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Nuclear Factor Erythroid 2-Related Factor 2 Versus Reactive Oxygen Species: Potential Therapeutic Approach on Fighting Liver Fibrosis

Lenny Setiawati[1,2,3], Isabella Kurnia Liem[1]*, Firda Asma'ul Husna[1]

[1]Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [2]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [3]Department of Anatomy, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

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***Correspondence:** Isabella Kurnia Liem, Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta Pusat 10430, DKI Jakarta, Indonesia. E-mail: bellajo04@gmail.com
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Abstract

Chronic liver disease (CLD) is a progressive deterioration of the liver due to exposure to viruses, drugs, fat accumulation, and toxicity which lead to an imbalance between extracellular matrix accumulation and degradation. Accumulation of the extracellular matrix is a normal liver response at the beginning of the injury. However, increasing extracellular matrix accumulation leads to fibrosis, cirrhosis, and organ failure. Until today, liver transplant is the gold standard therapy for end-stage CLD. Unfortunately, the liver transplant itself faces difficulties such as finding a compatible donor and dealing with complications after treatment. This review provides further information about nuclear factor erythroid 2-related factor 2 (Nrf2) as an alternative approach to fight liver fibrosis. Transformation of hepatic stellate cell (HSC) to myofibroblast has been known as the main mechanism that occurs in fibrosis while epithelial-mesenchymal transition (EMT) and mitochondrial dysfunction become the mechanism followed. In these conditions, oxidative stress is the great promoter which builds a vicious cycle leading to CLD progressivity. Hence, Nrf2 as antioxidant regulator becomes the potential target to break the cycle. While reactive oxygen species (ROS) in oxidative stress induce HSC activation, EMT, and mitochondrial dysfunction through activation of many signaling pathways, Nrf2 acts to diminish ROS directly by regulating secreted antioxidants and its scavenging action. Nrf2 also inactivates fibrosis signaling pathways and plays a role in maintaining mitochondrial health. Therefore, Nrf2 can be a potential target for liver fibrosis therapy.

Introduction

The utmost concern about chronic liver disease (CLD) is its progressivity leading to fibrosis, cirrhosis, organ failure, and death. Chronic exposure to causative agents such as viruses, drugs, fat accumulation, and toxic substances increases extracellular matrix accumulation more than its degradation, thereby resulting in damage to liver architecture, and impaired liver function which leads to organ failure. Global Burden Disease data in 2017 showed that cirrhosis caused about 2.4% of the total causes of death globally [1]. The data also showed that there was an increase in cirrhosis cases in 2017 by 30.5 million compared to 1990 [1]. Therefore, CLD therapies become urgently needed to improve patients' quality of life. Until now, liver transplant is the gold standard therapy for end-stage CLD, but finding a compatible donor and managing immunogenicity problems still become challenging issues [2].

The question which arises is whether there will be better alternative therapies for inhibiting the progressivity of CLD or even regenerating damaged liver. Therefore, understanding liver fibrosis mechanism is very important. The mechanism is started by prolonged exposure to causative agents on the liver will induce damage-associated molecular patterns (DAMPs) secretion, such as high-mobility-group-box 1 (HMGB1), S100 proteins, and heat shock protein-70 (HSP70) from damaged hepatocytes [3]. In this pattern, HMGB1 is the most studied DAMPs which have a role to promote endothelial-mesenchymal transition (EndoMT) and thereby become the other source of fibroblast besides hepatic stellate cell (HSC) activation [4]. DAMPs also activate Kupffer cells (KCs) to secrete chemotactic cytokines thus promoting the M1 pro-inflammatory monocytes recruitment and triggers a T-cell-mediated inflammatory process [5], [6].

From causative agent exposure until fibrogenesis, reactive oxygen species (ROS) become the key mediators involved in the mechanism. Damaged

hepatocytes will generate ROS which lead to oxidative stress, change the liver microenvironment, and cause mitochondrial dysfunction. Damaged mitochondria will generate more ROS, which make the damage getting worse. The vicious cycle between ROS, mitochondria dysfunction, and fibrosis will be greater over time unless the cycle is interrupted by an antioxidant system, the anti-ROS. Many studies revealed that the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), an antioxidant regulator, will reduce ROS, inflammation, and also inhibit the fibrosis signaling pathway. Nrf2 has been reported to have a role in inhibiting HSC activation, epithelial-mesenchymal transition – endothelial-mesenchymal transition (EMT-EndoMT), and mitochondrial dysfunction which are the important events induced by ROS in liver fibrosis (Figure 1) [7].

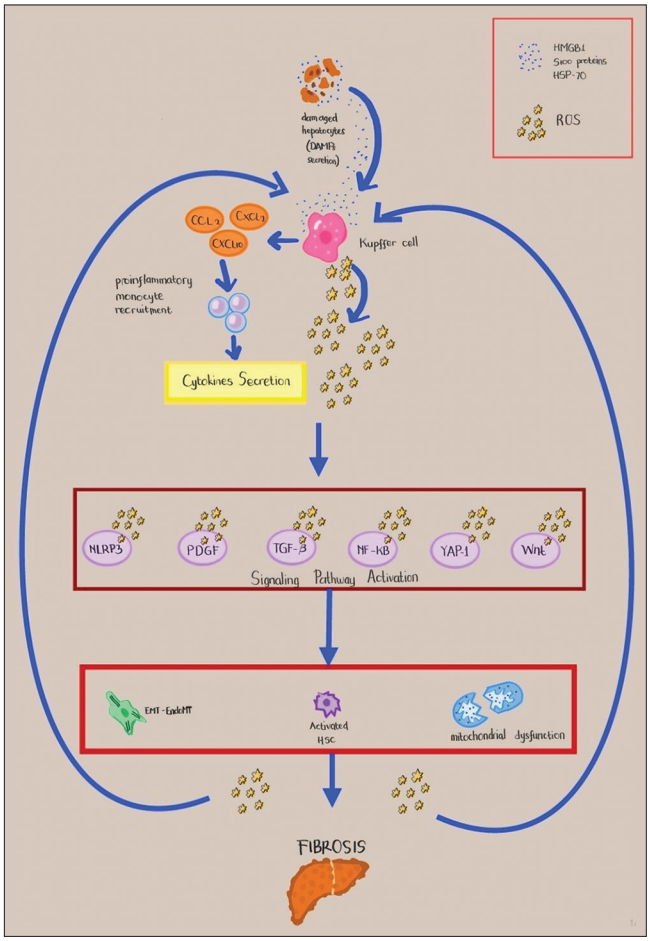

Figure 1: The role of reactive oxygen species in liver fibrosis

Reactive Oxygen Species in Hepatic Stellate Cell Activation and Epithelial-Mesenchymal Transition

Until today, HSC activation has been known as the major factor underlying liver fibrosis. For supporting its activation, activated HSC will secrete some other molecules such as all-trans retinoic acid (ATRA), a vitamin A metabolite, and thereby show morphological changes as reduced lipid droplets [8]. Recent studies showed that ATRA mediates crosstalk between HSC, KCs, and activated NODD-, LRR-and pyrin domain-containing protein3 (NLRP3) by upregulating ROS levels [8]. Activated HSC also secretes collagen triple helix repeat containing 1, a positive regulator of transforming growth factor beta (TGF-β) [9], and Kindlin-2, a TGF-β activator, which promote SMAD2/3 phosphorylation [10]. For supporting HSC migration, activated HSC have been known secreting nano-sized fibrogenic vesicles which promote HSC migration through platelet-derived growth factor (PDGF) and mammalian target of rapamycin signaling activation as well as autophagy inhibition [11]. This suggests that activated HSC has autocrine effects to promote self-activation.

In normal condition, ROS and antioxidant levels are balanced in the body. Physiologically, ROS can be generated from the electron transport chain in the mitochondria mainly by complexes I and III. The process of electron transport in mitochondria is mediated by NADPH Oxidase (NOX). Most of the NOX isoforms work using cytoplasmic NADPH as an electron donor which will produce superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) [12]. In this condition, only small amounts of ROS are generated and play a beneficial role in signaling pathways. Conversely, in oxidative stress, the increased ROS turn out to be detrimental. Increased ROS favor increased collagen and fibronectin which lead to fibrogenesis [13].

Oxidative stress also occurs in liver fibrosis (Figure 1). The TGF-β pathway has been known as the main pathway to activate HSC. TGF-β activates HSC through the canonical (SMAD2/3 dependent) and non-canonical pathways which interconnect TGF-β with other pathways such as PDGF, nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-KB), mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-kinase/Akt (PI3K/Akt) [14]. In TGF-β activation, ROS will cause conformational changes on latency-associated peptide (LAP), a protein which inactivates TGF-β, therefore inhibiting the binding between LAP and TGF-β (Figure 2). Free TGF-β will bind to its receptor and activate the TGF-β signaling pathway. Not only triggers TGF-β activation, ROS also amplify the effect of TGF-β in triggering fibrosis [15]. Furthermore, ROS also affect the upstream gene in the TGF-β pathway. Inhibition Annexin-A2-pseudogene 2 (Anxa2P2) gene [16] and Insulin-like growth factor 2 mRNA-binding protein 2 (Igf2bp2) gene [17] have been reported to inhibit the TGF-β pathway and thus attenuate fibrosis. Likewise, inhibition of ROS by exogenous compounds (Daphnetin, Physalin D, and Schisantherin A) has been shown to inhibit the TGF-β pathway, decrease HSC activation, and attenuate fibrosis [18], [19], [20].

However, other pathways such as PDGF, NF-KB, Yes-associated protein1 (YAP1), wingless Int

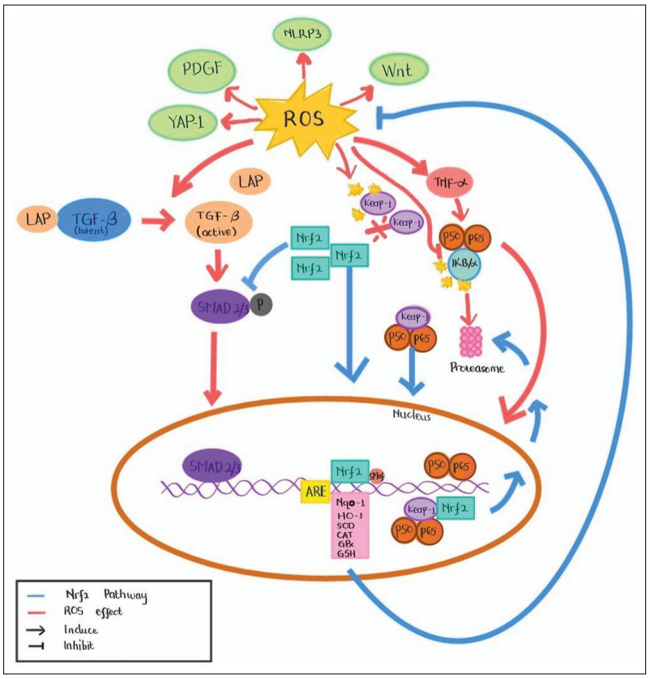

Figure 2: Reactive oxygen species activates transformation growth factor beta, Nuclear factor kappa-light-chain-enhancer of activated B cells, Wnt, platelet-derived growth factor, NODD-, LRR- and pyrin domain-containing protein3, and YAP1 signaling pathways as well as the nuclear factor erythroid 2-related factor 2 pathway. Nuclear factor erythroid 2-related factor 2 inhibits phosphorylation of SMAD2/3, thus inhibits transforming growth factor beta pathway, and acts as a transcription factor for antioxidant defense system regulation which neutralizes reactive oxygen species directly. Nevertheless, activation Nuclear factor kappa-light-chain-enhancer of activated B cells also compresses nuclear factor erythroid 2-related factor 2 by p50 and p65 binding with Keap1 to deliver Keap1 toward the nucleus and bind with nuclear factor erythroid 2-related factor 2. From this mechanism, nuclear factor erythroid 2-related factor 2 was released from ARE and was delivered to the cytoplasm toward proteasome degradation

(Wnt), and NLRP3 have been reported also be induced by ROS in fibrogenesis [14], [17], [21], [22], [23]. PDGF pathway is the important pathway in HSC differentiation and migration. In liver fibrosis, PDGF is highly expressed in macrophages, activated HSC, and injured endothelial cells. ROS will promote PDGF activation by stimulating Src family kinase to phosphorylate PDGF receptor alpha [24]. Increased ROS also maintain the activation of the PDGF pathway, thereby inhibiting HSC autophagy and increasing HSC migration [24].

NF-KB pathway, which is the main pathway of inflammation, is also influenced by ROS [25]. Activated NF-KB will mediate the immune response to infection, proliferation, and protection against UV rays. NF-KB itself is a protein complex consisting of p50 and p65 [25] and degraded in physiologic conditions by its binding with inhibitors of nuclear factor kappa-B kinase (IKK). In oxidative stress, excessive ROS will induce tumor necrosis factor alpha (TNF-A), the strongest activator for NF-KB activation. ROS also oxidize the Cys residue on IKK, thus inhibiting its binding to the NF-KB complex [25], [26]. As a result, the NF-KB complex is free to translocate to the nucleus and becomes the transcription factor (Figure 2).

ROS were also found to activate the Wnt pathway which plays a role in proliferation, regeneration, cell fate determination, and induce EMT [27], [28], [29]. Activating the Wnt pathway needs undegraded-B-catenin in the nucleus. For this purpose, in oxidative stress, ROS will induce the dissociation of Disheveled (Dvl) protein from Nucleoredoxin (NRX) through Ras-related C3 botulinum toxin substrate 1 (Rac1) activation and oxidized NRX. Free Dvl will be recruited by Frizzled protein to the plasma membrane, allowing Axin and glycogen synthase kinase-3 beta (GSK3B) to bind and phosphorylate lipoprotein receptor-related proteins 5/6 (LRP5/6), thereby preventing B-catenin degradation. At this time B-catenin will enter the nucleus and activate the antioxidant response element (ARE) [30].

The role of ROS in the fibrosis mechanism is also seen in Hippo/YAP1 signaling pathway. The hippo/YAP1 pathway plays a role in the proliferation and determination of organ size. The hippo pathway can interact with the TGF-β pathway, Wnt/B-catenin pathway, and NF-KB pathway through the MAPK p38 pathway [31]. On activation of the Hippo pathway, YAP1 protein expression is decreased and vice versa. ROS will induce YAP1 protein translocation to the nucleus. In many studies, increased YAP1 activation would lead to increased proliferation and fibrosis through the MAPK p38 pathway while YAP1 depletion would decrease fibrosis [31], [32].

Besides HSC activation, the epithelial or endothelial-to-mesenchymal transition is an important mechanism in fibrogenesis. It has been known that fibroblasts involved in fibrogenesis not only resulted from HSC-myofibroblast transformation but also the transition of hepatocytes and cholangiocytes into mesenchymal type. EMT can be interpreted as a cellular process that occurs when the epithelium or endothelium changes into a mesenchymal phenotype that has mesenchymal-like capacities. In general, EMT and EndoMT are similar processes with different origins [33]. The occurrence of EMT and EndoMT can be triggered by hypoxia, growth factors, metabolic changes, immune responses, and cytokines. An excessive free fatty acid found in Non-Alcoholic Steatohepatitis (NASH) has been reported to have a great role to induce EMT [29].

In this regard, TGF-β still becomes the main pathway of EMT. TGF-β is a potent inducer of EMT and EndoMT [34]. When TGF-β is activated, not only HSC but also the transition of hepatocytes and cholangiocytes are activated. A recent study confirmed that TGF-β activates EMT through its effect on promoted Y-box binding protein 1 (YB-1) binding to matrix metalloproteinase-9 (MMP9) promoter, thus increased MMP9 expression [35], while other study showed that ROS are the inducer of YB-1 secretion [36]. This relationship shows that when ROS induce TGF-β, ROS also induce YB-1 secretion, but, on the other hand, TGF-β promotes YB-1 binding to MMP9 promoter. Besides of it, the secretion of ROS-induced chemokine

(C-C motif) ligand-1 (CCL1) and its receptor, CXC chemokine receptor-2, will be increased by active TGF-β. These chemokines have been reported to induce mesenchymal markers such as N-cadherin, vimentin, and fibronectin, as well as at the same time decrease epithelial marker, E-cadherin [29].

Reactive Oxygen Species Induce Mitochondrial Dysfunction

The relationship between ROS and mitochondria is well-established. Mitochondria as a power house machine in cells will produce ROS in its regular physiological function in adenosine triphosphate (ATP) production. However, excessive ROS can result from exogenous and endogenous sources. The exogenous sources come from injury-causing agents [37]. The endogenous sources of ROS result from damaged cells, organelles, and also processes inside the body including from mitochondria itself [30]. Increasing ROS will induce lipid peroxidation, mitochondrial damage, and induction of NADPH Oxidase to produce more ROS. Therefore, mitochondrial autophagy (mitophagy) is increased to retain homeostasis. Nevertheless, prolonged increased ROS will lead to mitophagy system failure. ROS will cause lysosomal damage thus mitophagy inhibition has occurred [38], [39], [40].

Inside mitochondria, excessive ROS will inhibit cytochrome c oxidase which is a transmembrane protein involved in the electron transport chain and ATP production [29]. On the other hand, they also decreases mitochondrial cristae density by decreasing Optic atrophy1 (OPA1) protein, which plays a role in regulating mitochondrial fusion and architecture of the mitochondria cristae [38]. Depletion of OPA1 has been reported to produce more ROS, decrease complex I and IV, and reduce the surface area of the mitochondria inner membrane [38]. Thereby, ATP depletion will occur and give a signal to the body to increase mitochondria biogenesis. Unfortunately, a liver injury will also suppress mitochondria biogenesis, as shown in decreased transcription factor A, mitochondrial (TFAM), the main protein involved in the replication and transcription of the mitochondrial genome expression [38], [41].

Nuclear Factor Erythroid 2-Related Factor 2 in General

Nrf2 is a transcription factor of basic leucine zipper which belongs to the cap'n'collar family. It is encoded by the NFE2-like-BZIP transcription factor2 (NFE2L2) gene and plays an important role in maintaining cellular redox homeostasis, regulating antioxidant expression, anti-inflammatory, and cytoprotective genes [42]. Nrf2 under physiological conditions will be constitutively expressed in the cytoplasm and mostly bound to the Keap1 protein for degradation in the proteasome. Only a small amount of free Nrf2 compensates for physiological ROS levels [43].

Structurally, Nrf2 has 7 domains, namely, Neh1 to Neh7 which have different functions. Among the 7 domains, only Neh2 and Neh6 domains are needed for Nrf2 inactivation. Neh2 domain contains the ETGE and DLG motifs used by Nrf2 to bind to the Keap1 protein in the canonical pathway. The Neh6 domain is used by Nrf2 to bind to beta transducin repeat-containing protein (B-TrCP) in a non-canonical pathway [43]. Both Keap1 and B-TrCP are proteins that are negative regulators of Nrf2. However, the Keap1-dependent canonical pathway is the main pathway [43].

Keap1 protein is encoded by the Keap1 gene and has 4 domains including BTB, IVR, CTR, and DGR domains. BTB domain, which is needed for its dimerization, contains a Cys151 residue which is important for stress detection. IVR domain contains two cysteine residues, Cys273 and Cys288. Both BTB and IVR domains are important in the Nrf2 ubiquitination process as a binding site for CUL3/RBX1 complex [43]. CTR and DGR domains are the two domains that bind to the DLG (low affinity) and ETGE (high affinity) motifs on the Neh2 domain of Nrf2 [43], [44].

Through canonical pathways, oxidative stress induces conformational changes of cysteine residues (Cys151, Cys273, and Cys288) on Keap1 and leads to inhibition of the Nrf2 binding process. ROS also induce accumulation of p62 protein which compete with Nrf2 in binding to Keap1 and direct Keap1 degradation. Free Nrf2 will induce p62 expression [44] and translocate to the nucleus. It will form heterodimers with small musculoaponeurotic fibrosarcoma protein, then bind to ARE and activate various antioxidants and phase II enzyme genes including HO-1, NAD(P)H quinone dehydrogenase1 (Nqo1), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [45], [46]. The free Nrf2 has also been known to affect on apoptosis by the expression of B-cell lymphoma2 (Bcl2) and B-cell lymphoma-extra large (Bcl-xl) proteins that play a role in increasing apoptotic resistance that leads to tumorigenesis [47].

Nuclear Factor Erythroid 2-Related Factor 2 as Regulator of Reactive Oxygen Species Scavenger

As a master regulator of antioxidants, Nrf2 has a significant role in fighting ROS. ROS will trigger

Nrf2 to generate enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants such as SOD, catalase (CAT), and glutathione peroxidase (GPx) are the first-line antioxidants produced. They work to fight ROS by changing ROS into H2O in multistep processes. When the reduction of one electron from O2 molecules occurs, superoxide radical ($O2^{\cdot-}$) will be generated. SOD will convert $O2^{\cdot-}$ into H2O2 which is the substrate for CAT and GPx. Then, CAT or GPx will turn it into H2O and O2.

Non-enzymatic antioxidants such as thioredoxin and glutathione (GSH) are the second line antioxidants produced to suppress the free-radical chain reaction. In this case, GSH is a liver highly concentrated antioxidant. During the suppressing process, GSH will give an electron to stabilize ROS and then turn it into oxidized glutathione (GSSG). In this GSH cycle, Nrf2 has a role not only to produce GSH but also to reverse GSSG to GSH through its regulation of the glutathione reductase1 enzyme. Since GSSG is the oxidized form of GSH, therefore the ratio of GSH/GSSG will determine the oxidative stress states. This is supported by evidence that the decrease in Nrf2 was also followed by a decrease in GSH/GSSG ratio [48].

Nuclear Factor Erythroid 2-Related Factor 2 Inhibit Hepatic Stellate Cell Activation

Nevertheless, Nrf2 has a role to interfere with the TGF-β and NF-KB signaling pathways (Figure 2). Both of them are the main pathways involved in fibrogenesis and inflammation. The TGF-β pathway promotes not only HSC activation but also Nrf2 activation (Figure 2) [49]. The battle between HSC activation and Nrf2 will define the progressivity of fibrosis. When Nrf2 accumulation occurs, Nrf2 will inhibit SMAD2/3 phosphorylation, thus inhibiting the TGF-β canonical pathway [49]. Nrf2 has been reported able to increase SMAD7 expression, a negative regulator of TGF-β, by decreasing the expression of transformation growth factor beta receptor-1 (TGF-βRI). Inhibition of the TGF-β pathway through the activation of SMAD7 by Nrf2 has been shown to reduce the incidence of liver fibrosis [37], [50]. However, low Nrf2 has been found in increased HSC activation which is characterized by an increase in alpha-smooth muscle actin (α-SMA), Collagen type I, and IV [49].

So as TGF-β, Nrf2 has been reported as interfering with the NF-KB signaling pathway. NF-KB is an inducible transcription factor which plays a significant role in inflammation and oxidative stress [46]. When ROS are increased, ROS also stimulate Nrf2 activation by changing the Keap1-Nrf2 binding site, then increasing free Nrf2. Nrf2 has the ability to disrupt the NF-KB pathway by inhibiting phosphorylation of p65, a protein that forms NF-KB complex [23], [46], thus preventing NF-KB pathway activation. This mechanism can decrease TNF-alpha and stop the NF-KB activation [46]. Interestingly, a recent study has reported that in NF-KB activation, Nrf2 can be suppressed. This study has found that NF-KB subunits (p50 and p65) also bind to Keap1 directly. This Keap1-p50/p65 binding will lead to Keap1 translocation into the nucleus and promote dissociation of Nrf2 from ARE (Figure 2). Finally, this will stop the Nrf2 effect and support its degradation [51].

Nuclear Factor Erythroid 2-Related Factor 2 and Mitochondrial Damage

Nrf2 has an important role in maintaining mitochondrial homeostasis. For this purpose, Nrf2 not only reduces ROS directly, but also promotes mitochondrial biogenesis and eliminates damaged-mitochondria through mitophagy. Nrf2 regulates the p62/SQSTM1 and Parkin/PINK1 expression to activate mitophagy [15], [52]. Recent studies have shown that Nrf2 not only acts as a positive regulator of p62 and PTEN-induced (PINK1) gene expression but also maintains their basal expression levels. On the other side, Nrf2 also upregulates the mitochondrial biogenesis genes such as mitochondrial associated regulatory factor (Marf), dynamin-related protein 1, and peroxisome proliferators-activated receptor-gamma, and increases lysosome number needed for mitophagy [15], while knocked out Nrf2 has been reported followed by decrease of mitochondrial membrane potential, so does ATP production. The depletion of ATP production will give a signal to the body to start mitochondrial biogenesis. Nevertheless, the study with Nrf2-knockout-mice also reported a significant decrease in the mitochondrial DNA copy number, suggesting without Nrf2, mitochondrial biogenesis will be impaired (Figure 3) [25].

Nuclear Factor Erythroid 2-Related Factor 2 and Aggrephagy

In line with the mitophagy in mitochondria, aggrephagy can be determined as a selective autophagy for other damaged-proteins. Aggrephagy has been reported as a protective cellular response to shuttle aggregated proteins from the cytoplasm to lysosomes [53]. In normal condition, Nrf2 degradation is performed by proteasome as a degradation machine.

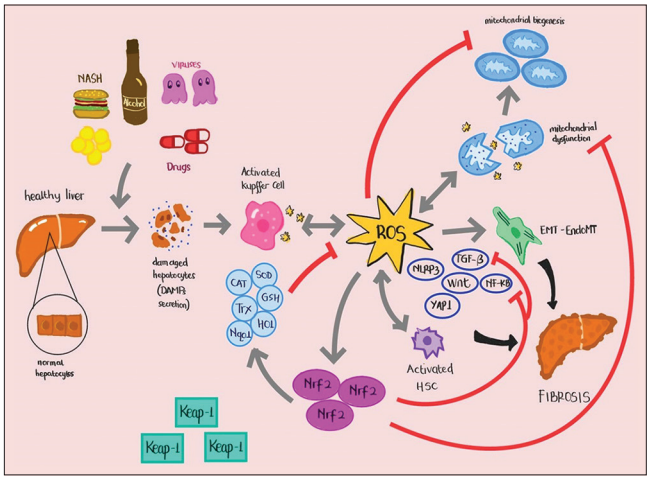

Figure 3: The interplay between reactive oxygen species, nuclear factor erythroid 2-related factor 2, and liver fibrosis. Reactive oxygen species activates hepatic stellate cell, promotes epithelial-mesenchymal transition – endothelial-mesenchymal transition and provokes mitochondrial dysfunction, but at the same time inhibits mitochondrial biogenesis

Proteasome is a multiprotein complex consisting of a 20S catalytic core and one or two 19S regulatory subunits which also degrades other proteins. In oxidative stress, redox alteration induce changes in proteasome function, thus proteasome failure might happen. Therefore, accumulating other damaged proteins and Nrf2 happen [54]. While the accumulation of damaged proteins acts as a cell stressor leads to cell necrosis and apoptosis, the accumulation of Nrf2 will activate aggrephagy through aggresome pathway [53]. This aggresome pathway refers to the compensation mechanism which involves the induction of p62 to assemble the polyubiquitinated proteins into micro aggregates through binding to the proteins' ubiquitin chain. As mentioned before, Nrf2 is a competitor and positive regulator of p62. Aggresome formation was found to be decreased when Nrf2 and p62 were downregulated and vice versa. Loss of Nrf2 resulted in aggrephagy failure and cell death. It is suggested that Nrf2-mediated activation p62 was required to decrease proteasomal stress-induced necrosis and apoptosis [53]. In accordance with this Nrf2 role to support autophagy especially aggrephagy, a study by Treger et al. showed that decreased autophagy was found in increased NAFLD severity thus aggravating fibrosis [54]. Therefore, the role of Nrf2 in autophagy promotion will improve fibrosis.

Nuclear Factor Erythroid 2-Related Factor 2 in Epithelial-Mesenchymal Transition Inhibition

The role of Nrf2 in EMT inhibition is related to the role of Nrf2 to inhibit TGF-β and NF-KB pathways. The recent study indicates that Nrf2 works to inhibit EMT through the Notch- TGF-β signaling. It was reported that Nrf2 is a regulator of Notch signaling which was activated by TGF-β in a ROS-dependent manner [34]. A study about the Notch signaling pathway in liver fibrosis shows a close relationship between Notch and fibrosis through increased Notch3/Jagged1 [55]. This finding is in accordance with the finding from another study which concluded the overexpression of Jagged1 will increase the expression of α-SMA and collagen 1 as well as EMT through TGF-β pathway activation [56]. Furthermore, TGF-β activation will induce activation of NF-KB through paracrine action in mesenchymal transition [57].

Nuclear Factor Erythroid 2-Related Factor 2 in Detoxification Process

As a vital organ, the liver has a role to neutralize toxic and turn them into undangerous molecules for the body. This function is very important to eliminate toxic or xenobiotic agents and maintain liver health. There are two phases of detoxification held by the liver. Phase I detoxification process consists of oxidation, reduction, and hydrolysis while phase II detoxification process consists of a conjugation process. Both of these phases will result in a water-soluble compound that can be easier removed from the body. For this function, Nrf2 has the role to regulate the expression of the phase II enzymes of detoxification such as Nqo1, SOD, HO-1, as well as CAT and GPx. Stimulation of Nrf2 activation such as stimulation by Physalin A, the main compound of *P. Alkekengi* which often use for the treatment of otitis media, sore throat, and renal disease, leads to the production of phase II enzymes of detoxification processes thus promoting liver detoxification function [58]. A study by Ahn *et al*. proved that phase II enzymes of detoxification expression have a role in cytoprotective function which was induced by translocation of Nrf2 through MAPK signaling [59].

Conclusions and Future Direction

Oxidative stress has a significant role to promote liver fibrosis. Nrf2 can promote ROS scavengers activities, interrupt fibrosis major pathways, and induce mitochondrial mitophagy, homeostasis, and biogenesis. Therefore, Nrf2 can be a potential target to fight liver fibrosis. Future studies can be carried out to challenge the Nrf2 effect on liver fibrosis or even liver regeneration.

References

1. GBD 2017 Cirrhosis Collaborators. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(3):245-66. https://doi.org/10.1016/S2468-1253(19)30349-8
   PMid:31981519
2. Craig EV, Heller MT. Complications of liver transplant. Abdom Radiol (NY). 2021;46(1):43-67. https://doi.org/10.1007/s00261-019-02340-5
   PMid:31797026
3. Kuramochi M, Izawa T, Pervin M, Bondoc A, Kuwamura M, Yamate J. The kinetics of damage-associated molecular patterns (DAMPs) and toll-like receptors during thioacetamide-induced acute liver injury in rats. Exp Toxicol Pathol. 2016;68(8):471-7. https://doi.org/10.1016/j.etp.2016.06.005
   PMid:27522298
4. Wei M, Zhang Y, Zhang H, Huang Z, Miao H, Zhang T, *et al*. HMGB1 induced endothelial to mesenchymal transition in liver fibrosis: The key regulation of early growth response factor 1. Biochim Biophys Acta Gen Subj. 2022;1866(10):130202. https://doi.org/10.1016/j.bbagen.2022.130202
   PMid:35820641
5. Han YH, Choi H, Kim HJ, Lee MO. Chemotactic cytokines secreted from kupffer cells contribute to the sex-dependent susceptibility to non-alcoholic fatty liver diseases in mice. Life Sci. 2022;306:120846. https://doi.org/10.1016/j.lfs.2022.120846
   PMid:35914587
6. Dai S, Liu F, Qin Z, Zhang J, Chen J, Ding WX, *et al*. Kupffer cells promote T-cell hepatitis by producing CXCL10 and limiting liver sinusoidal endothelial cell permeability. Theranostics. 2020;10(16):7163-77. https://doi.org/10.7150/thno.44960
   PMid:32641985
7. Shu G, Dai C, Yusuf A, Sun H, Deng X. Limonin relieves TGF-β-induced hepatocyte EMT and hepatic stellate cell activation *in vitro* and CCl4-induced liver fibrosis in mice via upregulating Smad7 and subsequent suppression of TGF-β/Smad cascade. J Nutr Biochem. 2022;107:109039. https://doi.org/10.1016/j.jnutbio.2022.109039
   PMid:35533902
8. Yu Z, Xie X, Su X, Lu H, Song S, Liu C, *et al*. ATRA-mediated-crosstalk between stellate cells and Kupffer cells inhibits autophagy and promotes NLRP3 activation in acute liver injury. J Cell Signal. 2022;93:110304. https://doi.org/10.1016/j.cellsig.2022.110304
   PMid:35278669
9. Li J, Wang Y, Ma M, Jiang S, Zhang X, Zhang Y, *et al*. Autocrine CTHRC1 activates hepatic stellate cells and promotes liver fibrosis by activating TGF-β signaling. EBioMedicine. 2019;40:43-55. https://doi.org/10.1016/j.ebiom.2019.01.009
   PMid:30639416
10. Yu J, Hu Y, Gao Y, Li Q, Zeng Z, Li Y, *et al*. Kindlin-2 regulates hepatic stellate cells activation and liver fibrogenesis. Cell Death Discov. 2018;4:34. https://doi.org/10.1038/s41420-018-0095-9
    PMid:30245857
11. Gao J, Wei B, de Assuncao TM, Liu Z, Hu X, Ibrahim S, *et al*. Hepatic stellate cell autophagy inhibits extracellular vesicle release to attenuate liver fibrosis. J Hepatol. 2020;73(5):1144-54. https://doi.org/10.1016/j.jhep.2020.04.044
    PMid:32389810
12. Burtenshaw D, Hakimjavadi R, Redmond EM, Cahill PA. Nox, reactive oxygen species and regulation of vascular cell fate. Antioxidants (Basel). 2017;6(4):90. https://doi.org/10.3390/antiox6040090
    PMid:29135921
13. Thannickal VJ, Zhou Y, Gaggar A, Duncan SR. Fibrosis: Ultimate and proximate causes. J Clin Invest. 2014;124(11):4673-7. https://doi.org/10.1172/JCI74368
    PMid:25365073
14. Han M, Liu X, Liu S, Su G, Fan X, Chen J, *et al*. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces hepatic stellate cell (HSC) activation and liver fibrosis in C57BL6 mouse via activating Akt and NF-KB signaling pathways. Toxicol Lett. 2017;273:10-9. https://doi.org/10.1016/j.toxlet.2017.03.013
    PMid:28302560
15. Gumeni S, Papanagnou ED, Manola MS, Trougakos IP. Nrf2 activation induces mitophagy and reverses Parkin/Pink1 knock down-mediated neuronal and muscle degeneration phenotypes. Cell Death Dis. 2021;12(7):671. https://doi.org/10.1038/s41419-021-03952-w
    PMid:34218254
16. Liao J, Zhang Z, Yuan Q, Luo L, Hu X. The mouse Anxa6/miR-9-5p/Anxa2 axis modulates TGF-β1-induced mouse hepatic stellate cell (mHSC) activation and CCl4-caused liver fibrosis. Toxicol Lett. 2022;362:38-49. https://doi.org/10.1016/j.toxlet.2022.04.004
    PMid:35483553
17. Xu Z, He B, Jiang Y, Zhang M, Tian Y, Zhou N, *et al*. Igf2bp2 knockdown improves CCl4-induced liver fibrosis and TGF-β-activated mouse hepatic stellate cells by regulating Tgfbr1. Int Immunopharmacol. 2022;110:108987. https://doi.org/10.1016/j.intimp.2022.108987
    PMid:35820364
18. Wang H, Che J, Cui K, Zhuang W, Li H, Sun J, *et al*. Schisantherin A ameliorates liver fibrosis through TGF-β1 mediated activation of TAK1/MAPK and NF-KB pathways *in vitro* and *in vivo*. Phytomedicine. 2021;88:153609. https://doi.org/10.1016/j.phymed.2021.153609
    PMid:34126414
19. Xiang D, Zou J, Zhu X, Chen X, Luo J, Kong L, *et al*. Physalin D attenuates hepatic stellate cell activation and liver fibrosis by blocking TGF-β/Smad and YAP signaling. Phytomedicine. 2020;78:153294. https://doi.org/10.1016/j.phymed.2020.153294
    PMid:32771890
20. Syed AM, Kundu S, Ram C, Kulhari U, Kumar K, Mugale MN, *et al*. Up-regulation of Nrf2/HO-1 and inhibition of TGF-β1/Smad2/3 signaling axis by daphnetin alleviates transverse aortic constriction-induced cardiac remodeling in mice. Free Radic Biol Med. 2022;186:17-30. https://doi.org/10.1016/j.phymed.2020.153294
    PMid:35513128
21. Wang Y, Sun Y, Zuo L, Wang Y, Huang Y. ASIC1a promotes high glucose and PDGF-induced hepatic stellate cell activation by inducing autophagy through CaMKKβ/ERK signaling pathway. Toxicol Lett. 2019;300:1-9. https://doi.org/10.1016/j.toxlet.2018.10.003
    PMid:30291941
22. Swiderska-Syn M, Xie G, Michelotti GA, Jewell ML, Premont RT, Syn WK, *et al*. Hedgehog regulates yes-associated protein 1 in regenerating mouse liver. Hepatology. 2016;64:232-44. https://doi.org/10.1002/hep.28542
    PMid:26970079
23. Accora BZ, Storm G, Bansal R. Inhibition of canonical WNT signaling pathway by B-catenin/CBP inhibitor ICG-001 ameliorates liver fibrosis *in vivo* through suppression of stromal CXCL12. Biochim Biophys Acta Mol Basis Dis. 2018;1864(3):804-18.

https://doi.org/10.1016/j.bbadis.2017.12.001
PMid:29217140

24. Kazlauskas A. PDGFs and their receptors. Gene. 2017;614:1-7. https://doi.org/10.1016/j.gene.2017.03.003
PMid:28267575

25. Pang Y, Zhang L, Liu Q, Peng H, He J, Jin H, *et al*. NRF2/PGC-1-mediated mitochondrial biogenesis contributes to T-2 toxin-induced toxicity in human neuroblastoma SH-SY5Y cells. Toxicol Appl Pharmacol. 2022;451:116167. https://doi.org/10.1016/j.taap.2022.116167
PMid:35842139

26. Arellanes-Robledo J, Reyes-Gordillo K, Ibrahim J, Leckey L, Shah R, Lakshman MR. Ethanol targets nucleoredoxin/dishevelled interactions and stimulates phosphatidylinositol 4-phosphate production *in vivo* and *in vitro*. Biochem Pharmacol. 2018;156:135-46. https://doi.org/10.1016/j.bcp.2018.08.021
PMid:30125555

27. Qin Q, Yang B, Liu Z, Xu L, Song E, Song Y. Polychlorinated biphenyl quinone induced the acquisition of cancer stem cells properties and epithelial-mesenchymal transition through Wnt/B-catenin. Chemosphere. 2021;263:128125. https://doi.org/10.1016/j.chemosphere.2020.128125
PMid:33297114

28. Staehlke S, Haack F, Waldner AC, Koczan D, Moerke C, Mueller P, *et al*. ROS dependent Wnt/β-catenin pathway and its regulation on defined micro-pillars – A combined *in vitro* and *in silico* study. Cells. 2020;9(8):1784. https://doi.org/10.1016/j.chemosphere.2020.128125
PMid:32726949

29. Kwapisz O, Górka J, Korlatowicz A, Kotlinowski J, Waligórska A, Marona P, *et al*. Fatty acids and a high-fat diet induce epithelial-mesenchymal transition by activating TGFβ and β-catenin in liver cells. Int J Mol Sci. 2021;22(3):1272. https://doi.org/10.3390/ijms22031272
PMid:33525359

30. Rharass T, Lemcke H, Lantow M, Kuznetsov SA, Weiss DG, Panáková D. Ca2+-mediated mitochondrial reactive oxygen species metabolism augments Wnt/β-catenin pathway activation to facilitate cell differentiation. J Biol Chem. 2014;289(40):27937-51. https://doi.org/10.1074/jbc.M114.573519
PMid:25124032

31. Salloum S, Jeyarajan AJ, Kruger AJ, Holmes JA, Shao T, Sojoodi M, *et al*. Fatty acids activate the transcriptional coactivator YAP1 to promote liver fibrosis via p38 mitogen-activated protein kinase. Cell Mol Gastroenterol Hepatol. 2021;12(4):1297-310. https://doi.org/10.1016/j.jcmgh.2021.06.003
PMid:34118488

32. Yu H, Yao Y, Bu F, Chen Y, Wu Y, Yang Y, *et al*. Blockade of YAP alleviates hepatic fibrosis through accelerating apoptosis and reversion of activated hepatic stellate cells. Mol Immunol. 2019;107:29-40. https://doi.org/10.1016/j.molimm.2019.01.004
PMid:30639476

33. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009;119(6):1429-37. https://doi.org/10.1172/JCI36183
PMid:19487819

34. Yazaki K, Matsuno Y, Yoshida K, Sherpa M, Nakajima M, Matsuyama M, *et al*. ROS-Nrf2 pathway mediates the development of TGF-β1-induced epithelial-mesenchymal transition through the activation of Notch signaling. Eur J Cell Biol. 2021;100(7-8):151181. https://doi.org/10.1016/j.ejcb.2021.151181
PMid:34763128

35. Guo Y, Xu X, Dong H, Shen B, Zhu J, Shen Z, *et al*. Loss of YB-1 alleviates liver fibrosis by suppressing epithelial-mesenchymal transition in hepatic progenitor cells. Biochim Biophys Acta Mol Basis Dis. 2022;1868(11):166510. https://doi.org/10.1016/j.bbadis.2022.166510
PMid:35926755

36. Guarino AM, Troiano A, Pizzo E, Bosso A, Vivo M, Pinto G, *et al*. Oxidative stress causes enhanced secretion of YB-1 protein that restrains proliferation of receiving cells. Genes (Basel). 2018;9(10):513. https://doi.org/10.3390/genes9100513
PMid:30360431

37. Chi Q, Xu T, He Y, Li Z, Tang X, Fan X, *et al*. Polystyrene nanoparticle exposure supports ROS-NLRP3 axis-dependent DNA-NET to promote liver inflammation. J Hazard Mater. 2022;439:129502. https://doi.org/10.1016/j.jhazmat.2022.129502
PMid:35868089

38. Franquesa AG, Perez PG, Kulis M, Szczepanowska K, Dahdah N, Gomez SM, *et al*. Remission of obesity and insulin resistance is not sufficient to restore mitochondrial homeostasis in visceral adipose tissue. Redox Biol. 2022;54:102353. https://doi.org/10.1016/j.redox.2022.102353
PMid:35777200

39. Xiao B, Cui Y, Li B, Zhang J, Zhang X, Song M, *et al*. ROS antagonizes the protection of Parkin-mediated mitophagy against aluminum-induced liver inflammatory injury in mice. Food Chem Toxicol. 2022;165:113126. https://doi.org/10.1016/j.fct.2022.113126
PMid:35569598

40. Yang B, Wang Y, Fang C, Song E, Song Y. Polybrominated diphenyl ether quinone exposure leads to ROS-driven lysosomal damage, mitochondrial dysfunction and NLRP inflammasome activation. Environ Pollut. 2022;311:119846. https://doi.org/10.1016/j.envpol.2022.119846
PMid:35944775

41. Chung KW, Dhillon P, Huang S, Sheng X, Shrestha R, Qiu C, *et al*. Mitochondrial damage and activation of the STING pathway lead to renal inflammation and fibrosis. Cell Metab. 2019;30(4):784-99.e5. https://doi.org/10.1016/j.cmet.2019.08.003
PMid:31474566

42. Meng Q, Chen R, Chen C, Su K, Li W, Tang L, *et al*. Transcription factors Nrf2 and NF-κB contribute to inflammation and apoptosis induced by intestinal ischemia-reperfusion in mice. Int J Mol Med. 2017;40:1731-40. https://doi.org/10.3892/ijmm.2017.3170
PMid:29039475

43. Zhou J, Zheng Q, Chen Z. The Nrf2 pathway in liver diseases. Front Cell Dev Biol. 2022;10:826204. https://doi.org/10.3389/fcell.2022.826204
PMid:35223849

44. Poon A, Saini H, Sethi S, O'Sullivan GA, Plun-Favreau H, Wray S, *et al*. The role of SQSTM1 (p62) in mitochondrial function and clearance in human cortical neurons. Stem Cell Rep. 2021;16(5):1276-89. https://doi.org/10.1016/j.stemcr.2021.03.030
PMid:33891871

45. Hao H, Xie F, Xu F, Wang Q, Wu Y, Zhang D. LipoxinA4 analog BML-111 protects podocytes cultured in high-glucose medium against oxidative injury via activating Nrf2 pathway. Int Immunopharmacol. 2022;111:109170. https://doi.org/10.1016/j.intimp.2022.109170
PMid:36007391

46. Rubio V, García-Pérez AI, Herráez A, Diez JC. Different roles of Nrf2 and NFKB in the antioxidant imbalance produced by esculetin or quercetin on NB4 leukemia cells. Chem Biol Interact. 2018;294:158-66. https://doi.org/10.1016/j.cbi.2018.08.015
PMid:30171828

47. Zhang Q, Zhang ZY, Du H, Li SZ, Tu R, Jia YF, *et al*. DUB3 deubiquitinates and stabilizes NRF2 in chemotherapy resistance

of colorectal cancer. Cell Death Differ. 2019;26:2300-13. https://doi.org/10.1038/s41418-019-0303-z

PMid:30778200

48. Tonelli C, Chio II, Tuveson DA. Transcriptional regulation by Nrf2. Antioxid Redox Signal. 2018;29:1727-45. https://doi.org/10.1089/ars.2017.7342

PMid:28899199

49. Prestigiacomo V, Suter-Dick L. Nrf2 protects stellate cells from Smad-dependent cell activation. PLoS One. 2018;13(7):e0201044. https://doi.org/10.1371/journal.pone.0201044

PMid:30028880

50. Sa'dyah NA, Putra A, Dirja BT, Hidayah N, Azzahara SY, Irawan RC. Suppression of transforming growth factor-β by mesenchymal stem-cells accelerates liver regeneration in liver fibrosis animal model. Univ Med. 2021;40(1):29-35. Available from: https://univmed.org/ejurnal/index.php/medicina/article/view/107957 [Last accessed on 2022 Oct 27].

51. Hu B, Wei H, Song Y, Chen M, Fan Z, Qiu R, *et al*. NF-KB and Keap1 interaction represses Nrf2-mediated antioxidant response in rabbit hemorrhagic disease virus infection. J Virol. 2020;94(10):e00016-20. https://doi.org/10.1128/JVI.00016-20

PMid:32161178

52. Fernandez-Gines R, Encinar JA, Hayes JD, Oliva B, Rodriguez-Franco MI, Rojo AI, *et al*. An inhibitor of interaction between the transcription factor NRF2 and the E3 ubiquitin ligase adapter B-TrCP delivers anti-inflammatory responses in mouse liver. Redox Biol. 2022;55:102428. https://doi.org/10.1016/j.redox.2022.102396

PMid:35839629

53. Qin S, Jiang C, Gao J. Transcriptional factor Nrf2 is essential for aggresome formation during proteasome inhibition. Biomed Rep. 2019;11(6):241-52. https://doi.org/10.3892/br.2019.1247

PMid:31798869

54. Galle-Treger L, Helou DG, Quach C, Howard E, Hurrell BP, Muench GR, *et al*. Autophagy impairment in liver CD11c+ cells promotes non-alcoholic fatty liver disease through production of IL-23. Nat Commun. 2022;13(1):1440. https://doi.org/10.1038/s41467-022-29174-y

PMid:35301333

55. Yue Z, Jiang Z, Ruan B, Duan J, Song P, Liu J, *et al*. Disruption of myofibroblastic Notch signaling attenuates liver fibrosis by modulating fibrosis progression and regression. Int J Biol Sci. 2021;17(9):2135-46. https://doi.org/10.7150/ijbs.60056

PMid:34239344

56. Tang G, Weng Z, Song J, Chen Y. Reversal effect of Jagged1 signaling inhibition on CCl4-induced hepatic fibrosis in rats. Oncotarget. 2017;8:60778-88. https://doi.org/10.18632/oncotarget.18484

PMid:28977825

57. Tian B, Zhao Y, Sun H, Zhang Y, Yang J, Brasier A. BRD4 mediates NF-kB-dependent epithelial-mesenchymal transition and pulmonary fibrosis via transcriptional elongation. Am J Physiol. 2016;311(6):L1183-201. https://doi.org/10.1152/ajplung.00224.2016

PMid:27793799

58. Shin JM, Lee KM, Lee HJ, Yun JH, Nho CW. Physalin A regulates the Nrf2 pathway through ERK and p38 for induction of detoxifying enzymes. BMC Complement Altern Med. 2019;19(1):101. https://doi.org/10.1186/s12906-019-2511-y

PMid:31072358

59. Ahn CB, Je JY, Kim YS, Park SJ, Kim BI. Induction of Nrf2-mediated phase II detoxifying/antioxidant enzymes *in vitro* by chitosan-caffeic acid against hydrogen peroxide-induced hepatotoxicity through JNK/ERK pathway. Mol Cell Biochem. 2017;424(1-2):79-86. https://doi.org/10.1007/s11010-016-2845-4

PMid:27743232