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Nuclear factor erythroid 2-related factor 2-mediated signaling and metabolic associated fatty liver disease

Bukke VN *et al.* NRF2 and MAFLD

Abstract

Oxidative stress is a key driver in the development and progression of several diseases, including metabolic associated fatty liver disease (MAFLD). This condition includes a wide spectrum of pathological injuries, extending from simple steatosis to inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Excessive buildup of lipids in the liver is strictly related to oxidative stress in MAFLD, progressing to liver fibrosis and cirrhosis. The nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of redox homeostasis. NRF2 plays an important role for cellular protection by inducing the expression of genes related to antioxidant, anti-inflammatory, and cyto-protective response. Consistent evidence demonstrates that NRF2 is involved in every step of MAFLD development, from simple steatosis to inflammation, advanced fibrosis, and initiation/progression of HCC. NRF2 activators regulate lipid metabolism and oxidative stress alleviating the fatty liver disease by inducing the expression of cytoprotective genes. Thus, modulating NRF2 activation is crucial not only in understanding specific mechanisms underlying MAFLD progression, but also to characterize effective therapeutic strategies. This review aims to give an outline of the current knowledge on the effects of NRF2 pathway, modulators, and mechanisms involved in the therapeutic implications of liver steatosis, inflammation, and fibrosis in MAFLD.

Key Words: Non-alcoholic fatty liver disease; Metabolic-associated fatty liver disease; Nuclear factor erythroid 2-related factor 2; Oxidative stress; Antioxidants; Liver injury

Bukke VN, Moola A, Serviddio G, Vendemiale G, Bellanti F. Nuclear factor erythroid 2-related factor 2-mediated signaling and metabolic associated fatty liver disease. *World J Gastroenterol* 2022; In press

Core Tip: This updated literature review contributes to the role of the nuclear factor erythroid 2-related factor 2 (NRF2) in combating inflammation, oxidative stress, steatosis, and fibrosis in metabolic associated fatty liver disease (MAFLD). There are several reviews that elucidate the advantages of NRF2 in human diseases, but this is the first review reporting the broad range of NRF2 modulators and their therapeutic implications in MAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease, affecting about 25% of the global population. Due to the reappraisal in its nomenclature, a group of experts changed the acronym NAFLD to Metabolic associated fatty liver disease (MAFLD), strengthening the link of this disease to metabolic alterations^[1]. MAFLD is defined as a condition where hepatic fat accumulation exceeds 5% of the liver weight, without alcohol consumption (< 30 g per day). It covers a wide spectrum of pathological conditions, extending from simple steatosis (deposit of fat in hepatocytes), to non-alcoholic steatohepatitis (NASH, characterized by the presence of 5% hepatic steatosis and inflammation with hepatocellular damage, whether or not any fibrosis), cirrhosis, and ultimately leading to hepatocellular carcinoma (HCC)^[2]. MAFLD is emerging with the prevalence of type 2 diabetes mellitus, obesity, and metabolic syndrome^[3]. Of note, patients with MAFLD and particularly with NASH exhibit an increased liver-related mortality rate and higher incidence of cardiovascular-related morbidity and mortality^[2].

MAFLD is considered as the hepatic expression of metabolic syndrome, but its pathogenesis is still not clearly known. Insulin resistance (IR) seems to play a key role in the initiation and progression of the disease from simple fatty liver to advanced forms^[4]. MAFLD pathogenesis is complex and multifactorial. The first theory was based on a two-hit hypothesis, where the first hit is liver steatosis, which is due to increased hepatic lipogenesis and reduced free fatty acid degradation caused by IR. This alteration is followed by the second hit of oxidative stress, which induces hepatocyte inflammation and cell death^[5,6]. However, this simplistic theory has been recently replaced by the multiple-hit hypothesis, where many factors including systemic and hepatic IR, intestinal microbiota, genetic predisposition, oxidative stress act simultaneously resulting in a cascade of detrimental effects such as hepatic inflammation, free radical production from

gut and adipose tissue, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and hepatocyte apoptosis^[7]. Among all the contributing factors of MAFLD, oxidative stress plays a major role. Oxidative stress promotes inflammation by activating Kupffer cells and stimulating the release of pro-inflammatory cytokines, directly leading to lipid, protein, and DNA/RNA damage. Nuclear factor erythroid 2-related factor 2 (NRF2) is the most important transcription factor in preserving redox homeostasis in the cell and counteracting oxidative or electrophilic stress by producing antioxidant and cytoprotective enzymes such as heme oxygenase 1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), and those involved in glutathione (GSH) metabolism^[8].

Thus, due to its antioxidative and detoxicant properties, it is currently accepted that NRF2 plays a pivotal role and has been recognized as a potential target to prevent the pathological spectrum of MAFLD. Even though the beneficial role of NRF2 in human diseases has been the topic of several recent reviews, the broad range of NRF2 modulators and their therapeutic implications in MAFLD were not completely summarized in recent literature. In this review, we describe the current knowledge on the effects of NRF2-dependent mechanisms involved in the therapeutic implications of liver steatosis, inflammation, and fibrosis in MAFLD.

NRF2 PATHWAY

NRF2 belongs to basic leucine zipper transcription factors in the Cap “n” Collar subfamily including seven functional domains, Nrf2-ECH homology (Neh) 1 to Neh7^[9]. Neh2 is important for interaction between NRF2 and Kelch-like ECH-associated protein 1 (Keap1), a negative modulator of NRF2^[10]. Keap1 is a substrate for Cullin based E3 ubiquitin ligase. During homeostatic conditions, Keap1 targets NRF2 that is localized in cytoplasm, causing its polyubiquitination and degradation. The binding and regulation of NRF2 by Keap1 has been defined as “hinge and latch model”^[11]. During oxidative stress, hyperactive cysteine residues of Keap1 undergo thiol modification and NRF2 is dissociated from Keap1, preventing ubiquitination and proteasomal degradation (Figure 1). The newly generated NRF2 escaped from Keap1 control translocates to the nucleus and heterodimerizes with the Maf proteins, promoting the expression of antioxidant response element (ARE) genes like HO-1, superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GSH-Px), NQO1, etc^[12].

Of note, emerging evidence revealed Keap1-independent novel mechanisms of NRF2 regulation. The phosphatidylinositol 3'-kinase (PI3K)/AKT pathway is protective against oxidative stress and is able to activate NRF2 signaling^[13]. PI3K-AKT-NRF2 signaling pathway involves the glycogen synthase kinase-3 β (GSK-3 β) as a key mediator. GSK-3 β can phosphorylate the NRF2 domain Neh6, containing serine residues that can be recognized by the β -transducin repeats-containing protein (β -TrCP). β -TrCP is a substrate receptor for ubiquitin ligase complex, which targets NRF2 for ubiquitination and proteasomal degradation^[14,15]. During autophagy, NRF2 is stabilized by the binding of p62 (autophagy substrate) to Keap1 at NRF2 binding site, resulting in the transcriptional activation of NRF2-target genes^[16,17]. Besides, oxidative stress-induced protein kinase C phosphorylates Neh2 at serine and threonine residue on Ser40, dissociating Keap1 homodimer and transferring NRF2 to the nucleus, thus binding to the ARE-mediated cytoprotective genes^[18] (Figure 2).

NRF2 IN THE PATHOGENESIS OF MAFLD

MAFLD is the most widespread chronic liver condition worldwide, potentially leading to end stage disease which requires liver transplantation^[19,20]. MAFLD is a lipotoxic disease characterized by both structural and functional mitochondria abnormalities and oxidative stress. Impairment in mitochondrial electron transport chain causes excessive production of reactive oxygen and nitrogen species (ROS and RNS)^[21]. ROS and RNS play a crucial role in cellular signaling, proliferation and differentiation, metabolism and immune defense mechanisms. Besides mitochondria, ROS and RNS are continuously produced by ER and peroxisomes as byproducts during their normal physiological processes. Oxidative stress is described as the imbalance between production of ROS/RNS and anti-oxidant systems^[22]. Oxidative stress is intrinsically linked to the pathogenesis of MAFLD, and NRF2 has been found to be a key regulator to protect against the hepatocellular injury. Since MAFLD development and progression are characterized by alterations of redox balance, NRF2 is involved in every stage of disease, from simple steatosis to inflammation, advanced fibrosis and initiation/progression of HCC^[8].

Nrf2 and liver steatosis

Accumulation of lipids in hepatocytes is the first step characterizing MAFLD development. This process is the result of increased fatty acid uptake/synthesis and decreased fatty acid oxidation/removal^[23]. Fatty acid oxidation in peroxisomes produces H₂O₂, which in turn decreases the expression of enzymes involved in fatty acid oxidation as carnitine palmitoyltransferase 1A, and acyl-CoA oxidase through their regulatory factor peroxisome proliferator activated receptor alpha. Besides, H₂O₂ promotes lipid accumulation by upregulating the expression of sterol regulatory element-binding protein-1c (SREBP-1c), which further activates fatty acid synthase (FAS) and stearoyl coenzyme-A desaturase 1 (SCD1), contributing to MAFLD pathogenesis^[24]. In addition, ER-stress activates SREBP-1c and increases the expression of hepatic very-low density lipoprotein receptor, leading to deposition of triglycerides (TG)^[12,24]. NRF2 is a key player in maintaining cellular homeostasis, suppressing MAFLD promotion and progression. A microarray analysis of mouse hepatic gene expression revealed that pharmacologic and genetic activation of NRF2 suppresses key enzymes involved in lipid synthesis and reduces hepatic lipid storage: indeed, NRF2^{-/-} mice fed a high-fat diet (HFD) are more prone to develop steatosis and oxidative stress than wild-type mice^[25]. Consistent to this, NRF2-knockout mice fed a methionine- and choline-deficient (MCD) diet developed a severe form of micro- and macrovesicular steatosis and neutrophil recruitment compared to wild-type mice^[26-28]. Studies on hepatic protein expression in NRF2-null and wild-type mice found two major groups of NRF2-modulated proteins. One group of proteins in NRF2 wild-type animals is implicated in phase II drug metabolism and antioxidant defense, while the other group of proteins in NRF2-null animals is involved in lipid and fatty acid synthesis and metabolism^[29]. Another study in NRF2-null 8-wk old mice revealed a higher expression of SREBP-1c and FAS than wild-type mice^[30]. Nonetheless, NRF2 has little effect on hepatic fatty acid metabolism in 12-25 wk old mice^[31,32].

In addition, the flavonoid glycoside scutellarin ameliorates MAFLD pathogenesis by reducing blood lipid levels and enhances antioxidant capacity by activating peroxisome proliferator-activated receptor gamma (PPAR-γ) and its cofactor-1α (PGC-1α), as well as NRF2-dependent enzymes HO-1 and GST. Moreover, scutellarin suppresses the nuclear factor κ B (NF-κB), and Keap1 mitigating MAFLD^[33]. Another study revealed that scutellarin contains breviscapine as its active component, exerting its antioxidant effects possibly through PI3K/AKT activation and subsequent enhancement of NRF2 nuclear

translocation, ² increasing the expression of HO-1 and NQO1. Thus, breviscapine could be used in MAFLD and hyperlipidemia due to its potential therapeutic effects^[34].

Besides, the food-derived compound apigenin is a modulator of PPAR- γ which attenuates NRF2-associated antioxidative response and hepatocyte lipid metabolism in MAFLD^[35]. The specific deletion of NRF2 in mice diminished the signs of MAFLD induced by high fat diet, decreasing the accumulation of TGs. Hepatic NRF2 deficiency dampens the expression of PPAR- γ , suggesting that the NRF2-dependent expression of PPAR- γ is critical in initiation and progression of MAFLD^[36].

Liver X receptors (LXRs) are a family of ⁷⁰ nuclear receptors implicated in the modulation of lipid homeostasis. Directly or *via* SREBP-1c, LXR α triggers ⁴⁰ the expression of lipogenic genes involved in the uptake and synthesis of fatty acids, TGs, cholesterol, and phospholipids. Treatment with the NRF2 activator sulforaphane suppresses T0901317-induced lipogenesis, promoting deacetylation of farnesoid X receptor (FXR) by competitive binding of p300, a protein necessary for the acetylation of FXR. FXRE ChIP assay confirmed that NRF2 may complex with p300 and, as a result, it gets dissociated from the FXR complex^[37-39]. Moreover, NRF2 activator inhibits SREBP-1c and lipogenic genes by promoting deacetylation of FXR and inducing small heterodimer partner (SHP), which accounts for the repression of LXR α -dependent gene transcription, protecting the liver from excessive fat accumulation^[40].

Nrf2 and liver inflammation

³ NRF2 is further involved in the regulation of pro- and ³⁶ anti-inflammatory mediators. NRF2 is known for its anti-inflammatory effects as it inhibits ³ the expression of pro-inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor (TNF), and inducible nitric oxide synthase. Moreover, NRF2-dependent antioxidant genes as HO-1, NQO1, glutamate cysteine ligase catalytic (GCLc) and modifier (GCLm) subunits inhibit ⁴⁷ the transcription of ³ pro-inflammatory mediators by blocking NF- κ B activation^[41-43]. Of note, NRF2 also triggers the NLR family pyrin domain containing 3 (NLRP3) inflammasome, which cleaves caspase-1 and initiates ³ the processing of pro-IL-1 β to mature IL-1 β ^[44]. NLRP3-dependent production of pro-inflammatory response can be inhibited by activation of NRF2 through dimethyl fumarate in alcoholic liver disease^[45], and 4-Acetylanthroquinol

⁵² B in mice fed with MCD diet^[46] inducing the expression of NQO1, which inhibits the ROS/RNS-dependent priming.

NRF2-KO mice fed the MCD diet lose the antioxidant and detoxification enzymes and show an increase in steatosis, inflammation, oxidative stress, lipid peroxidation, and fibrinogenesis^[26,28]. In line with these results, feeding the NRF2-KO mice with the HFD yield in significantly greater amounts of lipids and inflammation compared to wild type mice. NRF2-KO mice fed a diet containing 4% soyabean oil and 16% lard for 12 wk exhibit massive lipid accumulation, inflammation, oxidative stress, and iron accumulation when compared to their wild-type counterparts^[47]. NRF2-KO mice fed a diet containing 45 kcal% fat (0.02% cholesterol) for 24 wk displayed a higher MAFLD activity score compared to wild-type animals. In HFD-fed NRF2-KO mice, livers scored higher steatosis, ballooning, inflammation, and fibrosis when compared to Nrf2^{+/+} mice. The biochemical characterization studies of such mice revealed higher expression of sterol regulatory element binding transcription factor 1 (Srebf1), and 2 (Srebf2), and carbohydrate response element binding protein also known as MLX-interacting protein-like (Chrebp/Mlxip) in HFD-fed NRF2-KO mice, suggesting exaggerated lipogenic transcription^[48]. In another study, NRF2-KO mice fed a high-fat plus 30% fructose (HF30Fr) in drinking water exhibit a higher MAFLD score than wild-type. Moreover, these NRF2-KO mice overexpress lipogenic transcription factor Srebf1, FASN, SCD1, CD36, and also exhibited higher pro-inflammatory factors as NF- κ B p65 and p50 subunits^[49].

In another investigation, NRF2-KO mice fed a chow diet are subjected to scanty inflammation with minimal increase in IL-1 β , Cox2, and Nos2 mRNA^[26,28]. This is due to the compromised expression of zonula occludens-1 and claudin-1, which are responsible for the translocation of LPS from gut microbiota to the liver through portal vein. In addition, the phagocytic ability of Kupffer cells is diminished in NRF2-KO due to lower expression of macrophage receptor with collagenous structure that restricts TLR4 signaling and boosts the inflammatory response on exposure to LPS^[50].

²¹ *Nrf2 and liver fibrosis*

⁴ Liver fibrosis is a reversible wound healing response and degenerative condition caused by extensive deposition of extracellular matrix proteins like collagen fibrils^[51]. Mechanisms underlying liver fibrosis include the activation of both hepatic stellate cells

and Kupffer cells, resulting in functional and biological alterations^[52]. Oxidative stress is a serious process involved in liver damage, and the activation of KEAP1/NRF2 pathway plays a protective role in liver fibrosis^[12]. NRF2 activation triggers the reverse IR and attenuates liver fibrosis by inhibiting the hepatic steatosis. These noticeable effects during the NRF2 activation are due to the disruption of JAK2/STAT3 signaling and higher expression of suppressor of cytokine signaling 3 (SOCS3)^[53]. Moreover, administration of fibroblast growth factor 1 (FGF1) variant carrying substitutions of heparin-binding sites in 9-month-old mice inhibit activity and expression of lipogenic genes, improving both steatohepatitis and fibrosis^[54].

CCl₄-induced hepatic fibrosis is accompanied by elevated serum transaminases, alkaline phosphatase, bilirubin, decreased albumin and increased pro-inflammatory cytokines. Besides, CCl₄-intoxicated rats display increase in NF-κB, p65, malondialdehyde (MDA), and decrease in antioxidants. Bone marrow-derived mesenchymal stem cells show favorable effects in ameliorating the hepatic effects of CCl₄ through NRF2/HO-1 signaling, suppressing liver fibrosis, inflammation and oxidative stress^[55].

A major bioactive extract from the plant *Schisandra chinensis*, known as Schisandrin B, exerts anti-inflammatory, anti-tumor, antioxidative, and hepatoprotective properties. Schisandrin B effectively improves liver function and decreases collagen deposition in the CCl₄-induced liver fibrosis in rats, through the modulation of NRF2-ARE and TGF-β/Smad signaling pathways^[56]. Tanshinol, a water-soluble compound isolated from *Salvia miltiorrhiza* Bunge, is known to exert a variety of biological effects, including anti-fibrotic effects. Rats with CCl₄-induced liver fibrosis treated intraperitoneally with tanshinol show lower serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin, as well as circulating hyaluronic acid, laminin, type IV collagen, and procollagen III peptide as compared to controls. Tanshinol is also able to suppress the expression of inflammatory cytokines such as TGF-β, TNF, Cox-2, IL-1β, and IL-6 through regulation of the NF-κB pathway. In addition, tanshinol treatment is able to regulate the NRF2/HO-1 signaling pathway increasing SOD and GSH-Px and decreasing MDA levels. In this regard, tanshinol exerts protective effects on CCl₄-induced liver fibrosis by activating the NRF2 pathway^[57].

Asiatic acid (AA), a bioactive compound extracted from *Centella asiatica*, is known to have anti-inflammatory, antioxidative, and hepatoprotective properties^[19-22]. Fan *et al*^[34]

showed that treatment with AA in the CCl₄-induced liver fibrosis dramatically ameliorates oxidative stress, inflammation, and fibrosis in rats. The nuclear NRF2 levels were increased after AA treatment, and the NRF2-dependent proteins like HO-1, NQO-1, and GCLC were significantly increased to counteract oxidative stress. Furthermore, AA inhibited the NF-κB/IκBα and JAK1/STAT3 signaling pathway to suppress the activation of hepatic stellate cells and the production of inflammatory markers, suggesting that AA could be used for the treatment of liver fibrosis^[58]. Another water soluble compound, salvianolic acid A (SAA), extracted from a traditional Chinese herb *Radix Salvia miltiorrhiza*, was found to have anti-fibrotic effects. SAA is able to modulate the NRF2/HO-1, NF-κB/IκBα, p38 MAPK, and JAK1/STAT3 signaling pathways, and to ameliorate the CCl₄-induced liver fibrosis, improve morphology and attenuate collagen deposition in the fibrotic liver. Besides, SAA is able to increase the levels of SOD, GSH-Px, and decrease the MDA levels, indicating the effectiveness in preventing liver fibrosis by inhibiting inflammation and oxidative stress^[59]. Pharmacological stimulation of NRF2 by acetylenic tricyclic bis (cyano enone) TBE-31 reverses IR in wild-type mice, decreases liver steatosis by increasing hepatic fatty acid oxidation and reducing ER stress, and lessens markers of oxidative stress, apoptosis, and fibrosis. Of note, histology studies show that TBE-31 decreases the fibrosis score and MAFLD activity score^[59]. In another study, NRF2 activator NK-252 (1-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)-3-(pyridin-2-ylmethyl)urea), significantly reduces markers of fibrosis like COL1A1, TIMP-1, and TGF-β in rats, suggesting that this compound could be used as a therapeutic agent to reverse liver fibrosis. In addition, NK-252 attenuates the serum AST and ALT levels in male Fischer rats and upregulates NQO1 gene expression^[60].

THERAPEUTIC IMPLICATIONS OF NRF2 IN MAFLD

Currently, there is no medicine that can treat MAFLD, but some therapeutic agents are useful in managing the problems associated with the disease (Table 1). Thus, it is necessary to develop and test drugs for the prevention and treatment of the MAFLD, and it is conceivable that NRF2-activating compounds can attenuate MAFLD progression. Plant-derived compounds including resveratrol, curcumin, quercetin, and synthetic molecules like oltipraz, pirfenidone, could be used to prevent oxidative stress by modulating NRF2 pathway^[122].

Flavonoids represent a class of bioactive antioxidants extracted from vegetables, plants, and fruits known to exhibit therapeutic properties in MAFLD. The flavonoid 7-Mono-O-(β -hydroxyethyl)-rutoside activates NRF2 and improves the ratio of GSH/glutathione disulfide, increases the expression of HO-1 and GSH-Px³^[61,62]. The flavonoid scutellarin (4',5,6-trihydroxy flavonoid-7-glucuronide) increases NRF2 protein in C57BL/6J mice increases the expression of HO-1, GST, and NQO1, and inhibits both NF- κ B and Keap1^[33]. Furthermore, 7,8-dihydroxyflavone upregulates NRF2 activity to counteract alcohol-induced and HFD-induced liver toxicity^[63]. Apigenin (4',5,7-trihydroxyflavone), a flavonoid derived from fruits, inhibits lipid peroxidation and exerts protective effects against hepatic steatosis. Moreover, apigenin increases the activities of SOD, CAT, and GSH-Px^[35,64].

Gastrodin (GSTD) is a water-soluble extract of *Gastrodia elata* BI which exerts antioxidative activity and improves lipid metabolism in MAFLD mice by promoting NRF2 nuclear translocation^[65]. Clusterin, a glycoprotein extracted from ram rete testis fluid, improves steatosis and hepatitis induced by methionine and choline-deficient diet by triggering NRF2 and HO-1 expression^[66]. Osteocalcin treatment improves hepatic TG accumulation, promotes NRF2 nuclear translocation, and inhibits phosphorylation of c-Jun N-terminal kinase pathway^[67].

Besides, compounds like scutellarin containing breviscapine, hesperitin, apigenin, scoparone, Schisandrin B, tanshinol, AA and other tabulated compounds are known to exert antioxidative, and hepatoprotective activity by modulating NRF2 pathway.

CONCLUSION

Oxidative stress can be a potent inducer of inflammation and fibrosis in the spectrum of chronic liver diseases. Among them, MAFLD is the most widespread chronic liver condition worldwide. The transcription factor NRF2 has gained importance in recent years as a possible therapeutic target for the treatment of liver diseases. The expression of antioxidant protective genes through NRF2 pathway counteracts oxidative stress and prevents progression of liver damage in MAFLD. The different antioxidative molecules modulating the NRF2 pathway have exerted beneficial effects in ameliorating the liver damage. Currently, there is no efficient treatment to counteract the complex

pathophysiology of liver diseases. Thus, compounds having antioxidative properties could be useful candidates for the treatment of liver diseases by modulating the NRF2 signaling pathway. NRF2 activators could improve and prevent the advanced stages of MAFLD such as liver fibrosis and liver cirrhosis. Natural plant-derived and synthetic NRF2 activators require further experimental validation to be promoted as efficient therapeutic agents. Some drugs entered clinical trials and further attempts are ongoing to find NRF2 inducers with high bioavailability, safety, and specificity.

Figure Legends

Figure 1 Kelch-like ECH-associated protein 1-dependent nuclear factor-erythroid 2-related factor 2 signaling. During oxidative stress, nuclear factor-erythroid 2-related factor 2 detaches from kelch-like ECH-associated protein 1 and translocates to the nucleus to bind the target genes. In normal conditions, NRF2 is ubiquitinated and undergoes degradation. NRF2: Nuclear factor-erythroid 2-related factor 2; SMaf: Small musculoaponeurotic fibrosarcoma oncogene homologue; Keap1: Kelch-like ECH-associated protein 1; ARE: Antioxidant responsive element.

Figure 2 Kelch-like ECH-associated protein 1-independent nuclear factor-erythroid 2 signaling. During oxidative stress, selective autophagy substrate p62 could compete with nuclear factor-erythroid 2 (NRF2) to bind with kelch-like ECH-associated protein 1 (Keap1) as a consequence, NRF2 dissociates from Keap1 and translocates to the nucleus to induce target genes. Glycogen synthase kinase 3 β (GSK-3 β) phosphorylates the NRF2 subunit Nrf-ECH homology (Neh) 6, leading to degradation by β -transducin repeats containing protein, and phosphatidylinositol 3'-kinase-AKT signaling could inhibit GSK-3 β . Protein kinase C phosphorylates Ser40 in Neh2, inducing NRF2 translocation to the nucleus. NRF2: Nuclear factor-erythroid 2-related factor 2; SMaf: Small musculoaponeurotic fibrosarcoma oncogene homologue; Keap1: Kelch-like ECH-associated protein 1; ARE: Antioxidant responsive element; β -TrCP: β -transducin repeats containing protein; GSK-3 β : Glycogen synthase kinase 3 β .

Table 1 Modulators of nuclear factor erythroid 2-related factor 2 pathway in metabolic associated fatty liver disease

Compound name	Species	Diet/duration	Treatment	Key findings	Reference
MonoHER	Female C57BL/6J (Ldlr ^{-/-})	mice High fat and high cholesterol/13 wk	Administered subcutaneously at a dosage of 500 mg/kg of body weight (25 μ L/g of body weight)	daily NRF2 activation, \uparrow GSH/GSSG ratio, \uparrow HO-1, GSH-Px	[62]
Scutellarin	Male C57BL/6 hepaG2 cells Sprague-Dawley rats	mice High fat/10 wk High fat/12 wk	Administration of 12.5, 25.0, and 50.0 mg/kg per day Administered orally 50, 100, and 300 mg/kg/d	\uparrow PPAR γ , PGC-1 α , NRF2, HO-1, [33] NQO1, Keap1, NF- κ B NRF2, HO-1, NQO1; PI3K/AKT activation	[34]
Apigenin	Male C57BL/6J mice	High fat/16 wk	Injected intraperitoneally 30 mg/kg daily for 3 wk	NRF2 activation; inhibition; SOD, CAT, GSH-Px	[35]
7,8-dihydroxyflavone	Male wistar rats	High fat, ethanol/12 wk	Administered intraperitoneally at 5 mg/kg/d for 4 wk	Amelioration of liver architecture, vesicular changes, infiltration; restored serum biomarkers like AST, ALT, and TC; \uparrow NRF2; \downarrow NF- κ B	[63]
Resveratrol	Male C57BL/6 mice	High fat/16 wk	Supplemented with 0.4% resveratrol in HFD for 16 wk	Attenuated liver steatosis; \uparrow NRF2 activation; attenuated HFD induced methylation of NRF2	[68]

Quercetin	HepG2 cells	-	Treated with Quercetin at 5-50 μ M concentrations for 0, 10, 30, 60, 120, 240, and 1080 min	promoter; \downarrow oxidative stress	[69]
²⁹ Curcumin	Male C57BL/6 mice	High fat and high fructose/8 wk	Administered orally 50 and 100 mg/kg/d for 4 wk	\uparrow CYP3A, CYP7A; regulation of NRF2/FXR/LXR α pathway; \downarrow SREBP-1C, FAS	[70]
(1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione)	Male Sprague-Dawley rats	High fat/6 wk	Administered orally 50 mg/kg daily for 6 wk	\downarrow Steatosis and inflammation; \downarrow Serum aminotransferases, lipids, and insulin resistance; \downarrow TNF, IL-6, MDA; \uparrow NRF2, GSH, HO-1, SOD	[71]
Oltipraz	Male Fischer 344 rats	Choline-deficient L-amino acid-defined/10 wk	Administered orally at 60 mg/kg/d for 9 wk	\uparrow NRF2 activation; antifibrotic and anti-inflammatory; \downarrow AST and ALT; \uparrow NQO1 gene expression	[61]
GSTD	HL-7702 cells, male C57BL/6J, male Sprague-Dawley rats	Oleic acid (OA)/24 h, high fat/10 wk; high fat and high	Cells were treated with GSTD for 24 h, administered orally at 10, 20, 50 mg/kg per day for 10 wk, administered orally at 20, 50 mg/kg per	\uparrow NRF2, HO-1, SOD; activate AMPK/NRF2; \downarrow pro-inflammatory response, and hepatic steatosis; \downarrow MDA, ROS	[65]

			cholesterol/10 wk	day for 10 wk	
NK-252	1-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)-3-(pyridin-2-ylmethyl)urea	Male Fischer 344 rats	Choline-deficient amino acid-defined (CDAA)/10 wk	Administered orally at 20, 60 mg/kg/d for 9 wk	Attenuated abnormalities; ↑antifibrotic effects; ↓TGF-β1, collagen α1; NRF2 activation; ↑NQO1 expression [61]
Clusterin	a)	Male hCLU-tg mice	MCD/3 wk	Generated specific overexpression (hCLU-tg) mice and fed with MCD diet	↓Hepatic TGs; less infiltration of [66]
				hepatocyte-clusterin transgenic	↓TNF; ↑NRF2 activation and mRNA of HO-1
Osteocalcin		Male C57/BL6J mice	High fat/12 wk	Injected intraperitoneally at concentration 3 ng/μL/d for 12 wk	↓Hepatic TG accumulation; [67]
Orlistat		Male Sprague-Dawley rats	High fat/12 wk	Administered at 10 mg/kg/d for 12 wk	↑NRF2 activation; ↑CAT, SOD, GSH-Px; ↓JNK activation [72]
					protection against insulin resistance, hyperlipidaemia, oxidative stress,

<i>Garcinia Cambogia</i>	Male C57BL/6N mice	High fat/8 wk	Administered 200, 400 mg/kg/ d for 8 wk	and liver injury ↑NRF2 ⁶⁵ activation; production; suppressed lipogenic factors C/EBPα and PPARγ; ⁴⁵ suppressed apoptosis by normalizing Bcl-2/BAX ratio and PARP cleavage	↓ROS [73]
HTT	Male Sprague-Dawley rats, 3T3-L1 murine embryo fibroblast cells	High fat/4 wk, 3T3-L1 cells treated with 3T3-L1 ⁵⁶ cells treated with FBS/DMEM for 8 d	Administered orally HTT at 350, 700, and 1400 mg/kg/d, HTT at 500 μg/mL for 24 h or 48 h	↑NRF2-HO-1 activation, antioxidant activities; inhibited liver weight gain; reduced lipid profile; improved liver function; HTT promoted lipolysis and increased antioxidant activities in 3T3-L1 cells	[74]
Hesperitin	HepG2 cells, male wistar rats	OA/24 h, high fat/16 wk	Treated cells at 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 μM; administered 100 mg/kg in 0.5% CMC-Na	Alleviated hepatotoxicity and oxidative stress by increasing SOD, GSH-Px, GCLC, and HO-1; ↑NRF2 activation; suppressed OA induced inflammation; reduced TC, TGs, and LDLC in a dose-	[75]

dependent manner

Glucoraphan in	Male C57BL/6JSlc mice	High wk	fat/14	Administered glucoraphanin orally for 14 wk	0.3%	Decrease in weight gain; improved insulin resistance; reduced hepatic steatosis and oxidative stress; decrease in circulating LPS; ↑NRF2 activation; ↑energy expenditure and; UCP1 protein expression	[76]
<i>Scutellaria baicalensis</i> extract	Male KK-A ^y mice	1% Orotic acid and 33% Sugar/7 d	acid	Supplemented with diet for 7 d		Diminished increase in liver weight; attenuated hepatic steatosis; ↑NRF2 expression; suppress SREBP-1c gene and protein expression	[77]
Ginkgolide B	Male C57/BL6 ApoE ^{-/-} mice, HepG2 cells	High fat/5 wk, 100 palmitic acid (PA) and 200 μM OA/24 h	fat/5 wk, μM acid and 200 μM OA/24 h	Administered orally at 20, 30, μM and 1.3 mg/kg/d; treated cells at dosages 0, 1, 2, 4, 8, 16, and 32 μg/mL		NRF2 activation; inhibition of oxidative stress and lipid peroxidation through NRF2 pathway; increase in HO-1, GSH- Px4	[78]
Scoparone	Male C57BL/6 J mice, AML2 and RAW264.7 cells	Methionine- choline	Methionine- choline	Administered intraperitoneally for 4 wk	daily	Ameliorated inflammation; improved hepatic	[79]

deficient/4 wk; AML12/300 μ M PA and RAW264.7/10 μ M/20, 40, and 80 mg/kg; autophagy; suppressed inflammation by inhibiting ROS/P38/NRF2 axis and PI3K/AKT/mTOR pathway

RAW264.7/10 μ M/

Chloroquine

DA Male C57BL/6J HL7702 cells mice, High fat/12 wk, 0.6 mM OA/24 h Administered by gavage at 10, and 20 mg/kg/d for 9 wk; treated with 2.5, 5.0, and 10.0 μ M DA Ameliorated liver ferroptosis in [80] mice and cells; improved oxidative stress and lipid peroxidation *in vivo*; \uparrow NRF2-HO-1 expression; \uparrow GSH, GSH-Px4

Silibinin Male C57BL/6 NCTC-1469 cells mice, Methionine-choline-deficient/6 wk, OA plus PA/24 h Administered by gavage at 10, and 20 mg/kg/d for 6 wk, 0.25 mM/L PA and 0.5 mM/L OA/ 24 h Prevented CFLAR-JNK pathway; [81] \uparrow β -oxidation and; efflux of fatty acids; \uparrow expression of CAT, GSH, GSH-Px, and HO-1; \downarrow expression of CYP2E1 and CYP4A; \uparrow NRF2 activation

Chicoric acid Male C57BL/6 mice High fat/9 wk Administered by gavage at 15, 30 mg/kg/d for 9 wk Attenuated hyperglycemia, [82] dyslipidemia, and systemic inflammation; alleviated hepatic

lipid accumulation and oxidative stress; suppressed hepatic inflammation and NF-κB pathway; ↑NRF2/Keap1 activation; improved gut microbiota

Carbon monoxide releasing molecule-A1	Male C57BL/6J mice	High wk	fat/16	Administered intraperitoneally 2 mg/kg/d for 7 wk	↑NRF2/ARE activation; improved lipid homeostasis; ↑ATP production; improved mitochondrial biogenesis; ameliorated oxidative stress
					[83]

3 GSH: Reduced glutathione; GSSG: Oxidized glutathione; HO-1: Heme oxygenase-1; GSH-Px: Glutathione peroxidase; PPAR-γ: Peroxisome proliferator-activated receptor-γ; PGC-1α: Proliferator-activated receptor gamma coactivator-1α; NQO1: NAD(P)H quinone oxidoreductase 1; DA: Dehydroabietic acid; PA: Palmitic acid; NF-κB: Nuclear factor κ B; PI3K: Phosphatidylinositol 3'-kinase; SOD: Superoxide dismutase; CAT: Catalase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TC: Total cholesterol; HFD: High-fat diet; GCS: Glutamylcysteine-synthetase; MAPK: Mitogen-activated protein kinase; CYP3A: Cytochrome P450, family 3, subfamily A, CYP7A Cytochrome P450, family 7, subfamily A; FXR: Farnesoid-X-receptor; LXRA: Liver-X-receptor α; SREBP-1C: Sterol regulatory element-binding protein-1c; FAS: Fatty acid synthase; TNF: Tumor necrosis factor; IL-6: Interleukin-6; MDA: Malondialdehyde; AMPK: AMP kinase; ROS: Reactive oxygen species; TGF-β1: Transforming growth factor-β1; TG: Triglycerides; JNK: c-Jun N-terminal kinase; C/EBPα: CCAAT/enhancer binding protein α; Bcl-2: B-Cell Leukemia/Lymphoma 2; BAX: BCL2 associated X protein; PARP: Poly-ADP ribose polymerase; HTT: Hedansanqi Tiaozhi Tang; GCLC: Glutamate cysteine

ligase catalytic; LDLC: Low density lipoprotein cholesterol; LPS: Lipopolysaccharide; UCP1: Uncoupling protein 1; GSH-Px4: Glutathione peroxidase 4; Mtor: Mammalian target of rapamycin; CFLAR: CASP8 And FADD Like Apoptosis Regulator; CYP2E1: Cytochrome P450 Family 2 Subfamily E Member 1; CYP4A: Cytochrome P450 Family 4 Subfamily A; ARE: Antioxidant response element.

31 %

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