesis via CSE catalysis	↓		↓	↑ cell viability; ↑PI3K/Akt signaling pathway; ↑HO-1 expression via activation of Akt/Nrf2 pathway	[78]
HL-7702 cells	Gastrodin	A Chinese herbal medicine		↓	↓	↑ LKB1/AMPK and Nrf2 pathways.	[43]
HepG2 cells	Punicalagin	A polyphenol abundant in pomegranate				↓lipotoxicity through activating ERK/Nrf2 pathway	[86]
Huh-7.5 cells	NK-252	A biaryl urea compound; an Nrf2 activator			↓		[71]

AKT, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; CSE, cystathionine γ-lyase; HO-1, heme oxygenase-1; MEF, mouse embryonic fibroblast; NAFLD, nonalcoholic fatty liver disease; PPARα, peroxisome proliferator–activated receptor α; NF-κB, nuclear factor-κB; NQO1, NADPH quinone oxidoreductase-1; SREBP-1c, sterol regulatory element-binding protein 1c.

Figure 3



Paradoxical roles of Nrf2 in NAFLD progression. NAFLD consists of a series of metabolism disorders, including steatosis, inflammation, fibrosis and cirrhosis. In the stage of steatosis, triglycerides are accumulated in hepatocytes, which produce more ROS and damage the cells. Nrf2 is believed to help reduce the effect. Extra lipid formation may be caused by increased lipogenesis and decreased β-oxidation and weaken the ability of export with VLDL. However, the reported effect of Nrf2 was controversial. Steatosis and redox homeostasis in hepatocytes may recruit proinflammatory cytokines secreted by KCs and profibrotic factors secreted by HSCs. Upon now, research about the association between Nrf2 and fibrosis in NAFLD is not enough to draw the conclusion. NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HSCs, hepatic stellate cells; KCs, Kupffer cells; ROS, reactive oxygen species; VLDL, very-low-density lipoprotein.

NAFLD patients [72]. Exercising 3 to 4 times a week designed to achieve a heart rate of 60–75% of the age-based maximum not only helps weight loss, but also enhances cardiopulmonary function and has other independent beneficial effects, which will finally improve NAFLD [73]. However, for whatever reasons, only half of the patients with NAFLD can complete their weight loss task to alleviate NAFLD [74]. Thus, drug therapies for NAFLD are highly desired, even though there are no approved drugs in clinic yet.

It is clear that Nrf2 is a potential therapeutic target for NAFLD because of its crucial roles in oxidative stress management, control of anti-inflammatory response and lipid metabolism. Most studies of Nrf2 modulation therapies have been conducted in mouse models. In recent years, presumably to avoid synthetic toxicities, more and more researchers are looking to natural (from plants) and dietary Nrf2 inducers, such as nigaichigoside F1 [75], scutellarin [76], apigenin [42] and hydroxytyrosol [77]. Various natural compounds with Nrf2-activating effect have been tested in mouse or cell models. The results are summarized in Table 3.

One potential mechanism for the compounds against NAFLD is their ability to activate hepatic system against oxidative stress [42,43,71,75,76,78–83] and inflammation [43,71,77,79,80,82,84], which are at least in part in an Nrf2-dependent manner. In addition, these Nrf2 activators may affect lipid metabolism by influencing PPARα and SREBP1c, which are responsible for hepatic fatty acid oxidation and lipogenesis, respectively [42,43,77,81,82,85]. The most commonly used cell model for the test of Nrf2 activators in dealing with NAFLD is the HepG2 cells challenged with palmitate or oleate [75,78,86]. These *in vitro* studies have shown that Nrf2 may alleviate NALFD via affecting phosphatidylinositol 3′-kinase(PI3K)-Akt [78], extracellular signal regulated protein kinases (ERK) [86] and liver kinase B1 (LKB1)/AMPK pathways [43].

There are no Nrf2-ARE modulators in clinical trials for NAFLD treatment. The reason may be partially attributed to the cardiac disorders and gastrointestinal toxicities caused by synthetic Nrf2 inducers in previous clinical trials [87,88].

Conclusions and future perspectives

NAFLD is a group of chronic disorders involving impaired lipid metabolism, oxidative damage, inflammation, antioxidant and inflammatory responses and fibrosis. Considering the contribution of oxidative stress in these pathophysiologic events, Nrf2 has been extensively investigated as a potential therapeutic target of the disorders. Extensive studies with *Nrf2* transgenic mice and Nrf2 activators have shown that Nrf2 activation can improve lipid metabolism and protect the liver from oxidative stress and inflammation. However, many conundrums concerning Nrf2 and NAFLD still remain unclear.

Accumulating evidence indicates that Nrf2 may play paradoxical roles, which might be cell- and/or stage-specific, in the progression of NAFLD (Figure 3). The contradictory phenotypes of Nrf2 modifications on the development of NAFLD tested in various *in vivo* and *in vitro* models reveal the complexity of the disease and suggest that more precise studies are needed. Since NAFLD is a result of complex pathophysiological events concerning various types of liver cells, including hepatocytes, KCs and HSCs, Nrf2 in these cells may contribute differently to the progression of the disorders. As shown in Figure 3, NAFLD may be derived from the disturbance of lipid metabolism in hepatocytes, followed by activation of KCs and the release of cytokines. Sustained oxidative stress and inflammatory response may induce fibrosis via stimulation of HSCs to overproduce extracellular matrix. Here, we hypothesized that Nrf2 in the liver may play different roles in the progression of NAFLD. In the early stages of NAFLD, Nrf2 in hepatocytes plays crucial roles in regulating lipid metabolism and redox balance. With the advance to steatohepatitis, the role of Nrf2 in KCs become evident, which alleviates inflammation in the liver. At the end stage of NAFLD, Nrf2 in HSCs may be critical in modulating the formation of fibrosis.

To our knowledge, there is no research that concentrates on the specific roles of Nrf2 in KCs and HSCs in the development of NAFLD. Thus, more precise studies with proper animal and cell models are required to clarify the specific roles of Nrf2 in NAFLD-related cell types and assess its suitability as a therapeutic target to treat NAFLD.

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Conflict of interest

Nothing declared.

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