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Nrf2 and its Modulation in Inflammation

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Huai Deng
Editor

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Springer

Editor
Huai Deng
Department of Biology
University of Minnesota Duluth
Duluth
MN
USA

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Preface

Environmental toxins generated by modern industry significantly influence human health. Many of these toxins, when entering cells, can damage macromolecules including DNA, proteins, and lipids, either directly by themselves or indirectly via producing reactive oxidative species (ROS). Excess ROS levels can also be caused by dysfunctions of intracellular programs. It is believed that the oxidative and xenobiotic stimuli in different tissues account for the pathogeneses of many human diseases. Cellular factors that can respond to and eliminate oxidative and xenobiotic stresses are essential protective machineries against the environment toxin-induced pathogeneses.

Inflammation is a combinatory process that protects cells against the injuries caused by physical damage, bacterial/viral infections, or chemical toxins. This process includes recruitment of immune cells and release of molecular mediators such as cytokines. Unlike acute inflammation as a short-term natural healing process, chronic inflammation lasts for months or years and results in many diseases, including cardiovascular dysfunctions, metabolic syndrome and diabetes, neurodegeneration, cancer, and respiratory diseases. One mechanism whereby chronic inflammation leads to these pathologies is the enhanced ROS levels caused by the recruited mononuclear immune cells such as macrophages, neutrophils, and lymphocytes at the injured tissue. Chronic inflammation can also be directly induced by prolonged exposure to some environmental toxins. Therefore, oxidative and xenobiotic response pathways are important anti-inflammation mechanisms.

The NF-E2-related factor 2 (Nrf2) transcription factor is a central regulator that mediates transcriptional responses to oxidative and xenobiotic stimuli. Nrf2 can bind to antioxidant response elements (AREs), and it activates a battery of antioxidant and detoxifying genes. Most of the Nrf2-target genes code for enzymes that catalyze the metabolism or removal of ROS and other toxins. These include phase I enzymes (e.g., SOD, GPx), phase II enzymes (e.g., GST, NQO1), phase III transporters (e.g., MRP1), and other antioxidant proteins such as HO-1. The key regulator for Nrf2 activity in response to oxidative/xenobiotic stresses is kelch-like ECH-associated protein 1 (Keap1). Keap1 interacts with Nrf2 and induces its ubiquitination and degradation in the cytoplasm. According to the conventional models,

modification of cysteines on Keap1 by oxidative and xenobiotic compounds interferes with the Keap1–Nrf2 interaction and blocks Nrf2 degradation, which leads to nuclear accumulation of Nrf2 and activation of Nrf2-target genes.

Dysfunctions of Nrf2 and/or Keap1 were found to be related to many human diseases as well as inflammation. It is generally accepted that these pathogeneses are mediated by an imbalance redox hemostasis that are caused by the defected Keap1-Nrf2 pathway. However, the pathological roles of Nrf2 are complicated and sometimes paradoxical. For example, Nrf2 can both prevent and promote oncogenesis. Several models state that the levels of Nrf2 are critical for the distinct functions of Nrf2 in different aspects. Studies in the last 20 years have revealed a number of novel Nrf2-target genes and functions that are independent of redox regulation and detoxification, illuminating that Nrf2 can act more than a detoxifying factor. Studies in different model systems have identified the novel functions of Nrf2 in developmental programs, including cell proliferation, stem cell self-renewal and differentiation, apoptosis, autophage, lipid metabolism and adipogenesis, glucose metabolism, steroid hormone synthesis and responses, etc. It is likely that Nrf2 activates antioxidant and detoxifying genes in an inducible and global manner, and at the same time regulating developmental genes in a basal and tissue-specific manner.

Nrf2 also interacts with many other proteins and pathways, placing Nrf2 in a multi-layer network that regulates redox homeostasis as well as other cellular programs. The proteins that can directly interact with Nrf2 include small Mafs, NF-κB, AP-1, CBP/p300, MED16, CFTR, ATF3, ATF4, Bach1, ERα, PPARγ, RARα, p62, ARF, Brg1, CHD6, SMRT, p21, DJ-1, and PGAM5. These proteins can activate or suppress the Nrf2-mediated transcription through mechanisms such as dimerizing with Nrf2 at ARE or competing Nrf2 interaction with other proteins. Nrf2 can also be covalently modified by phosphorylation and acetylation, which could regulate Nrf2 stability, localization, and activity. In recent years, epigenetic functions of Nrf2 have been noticed. Nrf2 is able to interact with chromatin modifiers including ATP-dependent chromatin remodeling complexes, HATs, and HDACs. Cooperative roles of Nrf2 and some of its interacting proteins have been identified in a number of pathogeneses. Taken together, it is believed that the multiple interacting partners and biological functions of Nrf2 to some extent account for the complicated roles of Nrf2 in different diseases.

With the contributions from the experts in the field of Nrf2, this book comprehensively reviews the up-to-date discoveries for the roles of Nrf2 in several human diseases in the context of inflammation. In particular, the molecular mechanisms that mediate the functions of Nrf2 in inflammation and pathogenesis are explicated. Many of the chapters also summarize the research and therapeutic applications of Nrf2 activators and inhibitors as well as compounds that target Nrf2–protein interactions in different diseases.

In Chap. 1, Wang and Perez review the molecular functions of Nrf2 in inflammation, including the Nrf2-ARE-mediated antioxidant pathway, crosstalk between Nrf2 and the NF-κB inflammatory pathway, and Nrf2-target cytokine genes. In Chap. 2, Hohmann et al. in Verri's team describe the roles of Nrf2 in immune response during inflammation. This chapter demonstrates the molecular

mechanisms that mediate Nrf2 functions in both innate immunity and adaptive immunity, as well as the role of Nrf2 in different immune cells and relevant diseases. In Chap. 3, Carlson and Price in Deng's team review the roles of Nrf2 in airway inflammation and respiratory diseases. This chapter comprehensively summarizes the multiple regulatory proteins that interact with Nrf2 and the diverse target genes that mediate the novel functions of Nrf2, as well as the roles of Nrf2 and its interacting partners in respiratory diseases. In Chap. 4, Yamazaki and Itoh describe the molecular mechanisms that regulate Nrf2 activity in vascular endothelial cells, and the cofactors and target genes that mediate Nrf2 functions in protecting endothelial cells against ROS and inflammation, as well as the relevant endothelial dysfunctions, diseases, and aging. In Chap. 5, Mathis and Cui comprehensively review the roles of Nrf2 in the cardiovascular system and diseases, especially atherosclerosis, focusing on Nrf2 functions in apoptosis/necrosis, autophagy, proteasomal degradation, and other signaling pathways in the cardiovascular cells. In Chap. 6, Sarkar et al. in Sil's team discuss the complicated roles of Nrf2 in both the promotion and the suppression of inflammation-triggered carcinogenesis. This chapter reviews the multiple molecular mechanisms that control Nrf2 activity in normal cells versus cancer cells and that mediate the roles of Nrf2 in inflammation, genomic stability, and chemoprotection. In Chap. 7, Bayliak and Abrat focus on the role of Nrf2 in oxidative and inflammatory processes in obesity and metabolic diseases. This chapter describes the relationship between oxidative stress and inflammation in obesity, the functions of Nrf2 network in the regulation of adipocyte and energy metabolism, and the dual role of Nrf2 in the prevention and the aggravation of obesity and obesity-related inflammation. In Chap. 8, Braidy comprehensively summarizes the compounds that target Nrf2 or Nrf2–Keap1 interaction, including both Nrf2 activators and inhibitors. This chapter also reviews the mechanisms, functions, and therapeutic potential of these compounds in inflammation and diseases.

We believe that these chapters will fit together a puzzle to create a complete map for the established molecular mechanisms underlining Nrf2-associated inflammation and diseases. This book is expected to be a valuable reference for worldwide researchers conducting both mechanistic and therapeutic studies of Nrf2 and relevant factors. Finally, I wish to thank all the authors for kindly contributing their precious time and expertise to this book.

Duluth, MN, USA Huai Deng

The original version of this book was revised. This book was initially published with incorrect ISSN numbers which are corrected now. A correction to this chapter can be found at https://doi.org/10.1007/978-3-030-44599-7_9

Contents

Molecular Mechanisms of Nrf2 in Inflammation: Interactions Between Nrf2 and Inflammatory Mediators

Rong Wang and Viviana I. Perez

Abstract Inflammation is a common feature of chronic age-related diseases. Therefore, controlling inflammation by manipulation in the molecular mechanisms that cause the inflammatory process becomes critical to improve human health by preventing various diseases such as cancer, metabolic syndrome, neurodegenerative diseases, and many others. The transcription factor Nrf2 is essential for protection against oxidative/xenobiotic stress. However, Nrf2 also decreases inflammation through a cross talk with the NFκB signaling pathway. Although the mechanism by which Nrf2 mediates anti-inflammatory response is not fully elucidated, new insights have identified that in addition to regulating the expression of cytoprotective genes that have an established and well-known "antioxidant response element, ARE,", Nrf2 can also regulate over six hundred additional genes that do not have an ARE sequence, including genes coding for proinflammatory cytokines. In this chapter, we summarize and discuss the direct and indirect role of Nrf2 in the regulation of inflammatory response and the development of therapeutic targets by identifying new molecular mechanisms.

1 Introduction

Inflammation is a very complex response that underlies a variety of pathological and physiological processes, including the protection against pathogen invasion as well as participation in the development of metabolic disorders and diseases [1, 2]. In general, there are two types of inflammatory response, acute and chronic [3]. The better characterized acute inflammation is induced by noxious stimuli (infection or

R. Wang
Linus Pauling Institute, Oregon State University, Corvallis, OR, USA
e-mail: wang.rong@oregonstate.edu

V. I. Perez (✉)
Linus Pauling Institute, Oregon State University, Corvallis, OR, USA

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA
e-mail: Viviana.perez@oregonstate.edu

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injury); however, chronic inflammation is not induced by the classic instigators of acute inflammation, but rather it is believed to be induced by a variety of cellular and tissue malfunctions that with time shift negatively the homeostatic balance of several physiological systems. This type of inflammation is also known as "sterile inflammation," and in the case of age-related chronic inflammation, "inflamaging" [4].

While the molecular mechanisms involved in the development of acute inflammation are well known, the mechanisms involved in the induction of chronic inflammation are still poorly understood. In fact, some of the molecular participants are shared between them, for example, the production of cytokines and proinflammatory peptides. Moreover, although the pathological drivers of inflammation are well known, i.e., infection and tissue injury trigger the recruitment of leukocytes and plasma proteins to the affected tissue site, the physiology of inflammation is much less known. In general, we know that chronic inflammation is triggered by tissue or cell stress or malfunction, which induces an adaptive inflammatory response, and ultimately this is responsible for the chronic inflammatory conditions that are associated with most human diseases and aging [3].

Several molecular pathways that are activated by stress or cellular damaged are strongly linked to chronic inflammation. In this chapter, we will focus on the role of the Nrf2 molecular pathway in the regulation of chronic inflammation.

2 Nrf2 Signaling Pathway

The nuclear factor-erythroid-2 (NFE2)-related factor 2 (Nrf2), a member of the cap'n'collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors, was originally found to regulate the expression of a set of detoxification enzymes that metabolized drugs, e.g., glutathione S-transferase (GST) and NAD(P)H:quinoneoxidoreductase1(NQO1) [5] by binding to a common DNA sequence called antioxidant response element (ARE) that resembles the NFE2-binding motif [6].

Molecular and structural analyses of Nrf2 signaling revealed that Nrf2 activation is suppressed under basal conditions through binding to Keap1 protein in the cytoplasm, and it is activated by modification of critical cysteine thiols of Keap1, induced by oxidants and electrophiles; this results in the release of Nrf2 from Keap1 allowing its activation and translocation to the nucleus [7]. It is well known that by binding to the antioxidant redox element (ARE), Nrf2 regulates the expression of a wide variety of antioxidant stress genes and phase II detoxifying enzymes [8], and this appears to be the default mechanism by which Nrf2 regulates inflammation. A genomic-scale search for Nrf2 target genes identified a number of ARE-containing genes, so that it has been described that Nrf2 can regulate the induction of over 200 genes, including genes that regulate the synthesis and utilization of glutathione (GSH), the thioredoxin and peroxiredoxin systems, NADPH generation systems, iron metabolism, and an array of antioxidant and detoxification enzymes [9]. This is known as the cytoprotective role of Nrf2.

3 Nrf2 Signaling Pathway and Inflammation

Several lines of evidence coming from the use of Nrf2-deficient mice (Nrf2KO) have shown that Nrf2 also plays an anti-inflammatory function. For example, Nrf2KO mice tend to develop age-dependent autoimmune disease (lupus-like autoimmune nephritis), inflammatory lesions, and elevated cytokine levels in multiple tissues [10, 11]. Besides, increased inflammation is commonly observed in chemically induced pathology in Nrf2-deficient mice [12–17]. Also, numerous drugs or natural products that activate Nrf2 are potent anti-inflammatory agents, and their potency for induction of ARE genes correlates well with their potency for inhibition of inflammation [18–20].

The molecular events that underlie the interaction between Nrf2 and inflammatory response are still unclear; however, the accumulation of several lines of evidence in which the interaction of Nrf2 with different pathways associated with inflammation have been described, and here we will summarize each of them.

4 Nrf2 and the NFκB Signaling Pathway

The nuclear factor-κB (NFκB) is a family of transcription factors consisting of dimers of different proteins forming a complex, including RelA, RelB, p50, and p52 proteins [21]. NFκB regulates several pathways including aspects of development and cell proliferation; however, it's best characterized function is as a key mediator of the immune response to pathogens and inflammation [22]. Indeed, several proinflammatory cytokines such as tumor necrosis factor (TNF) α, interleukin (IL)-1β and IL-6, as well as bacterial lipopolysaccharide (LPS) are among the most potent NFκB activators [23]. Abnormal activation of NFκB has been connected to several inflammatory diseases, e.g., rheumatoid arthritis, asthma, and inflammatory bowel disease [24]. In the presence of an inflammatory stimuli, NFκB activation provides a rapid response by inducing a prompt activation of mitochondrial activity and NADPH oxidase expression, both of which represent the main source of free radicals in the cell [25, 26]. This provides the molecular link between NFκB and Nrf2.

Earlier studies indicated that activation of the Nrf2 pathway mediates the inhibition of both inflammation and the NFκB pathway; however, the molecular mechanism underlying these effects was blurred until recently. It is now clear that both pathways, NFκB and Nrf2, are essential for maintaining a coordinated cellular response involving initiation and resolution of the inflammatory status of the cell by multiple molecular interactions [27]. Many observations about the role of Nrf2 and NFκB interaction come from the use of Nrf2 knockout (KO) mice, where the lack of Nrf2 is associated with an increment of cytokine production [28]. Furthermore, different cell culture experiments in embryonic fibroblasts, astrocytes, and microglia from Nrf2KO mice showed a higher activation of the NFκB pathway after LPS treatment, compared with similar cells from wild-type mice, which results in the

elevation of cytokine production [29–31]. Since both transcription factors, NFκB and Nrf2, are redox sensitive, the absence of Nrf2 will increase oxidative and nitrosative stress which will allow the amplification of NFκB activation and increase the production of cytokines [32]. Furthermore, pre-activation of Nrf2 in primary peritoneal macrophages using sulforaphane, dampens the production of several cytokines (including TNFα, IL1β) in response to LPS; this effect was not observed in macrophages obtained from Nrf2KO mice [33].

Currently, we know that NFκB and Nrf2 can coordinate their respective activities at three stages; (1) Keap1 (the partner of Nrf2 in the cytosol) degrades IKKβ (the activator of NFκB) by ubiquitination, thus inhibiting the activity of NFκB [34]; (2) Also, inflammatory intermediaries, such as cycooxygenase2 (COX2), react with Keap1 releasing and activating Nrf2 and inhibiting NFκB pathway [35, 36]; (3) p65, a subunit of the NFκB complex, competes for the same transcriptional co-activator of Nrf2, CBP (CREB-binding protein); therefore, higher level of expression of p65 will decrease the availability of CBP for Nrf2 transcription, prioritizing the transcription of the NFκB-driven genes [27].

Another layer of Nrf2 and NFκB co-regulation has been described at the level of RAC1, a small GTPase protein that mediates the execution of the inflammatory response of the innate immune system by activating the NFκB pathway [37–39]. However, RAC1 also regulates Nrf2 activation via a completely independent pathway involving its partner Keap1. This regulation is through the activation of PI3K/AKT, which inhibits NFκB in a regulatory feedback loop [40, 41]. Although several other interactions and cross talk between Nrf2 and NFκB have been described, these interactions need further investigation to identify a clear mechanism. From this, we can conclude that the anti-inflammatory actions of many natural phytochemicals such sulforaphane and curcumin, might rely in their dual capacity to both activate the Nrf2 pathway and inhibit NFκB [27].

5 Nrf2 and the Inflammasome

The inflammasome is a multiple molecular pathways platform required for a functional innate immune system [42]. The inflammasome consists of a multi-protein complex of pathogen recognition receptors mainly NLRP3, which senses a wide variety of damage signals, such as pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and oxidative stress (ROS). Activation of the inflammasome pathway via NLRP3 activation triggers the activation of pro-caspase 1 and subsequently the secretion of proinflammatory cytokines such as IL-1β and IL-18 that ultimately induces a cell death process known as pyroptosis, which protects from further propagation of the pathogen [43]. Also, the inflammasome pathway can be activated by protein misfolding events that can occur in several diseases that involve proteinopathy, including well-characterized ones

such as Parkinson's disease and Alzheimer's disease, but also other diseases such as type 2 diabetes, atherosclerosis, and cancer [44–47].

Recently, observations from Dr. Hu's laboratory have indicated that activation of Nrf2 is negatively correlated with activation of the inflammasome, whereas Nrf2 inhibits NLRP3 in macrophages through the expression of NQO1 protein [48]. Similarly, a well-known activator of the Nrf2 pathway, tert-butylhydroquinone (tBHQ) negatively regulated NLRP3 transcription in an Nrf2-dependent manner [48]. Furthermore, a series of reports using mouse models of lupus disease have shown that other compounds that activate the Nrf2 pathway result in decreased NLRP3 activity [49, 50], with a consequent decrease in caspase-1, IL-1β, and TNFα expression [51].

In contrast to those studies, it has been observed that during atherogenesis, cholesterol crystals engulfed by macrophages initiate an inflammatory response mediated by inflammasomes [52]; interestingly, it was also observed that the cholesterol crystals activate the Nrf2 pathway, and this process is required for the cholesterol crystal-induced inflammasome response [53]. Therefore in this specific scenario, Nrf2 was activated as an adaptive response that enhances the activation of the inflammasome. Similarly, observations from studies done in macrophages obtained from Nrf2KO mice indicate that the Nrf2 pathway is necessary for the activation of NLPR3, but not the NLRC4 inflammasome. Overall, it is becoming increasingly clear that activation of Nrf2 and the inflammasome are tightly co-regulated, but the direction of this regulation appears to be context- and perhaps cell-dependent. Further studies are needed to reveal the exact role of Nrf2 in the regulation of the inflammasome pathway.

6 Nrf2 and the Activation of Heme Oxygenase 1 (HO-1)

Nrf2 is the main transcription factor responsible for inducing heme oxygenase-1 (HO-1) expression, and in fact HO-1 expression is widely used as one of the core control proteins to test Nrf2 activation. HO-1 levels are usually elevated in acute inflammatory disorders, and it has been suggested that increased HO-1 production confers strong anti-inflammatory effects against cellular or tissue injury [54]. Upregulation of HO-1 attenuates skin inflammation and accelerates wound healing after epithelial injury in mice [55, 56]. Several studies have demonstrated the anti-inflammatory effect of Nrf2-mediated HO-1 and its metabolites. HO1 is the rate-limiting enzyme in the pathway that catalyzes the degradation of heme, a toxic compound because it catalyzes an iron-dependent Fenton reaction that causes oxidative damage to macromolecules [57]. In this pathway, HO-1 catalyzes the degradation of heme moieties into carbon monoxide and free iron, which is rapidly sequestered by ferritin, a storage protein whose expression is also Nrf2 dependent. During the process, the reminder of the heme group, biliverdin is also converted into bilirubin, a potent antioxidant [58]. Therefore, the degradation of heme, a proinflammatory compound, and the generation of anti-inflammatory compounds, such

as carbon monoxide and bilirubin [59], represent the major protective effects of HO-1, having both antioxidant and anti-inflammatory properties.

Experiments using a rat liver transplantation model showed that HO-1 expression leads to an inhibition of NFκB, which is associated with a reduction in intestinal ischemia-reperfusion injury and tight-junction dysfunction [60]. Moreover, in mouse-derived C2C12 myoblasts, HO-1 expression protects against hydrogen peroxide cytotoxicity [61]. Using immune cells such as monocytes (RAW264.7 and mouse peritoneal macrophage-derived foam cells), expression of HO-1 reduced the inflammatory response induced by LPS, suggesting HO-1 might play an important role in the progression of atherosclerosis [62]. Similar results were observed in BV2 microglial cells and hippocampal Ht22 cells treated with LPS, where HO-1 helps against cell death [63]. Finally, using a smoke-induced inflammatory model, HO-1 activity influences regulatory T cells (Tregs) in the lungs and prevents smoke-induced B cell infiltrates [64]. HO-1 also increases IL10 (an anti-inflammatory cytokine) and TGF-β, leading to attenuation of airway inflammation [65].

In addition, by-products of HO-1 activity, such CO and bilirubin, have been shown to suppress autoimmune encephalomyelitis and hepatitis [66, 67] and protect mice and rats against endotoxin shock by preventing the generation of nitric oxide (NO) and inducible nitric oxidase synthase (iNOS) [68, 69]. Bilirubin was shown to protect against experimental autoimmune encephalomyelitis and autoimmune hepatitis [66, 67]. Furthermore, an increase in HO-1 activity in endothelial cells leads to the inhibition of NFκB-mediated transcription of adhesion molecules such as E-selectin and vascular cell adhesion 1 (VCAM-1), through the action of bilirubin and possibly by the decrease in free intracellular iron ions [70]. So in summary, several lines of evidence point to HO-1, a major downstream effector of Nrf2, as an important player in various anti-inflammatory processes.

7 Nrf2 and the Regulation of Expression of Cytokines and Other Proinflammatory Molecules

Different types of cells secrete cytokines in response to a stress. Cytokines are small proteins and polypeptides originally described as modulators of immune function and inflammation, but now well known to also regulate cell growth, cell differentiation, and wound healing [59]. While the list of known cytokines continues to grow, major groups include interleukins (ILs), interferons, tumor necrosis factor (TNF), growth factors, and chemokines. Chemokines are small cytokines whose major role is to guide the migration of inflammatory cells including leukocytes, monocytes, and neutrophils, to the site of inflammation. There are cytokines with proinflammatory functions (IL6, TNFα), while others have anti-inflammatory functions (IL10) [59].

As we discussed above, oxidative stress (oxidative or nitrosative stress) is able to activate the NFκB pathway, which will activate the secretion of proinflammatory cytokines, but activation of Nrf2 will dampen this response by inhibiting the NFκB

pathway and decrease the proinflammatory cytokine secretion [71]. For example, it is well known that Nrf2KO mice are more susceptible to a variety of oxidants, showing a higher inflammatory response [10, 11], and Nrf2 activation prevents LPS-induced transcriptional upregulation of proinflammatory cytokines [71]. In the case of Nrf2KO mice, the anti-inflammatory effect does not occur [72] and as a result, peritoneal neutrophils from Nrf2KO mice treated with LPS have significantly higher levels of proinflammatory cytokines (TNF-a and IL6) and chemokines (MCP1 and MIP20) than wild-type cells [72]. On the other hand, overexpression of Nrf2 protein in endothelial cells inhibits the overproduction of proinflammatory cytokines and chemokines, as well as the activation of NFκB [73, 74].

Upon stimulation of cytokine production, there is an increase in cell adhesion molecules (CAMs) in vascular endothelium and immune cells [59]. CAMs, such as ICAM-1 and VCAM-1, are proteins that bind to the cell surface, and they are involved in the recognition of the triggering signals that lead to cell activation, signal transduction, cell proliferation, and differentiation in response to inflammatory stimuli. For example, VCAM-1 mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vascular endothelium and contributes to leukocyte recruitment, which ultimately will lead to tissue damage due to oxidative stress [59, 75]. The expression of several CAMs is significantly higher in Nrf2KO mice [76]. It has been observed that Nrf2 represses the promotor of VCAM1 [31], and it is believed that this process is mediated by the expression of HO-1 [36, 71, 74, 77]. Similarly, upregulation of Nrf2 in human retinal pigment epithelial cells by lycophene is involved in the inhibition of TNFα, ICAM1, and NFκB expression [78].

Other factors involved in the inflammatory response are the metalloproteinase (MMPs), proteolytic enzymes that are involved in multiple physiological and pathological processes such as cell migration, wound healing, angiogenesis, apoptosis, and tumor metastasis. It has been observed that Nrf2 inhibits the expression of MMP9 and MMP7 in macrophages and epithelial cells, which is beneficial in the treatment of inflammatory bowel disease [79, 80]. Similarly, the protective effect of Nrf2 against UV damage in skin cells is mediated by the inhibition of both MPP9 expression and the NFκB pathway [81].

Therefore, so far it is evident that the Nrf2 pathway is essential for the control of inflammation, which is involved in the regulation of cytokines and MMPs expression. This regulation can be mediated by a direct action of the Nrf2 pathway, e.g., via increased expression of Nrf2 target genes through the ARE, and the elimination of ROS through expression of antioxidant proteins, or indirectly by the influence of Nrf2 on the NFκB pathway.

However, several reports have shown that Nrf2 regulates the expression of macrophage-specific genes that do not belong to the "anti-oxidative response elements, ARE." For instance, work from Harvey et al. [82] and Ishii et al. [83] has shown that genes encoding for MARCO (a scavenger receptor which is required for bacteria phagocytosis) and CD36 (a receptor for oxidized low-density lipoprotein related to atherosclerosis) are targets of Nrf2 but are not ARE-regulated genes. These data indicate that Nrf2 may regulate the expression of genes involved in inflammation in an ARE-independent manner. A recent report by Koboyashi et al.

[71] has demonstrated that indeed Nrf2 can regulate the expression of several genes, including IL6 and IL1b in an ARE-independent manner. Using microarrays and Nrf2 chromatin immunoprecipitation (ChIP) seq analysis in Nrf2 activated and Nrf2-depleted macrophages, they observed that Nrf2 binds to the proximity of pro-inflammatory cytokine genes and inhibits LPS-induced expression of these genes. This transcriptional interference mediated by NRf2 is not dependent of ARE sequences but rather by a disruption of the binding of RNA polymerase II to the corresponding cytokine loci (IL6 and IL1β). A similar observation was found using in vivo imaging analysis, where Nrf2 activation inhibits IL6 induction and alleviates the inflammatory phenotype in WIM-6 mice, a murine inflammatory model [71]. This data indicate that the Nrf2 pathway can regulate inflammatory responses in several ways, some of which are independent of the traditional ARE sequence.

8 Nrf2, Aging, and Cellular Senescence

Nrf2 has been described to play a role in slowing aging processes by mediating the beneficial effects of many manipulations that extend longevity and health span [84, 85]. There is an inverse correlation between aging and Nrf2 expression in several animal models [86, 87] and expression of several Nrf2 target genes decreases with aging as well, e.g., the levels of GSH and several antioxidant enzymes decrease with age [88–90]. Similarly, evidence has been found in vitro during replicative cellular senescence. For example, a decrease in both Nrf2 protein and mRNA levels (~65% and 45%, respectively) was found in senescence fibroblasts relative to their pre-senescent counterparts [91].

Cellular senescence is defined as an irreversible cell cycle arrest state where cells cannot divide but are still viable and metabolically active. Nowadays, it is well recognized that cellular senescence has an active role in aging, and it has been implicated in a number of pathologies including several age-related diseases [92, 93]. One characteristic of senescent cells is that they secrete a set of proinflammatory factors, including proteases, chemokines, proinflammatory cytokines, and miRNAs, which are believed to have a strong impact on the function of cells in their vicinity. It is believed that these secreted factors, better known as the senescence-associated secretory phenotype (SASP), disrupt the cellular microenvironment and alter the ability of adjacent cells to function properly, resulting in a compromised tissue structure and function [94, 95].

At a young age, Nrf2-KO mice show a diminished ability to respond to a wide variety of stressors and show symptoms associated with several age-related chronic diseases including emphysema, cancer, gastritis, retinopathy, and increased inflammation [96–98]. While the anti-aging effect of Nrf2 has been linked mostly to cytoprotection against stress and cancer prevention, silencing the Nrf2 pathway in human embryonic fibroblasts leads to premature cellular senescence [90]. Our own studies also demonstrated that human fibroblasts (WI38) that are deficient in Nrf2 have increased levels of senescent cells compared to wild-type cells, similar to what we found in vivo using the Nrf2KO mouse, where an increase in p16 (a marker of

senescent cells) and proinflammatory cytokines were found in tissues and plasma [99].

Likely, the decrease of Nrf2 levels with age and the downregulation of both its target genes and its direct regulation of inflammatory mediators (NFκB, cytokines expression) are contributing factors to the exacerbation of disease underlined by chronic inflammation observed during aging.

9 Nrf2 and Diseases

The first insights into the role of Nrf2 on disease came from the study of Nrf2 knockout mice, which exhibited symptoms of inflammation, tissue damage, and neurodegeneration [7, 8, 59]. Also, these animals are more susceptible to a series of inflammatory diseases, including models of emphysema, gastritis, colitis, cardiovascular disease, liver damage, and neurodegenerative disease [12, 59, 96]. Using ovalbumin complex to induce airway inflammation, it was observed that ablation of the Nrf2 pathway increased the allergen-mediated airway inflammation and asthma [14, 96, 100]. These studies demonstrated that Nrf2-mediated signaling pathways limit airway eosinophilia, mucus hypersecretion, and prevent airway hyperreactivity that prevents the development of asthma [100].

Deficiency or alteration of the Nrf2 pathway has been directly linked to several inflammatory diseases, such as pleurisy [101], atherosclerosis and myocarditis [102, 103], sickle cell disease [104], arthritis [105], LPS-induced uveitis [106], skin damage [107], and pneumonia, [31]. Also, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) disease, LPS-induced encephalitis [108–110] in all these diseases, activation of the Nrf2 signaling pathway reduces the inflammatory injury, alleviates the burden of inflammatory factors such as proinflammatory cytokines, and boosts the expression of detoxifying enzymes that help to minimize the inflammatory response.

Although the list of diseases where Nrf2 seems to be important is extensive, we will focus this review solely on what is known about the role of the Nrf2 signaling pathway in two major diseases, neurodegenerative diseases and cancer, which are among the most serious and prevalent health concerns in our society.

10 Nrf2 and Neurodegenerative Diseases

In general, most of the neurodegenerative diseases, if not all, are characterized by increased oxidative stress, mitochondrial dysfunction, and neuroinflammation; and the common molecular feature between them is a dysregulation of Nrf2 signaling pathway. As we described before, a significant amount of research has been dedicated to investigate the role of Nrf2 in inflammatory diseases. From this work, it has been identified that Nrf2 is a promising target to treat several neurodegenerative diseases that include a significant inflammatory component, such as amyotrophic

lateral sclerosis (ALS), multiple sclerosis, Parkinson's diseases (PD), Alzheimer's disease (AD), and Huntington's disease (HD) [27, 59].

In general, it has been observed that in these diseases, Nrf2 can reduce the number of overactive microglia and astrocytes, which are substantial contributors to central nervous system pathology [111–113]. It has been described that oxidative damage, mitochondrial dysfunction, and chronic inflammation are common pathological mechanisms observed in neurodegenerative diseases, and Nrf2 regulates all of them tightly [114]. Furthermore, in many of these diseases, Nrf2 levels are diminished. For example, in postmortem brains of AD patients the levels of Nrf2 protein were lower in hippocampal neurons, compared to age-matched controls [115]. Similarly, mouse transgenic AD models expressing mutated human APP and PS1 genes showed reduced expression of Nrf2 and target genes such as NQO1, glutathione synthetic enzymes, and HO-1 in hippocampal neurons [116]. Importantly, overexpression of Nrf2 in AD transgenic mice protects against the toxic effect of Aβ deposition and improves cognition [117]. Furthermore, it was discovered that GSK3b, a kinase that sensitizes neurons to oxidative stress and increases neuronal death, can phosphorylate Nrf2, inducing it exclusion from the nucleus and decreasing its activity [118, 119].

Similar features are found in other neurodegenerative diseases. For example, postmortem brain tissue from PD patients shows lower levels of Nrf2 signaling pathway [115], and one of the earliest parameters observed in PD is the depletion of GSH in the substantia nigra [120], which leads to a decrease in mitochondrial complex I activity, causing an increased in ROS, α-synuclein aggregation, and the death of dopaminergic neurons [121]. As for Huntington's disease, an increase in oxidative stress and mitochondrial dysfunction are observed in HD patients [122, 123]. In both cases, activation of the Nrf2 pathway protects against neurotoxicity in animal models for PD (neurotoxicity induced by 6-OHDA and MPTP) and HD (neurotoxicity induced by malonate) [122, 124].

In the case of ALS, a vast set of data have identified that mutations in SOD1 is the source of oxidative stress that causes mitochondrial dysfunction. This appears to be a major mechanism underlying the development of ALS [125–128]. Postmortem studies have shown that expression of Nrf2 (mRNA and protein levels) in motor neurons expressing mutant SOD1 from spinal cord from ALS patients are lower than those from healthy patients [129, 130], suggesting that deficiency of Nrf2 might play a role in this disease. In vitro studies using astrocytes overexpressing a disease-relevant mutant SOD1 showed that the expression of Nrf2 and related genes are downregulated [131, 132], and that induction of Nrf2 activity increases the survival of these cells. Some reports also indicate that induction of Nrf2 activity improves the survival of the ALS mouse model [133].

Activation of the Nrf2 signaling pathway has been pointed as a key potential target for the development of new drugs to treat/prevent neurodegenerative diseases. Following this venue, a flourishing number of natural products and polyphenolic compounds that activate Nrf2 pathway have been widely studied as possible therapeutic tools against these diseases. For example, curcumin has been used to treat AD in drosophila and mouse models of the disease [134, 135]. Ursolic acid and

oleanolic acid, members of the triterpenoid family, are being extensively studied as drugs to treat AD and PD [136, 137]. Another well-known Nrf2 activator is sulforaphane, a compound found in cruciferous vegetables, which has been widely used not only for neurodegenerative diseases [138, 139], but also for other diseases that involve increased oxidative stress, i.e., cancer (see below). Naringenin, a flavonoid found in grapefruit, activates the Nrf2 pathway, and it has been used to treat mouse models of AD and PD for its neuroprotective effects [124, 140, 141]. Also, an epidemiological study has linked a flavonoid-rich dietary intake with reduced PD risk [142]. Many of these compounds and derivatives have already been tested in both in vitro and in vivo (mouse models) and some of them are in clinical trials. For further review, please see Buendia et al., [114].

11 Nrf2 and Cancer

One of the most notorious phenotypes observed in Nrf2KO mice is the high susceptibility of these animals to develop cancer at young age [143]. From this initial study, the role of Nrf2 in carcinogenesis has been massively studied, and the evidence that Nrf2 has an anti-carcinogenesis effect is abundant [144–146]. For example, several chemopreventive drugs (i.e., sulforaphane, dithiolethiones) have the ability to activate the Nrf2 pathway, which activates several cytoprotective pathways including the expression of antioxidant enzymes and inhibition of proinflammatory pathways [147]. The effect of chemopreventive drugs is abolished in Nrf2KO mice, while mice that overexpress Nrf2 showed improved resistance to cell metastasis [148]. Many natural products that activate Nrf2, such as sulforaphane have been intensively studied as chemopreventive agents. Several putative protective mechanisms that might imply Nrf2 have been described, including activation of cytoprotective factors (antioxidant enzymes) as well as downregulation of the NFκB signaling pathway, and oncogenes fos and jun of the AP1 complex [149, 150]. The importance of Nrf2 activation mediated by chemopreventive agents has been reinforced multiple times, not only for cancer prevention but also in other diseases and conditions such as diabetes [151], obesity [152], and neurodegeneration [122].

However, activation of the Nrf2 pathway does not always have a good connotation, and in fact a higher activity of the Nrf2 pathway has been found in some cancer cells [147]. It has been discovered that specific mutations in Nrf2 itself can lead to its overactivation, and cancer cells that have this mutated version of Nrf2 have higher cytoprotection. Therefore, in this case, oncogenes can promote tumorogenesis in part through an Nrf2-induced favorable intracellular environment, enhancing cancer cell survival, and promoting tumor growth [153]. Moreover, common oncogenes such as KRAS, BRAF, and MYC, all increase the transcription of Nrf2, leading the cytoprotective activity in cancer cells, resulting in cells being more resistant to standard treatment. Therefore, it seems that an increase in Nrf2 activity is desirable in a premalignant state, when a cellular response to the developing

carcinogenesis might halt further progress in that direction, but is undesirable in later stages of tumorigenesis, when cells may become more resistant to treatment.

12 Conclusions

It is very clear that the Nrf2 signaling pathway regulates inflammation, notably by increasing the expression of cytoprotective genes whose expression is driven by binding to ARE sequences. However, new evidence has demonstrated that Nrf2 can also regulate the inflammatory response through cross talk with the NFκb pathway, and by a direct regulation of gene expression of cytokine genes that do not have an

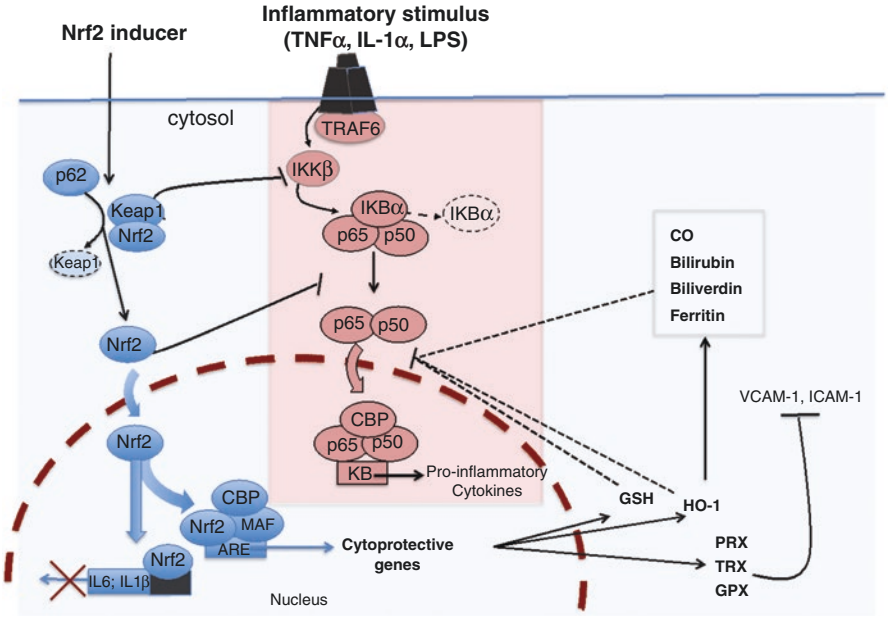

Fig. 1 Nrf2 and the regulation of inflammatory pathway. The activation of Nrf2 pathway allows Nrf2 transcription factor to translocate to the nucleus. In the nucleus, Nrf2 is able to bind to the ARE sequence and activate the expression of cytoprotective genes, such as antioxidant enzymes [peroxiredoxin (PRX); thioredoxin, TRX; glutaredoxin, (GPX)], key enzymes for synthesis of glutathione (GSH), heme oxygenase 1, and (HO-1) among others. HO-1 has anti-inflammatory action itself by modulating NfκB pathway (p65/p50 subunits) directly or indirectly by increasing the levels of carbon monoxide (CO), bilirubin, biliverdin that together decreases the oxidant levels. Data on literature indicate that peroxiredoxin (Prx), thioredoxin (Trx) and glutathione peroxidase (Gpx) are key enzymes to inhibit the activity of cellular adhesion proteins (CAMs) that participate in inflammation. On the other hand, Nrf2 also can bind directly to the DNA sequence of cytokines (IL6 and IL1-α) and inhibits their expression by impeding the binding of polymerase II to the DNA and inhibiting their transcription. At the cytosolic level, it has been described that Nrf2 can directly regulate NFκB pathway by redox-sensitive mechanism

ARE sequence. In fact, current studies using more advanced techniques, such ChIP-seq and big data analysis, have shown that Nrf2 regulates over 200 genes that encode for cytoprotective proteins [154], but also control the expression of other 600 additional genes associated with inflammation, cancer, neurodegeneration, and other major diseases [155].

Dimethyl fumarate (Tecfidera®), an Nrf2 activator that is approved by the FDA to treat the inflammatory disease multiple sclerosis is the best example of the potential of Nrf2 manipulation as a means to regulate inflammation [156]. Many ongoing studies targeting the Nrf2 pathway are devoted to develop specific therapeutic agents to control the symptoms of inflammation and prevent and treat many diseases, such as cancer, neurodegeneration, and other age-related chronic diseases. With this new data available, new highly specific activators of Nrf2 can be designed to minimize its pleiotropic effects, avoiding the pro-cancer effects, and probably extending the healthy portion of the human life span (Fig. 1).

References

1. Brooks-Worrell B, Palmer JP. Immunology in the clinic review series; focus on metabolic diseases: development of islet autoimmune disease in type 2 diabetes patients: potential sequelae of chronic inflammation. Clin Exp Immunol. 2012;167:40–6.
2. Stolp HB, Dziegielewska KM. Review: role of developmental inflammation and blood–brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. Neuropathol Appl Neurobiol. 2009;35:132–46.
3. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454:428–35.
4. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. The Journals of Gerontology: Series A. 2014;69:S4–9.
5. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–22.
6. Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. Annu Rev Pharmacol Toxicol. 2003;43:233–60.
7. Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells. 2011;16:123–40.
8. Kobayashi E, Suzuki T, Yamamoto M. Roles Nrf2 plays in myeloid cells and related disorders. Oxidative Med Cell Longev. 2013;2013:529219.
9. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as anticancer strategy. Nat Rev Drug Discov. 2013:931–47.
10. Yoh K, Itoh K, Enomoto A, Hirayama A, Yamaguchi N, Kobayashi M, Morito N, Koyama A, Yamamoto M, Takahashi S. Nrf2-deficient female mice develop lupus-like autoimmune nephritis. Kidney Int. 2001;60:1343–53.
11. Ma Q, Battelli L, Hubbs AF. Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. Am J Pathol. 2006;168:1960–74.
12. Johnson DA, Amirahmadi S, Ward C, Fabry Z, Johnson JA. The absence of the pro-antioxidant transcription factor Nrf2 exacerbates experimental autoimmune encephalomyelitis. Toxicol Sci. 2010;114:237–46.

13. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J Clin Investig. 2004;114:1248–59.
14. Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, Yamamoto M, Kensler TW, Tuder RM, Georas SN, Biswal S. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med. 2005;202:47–59.
15. Garbin U, Fratta Pasini A, Stranieri C, Cominacini M, Pasini A, Manfro S, Lugoboni F, Mozzini C, Guidi G, Faccini G. Cigarette smoking blocks the protective expression of Nrf2/ARE pathway in peripheral mononuclear cells of young heavy smokers favouring inflammation. PLoS One. 2009;4:e8225.
16. Cho HY, Reddy SP, Yamamoto M, Kleeberger SR. The transcription factor NRF2 protects against pulmonary fibrosis. FASEB J. 2004;18:1258–60.
17. He X, Ma Q. Disruption of Nrf2 synergizes with high glucose to cause heightened myocardial oxidative stress and severe cardiomyopathy in diabetic mice. J Diabetes Metab. 2012;002(Suppl 7)
18. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, et al. Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. Proc Natl Acad Sci U S A. 2005;102:4584–9.
19. Talalay P, Fahey JW, Healy ZR, Wehage SL, Benedict AL, et al. Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. Proc Natl Acad Sci U S A. 2007;104:17500–5.
20. Ma Q, Kinneer K. Chemoprotection by phenolic antioxidants. Inhibition of tumor necrosis factor α induction in macrophages. J Biol Chem. 2002;277:2477–84.
21. Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Ann Rev Immunol. 1996;14:649–83.
22. Smale ST. Hierarchies of NF-kappa B target-gene regulation. Nat Immunol. 2011;12:689–94.
23. Sakurai H, Suzuki S, Kawasaki N, Nakano H, Okazaki T, Chino A, Doi T, Saiki I. Tumor necrosis factor-alpha-induced IKK phosphorylation of NF-kappaB p65 on serine 536 is mediated through the TRAF2, TRAF5, and TAK1 signaling pathway. J Biol Chem. 2003;278(38):36916–23.
24. Perkins ND. The Rel/NF-kappa B family: friend and foe. Trends Biochem Sci. 2000;25:434–40.
25. Manea A, Manea SA, Gafencu AV, Raicu M. Regulation of NADPH oxidase subunit p22(phox) by NF-kB in human aortic smooth muscle cells. Arch Physiol Biochem. 2007;113:163–72.
26. Mauro C, Leow SC, Anso E, Rocha S, Thotakura AK, Tornatore L, Moretti M, De Smaele E, Beg AA, Tergaonkar V, et al. NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. Nat Cell Biol. 2011;13:1272–9.
27. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-kB response pathways. Biochem Soc Trans. 2015;43:621–6.
28. Pan H, Wang H, Wang X, Zhu L, Mao L. The absence of Nrf2 enhances NF-kappaB-dependent inflammation following scratch injury in mouse primary cultured astrocytes. Mediat Inflamm. 2012;217580
29. Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ, Ladner KJ, Bevan AK, Foust KD, Godbout JP, et al. Microglia induce motor neuron death via the classical NF-kappaB pathway in amyotrophic lateral sclerosis. Neuron. 2014;81:1009–23.
30. Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M, Liby KT, Risingsong R, Sporn M, Beal MF, Kiaei M. Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free Radic Biol Med. 2011;51:88–96.
31. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, Biswal S. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest. 2006;116:984–95.

32. Ganesh YV, Negi G, Sharma SS, Kumar A. Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-kappaB pathways in diabetic neuropathy. Redox Biol. 2013;1:394–7.
33. Lin W, Wu RT, Wu T, Khor TO, Wang H, Kong AN. Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. Biochem Pharmacol. 2008;76:967–73.
34. Lee DF, Kuo HP, Liu M, Chou CK, Xia W, Du Y, Shen J, Chen CT, Huo L, Hsu MC, Li CW, Ding Q, Liao TL, Lai CC, Lin AC, Chang YH, Tsai SF, Li LY, Hung MC. KEAP1 E3 ligase-mediated downregulation of NF-kappaB signaling by targeting IKKbeta, Mol. Cell. 2009;36:131–40.
35. Brigelius-Flohe R, Flohe L. Basic principles and emerging concepts in the redox control of transcription factors. Antioxid Redox Signal. 2011;15:2335–81.
36. Banning A, Brigelius-Flohe R. NF -kappaB, Nrf2, and HO-1 interplay in redox-regulated VCAM-1 expression. Antioxid Redox Signal. 2005;7:889–99.
37. Arbibe L, Mira JP, Teusch N, Kline L, Guha M, Mackman N, Godowski PJ, Ulevitch RJ, Knaus UG. Toll-like receptor 2-mediated NF-κB activation requires a Rac1-dependent pathway. Nat Immunol. 2000;1:533–40.
38. Bokoch GM. Regulation of innate immunity by rho GTPases. Trends Cell Biol. 2005;15:163–71.
39. Ohsawa K, Imai Y, Kanazawa H, Sasaki Y, Kohsaka S. Involvement of Iba1 in membrane ruffling and phagocytosis of macrophages/microglia. J Cell Sci. 2000;113:3073–84.
40. Salazar M, Rojo AI, Velasco D, de Sagarra RM, Cuadrado A. Glycogen synthase kinase-3β inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J Biol Chem. 2006;281:14841–51.
41. Cuadrado A, Martín-Moldes Z, Ye J, Lastres-Becker I. Transcription factors NRF2 and NF-kB are coordinated effectors of the rho family, GTP-binding protein RAC1 during inflammation. J Biol Chem. 2014;289:15244–58.
42. Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat Rev Immunol. 2010;10:210–5.
43. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V, Latz E. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol. 2009;183:787–91.
44. Wang H, Liu K, Geng M, Gao P, Wu X, Hai Y, Li Y, Li Y, Luo L, Hayes JD, Wang XJ, Tang X. RXRalpha inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2. Cancer Res. 2013;73:3097–108.
45. Shi F, Kouadir M, Yang Y. NALP3 inflammasome activation in protein misfolding diseases. Life Sci. 2015;135:9–14.
46. Kolb R, Liu GH, Janowski AM, Sutterwala FS, Zhang W. Inflammasomes in cancer: a double-edged sword. Protein Cell. 2014;5:12–20.
47. Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol. 2016;13:148–59.
48. Liu X, Zhang X, Ding Y, Zhou W, Tao L, Lu P, Wang Y, Hu R. Nuclear factor E2-related factor-2 negatively regulates NLRP3 inflammasome activity by inhibiting reactive oxygen species-induced NLRP3 priming. Antioxid Redox Signal. 2017;26:28–43.
49. Tsai PY, Ka SM, Chang JM, Chen HC, Shui HA, Li CY, Hua KF, Chang WL, Huang JJ, Yang SS, Chen A. Epigallocatechin-3-gallate prevents lupus nephritis development in mice via enhancing the Nrf2 antioxidant pathway and inhibiting NLRP3 inflammasome activation. Free Radic Biol Med. 2011;51:744–54.
50. Ka SM, Lin JC, Lin TJ, Liu FC, Chao LK, Ho CL, Yeh LT, Sytwu HK, Hua KF, Chen A. Citral alleviates an accelerated and severe lupus nephritis model by inhibiting the activation signal of NLRP3 inflammasome and enhancing Nrf2 activation. Arthritis Res Ther. 2015;17:331.

51. He L, Peng X, Zhu J, Chen X, Liu H, Tang C, Dong Z, Liu F, Peng Y. Mangiferin attenuate sepsis-induced acute kidney injury via antioxidant and anti-inflammatory effects. Am J Nephrol. 2014;40:441–50.
52. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010;464: 1357–61.
53. Freigang S, Ampenberger F, Spohn G, Heer S, Shamshiev AT, et al. Nrf2 is essential for cholesterol crystal-induced inflammasome activation and exacerbation of atherosclerosis. Eur J Immunol. 2011;41:2040–51.
54. Zhao C, Gillette DD, Li X, Zhang Z, Wen H. Nuclear factor E2-related factor-2 (Nrf2) is required for NLRP3 and AIM2 inflammasome activation. J Biol Chem. 2014;289(24):17020–9.
55. Yachie A, Toma T, Mizuno K, Okamoto H, Shimura S, Ohta K, Kasahara Y, Koizumi S. Heme oxygenase-1 production by peripheral blood monocytes during acute inflammatory illnesses of children. Exp Biol Med. 2003;228:550–6.
56. Pae HO, Ae Ha Y, Chai KY, Chung HT. Heme oxygenase-1 attenuates contact hypersensitivity induced by 2,4-dinitrofluorobenzene in mice. Immunopharmacol Immunotoxicol. 2008;30:207–16.
57. Patil K, Bellner L, Cullaro G, Gotlinger KH, Dunn MW, Schwartzman ML. Heme oxygenase-1 induction attenuates corneal inflammation and accelerates wound healing after epithelial injury. Invest Ophthalmol Vis Sci. 2008;49:3379–86.
58. Kim J, Surh YJ. The role of *Nrf2* in cellular innate immune response to inflammatory injury. Toxicological Research. 2009;25(4):159–73.
59. Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ, Figdor CG. Different faces of the heme-heme oxygenase system in inflammation. Pharmacol Rev. 2003;55: 551–71.
60. Ahmed SM, Luo L, Namani A, Wang XJ, Tang X. *Nrf2* signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta. 1863;2017:585–97.
61. Chi X, Yao W, Xia H, Jin Y, Li X, Cai J, Hei Z. Elevation of HO-1 expression mitigates intestinal ischemia-reperfusion injury and restores tight junction function in a rat liver transplantation model. Oxidative Med Cell Longev. 2015;986075
62. Choi H. The cytoprotective effect of isorhamnetin against oxidative stress is mediated by the upregulation of the Nrf2-dependent HO-1 expression in C2C12 myoblasts through scavenging reactive oxygen species and ERK inactivation. Gen Physiol Biophys. 2016;35:145–54.
63. Kuhn AM, Tzieply N, Schmidt MV, von Knethen A, Namgaladze D, Yamamoto M, Brune B. Antioxidant signaling via Nrf2 counteracts lipopolysaccharide-mediated inflammatory responses *in* foam cell macrophages. Free Radic Biol Med. 2011;50:1382–91.
64. Lee DS, Jeong GS. Arylbenzofuran isolated from Dalbergia odorifera suppresses lipopolysaccharide-induced mouse BV2 microglial cell activation, which protects mouse hippocampal HT22 cells death from neuroinflammation-mediated toxicity. Eur J Pharmacol. 2014;728:1–8.
65. Brandsma CA, Hylkema MN, van der Strate BW, Slebos DJ, Luinge MA, Geerlings M, Timens W, Postma DS, Kerstjens HA. Heme oxygenase-1 prevents smoke-induced B-cell infiltrates: a role for regulatory T cells? Respir Res. 2008;9:17.
66. Xia ZW, Xu LQ, Zhong WW, Wei JJ, Li NL, Shao J, Li YZ, Yu SC, Zhang ZL. Heme oxygenase-1 attenuates ovalbumin-induced airway inflammation by up-regulation of foxp3 T-regulatory cells, interleukin-10, and membrane-bound transforming growth factor-1. Am J Pathol. 2007;171:1904–14.
67. Liu Y, Zhu B, Wang X, Luo L, Li P, Paty DW, Cynader MS. *Bilirubin* as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis. J Neuroimmunol. 2003;139:27–35.
68. Sawada K, Ohnishi K, Kosaka T, Chikano S, Egashira A, Okui M, Shintani S, Wada M, Nakasho K. Shimoyama T exacerbated autoimmune *hepatitis* successfully treated with leukocytapheresis and *bilirubin* adsorption therapy. J Gastroenterol. 1997;32:689–95.
69. Kadl A, Pontiller J, Exner M, Leitinger N. Single bolus injection of *bilirubin* improves the clinical outcome in a mouse model of endotoxemia. Shock. 2007;28:582–8.

70. Lanone S, Bloc S, Foresti R, Almolki A, Taillé C, Callebert J, Conti M, Goven D, Aubier M, Dureuil B, El-Benna J, Motterlini R, Boczkowski J. Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. FASEB J. 2005;19:1890–2.
71. Soares MP, Seldon MP, Gregoire IP, Vassilevskaia T, Berberat PO, Yu J, Tsui TY, Bach FH. Heme oxygenase-1 modulates the expression of adhesion molecules associated with endothelial cell activation. J Immunol. 2004;172:3553–63.
72. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, Tanaka N, Moriguchi T, Motohashi H, Nakayama K, Yamamoto M. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624.
73. Thimmulappa RK, Scollick C, Traore K, Yates M, Trush MA, Liby KT, Sporn MB, Yamamoto M, Kensler TW, Biswal S. Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-Imidazolide. Biochem Biophys Res Commun. 2006;351:883–9.
74. Levonen AL, Inkala M, Heikura T, Jauhiainen S, Jyrkkänen HK, Kansanen E, Määttä K, Romppanen E, Turunen P, Rutanen J, Yla-Herttuala S. Nrf2 gene transfer induces antioxidant enzymes and suppresses smooth muscle cell growth in vitro and reduces oxidative stress in rabbit aorta in vivo. Arterioscler Thromb Vasc Biol. 2007;27:741–7.
75. Pae HO, Oh GS, Lee BS, Rim JS, Kim YM, Chung HT. 3-Hydroxyanthranilic acid, one of L-tryptophan metabolites, inhibits monocyte chemoattractant protein-1 secretion and vascular cell adhesion molecule-1 expression via heme oxygenase-1 induction in human umbilical vein endothelial cells. Atherosclerosis. 2006;187:274–84.
76. Yang L, Froio RM, Sciuto TE, Dvorak AM, Alon R, Luscinskas FW. ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow. Blood. 2005;106:584–92.
77. Chen XL, Dodd G, Thomas S, Zhang X, Wasserman MA, Rovin BH, Kunsch C. Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression. Am J Physiol Heart Circ Physiol. 2006:H1862–70.
78. Chen XL, Varner SE, Rao AS, Grey JY, Thomas S, Cook CK, Wasserman MA, Medford RM, Jaiswal AK, Kunsch C. Kunsch Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem. 2003;278(2):703–11.
79. Yang PM, Wu ZZ, Zhang YQ, Wung BS. Lycopene inhibits ICAM-1 expression and NF-kappaB activation by Nrf2-regulated cell redox state in human retinal pigment epithelial cells. Life Sci. 2016;155:94–101.
80. Lee SH, Sohn DH, Jin XY, Kim SW, Choi SC, Seo GS. Seo2',4′,6′-tris(methoxymethoxy) chalcone protects against trinitrobenzene sulfonic acid-induced colitis and blocks tumor necrosis factor-alpha-induced intestinal epithelial inflammation via heme oxygenase 1-dependent and independent pathways. Biochem Pharmacol. 2007;74:870–80.
81. Kim BC, Jeon WK, Hong HY, Jeon KB, Hahn JH, Kim YM, Numazawa S, Yosida T, Park EH, Lim CJ. The anti-inflammatory activity of Phellinus linteus (Berk. & M.a. Curt.) is mediated through the PKCdelta/Nrf2/ARE signaling to up-regulation of heme oxygenase-1. J Ethnopharmacol. 2007;113:240–7.
82. Gan FF, , Ling H , Ang X , Reddy SA , Lee SS , Yang H , Tan SH , Hayes JD , Chui WK , Chew EH A novel shogaol analog suppresses cancer cell invasion and inflammation, and displays cytoprotective effects through modulation of NF-kappaB and Nrf2-Keap1 signaling pathways, Toxicol Appl Pharmacol2013; 272: 852–862.
83. Harvey CJ, Thimmulappa RK, Sethi S, Kong X, Yarmus L, Brown RH, Feller-Kopman D, Wise R, Biswal S. Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. Sci Transl Med. 2011;3:78ra32.
84. Ishii T, Itoh K, Ruiz E, Leake DS, Unoki H, Yamamoto M, Mann GE. Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. Circ Res. 2004;94:609–16.

85. Sykiotis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev Cell. 2008;14:76–85.
86. Steinbaugh MJ, Sun LY, Bartke A, Miller RA. Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. Am J Physiol Endocrinol Metab. 2012;4:E488–95.
87. Onken B, Driscoll M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1. PLoS One. 2010;5:e8758.
88. Kode A, Rajendrasozhan S, Caito S, Yang SR, Megson IL, Rahman I. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2008;294:L478–88.
89. Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, Liu RM, Hagen TM. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proc Natl Acad Sci U S A. 2004;101:3381–6.
90. Duan W, Zhang R, Guo Y, Jiang Y, Huang Y, Jiang H, Li C. Nrf2 activity is lost in the spinal cord and its astrocytes of aged mice. In Vitro Cell Dev Biol Anim. 2009;45:388–97.
91. Kapeta S, Chondrogianni N, Gonos ES. Nuclear erythroid factor 2-mediated proteasome activation delays senescence in human fibroblasts. J Biol Chem. 2010;285:8171–84.
92. Cristofalo VJ. Ten years later: what have we learned about human aging from studies of cell cultures? The Cerontologist. 1996;36:737–41.
93. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol. 2013;75:685–705.
94. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, Van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011;479:232–6.
95. Coppé JP, Patil CK, Rodier F, Krtolica A, Beauséjour CM, Parrinello S, Hodgson JG, Chin K, Desprez PY, Campisi J. A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. PLoS One. 2010;5(2):e9188.
96. Ishii Y, Itoh K, Morishima Y, Kimura T, Kiwamoto T, Iizuka T, Hegab AE, Hosoya T, Nomura A, Sakamoto T, Yamamoto M, Sekizawa K. Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. J Immunol. 2005;175:6968–75.
97. Khor TO, Huang MT, Prawan A, Liu Y, Hao X, Yu S, Cheung WK, Chan JY, Reddy BS, Yang CS, Kong AN. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer Prev Res (Phila). 2008;1:187–91.
98. Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med. 2004;10:549–57.
99. Wang R, Yu Z, Sunchu B, Shoaf J, Dang I, Zhao S, Caples K, Bradley L, Beaver LM, Ho E, Löhr CV, Perez VI. *Rapamycin* inhibits the secretory phenotype of senescent cells by a Nrf2-independent mechanism. Aging Cell. 2017;16:564–74.
100. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J Clin Invest. 2004;114:1248–59.
101. Itoh K, Mochizuki M, Ishii Y, Ishii T, Shibata T, Kawamoto Y, Kelly V, Sekizawa K, Uchida K, Yamamoto M. Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2). Mol Cell Biol. 2004;24:36–45.
102. Freigang F, Ampenberger G, Spohn S, Heer AT, Shamshiev J, Kisielow M, Hersberger M, Yamamoto MF, Bachmann M. KopfNrf2 is essential for cholesterol crystal-induced inflammasome activation and exacerbation of atherosclerosis. Eur J Immunol. 2016;41:2040–51.
103. Xue WL, Bai X. Zhang L rhTNFR:fc increases Nrf2 expression via miR-27a mediation to protect myocardium against sepsis injury. Biochem Biophys Res Commun. 2016;464:855–61.
104. Keleku-Lukwete N, Suzuki M, Otsuki A, Tsuchida K, Katayama S, Hayashi M, Naganuma E, Moriguchi T, Tanabe O, Engel JD, Imaizumi M, Yamamoto M. Amelioration of inflamma-

tion and tissue damage in sickle cell model mice by Nrf2 activation. Proc Natl Acad Sci U S A. 2016;112:12169–74.
105. Maicas N, Ferrándiz ML, Brines R, Ibáñez L, Cuadrado A, Koenders MI, van den Berg WB, Alcaraz MJ. Deficiency of Nrf2 accelerates the effector phase of arthritis and aggravates joint disease. Antioxid Redox Signal. 2016;15:889–901.
106. Nagai N, Thimmulappa RK, Cano M, Fujihara M, Izumi-Nagai K, Kong X, Sporn MB, Kensler TW, Biswal S, Handa JT. Nrf2 is a critical modulator of the innate immune response in a model of uveitis. Free Radic Biol Med. 2009;47:300–6.
107. Braun S, Hanselmann C, Gassmann MG, auf dem Keller U, Born-Berclaz C, Chan K, Kan YW, Werner S. Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. Mol Cell Biol. 2002;22:5492–505.
108. Wang CY, Wang ZY, Xie JW, Cai JH, Wang T, Xu Y, Wang X, An L. CD36 upregulation mediated by intranasal LV-NRF2 treatment mitigates hypoxia-induced progression of Alzheimer's-like pathogenesis. Antioxid Redox Signal. 2016;21:2208–30.
109. Pareek TK, Belkadi A, Kesavapany S, Zaremba A, Loh SL, Bai L, Cohen ML, Meyer C, Liby KT, Miller RH, Sporn MB, Letterio JJ. Triterpenoid modulation of IL-17 and Nrf-2expression ameliorates neuroinflammation and promotes remyelination in autoimmune encephalomyelitis. Sci Rep. 2011;1:201.
110. Escartin C, Brouillet E. The Nrf2 pathway as a potential therapeutic target for Huntington disease a commentary on "Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington disease". Free Radic Biol Med. 2016;49:144–6.
111. Brandenburg LO, Kipp M, Lucius R, Pufe T, Wruck CJ. Sulforaphane suppresses LPS-induced inflammation in primary rat microglia. Inflamm Res. 2010;59:443–50.
112. Calkins MJ, Johnson DA, Townsend JA, Vargas MR, Dowell JA, Williamson TP, Kraft AD, Lee J-M, Li J, Johnson JA. The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease. Antioxid Redox Signal. 2009;11:497–508.
113. Innamorato NG, Rojo AI, Garcia-Yague AJ, Yamamoto M, de Ceballos ML, Cuadrado A. The transcription factor Nrf2 is a therapeutic target against brain inflammation. J Immunol. 2008;181:680–9.
114. Buendia I, Michalska P, Navarro E, Gameiro I, Egea J, León R. *Nrf2*-ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. Pharmacol Ther. 2016;157:84–104.
115. Ramsey CP, Glass CA, Montgomery MB, Lindl KA, Ritson GP, Chia LA, Hamilton RL, Chu CT, Jordan-Sciutto KL. Expression of Nrf2 in neurodegenerative diseases. J Neuropathol Exp Neurol. 2007;66:75–85.
116. Kanninen K, Malm TM, Jyrkkänen HK, Goldsteins G, Keksa-Goldsteine V, Tanila H, Yamamoto M, Ylä-Herttuala S, Levonen AL, Koistinaho J. Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. Mol Cell Neurosci. 2008;39:302–13.
117. Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, Cole GM. Author information:phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. Neurobiol Aging. 2001;22:993–1005.
118. Rojo AI, Sagarra MR, Cuadrado A. GSK-3beta down-regulates the transcription factor Nrf2 after oxidant damage: relevance to exposure of neuronal cells to oxidative stress. J Neurochem. 2008;105:192–202.
119. Salazar M, Rojo AI, Velasco D, de Sagarra RM, Cuadrado A. Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J Biol Chem. 2006;281:14841–51.
120. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med. 2004;10(Suppl):S18–25.

121. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
122. Gan L, Johnson JA. Oxidative damage and the Nrf2–ARE pathway in neurodegenerative diseases. Biochim Biophys Acta. 1842;2014:1208–18.
123. Colle D, Hartwig JM, Soares FA, Farina M. Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro. Brain Res Bull. 2012;87:397–405.
124. Lou H, Jing X, Wei X, Shi H, Ren D, Zhang X. Naringenin protects against 6-OHDA-induced neurotoxicity via activation of the Nrf2/ARE signaling pathway. Neuropharmacology. 2014;79:380–8.
125. Jin YN, Yu YV, Gundemir S, Jo C, Cui M, Tieu K, Johnson GV. Impaired mitochondrial dynamics and Nrf2 signaling contribute to compromised responses to oxidative stress in striatal cells expressing full-length mutant huntingtin. PLoS One. 2013;8:e57932.
126. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX. Mutations in cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;362:59–62.
127. Contestabile A. Amyotrophic lateral sclerosis: from research to therapeutic attempts and therapeutic perspectives. Curr Med Chem. 2011;18:5655–65.
128. Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Radic Biol Med. 2010;48:629–41.
129. Pehar VMR, Robinson KM, Cassina P, Díaz-Amarilla PJ, Hagen TM, Radi R, Barbeito L, Beckman JS. Mitochondrial superoxide production and nuclear factor erythroid 2-related factor 2 activation in p75 neurotrophin receptor-induced motor neuron apoptosis. J Neurosci. 2007;27:7777–85.
130. Sarlette A, Krampfl K, Grothe C, Neuhoff N, Dengler R, Petri S. Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 2008;67:1055–62.
131. Milani P, Ambrosi G, Gammoh O, Blandini F, Cereda C. SOD1 and DJ-1 converge at Nrf2 pathway: a clue for antioxidant therapeutic potential in neurodegeneration. Oxidative Med Cell Longev. 2013;836760
132. Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. J Neurosci. 2008;28:13574–81.
133. Barber SC, Mead RC, Shaw PG. Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target. Biochim Biophys Acta (BBA) - Mol Basis Dis. 2006;1762:1051–67.
134. Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, Rock CL, Pruitt MA, Yang F, Hudspeth B, Hu S, Faull KF, Teter B, Cole GM, Frautschy SA. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. Pharmacol Exp Ther. 2008;326(1):196–208.
135. Caesar I, Jonson M, Nilsson KP, Thor S, Hammarström P. Curcumin promotes Abeta fibrillation and reduces neurotoxicity in transgenic Drosophila. PLoS One. 2012;7(2):e31424.
136. Liu J, Wu Q, Lu YF, Pi J. New insights into generalized hepatoprotective effects of oleanolic acid: key roles of metallothionein and Nrf2 induction. Biochem Pharmacol. 2008;76:922–8.
137. Li L, Zhang X, Cui L, Wang L, Liu H, Ji H, Du Y. Ursolic acid promotes the neuroprotection by activating Nrf2 pathway after cerebral ischemia in mice. Brain Res. 2013;1497:32–9.
138. Kim V, Kim HY, Ehrlich HY, Choi SY, Kim DJ, Kim Y. Amelioration of Alzheimer's disease by neuroprotective effect of sulforaphane in animal model. Amyloid. 2013;20:7–12.
139. Zhang R, Miao QW, Zhu CX, Zhao Y, Liu L, Yang J. Sulforaphane ameliorates neurobehavioral deficits and protects the brain from amyloid beta deposits and peroxidation in mice with Alzheimer-like lesions. Am J Alzheimers Dis Other Dement. 2015;30:183–91.
140. Heo HJ, Kim DO, Shin SC, Kim MJ, Kim BG, Shin DH. Effect of antioxidant flavanone, naringenin, from *Citrus junoson* neuroprotection. J Agric Food Chem. 2004;52:1520–5.

141. Zbarsky V, Datla KP, Parkar S, Rai DK, Aruoma OI, Dexter DT. Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. Free Radic Res. 2005;39:1119–25.
142. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology. 2012;78:1138–45.
143. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–22.
144. Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, Li Y, Chen W, Yi X, Zheng W, Wondrak GT, Wong PK, Zhang DD. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis. 2008;29:1235–43.
145. Hayes JD, McMahon M, Chowdhry S, Dinkova-Kostova AT. Cancer chemoprevention mechanisms mediated through the Keap1–Nrf2 pathway. Antioxid Redox Signal. 2010;13:1713–48.
146. Hu R, Saw CL, Yu R, Kong AN. Regulation of NF-E2-related factor 2 signaling for cancer chemo-prevention: antioxidant coupled with anti-inflammatory. Antioxid Redox Signal. 2010;13:1679–98.
147. Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. Nat Rev Cancer. 2012;12:564–71.
148. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, Suzuki T, Winnard PT Jr, Raman V, Ebina M, Nukiwa T, Yamamoto M. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. Carcinogenesis. 2010;31:1833–43.
149. Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhauser C. Nuclear factor κB is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J Biol Chem. 2001;276(34):32008–15.
150. Dickinson SE, et al. Inhibition of activator protein-1 by sulforaphane involves interaction with cysteine in the cFos DNA-binding domain: implications for chemoprevention of UVB-induced skin cancer. Cancer Res. 2009;69:7103–10.
151. Negi G, Kumar A, Joshi RP, Sharma SS. Oxidative stress and *Nrf2* in the pathophysiology of diabetic neuropathy: old perspective with a new angle. Biochem Biophys Res Commun. 2011;408:1–5.
152. Shin S, Wakabayashi J, Yates MS, Wakabayashi N, Dolan PM, Aja S, Liby KT, Sporn MB, Yamamoto M, Kensler TW. Role of *Nrf2* in prevention of high-fat diet-induced obesity by synthetic triterpenoid CDDO-imidazolide. Eur J Pharmacol. 2009;620:138–44.
153. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA. Oncogene-induced *Nrf2* transcription promotes ROS detoxification and tumorigenesis. Nature. 2011;475(7354):106–9.
154. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci. 2016;39:199–218.
155. Papp D, Lenti K, Módos D, Fazekas D, Dúl Z, Türei D, Földvári-Nagy L, Nussinov R, Csermely P, Korcsmáros T. The NRF2-related interactome and regulome contain multifunctional proteins and fine-tuned autoregulatory loops. FEBS Lett. 2012;586:1795–802.
156. Gao B, Doan A, Hybertson BM. The clinical potential of influencing Nrf2 signaling in degenerative and immunological disorders. Clin Pharm. 2016;6:19–34.

Nrf2 in Immune Responses During Inflammation

Miriam S. Hohmann, Tiago H. Zaninelli, Larissa Staurengo-Ferrari, Marília F. Manchope, Stephanie Badaro-Garcia, Andressa de Freitas, Rubia Casagrande, and Waldiceu A. Verri Jr.

Abstract One of the most studied functions of Nrf2 is its role in the maintenance of redox homeostasis in cells during oxidant stress. However, the role of Nrf2 in tissue and organism homoeostasis goes far beyond protection during oxidative conditions. In this chapter, we provide an overview of the regulation of inflammation and immune responses by Nrf2. We discuss the regulatory functions and mechanisms of action of Nrf2 on innate and adaptive immune responses, immune surveillance, and immune response in inflammatory diseases, and experimental models of disease. We also summarize the consequences of the deficiency or deregulation of Nrf2 axis and how they are related to the development and aggravation of diseases.

1 Introduction

The nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) is a member of the cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors that play a key role in the redox homeostatic gene regulatory network [1–4].

M. S. Hohmann · T. H. Zaninelli · L. Staurengo-Ferrari · M. F. Manchope · S. Badaro-Garcia
W. A. Verri Jr. (✉)
Departamento de Ciências Patológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Paraná, Brazil
e-mail: waverri@uel.br

A. de Freitas
Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

R. Casagrande
Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

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The expression of Nrf2 is observed in many tissues, especially those exposed to the external environment (skin, lungs, and gastrointestinal tract) or associated with detoxification (liver and kidneys) [5]. Under basal condition, Nrf2 is suppressed within the cytosol by an adaptor protein, Kelch-like erythroid cell-derived protein with CNC homology- (ECH-) associated protein 1 (Keap1) and can be activated by oxidants and electrophiles via modification of critical cysteine thiols of Keap1 and Nrf2 [5, 6]. Cysteine residues within Keap1 serve as primary sensors of stress signals, and their modification leads to conformational changes in Keap1, thereby inhibiting ubiquitination of Nrf2 [7]. As a result, stabilized Nrf2 can translocate into the nucleus and bind to its target genes [2]. Nrf2 translocation to the nucleus can also be modulated by the activation of protein kinase C (PKC), phosphoinositide 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) ERK1/2, which phosphorylate and stabilize Nrf2 [8–11]. Further, down-regulation of Nrf2 transcription can also occur. For instance, glycogen synthase kinase 3-beta (GSK-3β) and p38 MAPK induce proteasome degradation of Nrf2 in a Keap-1 independent and dependent manner, respectively [12, 13].

Perhaps one of the most studied functions of Nrf2 is its role in resistance to oxidant stress [3, 9, 14]. Nrf2 binding to antioxidant response elements (ARE) in the nucleus drives the expression of Nrf2 target genes such as antioxidant and detoxification enzymes [15, 16] NAD(P)H:quinone oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), glutamate cysteine ligase (GCL), glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD), glutathione S-transferase (GST), and thioredoxin UDP-glucuronosyltransferase [16, 17]. Accordingly, Nrf2 knockout ($^{-/-}$) ($Nrf2^{-/-}$) mice show substantially increased susceptibility to chemical toxicity and disease conditions associated with oxidative stress [2, 3, 18]. Whereas pharmacological boosting of Nrf2 activity protects animals from oxidative damage and development of diseases [19]. Nonetheless, the role of Nrf2 in tissue and organism homoeostasis goes far beyond its protective role during oxidative conditions. In this chapter, we will discuss the emerging role of Nrf2 in the regulation of inflammation and the immune response during inflammation. We will focus on the regulatory role and mechanisms of action of Nrf2 on innate and adaptive immune responses, immune surveillance, and immune response in inflammatory diseases and experimental models of disease.

2 Nrf2 and Inflammation

2.1 Overview of Inflammation

Inflammation is the most common feature of many chronic diseases and complications. It is an adaptive response triggered by harmful stimuli and conditions (e.g., infection and tissue injury) in tissues that is orchestrated by immune cells and mediators of varied structures/classes derived from plasma proteins or secreted by immune and organ parenchymal cells [20, 21]. Acute inflammation is the initial

response toward noxious stimuli, which are sensed by innate immune receptors expressed by tissue-resident cells including macrophages and mast cells [22]. The activation of these receptors triggers signaling pathways (e.g., nuclear factor kappa B (NF-κB), MAPK, janus kinase (JAK), signal transducers, and activators of transcription (STAT)) [23, 24], which ultimately culminate in the production and release of various pro-inflammatory molecules such as chemokines, cytokines, vasoactive amines (histamine and serotonin), and eicosanoids (prostaglandins and leukotrienes) [25]. These mediators elicit local inflammatory exudate, a hallmark of acute inflammation. Plasma proteins and leukocytes (mainly neutrophils) gain access to the extravascular tissue and migrate to the site of inflammation [26]. At the inflammatory foci, neutrophils are activated and release reactive oxygen species (ROS) and enzymes as a mechanism to eliminate pathogens and necrotic tissue. Although this response is directed at containing tissue damage, collateral damage to the host may occur [25]. Thus, if inflammation is deregulated or inappropriately directed against self-organs, it can become detrimental and the cause of injury and disease [20, 25].

When the immune response is successful at eliminating the pathogen or damaged tissue, inflammation is followed by a resolution and repair phase. However, if the inflammatory stimulus persists, pathogen-specific immunologic effector pathways, i.e., adaptive immune responses to these stimuli, are put into place, and acute inflammation can progress to chronic inflammation. Adaptive immunity involves a tightly regulated interplay between antigen-presenting cells (APCs) and T and B lymphocytes. Recruited neutrophils are progressively replaced by macrophages and lymphocytes. Moreover, proliferation of blood vessels, fibrosis, and tissue destruction may also be present [21]. Considerable progress has been made in understanding the cellular and molecular events involved in the inflammatory response present during host defense, and the causes and mechanisms of localized and systemic chronic inflammation that occur in many diseases (including, chronic infection, autoimmune and cardiovascular diseases, type 2 diabetes, obesity, among others) [25]. However, current clinical therapies still need improvement and to find balance between the inhibition of inflammation and infection susceptibility increase, tissue damage, and drug side effects. Nevertheless, control of inflammation and the immune response is critical to prevent various chronic diseases [25].

2.2 Regulation of Inflammation by Nrf2

In addition to the central role in redox homeostasis, Nrf2 has been shown to attenuate inflammation [27–29]. Nrf2 activation in myeloid leukocytes alleviates inflammation [30]. Increased Nrf2 expression inhibits the expression of pro-inflammatory genes through down-regulation of the NF-κB pathway [31]. Nrf2-deficiency, on the other hand, causes an exacerbation of inflammation directed at inflammatory stimuli (e.g., lipopolysaccharide [LPS]), in sepsis, pleurisy, emphysema [29, 32–34], and autoimmune phenotypes in mice [35, 36]. In human clinical studies, an Nrf2

Table 1 Nrf2 activity in immune cells

Cell types	Activity
Neutrophils	Reduces ROS production and TNF-α, IL-6, MCP-1, and MIP-2 expression induced by LPS stimulus [33]
Macrophages	Mitigates LPS-induced TLR4 surface expression, NF-κB activation, ROS production, IL-6, TNF-α, and IFN-β expression [30] Enhances clearance of bacteria by modulating bacterial binding and phagocytosis [66] Inhibits TLR4 pathway activation by promoting PI3K/Akt and inactivating Foxo1 signaling [80]
Dendritic cells	Regulates GSH levels, co-stimulatory receptor expression, phagocytic functions, and antigen-specific CD8 T cell stimulation capacity [103] Regulates basal ROS levels, and MAPK and NF-κB signaling [103] Regulates the expression of MHC class II and cell surface co-stimulatory molecules CD86 and CD80 (94)
T lymphocytes	Promotes Th2 skewing of $CD4^+$, i.e., suppression of IFN-γ production while concurrently promoting the secretion of Th2 cytokines IL-4, IL-5, and IL-13, by repressing T-bet DNA binding and inducing GATA-binding protein 3 DNA binding [83] Reduces the sensitivity to apoptosis mediated by Fas and TNF-α by fine-tuning the intracellular redox equilibrium [108] Impairs the activation of primary human T helper by decreasing the expression of the activation makers CD25 and CD69 and IFN-γ and IL-2 levels [110]
Regulatory T lymphocytes	Promotes Treg by increasing fatty acid oxidation [96, 117] Enhances Treg activation via GPx, thioredoxin, NQO1, and HO-1 expression [118, 119] Increases Treg survival by conferring cellular protection against oxidants and other insults [113, 114, 117, 120]

inducer is being used in the treatment of multiple sclerosis [37, 38], in part due to its anti-inflammatory effect. These observations indicate that Nrf2 is essential for the control of inflammation [39].

In the past decade, numerous studies have demonstrated the role of Nrf2 in the regulation of immune cells and responses (Overview in Table 1). Immune cells and the immunological response are pivotal to orchestrating inflammation; therefore, in the next sections of this chapter we will discuss how Nrf2 regulates innate and adaptive immunity and how it can modulate acute and chronic inflammation.

3 Role of Nrf2 in Innate Immunity

3.1 Overview of Innate Immunity

Infection and injury have been defying the survival of species throughout evolution [40]. Innate immunity is an evolutionary ancient defensive frontline against infections and tissue lesions found in most multicellular organism [41, 42]. This early

recognition system possesses a repertoire of conserved pattern-recognition receptors (PRRs) encoded in the genome [40]. The PRRs are expressed on the cell surface, intracellular compartments, or secreted in bloodstream or tissue fluids [43]. Several families of PRRs compose the innate immunity, including Toll-like receptor (TLR), NOD-like receptor (NLR), and RIG-I-like receptor (RLR) [44], which are responsible for recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), presented in microbial infections or released after injury, respectively [43, 45]. PAMPs or DAMPs activate innate immunity, calling into action an appropriate metabolic, hemodynamic, immunological response to eliminate infections, facilitate healing, and initiate the transition to adaptive immunity [40].

PRRs recognize PAMPs expressed by microorganism that have been phagocytized by immune cells (mainly macrophages and neutrophils). The clearance of these microorganisms occurs in the phagolysosome via the oxidative burst, which is the production of ROS and nitrogen species (RNS) through NADPH oxidase and nitric oxide synthase [46–48]. In addition to the mechanism of phagocytosis, the activation of neutrophils induces the release of structures similar to a fibrous network composed of DNA fragments, chromatin and neutrophilic granule proteins, known as NETs (neutrophil extracellular traps). NETs are present in large amounts in inflammatory and infectious sites, acting directly on microorganisms, promoting an efficient extracellular microbicidal mechanism [49]. In the same line, tissue injury and necrosis release DAMPs that activate PRRs. HMGB-1, which is a DAMP, is a highly conserved and evolutionary protein that activates the immune response during sterile inflammation [40]. It activates leukocytes through TLR4 and receptor for advanced glycation end products (RAGE), activating NADPH oxidase to produce ROS, and inducing TNF-α production [40, 50–52].

Oxidative stress is the imbalance between oxidants and antioxidants. ROS and RNS react with macromolecules inducing damage to biomolecules, such as DNA, protein, and lipids [53, 54]. NF-κB is a redox-sensitive transcriptional factor, and its activation leads to the expression of cell adhesion molecules, receptors, cytokines, and chemokines [55–57]. NF-κB activation contributes to the production of free radicals and pro-inflammatory response [58, 59]. For cells to maintain the redox balance, a series of cytoprotective and antioxidant defense mechanisms, such as Nrf2 and its downstream targets are in play [60, 61]. The transcriptional factor Nrf2 appears to be an important regulator of innate immunity and subsequent inflammatory response by modulating oxidative/inflammatory stress (Overview in Fig. 1a).

3.2 Nrf2 and Innate Immunity

Innate immunity is responsible for early recognition and processing of pathogens. During many types of viral infection, ROS levels are found to be increased [62]. In Dengue virus (DENV) and respiratory syncytial virus (RSV) infections, similar observations have been made [63]. Besides oxidative stress, during DENV

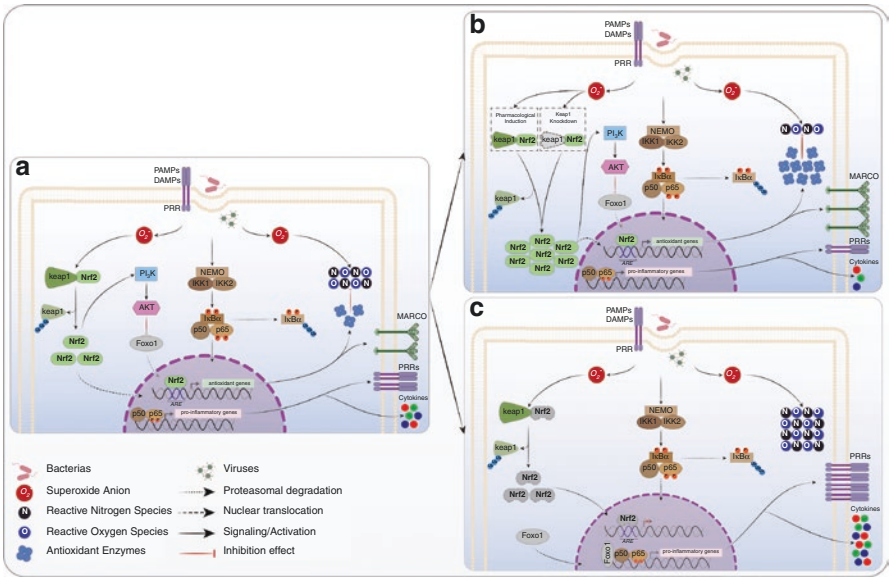

Fig. 1 Nrf2 in innate immunity. (**a**) Under physiological conditions: Pathogen-associated molecular patterns (PAMPs) expressed by viruses and bacteria and damage-associated molecular patterns (DAMPs) released during tissue damage activate pattern-recognition receptors (PRRs) to induce superoxide production. Superoxide anion, in turn, can produce other reactive oxygen species (ROS) and reactive nitrogen species (RNS). Simultaneously, PAMPs and DAMPs, via PPRs, also activate IκB kinase (IKK) complex, which phosphorylates IκBα, resulting in the ubiquitination and dissociation of IκBα from NF-κB. Activated NF-κB subunits (p50/p65) translocate to the nucleus and increase the expression of PRRs and pro-inflammatory cytokines. Superoxide anion induces Keap-1 inactivation, releasing Nrf2 from its inhibitor. Nrf2 activates PI_3K/AKT pathway, leading to the inhibition of Foxo1 translocation to the nucleus. Stabilized Nrf2 translocates to the nucleus and binds to antioxidant response element (ARE) gene promoter, inducing the expression of antioxidant enzymes and macrophage receptor with collagenous structure (MARCO) receptors. (**b**) Pharmacological induction of Nrf2 or conditional deletion of Keap-1: Pharmacological induction of Nrf2 or conditional deletion of Keap-1 increases the levels of activated Nrf2. Stabilized Nrf2 translocates to the nucleus and binds to ARE gene promoter, inducing the expression of antioxidant enzymes, which decrease ROS and RNS levels, and MARCO receptor. (**c**) Nrf2 disruption: The disruption of Nrf2 favors the translocation of Foxo1 to nucleus and enhances NF-κB-induced PRRs and pro-inflammatory cytokines expression. Additionally, there is reduced translocation of Nrf2 to the nucleus and subsequent expression of antioxidant enzymes and MARCO receptor. Thus, Nrf2 disruption increases the levels of PRRs, pro-inflammatory cytokines, ROS, and RNS

infection, inflammatory (*IFIT1*, *RSAD2*, *DDX58*, *CXCL10,* and *IFNb*) and apoptotic (*NOXA*, *BCLX*, and *RIPK1*) gene expression are also upregulated [63–65]. Studies have shown that Nrf2 is also central to maintaining adequate immune and apoptotic response. Proof of this is that slightly silencing Nrf2 increases DENV mRNA accumulation and DENV infectivity [63]. On the other hand, viral infections can inhibit Nrf2 activity, increasing oxidative stress and inflammation. RSV induces the deacetylation of Nrf2 and posterior degradation through the proteasome [64]. In RSV infection, $Nrf2^{-/-}$ mice present greater bronchoalveolar injury and

inflammation (i.e., increased leukocyte recruitment, notably neutrophils and eosinophils and IL-6, IL-13, and IL-18 production in bronchoalveolar lavage) compared to wild-type (WT) ($Nrf2^{+/+}$) mice [65]. Blocking histone deacetylase activity inhibits Nrf2 degradation, restoring Nrf2 activation and Nrf2-dependent gene transcription of NQO-1, superoxide dismutase (SOD)-1, and catalase in A549 epithelial cells and human small airway epithelial cells [64]. Treatment with Nrf2 inducer butylated hydroxyanisole inhibits oxidative stress in RSV infection *in vivo* and *in vitro* by increasing NQO1, SOD, catalase, and Nrf2 protein expression [64]. Sulforaphane, another Nrf2 inducer, inhibits RSV-induced oxidative stress and recruitment of neutrophils and eosinophils and increases the expression of Nrf2, NQO1, glutathione S-transferase P1 (GST-P1), HO-1, and GPx2 in mice [65]. Induction of Nrf2 by sulforaphane also reduces viral load during RSV infection [65], demonstrating that regulation of oxidative balance by Nrf2 is important for adequate response to viral infection.

During bacterial infection, Nrf2 disruption increases the susceptibility to infection and sepsis in mice [30, 66–68]. $Nrf2^{-/-}$ mice present more severe *Streptococcus pneumonia*-induced pneumonia, increased neutrophil recruitment, and TNF-α production in the lung when compared to WT mice [68]. Additionally, defect in bacterial clearance, systemic dissemination of the bacteria, increased disease score, severity of illness, and higher mortality rate were also observed in $Nrf2^{-/-}$ mice [68]. In agreement with these data, the disruption of Nrf2 inhibitor Keap-1 in myeloid leukocytes in mice increases peritoneal macrophage bacterial phagocytic activity when compared to macrophages from mice with conditional $Nrf2^{-/-}$ disruption in myeloid leukocytes [30]. Of note, Nrf2 up-regulates the expression of macrophage receptor with collagenous structure (MARCO) [66] (Fig. 1a), which is responsible for the recognition and clearance of bacteria [69]. Treatment with the Nrf2 inducer, sulforaphane, increases MARCO receptor expression (Fig. 1b), resulting in increased pulmonary clearance of *Haemophilus influenzae* and *Pseudomonas aeruginosa* by alveolar macrophages in WT mice but not in $Nrf2^{-/-}$ mice exposed to cigarette smoke [66].

The activation of the LPS/TLR4 signaling pathway increases ROS production in an NADPH oxidase-dependent manner [33]. ROS, on the other hand, can induce cytokine and chemokine expression by activating NF-κB [58, 59]. In $Nrf2^{-/-}$ peritoneal macrophages, the activation of TLR signaling pathways are increased when compared to WT macrophages. The TLR ligands (LPS-TLR4; LTA-TLR2, CpG-TLR9, and Poly[I:C]-TLR3) induce significantly greater expression of IL-6, TNF-α, and MCP-1 in the $Nrf2^{-/-}$ peritoneal macrophages [30] (Fig. 1c). LPS activation of TLR4 increases ROS production and augments TNF-α, IL-6, MCP-1, and MIP-2 gene expression in $Nrf2^{-/-}$ peritoneal neutrophils [33] (Fig. 1c). Additionally, ROS production appears to control TLR4 intracellular traffic between the Golgi and plasma membrane [30]. In fact, in $Nrf2^{-/-}$ peritoneal macrophages, LPS induces TLR4 transport from the Golgi to plasma membrane in an ROS-dependent manner [30]. Corroborating the effect of Nrf2 deficiency, the disruption in Keap-1, which enhances Nrf2 activity, inhibits TLR4 transport [30] (Fig. 1b). In the same line, treatment with CDDO-Im

(1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole), a potent inducer of Nrf2, protects WT, but not Nrf2$^{-/-}$ mice from LPS-induced septic shock by attenuating TNF-α, IL-6, MCP-1, and MIP-2 gene expression in the lung [33]. Moreover, the conditional disruption of Keap-1 in myeloid leukocytes decreases the mortality, lung injury, circulating levels of TNF-α, IL-6, MCP-1, and bacteremia induced by cecal ligation and puncture-induced sepsis compared to floxed WT mice, and increases all these parameters in mice with conditional disruption of Nrf2$^{-/-}$ in myeloid leukocyte [30]. Therefore, Nrf2 seems to regulate the innate immune response during bacterial infection by modulating leukocyte activity, host inflammatory response, and bacterial clearance.

In addition to regulating host response during infections, Nrf2 also plays an important role in sterile inflammatory conditions such as ischemia/reperfusion (I/R) injury [70]. TLR4 signaling is involved in several models of hepatic [71–74], pulmonary [75], brain [76, 77] myocardial [78], and renal [79] I/R injury. In hepatic I/R injury, the HMGB-1/TLR4 signaling pathway also seems to be involved [71]. During hepatic I/R injury in Nrf2$^{-/-}$ mice, liver injury and inflammation was exacerbated, as observed by increased neutrophil and macrophage recruitment and TNF-α and IL-1β production [80]. Treatment with cobalt protoporphyrin (CoPP) inhibits liver injury and inflammation in WT mice, but not Nrf2$^{-/-}$ mice, and activates PI_3K/AKT pathway that inhibits Foxo1 [80]. In bone marrow-derived macrophages (BMDM) CoPP induces Nrf2 in LPS-stimulated WT BMDM but not Nrf2$^{-/-}$ BMDM. CoPP also induces PI_3K/AKT/Foxo1 pathway, decreasing Foxo1, TLR4, and NF-κB protein levels in LPS-stimulated WT BMDM [80], suggesting that Nrf2 regulates TLR4 pathway through PI_3K/AKT/Foxo1 [80, 81] (Fig. 1a).

Collectively, the data in the literature suggest that Nrf2 is a major player in innate immunity and the control of oxidative/inflammatory stress in infectious and non-infectious diseases (Table 2). The redox balance is held through Nrf2 activation and the expression of its downstream targets. Besides, Nrf2 controls the expression of PRRs, down-regulates the expression of TLR4 activating PI_3K/AKT/Foxo1 signaling pathway, and up-regulates the MARCO receptor expression, which enhances bacterial clearance.

4 Nrf2 in Adaptive Immunity: Role of Dendritic Cells, Th1, Th2, Th17, and Tregs

Unlike the innate immune system, the adaptive immune system is highly specific to provide long-lasting protection in mammals. The adaptive immune response relies on white blood cells to carry out its tasks: B cells and T cells. Both B cells and T cells are lymphocytes derived from stem cells in the bone marrow and each type of cell follows different pathways to their final mature forms [82].

Focusing on subtypes of T cells, they can express the surface protein marker CD4 and are referred to as CD4$^+$ T cells or T “helper” (Th). These cells have no cytotoxic or phagocytic properties, but they can manage the immune response by

Table 2 Outcomes of pharmacological induction of Nrf2 or conditional deletion of Keap-1 and Nrf2 disruption

Pharmacological induction of Nrf2 or conditional deletion of Keap-1	Nrf2 disruption
Butylated hydroxyanisole increases NQO1, SOD, and catalase mRNA expression, and Nrf2 protein expression in A549 epithelial cells and human small airway epithelial cells (SAECs) [64]; Sulforaphane inhibits RSV-induced oxidative stress, neutrophil and eosinophil recruitment, and increases the expression of Nrf2, NQO1, glutathione S-transferase P1 (GST-P1), HO-1, and GPx2 in the lung of mice. Additionally, it reduces viral load during RSV infection [65]; Sulforaphane increases pulmonary clearance of *Haemophilus influenzae* and *Pseudomonas aeruginosa* in WT but not in Nrf2$^{-/-}$ mice. It also increases MARCO receptor gene expression in alveolar macrophages transfected with mock siRNA, but not in Nrf2 siRNA [66]; CDDO-Im (1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole) protects WT, but not Nrf2$^{-/-}$ mice from LPS-induced septic shock by attenuating TNF-α, IL-6, MCP-1, and MIP-2 gene expression in the lung [33]; Keap-1 deletion in myeloid leukocytes decreases the mortality, lung injury, circulating levels of TNF-α, IL-6, MCP-1, and bacteremia in mice with cecal ligation and puncture-induced sepsis [30]; The disruption of Nrf2 inhibitor Keap-1 in myeloid leukocytes in mice decreases LPS-induced ROS levels and TLR4 expression in peritoneal macrophages, when compared to conditional Nrf2 deletion in myeloid leukocytes and floxed peritoneal macrophage [23]; CoPP inhibits ischemia and reperfusion-induced liver injury and inflammation in WT mice but not Nrf2$^{-/-}$ mice [80] In bone marrow-derived macrophages (BMDM), CoPP treatment induces PI_3K/AKT/Foxo1 pathway, decreasing Foxo1, TLR4, and NF-κB protein levels [80]	In Nrf2$^{-/-}$ mice, respiratory syncytial virus (RSV) infection induces greater bronchoalveolar injury and increased neutrophil and eosinophil recruitment and IL-6, IL-13, and IL-18 production in the bronchoalveolar lavage compared to wild-type (WT) mice [65]; In Nrf2$^{-/-}$ mice, *Streptococcus pneumonia* induces more severe pneumonia, increased mortality rate, neutrophil recruitment, TNF-α production in the lung, defect in bacterial clearance, and systemic dissemination when compared to WT mice [68]; In Nrf2$^{-/-}$ peritoneal macrophages, TLR ligand stimuli induce significantly greater expression of IL-6, TNF-α, and MCP-1 (30); In Nrf2$^{-/-}$ peritoneal neutrophils, LPS/TLR4 stimulus increases ROS production and augments TNF-α, IL-6, MCP-1, and MIP-2 gene expression [33]; Conditional Nrf2 deletion in myeloid leukocytes increases the mortality, lung injury, circulating levels of TNF-α, IL-6, MCP-1, and bacteremia in mice with cecal ligation and puncture-induced sepsis [30]; Peritoneal macrophages from mice with conditional Nrf2 deletion in myeloid leukocytes after LPS stimulus increases TLR4 transport from the Golgi to plasma membrane in an ROS-dependent manner [30]; In Nrf2$^{-/-}$ mice, ischemia and reperfusion-induced liver injury and inflammation is exacerbated, as observed by increased neutrophil and macrophage recruitment and TNF-α and IL-1β production [80]

directing other cells to perform these actions [82]. For this reason, Th1/Th2 differentiation of $CD4^+$T cells is a critical process in tailoring an adaptive response to a specific pathogen or disease, which allows for flexibility in T cell function and downstream immune activity [83]. Besides the classical biphasic differentiation of

Th1 and Th2 cells, unexpected increases in the number of $CD4^+$T cells subsets, including Th17 and T regulatory (T reg) cells, have been recognized [84].

The pattern of activation in subtypes of $CD4^+$T cells in the adaptive immune system is a highly orchestrated process dictated by multiple cues from the immune system [85]. Differentiation of naïve Th cells into Th1, Th2, or Th17 effector cells occurs within a few days of a direct contact with APC in lymph nodes [82, 86]. Of note, APCs seem to have essential roles in both trafficking and presentation of immunogenic peptides via major histocompatibility (MHC) class II to shape the specific T cells response in accordance with the nature of invading pathogen or peptide [87]. The optimal activation of T cells requires a double signal that involves the engagement of the CD3-T cell receptors (TCRs) in association with co-stimulatory molecules such as B7, which interacts with CD28 on T cells [87–89].

In order to fully achieve the process of naïve Th cell decision-making, it is important to consider in a short term a variety of signaling cascade and molecular mechanisms that establish the draft of Th phenotype [87]. Cytokines are the most influential factor involved in T cell polarization [90, 91]. Nevertheless, the molecular mechanisms including control of gene expression by intracellular signaling cascades, chromatin remodeling, epigenetic molecules, and transcription factors have also been described [87, 92].

In parallel, T cell activation and proliferation is strongly dependent on the redox potential of the microenvironment [93, 94]. The phenotypes of Th cells appear to essentially build on the activation of redox-sensitive signaling cascades, where oxidative conditions support Th1 development while antioxidant conditions drive to Th2-type immune response [92, 93]. In this context, some studies suggest that Nrf2 activation may alter the shift in Th1/Th2 balance [83] (Fig. 2).

The pro-inflammatory cytokine IFN-γ plays a central role in the Th1-type immune response. IFN-γ signaling starts pathogen and tumor protection mechanisms in target cells and elicits ROS formation during the respiratory burst reaction [92, 95]. A pro-oxidant environment in the initial phase of an immune response might facilitate priming of Th1 cells. Conversely, regulation of the cellular redox level by Nrf2 in diseases models protect against a steep Th1 response by reducing the recruitment of immune cells and cytokine production, including TNF-α and IFN-γ [96]. This finding was substantiated by enhancing Nrf-2 pathway through generation of mice with genetic deletion of Keap1 [94].

In line with this proposition, a common food preservative, tert-butylhydroquinone (tBHQ), is able to activate Nrf2 in T cells and suppress IFN-γ production while concurrently promoting the secretion of Th2 cytokines IL-4, IL-5, and IL-13. This outcome is related to the Nrf2 repression of T-bet DNA binding and induction of GATA-binding protein 3 DNA binding [83]. Both intersecting T cell transcriptional factors enabled to define the genetic modules that control T cell polarity. The differentiation of Th1 and Th2 cells is regulated by the transcription factors T-bet and GATA3, respectively [97]. Further, $CD4^+$T cells from $Nrf2^{-/-}$ mice display a stronger ability to secret IFN-γ and restricted IL-4, IL-5, and IL-13 output compared to T cells from WT mice [83] (Fig. 2).

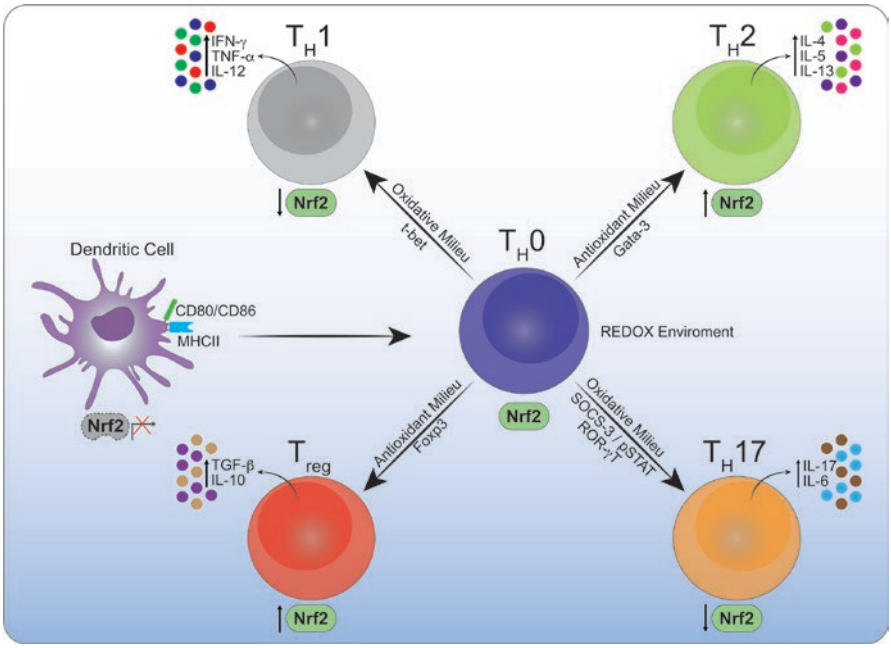

Fig. 2 Nrf2 in adaptive immunity: Role of dendritic cells, Th1, Th2, Th17, and Tregs. The activation of Nrf2 may alter the shift in Th1/Th2 balance in disease models by impairing Th1-driven response and reducing the recruitment of immune cells and cytokine production, including TNF-α, IFN-γ, and IL-12. This outcome is linked to the Nrf2 repression of T-bet DNA binding and induction of GATA-binding protein 3 DNA binding. Thus, the interrelationship of these molecules favors the secretion of Th2 cytokines IL-4, IL-5, and IL-13 in an antioxidant milieu. Besides the Th1/Th2 dichotomy, Nrf2 also modulates Th17 and Treg responses depending on the redox setting. Elevated oxidative damage exacerbates the differentiation of Th17 cells in the absence of Nrf2. Peculiarly, Nrf2 alters Th17 differentiation and related cytokines IL-17 and IL-6 by upregulating the expression of *suppressor of cytokine signaling* 3 (*Socs3)*. Nrf2, in turn, decreases the phosphorylation of Th17 transcription factor STAT3. Further, the effect of Nrf2 on the Th17 phenotype transcription factor ROR-γT is currently still unknown. There is a link between Th17 and Treg cells, pharmacological or genetic induction of Nrf2 in disease models evinces the reciprocal suppression of Th17 and expansion of Foxp3-expressing T cells, possibly in an IL-16R signaling-dependent manner. These populations are related to TGF-β and IL-10 secretion. In addition, the role of Nrf2 on Treg activation seems to be dependent on genes coding for antioxidants, such as GPx, thioredoxin, NQO1, and HO-1 and bestows to Tregs a reducing profile. Even with enhanced reducing properties, Tregs have high lipid oxidation mediated at least, in part, by Nrf2 through fatty acid oxidation and energy and power supply. Nrf2 can also modulate the T cell response by regulating oxidative stress-induced activation of dendritic cells (DCs). Specifically, $Nrf2^{-/-}$ DCs present augmented expression of MHC class II and the cells surface expression of co-stimulatory molecules CD86 and CD-80, which work in tandem to influence the behavior of Th cells

Assessment of disease severity in $Nrf2^{-/-}$ mice revealed that Nrf2 is able to regulate cellular redox level and molecular events that readily alter Th1/Th2 balance [98]. In an attempt to understand the role of Nrf2 in experimental autoimmune encephalomyelitis (EAE), mice were immunized with the myelin oligodendrocyte

glycoprotein peptide (MOG 35–55). The disruption of Nrf2 resulted in a more severe clinical course and rapid onset of the disease with glial cell activation via Th1 cytokines (IFN-γ, TNF-α, and IL-12) [99]. Similarly, Nrf2 has a protective role against the development of bleomycin-induced pulmonary inflammation and fibrotic response by attenuating the events that readily alter the Th1/Th2 commitment [98].

Regarding the ability of Nrf2 to respond to low levels of oxidative stress, Nrf2 plays an essential role in the response to environmental insults (e.g., airborne pollutants), indicating that it could be determinant to the susceptibility of developing Th2-related diseases [27, 28, 100]. Nrf2 is a critical determinant of susceptibility to allergen-induced asthma [27, 28], *M. tuberculosis*-induced granuloma development at the late stage and atopic dermatitis [101]. These findings support a pleiotropic role for Nrf2 in maintaining a balance between ROS production, antioxidant capacity, and T helper functions since most studies show that Nrf2 activation skews $CD4^+$T cells toward Th2 commitment [83].

Nrf2 can also modulate T cell response by regulating oxidative stress-induced activation of dendritic cells (DCs). DCs are a type of APC formers of a singular cellular network that shapes T helper response [102]. Nrf2 inhibition markedly alters the phenotype or murine DCs. In fact, $Nrf2^{-/-}$ DCs have impaired glutathione (GSH) levels, reduced phagocytic activity, and enhanced T cells stimulatory capacity [103]. Disrupting Nrf2 in DCs leads to an environment of oxidative stress associated with Th2-like response against endotoxin-free ambient particulate matter. Specifically, $Nrf2^{-/-}$ DCs present augmented expression of MHC class II and the cells surface expression of co-stimulatory molecules CD86 and CD80, which work in tandem to influence the behavior of Th cells [94] (Fig. 2).

The redox state of DCs plays an important role in the decline of Th1 immune response [104]. One possible explanation is that oxidative stress diminishes IL-12 and subsequent IFN-γ production in Th1 cells. Thus, restoring Nrf2 activity upregulates Th1 response in aging through a restoration of redox equilibrium [104]. A possible explanation is that Nrf2 via its effects on GSH synthesis and phase II detoxification enzymes could distort the signaling pathways that are required for DC maturation, cytokine production, and co-stimulatory receptor expression [104–107]. This could favor DC survival in an oxidative stress environment, in addition to contributing to increased viability in bystander T cells [104].

Evidence suggests that Nrf2 deficiency enhances the sensitivity to apoptosis mediated by Fas and TNF-α by fine-tuning the intracellular redox equilibrium [108]. In human T helper cells, some studies have assessed the expression and activity of Nrf2 at transcriptional levels [109]. In response to tBHQ, Nrf2 impairs the activation of primary human T helper by decreasing the expression of the activation makers CD25 and CD69 and IFN-γ and IL-2 levels [110].

The discovery of an IL-17-producing population of $CD4^+$T cells, termed Th17 cells, provided exciting new insights into adaptive immune regulation [111]. Recently, authors have shown that elevated oxidative damage exacerbates the differentiation of Th17 cells in the absence of Nrf2. Specifically, Nrf2 affects Th17 differentiation and related cytokines by upregulating *suppressor of cytokine signaling* 3 (*Socs3)* expression. Nrf2 in turn decreases the phosphorylation of Th17

transcription factor STAT3. In this sense, Nrf2 is a key modulator of Th17 by regulating SOCS3/pSTAT3 axis [112] (Fig. 2). Moreover, pharmacological or genetic induction of Nrf2 in experimental models focused on studying Th17 response show the reciprocal suppression of Th17 and expansion of Foxp3-expressing T cells [113]. Specifically, the Nrf2 nuclear translocation and subsequently increased HO-1 expression directly converted the differentiation of naïve T cells from Th17 cells to Treg cells, possibly in a dependent manner of IL-16R signaling [114]. However, the effect of Nrf2 on the Th17 phenotype transcription factor ROR-γT is still unknown.

The notion that T cells could suppress the function of other cells was popularized by Gershon and Kondo [115]. The detailed description for this T cell population called suppressor or regulatory T cell (Treg) came later in studies describing that Treg are generated from CD4+ T cells via activation of forkhead box P3 (FoxP3) transcription factor upon TGF-β stimulation [116]. However, it is still not completely understood how Tregs control other cells at the molecular level. The Nrf2 pathway may have a significant role in Treg function and activity since this pathway is involved in cell survival signaling and in response to oxidants and other insults to confer cellular protection [117].

The role of Nrf2 on Treg activation seems to be dependent on genes coding for antioxidants, such as GPx, thioredoxin, NQO1, and HO-1 [118, 119]. Such molecules would settle redox switches in a reduced (e.g., thiol or R–SH) position [93, 118]. In this context, some studies have shown that thiol levels are significantly greater in Treg populations with increased reducing power profile [118]. Consistent with this proposition, in models of autoimmune diseases such arthritis, uveitis, and acute kidney injury (AKI) disease, Nrf2 activity limits the break of immunological tolerance possibly by increasing Treg activity and the intracellular redox potential in this hostile milieu for lymphocytes [113, 114, 120] (Fig. 2). Another important point is that instability associated with Treg in inflammatory condition is closely linked to alterations in its metabolism [116].

Even with enhanced reducing properties, Tregs have high lipid oxidation rates *in vitro* and express low levels of Glut1, a glucose transporter, that facilitates entry of glucose into these cells, and low rated off fatty acid synthesis for this energy requirement. Further, these studies indicate that fatty acid oxidation is the essential metabolic process utilized for the generation of energy in Treg. A recent work proposed that Nrf2 increases fatty acid oxidation and it could explain, at least in part, the Nrf2 mechanism on Treg expansion [117]. Furthermore, Nrf2 alongside with mTOR and AMP kinase pathway could determine the metabolic pathway to be executed when T lymphocytes are activated [118].

Differentiation of T cells into effector versus regulatory pathways may be dictated by the intracellular redox potential, and this may be set to a large degree by Nrf2-sensitive genes [118]. The Nrf2 may affect the physiology and metabolism of $CD4^+$ cells by adjusting the redox balance. This issue is of cardinal importance since dysfunction in T cells physiology is presumed to be causative of autoimmune and inflammatory diseases that could be modulated by targeting the Nrf2 pathway.

5 Role of Nrf2 in Immune Surveillance

In addition to protecting against pathogen infections, the immune system also plays an important role in protecting the host from cancer [121]. The prevention of tumor development by the immune system can occur in three different manners. The immune system can protect the host from virus-induced tumors by eliminating or suppressing viral infections; it is able to eliminate pathogens and induce resolution of inflammation, preventing tumorigenesis; and can recognize and eliminate cancer cells according to the expression of tumor-specific antigens or cellular stress, a crucial process called immune surveillance [121]. Oxidative stress plays a role in cancer initiation and progression by inducing DNA damage, genome instability, and cell proliferation survival and migration [122].

Nrf2 plays a dual role in cancer surveillance acting as a tumor suppressor and as proto-oncogene. In early stages, Nrf2 is pivotal in the protection against chemical-induced carcinogenesis since it is capable of inhibiting ROS-induced DNA damage and tumor initiation and progression [123]. This protection is attributed to the increased expression of detoxifying enzymes that enhance the chemical hydrophilicity and elimination of the carcinogens, the removal of ROS, and the clearance of ROS-induced damage. Moreover, Nrf2 prevents oxidative stress during carcinogenesis by the regulation of antioxidant genes, such as glutamate cysteine ligase catalytic subunit (GCLC) and glutamate cysteine ligase modifier subunit (GCLM) [124].

Nrf2 protection against chemical carcinogen-induced tumor has been demonstrated in several studies. $Nrf2^{-/-}$ mice develop more gastric neoplasia induced by benzo(a)pyerene compared to WT mice [125]. $Nrf2^{-/-}$ mice also present greater incidence and size of colorectal cancers induced by azoxymethane. Moreover, more prolapsed rectum and rectal bleeding was observed in $Nrf2^{-/-}$ mice**,** indicating that Nrf2 also plays a critical role in diminishing the inflammation associated to this type of cancer [126]. Similar findings were observed for other types of tumors induced by chemical carcinogens, for example, bladder tumor induced by N-nitrosobutyl(4-hydroxybutyl)amine [127] and skin cancer after exposure to 7,12-dimethylbenz(a)anthracene or 12-O-tetradecanoylphorbol-13-acetate [128].

Nrf2 can also prevent cancer metastasis by maintaining the redox balance in the hematopoietic and immune systems. In the Lewis lung carcinoma model, Nrf2-deficient mice exhibit higher number of pulmonary metastatic nodules and an increase in inflammatory cells when compared with control [129]. The anti-cancer effect of Nrf2 may also be through immune-dependent pathways, mainly the IL-17D-dependent recruitment of NK cells. The Nrf2-mediated induction of IL-17D activates antitumor immunity to eliminate the tumor before Nrf2 shows its pro-tumor activity [130]. Moreover, the protective effect of Nrf2 has also been attributed to single-nucleotide polymorphism (SNP) in a promoter region of the *Nrf2* gene. Individuals with a single-nucleotide polymorphism in NRF2 upstream promoter region (rs6721961) showed reduced NRF2 gene expression and higher risk of lung cancer [131].

Although Nrf2 has been shown to have a protective role in cancer, Nrf2 can also act as a proto-oncogene. Similarly to the protective effect on normal cells, Nrf2 also promotes the survival and growth of cancer cells under unfavorable conditions, i.e., chronic oxidative stress, by inducing antioxidant pathways [130, 132]. In fact, higher expression of Nrf2 is indicative of a poor prognosis due to its ability to increase cancer cell proliferation and promote chemo-resistance and radio-resistance [133]. The overexpression of Nrf2 enhances antioxidant defenses, promoting cancer resistance to chemotherapies. In fact, patients who show high levels of Nrf2 are less responsive to common chemotherapeutic agents such as etoposide, carboplatin, cisplatin, 5-fluorouracil, and doxorubicin [134, 135]. In human type II endometrial cancer, Nrf2 overexpression was also implicated in the aggressiveness, resistance, and poor prognosis of the disease [135]. In agreement, ovarian cancer cells resistant to doxorubicin presented elevated levels of Nrf2 compared with controls and drug sensitivity was restored by the depletion of Nrf2 [136]. Nrf2 overexpression has also been observed in lung, breast, head, and neck carcinomas [133]. Moreover, Nrf2 pathway is necessary for the expression of Multidrug Resistance-Associated Protein 1 (MRP1) in H69 lung cancer cells [137]. Considering that resistance to chemotherapeutic treatment represents a major difficulty in the treatment of cancer, combined therapy using an Nrf2 inhibitor could be a new promising approach in cancer therapy.

Nrf2 regulates cancer cell proliferation by enhancing anabolic metabolism, maintenance of redox homeostasis, and activation of PI3K–Akt signaling and Nrf2-PI3K–Akt pathway positive feedback [138]. The loss of function of Keap1 also seems to favor Nrf2-mediated cancer cell growth. Several studies suggest that the loss of function of Keap1 prolongs Nrf2 activation and consequently promotes cancer cell growth [132].

It is noteworthy that increased Nrf2 expression is commonly observed in cancer. This can be attributed to several mechanisms, including somatic mutations in KEAP1, CUL3, or NRF2; epigenetic silencing of Keap1; aberrant accumulation of proteins that disrupt the interaction between Nrf2 and Keap1; transcriptional up-regulation of NRF2 through oncogene-dependent signaling; and modification of Keap1 by metabolic intermediates, which were widely discussed by Mitsuishi et al. (2012) [138].

Therefore, Nrf2 has a dual role in cancer: Nrf2 can reduce inflammation-associated induction of cancer, as well as protect cancer cells once cancer has developed.

6 Diseases and Experimental Models in Which Nrf2-Mediated Immune Response Plays an Important Role

Due to the broad action spectrum, Nrf2 is a valuable tool to assess tissue antioxidant capacity and inflammation in different diseases and inflammatory contexts. In most cases, Nrf2 deficiency is related to the aggravation of several pathologies in which

Table 3 Diseases and experimental models in which Nrf2-mediated immune response plays an important role

Diseases	Experimental model	Approach	Disease outcome	Reference
Acute kidney injury	I/R-induced	T cells from Keap1$^{-/-}$ mice	↓	[96]
Alzheimer	APP/PS1 transgenic mice	Nrf2-ARE induction by *tert*-butylhydroquinone activation and adenoviral Nrf2 target genes	↓	[152]
Amyotrophic lateral sclerosis	hSOD1 toxicity	GFAP-Nrf2 transgenic mice	↓	[153]
Asthma	Irritant-induced airway hyperresponsiveness	Nrf2$^{-/-}$ mice	↑	[149]
	Allergen-induced	Nrf2$^{-/-}$ mice	↑	[27]
Atherosclerosis	Age-accelerated atherosclerosis	LDLR$^{-/-}$ mice	↑	[156]
COPD	Elastase-induced	Nrf2$^{-/-}$ mice	↑	[29]
Emphysema	N/A	Assessment of the expression of Nrf2 protein in human pulmonary tissue samples and alveolar macrophages from patients with/without emphysema	N/A	[140]
Fibrosis	Bleomycin-induced	Nrf2$^{-/-}$ mice	↑	[150]
Liver injury	CCl_4-induced	Nrf2$^{-/-}$ mice	↑	[155]
Lupus nephritis	Pristine-induced	Nrf2$^{-/-}$ mice	↑	[145]
Parkinson	MPTP-induced	Nrf2$^{-/-}$ mice	↑	[151]

oxidative stress has a pivotal contribution. Thus, the role of this transcription factor has been studied in varied disease contexts in order to elucidate the paradoxical dysfunction of Nrf2 in non-malignant diseases and its relation with the immune system. This section will focus on discussing the role of Nrf2 in diseases and its relevance in immune cells (Table 3).

Ishii et al. (2005) have demonstrated the importance of Nrf2 in the protection against elastase-induced pulmonary inflammation and emphysema. The development of emphysema and depletion in antioxidant expression are exacerbated in Nrf2$^{-/-}$ mice. Macrophage infiltration is pivotal to the pathophysiology of this model, thus Nrf2$^{-/-}$ mice were transplanted with WT mouse bone marrow cells. The presence of Nrf2$^{+/+}$ macrophages markedly ameliorated inflammation and disease [29], highlighting the importance of this transcription factor in the front line immune system cells, regulating inflammation and oxidative stress.

Another stimulus that induces oxidative stress in the lungs is cigarette smoke. Long-term exposure leads to chronic obstructive pulmonary diseases (COPD) characterized by the presence of emphysema and/or chronic bronchitis. Nrf2$^{-/-}$ mice have been shown to be more susceptible to COPD development when chronically exposed to cigarette smoke. On the other hand, the exposure of WT mice to cigarette smoke combined to a pharmacological-inducer of Nrf2 activation ameliorates the outcome of COPD [139]. Corroborating these data, lung biopsies from patients with cigarette smoke-induced emphysema revealed increased levels of Keep 1 and Bach1 and decreased expression of Nrf2 and downstream ARE genes (HO-1, GPX2, and NQO1). Similar abnormalities in Nrf2, Keap1, and Bach1 expression and increased lipid peroxidation were found in the alveolar macrophages of these patients [140]. This evidence shows translational data supporting therapies to improve Nrf2 activity.

AKI is a kidney pathology with a high mortality rate [141]. T lymphocyte infiltration, inflammation, intense oxidative stress, apoptosis, and epithelial and endothelial cell dysfunction have been implicated in the pathophysiology of this disease [142, 143]. In order to study the relevance of T lymphocytes expressing Nrf2 in the context of the disease, Noel et al. (2015) chose I/R-induced AKI model in mice. Thereunto, Keap1 was knocked out exclusively in T cells. Enhanced Nrf2 expression in T lymphocytes promoted Treg population and decreased number of macrophages and pro-inflammatory cytokine (TNF-α, INF-γ, and IL-17) levels, which ameliorated functional and histological alterations in the kidney [96].

As discussed above, Treg cells have an important role in self-tolerance, avoiding the development of autoimmune diseases, and controlling the inflammatory process. Scurf mice (Treg-deficient mice) develop severe multi-organ inflammation with hyperactivation of autoreactive effector T cells and lethality of mice by 4 weeks of age. Systemic activation of Nrf2, through Keap1 knockdown, reduces the number of activated effector T cells, cytokines production and increase survival rate of scurf mice in a Treg-independent manner [144]. Moreover, in pristine-induced lupus nephritis, Nrf2$^{-/-}$ mice suffer from more renal damage and structural and functional pathological alterations in the kidney. Interestingly, treatment with the Nrf2 pharmacological-inducer sulforaphane also ameliorates renal function, oxidative injury, and the activation of NF-κB and TGF-β signaling pathway. Thus, Nrf2 plays an important role in preventing the progression and severity of immune response-mediated pathologies [145].

The integrity and adequate function of Nrf2 is age dependent [146]. Studies have investigated the decrease in Nrf2 expression in aging and the increase in susceptibility to oxidative stress and diseases. For example, aged Nrf2$^{-/-}$ female mice develop lupus-like autoimmune nephritis [35]. In this study, the animals were maintained in recommended conditions and no stimuli were used to generate the pathology. At 60 weeks of life glomeruli lesions could be observed in these mice [35]. In a similar context, Ma et al. (2006) assessed the manifestation of a lupus-like systemic autoimmune syndrome, characterized by multi-organ inflammatory lesions in Nrf2$^{-/-}$ mice. The syndrome predominantly affects females and

courses with the presence of anti-double-stranded DNA antibodies, intravascular deposition of immunoglobulin complexes, and premature death. The mechanism behind this syndrome is the increase in oxidative tissue lesions, enhanced proliferation of T $CD4^+$ cells and unbalanced ratios of $CD4^+$ and $CD8^+$ cellular population [36].

In humans, lower expression of Nrf2 was also observed in liver tissue of older donors, and this was attributed to the increased susceptibility to I/R injury. The decrease in Nrf2 expression during aging and its inhibition, secondary to Nf-κB activation during inflammation, leads to a paradoxical suppression of Nrf2 activity at a time the organism most needs its total functionality [147].

In contrast to previous studies revealing the positive effects of increasing Nrf2 activity, $Keap1^{-/-}$ mice show weaning-age (postnatal) lethality. The absence of Keep1 leads to the accumulation of Nrf2 in the cell's nucleus, therefore, increasing the expression of ARE genes. Although, increased Nrf2 activity is beneficial in most diseases settings, its constitutive activation leads to morphological alterations in the esophagus and forestomach, showing hyperkeratotic lesions. Two major conclusions were made. First, there is a subset of genes responsible for squamous cells differentiation in the ARE region; therefore, Nrf2 activates its transcription. Second, during desquamation, keratin oxidation increases the susceptibility of keratins to proteinases and the high amount of antioxidant proteins expressed constitutively through Nrf2, minimizes this processes, avoiding desquamation of these areas [148]. Therefore, it needs to be taken into account that induced Nrf2 and its counterparts are essential to regulate the antioxidant response and prevent disease. However, constitutive activation of Nrf2 can be prejudicial. Although pharmacological-induced expression of Nrf2 is largely used in murine experimental models, the side effects of this intervention need to be clarified, in order to be used for therapeutic purpose.

The most common experimental tool used to study Nrf2 consists in the employment of murine KOs for its encoding gene, *nrf2*. Mice lacking Nrf2 shows complete development, however high sensitivity to oxidative insults [146]. The use of $Nrf2^{-/-}$ mice allowed a deeper understanding of Nrf2 and its role in pulmonary diseases, such as asthma [27, 149], emphysema [140], fibrosis [150], and COPD [139]; neurodegenerative diseases, such as Parkinson [151], Alzheimer [152], and amyotrophic lateral sclerosis [153]; inflammatory disorders, bowel diseases [154], liver toxicity [155], atherosclerosis [156], autoimmune encephalomyelitis [157], and autoimmune inflammation [144] (Table 3 and Fig. 3).

The majority of studies with Nrf2-based experimental models are focused in determining the relationship between this transcription factor and the outcomes of the disease replicated in this model. In contrast, those models are also employed in the elucidation of pharmacological mechanisms using Nrf2 as a pivotal transcription factor in the reduction of determined phenomena. Therefore, as broad as the action spectrum of Nrf2 is the possibilities of its evaluation in a variety of experimental models.

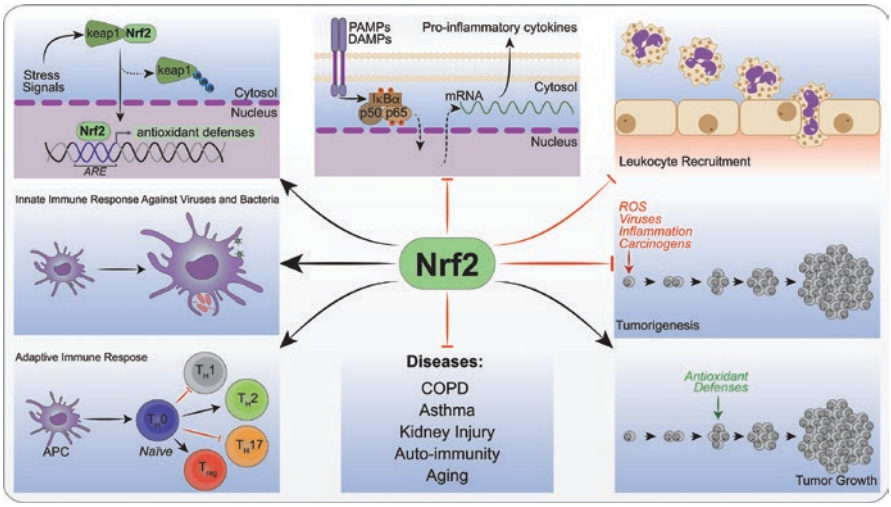

Fig. 3 Role of Nrf2 in the regulation of oxidative stress, inflammation, immune responses and surveillance, and diseases. *Redox homeostasis:* Cysteine residues within Keap1 sense stress signals (reactive oxidants and electrophilic agents), and their modification leads to conformational changes in Keap1, thereby inhibiting ubiquitination of Nrf2 by Keap1. Stabilized Nrf2 translocates into the nucleus and binds to the antioxidant response elements (ARE) and enhances transcription of antioxidants and detoxification enzymes responsible for restoring redox balance. *Inflammation:* Increased Nrf2 expression in leukocytes inhibits the expression of pro-inflammatory genes through down-regulation of the NF-κB pathway and inflammatory cell recruitment to the site of inflammation. *Immune responses:* Nrf2 is a major player in innate immunity and the control of oxidative/inflammatory stress in infectious and non-infectious diseases. Nrf2 controls the expression of pattern-recognition receptors (PRRs), clearance of pathogens, and activation of multiple intracellular pathways, for example, PI_3K/AKT/Foxo1 signaling pathway. In adaptive immunity, Nrf2 can modulate T cell differentiation by regulating oxidative stress-induced activation of dendritic cells (DCs). Additionally, Nrf2 displays important role in skewing Th responses, i.e., Th1, Th2, Th17, and T regulatory (Treg). Nrf2 activation impairs Th1- and favors Th2-driven responses. There is also a link between the induction of Nrf2 and suppression of Th17 and expansion of Foxp3-expressing T cells (Treg cells). *Immune surveillance:* Nrf2 has a dual role in cancer. Nrf2 is important to reduce reactive oxygen species (ROS), viral infection, and inflammation-induced tumorigenesis. However, upon the development of cancer, similarly to the protective effect on normal cells, Nrf2 also promotes the survival and growth of cancer cells under unfavorable conditions, i.e., chronic oxidative stress, by inducing antioxidant pathways. *Diseases and other conditions:* Due to the broad action spectrum, Nrf2 deficiency is related to the aggravation of diseases and conditions in which oxidative stress is pivotal, for example, chronic obstructive pulmonary diseases (COPD), asthma, kidney disease, and auto-immunity, and aging

7 Conclusion

Although Nrf2 is best known for its role in resistance to oxidant stress, it is also critical to the regulation of inflammation and immune responses. Varied immune cells express Nrf2 and its activation is essential to the regulatory role in inflammation and innate and adaptive immunity displayed by Nrf2 during infectious and

non-infectious diseases. Most of the regulatory functions exerted by Nrf2 involve the activation of its downstream targets and maintenance of redox homeostasis in cells. Due to the broad action spectrum, deficiency or deregulation of Nrf2 axis is related to the development and aggravation of several pathologies in which oxidative stress has a pivotal contribution. While it is tempting to propose pharmacological induction of Nrf2 as a potential therapeutic tool in diseases, adverse and off-target effects of this intervention still need to be clarified. In cancer, for example, Nrf2 has conflicting functions. Although it is an important protective mechanism in preventing the development of cancer, once it occurs, Nrf2 confers antioxidant protection to cancer cells and favors their proliferation. Moreover, constitutive activation of Nrf2 has been shown to be prejudicial to development. Therefore, further studies are still necessary in order to understand the consequences of modulating Nrf2 activity and propose it as a therapeutic target.

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References

1. Chan K, Han XD, Kan YW. An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. Proc Natl Acad Sci U S A. 2001;98(8):4611–6.
2. Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med. 2004;10(11):549–57.
3. Walters DM, Cho HY, Kleeberger SR. Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf2. Antioxid Redox Signal. 2008;10(2):321–32.
4. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci U S A. 1994;91(21):9926–30.
5. Itoh K, Mimura J, Yamamoto M. Discovery of the negative regulator of Nrf2, Keap1: a historical overview. Antioxid Redox Signal. 2010;13(11):1665–78.
6. Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells. 2011;16(2):123–40.
7. Suzuki T, Motohashi H, Yamamoto M. Toward clinical application of the Keap1-Nrf2 pathway. Trends Pharmacol Sci. 2013;34(6):340–6.
8. Bloom DA, Jaiswal AK. Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. J Biol Chem. 2003;278(45):44675–82.
9. Nguyen T, Sherratt PJ, Huang HC, Yang CS, Pickett CB. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. J Biol Chem. 2003;278(7):4536–41.

10. Manandhar S, Cho JM, Kim JA, Kensler TW, Kwak MK. Induction of Nrf2-regulated genes by 3H-1, 2-dithiole-3-thione through the ERK signaling pathway in murine keratinocytes. Eur J Pharmacol. 2007;577(1–3):17–27.
11. Dietrich C. Antioxidant functions of the aryl hydrocarbon receptor. Stem Cells Int. 2016;2016:7943495.
12. Keum YS, Yu S, Chang PP, Yuan X, Kim JH, Xu C, et al. Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. Cancer Res. 2006;66(17):8804–13.
13. Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene. 2013;32(32):3765–81.
14. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. 2013;1:45–9.
15. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236(2):313–22.
16. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284(20):13291–5.
17. Ruiz S, Pergola PE, Zager RA, Vaziri ND. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int. 2013;83(6):1029–41.
18. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol. 2007;47:89–116.
19. Talalay P, Dinkova-Kostova AT, Holtzclaw WD. Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. Adv Enzym Regul. 2003;43:121–34.
20. Kumar V, Abul Abbas A, Aster J. Cellular responses to stress and toxic insults: adaptation, injury, and death. In: Robbins, Cotran, editors. Pathologic basis of disease. 14th ed. Amsterdam: Elsevier; 2014.
21. Kumar V, Abul Abbas A, Aster J. Acute and chronic inflammation. In: Robbins, Cotran, editors. Pathologic basis of disease. 14th ed. Amsterdam: Elsevier; 2014.
22. Barton GM. A calculated response: control of inflammation by the innate immune system. J Clin Invest. 2008;118(2):413–20.
23. Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer. 2013;12:86.
24. Kaplan MH. STAT signaling in inflammation. JAKSTAT. 2013;2(1):e24198.
25. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428–35.
26. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. Immunity. 2014;41(5):694–707.
27. Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, et al. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med. 2005;202(1):47–59.
28. Sussan TE, Gajghate S, Chatterjee S, Mandke P, McCormick S, Sudini K, et al. Nrf2 reduces allergic asthma in mice through enhanced airway epithelial cytoprotective function. Am J Physiol Lung Cell Mol Physiol. 2015;309(1):L27–36.
29. Ishii Y, Itoh K, Morishima Y, Kimura T, Kiwamoto T, Iizuka T, et al. Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. J Immunol. 2005;175(10):6968–75.
30. Kong X, Thimmulappa R, Craciun F, Harvey C, Singh A, Kombairaju P, et al. Enhancing Nrf2 pathway by disruption of Keap1 in myeloid leukocytes protects against sepsis. Am J Respir Crit Care Med. 2011;184(8):928–38.

31. Chen XL, Dodd G, Thomas S, Zhang X, Wasserman MA, Rovin BH, et al. Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression. Am J Physiol Heart Circ Physiol. 2006;290(5):H1862–70.
32. Itoh K, Mochizuki M, Ishii Y, Ishii T, Shibata T, Kawamoto Y, et al. Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2). Mol Cell Biol. 2004;24(1):36–45.
33. Thimmulappa RK, Scollick C, Traore K, Yates M, Trush MA, Liby KT, et al. Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-Imidazolide. Biochem Biophys Res Commun. 2006;351(4):883–9.
34. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest. 2006;116(4):984–95.
35. Yoh K, Itoh K, Enomoto A, Hirayama A, Yamaguchi N, Kobayashi M, et al. Nrf2-deficient female mice develop lupus-like autoimmune nephritis. Kidney Int. 2001;60(4):1343–53.
36. Ma Q, Battelli L, Hubbs AF. Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. Am J Pathol. 2006;168(6):1960–74.
37. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmaj K, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med. 2012;367(12):1098–107.
38. Burness CB, Deeks ED. Dimethyl fumarate: a review of its use in patients with relapsing-remitting multiple sclerosis. CNS Drugs. 2014;28(4):373–87.
39. Jin W, Wang H, Yan W, Xu L, Wang X, Zhao X, et al. Disruption of Nrf2 enhances upregulation of nuclear factor-kappaB activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury. Mediat Inflamm. 2008;2008:725174.
40. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. Annu Rev Immunol. 2011;29:139–62.
41. Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev. 2000;173:89–97.
42. Anderson G, Maes M. Oxidative/nitrosative stress and immuno-inflammatory pathways in depression: treatment implications. Curr Pharm Des. 2014;20(23):3812–47.
43. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.
44. Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol. 2012;4(3)
45. Jounai N, Kobiyama K, Takeshita F, Ishii KJ. Recognition of damage-associated molecular patterns related to nucleic acids during inflammation and vaccination. Front Cell Infect Microbiol. 2012;2:168.
46. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197–223.
47. Clark RA. Activation of the neutrophil respiratory burst oxidase. J Infect Dis. 1999;179(Suppl 2):S309–17.
48. Iles KE, Forman HJ. Macrophage signaling and respiratory burst. Immunol Res. 2002;26(1–3):95–105.
49. Wartha F, Beiter K, Normark S, Henriques-Normark B. Neutrophil extracellular traps: casting the NET over pathogenesis. Curr Opin Microbiol. 2007;10(1):52–6.
50. Yang H, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, Li J, et al. A critical cysteine is required for HMGB1 binding to toll-like receptor 4 and activation of macrophage cytokine release. Proc Natl Acad Sci U S A. 2010;107(26):11942–7.
51. Fan J, Li Y, Levy RM, Fan JJ, Hackam DJ, Vodovotz Y, et al. Hemorrhagic shock induces NAD(P)H oxidase activation in neutrophils: role of HMGB1-TLR4 signaling. J Immunol. 2007;178(10):6573–80.
52. Tang D, Kang R, Zeh HJ 3rd, Lotze MT. High-mobility group box 1, oxidative stress, and disease. Antioxid Redox Signal. 2011;14(7):1315–35.
53. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007;35(Pt 5):1147–50.

54. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9–19.
55. Toledano MB, Leonard WJ. Modulation of transcription factor NF-kappa B binding activity by oxidation-reduction in vitro. Proc Natl Acad Sci U S A. 1991;88(10):4328–32.
56. Jamaluddin M, Wang S, Boldogh I, Tian B, Brasier AR. TNF-α-induced NF-κB/RelA Ser276 phosphorylation and enhanceosome formation is mediated by an ROS-dependent PKAc pathway. Cell Signal. 2007;19(7):1419–33.
57. Ndengele MM, Muscoli C, Wang ZQ, Doyle TM, Matuschak GM, Salvemini D. Superoxide potentiates NF-kappaB activation and modulates endotoxin-induced cytokine production in alveolar macrophages. Shock. 2005;23(2):186–93.
58. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-kappaB signaling. Cell Res. 2011;21(1):103–15.
59. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1(6):a001651-a.
60. Bryan HK, Olayanju A, Goldring CE, Park BK. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. 2013. 705–17
61. Gao B, Doan A, Hybertson BM. The clinical potential of influencing Nrf2 signaling in degenerative and immunological disorders. Clin pharmacol. 2014;6:19–34.
62. Schwarz KB. Oxidative stress during viral infection: a review. Free Radic Biol Med. 1996;21(5):641–9.
63. Olagnier D, Peri S, Steel C, van Montfoort N, Chiang C, Beljanski V, et al. Cellular oxidative stress response controls the antiviral and apoptotic programs in dengue virus-infected dendritic cells. PLoS Pathog. 2014;10(12):e1004566.
64. Komaravelli N, Tian B, Ivanciuc T, Mautemps N, Brasier AR, Garofalo RP, et al. Respiratory syncytial virus infection down-regulates antioxidant enzyme expression by triggering deacetylation-proteasomal degradation of Nrf2. Free Radic Biol Med. 2015;88(Pt B):391–403.
65. Cho HY, Imani F, Miller-DeGraff L, Walters D, Melendi GA, Yamamoto M, et al. Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease. Am J Respir Crit Care Med. 2009;179(2):138–50.
66. Harvey CJ, Thimmulappa RK, Sethi S, Kong X, Yarmus L, Brown RH, et al. Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. Sci Transl Med. 2011;3(78):78ra32.
67. Kong X, Thimmulappa R, Kombairaju P, Biswal S. NADPH oxidase-dependent reactive oxygen species mediate amplified TLR4 signaling and sepsis-induced mortality in Nrf2-deficient mice. J Immunol. 2010;185(1):569–77.
68. Gomez JC, Dang H, Martin JR, Doerschuk CM. Nrf2 modulates host defense during Streptococcus pneumoniae pneumonia in mice. J Immunol. 2016;197(7):2864–79.
69. Kraal G, van der Laan LJ, Elomaa O, Tryggvason K. The macrophage receptor MARCO. Microbes Infect. 2000;2(3):313–6.
70. Lin Q, Li M, Fang D, Fang J, Su SB. The essential roles of toll-like receptor signaling pathways in sterile inflammatory diseases. Int Immunopharmacol. 2011;11(10):1422–32.
71. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005;201(7):1135–43.
72. Zhai Y, Shen XD, O'Connell R, Gao F, Lassman C, Busuttil RW, et al. Cutting edge: TLR4 activation mediates liver ischemia/reperfusion inflammatory response via IFN regulatory factor 3-dependent MyD88-independent pathway. J Immunol. 2004;173(12):7115–9.
73. Wu HS, Zhang JX, Wang L, Tian Y, Wang H, Rotstein O. Toll-like receptor 4 involvement in hepatic ischemia/reperfusion injury in mice. Hepatobiliary Pancreat Dis Int. 2004;3(2):250–3.
74. Tsung A, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, et al. Hepatic ischemia/reperfusion injury involves functional TLR4 signaling in nonparenchymal cells. J Immunol. 2005;175(11):7661–8.

75. Shimamoto A, Pohlman TH, Shomura S, Tarukawa T, Takao M, Shimpo H. Toll-like receptor 4 mediates lung ischemia-reperfusion injury. Ann Thorac Surg. 2006;82(6):2017–23.
76. Cao CX, Yang QW, Lv FL, Cui J, Fu HB, Wang JZ. Reduced cerebral ischemia-reperfusion injury in toll-like receptor 4 deficient mice. Biochem Biophys Res Commun. 2007;353(2):509–14.
77. Caso JR, Pradillo JM, Hurtado O, Lorenzo P, Moro MA, Lizasoain I. Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. Circulation. 2007;115(12):1599–608.
78. Hua F, Ha T, Ma J, Li Y, Kelley J, Gao X, et al. Protection against myocardial ischemia/reperfusion injury in TLR4-deficient mice is mediated through a phosphoinositide 3-kinase-dependent mechanism. J Immunol. 2007;178(11):7317–24.
79. Wu H, Chen G, Wyburn KR, Yin J, Bertolino P, Eris JM, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. J Clin Invest. 2007;117(10):2847–59.
80. Huang J, Yue S, Ke B, Zhu J, Shen XD, Zhai Y, et al. Nuclear factor erythroid 2-related factor 2 regulates toll-like receptor 4 innate responses in mouse liver ischemia-reperfusion injury through Akt-forkhead box protein O1 signaling network. Transplantation. 2014;98(7):721–8.
81. Rao J, Qian X, Li G, Pan X, Zhang C, Zhang F, et al. ATF3-mediated NRF2/HO-1 signaling regulates TLR4 innate immune responses in mouse liver ischemia/reperfusion injury. Am J Transplant. 2015;15(1):76–87.
82. Janeway CA Jr. How the immune system protects the host from infection. Microbes Infect. 2001;3(13):1167–71.
83. Rockwell CE, Zhang M, Fields PE, Klaassen CD. Th2 skewing by activation of Nrf2 in CD4(+) T cells. J Immunol. 2012;188(4):1630–7.
84. Hirahara K, Nakayama T. CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. Int Immunol. 2016;28(4):163–71.
85. Jain A, Pasare C. Innate control of adaptive immunity: beyond the three-signal paradigm. J Immunol. 2017;198(10):3791–800.
86. Lafaille JJ. The role of helper T cell subsets in autoimmune diseases. Cytokine Growth Factor Rev. 1998;9(2):139–51.
87. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology. 2008;123(3):326–38.
88. Frauwirth KA, Thompson CB. Activation and inhibition of lymphocytes by costimulation. J Clin Invest. 2002;109(3):295–9.
89. Duncan DD, Swain SL. Role of antigen-presenting cells in the polarized development of helper T cell subsets: evidence for differential cytokine production by Th0 cells in response to antigen presentation by B cells and macrophages. Eur J Immunol. 1994;24(10):2506–14.
90. Grogan JL, Mohrs M, Harmon B, Lacy DA, Sedat JW, Locksley RM. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. Immunity. 2001;14(3):205–15.
91. Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. Nat Immunol. 2002;3(7):643–51.
92. Gostner JM, Becker K, Fuchs D, Sucher R. Redox regulation of the immune response. Redox Rep. 2013;18(3):88–94.
93. Kesarwani P, Murali AK, Al-Khami AA, Mehrotra S. Redox regulation of T-cell function: from molecular mechanisms to significance in human health and disease. Antioxid Redox Signal. 2013;18(12):1497–534.
94. Yan Z, Garg SK, Kipnis J, Banerjee R. Extracellular redox modulation by regulatory T cells. Nat Chem Biol. 2009;5(10):721–3.
95. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med. 1983;158(3):670–89.

96. Noel S, Martina MN, Bandapalle S, Racusen LC, Potteti HR, Hamad AR, et al. T lymphocyte-specific activation of Nrf2 protects from AKI. J Am Soc Nephrol. 2015;26(12): 2989–3000.
97. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol. 2013;13(11):777–89.
98. Kikuchi N, Ishii Y, Morishima Y, Yageta Y, Haraguchi N, Itoh K, et al. Nrf2 protects against pulmonary fibrosis by regulating the lung oxidant level and Th1/Th2 balance. Respir Res. 2010;11:31.
99. Johnson DA, Amirahmadi S, Ward C, Fabry Z, Johnson JA. The absence of the pro-antioxidant transcription factor Nrf2 exacerbates experimental autoimmune encephalomyelitis. Toxicol Sci. 2010;114(2):237–46.
100. Riedl MA, Nel AE. Importance of oxidative stress in the pathogenesis and treatment of asthma. Curr Opin Allergy Clin Immunol. 2008;8(1):49–56.
101. Mizuno S, Yamamoto M, Sugawara I. Significant reduction of granulomas in Nrf2-deficient mice infected with Mycobacterium tuberculosis. Indian J Tuberc. 2010;57(2):108–13.
102. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol. 2013;31:563–604.
103. Aw Yeang HX, Hamdam JM, Al-Huseini LM, Sethu S, Djouhri L, Walsh J, et al. Loss of transcription factor nuclear factor-erythroid 2 (NF-E2) p45-related factor-2 (Nrf2) leads to dysregulation of immune functions, redox homeostasis, and intracellular signaling in dendritic cells. J Biol Chem. 2012;287(13):10556–64.
104. Kim HJ, Barajas B, Wang M, Nel AE. Nrf2 activation by sulforaphane restores the age-related decrease of T(H)1 immunity: role of dendritic cells. J Allergy Clin Immunol. 2008;121(5):1255–61 e7.
105. Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. Proc Natl Acad Sci U S A. 1999;96(22):12731–6.
106. Wallet MA, Sen P, Tisch R. Immunoregulation of dendritic cells. Clin Med Res. 2005;3(3):166–75.
107. Kim HJ, Barajas B, Chan RC, Nel AE. Glutathione depletion inhibits dendritic cell maturation and delayed-type hypersensitivity: implications for systemic disease and immunosenescence. J Allergy Clin Immunol. 2007;119(5):1225–33.
108. Morito N, Yoh K, Itoh K, Hirayama A, Koyama A, Yamamoto M, et al. Nrf2 regulates the sensitivity of death receptor signals by affecting intracellular glutathione levels. Oncogene. 2003;22(58):9275–81.
109. Morzadec C, Macoch M, Sparfel L, Kerdine-Romer S, Fardel O, Vernhet L. Nrf2 expression and activity in human T lymphocytes: stimulation by T cell receptor activation and priming by inorganic arsenic and tert-butylhydroquinone. Free Radic Biol Med. 2014;71:133–45.
110. Turley AE, Zagorski JW, Rockwell CE. The Nrf2 activator tBHQ inhibits T cell activation of primary human CD4 T cells. Cytokine. 2015;71(2):289–95.
111. Romagnani S. Human Th17 cells. Arthritis Res Ther. 2008;10(2):206.
112. Zhao M, Chen H, Ding Q, Xu X, Yu B, Huang Z. Nuclear factor erythroid 2-related factor 2 deficiency exacerbates lupus nephritis in B6/lpr mice by regulating Th17 cell function. Sci Rep. 2016;6:38619.
113. Moon SJ, Park JS, Woo YJ, Lim MA, Kim SM, Lee SY, et al. Rebamipide suppresses collagen-induced arthritis through reciprocal regulation of th17/treg cell differentiation and heme oxygenase 1 induction. Arthritis Rheumatol. 2014;66(4):874–85.
114. Chen X, Su W, Wan T, Yu J, Zhu W, Tang F, et al. Sodium butyrate regulates Th17/Treg cell balance to ameliorate uveitis via the Nrf2/HO-1 pathway. Biochem Pharmacol 2017
115. Germain RN. Special regulatory T-cell review: a rose by any other name: from suppressor T cells to Tregs, approbation to unbridled enthusiasm. Immunology. 2008;123(1):20–7.
116. Newton R, Priyadharshini B, Turka LA. Immunometabolism of regulatory T cells. Nat Immunol. 2016;17(6):618–25.

117. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci. 2014;39(4):199–218.
118. Lu CY, de Albuquerque Rocha N. Oxidative stress and metabolism: the NF-erythroid 2 p45-related factor 2:Kelch-like ECH-associated protein 1 system and regulatory T lymphocytes in ischemic AKI. J Am Soc Nephrol. 2015;26(12):2893–5.
119. Mougiakakos D, Johansson CC, Jitschin R, Bottcher M, Kiessling R. Increased thioredoxin-1 production in human naturally occurring regulatory T cells confers enhanced tolerance to oxidative stress. Blood. 2011;117(3):857–61.
120. Smigiel KS, Srivastava S, Stolley JM, Campbell DJ. Regulatory T-cell homeostasis: steady-state maintenance and modulation during inflammation. Immunol Rev. 2014;259(1): 40–59.
121. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007;117(5):1137–46.
122. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010;49(11):1603–16.
123. Milkovic L, Zarkovic N, Saso L. Controversy about pharmacological modulation of Nrf2 for cancer therapy. Redox Biol. 2017;12:727–32.
124. Iida K, Itoh K, Maher JM, Kumagai Y, Oyasu R, Mori Y, et al. Nrf2 and p53 cooperatively protect against BBN-induced urinary bladder carcinogenesis. Carcinogenesis. 2007;28(11):2398–403.
125. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci U S A. 2001;98(6):3410–5.
126. Khor TO, Huang MT, Prawan A, Liu Y, Hao X, Yu S, et al. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer Prev Res. 2008;1(3): 187–91.
127. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci U S A. 2002;99(11):7610–5.
128. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, et al. Inhibition of 7,12-dimethylbenz(a) anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res. 2006;66(16):8293–6.
129. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, Suzuki T, et al. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. Carcinogenesis. 2010;31(10):1833–43.
130. Saddawi-Konefka R, Seelige R, Gross ET, Levy E, Searles SC, Washington A Jr, et al. Nrf2 induces IL-17D to mediate tumor and virus surveillance. Cell Rep. 2016;16(9):2348–58.
131. Suzuki T, Shibata T, Takaya K, Shiraishi K, Kohno T, Kunitoh H, et al. Regulatory nexus of synthesis and degradation deciphers cellular Nrf2 expression levels. Mol Cell Biol. 2013;33(12):2402–12.
132. Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD. Dual roles of Nrf2 in cancer. Pharmacol Res. 2008;58(5–6):262–70.
133. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013;27(20):2179–91.
134. Homma S, Ishii Y, Morishima Y, Yamadori T, Matsuno Y, Haraguchi N, et al. Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer. Clin Cancer Res. 2009;15(10):3423–32.
135. Jiang T, Chen N, Zhao F, Wang XJ, Kong B, Zheng W, et al. High levels of Nrf2 determine chemoresistance in type II endometrial cancer. Cancer Res. 2010;70(13):5486–96.
136. Shim GS, Manandhar S, Shin DH, Kim TH, Kwak MK. Acquisition of doxorubicin resistance in ovarian carcinoma cells accompanies activation of the NRF2 pathway. Free Radic Biol Med. 2009;47(11):1619–31.
137. Ji L, Li H, Gao P, Shang G, Zhang DD, Zhang N, et al. Nrf2 pathway regulates multidrug-resistance-associated protein 1 in small cell lung cancer. PLoS One. 2013;8(5):e63404.

138. Mitsuishi Y, Motohashi H, Yamamoto M. The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. Front Oncol. 2012;2:200.
139. Sussan TE, Rangasamy T, Blake DJ, Malhotra D, El-Haddad H, Bedja D, et al. Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. Proc Natl Acad Sci U S A. 2009;106(1):250–5.
140. Goven D, Boutten A, Lecon-Malas V, Marchal-Somme J, Amara N, Crestani B, et al. Altered Nrf2/Keap1-Bach1 equilibrium in pulmonary emphysema. Thorax. 2008;63(10):916–24.
141. Rewa O, Bagshaw SM. Acute kidney injury-epidemiology, outcomes and economics. Nat Rev Nephrol. 2014;10(4):193–207.
142. Jang HR, Ko GJ, Wasowska BA, Rabb H. The interaction between ischemia-reperfusion and immune responses in the kidney. J Mol Med (Berl). 2009;87(9):859–64.
143. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. Nephron Exp Nephrol. 2008;109(4):e102–7.
144. Suzuki T, Murakami S, Biswal SS, Sakaguchi S, Harigae H, Yamamoto M, et al. Systemic activation of NRF2 alleviates lethal autoimmune inflammation in scurfy mice. Mol Cell Biol 2017
145. Jiang T, Tian F, Zheng H, Whitman SA, Lin Y, Zhang Z, et al. Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-kappaB-mediated inflammatory response. Kidney Int. 2014;85(2):333–43.
146. Sykiotis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. Sci Signal. 2010;3(112):re3.
147. Zaman MB, Leonard MO, Ryan EJ, Nolan NP, Hoti E, Maguire D, et al. Lower expression of Nrf2 mRNA in older donor livers: a possible contributor to increased ischemia-reperfusion injury? Transplantation. 2007;84(10):1272–8.
148. Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, et al. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat Genet. 2003;35(3):238–45.
149. Ano S, Panariti A, Allard B, O'Sullivan M, McGovern TK, Hamamoto Y, et al. Inflammation and airway hyperresponsiveness after chlorine exposure are prolonged by Nrf2 deficiency in mice. Free Radic Biol Med. 2017;102:1–15.
150. Cho HY, Reddy SP, Yamamoto M, Kleeberger SR. The transcription factor NRF2 protects against pulmonary fibrosis. FASEB J. 2004;18(11):1258–60.
151. Burton NC, Kensler TW, Guilarte TR. In vivo modulation of the Parkinsonian phenotype by Nrf2. Neurotoxicology. 2006;27(6):1094–100.
152. Kanninen K, Malm TM, Jyrkkanen HK, Goldsteins G, Keksa-Goldsteine V, Tanila H, et al. Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. Mol Cell Neurosci. 2008;39(3):302–13.
153. Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. J Neurosci. 2008;28(50):13574–81.
154. Khor TO, Huang MT, Kwon KH, Chan JY, Reddy BS, Kong AN. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res. 2006;66(24):11580–4.
155. Xu W, Hellerbrand C, Kohler UA, Bugnon P, Kan YW, Werner S, et al. The Nrf2 transcription factor protects from toxin-induced liver injury and fibrosis. Lab Investig. 2008;88(10):1068–78.
156. Collins AR, Lyon CJ, Xia X, Liu JZ, Tangirala RK, Yin F, et al. Age-accelerated atherosclerosis correlates with failure to upregulate antioxidant genes. Circ Res. 2009;104(6):e42–54.
157. Larabee CM, Desai S, Agasing A, Georgescu C, Wren JD, Axtell RC, et al. Loss of Nrf2 exacerbates the visual deficits and optic neuritis elicited by experimental autoimmune encephalomyelitis. Mol Vis. 2016;22:1503–13.

Nrf2 and the Nrf2-Interacting Network in Respiratory Inflammation and Diseases

Jennifer Carlson, Lindsey Price, and Huai Deng

Abstract Atmospheric pollutants and cigarette smoke influence the human respiratory system and induce airway inflammation, injury, and pathogenesis. Activation of the NF-E2-related factor 2 (Nrf2) transcription factor and downstream antioxidant response element (ARE)-mediated transcriptions play a central role in protecting respiratory cells against reactive oxidative species (ROS) that are induced by airway toxins and inflammation. Recent studies have revealed that Nrf2 can also target and activate many genes involved in developmental programs such as cell proliferation, cell differentiation, cell death, and metabolism. Nrf2 is closely regulated by the interaction with kelch-like ECH-associated protein 1 (Keap1), while also directly interacts with a number of other proteins, including inflammatory factors, transcription factors, autophagy mediators, kinases, epigenetic modifiers, etc. It is believed that the multiple target genes and the complicated interacting network of Nrf2 account for the roles of Nrf2 in physiologies and pathogeneses. This chapter summarizes the molecular functions and protein interactions of Nrf2, as well as the roles of Nrf2 and the Nrf2-interacting network in respiratory inflammation and diseases, including acute lung injury (ALI), asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis (PF), cystic fibrosis (CF), viral/bacterial infections, and lung cancers. Therapeutic applications that target Nrf2 and its interacting proteins in respiratory diseases are also reviewed.

1 Introduction

Environmental toxins generated by modern industry significantly influence human health. The respiratory system is most susceptible to pollutants as it is directly exposed to atmospheric toxins such as airborne chemicals, O_3, particulate matter (PM), and cigarette smoke (CS). Respiratory diseases and relevant secondary symptoms, including ALI, asthma, COPD, bacterial/viral infections, lung fibrosis, and lung cancers, are among the most common causes of severe illness and death worldwide [1].

J. Carlson · L. Price · H. Deng (✉)
Department of Biology, University of Minnesota Duluth, Duluth, MN, USA
e-mail: dengh@d.umn.edu

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Most of the airway pathogeneses are related to xenobiotic and oxidative stresses occurring in different types of respiratory cells. These stresses can be triggered directly by the inhaled toxins (xenobiotics) or by excess reactive oxygen species (ROS) that are induced by many of these toxins. Intracellular accumulation of ROS may damage macromolecules including DNA, proteins, and lipids, thereby inducing cell defects or death. Imbalanced redox homeostasis and cell damage lead to inflammatory responses, which further enhance oxidative stress. Cellular factors that can respond to and reduce oxidative/xenobiotic stresses are essential protective machinery against airway pathogenesis.

NF-E2-related factor 2 (Nrf2), a cap 'n' collar (CNC)-bZIP family transcription factor, is a central regulator that protects cells against ROS and xenobiotics [2, 3]. Activated by oxidative and xenobiotic stimuli, Nrf2 binds to antioxidant response elements (AREs) and activates a battery of antioxidant and detoxifying genes [4]. Nrf2 also activates many developmental genes, allowing Nrf2 to regulate programs that are related with cell proliferation, cell differentiation, cell death, and metabolism [4]. In addition, Nrf2 interacts with many other proteins and pathways, providing a complicated regulatory network for redox homeostasis and other cellular programs. Nrf2 is identified as the key player and important drug target for a number of human diseases including respiratory diseases [5]. Fully understanding the molecular partners and downstream functions of Nrf2 will significantly aid in the development of Nrf2-targeting therapies. This chapter first reviews the molecular mechanisms of Nrf2, focusing on the multiple interacting partners of Nrf2. Next, the molecular functions and therapeutic applications of Nrf2 and the Nrf2-interacting network in respiratory inflammation and diseases are summarized.

2 Molecular Mechanisms of the Nrf2 Network

2.1 Keap1-Nrf2 Pathway

The key regulator of Nrf2 activity in response to oxidative and xenobiotic stimuli is kelch-like ECH-associated protein 1 (Keap1). As an adaptor of the Cul3-based E3 ligase, Keap1 confines Nrf2 to the cytoplasm through mediating Nrf2 ubiquitination and degradation [6]. The Keap1–Nrf2 interaction is mediated by the Kelch repeats of the Keap1 dimer and the N-terminal DLG and ETGE motifs of Nrf2. Cysteine residues (e.g., C151, C273, C288) on Keap1 can be modified by a broad range of oxidative and xenobiotic compounds [7, 8]. According to the established model, cysteine modifications alter the conformation of the Keap1 dimer and interfere with the Keap1–Nrf2 interaction, thereby blocking the proteasomal degradation of Nrf2. The stabilized Nrf2 proteins accumulate in the nucleus and activate transcriptions [9, 10]. After induction, Keap1 can enter the nucleus and shuttle Nrf2 back to the cytosol [11]. The genetic interaction of Keap1 and Nrf2 has been verified in vivo. *Keap1*$^{-/-}$ mice die before weaning due to a severe dysfunction of keratinocytes, while co-knockout of Nrf2 significantly rescues the viability of *Keap1*$^{-/-}$ mice [12].

2.2 *Nrf2-Downstream Genes and Functions*

2.2.1 Antioxidant and Detoxifying Genes

Microarray analyses based on Nrf2 activators and *Nrf2* knockout mice have identified many antioxidant and detoxifying genes that are controlled by Nrf2-ARE [4, 13–15]. Most of these genes code for enzymes that catalyze the metabolism or removal of ROS and other toxins, including: (1) phase I antioxidant enzymes such as superoxide dismutases (SODs), glutathione peroxidase (GPx), and glutathione reductase (GR); (2) phase II detoxifying enzymes such as glutathione-S-transferase (GST), NADP(H):quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligases (GCLM/GCLC); (3) phase III xenobiotic transporters such as multidrug resistance protein 1 (MRP1); and (4) other stress response proteins such as heme oxygenase-1 (HO-1). Protective roles of some of these enzymes against respiratory inflammation and damages have been demonstrated by several studies. For example, the lungs of *Sod2* mutant mice have enhanced sensitivity to hyperoxia [16], while overexpression of *Sod2* in the mouse airway reduces the hyperoxia-induced lung inflammation and injury [17]. Activation of the Nrf2-ARE antioxidant pathway is believed to be an efficient therapeutic strategy for redox-related lung diseases.

2.2.2 Other Nrf2-Target Genes and Functions

Recent studies in different model systems have revealed that Nrf2 can target genes independent of oxidative and xenobiotic responses, demonstrating that Nrf2 can act as more than just a detoxifying factor. For examples, a ChIP-seq study combining microarray assays using mouse embryonic fibroblasts with either enhanced or reduced levels of Nrf2 identified around 1000 Nrf2-target genes, more than half of which were involved in cell proliferation [4]. Nrf2 can control adipogenesis through binding to and activating *peroxisome proliferator-activated receptor γ* (*PPARγ*), *retinoid X receptor α* (*RXRα*), and *small heterodimer partner* (*SHP*) nuclear receptor genes as well as some lipid metabolism genes [18–21]. The Nrf2-activated *RXRα* can also control the differentiation of acute myeloid leukemia cells [22]. In addition, Nrf2 can regulate neuronal stem cell fate through activating genes that inhibit self-renewal or promote differentiation [23]. Another essential Nrf2-target gene is *p62*, which mediates the role of Nrf2 in the regulation of autophagy [24]. In a human lung cancer cell line, NRF2 activates transcripts of anti-apoptotic factor Bcl-2 and glucose metabolic enzymes [25, 26], indicating the promotive role of Nrf2 in cancer cell proliferation. It is notable that Nrf2 can target and inhibit some proinflammatory cytokine genes such as *IL6* through an ARE-independent manner, revealing a novel mechanism that mediates the anti-inflammation function of Nrf2 [27].

Structural and functional conservation of the Keap1-Nrf2 signaling has been revealed in mammals, zebrafish, and *Drosophila* [8, 28]. Studies using non-mamalian model organisms provide additional insights into the biological functions of Nrf2. In *Drosophila*, CncC and dKeap1 (homologs of Nrf2 and Keap1, respectively) regulate metamorphosis through targeting and activating ecdysteroid biosynthetic genes

and response genes in a tissue-specific manner [29], indicating a potential role of Nrf2 family proteins in the regulation of steroid hormones. CncC and dKeap1 also regulate intestinal stem cell proliferation in *Drosophila* [30]. It is believed that multiple developmental functions of Nrf2 and Keap1 in specific tissues contribute to their complicated roles in diseases [31, 32]. How Nrf2 selectively targets and activates developmental genes remains to be elucidated, but it is likely that it acts through mechanisms that differ from the classic ARE-dependent antioxidant pathway. Notably, recent studies showed that dKeap1 can directly bind to chromatin and function as a transcription coactivator with CncC through interaction with CncC at specific genomic loci [29, 33].

2.3 *Other Nrf2-Interacting Proteins*

Besides Keap1, a number of proteins that interact with Nrf2 have been identified. These protein partners can regulate the activity, subcellular localization, degradation, and chromatin-binding specificity of Nrf2. This places Nrf2 in the center of a multi-layer regulatory network for redox homeostasis and other cellular programs (Fig. 1). These interactions are believed to mediate the complicated roles of Nrf2 in pathogeneses, including respiratory diseases.

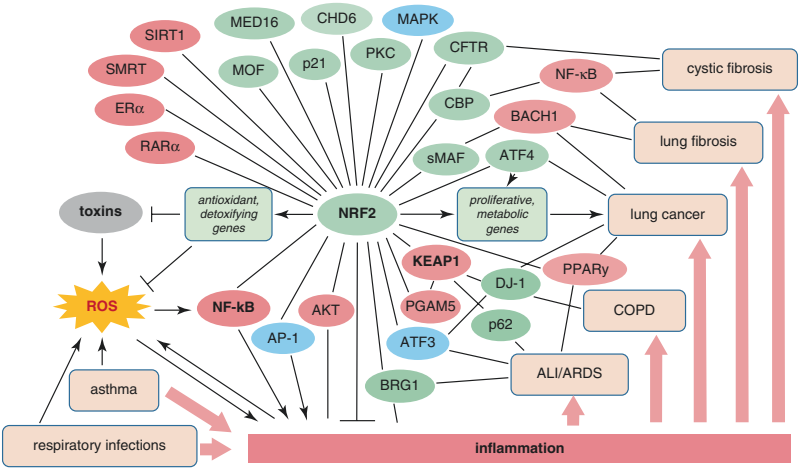

Fig. 1 Nrf2-interacting network and its correlation with respiratory inflammation and diseases. Nrf2 plays a protective role against respiratory inflammation and respiratory diseases through mediating the transcriptional responses to oxidative and xenobiotic stresses. Nrf2 can also promote lung oncogenesis through activating genes that facilitate cell proliferation. In addition, Nrf2 interacts with many proteins and pathways, placing Nrf2 in the center of a network that controls redox homeostasis, inflammation, and pathogenesis. The ovals represent proteins that can interact with Nrf2. The positive and negative regulators of Nrf2 activity are colored in green and red, respectively. Proteins in blue ovals can either activate or inhibit Nrf2 activity. The identified roles of Nrf2 and some of the Nrf2-interacting proteins in respiratory inflammation and diseases are represented with line connections

2.3.1 Inflammatory Factors

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) are classic transcription factors that induce inflammation in response to oxidative stress through the activation of genes encoding proinflammatory cytokines and chemokines. The essential roles of NF-κB and AP-1 proteins in inflammation and chronic lung diseases (e.g., asthma, IPF, and COPD) have been identified by many studies [34–36]. It is noted that oxidative stress can co-activate NF-κB and Nrf2 pathways. For example, PM exposures co-induce NF-κB and Nrf2-HO-1 in both mouse lungs and human bronchial epithelial cells [37, 38]. NF-κB can inactivate Nrf2 by competing with the CBP–Nrf2 interaction or by recruiting the transcription repressor HDAC3 to ARE [39]. The AP-1 subunit c-Jun can dimerize with Nrf2 at AREs and co-activate Nrf2-induced transcription, while another AP-1 subunit c-Fos can suppress Nrf2-induced transcription [40, 41]. Crosstalk between Nrf2 and these inflammatory factors establishes a mechanism whereby Nrf2 regulates inflammation.

2.3.2 Small Maf Transcription Factors

Nrf2 can form heterodimers with small Maf (sMaf) transcription factors, including MafF, MafG, and MafK [42]. These interactions are mediated by their leucine zipper domains. It has been well established that the Nrf2-sMaf complexes recognize ARE and the sMaf proteins can support Nrf2-mediated transcriptional activation [43]. Combinatory knockouts of MafF and MafG can rescue the lethality of *Keap1* null mice, suggesting the cooperative roles of sMaf proteins with Nrf2 in vivo [44]. ChIP-Seq analyses of Nrf2-MafG-binding sites indicate that Nrf2-sMaf heterodimers globally regulate antioxidant and metabolic genes [45].

2.3.3 ATF/CREB Transcription Factors and Cofactors

A number of transcription factors, coactivators, and mediators can form complexes with Nrf2 at AREs and control Nrf2-mediated transcription. The mediator MED16 directly interacts with Nrf2 and mediates 75% of Nrf2-dependent transcription [46]. CREB-binding protein (CBP) and its cofactor p300 can bind the Neh4/5 domains of Nrf2. It was observed that p300 and Nrf2 can be co-recruited to ARE in response to oxidative stress [47, 48]. The Nrf2–CBP interaction can be regulated by a $Cl^-/HCO3^-$ channel protein cystic fibrosis transmembrane conductance regulator (CFTR), which also interacts with Nrf2 and promotes Nrf2-dependent transcription in human bronchial epithelial cells [49]. Activating transcription factor 4 (ATF4) can dimerize with Nrf2 at ARE and co-activate *HO-1* [50]. Formation of the ATF3-Nrf2 dimer can compete with the Nrf2–CBP interaction at ARE and suppress Nrf2-induced transcription [51]. On the other hand, ATF3 depletion in human bronchoalveolar epithelial cells enhances NRF2 degradation through KEAP1 and DJ-1 pathways [52]. Therefore, ATF3 can either positively or negatively control Nrf2 activity.

2.3.4 Nuclear Receptors

Several nuclear receptors can enhance or inhibit Nrf2 activity through interactions with Nrf2. PPARγ can directly bind to Nrf2 [53], while Nrf2 and PPARγ also regulate the transcription of each other in the lungs of hyperoxia-induced mice [54, 55]. Estrogen receptor α (ERα) can form a complex with Nrf2 and suppress the Nrf2 pathway in a ligand-dependent manner [56]. Retinoic acid receptor α (RARα) can inhibit Nrf2 binding to ARE [57]. Direct interactions between DNA-binding domains of RARα and the Neh7 domain of NRF2 have been reported recently in acute promyelocytic leukemia, in which RARα fuses with promyelocytic leukemia protein (PML) [58].

2.3.5 p62 and Autophagy

Nrf2 can directly activate gene expression of p62, a selective adaptor that targets ubiquitinated cargos to the autophagosome during autophagy [24, 59]. In addition, phosphorylated p62 can interact with the Kelch repeats domain of Keap1 and compete with the Keap1–Nrf2 interaction, resulting in stabilization of Nrf2 and activation of antioxidant genes [24, 60, 61]. Inhibition of autophagy by arsenic can induce accumulation of autophagosomes and p62, resulting in prolonged activation of Nrf2 [62]. On the other hand, since p62 is an autophagy target, induction of autophagy can enhance the degradation of p62-Keap1 complexes, thereby activating the Nrf2 pathway [63].

2.3.6 Kinases and Nrf2 Phosphorylation

In response to specific compounds, Nrf2-ARE activity can be controlled by protein kinases such as PKC, Akt, ERK, and p38 MAPK in specific cell types, including pulmonary epithelial cells [64]. Nrf2 can be directly phosphorylated by PKC and MAP kinases [65, 66]. How this phosphorylation controls Nrf2 function remains to be elucidated. ERK/MAPK is required for the oxidative-induced NRF2 nuclear localization in HepG2 cells [67]. However, another study indicates that phosphorylation of Nrf2 by MAPK at multiple sites only moderately enhances Nrf2 nuclear accumulation [66]. The p38 MAPK inhibits Nrf2-mediated *HO-1* expression in HepG2 and MEF cells [68, 69]. In *C. elegans*, phosphorylation of the Nrf2 homolog SKN-1 by p38 MAPK or by Akt have opposite effect on SKN-1 nuclear accumulation and transcription activity [70, 71]. In *Drosophila*, CncC functions downstream of the Ras/ERK pathway to activate ecdysone-synthetic genes in the prothoracic gland [29, 72]. In addition, the constitutively activated Ras^{V12} modulates CncC binding at specific chromatic loci, indicating that MAPK may directly control the chromatin binding specificity of Nrf2 [29].

2.3.7 Acetylation of Nrf2

Nrf2 can be acetylated by CBP/p300 [48]. Upon oxidative stimuli, acetylation of Nrf2 at Lys-588/591 by CBP/p300 enhances the binding of Nrf2 to ARE promoters, supporting CBP/p300 as a coactivator of Nrf2 in oxidant responses [48]. Alternative reading frame (ARF), a tumor suppressor that triggers apoptosis, directly interacts with Neh1/3 domains of NRF2 in a human non-small cell lung carcinoma cell line [73]. It was proposed that the ARF–Nrf2 interaction prevents acetylation of NRF2 by CBP and suppresses Nrf2-mediated transcription [73, 74]. Males absent on the first (MOF), a histone acetyltransferase, can acetylate Nrf2 at Lys-588 and is required for Nrf2-induced transcription [75]. Nrf2 can be de-acetylated by sirtuin 1 (SIRT1) at Lys-588/591, which suppresses Nrf2-dependent transcription [76]. These studies indicate acetylation as a positive regulatory mechanism for Nrf2 activity. Alternatively, since both CBP/p300 and MOF are histone acetyltransferases [75, 77], these proteins may cooperate with Nrf2 indirectly through the epigenetic regulation of chromatin structure.

2.3.8 Epigenetic Machinery

Increasing lines of evidences reveal the interactions of Nrf2 with epigenetic modifiers. Brahma-related gene 1 (Brg1), the ATPase of SWI/SNF complex, forms a complex with Nrf2 selectively at the *HO-1* promoter and activates *HO-1* transcription through Brg1-mediated Z-DNA formation [78, 79]. Chromodomain helicase DNA-binding protein-6 (CHD6) interacts with the Neh3 domain of Nrf2 and facilitates Nrf2-induced *NQO1* expression [80]. Silencing mediator for retinoid and thyroid hormone receptor (SMRT), a transcriptional repressor that mediates histone deacetylation, can interact with Nrf2 Neh4/5 domains and inhibit Nrf2-induced *GSTA2* expression [81]. Notably, a recent study in *Drosophila* showed that depletion of CncC or dKeap1 suppresses pericentric silencing and decreases the level of heterochromatin marker H3K9me2, providing direct evidence in support of the epigenetic role of Keap1/Nrf2 family proteins in chromatin remodeling [82].

2.3.9 Other Nrf2-Interacting Proteins

BTB domain and CNC homolog 1 (Bach1) is a CNC family transcription factor that is essential in controlling heme level through the inhibition of *HO-1* gene. Bach1 can dimerize with sMaf and compete with the Nrf2–sMaf interaction at ARE sites and inhibit the activation of *HO-1* [83]. NRF2-induced *HO-1* expression requires inactivation of BACH1 [84]. BACH1 can promote invasion and metastasis by activating metastatic genes [85, 86]. Recent studies showed that Nrf2 activation can inhibit the F-box Protein 22 (Fbxo22)-induced Bach1 degradation, revealing a novel mechanism whereby the Nrf2-Bach1 pathway promotes carcinogenesis [87, 88].

The p21 tumor suppressor can interact with the DLG and ETGE motifs of Nrf2, compete with the Keap1–Nrf2 interaction and stabilize Nrf2 [89]. This discovery adds one more mechanism to the multiple functions of the p53-p21 pathway in cytoprotection and cell survival, indicating a potential role of the Nrf2-p21 network in oncogenesis [90].

DJ-1/PARK7 is a protein that plays multiple antioxidant and cytoprotective functions and is associated with cancer and neurodegeneration. DJ-1 can stabilize Nrf2 by inhibiting the Keap1–Nrf2 interaction and proteasomal degradation of Nrf2 [91]. High levels of both DJ-1 and NRF2 were seen in a large set of lung carcinomas [92].

Phosphoglycerate mutase 5 (PGAM5) is a mitochondrion-binding protein whose ESGE motif can interact with the Kelch domain of Keap1 [93]. It was proposed that a Keap1 dimer can simultaneously interact with one Nrf2 and one PGAM5 molecule. This PGAM5-Keap1-Nrf2 complex can be anchored to mitochondrial surface and likely serve as an antioxidant mechanism specifically targeting ROS leaked from mitochondria [94].

3 The Roles of Nrf2 and Nrf2-Interacting Proteins in Respiratory Diseases

3.1 ALI/ARDS

Acute lung injury (ALI) is a syndrome manifesting as lung edema, inflammation, and alveolar hemorrhaging. If not treated, ALI can progress into its severe form, acute respiratory distress syndrome (ARDS). ALI can be induced by aspiratory injuries caused by various stimuli. For example, mechanical ventilation (MV) along with hyperoxia is used as a therapy for patients with respiratory dysfunction, but can induce lung inflammation and ventilator-induced lung injury (VILI), which directly causes or exacerbates ALI. ROS play a central role in the pathogenesis of lung injury and ALI/ARDS [95]. Protective roles of Nrf2-ARE-activated genes against respiratory injury have been revealed in mouse models with induced lung injury. For examples, overexpression of SOD2 in airways of mice can reduce the hyperoxia-induced lung injury and inflammation [17, 96]. In support of a protective role of Nrf2 in the lungs, enhanced lung injuries and inflammatory responses to MV were found in *Nrf2*$^{-/-}$ mice compared with wild-type mice, and these responses can be restored by supplementing *Nrf2*$^{-/-}$ mice with antioxidant N-acetylcysteine (NAC) [97]. Sulforaphane (SFN), an activator of Nrf2, can reduce pulmonary injury in wild-type mice but not in *Nrf2*$^{-/-}$ mice [98]. CDDO-imidazolide (CDDO-Im), another Nrf2 activator, protects the lungs of mice against CS-induced oxidative stress and alveolar damage through the Nrf2 pathway [99]. These studies support the use of Nrf2-activating antioxidant therapeutics as a way to attenuate pulmonary inflammation and damage in ALI/ARDS patients.

Cooperative roles of Nrf2 with several interacting partners, including ATF3, PPARγ, Brg1, p62, and Akt, in lung inflammation and injury have been revealed. ATF3-deficient mice have enhanced susceptibility to ALI and VILI, which is associated with the recruitment of inflammatory cells and loss of junctions among resident cells [52, 100]. It was proposed that ATF3 can reduce lung injury through inhibiting Nrf2 degradation via Keap1 and DJ-1 pathways [52]. The protective role of Nrf2 in hypoxia-induced ALI can also be mediated by PPARγ, which can be transcriptionally induced by Nrf2 and activate several anti-inflammatory and antioxidant genes [55]. In a hepatic ischemia/reperfusion (HIR)-induced ALI mouse model, overexpression of Brg1 increases Nrf2 activity and reduces ROS and inflammatory factors in lung tissues [101]. Autophagy plays both a positive and negative role in ALI, and this dual role is suggested to be mediated by the Keap1-Nrf2-p62 pathway [102]. The protective role of the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway against lipopolysaccharide (LPS)-induced lung inflammation and injury was revealed in mice treated with desoxyrhapontigenin [103].

3.2 Asthma

Asthma is a lung disorder that is characterized by the swelling of airways, which causes breathing difficulty, coughing, wheezing, and chest tightness. The swelling of the airways can be initiated or exacerbated by environmental allergens (pollen, mold, etc.) and irritants (smoke, dust, gas, etc.). In a mouse model, challenging by the allergen ovalbumin increases inflammation and asthma symptoms by inducing the expression of proinflammatory factors and increasing mucus secretion and airway hyperresponsiveness. In these allergen-challenged mice, Nrf2 knockout resulted in a worsening of asthma symptoms, represented by a higher number of inflammatory cells in bronchoalveolar lavage (BAL) fluid and an increase in mucus-producing cells in the proximal airways [104]. When being exposed to acetylcholine, a molecule involved in asthma pathogenesis, the lungs of *Nrf2*$^{-/-}$ mice are more prone to asthmatic symptoms than the wild-type mice, represented by the less stretch and the greater resistance of air passage through the airways [104]. In humans, a recent study on childhood bronchial asthma in Egyptian children found that children with lower levels of NRF2 in the serum had more severe asthma [105].

Given the role of Nrf2 in protection against asthma, a number of drugs targeting Nrf2 have been tested in rats and mice and been found to reduce asthma symptoms. For example, polydatin treatment of asthmatic mice showed upregulated Nrf2 signaling and reduced oxidative stress in the lungs as well as attenuation of asthma symptoms [106]. Treatment of asthmatic rats with methanolic extract from Artemisia pallens increased Nrf2 levels in the lungs and reduced oxidative stress and inflammation [107]. Taken together, these studies suggest that Nrf2 plays an important role in protecting the lungs against asthma and can be a target for asthma treatment.

3.3 COPD

Chronic obstructive pulmonary disease (COPD) is usually caused by exposure to cigarette smoke (CS) or occupational exposure to various chemicals and smoke, and characterized by airway inflammation and emphysema. Emphysema is mainly caused by damage to alveolar walls and enlargement of air sacs, making it difficult to exhale and inhale. Several studies in mice and humans have indicated that Nrf2 appears to play a protective role against emphysema, and the loss of Nrf2 activity correlates with the progression of COPD. Emphysema can be induced in mice by elastase, which is an enzyme that breaks down elastin, a protein that is vital to the elasticity of the lungs. Although both wild-type and *Nrf2*$^{-/-}$ mice have alveolar wall damage and enlarged airspaces upon elastase treatment, these symptoms are exacerbated in the *Nrf2*$^{-/-}$ mice [108]. Elastase-treated mice had a significant increase in expression of Nrf2-regulated antioxidant genes in the alveolar macrophages. Transplantation of wild-type bone marrow to *Nrf2*$^{-/-}$ mice significantly reduced emphysema symptoms, indicating that the Nrf2-induced antioxidant pathway in alveolar macrophages plays a protective role against lung inflammation and emphysema [108]. In *Drosophila,* drug-induced increase in Nrf2/CncC activity partially rescued the flies that had been chronically exposed to CS [109]. Fisetin, a plant flavonoid compound, has been suggested as a potential therapeutic to COPD since it can activate the Nrf2 pathway and reduced the oxidative stress, inflammation, and lung damage caused by CS exposure to rats [110]. In human studies, it has been found that initial exposure to CS results in activation of NRF2 in alveolar macrophages. However, in older smokers with COPD, NRF2 expression was reduced in alveolar macrophages [111]. Genetic polymorphisms in NRF2-regulatory genes or NRF2-target genes have also been found to contribute to COPD [112]. These studies demonstrate the importance of Nrf2 in protecting the lungs against oxidative stress and damage from CS while suggesting Nrf2 as a potential therapeutic target in COPD treatment.

The role of Nrf2 dysfunction in COPD is likely mediated by the loss of DJ-1. In support of this model, DJ-1 overexpression activates Nrf2 and inhibits apoptosis of alveolar type II cells that are treated with CS extract, suggesting the protective role of the DJ-1-Nrf2 pathway against the CS-induced oxidative stress and inflammatory response [113]. Crosstalk of Nrf2 and NF-κB in inflammation and COPD have been revealed by many studies [35]. In particular, a number of nature products or pharmacological compounds that can simultaneously activate the Nrf2 pathway and inhibit the NF-κB pathway have shown therapeutic potency against respiratory inflammation and COPD [114–119].

3.4 Lung Fibrosis/IPF

Lung fibrosis is generally caused by the proliferation of fibroblasts from profibroblasts and the subsequent accumulation of extracellular matrix proteins. Idiopathic pulmonary fibrosis (IPF) is a disease that is characterized by progressive scarring

of the lung tissue around the alveoli. This results in thickening and stiffness of the alveolar walls and makes it difficult for oxygen to penetrate into the bloodstream, leading to symptoms that include dry coughing, breathing difficulty, and finger clubbing. Although the causes of IPF remain to be fully understood, oxidative stresses that are induced by stimuli such as CS are thought to be a contributing factor [120]. IPF patients have higher than normal levels of ROS in the BAL fluid and lower than normal levels of antioxidant enzymes (e.g., SOD) and antioxidants in the lungs. Bleomycin-induced pulmonary injury, inflammation, and fibrosis in mice has been used as a model to study lung fibrosis. *Nrf2*$^{-/-}$ mice have significantly higher sensitivity to the bleomycin-induced fibrogenesis than wild-type mice [121], suggesting that Nrf2 plays a protective role against pulmonary fibrosis.

It is well-known that the herbicide paraquat (PQ) can cause pulmonary fibrosis in animals and human [124]. Rapamycin is known to protect against PQ-induced lung injury and has been suggested as a potential treatment for pulmonary fibrosis [122]. Treatment of rats or mice with rapamycin led to an increase in Nrf2 levels and a decrease of PQ-induced ROS and fibrosis-related factors, and the effect of rapamycin can be reversed by an Nrf2 knockdown [123, 124]. In several in vitro assays, the PQ-induced fibroblast-to-myofibroblast transition (FMT) or epithelial-mesenchymal transition (EMT), both important cellular processes in fibrosis, were inhibited by treatment with rapamycin or Nrf2 activator [123, 124]. Rosavin, emodin, and pirfenidone are other drugs that have recently been proposed as treatments for pulmonary fibrosis [125–127]. Treatment of mice or rats with these compounds increases the expression and/or activity of Nrf2 and reduces the bleomycin-induced lung fibrosis. It is also noticed that these drugs can simultaneously inhibit NF-κB or Bach1, suggesting that the unbalanced Nrf2/NF-κB or Nrf2/Bach1 equilibrium may contribute to the development of IPF.

3.5 Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent and ATP-gated chloride channel that regulates epithelial surface fluid secretion in respiratory and gastrointestinal tracts. In the lung of a CF patient, the mucus blocks the airways and traps bacteria, leading to infections, inflammation, and respiratory dysfunctions. CF is commonly associated with the F508del mutation of CFTR. It was found that CFTR interacts with Nrf2 in human bronchial epithelial (hBE) cells, and that CFTR–NRF2 interaction decreases in CF hBE cells [49]. VX809 and VX661, the approved correctors for CFTR-F508del, restores Nrf2 phosphorylation and its interaction with CBP [49]. Excessive inflammation triggered by the activation of NF-κB by oxidative stress is highly related with the morbidity and mortality of CF [128]. Roles of CBP-Nrf2-NF-κB network in CF have been revealed in airway epithelial cells both in vitro and in vivo. Dramatic decrease

of Nrf2 activity is detected in CF cell lines as well as lungs and excised nasal epithelia of CF mice [129]. The defective Nrf2 in CF cells is associated with cAMP signaling, and inhibition of cAMP signaling can interfere in CBP interactions with Nrf2 and NF-κB, resulting in enhanced Nrf2 activity and reduced NF-κB activity in CF cells [130]. Combined, these studies implicate that reducing inflammation in CF by strengthening CBP–Nrf2 or weakening CBP–NF-κB interactions might be potential therapies for CF.

3.6 Respiratory Infections

There are many different viral and bacterial infections that can occur in airways. The most common pathogens are viruses, including respiratory syncytial virus (RSV), influenza A virus (IAV), adenovirus, coronavirus, etc. The less common but very present bacterial pathogens include *Streptococcus pneumoniae, Haemophilus influenzae,* and *Mycobacterium tuberculosis* (TB). Both viral and bacterial infections occur in the upper and lower respiratory tract as well as the esophagus. Infection-induced symptoms such as coughing and mucus build up can cause damage to the epithelial tissue and create ROS.

RSV targets the epithelial airway tissues and has been found to activate deacetylation and proteasomal degradation of Nrf2 [131]. *Nrf2*$^{-/-}$ mice that are infected with RSV show more severe lung inflammation and injury as well as attenuated viral clearance than wild-type mice [132]. IAV mainly targets the epithelial tissue and specifically alveolar type I and II epithelial cells. An in vitro study shows that infection of these cells by IAV increases NRF2/HO-1 level. NRF2 knockdown causes these cells to be more sensitive to IAV-induced injury, while overexpression of NRF2 shows a protective effect against IAV [133]. It was found that a NRF2 knockdown is correlated with an increase in viral entry and replication while SFN-induced activation of NRF2 in human nasal epithelial cells significantly decreases IAV entry and replication, directly supporting a protective role of Nrf2 against IAV infection [134]. The effectiveness of entry and replication of a virus can be altered with the cleavage of viral HA surface proteins, which allows the virus to enter the targeted host cell. It has been shown that an increase in NRF2 expression correlates with a lowered expression of the viral HA gene [134]. Several Nrf2 activators have shown protective effect against RSV and IAV. For example, SFN or emodin treatment significantly reduces virus-induced lung inflammation and viral replication [132, 135].

Streptococcus pneumoniae is the most common cause of pneumonia and sepsis. Nrf2 knockout has an increase of proinflammatory mediators and neutrophils present in lung digests from mice treated with *Streptococcus* [136]. Activation of NRF2 by resveratrol significantly reduced oxidative stress in human lung epithelial cells infected by *Streptococcus* [137]. Another common bacterium that infects the lungs is *Tuberculosis* (TB). The Nrf2 activator NAC has been shown

to decrease the bacteria counts and lung injury in guinea pig model infected by TB, suggesting that NAC could be used as an optional aid along with treatment [138].

3.7 *Lung Cancers*

The double-sided sword roles of Nrf2 in carcinogenesis has been revealed in many studies and discussed in several reviews [10, 139]. On the one hand, Nrf2-deficient mice treated with different carcinogens show increased severities of inflammation-associated carcinogenesis such as the AOM/DSS-induced colonic tumor [140]. On the other hand, enhanced levels of NRF2 or mutations that are predicted to activate NRF2 are found in many human cancers especially lung cancers [10, 141–144]. In support of the promotive role of Nrf2 in lung cancer, targeted deletion of Nrf2 reduces urethane-induced lung tumorigenesis in mice [145]. NRF2 depletion increases chemosensitivity of human lung cancer A549 cells both in vitro and in vivo [146]. Lung tumorigenesis induced by constitutively active K-Ras^{G12D} is suppressed in Nrf2-deficient mice [147]. It is proposed that Nrf2 facilitates cancer cell resistance to chemotherapeutic drugs and radiation through the activation of the antioxidant pathway. Enhanced NRF2-HO-1 pathway is increasingly seen in lung adenocarcinoma [148]. In addition, Nrf2 can promote cancer cell proliferation through activating proliferating and metabolic genes [139]. In human lung cancer cell lines, NRF2 activates transcriptions of anti-apoptotic factor Bcl-2 and glucose metabolic enzymes [25, 26], which to some extent accounts for the role of NRF2 in promoting lung cancers.

Recent studies have revealed that the Nrf2-interacting networks also contribute to the oncogenic role of Nrf2 in lung cancers. For example, single nucleotide polymorphisms (SNPs) in MAF-G have been found to be associated with lung cancers or airway transcription response to CS [149]. Enhanced levels of both MOF and NRF2-target transcriptions were revealed in human non-small cell lung cancer [75]. Co-upregulation of both DJ-1 and NRF2 were observed in a large set of lung carcinomas [92]. Depletion of KEAP1 in human non-small cell lung carcinomas cell lines increases their sensitivity to chemotherapeutic agents, which is likely mediated by enhanced PPARγ levels and its activation of differentiation genes [150]. Novel mechanisms of the Nrf2-Bach1 pathway in lung cancer metastasis has been recently identified [87, 88]. In normal cells, free heme inhibit Bach1 activity through Fbxo22-mediated ubiquitination. In cells with enhanced Nrf2, activation of HO-1 decreases the cellular level of heme thus stabilizing Bach1. Enhanced Bach1 promotes lung cancer metastasis through the activation of metastatic genes and glycolysis genes. Another protein that cooperates with Nrf2 in the promotion of lung cancer is ATF4. Nrf2-ATF4 dimer can directly target and activate *HO-1* gene [50]. Cooperative activations of *HO-1* by Nrf2 and ATF4 promotes fibrosarcoma lung colonization [151]. Nrf2 and ATF4 can also co-activate biosynthetic enzyme genes involved in serine/glycine metabolism, which plays a promotive role in non-small cell lung cancer [152].

4 Lung Disease Therapies Targeting Nrf2-Interacting Network

Given the protective role of Nrf2 in airway inflammation, injury and pathogenesis, designing drugs that target Nrf2 and its essential regulator Keap1 is valuable for lung disease therapies. Many nature products and pharmaceutical compounds have been identified as activators or inhibitors of Nrf2 and Keap1 [153]. Protective roles of these drugs against lung inflammation, injuries, and diseases have been verified in mouse models, and some drugs are in clinical trials. For example, SFN (an isothiocyanate isolated from cruciferous plants) treatment enhances bacteria removal and phagocytosis in the alveolar macrophages from both mice and COPD patients, supporting the therapeutic effect of SFN in treating COPD [154, 155]. A drug screen in human lung fibroblasts identified a group of synthetic small molecules (HPP-4382) that induced Nrf2-dependent HO-1 expression [156]. Among these compounds, HPP971 has completed phase 1 studies and shown potential therapeutic benefits in diseases, including respiratory injuries (vTv Therapeutics).

The roles of Nrf2 in respiratory inflammation and diseases can be mediated by many other proteins that interact with Nrf2 (Fig. 1). Therefore, based on knowledge of the molecular functions of the Nrf2 network in pathogeneses, drugs targeting the Nrf2-interacting networks can increase the efficiency and selectivity of therapies. Scientists are screening for compounds that directly target Nrf2-associated protein complexes especially the Keap1-Nrf2 complex. In addition, compounds that simultaneously target Nrf2 and its interacting partners have shown therapeutic potency for lung diseases.

4.1 Compounds Targeting Nrf2–Keap1 Interaction

Several groups have successfully identified compounds that directly interfere with the Nrf2–Keap1 interaction. These include ML334 by Hu and colleagues and monoacidic compounds by GlaxoSmithKline Pharmaceuticals [157, 158]. These compounds have been shown to induce the expression of NRF2-regulated genes in bronchial epithelial cells derived from COPD patients and reverse ozone-induced lung inflammation in rats [158].

4.2 Compounds Co-Targeting Nrf2 and NF-κB

NF-κB is an essential factor and therapy target for airway inflammation and chronic lung diseases [159]. Natural products that have both antioxidant and anti-inflammatory effects were seen in recent studies as potential therapies for

inflammation-related lung injuries and diseases. For example, tyrosol (a natural phenolic antioxidant) treatment of LPS-induced ALI mice significantly inhibited NF-κB and AP-1 and reduced inflammatory cytokines, while at the same time activating Nrf2 and HO-1 [160]. Oridonin (isolated from *Rabdosia rubescens*) and bardoxolone (a synthetic triterpenoid based on oleanolic acid) inhibit the NF-κB pathway and activate the Nrf2 pathway in LPS-induced ALI mice [161, 162], supporting the antioxidant and anti-inflammatory effects of these compounds for ALI protection and therapy. Investigations of several compounds targeting the Nrf2-NF-κB network in both mouse and rat models have shown their potential therapeutic effects against CS-induced inflammation, emphysema, and COPD. These include natural products such as forsythiaside (a hydroxycinnamic acid from *Forsythia suspensa*) [114], eucalyptol (a monoterpenoid oil isolated from *Eucalyptus*) [115], platycodin D (a saponin from the root of *Platycodon grandiflorus*) [116], and isoliquiritigenin (a flavonoid from *Glycyrrhizae* species) [117], as well as pharmacological compounds such as Sul-121 [118] and 15d-PGJ2 [119]. All these drugs can reduce respiratory inflammation and CS-induced emphysema through both the activation of the Nrf2 pathway and the down-regulation of the NF-κB pathway. In addition, rosavin (found in *Rhodiola rosea*) and emodin (isolated from rhubarb *Rheum palmatum*), nature products that activate Nrf2 and inhibit NF-κB, have recently been proposed as treatments for pulmonary fibrosis [125, 126]. Dimethyl fumarate (DMF) was approved by the FDA for the treatment of multiple sclerosis. It was found that DMF can both activate Nrf2 and inhibit the NF-κB pathway and prevent the development of bleomycin-induced lung fibrosis in mice [163]. Notably, DMF can control Nrf2 activity in a dose-dependent manner. In several cancer cells, low concentrations of DMF activate Nrf2, while high concentrations of DMF reduce the nuclear Nrf2 level, likely through the down-regulation of the Nrf2 stabilizer DJ-1 [164].

4.3 Compounds Targeting Nrf2 Phosphorylation Pathways

Desoxyrhapontigenin (isolated from rhubarb plants), a compound that shows significant anti-inflammatory and antioxidant effects, can inhibit MAPK, Akt, and NF-κB and activate Nrf2 [103, 165]. It was found that desoxyrhapontigenin pretreatment reduces LPS-induced lung inflammation and injury in mice through the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway [103]. Metformin reduces the risk of lung cancer during anti-diabetic treatment [166]. A recent study showed that metformin suppresses proliferation and induces apoptosis of the human lung carcinoma A549 cells through the inhibition of the Akt and ERK1/2 signaling pathways and activation of Nrf2 [167]. These studies indicate the potency of Nrf2 phosphorylation pathways as therapeutic targets for lung diseases.

4.4 Other Compounds Targeting Nrf2 Network

Interacting with the Keap1-Nrf2 pathway, autophagy can play both positive and negative roles in chronic lung diseases, CS exposure, and ALI [102]. It was found that vitamin D can protect against PM-induced lung injury in mice through the degradation of p62 and Keap1 and the activation of the Nrf2 pathway [168]. Pirfenidone (PFD), an approved drug for IPF treatment, can suppress bleomycin-induced lung fibrosis through both the activation of the Nrf2 pathway and the inhibition of Bach1 [127, 169].

5 Conclusions

As a master antioxidant factor, the protective roles of Nrf2 against respiratory inflammation and pathogenesis, as well as the brilliant potency of Nrf2-targeting therapies for lung diseases, have been verified and supported by numerous studies. However, with the revealing of more and more Nrf2-interacting proteins/pathways and Nrf2-targeting genes/functions, the complexity of the Nrf2 network in physiology and pathology needs to be considered when designing therapies targeting Nrf2. On the bright side, selectively targeting Nrf2-related protein interactions or regulatory networks could increase the efficiency and specificity of therapies. On the negative side, off-target effects that are related to the multiple interacting partners and biological functions of Nrf2 need to be considered when modifying Nrf2 level or activity in different types of cells. Many recent studies have focused on the potential application of natural antioxidant-rich foods that have limited side effects. Although the protective effects of many of the natural products against lung inflammation and pathogenesis have been verified in animal models, their therapeutic efficiency for lung diseases as well as long-term biological effects in humans remain to be evaluated.

Given that activation of the Nrf2 pathway is a strategy for treating most of the respiratory diseases, we should be mindful of the tumorigenic risk of Nrf2 activators. Chemoprevention effects of some Nrf2 inducers, such as SFN and CDDO-Im, have been studied in both animal models and clinical trials [170, 171]. It is necessary to determine the safe thresholds of Nrf2 level in specific cells as well as the safe doses of Nrf2 activators that will not induce oncogenesis. It would also be useful to design therapies that differentially target the oncogenic role and protective role of Nrf2, which may be achieved by targeting specific Nrf2-related protein interactions in the future. Doubtlessly, revealing the complete range of molecular mechanisms and biological functions of the Nrf2 network will contribute to the development of Nrf2-targeting therapies.

Competing Interest Statement The authors declare that they have no competing interest.

References

1. Mortality, G. B. D. & Causes of Death, C. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the global burden of disease study 2015. Lancet. 2016;388:1459–544. https://doi.org/10.1016/S0140-6736(16)31012-1.
2. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol. 2007;47:89–116. https://doi.org/10.1146/annurev.pharmtox.46.120604.141046.
3. Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab Rev. 2006;38:769–89. https://doi.org/10.1080/03602530600971974.
4. Malhotra D, et al. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Res. 2010;38:5718–34. https://doi.org/10.1093/nar/gkq212.
5. Sykiotis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. Sci Signal. 2010;3:re3. https://doi.org/10.1126/scisignal.3112re3.
6. Kobayashi A, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004;24:7130–9. https://doi.org/10.1128/MCB.24.16.7130-7139.2004.
7. Eggler AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD. Modifying specific cysteine's of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc Natl Acad Sci U S A. 2005;102:10070–5. https://doi.org/10.1073/pnas.0502402102.
8. Kobayashi M, et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. Mol Cell Biol. 2009;29:493–502. https://doi.org/10.1128/MCB.01080-08.
9. Itoh K, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13:76–86. https://doi.org/10.1101/gad.13.1.76.
10. Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells. 2011;16:123–40. https://doi.org/10.1111/j.1365-2443.2010.01473.x.
11. Sun Z, et al. KPNA6 (Importin {alpha}7)-mediated nuclear import of Keap1 represses the Nrf2-dependent antioxidant response. Mol Cell Biol. 2011;31:1800–11. https://doi.org/10.1128/MCB.05036-11.
12. Wakabayashi N, et al. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat Genet. 2003;35:238–45. https://doi.org/10.1038/ng1248.
13. Thimmulappa RK, et al. Identification of Nrf2-regulated genes induced by the chemo preventive agent sulforaphane by oligonucleotide microarray. Cancer Res. 2002;62:5196–203.
14. Kwak MK, et al. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. J Biol Chem. 2003;278:8135–45. https://doi.org/10.1074/jbc.M211898200.
15. Hu R, et al. Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray. Life Sci. 2006;79:1944–55. https://doi.org/10.1016/j.lfs.2006.06.019.
16. Asikainen TM, et al. Increased sensitivity of homozygous Sod2 mutant mice to oxygen toxicity. Free Radic Biol Med. 2002;32:175–86. https://doi.org/10.1016/s0891-5849(01)00776-6.
17. Folz RJ, Abushamaa AM, Suliman HB. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. J Clin Invest. 1999;103:1055–66. https://doi.org/10.1172/JCI3816.
18. Pi J, et al. Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity. J Biol Chem. 2010;285:9292–300. https://doi.org/10.1074/jbc.M109.093955.

19. Chorley BN, et al. Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. Nucleic Acids Res. 2012;40:7416–29. https://doi.org/10.1093/nar/gks409.
20. Huang J, Tabbi-Anneni I, Gunda V, Wang L. Transcription factor Nrf2 regulates SHP and lipogenic gene expression in hepatic lipid metabolism. Am J Physiol Gastrointest Liver Physiol. 2010;299:G1211–21. https://doi.org/10.1152/ajpgi.00322.2010.
21. Kitteringham NR, et al. Proteomic analysis of Nrf2 deficient transgenic mice reveals cellular defence and lipid metabolism as primary Nrf2-dependent pathways in the liver. J Proteome. 2010;73:1612–31. https://doi.org/10.1016/j.jprot.2010.03.018.
22. Bobilev I, et al. The Nrf2 transcription factor is a positive regulator of myeloid differentiation of acute myeloid leukemia cells. Cancer Biol Ther. 2011;11:317–29. https://doi.org/10.4161/cbt.11.3.14098.
23. Khacho M, et al. Mitochondrial dynamics impacts stem cell identity and fate decisions by regulating a nuclear transcriptional program. Cell Stem Cell. 2016;19:232–47. https://doi.org/10.1016/j.stem.2016.04.015.
24. Jain A, et al. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. J Biol Chem. 2010;285:22576–91. https://doi.org/10.1074/jbc.M110.118976.
25. Niture SK, Jaiswal AK. Nrf2 protein up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis. J Biol Chem. 2012;287:9873–86. https://doi.org/10.1074/jbc.M111.312694.
26. Mitsuishi Y, et al. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell. 2012;22:66–79. https://doi.org/10.1016/j.ccr.2012.05.016.
27. Kobayashi EH, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624. https://doi.org/10.1038/ncomms11624.
28. Sykiotis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev Cell. 2008;14:76–85. https://doi.org/10.1016/j.devcel.2007.12.002.
29. Deng H, Kerppola TK. Regulation of Drosophila metamorphosis by xenobiotic response regulators. PLoS Genet. 2013;9:e1003263. https://doi.org/10.1371/journal.pgen.1003263.
30. Hochmuth CE, Biteau B, Bohmann D, Jasper H. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. Cell Stem Cell. 2011;8:188–99. https://doi.org/10.1016/j.stem.2010.12.006.
31. Slocum SL, Kensler TW. Nrf2: control of sensitivity to carcinogens. Arch Toxicol. 2011;85:273–84. https://doi.org/10.1007/s00204-011-0675-4.
32. Deng H. Multiple roles of Nrf2-Keap1 signaling: regulation of development and xenobiotic response using distinct mechanisms. Fly (Austin). 2014;8:7–12. https://doi.org/10.4161/fly.27007.
33. Deng H, Kerppola TK. Visualization of the Drosophila dKeap1-CncC interaction on chromatin illumines cooperative, xenobiotic-specific gene activation. Development. 2014;141:3277–88. https://doi.org/10.1242/dev.110528.
34. Rahman I, Adcock IM. Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J. 2006;28:219–42. https://doi.org/10.1183/09031936.06.00053805.
35. Schuliga M. NF-kappaB signaling in chronic inflammatory airway disease. Biomol Ther. 2015;5:1266–83. https://doi.org/10.3390/biom5031266.
36. Birnhuber A, Biasin V, Schnoegl D, Marsh LM, Kwapiszewska G. Transcription factor Fra-2 and its emerging role in matrix deposition, proliferation and inflammation in chronic lung diseases. Cell Signal. 2019;64:109408. https://doi.org/10.1016/j.cellsig.2019.109408.
37. Cattani-Cavalieri I, et al. Acute exposure to diesel-biodiesel particulate matter promotes murine lung oxidative stress by Nrf2/HO-1 and inflammation through the NF-kB/TNF-alpha pathways. Inflammation. 2019;42:526–37. https://doi.org/10.1007/s10753-018-0910-8.
38. Leclercq B, et al. Air pollution-derived PM2.5 impairs mitochondrial function in healthy and chronic obstructive pulmonary diseased human bronchial epithelial cells. Environ Pollut. 2018;243:1434–49. https://doi.org/10.1016/j.envpol.2018.09.062.

39. Liu GH, Qu J, Shen X. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. Biochim Biophys Acta. 2008;1783:713–27. https://doi.org/10.1016/j.bbamcr.2008.01.002.
40. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A. 1996;93:14960–5. https://doi.org/10.1073/pnas.93.25.14960.
41. Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. Oncogene. 1998;17:3145–56. https://doi.org/10.1038/sj.onc.1202237.
42. Katsuoka F, Yamamoto M. Small Maf proteins (MafF, MafG, MafK): history, structure and function. Gene. 2016;586:197–205. https://doi.org/10.1016/j.gene.2016.03.058.
43. Katsuoka F, et al. Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. Mol Cell Biol. 2005;25:8044–51. https://doi.org/10.1128/MCB.25.18.8044-8051.2005.
44. Motohashi H, Katsuoka F, Engel JD, Yamamoto M. Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1-Nrf2 regulatory pathway. Proc Natl Acad Sci U S A. 2004;101:6379–84. https://doi.org/10.1073/pnas.0305902101.
45. Hirotsu Y, et al. Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res. 2012;40:10228–39. https://doi.org/10.1093/nar/gks827.
46. Sekine H, et al. The mediator subunit MED16 transduces NRF2-activating signals into antioxidant gene expression. Mol Cell Biol. 2016;36:407–20. https://doi.org/10.1128/MCB.00785-15.
47. Katoh Y, et al. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes Cells. 2001;6:857–68.
48. Sun Z, Chin YE, Zhang DD. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol Cell Biol. 2009;29:2658–72. https://doi.org/10.1128/MCB.01639-08.
49. Borcherding DC, et al. Clinically-approved CFTR modulators rescue Nrf2 dysfunction in cystic fibrosis airway epithelia. J Clin Invest. 2019;129:3448–63. https://doi.org/10.1172/JCI96273.
50. He CH, et al. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. J Biol Chem. 2001;276:20858–65. https://doi.org/10.1074/jbc.M101198200.
51. Brown SL, Sekhar KR, Rachakonda G, Sasi S, Freeman ML. Activating transcription factor 3 is a novel repressor of the nuclear factor erythroid-derived 2-related factor 2 (Nrf2)-regulated stress pathway. Cancer Res. 2008;68:364–8. https://doi.org/10.1158/0008-5472.CAN-07-2170.
52. Shan Y, et al. ATF3 protects pulmonary resident cells from acute and ventilator-induced lung injury by preventing Nrf2 degradation. Antioxid Redox Signal. 2015;22:651–68. https://doi.org/10.1089/ars.2014.5987.
53. Ikeda Y, et al. Suppression of rat thromboxane synthase gene transcription by peroxisome proliferator-activated receptor gamma in macrophages via an interaction with NRF2. J Biol Chem. 2000;275:33142–50. https://doi.org/10.1074/jbc.M002319200.
54. Cho HY, Reddy SP, Debiase A, Yamamoto M, Kleeberger SR. Gene expression profiling of NRF2-mediated protection against oxidative injury. Free Radic Biol Med. 2005;38:325–43. https://doi.org/10.1016/j.freeradbiomed.2004.10.013.
55. Cho HY, et al. Nrf2-regulated PPAR{gamma} expression is critical to protection against acute lung injury in mice. Am J Respir Crit Care Med. 2010;182:170–82. https://doi.org/10.1164/rccm.200907-1047OC.
56. Ansell PJ, et al. Repression of cancer protective genes by 17beta-estradiol: ligand-dependent interaction between human Nrf2 and estrogen receptor alpha. Mol Cell Endocrinol. 2005;243:27–34. https://doi.org/10.1016/j.mce.2005.08.002.

57. Wang XJ, Hayes JD, Henderson CJ, Wolf CR. Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha. Proc Natl Acad Sci U S A. 2007;104:19589–94. https://doi.org/10.1073/pnas.0709483104.
58. Li Y, Wang H, Wang XJ, Tang X. The short isoform of PML-RARalpha activates the NRF2/HO-1 pathway through a direct interaction with NRF2. FEBS Lett. 2017;591:2859–68. https://doi.org/10.1002/1873-3468.12779.
59. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy. 2011;7:279–96. https://doi.org/10.4161/auto.7.3.14487.
60. Komatsu M, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol. 2010;12:213–23. https://doi.org/10.1038/ncb2021.
61. Ichimura Y, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell. 2013;51:618–31. https://doi.org/10.1016/j.molcel.2013.08.003.
62. Lau A, et al. Arsenic inhibits autophagic flux, activating the Nrf2-Keap1 pathway in a p62-dependent manner. Mol Cell Biol. 2013;33:2436–46. https://doi.org/10.1128/MCB.01748-12.
63. Taguchi K, et al. Keap1 degradation by autophagy for the maintenance of redox homeostasis. Proc Natl Acad Sci U S A. 2012;109:13561–6. https://doi.org/10.1073/pnas.1121572109.
64. Xu Y, et al. Pseudomonas aeruginosa pyocyanin activates NRF2-ARE-mediated transcriptional response via the ROS-EGFR-PI3K-AKT/MEK-ERK MAP kinase signaling in pulmonary epithelial cells. PLoS One. 2013;8:e72528. https://doi.org/10.1371/journal.pone.0072528.
65. Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J Biol Chem. 2002;277:42769–74. https://doi.org/10.1074/jbc.M206911200.
66. Sun Z, Huang Z, Zhang DD. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. PLoS One. 2009;4:e6588. https://doi.org/10.1371/journal.pone.0006588.
67. Zipper LM, Mulcahy RT. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. Toxicol Sci. 2003;73:124–34. https://doi.org/10.1093/toxsci/kfg083.
68. Keum YS, et al. Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. Cancer Res. 2006;66:8804–13. https://doi.org/10.1158/0008-5472.CAN-05-3513.
69. Naidu S, Vijayan V, Santoso S, Kietzmann T, Immenschuh S. Inhibition and genetic deficiency of p38 MAPK up-regulates heme oxygenase-1 gene expression via Nrf2. J Immunol. 2009;182:7048–57. https://doi.org/10.4049/jimmunol.0900006.
70. Inoue H, et al. The C. elegans p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. Genes Dev. 2005;19:2278–83. https://doi.org/10.1101/gad.1324805.
71. Tullet JM, et al. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell. 2008;132:1025–38. https://doi.org/10.1016/j.cell.2008.01.030.
72. Rewitz KF, Yamanaka N, Gilbert LI, O'Connor MB. The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. Science. 2009;326:1403–5. https://doi.org/10.1126/science.1176450.
73. Chen D, et al. NRF2 is a major target of ARF in p53-independent tumor suppression. Mol Cell. 2017;68:224–232 e224. https://doi.org/10.1016/j.molcel.2017.09.009.
74. Hayes JD, Dinkova-Kostova AT. Epigenetic control of NRF2-directed cellular antioxidant status in dictating life-death decisions. Mol Cell. 2017;68:5–7. https://doi.org/10.1016/j.molcel.2017.09.023.
75. Chen Z, et al. The histone acetyltranseferase hMOF acetylates Nrf2 and regulates anti-drug responses in human non-small cell lung cancer. Br J Pharmacol. 2014;171:3196–211. https://doi.org/10.1111/bph.12661.

76. Kawai Y, Garduno L, Theodore M, Yang J, Arinze IJ. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. J Biol Chem. 2011;286:7629–40. https://doi.org/10.1074/jbc.M110.208173.
77. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell. 1996;87:953–9. https://doi.org/10.1016/s0092-8674(00)82001-2.
78. Zhang J, et al. BRG1 interacts with Nrf2 to selectively mediate HO-1 induction in response to oxidative stress. Mol Cell Biol. 2006;26:7942–52. https://doi.org/10.1128/MCB.00700-06.
79. Ge M, et al. Brg1-mediated Nrf2/HO-1 pathway activation alleviates hepatic ischemia-reperfusion injury. Cell Death Dis. 2017;8:e2841. https://doi.org/10.1038/cddis.2017.236.
80. Nioi P, Nguyen T, Sherratt PJ, Pickett CB. The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. Mol Cell Biol. 2005;25:10895–906. https://doi.org/10.1128/MCB.25.24.10895-10906.2005.
81. Ki SH, Cho IJ, Choi DW, Kim SG. Glucocorticoid receptor (GR)-associated SMRT binding to C/EBPbeta TAD and Nrf2 Neh4/5: role of SMRT recruited to GR in GSTA2 gene repression. Mol Cell Biol. 2005;25:4150–65. https://doi.org/10.1128/MCB.25.10.4150-4165.2005.
82. Carlson J, Swisse T, Smith C, Deng H. Regulation of position effect variegation at pericentric heterochromatin by Drosophila Keap1-Nrf2 xenobiotic response factors. Genesis. 2019;57:e23290. https://doi.org/10.1002/dvg.23290.
83. Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. J Biol Chem. 2005;280:16891–900. https://doi.org/10.1074/jbc.M500166200.
84. Reichard JF, Motz GT, Puga A. Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. Nucleic Acids Res. 2007;35:7074–86. https://doi.org/10.1093/nar/gkm638.
85. Liang Y, et al. Transcriptional network analysis identifies BACH1 as a master regulator of breast cancer bone metastasis. J Biol Chem. 2012;287:33533–44. https://doi.org/10.1074/jbc.M112.392332.
86. Lee U, et al. A prognostic gene signature for metastasis-free survival of triple negative breast cancer patients. PLoS One. 2013;8:e82125. https://doi.org/10.1371/journal.pone.0082125.
87. Lignitto L, et al. Nrf2 activation promotes lung cancer metastasis by inhibiting the degradation of bach1. Cell. 2019;178:316–329 e318. https://doi.org/10.1016/j.cell.2019.06.003.
88. Wiel C, et al. BACH1 stabilization by antioxidants stimulates lung cancer metastasis. Cell. 2019;178:330–345 e322. https://doi.org/10.1016/j.cell.2019.06.005.
89. Chen W, et al. Direct interaction between Nrf2 and p21(Cip1/WAF1) upregulates the Nrf2-mediated antioxidant response. Mol Cell. 2009;34:663–73. https://doi.org/10.1016/j.molcel.2009.04.029.
90. Chen W, et al. Does Nrf2 contribute to p53-mediated control of cell survival and death? Antioxid Redox Signal. 2012;17:1670–5. https://doi.org/10.1089/ars.2012.4674.
91. Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc Natl Acad Sci U S A. 2006;103:15091–6. https://doi.org/10.1073/pnas.0607260103.
92. Merikallio H, Paakko P, Kinnula VL, Harju T, Soini Y. Nuclear factor erythroid-derived 2-like 2 (Nrf2) and DJ1 are prognostic factors in lung cancer. Hum Pathol. 2012;43:577–84. https://doi.org/10.1016/j.humpath.2011.05.024.
93. Lo SC, Hannink M. PGAM5, a Bcl-XL-interacting protein, is a novel substrate for the redox-regulated Keap1-dependent ubiquitin ligase complex. J Biol Chem. 2006;281:37893–903. https://doi.org/10.1074/jbc.M606539200.
94. Lo SC, Hannink M. PGAM5 tethers a ternary complex containing Keap1 and Nrf2 to mitochondria. Exp Cell Res. 2008;314:1789–803. https://doi.org/10.1016/j.yexcr.2008.02.014.

95. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20:1126–67. https://doi.org/10.1089/ars.2012.5149.
96. Danel C, et al. Gene therapy for oxidant injury-related diseases: adenovirus-mediated transfer of superoxide dismutase and catalase cDNAs protects against hyperoxia but not against ischemia-reperfusion lung injury. Hum Gene Ther. 1998;9:1487–96. https://doi.org/10.1089/hum.1998.9.10-1487.
97. Papaiahgari S, et al. Genetic and pharmacologic evidence links oxidative stress to ventilator-induced lung injury in mice. Am J Respir Crit Care Med. 2007;176:1222–35. https://doi.org/10.1164/rccm.200701-060OC.
98. Cho HY, et al. Sulforaphane enriched transcriptome of lung mitochondrial energy metabolism and provided pulmonary injury protection via Nrf2 in mice. Toxicol Appl Pharmacol. 2019;364:29–44. https://doi.org/10.1016/j.taap.2018.12.004.
99. Sussan TE, et al. Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. Proc Natl Acad Sci U S A. 2009;106:250–5. https://doi.org/10.1073/pnas.0804333106.
100. Akram A, et al. Activating transcription factor 3 confers protection against ventilator-induced lung injury. Am J Respir Crit Care Med. 2010;182:489–500. https://doi.org/10.1164/rccm.200906-0925OC.
101. Ge M, et al. Overexpression of Brg1 alleviates hepatic ischemia/reperfusion-induced acute lung injury through antioxidative stress effects. Oxidative Med Cell Longev. 2017;2017:8787392. https://doi.org/10.1155/2017/8787392.
102. Rojo de la Vega M, et al. Role of Nrf2 and autophagy in acute lung injury. Curr Pharmacol Rep. 2016;2:91–101. https://doi.org/10.1007/s40495-016-0053-2.
103. Joo Choi R, Cheng MS, Shik Kim Y. Desoxyrhapontigenin up-regulates Nrf2-mediated heme oxygenase-1 expression in macrophages and inflammatory lung injury. Redox Biol. 2014;2:504–12. https://doi.org/10.1016/j.redox.2014.02.001.
104. Rangasamy T, et al. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med. 2005;202:47–59. https://doi.org/10.1084/jem.20050538.
105. Youness ER, Shady M, Nassar MS, Mostafa R, Abuelhamd W. The role of serum nuclear factor erythroid 2-related factor 2 in childhood bronchial asthma. J Asthma. 2019:1–6. https://doi.org/10.1080/02770903.2019.1571081.
106. Zeng H, et al. Polydatin attenuates reactive oxygen species-induced airway remodeling by promoting Nrf2-mediated antioxidant signaling in asthma mouse model. Life Sci. 2019;218:25–30. https://doi.org/10.1016/j.lfs.2018.08.013.
107. Mukherjee AA, Kandhare AD, Rojatkar SR, Bodhankar SL. Ameliorative effects of Artemisia pallens in a murine model of ovalbumin-induced allergic asthma via modulation of biochemical perturbations. Biomed Pharmacother. 2017;94:880–9. https://doi.org/10.1016/j.biopha.2017.08.017.
108. Ishii Y, et al. Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. J Immunol. 2005;175:6968–75. https://doi.org/10.4049/jimmunol.175.10.6968.
109. Prange R, et al. A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention. Aging (Albany NY). 2018;10:2122–35. https://doi.org/10.18632/aging.101536.
110. Hussain T, et al. The plant flavonoid, fisetin alleviates cigarette smoke-induced oxidative stress, and inflammation in Wistar rat lungs. J Food Biochem. 2019;43:e12962. https://doi.org/10.1111/jfbc.12962.
111. Suzuki M, et al. Down-regulated NF-E2-related factor 2 in pulmonary macrophages of aged smokers and patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2008;39:673–82. https://doi.org/10.1165/rcmb.2007-0424OC.
112. Korytina GF, et al. Associations of the NRF2/KEAP1 pathway and antioxidant defense gene polymorphisms with chronic obstructive pulmonary disease. Gene. 2019;692:102–12. https://doi.org/10.1016/j.gene.2018.12.061.

113. Bahmed K, et al. DJ-1 modulates nuclear Erythroid 2-related Factor-2-mediated protection in human primary alveolar type II cells in smokers. Am J Respir Cell Mol Biol. 2016;55:439–49. https://doi.org/10.1165/rcmb.2015-0304OC.
114. Cheng L, Li F, Ma R, Hu X. Forsythiaside inhibits cigarette smoke-induced lung inflammation by activation of Nrf2 and inhibition of NF-kappaB. Int Immunopharmacol. 2015;28:494–9. https://doi.org/10.1016/j.intimp.2015.07.011.
115. Kennedy-Feitosa E, et al. Eucalyptol promotes lung repair in mice following cigarette smoke-induced emphysema. Phytomedicine. 2019;55:70–9. https://doi.org/10.1016/j.phymed.2018.08.012.
116. Gao W, Guo Y, Yang H. Platycodin D protects against cigarette smoke-induced lung inflammation in mice. Int Immunopharmacol. 2017;47:53–8. https://doi.org/10.1016/j.intimp.2017.03.009.
117. Yu D, Liu X, Zhang G, Ming Z, Wang T. Isoliquiritigenin inhibits cigarette smoke-induced COPD by attenuating inflammation and oxidative stress via the regulation of the Nrf2 and NF-kappaB signaling pathways. Front Pharmacol. 2018;9:1001. https://doi.org/10.3389/fphar.2018.01001.
118. Han B, et al. The novel compound Sul-121 inhibits airway inflammation and hyperresponsiveness in experimental models of chronic obstructive pulmonary disease. Sci Rep. 2016;6:26928. https://doi.org/10.1038/srep26928.
119. Li XY, Luo BL, Wang LJ, Zhang WD, Liu ZG. 15-Deoxy-prostaglandin J2 anti-inflammation in a rat model of chronic obstructive pulmonary disease and human bronchial epithelial cells via Nrf2 activation. Genet Mol Res. 2015;14:14037–42. https://doi.org/10.4238/2015.October.29.22.
120. Walters DM, Cho HY, Kleeberger SR. Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf2. Antioxid Redox Signal. 2008;10:321–32. https://doi.org/10.1089/ars.2007.1901.
121. Cho HY, Reddy SP, Yamamoto M, Kleeberger SR. The transcription factor NRF2 protects against pulmonary fibrosis. FASEB J. 2004;18:1258–60. https://doi.org/10.1096/fj.03-1127fje.
122. Chen D, et al. The mechanism of rapamycin in the intervention of paraquat-induced acute lung injury in rats. Xenobiotica. 2015;45:538–46. https://doi.org/10.3109/00498254.2014.995149.
123. Xu Y, et al. Rapamycin protects against paraquat-induced pulmonary fibrosis: activation of Nrf2 signaling pathway. Biochem Biophys Res Commun. 2017;490:535–40. https://doi.org/10.1016/j.bbrc.2017.06.074.
124. Tai W, et al. Rapamycin attenuates the paraquat-induced pulmonary fibrosis through activating Nrf2 pathway. J Cell Physiol. 2019; https://doi.org/10.1002/jcp.29094.
125. Xin X, et al. Protective effects of Rosavin on bleomycin-induced pulmonary fibrosis via suppressing fibrotic and inflammatory signaling pathways in mice. Biomed Pharmacother. 2019;115:108870. https://doi.org/10.1016/j.biopha.2019.108870.
126. Tian SL, Yang Y, Liu XL, Xu QB. Emodin attenuates bleomycin-induced pulmonary fibrosis via anti-inflammatory and anti-oxidative activities in rats. Med Sci Monit. 2018;24:1–10. https://doi.org/10.12659/msm.905496.
127. Liu Y, Lu F, Kang L, Wang Z, Wang Y. Pirfenidone attenuates bleomycin-induced pulmonary fibrosis in mice by regulating Nrf2/Bach1 equilibrium. BMC Pulm Med. 2017;17:63. https://doi.org/10.1186/s12890-017-0405-7.
128. Bodas M, Vij N. The NF-kappaB signaling in cystic fibrosis lung disease: pathophysiology and therapeutic potential. Discov Med. 2010;9:346–56.
129. Chen J, et al. Dysfunction of Nrf-2 in CF epithelia leads to excess intracellular H2O2 and inflammatory cytokine production. PLoS One. 2008;3:e3367. https://doi.org/10.1371/journal.pone.0003367.
130. Ziady AG, et al. Interaction with CREB binding protein modulates the activities of Nrf2 and NF-kappaB in cystic fibrosis airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2012;302:L1221–31. https://doi.org/10.1152/ajplung.00156.2011.

131. Komaravelli N, et al. Respiratory syncytial virus infection down-regulates antioxidant enzyme expression by triggering deacetylation-proteasomal degradation of Nrf2. Free Radic Biol Med. 2015;88:391–403. https://doi.org/10.1016/j.freeradbiomed.2015.05.043.
132. Cho HY, et al. Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease. Am J Respir Crit Care Med. 2009;179:138–50. https://doi.org/10.1164/rccm.200804-535OC.
133. Kosmider B, et al. Nrf2 protects human alveolar epithelial cells against injury induced by influenza a virus. Respir Res. 2012;13:43. https://doi.org/10.1186/1465-9921-13-43.
134. Kesic MJ, Simmons SO, Bauer R, Jaspers I. Nrf2 expression modifies influenza a entry and replication in nasal epithelial cells. Free Radic Biol Med. 2011;51:444–53. https://doi.org/10.1016/j.freeradbiomed.2011.04.027.
135. Dai JP, et al. Emodin inhibition of influenza a virus replication and influenza viral pneumonia via the Nrf2, TLR4, p38/JNK and NF-kappaB pathways. Molecules. 2017;22 https://doi.org/10.3390/molecules22101754.
136. Gomez JC, Dang H, Martin JR, Doerschuk CM. Nrf2 modulates host defense during Streptococcus pneumoniae pneumonia in mice. J Immunol. 2016;197:2864–79. https://doi.org/10.4049/jimmunol.1600043.
137. Zahlten J, et al. Streptococcus pneumoniae-induced oxidative stress in lung epithelial cells depends on pneumococcal autolysis and is reversible by resveratrol. J Infect Dis. 2015;211:1822–30. https://doi.org/10.1093/infdis/jiu806.
138. Palanisamy GS, et al. Evidence for oxidative stress and defective antioxidant response in Guinea pigs with tuberculosis. PLoS One. 2011;6:e26254. https://doi.org/10.1371/journal.pone.0026254.
139. Taguchi K, Yamamoto M. The KEAP1-NRF2 system in Cancer. Front Oncol. 2017;7:85. https://doi.org/10.3389/fonc.2017.00085.
140. Khor TO, et al. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer Prev Res (Phila). 2008;1:187–91. https://doi.org/10.1158/1940-6207.CAPR-08-0028.
141. Singh A, et al. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Med. 2006;3:e420. https://doi.org/10.1371/journal.pmed.0030420.
142. Padmanabhan B, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell. 2006;21:689–700. https://doi.org/10.1016/j.molcel.2006.01.013.
143. Ohta T, et al. Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. Cancer Res. 2008;68:1303–9. https://doi.org/10.1158/0008-5472.CAN-07-5003.
144. Takahashi T, et al. Mutations in Keap1 are a potential prognostic factor in resected non-small cell lung cancer. J Surg Oncol. 2010;101:500–6. https://doi.org/10.1002/jso.21520.
145. Bauer AK, et al. Targeted deletion of Nrf2 reduces urethane-induced lung tumor development in mice. PLoS One. 2011;6:e26590. https://doi.org/10.1371/journal.pone.0026590.
146. Bialk P, Wang Y, Banas K, Kmiec EB. Functional gene knockout of NRF2 increases chemosensitivity of human lung cancer A549 cells in vitro and in a xenograft mouse model. Mol Ther Oncolytics. 2018;11:75–89. https://doi.org/10.1016/j.omto.2018.10.002.
147. DeNicola GM, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature. 2011;475:106–9. https://doi.org/10.1038/nature10189.
148. Tsai JR, et al. High expression of heme oxygenase-1 is associated with tumor invasiveness and poor clinical outcome in non-small cell lung cancer patients. Cell Oncol (Dordr). 2012;35:461–71. https://doi.org/10.1007/s13402-012-0105-5.
149. Wang X, et al. Genetic variation and antioxidant response gene expression in the bronchial airway epithelium of smokers at risk for lung cancer. PLoS One. 2010;5:e11934. https://doi.org/10.1371/journal.pone.0011934.
150. Zhan L, et al. Regulatory role of KEAP1 and NRF2 in PPARgamma expression and chemoresistance in human non-small-cell lung carcinoma cells. Free Radic Biol Med. 2012;53:758–68. https://doi.org/10.1016/j.freeradbiomed.2012.05.041.
151. Dey S, et al. ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. J Clin Invest. 2015;125:2592–608. https://doi.org/10.1172/JCI78031.

152. DeNicola GM, et al. NRF2 regulates serine biosynthesis in non-small cell lung cancer. Nat Genet. 2015;47:1475–81. https://doi.org/10.1038/ng.3421.
153. Cuadrado A, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov. 2019;18:295–317. https://doi.org/10.1038/s41573-018-0008-x.
154. Harvey CJ, et al. Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. Sci Transl Med. 2011;3:78ra32. https://doi.org/10.1126/scitranslmed.3002042.
155. Bewley MA, et al. Opsonic phagocytosis in chronic obstructive pulmonary disease is enhanced by Nrf2 agonists. Am J Respir Crit Care Med. 2018;198:739–50. https://doi.org/10.1164/rccm.201705-0903OC.
156. Attucks OC, et al. Induction of heme oxygenase I (HMOX1) by HPP-4382: a novel modulator of Bach1 activity. PLoS One. 2014;9:e101044. https://doi.org/10.1371/journal.pone.0101044.
157. Hu L, et al. Discovery of a small-molecule inhibitor and cellular probe of Keap1-Nrf2 protein-protein interaction. Bioorg Med Chem Lett. 2013;23:3039–43. https://doi.org/10.1016/j.bmcl.2013.03.013.
158. Davies TG, et al. Monoacidic inhibitors of the Kelch-like ECH-associated protein 1: nuclear factor erythroid 2-related factor 2 (KEAP1:NRF2) protein-protein interaction with high cell potency identified by fragment-based discovery. J Med Chem. 2016;59:3991–4006. https://doi.org/10.1021/acs.jmedchem.6b00228.
159. Brasier AR. Therapeutic targets for inflammation-mediated airway remodeling in chronic lung disease. Expert Rev Respir Med. 2018;12:931–9. https://doi.org/10.1080/17476348.2018.1526677.
160. Wang WC, et al. Protective effects of tyrosol against LPS-induced acute lung injury via inhibiting NF-kappaB and AP-1 activation and activating the HO-1/Nrf2 pathways. Biol Pharm Bull. 2017;40:583–93. https://doi.org/10.1248/bpb.b16-00756.
161. Yang H, Lv H, Li H, Ci X, Peng L. Oridonin protects LPS-induced acute lung injury by modulating Nrf2-mediated oxidative stress and Nrf2-independent NLRP3 and NF-kappaB pathways. Cell Commun Signal. 2019;17:62. https://doi.org/10.1186/s12964-019-0366-y.
162. Pei X, Zhang XJ, Chen HM. Bardoxolone treatment alleviates lipopolysaccharide (LPS)-induced acute lung injury through suppressing inflammation and oxidative stress regulated by Nrf2 signaling. Biochem Biophys Res Commun. 2019;516:270–7. https://doi.org/10.1016/j.bbrc.2019.06.006.
163. Grzegorzewska AP, et al. Dimethyl Fumarate ameliorates pulmonary arterial hypertension and lung fibrosis by targeting multiple pathways. Sci Rep. 2017;7:41605. https://doi.org/10.1038/srep41605.
164. Saidu NE, et al. Dimethyl fumarate controls the NRF2/DJ-1 axis in cancer cells: therapeutic applications. Mol Cancer Ther. 2017;16:529–39. https://doi.org/10.1158/1535-7163.MCT-16-0405.
165. Choi RJ, Chun J, Khan S, Kim YS. Desoxyrhapontigenin, a potent anti-inflammatory phytochemical, inhibits LPS-induced inflammatory responses via suppressing NF-kappaB and MAPK pathways in RAW 264.7 cells. Int Immunopharmacol. 2014;18:182–90. https://doi.org/10.1016/j.intimp.2013.11.022.
166. Lai SW, et al. Antidiabetes drugs correlate with decreased risk of lung cancer: a population-based observation in Taiwan. Clin Lung Cancer. 2012;13:143–8. https://doi.org/10.1016/j.cllc.2011.10.002.
167. Zhang J, Jiao K, Liu J, Xia Y. Metformin reverses the resistance mechanism of lung adenocarcinoma cells that knocks down the Nrf2 gene. Oncol Lett. 2018;16:6071–80. https://doi.org/10.3892/ol.2018.9382.
168. Tao S, et al. Vitamin D protects against particles-caused lung injury through induction of autophagy in an Nrf2-dependent manner. Environ Toxicol. 2019;34:594–609. https://doi.org/10.1002/tox.22726.

169. Margaritopoulos GA, Vasarmidi E, Antoniou KM. Pirfenidone in the treatment of idiopathic pulmonary fibrosis: an evidence-based review of its place in therapy. Core Evid. 2016;11:11–22. https://doi.org/10.2147/CE.S76549.
170. Cheung KL, Kong AN. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. AAPS J. 2010;12:87–97. https://doi.org/10.1208/s12248-009-9162-8.
171. Liby K, et al. The synthetic triterpenoids CDDO-methyl ester and CDDO-ethyl amide prevent lung cancer induced by vinyl carbamate in a/J mice. Cancer Res. 2007;67:2414–9. https://doi.org/10.1158/0008-5472.CAN-06-4534.

Nrf2 in the Regulation of Endothelial Cell Homeostasis During Inflammation

Hiromi Yamazaki and Ken Itoh

Abstract Vascular endothelial cells line the inner surface of blood vessels, functioning as the selective barrier between the blood and internal organs. Inflammation in endothelial cells impairs vascular functions, such as barrier function and the control of blood pressure, and enhances the recruitment of leukocytes, resulting in cardiovascular disease or cerebrovascular disease. Vascular inflammation is often initiated by enhanced generation of reactive oxygen species (ROS) and provokes additional oxidative stress. The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) coordinately activates the expression of antioxidant and xenobiotic detoxifying genes, thereby protecting the vascular cells from oxidative stress. A number of studies have investigated the role of Nrf2 in endothelial cells and revealed that endothelial Nrf2 is activated not only by well-known electrophilic Nrf2 inducers such as sulforaphane in broccoli but also by mechanical shear stress and by circulating insulin-like growth factor (IGF)-1. Nrf2 is activated in the endothelial cells of straight segments of vessels that are exposed to unidirectional laminar flow (L-flow), thus contributing to the atheroprotective property of these areas. In contrast, Nrf2 activation is defective and unactivated in cells in branched segments that are exposed to disturbed flow; these areas are atheroprone. We review the mechanisms for endothelial Nrf2 activation via physiological stimuli and its effects on vascular biology, and we discuss the roles of Nrf2 target genes.

1 Function of Endothelial Cells

Vascular endothelial cells are a type of epithelial cells that line the cardiovascular system from the heart to the capillaries. A defective function of the endothelium is a key risk factor for cardiovascular disease (CVD) and initiates the development of atherosclerosis [1–3]. Endothelial cells have unique functions in vascular biology, as described below (Fig. 1).

H. Yamazaki · K. Itoh (✉)
Department of Stress Response Science, Center for Advanced Medical Research, Hirosaki University Graduate School of Medicine, Hirosaki, Japan
e-mail: itohk@hirosaki-u.ac.jp

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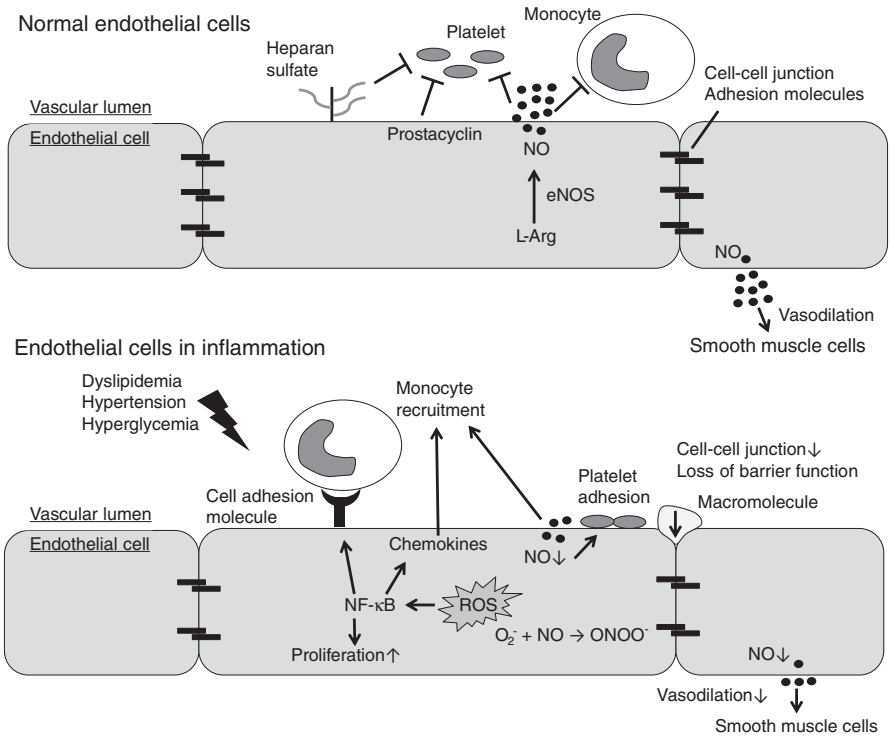

Fig. 1 Endothelial functions. The functions of normal endothelial cells and cells involved in inflammation are shown. See text for details

1.1 Barrier Functions

Endothelial cells form a physical barrier that separates blood from each tissue. Communication between blood and tissue occurs through the delivery of molecules and circulating substances across a single layer of endothelial cells by bidirectional transport, either through endothelial transporters or the interspace between cells [4].

1.2 Control of Blood Pressure

Endothelial cells produce and secrete endothelium-derived relaxing factor (EDRF) (i.e., nitric oxide (NO)) and endothelium-derived contracting factor (EDCF) (i.e., endothelin) in response to various stimuli to adjust blood pressure [5–7]. In particular, NO is generated by the conversion of the amino acid L-arginine to NO and L-citrulline by the endothelial NO synthase (eNOS) [8, 9]. Once produced, NO rapidly diffuses across smooth muscle cells and induces relaxation by activating guanylate cyclase to produce cGMP [8, 9]. NO also protects against vascular injury, inflammation, and thrombosis, as mentioned below.

1.3 Thrombosis and Fibrinolysis

Endothelial cells are normally anticoagulant and antithrombotic because they produce and secrete antiplatelet agents, such as prostacyclin and NO [10]. Endothelial cells express heparan sulfate on the cell surface, which acts as a cofactor for activating antithrombin [11]. Endothelial cells are also involved in fibrinolysis by secreting tissue-type plasminogen activator that converts plasminogen to the active enzyme plasmin through constitutive and regulated pathways [10].

1.4 Angiogenesis

Vascular endothelial growth factor (VEGF) is an inducer of angiogenesis [12]. VEGF binds to the tyrosine kinase receptor VEGE receptor 2 (VEGFR2) of endothelial cells and stimulates the production of factors that increase vessel permeability (eNOS), proliferation/survival and migration. The repair of damaged organ and wound healing are achieved by regulated angiogenesis.

1.5 Leukocyte Recruitment

The interaction of endothelial cells and leukocytes is usually inhibited by basal levels of endothelial NO but activated in response to various stimuli, such as infection, certain mechanical stress, and ischemia reperfusion through the downregulation of NO or upregulation of adhesion molecules [2, 3, 13]. Proper leukocyte recruitment is necessary for regulated inflammation, while excessive or persistent reactions result in tissue-damaging inflammation that associated with the dysfunction of endothelial cells.

2 Inflammation in Endothelial Cells

2.1 Triggers of Inflammation in Endothelial Cells

Inflammation of endothelial cells is evoked by various pathological states, such as dyslipidemia, hypertension, and hyperglycemia, which are closely linked with lifestyle as well as infection (Fig. 1) [1–3]. In dyslipidemia, low-density lipoprotein (LDL) undergoes oxidative modification and is converted to oxidized LDL (oxLDL). oxLDL elicits the recruitment of leukocytes to the endothelial cells by decreasing intracellular NO, inducing the expression of adhesion molecules, chemokines, and proinflammatory cytokines [1–3]. On the other hand, the overexpression of angiotensin II (AII) is the cause of vascular dysfunction in renovascular hypertension. AII

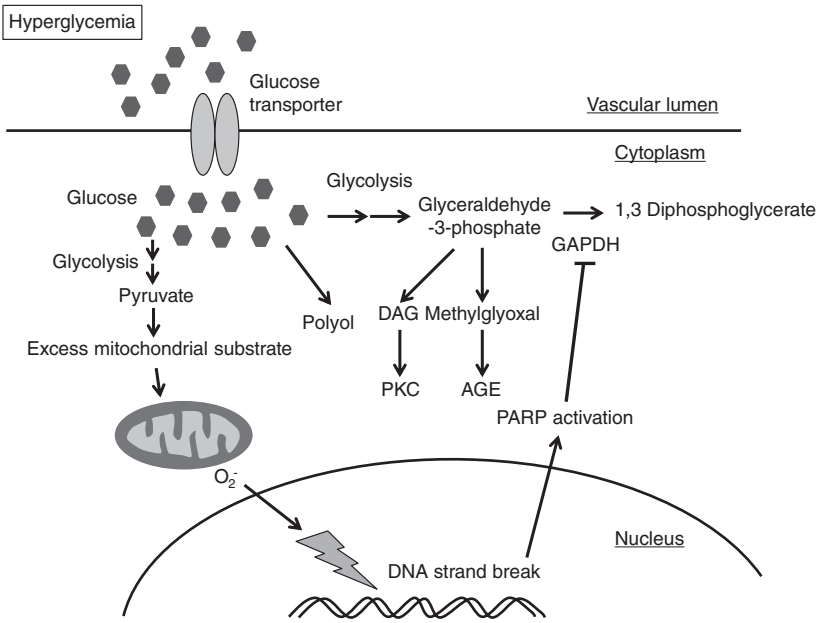

Fig. 2 The molecular mechanisms for ROS production by hyperglycemia. See text for details

induces the production of superoxide anion (O_2^-) and activates the expression of the proinflammatory cytokines, monocyte chemoattractant protein-1 (MCP-1), and vascular cell adhesion molecule-1 (VCAM-1). In the case of hyperglycemia, advanced glycation end products (AGEs) bind to receptors for AGEs (RAGEs) and induce the production of proinflammatory cytokines in endothelial cells by activating the transcription factor NF-κB, the master regulator of inflammation. Hyperglycemia also promotes oxidative stress by enhancing the generation of reactive oxygen species (ROS) by multiple mechanisms [1–3, 14–17]. Importantly, the greater amount of glucose influx to endothelial cells results in increased flux of mitochondrial respiration substrates, leading to production of mitochondrial O_2^- [14–16] (Fig. 2). O_2^- activates poly (ADP-ribose) polymerase (PARP) by inducing DNA double-strand break to inhibit the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), one of glycolytic enzyme, by polyADP-ribosylation resulting in the production of AGEs, activation of PKC, and polyol pathway. Importantly, ROS such as O_2^- reduces NO bioavailability by directly reacting with NO leading to peroxynitrite ($ONOO^-$) production or inducing eNOS uncoupling [18, 19].

2.2 *Consequences of Inflammation in Endothelial Cells*

Inflammation in endothelial cells is accompanied by the activation of NF-κB. NF-κB transcriptionally activates several genes such as cell adhesion molecules, chemokines and cytokines, resulting in recruitment of monocyte and cell proliferation [3].

Inflammation in endothelial cells promotes macromolecular transport by decreasing cell-to-cell and cell-to-matrix adhesion and by increasing centripetally directed tension (i.e., cell shrinkage), resulting in the formation of intercellular gaps [4]. Inflammation may also increase the selective transport of macromolecules through cells [4]. Such dysfunction of endothelial cells in coronary arteries results in diminished NO production, leading to impaired vasodilation and myocardial perfusion, which cause myocardial ischemia [1, 2].

3 Shear Stress

The vascular endothelial cells are constantly exposed to hemodynamic forces. Endothelial cells can sense hemodynamic forces because they have receptors that sense the flow and transduce mechanical signals [20–23]. The nature of the endothelial mechanical stress that occurs from the exposure to blood flow varies depending on the arterial architecture [21–24]. Steady laminar flow (L-flow) or pulsatile flow that occurs in the straight segments of the arterial tree is atheroprotective, whereas disturbed oscillatory flow (O-flow) in branch points are atheroprone because the respective shear stress has different effects on endothelial cells. L-flow confers pro-survival, cell-statics, and barrier function on endothelial cells, and it suppresses coagulation and leukocyte adhesion. On the other hand, O-flow gradually alters the endothelial cells toward proliferative and procoagulant properties, reduces barrier function, and increases the adhesion of leukocytes to endothelial cells. These differences are mainly attributable to the alteration of transcriptional regulation in endothelial cells through receptors for mechanical stress [20–23]. During L-flow, the transcription factor Krüppel-like transcription factor 2 (KLF2) is activated in endothelial cells, and KLF2 directly upregulates eNOS expression, resulting in increased NO production [25, 26]. In contrast, in endothelial cells that are exposed to O-flow, decreased KLF2 expression reduces eNOS-mediated NO production and results in inflammation and oxidative stress in these cells [27]. In addition to KLF2, increasing observations have demonstrated that Nrf2 (nuclear factor erythroid 2-related factor 2) is also a key player that acts as an endothelial mechanosensitive transcription factor.

4 Nrf2 Activation in Endothelial Cells

4.1 Transcription Factor Nrf2

The transcription factor Nrf2 is a master regulator of cytoprotective response against oxidative stress and xenobiotics (Fig. 3). Under unstressed conditions, Nrf2 is ubiquitinated by the Keap1 (Kelch-like ECH-associated protein 1)-Cul3 E3 ligase and degraded by the proteasome [28–31]. Upon exposure to electrophiles or oxidants, Keap1-dependent Nrf2 degradation is attenuated because of the modification of

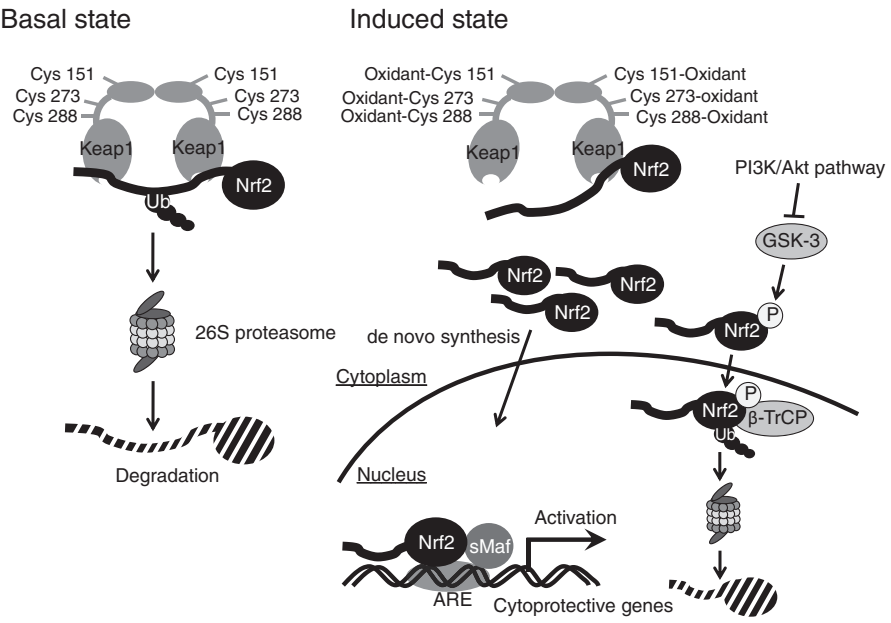

Fig. 3 Regulation of Nrf2. The molecular mechanisms for Nrf2 activation are shown. Nrf2 is repressed by Keap1 via ubiquitin-mediated proteosomal degradation in basal states. Upon oxidative stress, the reactive cysteine residues of Keap1 are modified by oxidants. Subsequently, Nrf2 degradation is attenuated, and newly synthesized Nrf2 translocates to the nucleus and activates its target genes. Nrf2 is also degraded by the GSK-3β-TrCP-dependent pathway, while the PI3K/Akt pathway suppresses the degradation and enhances Nrf2 activity

reactive cysteine residues of Keap1, resulting in Nrf2 accumulation in the nucleus [32–37]. In addition, the glycogen synthase kinase 3 (GSK-3) phosphorylates Nrf2. Then, phosphorylated Nrf2 binds to the β-TrCP-Cul1 E3 ligase and is degraded via the ubiquitin-proteasome pathway in the nucleus [38, 39]. Because the phosphoinositide 3-kinase (PI3K)/Akt pathway, which is the intracellular signaling pathway related to cellular proliferation and survival, inhibits GSK-3 activity by phosphorylation, the protein level of Nrf2 is often augmented in proliferating cells that exhibit increased PI3K/Akt pathway activation [40, 41]. Accumulated Nrf2 in the nucleus heterodimerizes with small Maf proteins (sMaf) and binds to antioxidant or electrophile response elements (AREs/EpREs, respectively) in the gene regulatory region of many cytoprotective genes [42–45]. Nrf2 activates genes involved in the synthesis and conjugation of glutathione (GSH), antioxidant proteins/enzymes, drug-metabolizing enzymes, drug transporters, proteasome subunits, pentose phosphate pathway enzymes, and enzymes involved in nucleotide synthesis [42, 43, 46]. To examine the physiological effects of Nrf2 in vivo, Nrf2 gene targeting was performed [42]. *Nrf2* knockout (KO) mice are viable and fertile under normal conditions, but these mice are susceptible to various environmental chemicals that provoke oxidative stress or bacterial endotoxin [42, 47, 48]. In

contrast, Nrf2 activation by compounds that modify reactive cysteine of Keap1 confers protection against various stressors and diseases in mice [37, 49–54]. The anti-inflammatory activity of Nrf2 is often explained by its antioxidant protein/enzyme-inducing property because the exacerbated inflammation in *Nrf2* KO mice is often alleviated by N-acetyl cysteine (NAC) [47]. However, it has been recently shown that Nrf2 represses interleukin (IL)-6 or IL-1β transcription by directly binding to their regulatory regions although the precise mechanisms are presently unclear [55].

4.2 Shear Stress and Nrf2

cDNA subtraction and microarray analysis revealed that L-flow activates ARE-regulated genes, such as NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), ferritin, microsomal epoxide hydrolase, glutathione *S*-transferase, γ-glutamylcysteine ligase, and solute carrier family 7, member 11 in human aortic endothelial cells (HAECs) [56] or human umbilical vein endothelial cells (HUVECs) [57]. Exposure to L-flow induces the nuclear translocation of Nrf2 in endothelial culture cells [58–61] and in endothelial cells in the straight segments of the arterial tree [62, 63].

Several possible mechanisms have been suggested for Nrf2 activation by L-flow. We showed that the cyclooxygenase-2 (COX-2)-dependent generation of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), which belongs to the cyclopentenone-type prostaglandins with electrophilic character, is essential for the induction of Nrf2 target genes in endothelial cells in response to L-flow (Fig. 4a, b). L-flow induces Nrf2 accumulation, the increased binding of it to the ARE of NQO1, and increases target gene expression, such as NQO1, HO-1, and ferritin heavy chain (Fig. 4b–e). Exposure of endothelial cells to L-flow enhances the production of prostaglandin D_2 (PGD_2) and 15d-PGJ_2 through the upregulation of cytosolic phospholipase A_2, COX-2, and lipocalin-type PGD synthase [64–66], and specific COX-2 inhibitors (Nimesulide and NS-398) repress the production of PGD_2 and Nrf2 activation. Of note, it was reported that 15d-PGJ_2 covalently binds to Cys273 of Keap1 and suppresses Nrf2 degradation [34–36, 58, 67, 68]. Thus, although it is well known that 15d-PGJ_2 possesses anti-inflammation roles by activating PPARγ and repressing NF-κB, the novel function involving Nrf2 adds to the anti-inflammatory function of 15d-PGJ_2.

Exposure of HUVECs to L-flow induces Nrf2 nuclear translocation, which is inhibited by LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor and calphostin C, a protein kinase C (PKC) inhibitor [60, 62] (Fig. 5). These results demonstrated that PI3K and PKC are involved in the signaling pathway that leads to the nuclear translocation of Nrf2 in response to L-flow. As mentioned above, Nrf2 activity is enhanced by the activation of the PI3K/Akt pathway by inhibiting β-TrCP-dependent degradation in the nucleus [40, 41]. It was also reported that PKC phosphorylates Nrf2 and regulates Nrf2 target gene expression in response to

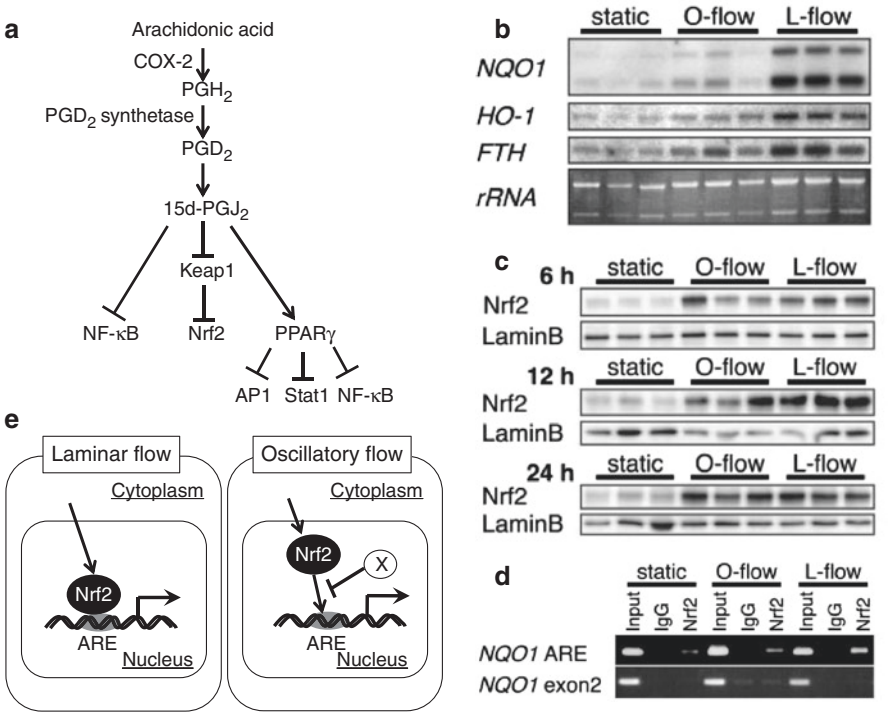

Fig. 4 L-flow activates Nrf2 by producing 15d-PGJ_2. (**a**) The metabolic pathway of arachidonic acid is shown. 15d-$PGGJ_2$ exerts anti-inflammatory roles through the activation of PPARγ and the repression of Nrf2 and NF-κB. (**b**) The mRNA levels of Nrf2 target genes of HAECs exposed to L-flow, O-flow, or kept under a static condition for 24 h. *FTH*; ferritin heavy chain. (**c**) HAECs were exposed to respective flows, and their nuclear extracts were analyzed by Western blot analysis using the anti-Nrf2 antibody. (**d**) HAECs were exposed to respective flows for 24 h. A chromatin immunoprecipitation (ChIP) assay was carried out with the anti-Nrf2 antibody or normal rabbit IgG. (**b**–**d**) were reprinted from an already published report [58]. (**e**) Mechanism of Nrf2 activation by blood flow. Although both L-flow and O-flow induce Nrf2 accumulation in the nucleus, only L-flow, but not O-flow activates Nrf2 binding to ARE of target genes and activates their expression. One plausible explanation is the presence of factor X that is inducible by O-flow and inhibits Nrf2 binding to ARE

oxidative stress [69, 70]. Such mechanisms may be involved in the regulation of Nrf2 activity in endothelial cells under L-flow condition.

KLF2, a master regulator of endothelial cells, is also involved in Nrf2 activation [59] (Fig. 5). The siRNA-mediated knockdown of KLF2 partially decreases NQO1 expression under L-flow conditions, whereas KLF2 overexpression increases NQO1 expression both in the absence and presence of the Nrf2 inducer tert-butylhydroquinone (tBHQ). These results suggest that the concomitant activation of both the transcription factor Nrf2 and KLF2 might be required for efficient Nrf2 target gene expression.

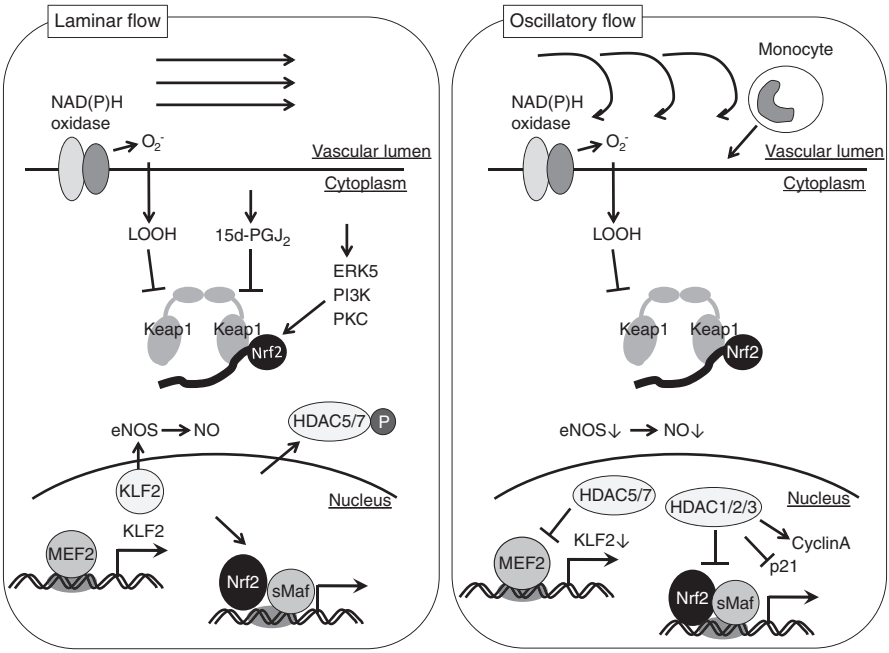

Fig. 5 Differential regulation of Nrf2 activity by blood flows. The left and right schemes show the several mechanisms for Nrf2 regulation in endothelial cells exposed to L-flow and O-flow, respectively. Erk5 also regulates KLF2 expression via MEF2 although it is not shown in the fig [71]

Although extracellular signal-regulated kinase 5 (ERK5) has been reported to regulate endothelial integrity to protect endothelial cells from vascular dysfunction by activating the KLF2 pathway [71–74], ERK5 is also involved in Nrf2 activation [75] (Fig. 5). ERK5 inhibition either by knockdown using siRNA or by biochemical inactivation with a specific compound inhibits Nrf2 target gene expression in response to L-flow, whereas the activation of ERK5 increases the transcriptional activity and nuclear translocation of Nrf2. Furthermore, the direct interaction between ERK5 and Nrf2 under L-flow is observed. This interaction depends on the active state of ERK5, suggesting that ERK5 binds to Nrf2 and phosphorylates Nrf2, which may result in the dissociation of Nrf2 from the Keap1-Cul3 complex.

As a number of reports have shown ROS activates Nrf2, endothelial Nrf2 is also activated by ROS during exposure to L-flow (Fig. 4). Several reports have examined the intracellular ROS level using dichloro-dihydro-fluorescein diacetate (DCFH-DA). DCFH-DA is oxidized to the fluorescent dichlorofluorescein (DCF) by potent oxidants, such as hydroxyl radical ($^{\cdot}$OH), hydrogen peroxide (H_2O_2), O_2^-, nitric oxide (NO), and $ONOO^-$ [76]. DCF fluorescence was increased 30 min to 1 h after L-flow stimulation in HUVECs and HMVECs [77, 78]. O_2^- production detected by electron spin resonance (ESR) was also increased 1 h after L-flow stimulation, whereas it was decreased by 18 h after stimulation [79]. The xanthine oxidase inhibitor oxypurinol and the flavoprotein inhibitor diphenyleneiodonium, which inhibits both NAD(P)H

oxidase and the mitochondrial respiratory chain, markedly suppressed the expression of Nrf2 target genes [77]. The deficiency of the NAD(P)H oxidase component p47 impaired O_2^- production in endothelial cells under L-flow [79]. Additionally, diphenylpyrenlphosphine (DPPP), a reducing compound of lipid hydroperoxides, also significantly suppressed Nrf2-regulated gene expression [77]. Such L-flow-induced ROS might directly attack the cysteine residues of Keap1 and repress the ubiquitin-proteasome-mediated degradation of Nrf2. NAC treatment also significantly attenuated the Nrf2 activation induced by L-flow, suggesting that oxidative stress is involved in the activation of Nrf2 [77]. Such L-flow-induced ROS might directly attack the cysteine residues of Keap1 and repress the ubiquitin-proteasome-mediated degradation of Nrf2. Although O-flow induces ROS production via NAD(P)H oxidase [79, 80] as well as L-flow, O_2^- accumulation persisted 18 h after exposure to O-flow [79]. Although Nrf2 accumulated in the nucleus with O-flow, this increase was concomitant with increases in ROS; however, Nrf2 target genes were not induced by O-flow [58] (Fig. 4b, c). Chromatin immunoprecipitation (ChIP) using the anti-Nrf2 antibody showed that Nrf2 binds to the NQO1 ARE in response to L-flow, but it is impaired under O-flow (Fig. 4d). This finding suggests the presence of certain factor(s) that could be induced by O-flow to prevent Nrf2 binding to the NQO1 ARE. Lee and colleagues provided several results that indicate these factors may be class I histone deacetylases (HDACs) [81](Fig. 5). The nuclear accumulation of class I and class II HDACs (HDAC-1/2/3 and HDAC-5/7, respectively) was observed in cultured endothelial cells under O-flow and rat aortic arch. O-flow induced the association of HDAC-1/2/3 with Nrf2 and Nrf2 deacetylation, probably by HDAC-1/2/3, and thus repressed Nrf2 binding to ARE of target genes and their transcription. O-flow also repressed KLF2 transcription by inducing the association of HDAC-3/5/7 and myocyte enhancer factor-2 (MEF2), a positive regulator of KLF2, and the deacetylation of MEF2. O-flow-mediated HDAC-1/2/3 accumulation also activated cyclin A and repressed p21CIP1 in endothelial cells, inducing their proliferation, although the precise mechanisms are yet to be determined. The O-flow-induced HDAC signaling is mediated by the PI3K/Akt pathway because HDAC induction was repressed by PI3K inhibitor LY294002. On the other hand, L-flow did not induce HDAC-1/2/3 accumulation in the nucleus, induced the phosphorylation of HDAC-5/7, and exported HDAC-5/7 from the nucleus. These findings demonstrate the importance of HDACs in regulating the oxidative, inflammatory, and proliferative responses of endothelial cells to O-flow.

4.3 Aging and Nrf2

Over three quarters of deaths from CVD occur among people older than the age of 65 years. In young organisms, the homeostatic response mediated by Nrf2 serves to attenuate vascular oxidative stress and limits the cellular damage caused by increased free radical production, diabetic conditions, and oxidized phospholipids [82, 83]. Ungvari et al. showed the carotid arteries of aged rhesus macaques exhibit

significant oxidative stress, which was indicated by the increased 8-iso-PGF2α and 4-hydroxynonenal (HNE) content and decreased glutathione and ascorbate levels, compared with vessels of young macaques [84]. Oxidative stress in aged rhesus macaques induced inflammation phenotype characterized by NF-κB, IL-6, inducible NOS (iNOS), and intercellular adhesion molecule-1 (ICAM-1) activation but did not activate Nrf2 target genes, such as glutamate–cysteine ligase, catalytic subunit (GCLC), NQO1, and HO-1. Although these results were examined in carotid arteries that contain a variety of cells (e.g., endothelial cells, smooth muscle cells and migrated leukocytes), endothelial cells might be one of the responsible cells.

Circulating levels of insulin-like growth factor (IGF)-1, which is produced in the liver, decline during aging, which significantly increases the risk for CVD in humans. The liver-specific knockdown of the *Igf1* gene in mice exhibited decreased expression of Nrf2 mRNA and the Nrf2 target genes GCLC, NQO1, and HO-1, in vascular vessels, whereas IGF-1 treatment in cultured primary human coronary arterial endothelial cells (CAECs) activate the Nrf2 pathway [85]. This report also provided evidence that in endothelial cells, IGF-1 activates Nrf2 via an Akt1-dependent pathway. The decrease of Nrf2 activity because of IGF-1 hypofunction during aging may contribute to vascular impairments in aging.

5 The Effect of Nrf2 Activation in Endothelial Cells

The scavenging of ROS by Nrf2 would be one of its critical roles in endothelial cells because endothelial ROS leads to monocyte adhesion that results in the initiation of inflammation [79]. Nrf2 is also involved in the regulation of the adhesion molecule VCAM-1 [63]. Nrf2-mediated scavenging of ROS results in the activation of MAP kinase phosphatase-1 (MKP-1), followed by the inactivation of p38 mitogen-activated protein (MAP) kinase that positively regulates VCAM-1. The activation of Nrf2 by sulforaphane suppresses p38 activation and VCAM-1 expression in the susceptible regions of the aorta, suggesting the possible future clinical use for prevention of atherosclerosis. Nrf2 or NQO1 suppresses tumor necrosis factor-α (TNF-α)-induced activation of VCAM-1 gene expression [56], indicating that the increased expression of ARE-mediated genes has a potential atheroprotective effect and exerts anti-inflammatory functions in endothelial cells. Of note, HO-1 is a well-known Nrf2 target gene that has anti-inflammatory roles [86] (Fig. 6). HO-1 is a rate-limiting enzyme that catalyze the conversion of heme into biliverdin, iron, and carbon monoxide (CO) [87]. Biliverdin is rapidly converted by biliverdin reductase to the antioxidant bilirubin [88]. The heme-derived ferrous iron is directly sequestered and inactivated by co-induced ferritin [88]. Ferritin limits the generation of ROS by binding free labile iron that would otherwise participate in a Fenton reaction to promote the generation of ROS [89]. CO is a vasodilator and has profound effects on intracellular signaling processes, which culminate in anti-inflammatory, antiproliferative, antiapoptotic, and anticoagulative effects through modulating signaling pathways such as p38 MAPK, ERK, and JNK or activating guanylate cyclase [88].

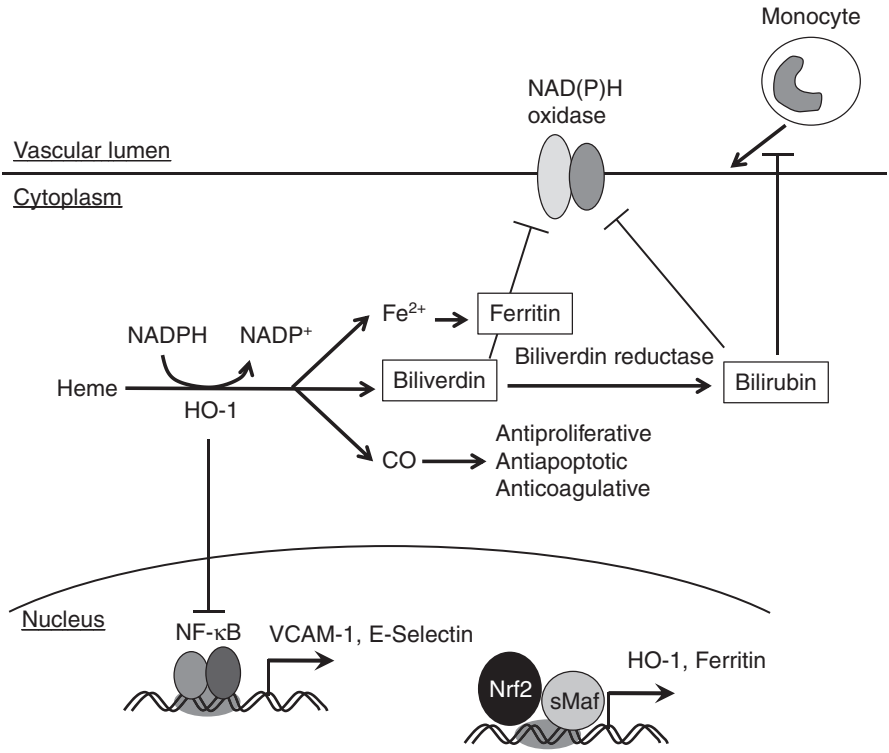

Fig. 6 Anti-inflammatory function of HO-1 in endothelial cells. HO-1 exerts anti-inflammatory functions through the depicted pathways. Enclosed factors act as antioxidants by themselves

Bilirubin and biliverdin scavenge ROS/reactive nitrogen species (RNS) and reduce ROS production through the direct inhibition of NAD(P)H oxidase [90]. Bilirubin inhibits the activation of O_2^- -producing NAD(P)H oxidase in a neutrophil cell-free system [91, 92]. Furthermore, HO-1 is proposed to inhibit eNOS uncoupling by reducing eNOS expression when its cofactor tetrahydrobiopterin availability is limited [93]. Humans with HO-1 deficiency showed hemolytic anemia characterized by marked erythrocyte fragmentation and intravascular hemolysis, with a paradoxical increase in serum haptoglobin and low bilirubin [94]. An abnormal coagulation/fibrinolysis system, associated with elevated thrombomodulin and von Willebrand factor, indicated the presence of severe and persistent endothelial damage. The expression of HO-1 induced by adenovirus or hemin inhibits the expression of pro-inflammatory genes, such as E-selectin and VCAM-1, by the inhibition of NF-κB activation [95]. Curcumin, a spice and coloring agent in food that activates Nrf2, induces HO-1 expression and confers cytoprotective effect against oxidative stress in bovine aortic endothelial cells [96]. This cytoprotective effect was considerably attenuated by protoporphyrin IX, an inhibitor of HO, indicating the importance of HO-1. Additional evidence for the role of HO in vasoregulation is provided by

ex vivo models. The overexpression of HO-1 by adenovirus infection of the vessels inhibited phenylephrine-dependent vasoconstriction in isolated aortic rings, also in a fashion dependent on heme degradation but independent of NO production [97]. Hemin-induced HO-1 activation in microvascular endothelial cells exhibits protection against oxidative stress and leukocyte adhesion by a mechanism that depends on the antioxidant role of bilirubin [98]. In vivo Nrf2 activation in the atherosensitive region of the aorta by sulforaphane induced HO-1 expression and suppressed p38-VCAM-1 signaling in an Nrf2-dependent manner [63]. The inhibition of HO-1 activity using zinc protoporphyrin did not influence the VCAM-1 suppression caused by sulforaphane in HUVECs. These results indicate that HO-1 is not essential for the p38-suppressing effects of sulforaphane and the other Nrf2-induced antioxidants can compensate for the absence of HO-1 activity. It has also been reported that Nrf2 and Oct-1 corporately suppress the transcription of the NAD(P)H oxidase 4 (Nox4) gene under L-flow condition, leading to the suppression of O_2^- production [61].

6 Vascular Diseases and Nrf2

Although dyslipidemia and hyperglycemia provokes inflammation in endothelial cells (See Sect. 2.1), Nrf2 activation is protective against these stresses. oxLDL induced the expression of MCP-1, VCAM-1, and ICAM-1 in HUVECs and also provokes cell death. Pretreatment of polyphenol genistein (4′,5,7-trihydroxyisoflavone) increased HO-1 expression and simultaneously suppressed MCP-1, VCAM-1, and ICAM-1 expression and restored the cell viability in an Nrf2-dependent manner [99]. Mimicking hyperglycemia in human microvascular endothelial cells (HMEC-1) increased the generation of ROS, which was prevented by Nrf2 inducer sulforaphane [100]. ROS accumulation was increased further by either knockdown of Nrf2 or transketolase, one of the pentose phosphate pathway (PPP) enzymes. PPP generates not only ribose 5-phosphate (R5P), a critical substrate for the nucleotide synthesis, but also NADPH as reducing equivalents. These results suggest that Nrf2-driven PPP activation generates NADPH, which contributes to scavenging of ROS induced by hyperglycemia. Additionally, Nrf2-mediated antioxidant defense has a critical role in fetal endothelial cells that are exposed to hyperglycemia [101]. HUVECs cultured from gestational diabetes mellitus (DM) pregnancies showed reduced rates of cell proliferation to reach confluency [102]. Further proteomic analysis revealed that proteins involved in redox homeostasis were significantly altered in gestational DM and associated with increased mitochondrial superoxide generation, protein oxidation, DNA damage, and diminished GSH synthesis [101]. In accordance, induction of Nrf2 was impaired in response to 4-HNE. Decreased DJ-1, involved in Nrf2 protein stability, and increased GSK3β phosphorylation in gestational DM cells may account for the deficits in Nrf2 nuclear accumulation. These results suggest that forced expression of Nrf2 might protect fetal endothelial cells from hyperglycemia. Of note, the therapeutic potential of sulforaphane against

diabetic endothelial dysfunction of Goto-Kakizaki (GK) rats, an animal model of non-obese type 2 diabetes, was reported [103]. Although GK rats exhibited significantly lower levels of Nrf2 in endothelial cells in aorta and mesenteric arteries and higher levels of oxidative stress and endothelial dysfunction, administration of sulforaphane improved NO bioavailability and decreased oxidative damage, AGEs, and HbA1c levels. In addition, sulforaphane taken as broccoli sprout concentrates decreases fasting glucose levels and glycated HbA1c in obese patients with dysregulated type 2 diabetes. Mechanistically, administration of sulforaphane improved glucose tolerance and insulin sensitivity of high fat diet-treated rats and suppressed glucose production via suppressing gene expression involved in gluconeogenesis [104]. Collectively, these data suggested the potential role of Nrf2 both in preventing type 2 diabetes and hyperglycemia-mediated vascular injuries associated with diabetes.

7 Conclusion

Many reports have demonstrated that L-flow activates endothelial Nrf2, which preserves vascular homeostasis and confers resistance to atherosclerosis. Nrf2 activation provides endothelial cells with various functions and outcomes, such as antioxidant defense against ROS, suppression of ROS production and leukocyte recruitment, and protection from endothelial dysfunction. Although Nrf2 is not induced by O-flow, the forced activation of endothelial Nrf2 by phytochemicals such as sulforaphane reduces endothelial inflammation in atheroprone areas, suggesting Nrf2 as a potential therapeutic target for preventing vascular diseases.

References

1. Libby P. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135–43. https://doi.org/10.1161/hc0902.104353.
2. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, et al. The vascular endothelium and human diseases. Int J Biol Sci. 2013;9(10):1057–69. https://doi.org/10.7150/ijbs.7502.
3. Gimbrone MA Jr, Garcia-Cardena G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res. 2016;118(4):620–36. https://doi.org/10.1161/CIRCRESAHA.115.306301.
4. Stevens T, Garcia JG, Shasby DM, Bhattacharya J, Malik AB. Mechanisms regulating endothelial cell barrier function. Am J Physiol Lung Cell Mol Physiol. 2000;279(3):L419–22. https://doi.org/10.1152/ajplung.2000.279.3.L419.
5. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci U S A. 1989;86(9):3375–8.
6. Gardiner SM, Compton AM, Bennett T, Palmer RM, Moncada S. Control of regional blood flow by endothelium-derived nitric oxide. Hypertension. 1990;15(5):486–92.

7. Masaki T. Historical review: Endothelin. Trends Pharmacol Sci. 2004;25(4):219–24. https://doi.org/10.1016/j.tips.2004.02.008.
8. Moncada S, Higgs EA, Hodson HF, Knowles RG, Lopez-Jaramillo P, McCall T, Palmer RMJ, Radomski MW, Rees DD, Schulz R. The l-arginine: nitric oxide pathway. J Cardiovasc Pharmacol. 1991;17(Suppl.3):S1–9.
9. Huang PL. eNOS, metabolic syndrome and cardiovascular disease. Trends Endocrinol Metab. 2009;20(6):295–302. https://doi.org/10.1016/j.tem.2009.03.005.
10. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovasc Disord. 2015;15:130. https://doi.org/10.1186/s12872-015-0124-z.
11. Weitz JI. Heparan sulfate: antithrombotic or not? J Clin Invest. 2003;111(7):952–4. https://doi.org/10.1172/JCI200318234.
12. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev. 2004;56(4):549–80. https://doi.org/10.1124/pr.56.4.3.
13. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A. 1991;88(11):4651–5.
14. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404(6779):787–90. https://doi.org/10.1038/35008121.
15. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, et al. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. J Clin Invest. 2003;112(7):1049–57. https://doi.org/10.1172/jci18127.
16. Reusch JE. Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose? J Clin Invest. 2003;112(7):986–8. https://doi.org/10.1172/jci200319902.
17. Satta S, Mahmoud AM, Wilkinson FL, Yvonne Alexander M, White SJ. The role of Nrf2 in cardiovascular function and disease. Oxidative Med Cell Longev. 2017;2017:9237263. https://doi.org/10.1155/2017/9237263.
18. Förstermann U. Janus-faced role of endothelial NO synthase in vascular disease: uncoupling of oxygen reduction from NO synthesis and its pharmacological reversal. Biol Chem. 2006;387(12):1521–33. https://doi.org/10.1515/BC.2006.190.
19. Pierini D, Bryan NS. Nitric oxide availability as a marker of oxidative stress. Methods Mol Biol. 2015;1208:63–71. https://doi.org/10.1007/978-1-4939-1441-8_5.
20. Chistiakov DA, Orekhov AN, Bobryshev YV. Effects of shear stress on endothelial cells: go with the flow. Acta Physiol. 2017;219(2):382–408. https://doi.org/10.1111/apha.12725.
21. Resnick N, Yahav H, Shay-Salit A, Shushy M, Schubert S, Zilberman LCM, et al. Fluid shear stress and the vascular endothelium: for better and for worse. Prog Biophys Mol Biol. 2003;81(3):177–99. https://doi.org/10.1016/s0079-6107(02)00052-4.
22. Barakat A, Lieu, D. Differential responsiveness of vascular endothelial cells to different types of fluid mechanical shear stress. Cell Biochem Biophys. 2003;38(3):323–43. https://doi.org/10.1385/cbb:38:3:323.
23. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev. 2011;91(1):327–87. https://doi.org/10.1152/physrev.00047.2009.
24. Napoli C, Williams-Ignarro S, De Nigris F, Lerman LO, Rossi L, Guarino C, Mansueto G, Di Tuoro F, Pignalosa O, De Rosa G, Sica V, Ignarro LJ. Long-term combined beneficial effects of physical training and metabolic treatment on atherosclerosis in hypercholesterolemic mice. Proc Natl Acad Sci U S A. 2004;101(52):18262. https://doi.org/10.1073/pnas.0409060101.
25. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by

steady laminar shear stress. Proc Natl Acad Sci U S A. 1996;93(19):10417–22. https://doi.org/10.1073/pnas.93.19.10417.
26. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, et al. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. J Exp Med. 2004;199(10):1305–15. https://doi.org/10.1084/jem.20031132.
27. Wang N, Miao H, Li YS, Zhang P, Haga JH, Hu Y, et al. Shear stress regulation of Kruppel-like factor 2 expression is flow pattern-specific. Biochem Biophys Res Commun. 2006;341(4):1244–51. https://doi.org/10.1016/j.bbrc.2006.01.089.
28. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13(1):76–86. https://doi.org/10.1101/gad.13.1.76.
29. Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, Yamamoto M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells. 2003;8(4):379–91. https://doi.org/10.1046/j.1365-2443.2003.00640.x.
30. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004;24(16):7130–9. https://doi.org/10.1128/MCB.24.16.7130-7139.2004.
31. Katoh Y, Iida K, Kang MI, Kobayashi A, Mizukami M, Tong KI, et al. Evolutionary conserved N-terminal domain of Nrf2 is essential for the Keap1-mediated degradation of the protein by proteasome. Arch Biochem Biophys. 2005;433(2):342–50. https://doi.org/10.1016/j.abb.2004.10.012.
32. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent Ubiquitination of Nrf2 and for stabilization of Nrf2 by Chemopreventive agents and oxidative stress. Mol Cell Biol. 2003;23(22):8137–51. https://doi.org/10.1128/mcb.23.22.8137-8151.2003.
33. Yamamoto T, Suzuki T, Kobayashi A, Wakabayashi J, Maher J, Motohashi H, et al. Physiological significance of reactive cysteine residues of Keap1 in determining Nrf2 activity. Mol Cell Biol. 2008;28(8):2758–70. https://doi.org/10.1128/MCB.01704-07.
34. Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, Nakayama Y, et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. Mol Cell Biol. 2009;29(2):493–502. https://doi.org/10.1128/MCB.01080-08.
35. Takaya K, Suzuki T, Motohashi H, Onodera K, Satomi S, Kensler TW, et al. Validation of the multiple sensor mechanism of the Keap1-Nrf2 system. Free Radic Biol Med. 2012;53(4):817–27. https://doi.org/10.1016/j.freeradbiomed.2012.06.023.
36. Saito R, Suzuki T, Hiramoto K, Asami S, Naganuma E, Suda H, et al. Characterizations of three major cysteine sensors of Keap1 in stress response. Mol Cell Biol. 2015;36(2):271–84. https://doi.org/10.1128/MCB.00868-15.
37. Suzuki T, Yamamoto M. Stress-sensing mechanisms and the physiological roles of the Keap1-Nrf2 system during cellular stress. J Biol Chem. 2017;292(41):16817-24. https://doi.org/10.1074/jbc.R117.800169.
38. Rada P, Rojo AI, Chowdhry S, McMahon M, Hayes JD, Cuadrado A. SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol Cell Biol. 2011;31(6):1121–33. https://doi.org/10.1128/MCB.01204-10.
39. Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene. 2013;32(32):3765–81. https://doi.org/10.1038/onc.2012.388.
40. Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, et al. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell. 2012;22(1):66–79. https://doi.org/10.1016/j.ccr.2012.05.016.

41. Taguchi K, Hirano I, Itoh T, Tanaka M, Miyajima A, Suzuki A, et al. Nrf2 enhances cholangiocyte expansion in Pten-deficient livers. Mol Cell Biol. 2014;34(5):900–13. https://doi.org/10.1128/MCB.01384-13.
42. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236(2):313–22.
43. Itoh K, Tong KI, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med. 2004;36(10):1208–13. https://doi.org/10.1016/j.freeradbiomed.2004.02.075.
44. Katsuoka F, Motohashi H, Ishii T, Aburatani H, Engel JD, Yamamoto M. Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. Mol Cell Biol. 2005;25(18):8044–51. https://doi.org/10.1128/MCB.25.18.8044-8051.2005.
45. Yamazaki H, Katsuoka F, Motohashi H, Engel JD, Yamamoto M. Embryonic lethality and fetal liver apoptosis in mice lacking all three small Maf proteins. Mol Cell Biol. 2012;32(4):808–16. https://doi.org/10.1128/MCB.06543-11.
46. Suzuki T, Motohashi H, Yamamoto M. Toward clinical application of the Keap1-Nrf2 pathway. Trends Pharmacol Sci. 2013;34(6):340–6. https://doi.org/10.1016/j.tips.2013.04.005.
47. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest. 2006;116(4):984–95. https://doi.org/10.1172/JCI25790.
48. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol. 2007;47:89–116. https://doi.org/10.1146/annurev.pharmtox.46.120604.141046.
49. Taguchi K, Maher JM, Suzuki T, Kawatani Y, Motohashi H, Yamamoto M. Genetic analysis of cytoprotective functions supported by graded expression of Keap1. Mol Cell Biol. 2010;30(12):3016–26. https://doi.org/10.1128/MCB.01591-09.
50. Ohkoshi A, Suzuki T, Ono M, Kobayashi T, Yamamoto M. Roles of Keap1-Nrf2 system in upper aerodigestive tract carcinogenesis. Cancer Prev Res. 2013;6(2):149–59. https://doi.org/10.1158/1940-6207.CAPR-12-0401-T.
51. Miyazaki Y, Shimizu A, Pastan I, Taguchi K, Naganuma E, Suzuki T, et al. Keap1 inhibition attenuates glomerulosclerosis. Nephrol Dial Transplant. 2014;29(4):783–91. https://doi.org/10.1093/ndt/gfu002.
52. Satoh H, Moriguchi T, Saigusa D, Baird L, Yu L, Rokutan H, et al. NRF2 intensifies host defense systems to prevent lung carcinogenesis, but after tumor initiation accelerates malignant cell growth. Cancer Res. 2016;76(10):3088–96. https://doi.org/10.1158/0008-5472.CAN-15-1584.
53. Keleku-Lukwete N, Suzuki M, Otsuki A, Tsuchida K, Katayama S, Hayashi M, et al. Amelioration of inflammation and tissue damage in sickle cell model mice by Nrf2 activation. Proc Natl Acad Sci U S A. 2015;112(39):12169–74. https://doi.org/10.1073/pnas.1509158112.
54. Yamazaki H, Tanji K, Wakabayashi K, Matsuura S, Itoh K. Role of the Keap1/Nrf2 pathway in neurodegenerative diseases. Pathol Int. 2015;65(5):210–9. https://doi.org/10.1111/pin.12261.
55. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624. https://doi.org/10.1038/ncomms11624.
56. Chen XL, Varner SE, Rao AS, Grey JY, Thomas S, Cook CK, et al. Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem. 2003;278(2):703–11. https://doi.org/10.1074/jbc.M203161200.
57. Warabi E, Wada Y, Kajiwara H, Kobayashi M, Koshiba N, Hisada T, et al. Effect on endothelial cell gene expression of shear stress, oxygen concentration, and low-density lipoprotein as

studied by a novel flow cell culture system. Free Radic Biol Med. 2004;37(5):682–94. https://doi.org/10.1016/j.freeradbiomed.2004.05.020.
58. Hosoya T, Maruyama A, Kang MI, Kawatani Y, Shibata T, Uchida K, et al. Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. J Biol Chem. 2005;280(29):27244–50. https://doi.org/10.1074/jbc.M502551200.
59. Fledderus JO, Boon RA, Volger OL, Hurttila H, Yla-Herttuala S, Pannekoek H, et al. KLF2 primes the antioxidant transcription factor Nrf2 for activation in endothelial cells. Arterioscler Thromb Vasc Biol. 2008;28(7):1339–46. https://doi.org/10.1161/ATVBAHA.108.165811.
60. Hsieh CY, Hsiao HY, Wu WY, Liu CA, Tsai YC, Chao YJ, et al. Regulation of shear-induced nuclear translocation of the Nrf2 transcription factor in endothelial cells. J Biomed Sci. 2009;16:12. https://doi.org/10.1186/1423-0127-16-12.
61. Goettsch C, Goettsch W, Brux M, Haschke C, Brunssen C, Muller G, et al. Arterial flow reduces oxidative stress via an antioxidant response element and Oct-1 binding site within the NADPH oxidase 4 promoter in endothelial cells. Basic Res Cardiol. 2011;106(4):551–61. https://doi.org/10.1007/s00395-011-0170-3.
62. Dai G, Vaughn S, Zhang Y, Wang ET, Garcia-Cardena G, Gimbrone MA Jr. Biomechanical forces in atherosclerosis-resistant vascular regions regulate endothelial redox balance via phosphoinositol 3-kinase/Akt-dependent activation of Nrf2. Circ Res. 2007;101(7):723–33. https://doi.org/10.1161/CIRCRESAHA.107.152942.
63. Zakkar M, Van der Heiden K, Luong le A, Chaudhury H, Cuhlmann S, Hamdulay SS et al. Activation of Nrf2 in endothelial cells protects arteries from exhibiting a proinflammatory state. Arterioscler Thromb Vasc Biol 2009;29(11):1851–7. https://doi.org/10.1161/ATVBAHA.109.193375.
64. Pearce MJ, McIntyre TM, Prescott SM, Zimmerman GA, Whatley RE. Shear stress activates cytosolic phospholipase A2 (cPLA2) and MAP kinase in human endothelial cells. Biochem Biophys Res Commun. 1996;218(2):500–4. https://doi.org/10.1006/bbrc.1996.0089.
65. Taba Y, Sasaguri T, Miyagi M, Abumiya T, Miwa Y, Ikeda T, Mitsumata M. Fluid shear stress induces lipocalin-type prostaglandin D(2) synthase expression in vascular endothelial cells. Circ Res. 2000;86(9):967–73. https://doi.org/10.1161/01.res.86.9.967.
66. Inoue H. Transcriptional and posttranscriptional regulation of Cyclooxygenase-2 expression by fluid shear stress in vascular endothelial cells. Arterioscler Thromb Vasc Biol. 2002;22(9):1415–20. https://doi.org/10.1161/01.atv.0000028816.13582.13.
67. McMahon M, Lamont DJ, Beattie KA, Hayes JD. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. Proc Natl Acad Sci U S A. 2010;107(44):18838–43. https://doi.org/10.1073/pnas.1007387107.
68. Kansanen E, Bonacci G, Schopfer FJ, Kuosmanen SM, Tong KI, Leinonen H, et al. Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. J Biol Chem. 2011;286(16):14019–27. https://doi.org/10.1074/jbc.M110.190710.
69. Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J Biol Chem. 2002;277(45):42769–74. https://doi.org/10.1074/jbc.M206911200.
70. Bloom DA, Jaiswal AK. Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. J Biol Chem. 2003;278(45):44675–82. https://doi.org/10.1074/jbc.M307633200.
71. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J Clin Invest. 2006;116(1):49–58. https://doi.org/10.1172/JCI24787.
72. Woo CH, Shishido T, McClain C, Lim JH, Li JD, Yang J, et al. Extracellular signal-regulated kinase 5 SUMOylation antagonizes shear stress-induced anti-inflammatory response and endothelial nitric oxide synthase expression in endothelial cells. Circ Res. 2008;102(5):538–45. https://doi.org/10.1161/CIRCRESAHA.107.156877.
73. Ohnesorge N, Viemann D, Schmidt N, Czymai T, Spiering D, Schmolke M, et al. Erk5 activation elicits a vasoprotective endothelial phenotype via induction of Kruppel-like factor 4 (KLF4). J Biol Chem. 2010;285(34):26199–210. https://doi.org/10.1074/jbc.M110.103127.

74. Clark PR, Jensen TJ, Kluger MS, Morelock M, Hanidu A, Qi Z, et al. MEK5 is activated by shear stress, activates ERK5 and induces KLF4 to modulate TNF responses in human dermal microvascular endothelial cells. Microcirculation. 2011;18(2):102–17. https://doi.org/10.1111/j.1549-8719.2010.00071.x.
75. Kim M, Kim S, Lim JH, Lee C, Choi HC, Woo CH. Laminar flow activation of ERK5 protein in vascular endothelium leads to atheroprotective effect via NF-E2-related factor 2 (Nrf2) activation. J Biol Chem. 2012;287(48):40722–31. https://doi.org/10.1074/jbc.M112.381509.
76. Hempel SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2′,7′-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. Free Radic Biol Med. 1999;27(1–2):146–59.
77. Warabi E, Takabe W, Minami T, Inoue K, Itoh K, Yamamoto M, et al. Shear stress stabilizes NF-E2-related factor 2 and induces antioxidant genes in endothelial cells: role of reactive oxygen/nitrogen species. Free Radic Biol Med. 2007;42(2):260–9. https://doi.org/10.1016/j.freeradbiomed.2006.10.043.
78. Jones CI 3rd, Zhu H, Martin SF, Han Z, Li Y, Alevriadou BR. Regulation of antioxidants and phase 2 enzymes by shear-induced reactive oxygen species in endothelial cells. Ann Biomed Eng. 2007;35(5):683–93. https://doi.org/10.1007/s10439-007-9279-9.
79. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, et al. Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. J Biol Chem. 2003;278(47):47291–8. https://doi.org/10.1074/jbc.M305150200.
80. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol. 2003;285(6):H2290–7. https://doi.org/10.1152/ajpheart.00515.2003.
81. Lee DY, Lee CI, Lin TE, Lim SH, Zhou J, Tseng YC, et al. Role of histone deacetylases in transcription factor regulation and cell cycle modulation in endothelial cells in response to disturbed flow. Proc Natl Acad Sci U S A. 2012;109(6):1967–72. https://doi.org/10.1073/pnas.1121214109.
82. Ungvari Z, Bailey-Downs L, Gautam T, Jimenez R, Losonczy G, Zhang C, et al. Adaptive induction of NF-E2-related factor-2-driven antioxidant genes in endothelial cells in response to hyperglycemia. Am J Physiol Heart Circ Physiol. 2011;300(4):H1133–40. https://doi.org/10.1152/ajpheart.00402.2010.
83. Afonyushkin T, Oskolkova OV, Philippova M, Resink TJ, Erne P, Binder BR, et al. Oxidized phospholipids regulate expression of ATF4 and VEGF in endothelial cells via NRF2-dependent mechanism: novel point of convergence between electrophilic and unfolded protein stress pathways. Arterioscler Thromb Vasc Biol. 2010;30(5):1007–13. https://doi.org/10.1161/ATVBAHA.110.204354.
84. Ungvari Z, Bailey-Downs L, Gautam T, Sosnowska D, Wang M, Monticone RE, et al. Age-associated vascular oxidative stress, Nrf2 dysfunction, and NF-{kappa}B activation in the nonhuman primate Macaca mulatta. J Gerontol A Biol Sci Med Sci. 2011;66(8):866–75. https://doi.org/10.1093/gerona/glr092.
85. Bailey-Downs LC, Mitschelen M, Sosnowska D, Toth P, Pinto JT, Ballabh P, et al. Liver-specific knockdown of IGF-1 decreases vascular oxidative stress resistance by impairing the Nrf2-dependent antioxidant response: a novel model of vascular aging. J Gerontol A Biol Sci Med Sci. 2012;67(4):313–29. https://doi.org/10.1093/gerona/glr164.
86. Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. Trends Immunol. 2003;24(8):449–55. https://doi.org/10.1016/s1471-4906(03)00181-9.
87. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc Natl Acad Sci U S A. 1968;61(2):748–55.
88. Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev. 2006;86(2):583–650. https://doi.org/10.1152/physrev.00011.2005.

89. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM. Ferritin: a cytoprotective antioxidant strategem of endothelium. J Biol Chem. 1992;267(25):18148–53.
90. Kwak JY, Takeshige K, Cheung BS, Minakami S. Bilirubin inhibits the activation of superoxide-producing NADPH oxidase in a neutrophil cell-free system. Biochim Biophys Acta. 1991;1076(3):369–73.
91. Jansen T, Hortmann M, Oelze M, Opitz B, Steven S, Schell R, et al. Conversion of biliverdin to bilirubin by biliverdin reductase contributes to endothelial cell protection by heme oxygenase-1-evidence for direct and indirect antioxidant actions of bilirubin. J Mol Cell Cardiol. 2010;49(2):186–95. https://doi.org/10.1016/j.yjmcc.2010.04.011.
92. Fujii M, Inoguchi T, Sasaki S, Maeda Y, Zheng J, Kobayashi K, et al. Bilirubin and biliverdin protect rodents against diabetic nephropathy by downregulating NAD(P)H oxidase. Kidney Int. 2010;78(9):905–19. https://doi.org/10.1038/ki.2010.265.
93. Heiss EH, Schachner D, Werner ER, Dirsch VM. Active NF-E2-related factor (Nrf2) contributes to keep endothelial NO synthase (eNOS) in the coupled state: role of reactive oxygen species (ROS), eNOS, and heme oxygenase (HO-1) levels. J Biol Chem. 2009;284(46):31579–86. https://doi.org/10.1074/jbc.M109.009175.
94. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, Koizumi S. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J Clin Invest. 1999;103(1):129–35. https://doi.org/10.1172/JCI4165.
95. Soares MP, Seldon MP, Gregoire IP, Vassilevskaia T, Berberat PO, Yu J, et al. Heme oxygenase-1 modulates the expression of adhesion molecules associated with endothelial cell activation. J Immunol. 2004;172(6):3553–63. https://doi.org/10.4049/jimmunol.172.6.3553.
96. Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic Biol Med. 2000;28(8):1303–12.
97. Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, Clinton Webb R, Lee ME, Nabel GJ, Nabel EG. Heme oxygenase-1 protects against vascular constriction and proliferation. Nat Med. 2001;693–8(7):6. https://doi.org/10.1038/89068.
98. Hayashi S, Takamiya R, Yamaguchi T, Matsumoto K, Tojo SJ, Tamatani T, Kitajima M, Makino N, Ishimura Y, Suematsu M. Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: role of bilirubin generated by the enzyme. Circ Res. 1999;85(8):663–71.
99. Zhang HP, Zheng FL, Zhao JH, Guo DX, Chen XL. Genistein inhibits ox-LDL-induced VCAM-1, ICAM-1 and MCP-1 expression of HUVECs through heme oxygenase-1. Arch Med Res. 2013;44(1):13–20. https://doi.org/10.1016/j.arcmed.2012.12.001.
100. Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes. 2008;57(10):2809–17. https://doi.org/10.2337/db06-1003.
101. Cheng X, Chapple SJ, Patel B, Puszyk W, Sugden D, Yin X, Mayr M, Siow RC, Mann GE. Gestational diabetes mellitus impairs Nrf2-mediated adaptive antioxidant defenses and redox signaling in fetal endothelial cells in utero. Diabetes. 2013;62(12):4088–97. https://doi.org/10.2337/db13-0169.
102. Sobrevia L, Cesare P, Yudilevich DL, Mann GE. Diabetes-induced activation of system y+ and nitric oxide synthase in human endothelial cells: association with membrane hyperpolarization. J Physiol. 1995;489(Pt 1):183–92.
103. Pereira A, Fernandes R, Crisostomo J, Seica RM, Sena CM. The Sulforaphane and pyridoxamine supplementation normalize endothelial dysfunction associated with type 2 diabetes. Sci Rep. 2017;7(1):14357. https://doi.org/10.1038/s41598-017-14733-x.
104. Axelsson AS, Tubbs E, Mecham B, Chacko S, Nenonen HA, Tang Y, Fahey JW, Derry JMJ, Wollheim CB, Wierup N, Haymond MW, Friend SH, Mulder H, Rosengren AH. Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. Sci Transl Med. 2017;9(394):eaah4477. https://doi.org/10.1126/scitranslmed.aah4477.

The Role of Nrf2 in the Cardiovascular System and Atherosclerosis

Bryan J. Mathis and Taixing Cui

Abstract The cardiovascular system is an important transport system comprised of the heart and associated blood vessels. Within this transport network, various biochemical reactions (including gas transfer, immune modulation, waste transport, and fluid transfer) take place within a three-layered vascular structure that is highly susceptible to damage from bacterial polysaccharides, elevated blood lipids, immune by-products, and reactive oxygen species (ROS). Recently, ROS have come to the forefront of translational research as reports show a key role for ROS and unchecked vascular cell proliferation in the development of atherosclerotic plaques as well as damage to the myocardium. Of prime importance in the maintenance of homeostasis against these insults is the body's innate antioxidant system which is controlled almost entirely by the master transcription factor Nrf2. This chapter will explain the molecular mechanism behind the regulation of Nrf2, explore the impact of Nrf2 on the cardiovascular system and probable link to autophagy, and explicate the large number of exogenous chemical regulators of Nrf2 that are available for use in both in vitro and in vivo cardiovascular studies.

1 Introduction and Summary of Nrf2/Keap1 Axis

The flux between constitutively expressed nuclear factor erythroid 2-related factor 2 (Nrf2) and its regulator Kelch-like erythroid cell-derived protein with CNC homology-associated protein 1 (Keap1) are responsible for homeostatic control of reactive oxygen species (ROS) generated as natural by-products of the normal metabolism of oxygen or by exogenous insults. Nrf2 is in the basic leucine zipper (bZip) family of Cap "n" Collar (CNC) transcription factors and is known to be constitutively expressed and found in the cytoplasm where it is regulated by Keap1 which contains two ß-propeller spheres which bind to Nrf2 and facilitate its

B. J. Mathis
Faculty of Medicine, University of Tsukuba Affiliated Hospital, Ibaraki, Japan

T. Cui (✉)
Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, Columbia, SC, USA
e-mail: Taixing.Cui@uscmed.sc.edu

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K48-linked polyubiquitination and subsequent degradation in the proteasome by E3 ubiquitin ligase complex Cullin3 [1–4]. Nrf2 directly controls up to 1% of total gene expression (antioxidant response, metabolism, DNA repair, etc.) and plays a critical role in cellular defense [5]. Although Nrf2 itself has no sensitivity for ROS, the exquisite sensitivity of Nrf2 suppressor Keap1's cysteine residues, especially Cys273 and Cys288, to oxidative attack by reactive oxygen electrophiles plays a critical role in rapid response to electrophilic insults [6]. Upon attack by ROS, these residues cause the spherical propellers of Keap1 to undergo a conformational change to release Nrf2, which translocates to the nucleus, interacts with adaptor protein Maf2 and attracts Creb and p300 to form a transcriptional activator complex on the upstream antioxidant response element (ARE) that upregulates genes such as heme oxygenase 1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GPX) that form the cornerstone of the homeostatic response to ROS [1, 7–9]. As Nrf2 has a relatively short half-life of about 20–30 min vs. 12.7 h for Keap1, the cost to the cell of constantly turning over Nrf2 is compensated by the ability to rapidly respond to ROS that occurs as a normal part of mitochondrial metabolism [7, 10]. As ROS are part and parcel of aerobic metabolism, mammalian cells have evolved an elegant and multi-tiered response system where antioxidant/detoxification enzymes are regulated in phases, with Phase I genes under control of Aryl hydrocarbon receptors (AhR) or pregnane X receptors, Phase II genes under control of Nrf2 and Phase III genes such as P-glycoprotein mainly serving a barrier role [11, 12]. Translational science has therefore focused on these defense systems, especially Phase II, at a mechanistic level to determine whether or not exogenous control could provide better patient outcomes in cases of diseases that are known to result from or present large amounts of chronic oxidative stress such as atherosclerosis, diabetes, or heart failure.

Because it is the keystone transcription factor for antioxidant defense, it is constitutively expressed in most somatic cells (removing the risk of inserting chimeric genes into a patient), and there are a plethora of reagents and molecular tools to study it, Nrf2 has become an intensely studied gene that carries potential for direct intervention in oxidative diseases and the aging process. This chapter will briefly cover known characteristics of Nrf2, outline its regulation and role within the body (with a focus on the cardiovascular system), and examine the many exogenous regulators that are used in translational studies to dissect the precise position of Nrf2 in the machinery of oxidative disease. Additionally, recent evidence has shown links between Nrf2 and the autophagic system in chronic heart disease and this evidence will be analyzed for new insights into late-stage therapies for improved treatment outcomes.

Table 1 The molecular characteristics of Nrf2

Nrf2 Region Name	Function [6, 8, 15]
Neh1	CNC-bZIP dimerization domain for Maf and NLS for nuclear localization
Neh2	Contains motifs to bind Kelch sequences on Keap1 and lysine residues receive polyubiquitination for degradation. Sensitive aa 69–84 on Nrf2 ETGE region bind Keap1 even if as low as 5 nM and DLG region of Neh2 can provide secondary binding.
Neh3	Contains CHD6 transcriptional coactivator. Fyn binding region.
Neh4	Contains cAMP response element binding (Creb) protein (CBP) (CH3 domain-binding)
Neh5	Binding region for CBP (CH3 domain-binding)
Neh6	Degradation region (degrons) containing redox-sensitive sequences. Targeted by E3 ubiquitin ligase ß-TrCP
Neh7	Contains regulatory elements for fatty acid metabolism-associated retinoic X receptor alpha (RXR-alpha) binding

1.1 Characteristics of Nrf2

1.1.1 Molecular Characteristics

Nrf2 is a 67.8 kDa protein of 605 aa whose 6 exon coding region on human chromosome 2 (2q31) translates to a 2859 bp mRNA [9]. Nrf2 has been resolved by X-ray diffraction to 1.5A resolution [13]. Three natural variants have been reported with R43Q, S99P, and V268M [13, 14]. Multiple functional polymorphisms of and mutations within the Nrf2 coding sequence have been discovered and these may lead to both increased disease risk or resistance of cancers to chemo- and radiotherapy [9]. Under basal conditions, Nrf2 levels are highest in the esophagus, with high levels in every tissue (except lipids) and the pancreas [13, 14]. Nrf2 is highly conserved in lesser organisms, including mice and zebrafish, which serve as animal models to study Nrf2 influence on both redox homeostasis and metabolism in general.

Nrf2 contains 6 exons that code for 7 "Nrf2-ECH homology domains" or Neh1 through 7 (Table 1).

1.1.2 Mechanism of Induction

The canonical model for Nrf2 stress response has four discrete stages: basal, pre-induction, induction, and post-induction [16].

Stage 1: Basal

At this stage, Nrf2 is constitutively expressed, bound to Keap1 and degraded via K48-linked polyubiquitination via the Cul3 complex (see Characteristics of Keap1 below for details on this interaction) and subsequent digestion by the 26S proteasome. Nrf2 binds Keap1 at the ETGE/DLG regions [6, 8, 15].

Stage 2: Pre-Induction

During this stage, suppressors of Nrf2 are exported from the nucleus and degraded via phosphorylation of tyrosine residues on Bach1, INrf2, and Fyn [16].

Stage 3: Induction

Once freed from Keap1, Nrf2 has been shown to translocate to the nucleus via importin a5 and B1 that recognize nuclear localization signals (NLS) located on both the N-terminal (aa 587–593) and N-terminal regions (aa 42–53) [17]. Once localized, adaptor protein complexes form around Nrf2. These include: Creb, MafK, and p300 [1, 18, 19]. Transcription of antioxidant defense genes such as HO-1, NQO1, and GPX begins.

Stage 4: Post-Induction

Intranuclear control of Nrf2 is accomplished by the delayed accumulation of Fyn kinase that phosphorylates Y568 on Nrf2, forcing its export via chromosomal region maintenance-1 (Crm-1) 4–5 h after initiation of antioxidant transcription [20]. There is some evidence that Keap1 also plays a role in post-inductive repression as it contains a leucine/isoleucine-enriched nuclear export signal carrier that interacts with Crm1/exportin [21, 22]. However, there are also reports that a redox-insensitive Nrf2 nuclear export signal on the leucine zipper (bZIP) located in Neh1 also facilitates export [23, 24].

1.2 Characteristics of Keap1

Keap1, discovered by Itoh and colleagues in 1999, consists of a Broad complex, Tramtrack, and Bric-a-brac region (BTB) which is spaced from C-terminal Kelch repeats by an intervening region (IVR) [2, 25–27]. It has been found to localize (80%) in the cytoplasm [17]. It is the Kelch repeats that provide for Nrf2 binding and these form a six-bladed ß-propeller structure whose DA and BC loops are capable of binding Nrf2 [6]. Described as a “hinge and latch” model, the BTB regions of 2 Keap1 molecules dimerize and the Kelch domains of each will bind to the ETGE (primary hinge) and DLG (secondary latch) regions of Nrf2-Neh2 to form a pyramidal complex that exposes the Nrf2 lysine residues to ubiquitin ligation [6, 8, 16].

The generally accepted model for Keap1 regulation of Nrf2 in all tissues is the Cul3 thiol modification model that relies entirely on 25 (murine) or 27 (human) cysteine residues within Keap1 that are highly susceptible to electrophilic attack [27]. The BTB regions bind Cul3, which in turn binds a RING ligase (Rbx1) and this complex then links polyubiquitin to specific lysine residues in the Neh2 region of Nrf2 in a K48 fashion, facilitating its degradation [4, 6]. On the other hand, under high ROS conditions, electrophilic attack on C151 in the BTB as well as C273 and C288 in the IVR force a conformational change and the release of ubiquitinating adaptor protein Cul3 from the Keap1-Nrf2 complex, releasing Nrf2 to translocate to the nucleus [10, 27].

There is evidence that the Kelch repeats of Keap1 interact with importin alpha7 (KPNA6) to shuttle it into the nucleus where it is capable of repressing Nrf2

transcriptional activity as well as facilitating export using a nuclear export signal (NES) [21, 28].

1.3 Characteristics of ARE and Functional Mechanism of AOX Activation

The antioxidant response element (ARE) is a 16 nt sequence found upstream of detoxification and antioxidant proteins that acts as a cis regulator of transcription [15]. The aforementioned Nrf2-Creb-Maf-p300 complex then binds to this area to recruit and activate RNA polymerases through factors such as RNA polymerase II kinase (Cdk12) to drive transcription [29–32].

2 Links Between Nrf2 and Pathways Outside of Redox Homeostasis

The canonical pathway of Nrf2, both in its regulation and its downstream effects, has been extensively detailed and reviewed in the literature. However, as befits its position as a central regulator of transcription, there has been a wealth of experimental evidence that points to connections with other signaling networks. As redox homeostasis is an expensive proposition, errant activation may constitute lost opportunity costs for other needs, necessitating tight and redundant control of Nrf2 and it targets to prevent this from happening. What follows is a brief summary of some of the more well-known links that Nrf2 has outside of its own place as a Phase II activator.

2.1 Links to Apoptosis/Necrosis

Nrf2 has been investigated as a regulatory factor in cellular apoptosis and necrosis. In studies linked to the Nrf2 exogenous chemical regulator bardoloxone (CDDO, to be discussed later), Nrf2 was found to have an upregulatory effect on apoptotic factors such as slug, Bax, TCF-ZEB1, Snail, and downregulate specific caspases essential to necrosis such as caspases 3,8 and 9 while also forcing iκB kinase disruption to downregulate NF-κB activation and push cells to undergo apoptosis [30, 33–35]. Although Nrf2's primary role is to maintain redox homeostasis, it may play a role as a key cog in the apoptotic failsafe machinery to prevent the tissue damage and edema that result from release of necrotic debris such as mitochondrial cytochrome c or other nitrosamines [34, 36]. However, a recent experimental report has tied Nrf2 to enhancement of cardiac necrosis in an environment of autophagic

dysfunction and studies have shown that late-stage apoptotic cells that are not cleared quickly by APCs may shift to necrotic death [37, 38]. This new evidence may require a revisiting of Nrf2's role in necrosis avoidance with respect to the stages of apoptosis and intricate trigger mechanisms for the pathways involved.

2.2 Links to Autophagy

Autophagy, or "self-eating" is a mechanism by which damaged or aggregate proteins can be digested and recycled. It is inversely linked to the ubiquitin/proteasome (Ub/Prot) system and differs in that only autophagy can recycle entire organelles (such as damaged mitochondria) and uses selective autophagic receptors (SAR) while the Ub/Prot system functions only on ubiquitinated proteins of small to moderate size [39]. Under basal conditions, the Ub/Prot system maintains protein clearance, but, under starvation conditions or if the Ub/Prot system is inactivated, the autophagic machinery is activated in a compensatory manner by autophagic adaptor p62 and activation of normally suppressed mTORC1 [40]. This indicates an extensive amount of crosstalk and contraregulation between the two systems. For example, in retinal pigment epithelial cells, it was reported that autophagic proteins LC-I, LC-II, LAMP-1, and p62 were regulated by ubiquitination [41]. Of interest to this review, however, is the discovery of the p62-Keap1-Nrf2 axis that plays a key role in the regulation of cellular redox stress. Keap1 has been experimentally shown to interact directly with p62 which forces it to release Nrf2 and be degraded in the autophagosomes while Nrf2 itself has been shown to directly increase p62 transcription, establishing an upregulatory feedback loop between Nrf2 and autophagic induction [40, 42]. The PI3K/AKT pathway that regulates autophagy is also required for Nrf2 activation [43]. In addition, there is experimental evidence that Nrf2 directly regulates sestrin2, which, in turn, inhibits mTORC1 and autophagic induction while acting as a cofactor for p62/Keap1 binding [44, 45]. It is therefore clear from the literature that Nrf2 is intimately tied into the autophagic system but its role within a damaged or nonfunctional autophagic system has yet to be fully explored within the cardiovascular system [37].

2.3 Links to Proteasomal Regulation

The proteasomal degradation system has been extensively studied for its role in controlling protein turnover and misfolded protein clearance by ubiquitin tagging, and there are myriad reviews and experimental reports that dissect the complete proteasomal mechanism and its targets. Canonically, K48-linked polyubiquitination and subsequent 26S proteasomal degradation is the primary controller of Nrf2 levels within the cytoplasm. However, there is some evidence that, at least within a developmental context, Nrf2 is able to control the proteasomal machinery by

colocalization with OCT4 and NANOG as well as proteasome maturation protein (POMP) [46]. There is also evidence that, Nrf2, due to its link with autophagic switch p62 and interaction via Ser40 with the BIP/IRE1/PERK pathways of the unfolded protein response (UPR), is able to function as a central decision branch that activates either autophagy or the proteasome in response to misfolded proteins [47]. The question of differential modification of Nrf2 residues (serine versus lysine) leading to clearly different outcomes of Nrf2 activation (proteasomal activation versus detoxification) has therefore become another factor that complicates clear and precise mechanistic understanding of Nrf2 within the cell.

2.4 Links to Other Key Signaling Pathways

Nrf2 has multiple ties to diverse other signaling pathways and these ties have been experimentally determined in diverse somatic cell types such as macrophages, endothelial cells, and osteocytes. Of primary importance is the link between Nrf2 and the p38-MAPK family of factors that control Nrf2 transcription such as MEK, BRAF, c-jun, c-fos, and c-myc as well as genes like CYLD that were recently found to also affect the p38/AP-1 axis to suppress Nrf2 [48]. Evidence of direct phosphorylation of Nrf2 by MAPK in a Keap1-dependent manner has also been reported, cementing the link between the two pathways [49, 50]. There is also ample evidence of ties to other systems such as RANKL in osteocytes, regulation of Nrf2 by SUMOylation, regulation of Nrf2 by endogenous miRNAs, and increase of Keap1 binding by cofactor p21 [51–55]. There is also a connection between Wnt and Nrf2, as WNT-3A was found in hepatocytes to regulate an Axin/Nrf2 transcriptional complex and is involved in PPARγ interaction with Nrf2/Wnt/ß-catenin and forkhead box proteins such as FOXO [56, 57]. Additionally, Nrf2 crosstalk with Notch and evidence of angiogenesis being regulated by this axis has been reported; Wakabayashi and colleagues have extensively reviewed the Nrf2/Notch link, especially in hepatocytes [58, 59]. Interestingly, there is experimental evidence that Nrf2 could act as a counterweight to AngII upregulation of MAPK factors that cause cardiac hypertrophy by upregulating antioxidant response that protects against the downregulation of cyclin-dependent kinase inhibitor p27(kip1) although direct mechanisms of AngII/Nrf2 interaction are not yet known [60]. This raises the question as to whether or not established pathways such as Wnt, AngII, and Notch may have even deeper involvement with Nrf2 than previously believed.

Nrf2 is usually seen as an antioxidant transcription factor and is often studied by assuming isolation from other cellular systems. However, this approach may lead to paradoxical or otherwise confusing results as ample evidence of Nrf2 ties to critical signaling systems as diverse as Notch, Wnt, AngII, and PPARγ have been reported. Nrf2 could be thought of as an epigenetic regulator in this regard and future studies would therefore have to take into account the far-reaching effects that exogenous Nrf2 manipulation may cause in experimental animal and cell line-based models.

3 The Role of Nrf2 in the Cardiovascular System

The cardiovascular system is comprised of a vast network of tri-layered vessels that are either elastic and large (aorta, carotid arteries, etc.) bodies that equalize blood pressure between systole and diastole or muscular, flexible vessels (e.g., femoral arteries) that flex to adapt their tone to the mechanical force generated by the rushing blood from the heart [61]. The heart itself is a four-chambered, muscular organ that self-regulates its pumping action, adapts to oxygen demand from the musculature and is richly oxygenated by a network of coronary arteries. An adult heart pumps an average of 7200 L of blood every 24 hours and as every cell in the body must receive energy (glucose) and electron acceptors (oxygen) to maintain homeostasis, cardiovascular health impacts every other organ system [62]. There are three key elements to the cardiovascular system that allow it to function: the morphology of the blood vessels, the composition of blood and its various fractions, and the myocardium itself with its timed pumping action. This section will detail these key elements as well as the role that Nrf2 plays within each system and in the homeostasis of the cardiovascular system as a whole. A short review of the literature concerning various cardiovascular diseases and the role that Nrf2 has played in experimental studies of these maladies will follow.

3.1 The Role of Nrf2 in Blood Vessels

Blood vessels are formed of a mesh composite of layers consisting of the outermost adventitia (fibrous, collagen-enriched structural support), the media (a band of smooth muscle-enriched tissue that provides elasticity), and the inner intima layer (endothelial cells that provide a smooth, snagless lining) [63]. Each layer is uniquely constructed to provide a structural role (support, tone, lining) as well as a biochemical/cellular milieu for handling repairs and maintaining integrity after adverse events. The adventitia, composed of fibroblasts, nerve innervation points, and connective tissue, is generally thought to function as structural support, harbor a niche population of stem/progenitor cells, serve as an immune trafficking interface (for APC extravasation, etc.) and, most importantly, nourish the medial layer through the microvasculature of the vasa vasorum [64]. The medial layer, as the key element that maintains vascular plasticity, consists of mature smooth muscle cells expressing proteins such as smooth muscle actin, elastin, matrix metalloproteases (that modulate collagen and elastin), and myosin heavy chain, and these cells also benefit from endothelially produced endogenous nitrous oxide (NO) as a signaling mechanism to maintain their elasticity [61, 63, 65]. The intimal layer consists of a multilayered structure that connects to the media with a thin connective layer, and this underlies the stratum of endothelial cells that interface with the lumen and produce endogenous nitrous oxide synthase (eNOS) and NO to maintain tone [66]. Production of eNOS is this layer's greatest contribution to vascular stability, as

eNOS serves to regulate tone, prevent thrombosis, regulate blood flow, and prevent errant proliferation of vascular stem cell progenitors, all of which may cause vascular disease [67]. The pressure within the vessels themselves is regulated in real time by the renin-angiotenin-aldosterone axis (RAAS), which consists of the rate-limiting conversion of the precursor angiotensinogen (Ang I) to active angiotensin (Ang II) which then activates AngII receptor AT1 to control osmotic blood pressure with the electrolyte-regulating hormone aldosterone [68].

This unique composite morphology gives the blood vessel network two main characteristics: elasticity and capacity for self-repair and Nrf2 has been found experimentally to play a large supporting role in these two functions. Elasticity of blood vessels relies mainly on endogenous nitrous oxide, and there has been much research done on the regulation of this critical blood gas. It has been reported that Nrf2, through transcription of HO-1 whose enzymatic activity reduces eNOS levels, activates under redox attack when levels of eNOS reaction-limiting cofactor tetrahydrobiopterin are low to modulate eNOS levels [69]. A report by Luo and colleagues has also found that Nrf2 can transduce through the DDAH/PPARγ pathway to activate eNOS transcription as eNOS does contain putative ARE regions [70]. In a reciprocal fashion, the mechanical shear stress of the blood vessels has been found to generate ROS through a lipid oxidation by xanthine oxidase/flavoprotein, and this maintains transcription of Nrf2 [71–73]. The capacity for self-repair is a critical mechanism to maintain the integrity of the vascular wall and Nrf2 also plays a role in this machinery. Recent experimental evidence in a murine model of vascular injury points to Nrf2 acting on VSMC apoptotic mechanisms to prevent neointimal hyperplasia while generation of antioxidant factors such as HO-1 may defend against further injury by neutralizing ROS generated by local immune responses [74, 75]. Furthermore, there is evidence of stem cell proliferation and errant angiogenesis being regulated by Nrf2 through its upregulation of IL-8 and HO-1, which has been found to interact with VEGF and reduce ROS to the lower levels where it may act as a critical second messenger for proliferation [76–78]. Other pathways known to play a role in stem/progenitor proliferation (such as Notch, Wnt, etc.) may see Nrf2 play a secondary role in regulation through upregulation of its downstream targets [79].

3.2 The Role of Nrf2 in Blood

Blood is a catchall term for the cardiovascular fluid and is comprised of multiple fractions: red blood cells (anuclear cells containing hemoglobin for oxygen exchange), serum (proteins, electrolytes, hormones, and non-fibrinogenic factors), plasma (proteins such as albumin, CO_2, glucose, and water), and various immune and clotting cells (monocytes, thrombocytes, leukocytes, etc.). Oxygen exchange from red blood cell to somatic cells within the capillary beds has been well-studied and is outside the scope of this review. However, it is worth noting that experimental evidence showing Nrf2 protecting erythrocytes and their progenitor hematopoietic

stem cells from oxidative stress has been reported as well as fate determination of these stem cells by the Keap1-Nrf2 axis [80, 81]. Of primary importance to cardiovascular research is the buffy coat, or immune cell-enriched fraction, that contains a milieu of both inactive cells (e.g., macrophages) and progenitors (such as monocytes) that can be stimulated by cellular debris or necrosis released by vascular injury. In these cases, it can be expected that inflammation resulting from upregulation of induced NOS (iNOS), superoxide anions, cytokines (TNF, IL-6) and degranulation of mast cells will wreak havoc on the surrounding tissue. In this case, the primary mode of Nrf2 action in neutralizing this excess ROS via upregulation of antioxidant defense has been proven in the literature beyond any doubt. However, there is also recent evidence that Nrf2 may act to directly suppress inflammatory cytokines such as IL-6 and IL1β in macrophages by binding to their upstream regions that recruit RNA Pol II and block transcription [82]. This effect has also been shown in IL-12 suppression by Nrf2 in murine dendritic cells sensitized by arsenic [83]. Clearly, this dual role in both direct inhibition of vessel-damaging immune cells and neutralization of the induced ROS/NOS by Nrf2 makes it a powerful mediator of the immune system which can be exploited in cardiovascular disease studies.

3.3 The Role of Nrf2 in the Myocardium

The heart is the keystone organ of the cardiovascular system and contains four chambers (two atria for collecting blood and two ventricles for pumping it to the lungs and body) along with a dedicated vessel system (pulmonary vein and artery) to exchange oxygen in the lungs. The myocardium is the functional muscle unit of the heart and is comprised of multiple cardiomyocytes arranged in striations and with a fractional amount in the sinoatrial node (about 1%) capable of producing spontaneous electrical signals to coordinate the pumping efforts of their brethren. The heart itself continuously pumps and the resultant large demand for oxygen and energy is supplied to the myocardium via two coronary arteries and a network of branches that prevents ischemic insult during peak demand.

Within the cardiomyocytes, the demand for Nrf2 is high as free radicals such as nitric oxide, superoxide anion and peroxides are produced in large quantities by the constant nature of the pumping [84]. It also has the effect of controlling errant hypertrophy (and subsequent decompensation) due to pressure increases by the RAAS axis while responding to ROS generated by exercise that could cause myocardial damage [85]. Interestingly, increases in Nrf2 during exercise do not cause hypertrophy and maladaptive remodeling as in cases of heart disease, most likely due to a functional autophagy system in healthy hearts [37, 86, 87]. It is therefore generally accepted that, in healthy hearts, Nrf2 is a beneficial gatekeeper that downregulates levels of ROS before they can damage the myocardium.

3.4 The Role of Nrf2 in Cardiovascular Diseases

As the cardiovascular system is a complex and multi-layered organ that is interconnected by nature with every other system and intraconnected by the blood it carries, it stands to reason that diseases affecting this system would be equally complex and varied. The World Health Organization (www.who.int) estimates that 17.5 million people die each year from cardiovascular disease (which amounts to 31% of total deaths), and 80% of these deaths are from heart failure or blood vessel blockages (i.e., atherosclerotic strokes). Diseases of the cardiovascular system are therefore the global number one killer of humans and are thought to be largely preventable by diet and exercise. However, cumulative risk factors such as smoking, high blood lipids, and obesity may, over time, cause a cascade of damage that leads to the chief triad of diabetes, atherosclerosis, and heart failure. Most cardiovascular disease results from chronic exposure to both environmental insults (cigarette smoke, heavy metals such as arsenic and mercury) and dietary factors such as chronically high blood sugar that damages the delicate intimal endothelium with glycation end products that overwhelm antioxidant defenses. It is for this reason that Nrf2 has been examined extensively in the context of both maintaining the defense redox barrier and imparting additional protection in compromised vessels and hearts. What follows is a short examination of the three chief cardiovascular diseases and the role that Nrf2 has been found to play in their prevention and pathogenesis.

3.4.1 Diabetic Vasculopathy and Nrf2

Diabetes is a disease of unchecked blood sugar regulation that is generally split into Type I (no functional insulin production) and Type II adult onset (insulin resistance leading to hyperglycemia) and no cure currently exists for either. The WHO indicates that 8.5% of the global population in 2014 suffer from diabetes and it caused a total estimated death toll of 3.7 million people. From a cardiovascular standpoint, Type II diabetes has an intimate association with disease from osmotic and AngII-mediated increases in blood pressure, cardiomyocyte toxicity from hyperglycemia, atherosclerosis, vascular inflammation, kidney disease, necrosis of blood vessels in the extremities (necessitating amputation) and heart failure. This means that diabetic patients eventually become cardiovascular/heart failure patients, necessitating the search for interventions that can reduce both diabetic and cardiovascular sequelae. The main factor in these cases is chronic hyperglycemia, which reduces the amount of endogenous NO and increases vasoconstriction endothelin-1 as well as upregulating insulin, which in turn upregulates the triglyceride production in the liver [88]. Hyperglycemic patients have also been found with higher levels of inflammatory cytokines such as TNFα, IL-1β, and IL-6 in a fashion similar to obese patients [88]. Furthermore, recent clinical evidence has shown elevated levels of the inducible inflammation upregulator COX-2 protein in diabetic heart patients after cardiopulmonary bypass [89]. Hyperglycemia can additionally affect even the

response of vessels to contractile signals by upregulating PKCα, PKCβ, and nitrotyrosine as was found in clinical studies of poorly controlled diabetes where arterioles of poorly controlled diabetics could not respond as well to endothelial vasoconstrictors ADP and substance P while expressing high levels of PKCα, PKCβ, and nitrotyrosine [90]. It is this constant attack on the vasculature, including the coronary arteries, that can lead to atherosclerosis/heart failure and Nrf2 intervention would be expected to attack diabetic sequelae by ameliorating damage due to ROS induced by oxidized blood lipids and inflammatory cytokines.

Nrf2 has been found to protect against diabetic sequelae in several systems, including the cardiovascular system. It was recently found, for example, to mediate the production of downstream metallothionein production in cardiomyopathy as well as downregulate formation of foamy macrophages [91, 92]. It also protects pancreatic cells from apoptosis by downregulating TRAIL and protecting transcription of proteasomal genes, which could maintain a critical source of insulin to control blood sugar [93]. In fact, multiple studies have shown that 4-hydroxy oxidized lipids such as 4-hydroxynonenal and 4-hydroxy hexenal can activate Nrf2 to protect against redox insult, proving that a robust Nrf2 response is a first line defense against hyperglycemic-mediated triglycerides and advanced glycation end products [74, 94, 95]. The effects of Nrf2 are indeed profound in organs affected by diabetes; Shelton and colleagues found that bardoloxone-methyl intraperitoneal injections into Nrf2 knockout mice caused alterations in 2561 transcripts and 240 proteins in the kidneys while WT mice saw changes in 3122 transcripts and 68 proteins, including redox balancers (GPX), and Nqo1 [96]. It could be correctly assumed that Nrf2 would have a similar effect in the cardiovascular system but several clinical trials (NTC01053936, NCT00811889, clinicaltrials.gov) that were completed 5–10 years ago have had no comprehensive study results published. In fact, there is some thought that the harmful nature of Nrf2 in diabetes may be due to ROS attenuation to the point where is no longer an effective secondary messenger to regulate protein turnover [97]. As Nrf2 upregulates CD36 (as a backup to PPAR) and as Type 2 diabetic rodents display enhanced cell surface expression of CD36 on the plasma membrane, Nrf2 may act as a negative modulator of glucose usage in favor of fat oxidation, complicating vascular health via oxidative damage [97–99]. It is, again, the dual nature of Nrf2 that seems to have BOTH cardiovascular protective and deleterious effects in diabetics, and this effect in animals has been well studied and reviewed [97]. However, the precise mechanism of switching on Nrf2's harmful or helpful effects in diabetic humans has yet to be fully explored and studying Nrf2 links to other regulatory pathways (such as Notch or Wnt) may prove fruitful in helping discover the true efficacious nature of Nrf2.

3.4.2 Atherosclerosis and Nrf2

Atherosclerosis is a narrowing of the coronary arteries (that oxygenate the myocardium) that causes chronic ischemia and a reduction in glucose supply to the cardiomyocytes. Narrowing of these arteries has been determined to result from plaques of fatty deposits enriched with immune cells that sustain an inflammatory cascade that traps additional lipids [100]. Basically, injury to the intimal endothelium (from hyperglycemia, mechanical injury, LPS, inflammation, etc.) causes lipids to stick and the resultant inflammation attracts macrophages and dendritic cells that consume cholesterol-laden debris until they become a perpetually activated foamy cell that infiltrates the intimal and medial layers to saturate them with inflammatory cytokines that cause ROS damage as well as signaling for vascular stem cell proliferation [63]. This plaque is thought to be responsible for the phenomenon of ischemia/reperfusion injury. It has been well studied in the literature that chronic ischemia causes an increase in ROS but reperfusion damage occurs if blood flow is spontaneously restored by plaque rupture or after bypass surgery [101]. During ischemia, it is generally accepted that mitochondrial dysfunction resulting from depolarization of membranes during errant calcium overload as well as conformational changes in oxidizing enzymes such as xanthine oxidase (which generates deleterious oxidized lipids) are compounded with the inflammatory response from reperfusion that bathes the myocardium in a sea of activated immune cells [102, 103]. Additionally, ROS from both ischemia and the Ang II system (via NOX2/4) play an important second messenger role in vascular proliferation by activating Src, CDK1, PDGFR, PKC, and other proliferative factors [104]. Although the typical hyperglycemia/cholesterol/high blood pressure suite of risk factors is usually blamed for atherosclerosis, it is also important to note that this progressive degradation of vascular integrity can occur from sequelae resulting from chronic kidney disease (CKD) which exacerbates the effect of the RAAS and injurious mechanical force on the coronary arteries [105]. This "cardiorenal anemia syndrome," with the kidneys as a bellwether for vascular health, represents a progressively debilitating cascade of alternating renal and heart stress that can reduce survival rates in CKD patients due to atherosclerosis [106]. Clearly, then, therapeutic intervention must address vascular issues both in the myocardium and the kidneys. It is for this reason that Nrf2 has been extensively studied in both organs.

The mechanisms by which Nrf2 can reduce stress in both coronary and renal arteries have been detailed in a previous section. The main mode of expected action in this case would be suppression of ROS and vascular proliferation, but it seems as if clinical studies using exogenous regulators have introduced an entirely new set of questions. The experimental oleanic compound bardoloxone (CDDO) and derivatives has been proven in multiple *animal* studies to upregulate Nrf2 but several Phase I human trials of CDDO for cancer reduction were discontinued because of adverse cardiovascular effects [107]. As the Nrf2 clinical field is primarily interested in chemotherapeutic considerations, there are very few clinical cardiovascular trials in which Nrf2 has been studied but several human trials have found increases in glomerular filtration rate which could contribute to defense against cardiorenal

anemia syndrome but will still harm the myocardium [107]. As an example, clinical trial NCT01351675, sponsored by Reata Pharmaceuticals for bardoloxone-methyl treatment in Stage 4 kidney patients, was terminated after a 4-year period because of strong evidence of cardiovascular death [108]. It has been well reported that Nrf2 is anti-atherogenic, with upregulation of endogenous Nrf2 being linked to a reduction in atherosclerosis via upregulation of HO-1 and antioxidant defense [74, 75, 109]. However, there are several reports in ApoE knockout mice that show increased Nrf2 production as atherogenic and this is thought to be based on interactions between the lipoprotein levels and immune scavenging as mediated by CD36 [110–112]. This hints at the dual nature of Nrf2 and that "more" isn't always "better," as endogenous Nrf2 is regulated at multiple junctions to prevent atherogenic effects but exogenous regulation ignores the lipoprotein and immune status in the vascular microenvironment. It is therefore obvious that the intricate regulatory network outside the canonical Nrf2 pathway are at work in these human trials and further work will be needed to see how Nrf2's relationship with other major pathways (angiogenesis, stem cell proliferation, etc.) are affecting its therapeutic potential for atherosclerosis.

3.4.3 Cardiac Disease and Nrf2

Heart failure is a progressive disease of the myocardium, where biochemical and mechanical pressures trigger a cascade of maladaptive hypertrophy with a gradual decline in pumping output. Ischemia/reperfusion injuries from atherosclerosis as well as infections (pericarditis) and kidney disease or diabetic complications can all trigger the failure cascade. There are four stages of heart failure as classified by the American Heart Association: Stage A, B, C, and D. In Stage A, risk factors such as hypertension, smoking, and high blood triglycerides set a patient up for Stage B, where compensation sequelae such as hypertrophy of the left ventricle, lower ejection fraction, and systolic dysfunction occur [113]. Stage C is a transitory stage to eventual collapse of cardiac function and sees a microenvironment of cardiac necrosis, maladaptive cardiomegaly, increased ROS, and defective autophagy within the cardiomyocytes [48, 114]. At Stage D, patient prognosis shifts to symptomatic relief and transplant as the myocardium is now a scarred, stiff mass that consists mostly of dying cells, inflammation and greatly reduced pumping capacity. It is within this framework of classification that the greatest effort of translational science has focused its efforts to catalog, analyze, and dissect the precise mechanisms by which a stressed heart eventually fails without direct intervention.

Within the failing heart, several mechanisms that contribute to the irreversibility of the disease: First, excessive ROS generated by cardiomyocyte mitochondria, eNOS neutralization, and secondary factors such as bacterial lipopolysaccharides damage cells and initiate inflammatory immune cascades that cause further damage [115, 116]. Second, failures in molecular machinery such as calmodulin/caveolin transport of critical calcium Ca^{2+} to B-adrenergic receptors that stimulate excitation–contraction and autophagy that clears aggregate proteins occurs [117–120].

There is some probable connection between calmodulin and Nrf2, as the calmodulin/CaMKII/ERK pathway has been experimentally shown to induce Nrf2 as a protective measure in macrophages [121]. However, an interesting new report from Qin and colleagues has found a dual nature of Nrf2 that relies on functional autophagy to ameliorate a murine model of heart disease but causes maladaptive remodeling if autophagy is nonfunctional [37]. Autophagy, as discussed earlier, is an important cellular recycling system for clearing damaged and aggregate proteins as well as damaged organelles capable of generating excessive ROS, such as mitochondria. As the mitochondria of cardiomyocytes are in constant demand, it stands to reason that autophagy, more so than the proteasome, is the critical recycling mechanism in heart homeostasis [122]. Within the phases of heart disease, however, detection of functional autophagy is constrained by use of electron microscopy and protein surveys for ATG5, Beclin-I, and LC-I and -II on heart biopsies and precise determinations of autophagic level may widely vary between studies [123]. There is some controversy as to whether or not the drop in mechanical force due to failure of different parts of the heart could affect autophagy in those areas, i.e., the mechanical load of pumping is required for autophagy [123]. It is therefore not clear which comes first: the failure of autophagy that causes rapid cell death and decompensation or a decompensation that causes mechanical stress to lessen and downregulate autophagy. This becomes a very interesting question in the context of Nrf2 as the aforementioned results from Qin and colleagues saw a very clear upregulation of Ang I (angiotensinogen) most likely caused by the switching off of Fyn export of Nrf2 from the nucleus [37]. As the activation of the RAAS axis increases blood pressure, the expectation would be for autophagy to increase in a compensatory manner but that assumes an intact machinery in a failing heart. At advanced stages of disease where vast areas of myocardial decompensation/autophagic failure may be present, higher pressure cannot activate autophagy and simply dooms the myocardium to a terminal loop of decompensation, decreased autophagy and death. This data challenges the dogma of Nrf2 as "always helpful" by introducing a dichotomy where the helpful/hurtful role of Nrf2 is actually dependent more on surrounding cellular functionality and the regulatory microenvironment than simple activation or deactivation.

Although Nrf2, by virtue of its vast links to multiple organs and signaling pathways, has been extensively studied, key questions still remain as to the precise nature of Nrf2 and its relationship with other homeostatic mechanisms such as the Ub/Prot system and myocardial autophagy. Due to the reports of paradoxical effects of Nrf2 in diabetes and atherosclerosis, a new model that focuses on Nrf2 within the context of other functional cell machinery (such as autophagy) is needed. Translational science, for the purpose of mechanistic determination, has usually treated both diseases and genes of interest in an isolated context. However, the new paradigm for both research and clinical treatment is to see all three chief diseases as interrelated and dependent on each other. For example, high blood pressure from diabetes causes atherosclerosis and ischemic insult in the coronary and renal arteries which triggers cardiorenal anemia symptoms that worsen atherosclerosis and finally trigger the heart failure cascade. If Nrf2 is to have a protective or intervention

effect in stopping this lethal triad, then it is critical to study the diseases holistically and not as isolated units. Additionally, it is also important to revise the single knockout or loss-of-function model in favor of integrating Nrf2 function within the interrelated context in which it functions. For example, if local levels of cellular metabolic genes are changed in late-stage heart failure, what effect could Nrf2 have on the health of these cells and their functional state?

4 Exogenous Nrf2 Regulation

As Nrf2 is a prominent target for studies in the cardiovascular system due to its keystone role in homeostasis, there has been a great interest in the discovery and development of novel compounds that allow for control of Nrf2 for therapeutic purposes without needing cellular ROS or toxin damage that usually activates the Nrf2 machinery. The body's native defense system consists of Phase I, II, and III enzymes, with Nrf2 classified as Phase II. The challenge for development of exogenous mediators is therefore to be accurate enough to target only Nrf2 (as opposed to Phase I or III), and carry enough precision to activate Nrf2 at the same level each time. However, there is mounting evidence that high, sustained levels of Nrf2 activation are undesirable, particularly in late-stage heart disease, post-exercise, post-balloon angioplasty, and in solid tumors, where antioxidant generation would provide protection against chemotherapeutic agents and increased metabolic ROS [37, 87, 124–127]. Additionally, electronegative antioxidants such as epigallocatechin gallate (EGCG) and Vitamin C have been hypothesized to damage the sugar phosphate backbone of nuclear DNA by reductive attack, compounding the damage that excess Nrf2 can do [127, 128]. Therefore, it is critical that exogenous downregulators be developed that allow for fine tuning of Nrf2 levels based on both the microenvironment and the condition of the patient. What follows is a survey of the current literature on compounds that have been discovered, modified, and created specifically to affect Nrf2 levels in the body.

4.1 Exogenous Activators of Nrf2

Aside from the natural activation of Nrf2 by ROS attack on Keap1, there are multiple exogenous chemical activators that have been reported in the literature. Many of these are either natural compounds or derivations of natural compounds with functional groups added. A common feature of natural compounds that can activate Nrf2 is the ability to react with thiolates, leading to screening searches for compounds such as isothiocynates, chalcones, terpenoids, and curcumins that can react in a microplate assay that measures induction of NAD(P)H by quinone oxidoreductase 1 (QR1) [129]. Note that exogenous upregulators of Nrf2 upstream regulatory elements (such as PI3K/Akt) will also increase Nrf2, but will lack the specificity of

the following compounds and are outside the scope of this review. Autophagic inhibitors (including bafilomycin A1, chloroquine, LY294002, etc.) would also increase levels of Nrf2 indirectly by removing the effect of constant proteasomal degradation on cytoplasmic Nrf2 and their effects have been extensively reviewed elsewhere [130, 131].

CDDO and Oleanic Acid Derivatives—The most well-studied and reviewed of the natural activators is oleanic acid derivative 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO/bardoloxone) which has been shown in multiple studies since the 1990s to decrease inflammation by increasing Nrf2 expression via conformational change of Keap1 on residue C151 and subsequent release of Nrf2 to the nucleus [1, 132, 133]. Although this compound was found to improve disease conditions in multiple organs, including the liver, kidneys, retina, bone marrow, and the lungs, it had a negative impact on cardiovascular disease and cancer [1].

Alpha Lipoic Acid—Another frequently studied and well-researched natural compound is alpha lipoic acid (ALA), which is known to regenerate innate Phase II defenses (such as glutathione) by stimulating Nrf2 transcription via the PI3K pathway, which increases Nrf2 translocation to the nucleus and upregulation of antioxidant recycling enzymes [134, 135]. This well-reviewed natural compound has seen some use in cardiovascular research where it was shown to reduce both infarct size and protect cardiac function in a rat model of coronary artery ligation [136]. It has also been studied to improve blood pressure and vascular endothelial function in Type 2 diabetic patients with some success [137]. As it does increase levels of Nrf2 that could combat LDL oxidation that leads to atherosclerosis, there have been several studies reviewed by Wollin that found some basis to recommending ALA for protection against cardiac and vascular diseases [138].

Plant-Derived Compounds (Coffee/Tea, Vegetables, Wine, and Fruits)—In 2011, Boettler and colleagues reported that several chemical constituents of roasted coffee formed from chlorogenic acid, including 5-O-caffeoylquinic acid (CGA) and N-methylpyridinium ion (NMP), could increase Nrf2 nuclear translocation [139]. Further work on this topic has revealed cafestol and kahweol as potential Nrf2 activators after metabolism in the liver [129]. In green tea, on the other hand, catechins such as green tea polyphenol (GTP-1) and EGCG have been shown to induce Nrf2 translocation to the nucleus [11, 140]. Other important organic sulfur-based activators such as allicins, indoles, epithionitriles, and sulforaphanes (as well as derivatives such as Oltipraz) have been discovered in vegetables such as broccoli, garlic, and watercress, and these are converted either by intestinal flora or mixing of thioglucoside conjugate with a myrosinase that converts them into active forms [15, 129, 141–144]. These compounds have proven effective in preventing cardiovascular disorders resulting from diabetes in db/db mouse models by direct increase in Nrf2 transcriptional activity [143]. *Humulus lupulus* (hops), used in brewing beer contains a chalcone compound known as xanthohumol that has been investigated as both a cholesterol synthesis inhibitor and a small molecule activator of Nrf2 and has been shown to reduce hypercholesterolemia, atherosclerosis, and fatty liver in ApoE−/− mice [145, 146]. Quinone/food preservative derivatives such as tert-butylhydroquinone (tBHQ) are also used extensively in the lab as reference Nrf2

inducers because their oxidative ability attacks Keap1 to release Nrf2 [83]. Additionally, much work has been done on resveratrol, contained in red wine and plants such as *Fallopia japonica* (Japanese knotweed), which is capable of not only its own antioxidant capacity but has been shown to activate Nrf2 pathways to prevent high-mobility group box 1 (HMGB1)-mediated mitochondrial damage in a pulmonary injury mouse model [147]. It does so by increasing NAD-dependent deacetylase sirtuin-1 (SIRT1) which increases transcription of Nrf2 [148]. Several studies have noted that quercetin, a plant polyphenol found in buckwheat, herbs, and apple skins, can upregulate Nrf2 nuclear translocation and subsequent transcription of glutathione genes [149]. Of interest to vascular researchers is quercetin's ability to downregulate cellular adhesion factors such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin by upregulation of Nrf2 via the p38-MAPK axis and also through SIRT1 [32, 150]. There is also recent evidence of further anti-inflammatory and potentially cardioprotective effect by activation of Dab2 which inhibits the professional antigen-presenting cell (APC) known as the dendritic cell (DC) in ApoE −/− mice [151]. These effects make quercetin a potent anti-atherosclerotic agent and a compound of great potential interest to ameliorate vascular disease. Lutein, a xanthophyllic carotenoid and natural antioxidant, has been found to increase PI3K/IKK/AKT activity and subsequent Nrf2 upregulation in BALB/c mice and also in an acute lung injury mouse model [43, 152]. The ability of lutein to effectively treat hypoxia was found to depend on this PI3K/AKT/Nrf2 axis, but it was also found to cause problems post-injury due to PI3K expression promoting eosinophilic damage in the absence of Nrf2-mediated antioxidant effects [43]. Isoflavone derivate 7-hydroxy-3-(49-hydroxyphenyl)-chroman (equol), found in soybeans and other legumes, was recently reported to be differentially produced by the metabolism of 30–50% of the population and, in an ApoE−/− high fat diet mouse model, could reduce atherosclerotic lesions through activation of Nrf2 through a possible estrogen receptor B or ERK1/2 mechanism [153].

Animal, Molecular and Inorganic Compounds—Although the main focus of the literature is on discovering or modifying plant-derived compounds to modulate Nrf2, some reports of non-plant-derived Nrf2 regulators exist. Docosahexaenoic acid (DHA), an Omega-3 fatty acid found only in photosynthetic microalgae or cold water fish, was found to increase p65 and the MAPK/IKK pathway to upregulate transcription of Nrf2 and also act on oxidized lipids (such as 1-palmitoyl-2-(epoxyisoprostane-E2)-sn-glycero-3-phosphorylcholine or 4-hydroxynonenal) that are agonists of Nrf2 [94, 95, 154, 155]. There is at least some interest in using advances in molecular cloning and gene insertion via viral or other vectors to manipulate levels of Nrf2 within the body and although this has been extensively reviewed in neurological systems, there have not yet been any reports of the feasibility of viral vector therapy to manipulate Nrf2 in a cardiovascular-specific clinical context [156]. An extensive array of plasmids to overexpress Nrf2 either constitutively or inducibly are commercially available and have been used in studies too numerous to mention although Nrf2-overexpressing murine models are not yet available.

Lithium chloride (LiCl) has been studied in the cardiovascular system as an activator of Nrf2 through suppression of GSK-3 [157, 158]. In a myocardial infarction model using Wistar rats, Lee and colleagues found the post-infarctive milieu to be dominated by oxidative and nitrosative stress and direct inhibition of nerve growth factor (NGF) was accomplished by supplementation with LiCl that increased Nrf2 levels through a PI3K pathway [157]. Lithium is therefore thought to act on GSK-3 primarily and PI3K tangentially to increase Nrf2 levels by suppressing its inhibitor and spurring transcription [5, 159]. Arsenic in low doses was reported to increase Nrf2 expression by oxidative damage which releases it from Keap1 [83]. Other toxic compounds that induce Phase II enzymes could be expected to act in much the same way as arsenic and may be unsuitable for in vivo studies due to sequelae from chronic exposure.

4.2 Exogenous Suppressors of Nrf2

Since upregulation of Nrf2 has been generally accepted in the literature as a beneficial phenomenon, a majority of the research into exogenous regulators of Nrf2 have focused on upregulation and sustained output of Nrf2 transcription. However, for loss-of-function studies and chemotherapeutic boosters, reliable suppressors of Nrf2 have also been investigated that could be of use in cardiovascular studies. Note that exogenous downregulators of Nrf2 upstream regulatory elements will also downregulate Nrf2 but may lack specificity and are outside the scope of this review.

4.2.1 Chemical Suppressors of Nrf2

It has been well established in the literature that Nrf2 has a dual role in solid tumors. It both suppresses tumors by defending against redox insults but it also lowers the efficacy of chemotherapy by imparting oxidative resistance to the transformed cells that comprise the tumor mass [160]. To this end, several novel compounds have been studied both separately and in combination with chemotherapeutic agents.

Trigonelline and Coffee—A well-studied Nrf2 suppressor is the alkaloid trigonelline, derived from green coffee, that disrupts the nuclear localization of Nrf2, which renders it vulnerable to circulating Keap1 in the cytoplasm [8, 93]. This would be expected to be deleterious to vascular health, however, high roasting temperatures creates a large number of pyridine derivatives of trigonelline (including nicotinic acid) that, in a study of thrombotic rats, decreased thrombus size [161]. Trigonelline used for research purposes should therefore be obtained in refined form to minimize paradoxical effects of degradation by-products. Trigonelline may have side effects not related to Nrf2 suppression: a study in an induced Type 2 diabetic hyperglycemia model in Wistar rats found that trigonelline treatment suppressed glycemia-induced cardiomyopathy by downregulating myocardial enzymes such as creatine kinase, lactose dehydrogenase, and aspartate aminotransferase [162]. In

spite of its apparent usefulness as an Nrf2 inhibitor, however, cardiovascular studies with trigonelline are much less in number than cancer studies. A report by Lee and colleagues on the antiarrhythmic effect of lithium on infarction in a rat model used trigonelline to suppress Nrf2 as a loss-of-function study is one of the few reports from the vascular field [157]. After the exact mechanism of trigonelline is elucidated, this compound may become an important tool to study Nrf2 in the cardiovascular system.

Other Natural Suppressors of Nrf2—A review by No and colleagues lists ascorbic acid, quassinoids such as brusatol and several flavonoids (chrysin, apigenin, luteolin) along with suggested concentrations for cell line use [163]. These agents either downregulate Nrf2 upstream pathways such as PI3K (chrysin), directly reduce Nrf2 protein levels independently of Keap1 (brusatol) or affect Nrf2 DNA binding ability (ascorbic acid), which may introduce variability in study results [163]. These substances have been used in cardiovascular studies, with chrysin inhibiting VSMC proliferation in a hypoxia-induced hypertension rat model and brusatol being found to inhibit hypoxia-inducible factor 1(HIF-1), a protein found to extensively crosstalk with Nrf2 [164, 165]. Ascorbic acid, although a putative suppressor of Nrf2, is thought to have a variable effect based on organ system, with positive effect in ischemic insult and vascular responsiveness but a negative effect in lung injury [166, 167]. Clearly, as the reports from the literature are contradictory, any use will have to take into account possible side reactions and unwanted side effects.

4.2.2 Molecular Suppressor of Nrf2

Molecular Suppressors of Nrf2—As every chemical modifier of Nrf2 carries the potential risk of unwanted effects based on the cell line/model organism, age-related oxidative milieu, etc., molecular manipulation of Nrf2 using genetic-based approaches have also been studied. B6.129X1-Nfe^{tm1Ywk} /J (Jackson Labs) and NRF2 knockout rats are available for use to study, while various plasmids exist to knock down Nrf2 in vitro and are commercially available for use in lentiviral or other transduction systems [168, 169]. CRISPR/Cas9 knockout systems have also become recently available from commercial suppliers and have been used in studying inflammatory responses [82]. RNA approaches using Nrf2 or Keap1 siRNA are also available commercially and have been used in cell-based gene profiling studies [170]. One report by Liu and colleagues found that suppression of Nrf2 negative competitive regulator Bach1 via an siRNA approach could increase Nrf2 levels; it stands to reason that overexpression of Bach1 may provide a natural suppressive effect on Nrf2 [171, 172]. The strategy of "boosting the suppressor" may not always be appropriate; as Keap1 was found to rely on cytoskeletal actin for its sensor function, overexpression of Keap1 may not necessarily provide adequate suppression of Nrf2 if actin filaments are already saturated [25, 173]. However, a recent report showed that bromodomain protein 4 (BRD4) is a key regulator of Keap1 and this could be exploited in vascular studies to suppress Nrf2 [174]. Using rapid screening technologies such as Neh2-luciferase assays and SPR-based solution competition assay, the search for novel inhibitors of Nrf2 is unceasing [15].

The past 15 years have seen an explosive growth in the number of compounds to regulate Nrf2 both in vitro and in vivo. Data from these studies has given a solid picture of Nrf2's role in redox metabolism and a detailed mechanism with regards to Keap1. This is important, as current opinion regards high antioxidant levels as beneficial. In theory, constant upregulation of Nrf2 by supplementation with any of these compounds should result in improved patient outcomes, but in the case of the cardiovascular system, compounds like CDDO that drive high Nrf2 levels have proven deleterious. Additionally, it should be noted that, with respect to vascular and cardiovascular diseases, multiple studies and meta studies have indicated that ROS scavenging and antioxidant supplementation to be ineffective [175–177]. As current scientific methodology is limited in the scope of mechanistic studies, able to focus on only a few genes at a time, it is clear that future research must use cutting edge epigenetic and –omics technologies to focus on how Nrf2 upregulation fits into the holistic system of a living body. However, having a toolbox of ready compounds to regulate Nrf2 in epigenetic or –omic studies will greatly assist in accurate dissection of Nrf2-regulated redox homeostasis mechanisms.

5 Conclusion

Over the past 25 years, Nrf2 has taken a front-stage role as the master antioxidant transcription factor in the entire body and most research has been devoted to dissecting its function in various organs. It is responsible for the activation of numerous detoxification and antioxidant genes and is the conserved, constitutively expressed, keystone regulator of redox homeostasis in mammals. As expected, this importance comes at a high regulatory price, with the co-constitutively expressed repressor Keap1 and constant degradation by the ubiquitin/proteasome system needed to regulate Nrf2 levels under basal redox conditions. However, this also allows for rapid action by Nrf2 to fluctuate in response to reactive oxygen and nitrosamines formed as a result of both mitochondrial metabolism and environmental stress. It would therefore not be inappropriate to call Nrf2 the modulator of the "antioxidant immune system," resplendent with the intricate up- and downstream molecular mechanisms inherently needed to control such an important keystone.

In this review, the role of Nrf2 in the cardiovascular system and an extensive analysis of exogenous mediators with a focus on those compounds useful for vascular studies were completed. It is well-known that chronic conditions such as Type II diabetes and metabolic syndrome mediate the development of atherosclerosis and subsequent heart disease. It is also well-known that Nrf2, at least in lab animals is highly protective against this triad of diseases by diverse molecular mechanisms. However, despite decades of intensive study and manipulation, several questions have arisen that remain unanswered. Chiefly among them is the knowledge that Nrf2 has dual roles in several somatic systems. For example, it carries both a tumor suppressor and enhancer role that seems paradoxical: suppression of ROS damage prevents tumors but also provides solid tumor cells with defenses against

chemotherapeutic agents. This paradox seems to carry over to the heart as well, with strong evidence that unchecked upregulation of Nrf2 for cardiotherapeutic purposes may carry unintended negative side effects if the microenvironmental milieu is ignored. For example, in the absence of functional autophagy, sustained Nrf2 transcription of Agt due to an absence of Fyn-operated Nrf2 nuclear export causes cardiac maladaptation yet early stage heart disease sees a substantial benefit from Nrf2 upregulation [1, 37]. There are also reports from multiple Phase I and II human trials that have been conducted where exogenous upregulation of Nrf2 to combat cancer has caused unexpected cardiac irregularities in addition to a failure to significantly shrink the tumors [1]. Additionally, epigenetic connections to the autophagic pathway (mTOR, p62) and other master signaling pathways such as PI3K/Akt, Notch, Wnt, AngII, p38-MAPK, Snail, and Bax decrease the signal to noise ratio of experimental results and therefore makes isolation of Nrf2 mechanisms difficult [1]. To this end, extensive effort has been invested into chemical, genetic, and inorganic regulators of Nrf2 to attempt to dissect out the specific roles and functions of Nrf2 within cell lines and laboratory animals. So far, these agents have proven useful in obtaining data on the pathology of certain oxidative diseases and in cause/effect studies in animal models of organ diseases and cancer. They have also extended understanding of the various cellular phenotypes and organ pathologies that result from manipulation of Nrf2. However, the next step in fully elucidating the relationship between the systemic (epigenetic) and specific (redox homeostasis) roles of Nrf2 will most likely require the use of powerful new technologies such as next-generation sequencing, proteomics, transcriptomics, and epigenetic surveys.

Nrf2 has been studied for over 20 years and although much has been discovered, there are still many issues blocking its widespread usage as a therapeutic target. Nrf2 has been proven to be beneficial in prevention of atherosclerosis due to metabolic disorders such as diabetes and also plays a role in defense against oxidative stress in aging but only if chemically stimulated. Additionally, the paradigm of "always on, always helpful" with regard to Nrf2 transcription has been upended by several experimental reports of enhanced cardiac necrosis and maladaptation in late-stage heart disease as well as multiple discontinued Nrf2 upregulator trials due to cardiac side effects. It seems as if Nrf2, acting alone, has its canonical effect but when activated in situations with dysfunctional cellular machinery (e.g., loss of autophagy), it plays an entirely different role due to the multiple epigenetic interactions with disease-enhancing pathways. Two conclusions with regard to cardiovascular disease and Nrf2 can therefore be made: First, Nrf2 is useful in a limited scope as a regulator of redox homeostasis before disease pathology is present and second, Nrf2 must be tightly controlled and possibly even downregulated exogenously after disease pathology robs cells of internal machinery such as autophagy. There seems to be a very thin line between help and harm in the Nrf2 pathway and the exact stage or time point where Nrf2 crosses this line may be entirely dependent on the microenvironment unique to each cell/organ type. To this end, precise elucidation of the effect of Nrf2 at each stage of vascular and cardiac disease with a bias towards detailed time points and possibly integrating Keap1 manipulators into experimental

plans might be needed to formulate a general concept of exactly when Nrf2 switches to harmful from helpful. Additionally, much work remains to be done in elucidating the exact condition of intracellular machinery (transcription, translation, autophagy, ubiquitin ligation, etc.) within the heart and vascular system at each stage of disease to provide a context for Nrf2 downstream effects.

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Memorium: B.J.M. wishes to dedicate this manuscript to the memory of Charles Edward Milliken who was a great scientist, friend, and mentor.

References

1. Mathis BJ, Cui T. CDDO and its role in chronic diseases. Adv Exp Med Biol. 2016;929:291–314.
2. Cleasby A, Yon J, Day PJ, Richardson C, Tickle IJ, Williams PA, et al. Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. PLoS One. 2014;9(6):e98896.
3. Sussan TE, Rangasamy T, Blake DJ, Malhotra D, El-Haddad H, Bedja D, et al. Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. Proc Natl Acad Sci U S A. 2009;106(1):250–5.
4. Ogura T, Tong KI, Mio K, Maruyama Y, Kurokawa H, Sato C, et al. Keap1 is a forked-stem dimer structure with two large spheres enclosing the intervening, double glycine repeat, and C-terminal domains. Proc Natl Acad Sci U S A. 2010;107(7):2842–7.
5. Cuadrado A. Structural and functional characterization of Nrf2 degradation by glycogen synthase kinase 3/??-TrCP. Free Radic Biol Med. 2015;88(Part B):147–57.
6. Canning P, Sorrell FJ, Bullock AN. Structural basis of Keap1 interactions with Nrf2. Free Radic Biol Med. 2015;88(Part B):101–7.
7. Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, Yamamoto M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells. 2003;8(4):379–91.
8. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013;27(20):2179–91.
9. Cho H-Y, Marzec J, Kleeberger SR. Functional polymorphisms in NRF2: implications for human disease. Free Radic Biol Med. 2015;88:1–10.
10. Taguchi K, Fujikawa N, Komatsu M, Ishii T, Unno M, Akaike T, et al. Keap1 degradation by autophagy for the maintenance of redox homeostasis. Proc Natl Acad Sci. 2012;109(34):13561–6.
11. Xu C, Li CY-T, Kong A-NT. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res. 2005;28(3):249–68.
12. Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. Prog Neurobiol. 2013;100(1):30–47.
13. Consortium TU. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017;45:D158–69.
14. Brown GR, Hem V, Katz KS, Ovetsky M, Wallin C, Ermolaeva O, et al. Gene: a gene-centered information resource at NCBI. Nucleic Acids Res. 2015;43(D1):D36–42.
15. Abed DA, Goldstein M, Albanyan H, Jin H, Hu L. Discovery of direct inhibitors of Keap1–Nrf2 protein–protein interaction as potential therapeutic and preventive agents. Acta Pharm Sin B. 2015;5(4):285–99.

16. Shelton P, Jaiswal AK. The transcription factor NF-E2-related factor 2 (nrf2): a protooncogene? FASEB J. 2013;27:414–23.
17. Theodore M, Kawai Y, Yang J, Kleshchenko Y, Reddy SP, Villalta F, et al. Multiple nuclear localization signals function in the nuclear import of the transcription factor Nrf2. J Biol Chem. 2008;283(14):8984–94.
18. Katoh Y, Itoh K, Yoshida E, Miyagishi M, Fukamizu A, Yamamoto M. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes Cells. 2001;6(10):857–68.
19. Bryan HK, Olayanju A, Goldring CE, Park BK. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. Biochem Pharmacol. 2013;85(6):705–17.
20. Kaspar JW, Jaiswal AK. Tyrosine phosphorylation controls nuclear export of Fyn, allowing Nrf2 activation of cytoprotective gene expression. FASEB J. 2011;25(3):1076–87.
21. Velichkova M, Hasson T. Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a Crm1-dependent nuclear export mechanism. Mol Cell Biol. 2005;25(11):4501–13.
22. Sun Z, Zhang S, Chan JY, Zhang DD. Keap1 controls Postinduction repression of the Nrf2-mediated antioxidant response by escorting nuclear export of Nrf2. Mol Cell Biol. 2007;27(18):6334–49.
23. Li W, Jain MR, Chen C, Yue X, Hebbar V, Zhou R, et al. Nrf2 possesses a redox-insensitive nuclear export signal overlapping with the Leucine zipper motif. J Biol Chem. 2005;280(31):28430–8.
24. Jain AK, Bloom DA, Jaiswal AK. Nuclear import and export signals in control of Nrf2. J Biol Chem. 2005;280(32):29158–68.
25. Kobayashi A, Kang M-I, Okawa H, Ohtsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004;24(16):7130–9.
26. Itoh K, Mimura J, Yamamoto M. Discovery of the negative regulator of Nrf2, Keap1: a historical overview. Antioxid Redox Signal. 2010;13(11):1665–78.
27. Kansanen E, Kuosmanen SM, Leinonen H, Levonen A-L. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. 2013;1(1):45–9.
28. Sun Z, Wu T, Zhao F, Lau A, Birch CM, Zhang DD. KPNA6 (Importin 7)-mediated nuclear import of Keap1 represses the Nrf2-dependent antioxidant response. Mol Cell Biol. 2011;31(9):1800–11.
29. Klaassen CD, Reisman S. a. Nrf2 the rescue: effects of the antioxidative/electrophilic response on the liver. Toxicol Appl Pharmacol. 2010;244(1):57–65.
30. Xing Y, Niu T, Wang W, Li J, Li S, Janicki JS, et al. Triterpenoid dihydro-CDDO-trifluoroethyl amide protects against maladaptive cardiac remodeling and dysfunction in mice: a critical role of Nrf2. PLoS One. 2012;7(9):e44899.
31. Leinonen HM, Kansanen E, Pölönen P, Heinäniemi M, Levonen A-L. Role of the Keap1-Nrf2 pathway in cancer. Adv Cancer Res. 2014;122:281–320.
32. Li X, Chatterjee N, Spirohn K, Boutros M, Bohmann D. Cdk12 is a gene-selective RNA polymerase II kinase that regulates a subset of the transcriptome, including Nrf2 target genes. Sci Rep. 2016;6(1):21455.
33. Wang Y-Y, Yang Y-X, Zhao R, Pan S-T, Zhe H, He Z-X, et al. Bardoxolone methyl induces apoptosis and autophagy and inhibits epithelial-to-mesenchymal transition and stemness in esophageal squamous cancer cells. Drug Des Devel Ther. 2015;9:993–1026.
34. Bonay M, Deramaudt TB. Nrf2: new insight in cell apoptosis. Cell Death Dis. 2015;6(10):e1897.
35. Valdecantos MP, Prieto-Hontoria PL, Pardo V, Módol T, Santamaría B, Weber M, et al. Essential role of Nrf2 in the protective effect of lipoic acid against lipoapoptosis in hepatocytes. Free Radic Biol Med. 2015;84:263–78.

36. Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, et al. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif. 2012;45(15):487–98.
37. Qin Q, Qu C, Niu T, Zang H, Qi L, Lyu L, et al. Nrf2-mediated cardiac maladaptive remodeling and dysfunction in a setting of autophagy insufficiency. Hypertension. 2016;67(1):107–17.
38. Strzyz P. Cell death: pulling the apoptotic trigger for necrosis. Nat Rev Mol Cell Biol. 2017;18(2):72.
39. Farré J-C, Subramani S. Mechanistic insights into selective autophagy pathways: lessons from yeast. Nat Rev Mol Cell Biol. 2016;17(9):537–52.
40. Dikic I. Proteasomal and autophagic degradation systems. Annu Rev Biochem. 2017;86:1–32.
41. Zhan J, He J, Zhou Y, Wu M, Liu Y, Shang F, et al. Crosstalk between the autophagy-lysosome pathway and the ubiquitin-proteasome pathway in retinal pigment epithelial cells. Curr Mol Med. 2016;16(5):487–95.
42. Jiang T, Harder B, Rojo De La Vega M, Wong PK, Chapman E, Zhang DD. P62 links autophagy and Nrf2 signaling. Free Radic Biol Med. 2015;88(Part B):199–204.
43. Reddy NM, Potteti HR, Vegiraju S, Chen HJ, Tamatam CM, Reddy SP. PI3K-AKT signaling via Nrf2 protects against hyperoxia-induced acute lung injury, but promotes inflammation post-injury independent of Nrf2 in mice. PLoS One. 2015;10(6):1–13.
44. Bae SH, Sung SH, Oh SY, Lim JM, Lee SK, Park YN, et al. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. Cell Metab. 2013;17(1):73–84.
45. Rhee SG, Bae SH. The antioxidant function of sestrins is mediated by promotion of autophagic degradation of Keap1 and Nrf2 activation and by inhibition of mTORC1. Free Radic Biol Med. 2015;88(Part B):205–11.
46. Jang J, Wang Y, Kim H-S, Lalli MA, Kosik KS. Nrf2, a regulator of the proteasome, controls self-renewal and pluripotency in human embryonic stem cells. Stem Cells. 2014;32(10):2616–25.
47. Pajares M, Cuadrado A, Rojo AI. Modulation of proteostasis by transcription factor NRF2 and impact in neurodegenerative diseases. Redox Biol. 2017;11:543–53.
48. Wang H, Lai Y, Mathis BJ, Wang W, Li S, Qu C, et al. Deubiquitinating enzyme CYLD mediates pressure overload-induced cardiac maladaptive remodeling and dysfunction via downregulating Nrf2. J Mol Cell Cardiol. 2015;84:143–53.
49. Sun Z, Huang Z, Zhang DD. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. PLoS One. 2009;4(8):e6588.
50. Krall EB, Wang B, Munoz DM, Ilic N, Raghavan S, Niederst MJ, et al. KEAP1 loss modulates sensitivity to kinase targeted therapy in lung cancer. elife. 2017;1:6.
51. Chen W, Sun Z, Wang X-J, Jiang T, Huang Z, Fang D, et al. Direct interaction between Nrf2 and p21Cip1/WAF1 upregulates the Nrf2-mediated antioxidant response. Mol Cell. 2009;34(6):663–73.
52. Villeneuve NF, Lau A, Zhang DD. Regulation of the Nrf2-Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases. Antioxid Redox Signal. 2010;13(11):1699–712.
53. Kanzaki H, Shinohara F, Kajiya M, Fukaya S, Miyamoto Y, Nakamura Y. Nuclear nrf2 induction by protein transduction attenuates osteoclastogenesis. Free Radic Biol Med. 2014;77:239–48.
54. Woo C-H, Shishido T, McClain C, Lim JH, Li J-D, Yang J, et al. Extracellular signal-regulated kinase 5 SUMOylation antagonizes shear stress-induced antiinflammatory response and endothelial nitric oxide synthase expression in endothelial cells. Circ Res. 2008;102(5):538–45.
55. Malloy MT, McIntosh DJ, Walters TS, Flores A, Goodwin JS, Arinze IJ. Trafficking of the transcription factor Nrf2 to promyelocytic leukemia-nuclear bodies: implications for degradation of nrf2 in the nucleus. J Biol Chem. 2013;288(20):14569–83.

56. Polvani S, Tarocchi M, Galli A. PPAR and oxidative stress: con() catenating NRF2 and FOXO. PPAR Res. 2012;2012:1–15.
57. Rada P, Rojo AI, Offergeld A, Feng GJ, Velasco-Martín JP, González-Sancho JM, et al. WNT-3A regulates an Axin1/NRF2 complex that regulates antioxidant metabolism in hepatocytes. Antioxid Redox Signal. 2015;22(7):555–71.
58. Wei Y, Gong J, Thimmulappa RK, Kosmider B, Biswal S, Duh EJ. Nrf2 acts cell-autonomously in endothelium to regulate tip cell formation and vascular branching. Proc Natl Acad Sci U S A. 2013;110(41):E3910–8.
59. Wakabayashi N, Chartoumpekis DV, Kensler TW. Crosstalk between Nrf2 and notch signaling. Free Radic Biol Med. 2015;88(Part B):158–67.
60. Li J, Zhang C, Xing Y, Janicki JS, Yamamoto M, Wang XL, et al. Up-regulation of p27kip1 contributes to Nrf2-mediated protection against angiotensin II-induced cardiac hypertrophy. Cardiovasc Res. 2011;90(2):315–24.
61. Shirwany NA, Zou M. Arterial stiffness: a brief review. Acta Pharmacol Sin. 2010;31(10):1267–76.
62. Barnes S, Curtis H. Biology: 5th Ed. 5th ed. New York City: W.H. Freeman & Co.; 1989. 756 p.
63. Mathis BJ, Lai Y, Qu C, Janicki JS, Cui T. CYLD-mediated signaling and diseases. Curr Drug Targets. 2015;16(4):284–94.
64. Majesky MW, Dong XR, Hoglund V, Mahoney WM, Daum G. The adventitia: a dynamic interface containing resident progenitor cells. Arterioscler Thromb Vasc Biol. 2011;31(7):1530–9.
65. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006;113(13):1708–14.
66. Moise M, Fairman RM. Atluri P, Karakousis G, Porrett P, Kaiser L, editors. Vascular disease and vascular surgery. 2nd ed. New York City: Lippincott Williams & Wlkins. p. 344–5.
67. Qian J, Fulton D. Post-translational regulation of endothelial nitric oxide synthase in vascular endothelium. Front Physiol 2013;4.
68. Ruster C, Wolf G. Renin-angiotensin-aldosterone system and progression of renal disease. J Am Soc Nephrol. 2006;17(11):2985–91.
69. Heiss EH, Schachner D, Werner ER, Dirsch VM. Active NF-E2-related factor (Nrf2) contributes to keep endothelial NO synthase (eNOS) in the coupled state: role of reactive oxygen species (ROS), eNOS, and heme oxygenase (HO-1) levels. J Biol Chem. 2009;284(46):31579–86.
70. Luo Z, Aslam S, Welch WJ, Wilcox CS. Activation of nuclear factor Erythroid 2-related factor 2 coordinates dimethylarginine dimethylaminohydrolase/PPAR-γ/endothelial nitric oxide synthase pathways that enhance nitric oxide generation in human glomerular endothelial cells. Hypertension. 2015;65(4):896–902.
71. Warabi E, Takabe W, Minami T, Inoue K, Itoh K, Yamamoto M, et al. Shear stress stabilizes NF-E2-related factor 2 and induces antioxidant genes in endothelial cells: role of reactive oxygen/nitrogen species. Free Radic Biol Med. 2007;42(2):260–9.
72. Kim M, Kim S, Lim JH, Lee C, Choi HC, Woo CH. Laminar flow activation of ERK5 protein in vascular endothelium leads to atheroprotective effect via NF-E2-related factor 2 (Nrf2) activation. J Biol Chem. 2012;287(48):40722–31.
73. McSweeney SR, Warabi E, Siow RCM. Nrf2 as an endothelial mechanosensitive transcription factor: going with the flow. Hypertension. 2016;67(1):20–9.
74. Chen B, Lu Y, Chen Y, Cheng J. The role of Nrf2 in oxidative stress-induced endothelial injuries. J Endocrinol. 2015;225(3):R83–99.
75. Ashino T, Yamamoto M, Numazawa S. Nrf2/Keap1 system regulates vascular smooth muscle cell apoptosis for vascular homeostasis: role in neointimal formation after vascular injury. Sci Rep. 2016;6(April):26291.
76. Florczyk U, Jazwa A, Maleszewska M, Mendel M, Szade K, Kozakowska M, et al. Nrf2 regulates angiogenesis: effect on endothelial cells, bone marrow-derived proangiogenic cells and hind limb ischemia. Antioxid Redox Signal. 2014;20(11):1693–708.

77. Li L, Pan H, Wang H, Li X, Bu X, Wang Q, et al. Interplay between VEGF and Nrf2 regulates angiogenesis due to intracranial venous hypertension. Sci Rep. 2016;6(1):37338.
78. Levonen AL, Inkala M, Heikura T, Jauhiainen S, Jyrkkänen HK, Kansanen E, et al. Nrf2 gene transfer induces antioxidant enzymes and suppresses smooth muscle cell growth in vitro and reduces oxidative stress in rabbit aorta in vivo. Arterioscler Thromb Vasc Biol. 2007;27(4):741–7.
79. Murakami S, Motohashi H. Roles of Nrf2 in cell proliferation and differentiation. Free Radic Biol Med. 2015;88(Part B):168–78.
80. Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis. Antioxid Redox Signal. 2008;10(11):1923–40.
81. Murakami S, Shimizu R, Romeo P-H, Yamamoto M, Motohashi H. Keap1-Nrf2 system regulates cell fate determination of hematopoietic stem cells. Genes Cells. 2014;19(3):239–53.
82. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624.
83. Macoch M, Morzadec C, Genard R, Pallardy M, Kerdine-Romer S, Fardel O, et al. Nrf2-dependent repression of interleukin-12 expression in human dendritic cells exposed to inorganic arsenic. Free Radic Biol Med. 2015;88(Part B):381–90.
84. Zhou S, Sun W, Zhang Z, Zheng Y. The role of Nrf2-mediated pathway in cardiac remodeling and heart failure. Oxidative Med Cell Longev. 2014;2014:260429.
85. Narasimhan M, Rajasekaran NS. Exercise, Nrf2 and antioxidant signaling in cardiac aging. Front Physiol. 2016;7(JUN):1–8.
86. Li J, Ichikawa T, Villacorta L, Janicki JS, Brower GL, Yamamoto M, et al. Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. Arterioscler Thromb Vasc Biol. 2009;29(11):1843–50.
87. Li S, Wang W, Niu T, Wang H, Li B, Shao L, et al. Nrf2 deficiency exaggerates doxorubicin-induced cardiotoxicity and cardiac dysfunction. Oxidative Med Cell Longev. 2014;2014:748524.
88. Dokken BB. The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. Diabetes Spectr. 2008;21(3):160–5.
89. Feng J, Anderson K, Singh AK, Ehsan A, Mitchell H, Liu Y, et al. Diabetes upregulation of cyclooxygenase 2 contributes to altered coronary reactivity after cardiac surgery. Ann Thorac Surg 2017 18.
90. Feng J, Liu Y, Chu LM, Singh AK, Dobrilovic N, Fingleton JG, et al. Changes in microvascular reactivity after cardiopulmonary bypass in patients with poorly controlled versus controlled diabetes. Circulation. 2012;126(11 Suppl 1):S73–80.
91. Gu J, Cheng Y, Wu H, Kong L, Wang S, Xu Z, et al. Metallothionein is downstream of Nrf2 and partially mediates sulforaphane prevention of diabetic cardiomyopathy. Diabetes. 2017;66(2):529–42.
92. Lu J, Guo S, Xue X, Chen Q, Ge J, Zhuo Y, et al. Identification of a novel series of anti-inflammatory and anti-oxidative phospholipid oxidation products containing Cyclopentenone moiety in vitro and in vivo: implication in atherosclerosis. J Biol Chem. 2017;292(13):5378–91.
93. Arlt A, Sebens S, Krebs S, Geismann C, Grossmann M, Kruse ML, et al. Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity. Oncogene. 2013;32(40):4825–35.
94. Ishikado A, Morino K, Nishio Y, Nakagawa F, Mukose A, Sono Y, et al. 4-Hydroxy Hexenal derived from docosahexaenoic acid protects endothelial cells via Nrf2 activation PLoS One. 2013;8(7).
95. Lopez-Bernardo E, Anedda A, Sanchez-Perez P, Acosta-Iborra B, Cadenas S. 4-Hydroxynonenal induces Nrf2-mediated UCP3 upregulation in mouse cardiomyocytes. Free Radic Biol Med. 2015;88(Part B):427–38.

96. Shelton LM, Lister A, Walsh J, Jenkins RE, Wong MHL, Rowe C, et al. Integrated transcriptomic and proteomic analyses uncover regulatory roles of Nrf2 in the kidney. Kidney Int. 2015;88(6):1261–73.
97. Chen J, Zhang Z, Cai L. Diabetic cardiomyopathy and its prevention by nrf2: current status. Diabetes Metab J. 2014;38(5):337–45.
98. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation. 2007;115(25):3213–23.
99. Olagnier D, Lavergne R-A, Meunier E, Lefèvre L, Dardenne C, Aubouy A, et al. Nrf2, a PPARγ alternative pathway to promote CD36 expression on inflammatory macrophages: implication for malaria. Mota MM, editor. PLoS Pathog. 2011;7(9):e1002254.
100. Lutgens E. Atherosclerotic plaque rupture: local or systemic process? Arterioscler Thromb Vasc Biol. 2003;23(12):2123–30.
101. Raedschelders K, Ansley DM, Chen DDY. The cellular and molecular origin of reactive oxygen species generation during myocardial ischemia and reperfusion. Pharmacol Ther. 2012;133(2):230–55.
102. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012;298:229–317.
103. Kalogeris T, Bao Y, Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol. 2014;2:702–14.
104. Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. Cardiovasc Res. 2006;71:216–25.
105. Olechnowicz-Tietz S, Gluba A, Paradowska A, Banach M, Rysz J. The risk of atherosclerosis in patients with chronic kidney disease. Int Urol Nephrol. 2013;45(6):1605–12.
106. Thomas R, Kanso A, Sedor JR. Chronic kidney disease and its complications. Prim Care Clin Off Pract. 2008;35(2):329–44.
107. Wang Y-Y, Yang Y-X, Zhe H, He Z-X, Zhou S-F. Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. Drug Des Devel Ther. 2014;8:2075–88.
108. Hybertson B, Gao B, Doan A. The clinical potential of influencing Nrf2 signaling in degenerative and immunological disorders. Clin Pharmacol Adv Appl 2014;19.
109. Kim JH, Choi YK, Lee KS, Cho DH, Baek YY, Lee DK, et al. Functional dissection of Nrf2-dependent phase II genes in vascular inflammation and endotoxic injury using Keap1 siRNA. Free Radic Biol Med. 2012;53(3):629–40.
110. Sussan TE, Jun J, Thimmulappa R, Bedja D, Antero M, Gabrielson KL, et al. Disruption of Nrf2, a key inducer of antioxidant defenses, attenuates ApoE-mediated atherosclerosis in mice. PLoS One. 2008;3(11):1–9.
111. Barajas B, Che N, Yin F, Rowshanrad A, Orozco LD, Gong KW, et al. NF-E2-related factor 2 promotes atherosclerosis by effects on plasma lipoproteins and cholesterol transport that overshadow antioxidant protection. Arterioscler Thromb Vasc Biol. 2011;31(1):58–66.
112. Howden R. Nrf2 and cardioascular defense. Oxidative Med Cell Longev. 2013;2013:1–10.
113. Goldberg LR, Stage B. Heart failure: management of asymptomatic left ventricular systolic dysfunction. Circulation. 2006;113(24):2851–60.
114. Kung G, Konstantinidis K, Kitsis RN. Programmed necrosis, not apoptosis, in the heart. Circ Res. 2011;108(8):1017–36.
115. Sawyer DB, Colucci WS. Mitochondrial oxidative stress in heart failure : “oxygen wastage” revisited. Circ Res. 2000;86(2):119–20.
116. Sugamura K, Keaney JF. Reactive oxygen species in cardiovascular disease. Free Radic Biol Med. 2011;51(5):978–92.
117. Wallukat G. The beta-adrenergic receptors. Herz. 2002;27(7):683–90.
118. Nishida K, Kyoi S, Yamaguchi O, Sadoshima J, Otsu K. The role of autophagy in the heart. Cell Death Differ. 2009;16(1):31–8.
119. Lompre A-M, Hajjar RJ, Harding SE, Kranias EG, Lohse MJ, Marks AR. Ca2+ cycling and new therapeutic approaches for heart failure. Circulation. 2010;121(6):822–30.

120. Shirakabe A, Ikeda Y, Sciarretta S, Zablocki DK, Sadoshima J. Aging and autophagy in the heart. Circ Res. 2016;118(10):1563–76.
121. Kim AN, Jeon W-K, Lee JJ, Kim B-C. Up-regulation of heme oxygenase-1 expression through CaMKII-ERK1/2-Nrf2 signaling mediates the anti-inflammatory effect of bisdemethoxycurcumin in LPS-stimulated macrophages. Free Radic Biol Med. 2010;49(3):323–31.
122. Tannous P, Zhu H, Johnstone JL, Shelton JM, Rajasekaran NS, Benjamin IJ, et al. Autophagy is an adaptive response in desmin-related cardiomyopathy. Proc Natl Acad Sci U S A. 2008;105(28):9745–50.
123. Xie M, Morales CR, Lavandero S, Hill JA. Tuning flux: autophagy as a target of heart disease therapy. Curr Opin Cardiol. 2011;26(3):216–22.
124. Kannan S, Muthusamy VR, Whitehead KJ, Wang L, Gomes AV, Litwin SE, et al. Nrf2 deficiency prevents reductive stress-induced hypertrophic cardiomyopathy. Cardiovasc Res. 2013;100(1):63–73.
125. de Haan JB. Limiting reductive stress for treating in-stent stenosis: the heart of the matter? J Clin Invest. 2014;124(12):5092–4.
126. Margaritelis NV, Kyparos A, Paschalis V, Theodorou AA, Panayiotou G, Zafeiridis A, et al. Reductive stress after exercise: the issue of redox individuality. Redox Biol. 2014;2:520–8.
127. Seifirad S, Ghaffari A, Amoli MM. The antioxidants dilemma: are they potentially immunosuppressants and carcinogens? Front Physiol. 2014;5:245.
128. Bouayed J, Bohn T. Exogenous antioxidants — double-edged swords in cellular redox state health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative Med Cell Longev. 2010;3(4):228–37.
129. Eggler AL, Savinov SN. In: Gang DR, editor. 50 years of phytochemistry research, vol. 2. Cham: Springer; 2013. p. 1–34.
130. Pasquier B. Autophagy inhibitors. Cell Mol Life Sci. 2016;73(5):985–1001.
131. Solitro AR, MacKeigan JP. Leaving the lysosome behind: novel developments in autophagy inhibition. Future Med Chem. 2016;8(1):73–86.
132. Liu J, Liu Y, Madhu C, Klaassen CD. Protective effects of oleanolic acid on acetaminophen-induced hepatotoxicity in mice. J Pharmacol Exp Ther. 1993;266(3):1607–13.
133. Honda T, Rounds BV, Gribble GW, Suh N, Wang Y, Sporn MB. Design and synthesis of 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, a novel and highly active inhibitor of nitric oxide production in mouse macrophages. Bioorg Med Chem Lett. 1998;8(19):2711–4.
134. Shay KP, Michels AJ, Li W, Kong A-NT, Hagen TM. Cap-independent Nrf2 translation is part of a lipoic acid-stimulated detoxification stress response. Biochim Biophys Acta, Mol Cell Res. 2012;1823(6):1102–9.
135. Zhang J, Zhou X, Wu W, Wang J, Xie H, Wu Z. Regeneration of glutathione by α-lipoic acid via Nrf2/ARE signaling pathway alleviates cadmium-induced HepG2 cell toxicity. Environ Toxicol Pharmacol. 2017;51:30–7.
136. Deng C, Sun Z, Tong G, Yi W, Ma L, Zhao B, et al. α-Lipoic acid reduces infarct size and preserves cardiac function in rat myocardial ischemia/reperfusion injury through activation of PI3K/Akt/Nrf2 pathway. Katare RG, editor. PLoS One. 2013;8(3):e58371.
137. Heinisch BB, Francesconi M, Mittermayer F, Schaller G, Gouya G, Wolzt M, et al. Alpha-lipoic acid improves vascular endothelial function in patients with type 2 diabetes: a placebo-controlled randomized trial. Eur J Clin Investig. 2010;40(2):148–54.
138. Wollin SD, Jones PJH. Alpha-lipoic acid and cardiovascular disease. J Nutr. 2003;133(11):3327–30.
139. Boettler U, Sommerfeld K, Volz N, Pahlke G, Teller N, Somoza V, et al. Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. J Nutr Biochem. 2011;22(5):426–40.
140. Wang Y, Wang B, Du F, Su X, Sun G, Zhou G, et al. Epigallocatechin-3-Gallate attenuates oxidative stress and inflammation in obstructive nephropathy via NF-?B and Nrf2/HO-1 signalling pathway regulation. Basic Clin Pharmacol Toxicol. 2015;117(3):164–72.

141. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. Free Radic Biol Med. 2015;88(Part B):179–88.
142. Tai H-C, Chung S-D, Chien C-T, Yu H-J. Sulforaphane improves ischemia-induced detrusor overactivity by downregulating the enhancement of associated endoplasmic reticulum stress, autophagy, and apoptosis in rat bladder. Sci Rep. 2016;6(August 2015):36110.
143. Xu Z, Wang S, Ji H, Zhang Z, Chen J, Tan Y, et al. Broccoli sprout extract prevents diabetic cardiomyopathy via Nrf2 activation in db/db T2DM mice. Sci Rep. 2016;6:30252.
144. Cominacini L, Mozzini C, Garbin U, Pasini A, Stranieri C, Solani E, et al. Endoplasmic reticulum stress and Nrf2 signaling in cardiovascular diseases. Free Radic Biol Med. 2015;88(Part B):233–42.
145. Yao J, Zhang B, Ge C, Peng S, Fang J. Xanthohumol, a polyphenol chalcone present in hops, activating Nrf2 enzymes to confer protection against oxidative damage in PC12 cells. J Agric Food Chem. 2015;63(5):1521–31.
146. Doddapattar P, Radovi B, Patankar JV, Obrowsky S, Jandl K, Nusshold C, et al. Xanthohumol ameliorates atherosclerotic plaque formation , hypercholesterolemia , and hepatic steatosis in ApoE -deficient mice. Mol Biol Rep. 2013;57(10):1718–28.
147. Dong WW, Liu YJ, Lv Z, Mao YF, Wang YW, Zhu XY, et al. Lung endothelial barrier protection by resveratrol involves inhibition of HMGB1 release and HMGB1-induced mitochondrial oxidative damage via an Nrf2-dependent mechanism. Free Radic Biol Med. 2015;88(Part B):404–16.
148. Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K, et al. Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. AJP Hear Circ Physiol. 2010;299(1):H18–24.
149. Arredondo F, Echeverry C, Abin-Carriquiry JA, Blasina F, Antúnez K, Jones DP, et al. After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult. Free Radic Biol Med. 2010;49(5):738–47.
150. Hung C, Chan S, Chu P, Tsai K. Quercetin is a potent anti-atherosclerotic compound by activation of SIRT1 signaling under oxLDL stimulation. Mol Nutr Food Res. 2015;59(10):1905–17.
151. Lin W, Wang W, Wang D, Ling W. Quercetin protects against atherosclerosis by inhibiting dendritic cell activation. Mol Nutr Food Res. 2017. (Epub ahead of print).
152. Kim JH, Na HJ, Kim CK, Kim JY, Ha KS, Lee H, et al. The non-provitamin a carotenoid, lutein, inhibits NF-??B-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-??B-inducing kinase pathways: role of H2O2 in NF-??B activation. Free Radic Biol Med. 2008;45(6):885–96.
153. Zhang T, Hu Q, Shi L, Qin L, Zhang Q, Mi M. Equol attenuates atherosclerosis in apolipoprotein E-deficient mice by inhibiting endoplasmic reticulum stress via activation of Nrf2 in endothelial cells. PLoS One. 2016;11(12):1–15.
154. Yang YC, Lii CK, Wei YL, Li CC, Lu CY, Liu KL, et al. Docosahexaenoic acid inhibition of inflammation is partially via cross-talk between Nrf2/heme oxygenase 1 and IKK/NF-??B pathways. J Nutr Biochem. 2013;24(1):204–12.
155. Gruber F, Ornelas CM, Karner S, Narzt MS, Nagelreiter IM, Gschwandtner M, et al. Nrf2 deficiency causes lipid oxidation, inflammation, and matrix-protease expression in DHA-supplemented and UVA-irradiated skin fibroblasts. Free Radic Biol Med. 2015;88(Part B):439–51.
156. Kanninen KM, Pomeshchik Y, Leinonen H, Malm T, Koistinaho J, Levonen AL. Applications of the Keap1-Nrf2 system for gene and cell therapy. Free Radic Biol Med. 2015;88(Part B):350–61.
157. Lee TM, Lin SZ, Chang NC. Antiarrhythmic effect of lithium in rats after myocardial infarction by activation of Nrf2/HO-1 signaling. Free Radic Biol Med. 2014;77:71–81.
158. Kerr F, Sofola-Adesakin O, Ivanov DK, Gatliff J, Gomez Perez-Nievas B, Bertrand HC, et al. Direct Keap1-Nrf2 disruption as a potential therapeutic target for Alzheimer?S disease. Lu B, editor. PLoS Genet. 2017;13(3):e1006593.

159. Castillo-Quan J, Li L, Kinghorn K, Ivanov D, Tain L, Slack C, et al. Lithium promotes longevity through GSK3/NRF2-dependent Hormesis. Cell Rep. 2016;15(3):638–50.
160. Menegon S, Columbano A, Giordano S. The dual roles of NRF2 in cancer. Trends Mol Med. 2016;22(7):578–93.
161. Kalaska B, Piotrowski L, Leszczynska A, Michalowski B, Kramkowski K, Kaminski T, et al. Antithrombotic Effects of pyridinium compounds formed from trigonelline upon coffee roasting. J Agric Food Chemestry. 2014;62:2853–60.
162. Kamble HV, Bodhankar SL. Cardioprotective effect of concomitant administration of trigonelline and sitagliptin on cardiac biomarkers, lipid levels, electrocardiographic and haemodynamic modulation on cardiomyopathy in diabetic Wistar rats. Biomed Aging Pathol. 2014;4(4):335–42.
163. No JH, Kim Y-B, Song YS. Targeting nrf2 signaling to combat chemoresistance. J cancer Prev. 2014;19(2):111–7.
164. Li X-W, Wang X-M, Li S, Yang J-R. Effects of chrysin (5,7-dihydroxyflavone) on vascular remodeling in hypoxia-induced pulmonary hypertension in rats. Chin Med. 2015;10(1):4.
165. Lu Y, Wang B, Shi Q, Wang X, Wang D, Zhu L. Brusatol inhibits HIF-1 signaling pathway and suppresses glucose uptake under hypoxic conditions in HCT116 cells. Sci Rep. 2016;6(1):39123.
166. Mehta D, Ravindran K, Kuebler WM. Novel regulators of endothelial barrier function. AJP Lung Cell Mol Physiol. 2014;307(12):L924–35.
167. Oudemans-van Straaten HM, Man AMS, de Waard MC. Vitamin C revisited. Crit Care. 2014;18(4):460.
168. Chan K, Lu R, Chang JC, Kan YW. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proc Natl Acad Sci U S A. 1996;93(24):13943–8.
169. Priestley JRC, Kautenburg KE, Casati MC, Endres BT, Geurts AM, Lombard JH. The NRF2 knockout rat: a new animal model to study endothelial dysfunction, oxidant stress, and microvascular rarefaction. Am J Physiol Heart Circ Physiol. 2016;310(4):H478–87.
170. Fourtounis J, Wang I-M, Mathieu M-C, Claveau D, Loo T, Jackson AL, et al. Gene expression profiling following NRF2 and KEAP1 siRNA knockdown in human lung fibroblasts identifies CCL11/Eotaxin-1 as a novel NRF2 regulated gene. Respir Res. 2012;13(1):92.
171. Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. J Biol Chem. 2005;280(17):16891–900.
172. Liu Y, Zheng Y. Bach1 siRNA attenuates bleomycin-induced pulmonary fibrosis by modulating oxidative stress in mice. Int J Mol Med. 2016:91–100.
173. Kang M-I, Kobayashi A, Wakabayashi N, Kim S-G, Yamamoto M. Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. Proc Natl Acad Sci. 2004;101(7):2046–51.
174. Hussong M, Börno ST, Kerick M, Wunderlich A, Franz A, Sültmann H, et al. The bromodomain protein BRD4 regulates the KEAP1/NRF2-dependent oxidative stress response. Cell Death Dis. 2014;5(4):e1195.
175. Gounder SS, Kannan S, Devadoss D, Miller CJ, Whitehead KS, Odelberg SJ, et al. Impaired transcriptional activity of Nrf2 in age-related myocardial oxidative stress is reversible by moderate exercise training. PLoS One. 2012;7(9).
176. Myung S-K, Ju W, Cho B, Oh S-W, Park SM, Koo B-K, et al. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. BMJ. 2013;346(jan18 1):f10.
177. Conti V, Izzo V, Corbi G, Russomanno G, Manzo V, De Lise F, et al. Antioxidant supplementation in the treatment of aging-associated diseases. Front Pharmacol. 2016;7(February):1–11.

Nrf2 and Inflammation-Triggered Carcinogenesis

Sayanta Sarkar, Noyel Ghosh, Mousumi Kundu, and Parames C. Sil

Abstract The nuclear factor erythroid 2(NFE-2)-related factor 2 (Nrf2), a transcription factor, is a major player in antioxidant and detoxification system implied by the cells in normal physiological condition as well as during intrinsic or extrinsic cellular stress. During tumorigenesis, activation of Nrf2 could be tumor suppressive or oncogenic. Aberrant Nrf2 signaling makes tumor cells resistant to chemotherapy and radiation. However, Nrf2 can also be cytoprotective in nature where it acts independently or in crosstalk with other cellular signaling pathways to maintain cellular homeostasis. In this book chapter, we highlight the role of Nrf2 in various types of cancers describing Nrf2 signaling in inflammation and oxidative stress to support either cancer cell survival or death. Current status of Nrf2 as a target in cancer therapy is also discussed.

1 Introduction

Reactive oxygen species (ROS) are continuously generated in our body due to internal metabolism as well as external environmental exposure. In physiological conditions, cells produce ROS for useful purposes and ROS signaling protects cells during inflammation and stress response. Moreover, it plays crucial role during cell division and autophagy to propagate and maintain homeostasis [1]. However, ROS generation and its accumulation in excess result in oxidative stress which is harmful for many cellular processes. Oxidative stress leads to inflammation, immune disorders, and many diseases like cancer and aging [2].

Nrf2 is a transcription factor that belongs to cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors. Nrf2 was first cloned as a factor bound to NFE2-binding motif in β-globin gene responsible for erythropoiesis development and platelet development [3]. Although Nrf2 is not necessary for differentiation of hematopoetic cells, it was found to induce a group of drug-metabolizing enzymes (DMEs), such as NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione S-transferase (GST) by means of antioxidants and

S. Sarkar · N. Ghosh · M. Kundu · P. C. Sil (✉)
Division of Molecular Medicine, Bose Institute, Kolkata, India
e-mail: parames@jcbose.ac.in

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electrophiles [4, 5]. Induction of such proteins promotes detoxification and elimination of several endogenous as well exogenous chemicals and thereby Nrf2 regulates oxidative stress response caused by inflammation [6].

Over past few decades, from several studies, the major role of Nrf2 has been shown to produce resistance to oxidant stress. While searching for genome-wide targets of Nrf2, many antioxidant response element (ARE) containing genes responsible for antioxidant homeostasis and drug detoxification have been identified [7]. Cells maintain concentration of Nrf2 in a very controlled manner, where Nrf2 is repressed by Keap1 (Kelch-like erythroid cell-derived protein with CNC homology-associated protein 1). Under normal cellular condition, Keap1 mediates ubiquitination and subsequent proteosomal degradation of Nrf2 [8]. However, the protective nature of Nrf2 can also be exploited by tumor cells to build a prosurvival niche for further tumor progression and drug resistance [9]. In this context, the emerging molecular mechanism and function of Nrf2 in the regulation of inflammation-mediated cancer are described below.

The function of Nrf2 signaling in cancer cells, in response to toxic chemicals and radiations (ionizing or ultraviolet radiation) has been well established [10, 11]. Cancer progression has been found to be prevented by Nrf2 through several pathways such as by generation of many antioxidants and detoxifying enzymes to suppress the reactive oxygen species (ROS). Recently, it has been found that cancer cells overexpress Nrf2 which ultimately leads to cancer progression [4, 12] and metastasis [13]. With ongoing research, the functions of Nrf2 in various phases of cancer progression have been highlighted deeply.

Although Nrf2 activity is majorly regulated by its inhibitor Keap1, many other regulatory mechanisms are also in existence. Nrf2 activity is regulated at the level of transcription, translation, and post-translational phase independent of Keap1-mediated regulation. Nrf2 is regulated by protein kinases like JNK, GSK3β, PKC, and Akt [14]. Nrf2 binding to caveolin-1 or p21 also alters its functioning. Moreover, Nrf2 could be regulated at epigenetic level as well by various microRNAs like miRNA-28, 144, and -200a [15]. The above-mentioned processes are equally vital factors for the activation of Nrf2 to keep the cellular redox potential in a balanced state to reduce the possibility of inflammation and thereby progression to cancer development.

2 Regulation of Nrf2 and Keap1

2.1 Keap1-Dependent Regulation

Keap1 plays an important role as redox sensor and involved in cellular defense by regulating the Nrf2 protein under normal as well as stressed conditions. In inactive state, Keap1 forms a homodimer to sequester Nrf2 in the cytosol and prevents Nrf2 translocation in the nucleus in association with the actin cytoskeleton [16]. In addition, Keap1 mediates the Cul3-based poly-ubiquitination and proteasomal degradation of Nrf2.

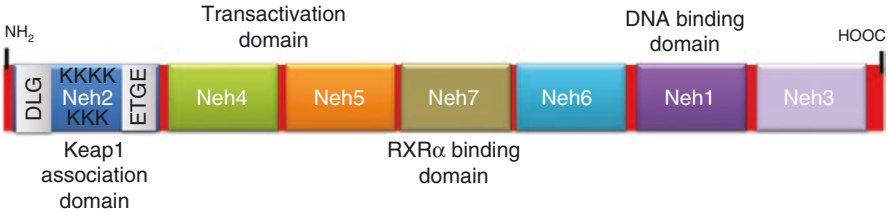

Fig. 1 Domain structure of Nrf2. The Nrf2 protein contains seven conserved Nrf2–ECH homology (Neh) domains. Neh1 domain binds to DNA and dimerizes with transcription factors; Neh2 binds to Keap1 through DLG and ETGE motif and contains seven lysine residues for ubiquitin ligation; Neh3 is needed for transcriptional activation; Neh4 and Neh5 are transactivation domains; Neh6 regulates Nrf2 stability; Neh7 is responsible for RXR/binding

2.2 Keap1-Dependent Proteasomal Degradation of Nrf2

The continuous activation of oxidative stress could be prevented by maintaining Nrf2 at very low level [17, 18]. Proteasomal degradation of Nrf2 promoted by Keap1 protein makes Nrf2 a high turnover protein with a half-life of 10–20 min only. Nrf2-Keap1 binding is based on double glycine repeat domain (DLG domain) in Keap1 and Neh2 domain in Nrf2 [19]. Two motifs within the Neh2 domain exhibit different affinity for Keap1; one binds strongly whereas, the other binds with low affinity. In normal cellular conditions, Nrf2 concentration is kept lowered through weak binding of Nrf2-Keap1 and thus targeting Nrf2 for ubiquitination by Cul3-E3 ligase and thereby degradation by 26S proteasomal pathway [20]. (Fig. 1).

2.3 Keap1 Degradation by Autophagy

The proteasome is a subcellular structure that is utilized for the degradation of specific proteins targeted for destruction. However, autophagy is generally considered as degradation pathway for nonspecific proteins, unfolded proteins, and cellular organelles. Findings from past few decades suggest that the autophagic degradation pathway is also capable of degrading specific, targeted proteins [21–23]. By binding to poly-ubiquitinated protein marked for degradation and to autophagosome, sequestosome1 (p62), a substrate adaptor via its multiple protein–protein interaction capability targets specific cellular proteins to undergo degradation via autophagosome [23, 24]. From some studies it has been elucidated that between Keap1 and p62 there is a significant relationship, where p62 regulates degradation of Keap1 by autophagy. A reduced expression of Keap1 has been observed in a group of cell line where p62 was overexpressed. On the other hand, when p62 was knocked out by siRNA, a high level of Keap1 protein expression with decreased protein level of Nrf2- and Ner2-regulated gene were observed [25]. When p62 is absent in a cell, the cellular Keap1 concentration is maintained twice as compared to normal level. When p62–Keap1 interaction mechanism was thoroughly studied, a concept has arrived which states that, under oxidative stress, Keap1 bound with Nrf2 undergoes

conformational change, which allows release of Nrf2, making an empty site in Keap1 protein where p62 can bind by STGE motif which can be compared with ETGE motif of Nrf2. Binding of p62 with Keap1 allows LC3 to bind which was already bound to autophagosome membrane and allow Keap1 for degradation [26].

3 Keap1-Independent Regulation

From many studies, it has been found that Keap1 is not only one candidate for regulation of Nf2. Sulforaphane, an Nrf2 inducer, overexpressed without hampering the binding between Nrf2 and Keap1. This explains the presence of alternative pathways for Nrf2 activation [27]. The expression and activity of a protein can be regulated at various time scale including transcriptional, post-transcriptional, protein abundance, post-translational modification, and subcellular localization. The phosphorylation of Nrf2 by several signaling machinery and the involvement of epigenetic factors such as microRNAs may also play a role in Nrf2 activation. In this section, alternative processes of Nrf2 regulation are thoroughly described.

3.1 Auto-Regulation and Transcriptional Regulation

Nrf2 acts as a transcription factor by up regulating the expression of genes that contain antioxidant response element (ARE) sequence in the promoter region. Likewise, the aryl hydrocarbon receptor [28] up regulates production of a number of phase I antioxidant enzymes such as Cytochrome p450 which contains xenobiotic response element (XRE) at their promoter site [29] which mediates the group of reactive intermediates, and these reactive intermediates can deliberately activate the antioxidant signaling pathway through the ARE. In another study, a compound TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) an inducer for both Nrf2 and AHR can induce all those genes which contain both ARE and XRE on their promoter region [30]. For some genes, which contain promoter where both Nrf2 and AHR can bind and induce gene expression. Interestingly, in Nrf2 promoter the ARE/XRE sequence has been found suggesting the possibility of auto-regulation of Nrf2 at transcriptional level. The XRE and ARE regions are present in close vicinity within the promoter region of Nrf2 suggesting the possibility of Nrf2 to control its own transcription that is auto-regulation. In support of this another group showed that the D3T (3H-1, 2-dithiole-3-thione), an inducer of ARE, increases Nrf2 at both the RNA and protein levels, and this overexpression can be inhibited by cyclohexamide, a transcriptional inhibitor. When ARE sequence is inserted at the promoter region of luciferase enzyme gene, its overexpression in the presence of Nrf2 is the evidence of the auto-regulation of Nrf2, allowing a positive feedback loop providing cellular defense against oxidative stress [31]. Many chemo-preventive agents are capable of trigger Nrf2 gene expression suppressing NF-κB activity. NF-κB can repress transcription

of genes which contain ARE at their promoter region. In the promoter sequence of Nrf2, there is a region where NF-κB can bind and down regulate those genes which are expressed by Nrf2. This finding suggests that a crosstalk between NF-κB and Nrf2 regulate cellular response against oxidative stress [32].

3.2 Post-Transcriptional Modulator: microRNAs

Over the past decades, microRNAs acquire attention for their function to control the modification of many signaling pathways, as well as the Nrf2 pathway. microRNAs including miR-28 [33], miR-144 [34], miR-200 [15], and miR-34 [35] play a crucial part for Nrf2 signaling in response to oxidative stress. In erythroid cells, an inhibitory effect of miR-144 on Nrf2 signaling pathway has been found. When expression of miR-144 is activated in erythroid cells, a significant loss of Nrf2 protein level with subsequent decrease of restoration of glutathione and modification of antioxidant activity proves the inverse effect of miR-144 on Nrf2 pathway [34]. In breast epithelial cells, a similar relationship was observed within miR-28 and Nrf2. MiR-28 has been seen in various cancers, e.g., lymphoma, glioma, squamous carcinoma, etc. and it is found to bind with Nrf2 mRNA resulting in its degradation along with induction of Nrf2 protein degradation. Neither the induction of Keap1 protein nor the binding between Nrf2 and Keap1 has been affected in the presence of miR-28 explaining that miR-28 function as Nrf2 regulator which is independent of Keap1 [33].

3.3 Post-Translational Modifications: Phosphorylation/ Acetylation

In response to oxidative stress, Nrf2 as transcription factor, move in the nucleus to activate various proteins to reduce cellular ROS. After maintaining cellular redox state when the activation of Nrf2 as transcription factor is no longer needed, the nuclear Nrf2 is phosphorylated which leads Nrf2 for nuclear export thereby degradation. Various pathways like extracellular signal-regulated kinase (ERK), glycogen synthase kinase 3 beta (GSK3β), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K/AKT), mitogen-activated protein kinase (MAPK) signaling pathways, etc. play crucial roles in this regulation. In response to oxidative stress, protein kinase C (PKC) phosphorylate at Ser40 of Neh2 domain of Nrf2 interrupts the binding of Keap1 with Nrf2; the free Nrf2 then moves into the nucleus [14]. PKC belongs to a family of serine/threonine kinases which can be subdivided into three classes: classical, novel, and atypical. In a study, the atypical class of PKC phosphorylates Nrf2 which causes Nrf2 to transport into the nucleus. The translocated Nrf2 then acts as transcription factor to allow the expression of phase II antioxidant enzymes in response to oxidative stress [36]. The nuclear localization sequences

(NLSs) and nuclear export sequences [27] in Nrf2 have been identified and confirmed that after released from Keap1, the free Nrf2 cannot move into the nucleus by its own. For translocation of Nrf2 into the nucleus, certain adaptor proteins like importin forms Nrf2-Importin complex and moves it through nuclear envelope [37]. Casein kinase 2 (CK2) targets approximately 13 potential phosphorylation sites within Nrf2 sequence [38] These phosphorylations by CK2 help Nrf2 for nuclear translocation, which can be repressed by inhibitors of CK2 [39]. The interplay between many signaling pathways regulating post-translational modification of Nrf2 will help to understand cell defense mechanism against oxidative stress.

3.4 Maintaining Cellular Homeostasis by Nrf2

To maintain normal cellular homeostasis, the function of Nrf2 is regulated by several ways. To bind with ARE/EpRE sequence, Nrf2 heterodimerization occurs with musculoaponeurotic fibrosarcoma oncogene homolog (Maf) protein. This Maf protein, which is bZIP type transcription factors, is also required for transactivation of Nrf2 [40–42]. In human Nrf2 protein, seven highly conserved regions known as Nrf2-ECH homology (Neh) domains have been found. Among the family of Neh domains, the Neh1 domain contains a CNC–bZIP structure which mediates heterodimerization with Maf [43]. Bach which is an ARE/EpRE-binding protein acts as a transcriptional repressor of Nrf2 especially in heme oxygenase-1 gene expression involved in oxidative stress response [44]. Neh4 and Neh5 domains play an important role in the transactivation activity of Nrf2. After binding with CREB-binding protein (CBP), the potency of Nrf2 as transcriptional activator increases by nearly 100-fold [45–47].

Nrf2 can be stabilized by phase II inducers. Till now, nine structurally diverse chemical groups have been identified as inducers those are involved in activation of phase II genes by Nrf2 [48]. Within a cell, primary sensors are found and they have certain cysteine residues in the polypeptide chain which actively participate in redox sensing. These phase 2 inducers can all modify sulfhydryl groups of reactive cysteine residues by alkylation, oxidation, or reduction. Interestingly, Keap1 has 27 cysteine residues which are highly reactive; as a result, phase II inducers sense those reactive cysteine residues of Keap1 and target Keap1 and allow the release of Nrf2. Dexamethasone mesylate, a sulfhydryl reactive inducer, directly reacts with cysteine amino acids of Keap1 and triggers dissociation of Keap1 from Nrf2 [49]. The phase 2 inducer 15-Deoxy-delta-12, 14-prostaglandin J2 (15d- PGJ2) interacts directly with Keap1 inducing Nrf2 [50]. Among the most reactive cysteine residues, mutations in Cys273 or Cys288 make Keap1 incapable of suppressing Nrf2 activity [51, 52]. Phase 2 inducers are also found to be activating some protein kinases as mediators of oxidative stress, for example, extracellular signal-regulated kinases (ERK) [53–55], MAPK/ERK kinase-1 [56], MEK kinase 1 [57], p38 mitogen-activated protein kinase (MAPK) [54, 55], PKR-like endoplasmic reticulum kinase (PERK) [58], phosphatidylinositol 3-kinase (PI3K) [59, 60], and protein kinase C

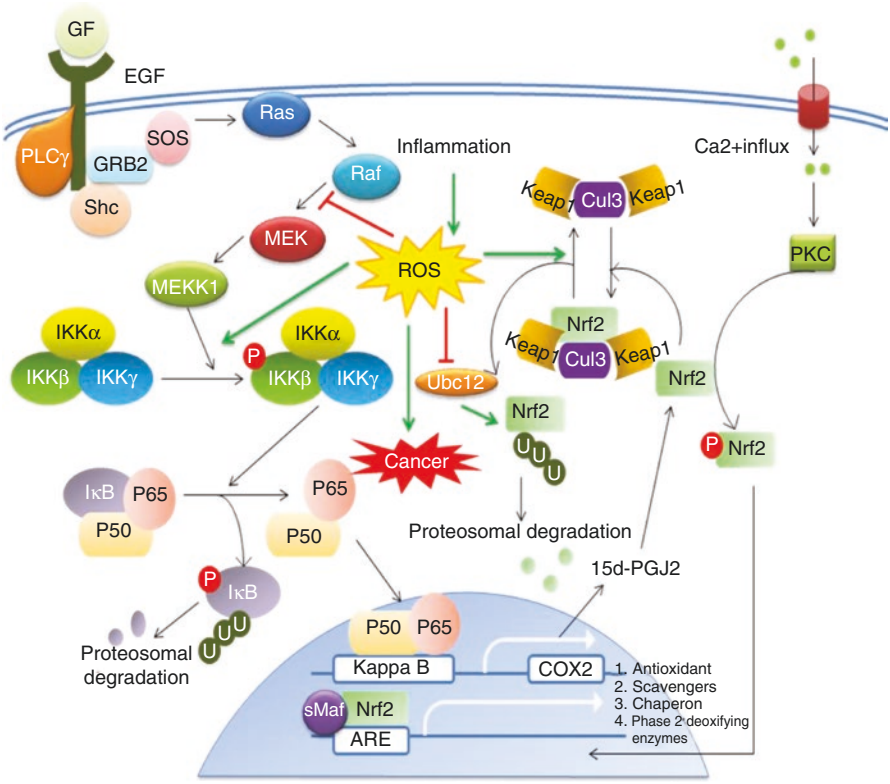

Fig. 2 Maintaining cellular homeostasis by Nrf2. Reactive oxygen species (ROS), either from external sources or generated within cell during inflammation, causes cancer. Nrf2 in response to ROS induces a group of antioxidants phase 2 scavengers, detoxifying enzymes which play an important role in fighting against cancer. Many other pathways like NF-κB, MAPK, and PKC also regulate Nrf2 signaling

(PKC) [36, 61, 62]. Furthermore, specific kinase inhibitors are capable of blocking the phase II antioxidant enzymes and ARE/XRE-triggered gene induction [14, 62]. It is possible that PKC and PERK and also their upstream signaling molecules may be playing some role as sensors for oxidative stress (Fig. 2).

4 Nrf2 Signaling

Nrf2 regulates expression of an array of antioxidant genes upon oxidative stress. Of many oxidative stress-induced pathways, Nrf2-Keap1 signaling plays a pivotal protective role for normal cells. Moreover, since Nrf2-deficient mice are more prone to carcinogenesis [63, 64] and Nrf2 loss leads to enhanced metastasis, it is thought to act as a tumor suppressor [65, 66]. However, recent studies have indicated an oncogenic function of Nrf2. Cancer cells are consistently under oxidative stress and to

overcome this problem, Nrf2 has been found to highly express in these cells thereby enhancing their therapeutic resistance. In the following section, we will elaborate signaling of Nrf2 in normal and cancer cells as well.

4.1 Nrf2 Signaling in Normal Cells

In physiological conditions, Nrf2 remains in cytosol and binds to Kelch/DGR domain of its inhibitor Keap1 with ETGE and DLG motifs present in Neh2 domain of Nrf2. Upon binding to these two sites, Keap1 targets Nrf2 for degradation. Keap1-dependent degradation is Culin3 mediated which is E3-ubiquitin ligase and conjugates ubiquitin moieties at the seven lysine residues present in the Neh2 domain of Nrf2. As a result, in the absence of any stress, Nrf2 is degraded in the cytosol and unable to translocate to the nucleus [67, 68].

However, cellular stress alters Nrf2 binding to Keap1 by its DLG domain. Stress changes Keap1 conformation by oxidizing cysteine residues present in its intervening region (IVR). Conformationally modified Keap1 is unable to bind Nrf2 at DLG domain. Now Keap1 remains bind to that Nrf2 which is not further subjected to poly-ubiquitination and degradation. Thereby newly translated Nrf2 translocates to the nucleus. In nucleus, Nrf2 binds to antioxidant response element (ARE) of a number of genes which are involved in cytoprotection. Nrf2 binding to ARE is mediated by masculoaponeurotic fibrosarcoma (Maf) family proteins. By dimerizing with Nrf2, Maf family proteins have two functions: (1) to facilitate Nrf2 binding to ARE, (2) to recruit co-activator for transcriptional activation of genes. Genes whose transcription is activated by Neh2 binding are related to antioxidant response, phase II detoxifying enzymes, glutathione synthesis, drug metabolism, drug resistance, HIPK2 signaling, etc. (Fig. 3) [67, 68].

4.2 Nrf2 Signaling in Tumor Suppression

It is a known that Nrf2 regulates expression level of genes important for antioxidant functions. These genes exert cytoprotective effects by their anti-inflammatory role but if inflammation persists, it results into carcinogenesis via formation of tumor-supportive microenvironment [69]. During carcinogenesis when a normal cell changes into a transformed cell, Nrf2 prevents this process by its antioxidant and anti-inflammatory functions [70]. Specific mutations in Nrf2 or its suppression during this process leads to carcinogenesis. Hence, it is thought that Nrf2 acts as tumor suppressor and tumor suppressive role of Nrf2 has been investigated thoroughly. By studying mutations in Nrf2 or knocking down Nrf2 gene, several reports have shown that Nrf2 is a tumor suppressor and its deletion/suppression leads to cervical, colorectal, oral, head and neck, lung, pancreatic, hepatic, and skin carcinogenesis [71–73]. In Nrf2 null mice, there is decreased expression of HO-1, NQO1, GST, GCL (glutamate-cysteine ligase), and UGT (UDP-glucuronosyltransferase) in

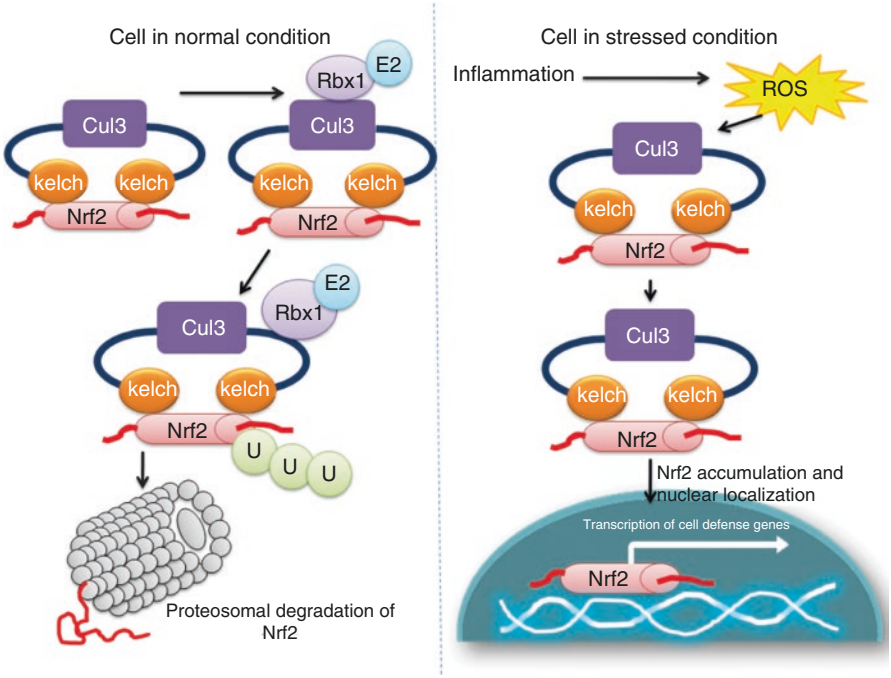

Fig. 3 Nrf2 signaling in normal and stressed conditions. In physiological conditions, Nrf2 undergoes Keap1-mediated proteasomal degradation by Cul3-ubiquitin ligase. In cellular stress, it dissociates with Keap1 and translocates into the nucleus and induce expression of various genes that are involved in maintaining cellular homeostasis

comparison to Nrf2 wild type mice. Moreover, a single nucleotide polymorphism at rs6721961 locus in human Nrf2 promoter results in its decreased levels which enhances the risk for non-small-cell lung cancer (NSCLC) [50]. In the first stage of metal-induced carcinogenesis, Nrf2 acts as anti-oncogenic protein and inhibits malignant cell transformation. Nrf2 is present at higher level in lung adenocarcinoma tissues. However, its knockdown enhances arsenic-induced transformation in BEASE-2B cell in the process of lung carcinogenesis [74]. Nrf2 knockdown results in decreased level of ROS which increases cell survival and proliferation during transformation and leads to tumorigenesis. Therefore, antioncogenic function of Nrf2 is due to its antioxidant gene regulation which reduces ROS level and thereby cell transformation. Likewise, exposure of BEAS-2BR cells with cadmium also leads to lung carcinogenesis. In this process, cadmium exposure leads to ROS generation in untransformed cells. ROS generation leads to increased level of TNF-α which activates NF-κB and COX-2 ultimately resulting in tumor forming inflammatory microenvironment. Overexpression of Nrf2 downstream targets resulted in reduced ROS level and inhibition of cadmium induced malignant cell transformation [70]. Additionally, Nrf2 null mice are highly prone to chemical (DMBA or TPA)-induced skin carcinogenesis. Mice having dominant-negative mutant of Nrf2 (dnNrf2) in basal keratinocytes of the epidermis show a prolonged inflammatory

response following skin injury and develop skin carcinomas. Here, the basal level of Nrf2 target genes is needed to prevent skin tumorigenesis. Hence, reduced chemical-induced detoxification as well as increased oxidative damage increases susceptibility of dnNrf2 mice for skin tumorigenesis [73]. Nrf 2 has an important role in colon carcinogenesis also. Nrf2 knockout results in chronic inflammation with higher expression of inflammatory marker genes such as COX-2, cPLA (cytosolic phospholipase A2), and LTB_4 (leukotriene B4), along with it there is a higher level of PCNA and cMyc in intestinal tissue of respective mice. These Nrf2 knockout mice develop higher number of tumors upon DSS treatment [75]. Collectively, Nrf2 knockout inhibits oxidative stress response pathway, augments inflammation and also increases proliferation of intestinal crypt cells resulting in intestinal carcinogenesis. In significant number of breast cancers cell lines, Nrf2 is present in low level. This is due to increased Cul3-mediated proteasomal degradation of Nrf2.

4.3 Nrf2 Signaling in Tumor Promotion

In 2004, Ikeda et al. highlighted the oncogenic function of Nrf2 in liver carcinoma for the first time [76]. In subsequent years, increasing pieces of evidence suggested that Nrf2 could act as oncogene as well. Constitutive Nrf2 activation results in many types of cancers.

During carcinogenesis, as a cell gets transformed it has higher proliferation capacity. Nrf2 has been found to be involved in this increased proliferation capacity in many cell types. Nrf2 does so by up regulating expression of genes involved in NADPH formation such as transaldolase 1 (TALDO1), phosphogluconate dehydrogenase (PGD), transketolase (TKT), and glucose-6-phosphate dehydrogenase (G6PD). In addition, transcription of some genes of metabolic pathway like isocitrate dehydrogenase 1 (IDH1), phosphoribosyl pyrophosphate amidotransferase (PPAT), methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), and malic enzyme 1 (ME1) is also enhanced. This leads to increased cellular level of purines which is a raw material for DNA and RNA synthesis. Hence, transformed cells proliferate at a higher rate than their normal counterparts [68, 77].

In some tumors, Nrf2 is hyperactive and protects cancer cells from oxidative stress. This hyperactivation of Nrf2 could be due to mutation, increased transcription, or post-translational modification. Hyperactive Nrf2 increases tumorigenesis by positive feedback loop between Nrf2 signaling and histone deacetylase 4 (HDAC4) and pentose phosphate pathway (PPP). Nrf2 directly regulates expression of apoptotic genes like Bcl-2 and Bcl-xL [68].

During arsenic-induced carcinogenesis, Nrf2 is responsible for protection of transformed cells by lowering the level of ROS and apoptosis in BEAS-2B cells. This lowered ROS level is due to increased expression of antioxidant genes like catalase and superoxide dismutase-2 and apoptosis resistance was due to direct regulation of Bcl-2 and Bcl-xL gene expression by Nrf2 [74]. Thus in lung adenocarcinoma, increased level of Nrf2 and its downstream targets helps in malignant cell transformation and tumorigenesis.

Likewise, cadmium also induces lung carcinogenesis. In cadmium-transformed BEAS-2BR cells, there is inhibition of autophagy which leads to increased level of p62. P62 is a competitive inhibitor of Nrf2 for Keap1 binding. Hence, it binds to Keap1 thereby increasing the level of Nrf2 into nucleus. In nucleus, Nrf2 increases expression of catalase and superoxide dismutase resulting in decreased ROS level. It also increases expression of anti-apoptotic genes like Bcl-2 and Bcl-xL, promoting resistance to cell death [70].

Overexpression of Nrf2 is also related to breast carcinogenesis. In MCF7 and MDA-MB-231 cell lines, NRF2 knockdown by siRNA results in decreased cell proliferation and cell migration during metastasis process [78]. Nrf2 regulates breast cancer metastasis by positively regulating RhoA GTPase which is important in cell migration [79]. RhoA along with its downstream effector proteins enhances stress fiber and focal adhesion formation which is necessary in breast cancer metastasis. Additionally, Nrf2 binds and silences estrogen-related receptor-α (ERR1) expression which is a negative regulator of Rho protein. Moreover, Nrf2 enhances breast cancer progression by activating HIF1-α expression which is a key transcription factor sensing hypoxia stress and regulating genes involved in angiogenesis, apoptosis, cell proliferation, and survival [78].

In cervical cancers, nuclear expression of Nrf2 is increased. Higher Nrf2 level is associated with reduced Keap1 expression due to hypermethylation of its gene. Moreover, increased Nrf2 correlates with decreased apoptosis, enhanced cell proliferation, induced cell migration, and invasion of SiHa (cervical cancer cells) cells [71].

Nrf2 protein level constantly remains high in head and neck squamous cell carcinoma (HNSCC) due to gain-of-function mutation in Nrf2 gene [80]. Likewise, Nrf2 expression is higher in oral squamous cell carcinoma (OSCC) in comparison to normal squamous mucosa [81].

5 Nrf2 Signaling in Maintaining Genomic Stability

Since genomic alterations are one of the basic needs for a cell to get transformed into cancerous cell, any lesion that causes genomic instability leads to cancer. Reactive oxygen species, ultraviolet radiation, X-rays, ionizing radiation, and most of the chemotherapeutic drugs tend to deregulate genomic stability either to develop cancer or to treat it. Various checkpoints act at specific places in a cell as surveillance to inhibit progression of this instability. Along with checkpoints, many repair pathways are also present to rectify genomic alterations. Up regulation of repair genes or down regulation of checkpoint proteins is the mechanism deployed by cancer cells to show drug or chemo-resistance or to maintain their growth. Cancer cells override the barrier of checkpoints or enhance repair capacity for their unperturbed cell cycle progression or to develop drug resistance. They do so by incorporating mutations in genes of DNA repair pathway and checkpoint pathway proteins. Nrf2 alters expression of genes involved in base excision repair, non-homologous end joining, and homologous recombination. One of the enzymes in base excision repair pathway, 8-oxoguanine DNA glycosylase (OGG1), rectifies oxidized lesions

at guanine bases induced by reactive oxygen species. Nrf2 regulates expression of OGG1 in mitochondria as well as in nucleus to enhance base excision repair capacity of cells during oxidative stress and thereby protects them from programmed cells death due to unrepaired bases [82]. During radiation therapy, cancer cells activate expression of Nrf2 which in turn up regulates p53-binding protein 1 (53BP1) expression. Elevated level of 53BP1 enhances non-homologous end joining capacity of cancer cells and thereby helps to develop radiation resistance [83]. Moreover, many genes in DNA repair pathway contain AREs, their regulation by Nrf2 is yet to be resolved clearly. However, Nrf2 regulates ionizing radiation-induced Rad51 foci formation which is involved in homologous recombination repair. It does so by increasing mRNA level of Rad51 and thereby increasing radiation resistance in cancer cells [84]. To increase homologous recombination repair capacity of cancer cells, BRCA1, whose gene is up regulated in breast and ovarian cancers, binds to Nrf2 and protects it by Keap1-dependent degradation. This enhances survival of cancer cells in oxidative stress conditions (Fig. 4) [85].

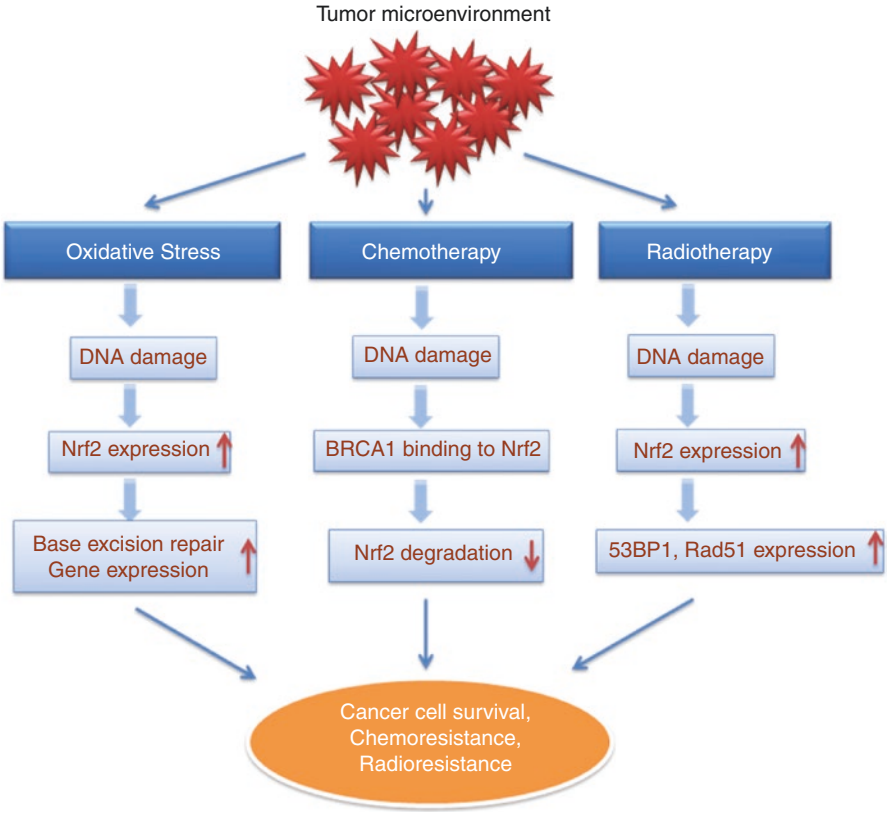

Fig. 4 Nrf2 signaling in maintaining genomic stability. Following DNA damage either by tumor oxidative stress microenvironment or by chemo- or radiotherapy, cells activate Nrf2 expression which thereby increases cells' ability to repair DNA damage that results in their survival and resistance to therapy

6 Nrf2 Signaling in Autophagy

Autophagy is a vital process for cells to maintain cellular homeostasis. It is necessary for nucleic acid synthesis, degradation of aggregated proteins, unfolded proteins, and removal of damaged mitochondria in physiological conditions [86]. However, autophagy could be a result of cellular stress like starvation, oxidative stress, and metabolic stress also [87]. P62 protein is a key player in autophagic signaling as it localizes to autophagosome and acts as a receptor for molecules to be undergoing autophagy. Nrf2 remains in positive feedback loop with p62. In mitochondrial stress, Ser403 residue of p62 gets phosphorylated by CK2 (casein kinase 2) or TBK1 (TANK-binding kinase 1). S403 phosphorylated targets p62 to the ubiquitinated cargos. Then, mTORC1-dependent phosphorylation of p62 at S351 takes place which enhances binding of p62 with Keap1. Keap1 binding to p62 located at autophagic cargos sequesters it at autophagosome and thereby targets it for degradation [88]. Resulting Nrf2 translocates to the nucleus and exerts its protective effects to the cells. This mechanism is utilized by hepatocellular carcinoma cells to protect them against oxidative stress and also to develop chemo-resistance.

K67 (the acetonyl naphthalene derivative) inhibits protein–protein interaction between S349 phosphorylated p62 and Keap1. It binds to DC pocket of Keap1 that is the site responsible for p62 binding and for Nrf2 binding as well. Treatment of hepatocellular carcinoma with inhibitor K67 reduces proliferation of HCC cells and also confers resistance to cisplatin and sorafenib [89]. Quercetin also activates Keap1-Nrf2-p62 axis and thereby reduces hepatic toxicity by various toxicants [89].

Moreover, during oxidative stress conditions, trehalose activates autophagy-mediated Nrf2- Keap1 regulation. Overexpression of p62 mimics Nrf2 activation [90].

Curcumin reduces inflammation, oxidative stress, and cytotoxic to cancer cells. Dietary supplementation of curcumin reduces AFB1-mediated inflammation by decreasing the level of inflammatory cytokines (NF-κB, IL-6, IL-8, IL-10, etc.) and increasing pro-inflammatory cytokines. This results into decreased AFB1 mediated inflammation. Curcumin also does so by up regulating Nrf2 and thereby its downstream gene, HO-1 in broiler hepatocytes [91].

In breast cancer MCF7 and T47D cells, Nrf2 signaling augments antioxidant response in glucose deprivation and increased autophagy conditions. This confers protection to breast cancer cells even in low glucose means in metabolic stressed conditions [92].

During protein synthesis, formation of misfolded protein is a general phenomenon and about 30% of total synthesized proteins become misfolded. These misfolded proteins do not have any function in cells; therefore, they are degraded by either proteasome or by autophagy-mediated processes. If these proteins remain in cells being unprocessed, it induces cytotoxicity. So for cells to increase their life span, it is very important to remove un/misfolded proteins as they form to inhibit their accumulation. These protein aggregates could be formed inside the cells. One could be due to inhibition of degradation pathway, leading to protein aggregate accumulation which is called aggresomes. Second types of aggregates are generated

during cellular stress like oxidative stress, heat shock named as aggresome-like induced structures (ALIS). Both types of aggregates are degraded by selective autophagy called as aggrephagy. TRIM (Triplicate motif protein), during oxidative stress conditions, forms a positive feedback loop having Nrf2, Keap1, p62, and TRIM16 proteins by regulating expression level of these proteins [93, 94].

Upon traumatic brain injury (TBI), inflammation and microglial activation takes place to reduce cytotoxicity. Valproic acid (VPA) up regulates p62-Keap1-Nrf2 signaling axis following brain injury and causes increased acetylation of histones. These results in VPA-mediated up regulation in antioxidant and autophagy response in TBI and it protects from neurodegeneration [95].

7 Nrf2 Signaling in Inflammation

Inflammasomes are the complexes that are made up of upstream caspase-1, NLRP3 (nucleotide-binding oligomerization domain (NOD)-like receptor containing pyrin domain 3) and ASC (apoptosis-associated speck-like protein containing a CARD). Here, NLRP3 acts as a sensor protein; ASC acts as adaptor protein and caspase-1 acts as effector protein. In addition to NLRP3, AIM2 (absent in melanoma 2), NLRP1, and NLRC4 (NLR family CARD domain containing protein 4) also act as a sensor for inflammosome. Like Nrf2 signaling, inflammosome pathway is also activated upon cellular stress. Inflammosome activation induces inflammation which causes cell death. Nrf2 and inflammosome pathway are activated in acute as well as in chronic inflammation, upon generation of ROS, following induction of autophagy. Since both of these pathways are activated upon same stress conditions, there is an obvious crosstalk between these two pathways [96]. Nrf2 regulates expression of genes that are present in inflammosome pathway as well. Following stress, sensor proteins in inflammosome pathway sense the stress and induce ASC oligomerization forming ASC specks. Then, caspase-1 binding activates proteases which ultimately induce pro-inflammatory cytokines such as IL-1 and IL-18. Pro-inflammatory cytokines initiate inflammation. TXNIP (thioredoxin-interacting protein), generated by ROS, also interacts inflammosome sensor proteins and activates inflammosome assembly. Nrf2 activation by ROS induction augments inflammosome activation in mouse cells and in human skin cells as well. Several studies suggest that Nrf2 activation reduces NF-κB activation which is required for NLRP3 priming to inflammasomes and thereby inhibits inflammasomes activation. This occurs due to the fact that Nrf2 activation causes cytoprotection whereas inflammosome activation results in cell death. Hence, Nrf2-inflammosome crosstalk occurs antagonistically. Both pathways are involved in inflammatory stress and nrf2 is also involved in cancer, it is relevant to study crosstalk and its significance in cancer cells also [96].

Moreover, in inflammatory conditions, lung injury is induced which is related to a high rate of mortality. This inflammation-induced lung injury could be a result of intestinal ischemia-reperfusion (I/R). In such conditions, Nrf2 down regulates

TLR4, inflammation, apoptosis, and regulates Akt activation. In murine lung epithelial cells, Nrf2 knockdown up regulates TLR4, HO-1, autophagy, and apoptosis in glucose-deprived conditions. In addition, Akt signaling is also down regulated [97].

8 Nrf2 Signaling in Chemo-Resistance

Moreover, cancer stem cells (CSCs) are population of cancer cells which has self-renewal properties like other stem cells. Cancer stem cells are responsible for tumor relapse after treatment of various types of cancers like lung, brain, and breast. They have low level of ROS like that of adult stem cells. Due to low ROS level, CSCs develop resistance to oxidative stress. In comparison to normal cells, CSCs present in breast cancer express increased levels of antioxidant proteins like catalase, superoxide dismutase, and glutathione peroxidase. This creates lower level of cellular-free radicals by increasing ROS metabolism in oxidative environment during radiation-induced DNA damage. In breast cancer cell lines like MCF7, there is a high level of cluster of differentiation 44 (CD44) proteins. CD44 is a glycoprotein and acts as receptor for proteins of extracellular matrix, hyaluronic acid which is a well-known marker of CSCs in various cancers. Increased expression of CD44 correlates with tumor initiation and progression due to increased motility and invasion capacity of cancer cells. Higher CD44 levels are also found to correlate with higher Nrf2 level in breast CSCs. This ultimately contributes to maintenance of lower ROS level upon radiation therapy and leads to radiation resistance against anticancer therapy through high CD44-high Nrf2 axis in breast tumors [98].

In gastric cancer, Nrf2-P-glycoprotein axis is responsible for multidrug resistance. During chemotherapy, some cancers develop drug resistance. This can be due to reduction in drug accumulation by membrane transporters. P-glycoprotein (P-gp), which is an efflux pump, plays a crucial role in drug resistance via decreasing drug concentration by causing drug efflux out of the cancer cells. There is a positive correlation between Nrf2 and p-gp in gastric cancer cells. Cumulative results of higher expression of Nrf2-dependent phase II detoxifying enzymes, antioxidant enzymes, and P-gp-dependent efflux of anticancer drugs creates tumor-supportive environment and leads to tumor drug resistance in gastric cancer [99].

Moreover, increased Nrf2 expression in K-Ras^{G12V} (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutated cells confers drug resistance. Majority of cells undergo cancerous transformation due to K-ras^{G12V} mutation. Nrf2 levels increase in such transformed cells which is responsible for cytoprotection and drug resistance through up regulation of its downstream target gene-NQO1. Nrf2 knockout in Ras transformed cells leads to increased apoptosis, lower cellular proliferation, and less tumor growth. This is due in part by lowed activation of antioxidant genes in Nrf2 knockout cells and increased ROS generation [100].

In case of ovarian cancer stem cell like cells (CSCs), aldehyde dehydrogenase (ALDH)-dependent up regulation of Nrf2 results in drug resistance. Highly active ALDH, an enzyme that causes aldehyde oxidation to their respective acids, is one of

the hallmarks of CSC. In 1258 ovarian cancer meta-analysis, ALDH has been found to be up regulated and it correlates with tumor survival and drug resistance. However, ALDH up regulation accompanies an increase in expression of a multidrug resistance protein which induces efflux of drug. ALDH-dependent Nrf2 signaling activation leads to Nrf2 translocation into the nucleus, increased transcription of NQO-1 and AKR1C1 genes. Also the total cellular Nrf2 level increases along with its nuclear localization. Higher nuclear localization is a result of increase in p62 level which causes dissociation of Nrf2 with Keap1, its release, and translocation into the nucleus. Therefore, ALDH-dependent Nrf2 activation through p62-related signaling in ovarian cells leads to CSC promoting properties and anticancer drug resistance [23].

In colon cancer, drug resistance is developed by Her2-Nrf2 signaling pathway. Somatic mutation in Her2, a member of receptor tyrosine kinase family of proteins, results in ovarian cancer like that of breast cancer. Resulting Her2 overexpression leads to resistance against anticancer drug like oxaliplatin in many cancers including colon cancer. Nrf2 inhibition in Her2-mutated colon cancer increases oxaliplatin-induced apoptosis and drug sensitivity in LS174T colon cancer cell line. Moreover, HCT116 colon cancer cells spheres, Nrf2 is highly expressed giving rise to doxorubicin treatment resistance in these cells [101].

In hepatocellular carcinoma (HCC), cisplatin resistance is induced by NRAL/miR-340-5p/Nrf2 signaling. NRAL (Nrf2 regulation-associated lncRNA) is a 495 nucleotide long non-coding RNA (lncRNA-ENST00000412153) present near 2722 kb from Nrf2 gene. CDDP depletes antioxidant ability of tumor cells thereby causing their apoptosis. NRAL develops resistance to CDDP in hepatocellular carcinoma by regulating Nrf2. It does so by directly interacting miR-340-5p which is an endogenous miRNA and targets Nrf2 mRNA for degradation. Therefore, NRAL acts as competing endogenous RNA (ceRNA) for miR-340-5p and thereby increasing Nrf2 level which ultimately results antioxidant capacity of cells and develops in CDDP, cisplatin resistance in HCC [102].

Nrf2 also confers drug resistance in bladder cancers. In bladder cancer, there is a positive feedback loop between Nrf2 and YAP (Yes-associated protein) that protects tumor cell against oxidative stress and apoptosis. YAP regulates expression of FOXM1 which is a transcription factor involved in Nrf2 transcription. Moreover, expression of YAP is also regulated by Nrf2 which creates a positive loop between Nrf2 and YAP. This regulates cellular oxidative homeostasis via GSH and develops cisplatin resistance in bladder cancers [103].

9 Aberrant Activation of Nrf2

Aberrant activation of Nrf2 in tumor cells confers malignancy. Many immunohistochemical and clinicopathological studies show strong correlations between Nrf2 activation and tumor progression [104–108]. These evidences make Nrf2 an important prognostic factor in a wide range of cancers. From many case studies, it has been found that Nrf2 have been constitutively activated due to changes in DNA

structure, RNA expression, or due to alteration in protein–protein interactions. Somatic mutations of Keap1 and Nrf2 genes generally found in the head and neck cancer, lung cancer, and bladder cancer. From the crystal structure, it has been found that most mutations of Keap1 are in the coding region and in case of Nrf2 the DLG/ETGE motifs are found to be the mutation-prone sites [109, 110]. Mutated Nrf2, resulted from alternative splicing of Nrf2 with exon2 skipping, is not able to bind to Keap1. This type of mutations are commonly found in lung, head and neck squamous cell carcinoma, and hepatocellular cancer with permanent localization of Nrf2 in the nucleus [111]. An adaptor protein p62 selects ubiquitinated Keap1 for autophagy resulting in accumulation of free Nrf2. In hepatocellular carcinoma, constitutive activation of Nrf2 is often observed due to the overexpression of phosphorylated p62 [88]. In fumarate hydratase deficiency, accumulated fumarate alkylates some thiol groups of Keap1 thereby stabilizing Nrf2 [28]. K-ras mutant mice induce overexpression of Myc through activation of Ras signaling. This overexpressed Myc then binds to the promoter of Nrf2 up regulating its transcription and thereby induces tumorigenesis [112].

10 Nrf2 Activators

Among many plant-derived photochemicals, curcumin, epigallocatechin-3-gallate, resveratrol, cafestol, lycopene, kahweol, cinnamonyl-based compounds, sulforaphane (SF), garlic organosulfur compounds, zerumbone, and carnosol are found to act as Nrf2 activators. Sulforaphane, an isothiocyanate, found in broccoli shows protection not only against skin tumor and tobacco-derived lung cancer but also shows reduction of abnormal crypt foci following exposure of N-nitrosobis (2 oxopropyl) amine (BOP) [113, 114]. Curcumin, another chemo-preventive natural product, also has been identified to activate Nrf2. In benzo(a)pyrene-induced murine lung and liver carcinoma, curcumin has been shown to increase Nrf2 expression with hampered binding to DNA, increased oxidative stress, and inflammation [115]. Dimethyl fumarate (DMF) causes alkylation of Keap1 on critical cysteine residue to inhibit Nrf2 ubiquitination, and thereby increases its stabilization and expression of various Nrf2 target genes [116]. DMF which is approved by Food and Drug Administration (FDA) for multiple sclerosis (MS), also shows some potential as an anticancer drug in various types of cancers like glioblastoma, head and neck cancer, melanoma, and colon cancer [117].

11 Nrf2 Inhibitors

In case of cancers with consecutive Nrf2 expression, inhibition of Nrf2 signaling by pharmacological modulators has been appeared as a potential therapeutic intervention. Molecules like ascorbic acid, luteolin, trigonelline, ochratoxin A, and all-trans retinoic acid (ATRA) are able to suppress the Nrf2 pathway either by inhibiting

Nrf2 binding to DNA or decreasing Nrf2 mRNA and protein expression [118]. Retinoic acid receptor α (RARα) and ATRA mimetic hamper both basal and inducible Nrf2 activity both in in vitro and in vivo [119]. In the presence of ATRA, Nrf2 forms a complex with RARα. This Nrf2 and RARα complex inhibits binding of Nrf2 on the ARE thereby makes Nrf2 unable to induce expression of its target genes.

12 Conclusion

From a number of studies, it has been cleared that inflammation provides a cell, an environment for tumor development. Aberrant production of ROS causes inflammation in our body. During inflammation, various types of chemokines and cytokines are produced that give rise to an ideal condition for tumor generation. In these cells, a constant increase in ROS generally observed which maintains tumor progression. If not controlled, the excessive ROS causes more mutations and ultimately drives tumor cell migration and invasion. In response to ROS-mediated oxidative stress, a group of cytoprotective enzymes are expressed by transcription factor Nrf2, thereby reducing ROS production. For cancer prevention in which oxidative stress contributes to the pathogenesis, elevating Nrf2 activity remains an important approach.

Conflicts of Interest There are no conflicts to declare.

References

1. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol. 2011;194:7–15.
2. Ma Q. Transcriptional responses to oxidative stress: pathological and toxicological implications. Pharmacol Ther. 2010;125:376–93.
3. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci U S A. 1994;91:9926–30.
4. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–22.
5. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A. 1996;93:14960–5.
6. Ma Q. Xenobiotic-activated receptors: from transcription to drug metabolism to disease. Chem Res Toxicol. 2008;21:1651–71.
7. Hayes JD, McMahon M, Chowdhry S, Dinkova-Kostova AT. Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. Antioxid Redox Signal. 2010;13:1713–48.
8. Hamada S, Taguchi K, Masamune A, Yamamoto M, Shimosegawa T. Nrf2 promotes mutant K-ras/p53-driven pancreatic carcinogenesis. Carcinogenesis. 2018;38:661–70.
9. Kensler TW, Wakabayashi N. Nrf2: friend or foe for chemoprevention? Carcinogenesis. 2010;31:90–9.

10. Lau A, Zheng Y, Tao S, Wang H, Whitman SA, White E, et al. Arsenic inhibits autophagic flux, activating the Nrf2-Keap1 pathway in a p62-dependent manner. Mol Cell Biol. 2013;33:2436–46.
11. Long M, Tao S, Rojo de la Vega M, Jiang T, Wen Q, Park SL, et al. Nrf2-dependent suppression of azoxymethane/dextran sulfate sodium-induced colon carcinogenesis by the cinnamon-derived dietary factor cinnamaldehyde. Cancer Prev Res. 2015;8:444–54.
12. Tao S, Rojo de la Vega M, Chapman E, Ooi A, Zhang DD. The effects of NRF2 modulation on the initiation and progression of chemically and genetically induced lung cancer. Mol Carcinog. 2018;57:182–92.
13. Wang H, Liu X, Long M, Huang Y, Zhang L, Zhang R, et al. NRF2 activation by antioxidant antidiabetic agents accelerates tumor metastasis. Sci Transl Med. 2016;8:334ra51.
14. Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J Biol Chem. 2002;277:42769–74.
15. Eades G, Yang M, Yao Y, Zhang Y, Zhou Q. miR-200a regulates Nrf2 activation by targeting Keap1 mRNA in breast cancer cells. J Biol Chem. 2011;286:40725–33.
16. Kang MI, Kobayashi A, Wakabayashi N, Kim SG, Yamamoto M. Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. Proc Natl Acad Sci U S A. 2004;101:2046–51.
17. Alam J, Killeen E, Gong P, Naquin R, Hu B, Stewart D, et al. Heme activates the heme oxygenase-1 gene in renal epithelial cells by stabilizing Nrf2. Am J Physiol Renal Physiol. 2003;284:F743–52.
18. Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, Yamamoto M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells. 2003;8:379–91.
19. Tong KI, Kobayashi A, Katsuoka F, Yamamoto M. Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism. Biol Chem. 2006;387:1311–20.
20. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004;24:7130–9.
21. Deretic V. Autophagy as an immune defense mechanism. Curr Opin Immunol. 2006;18:375–82.
22. Kaniuk NA, Kiraly M, Bates H, Vranic M, Volchuk A, Brumell JH. Ubiquitinated-protein aggregates form in pancreatic beta-cells during diabetes-induced oxidative stress and are regulated by autophagy. Diabetes. 2007;56:930–9.
23. Kim D, Choi BH, Ryoo IG, Kwak MK. High NRF2 level mediates cancer stem cell-like properties of aldehyde dehydrogenase (ALDH)-high ovarian cancer cells: inhibitory role of all-trans retinoic acid in ALDH/NRF2 signaling. Cell Death Dis. 2018;9:896.
24. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem. 2007;282:24131–45.
25. Copple IM, Lister A, Obeng AD, Kitteringham NR, Jenkins RE, Layfield R, et al. Physical and functional interaction of sequestosome 1 with Keap1 regulates the Keap1-Nrf2 cell defense pathway. J Biol Chem. 2010;285:16782–8.
26. Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol. 2010;12:213–23.
27. Li Y, Paonessa JD, Zhang Y. Mechanism of chemical activation of Nrf2. PLoS One. 2012;7:e35122.
28. Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, Lockstone H, et al. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. Cancer Cell. 2011;20:524–37.
29. Rushmore TH, Kong AN. Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. Curr Drug Metab. 2002;3:481–90.

30. Loignon M, Miao W, Hu L, Bier A, Bismar TA, Scrivens PJ, et al. Cul3 overexpression depletes Nrf2 in breast cancer and is associated with sensitivity to carcinogens, to oxidative stress, and to chemotherapy. Mol Cancer Ther. 2009;8:2432–40.
31. Kwak MK, Itoh K, Yamamoto M, Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cell Biol. 2002;22:2883–92.
32. Nair S, Doh ST, Chan JY, Kong AN, Cai L. Regulatory potential for concerted modulation of Nrf2- and Nfkb1-mediated gene expression in inflammation and carcinogenesis. Br J Cancer. 2008;99:2070–82.
33. Yang M, Yao Y, Eades G, Zhang Y, Zhou Q. MiR-28 regulates Nrf2 expression through a Keap1-independent mechanism. Breast Cancer Res Treat. 2011;129:983–91.
34. Sangokoya C, Telen MJ, Chi JT. microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. Blood. 2010;116:4338–48.
35. Li N, Muthusamy S, Liang R, Sarojini H, Wang E. Increased expression of miR-34a and miR-93 in rat liver during aging, and their impact on the expression of Mgst1 and Sirt1. Mech Ageing Dev. 2011;132:75–85.
36. Numazawa S, Ishikawa M, Yoshida A, Tanaka S, Yoshida T. Atypical protein kinase C mediates activation of NF-E2-related factor 2 in response to oxidative stress. Am J Physiol. 2003;285:C334–42.
37. Theodore M, Kawai Y, Yang J, Kleshchenko Y, Reddy SP, Villalta F, et al. Multiple nuclear localization signals function in the nuclear import of the transcription factor Nrf2. J Biol Chem. 2008;283:8984–94.
38. Pi J, Bai Y, Reece JM, Williams J, Liu D, Freeman ML, et al. Molecular mechanism of human Nrf2 activation and degradation: role of sequential phosphorylation by protein kinase CK2. Free Radic Biol Med. 2007;42:1797–806.
39. Apopa PL, He X, Ma Q. Phosphorylation of Nrf2 in the transcription activation domain by casein kinase 2 (CK2) is critical for the nuclear translocation and transcription activation function of Nrf2 in IMR-32 neuroblastoma cells. J Biochem Mol Toxicol. 2008;22:63–76.
40. Itoh K, Igarashi K, Hayashi N, Nishizawa M, Yamamoto M. Cloning and characterization of a novel erythroid cell-derived CNC family transcription factor heterodimerizing with the small Maf family proteins. Mol Cell Biol. 1995;15:4184–93.
41. Kataoka K, Igarashi K, Itoh K, Fujiwara KT, Noda M, Yamamoto M, et al. Small Maf proteins heterodimerize with Fos and may act as competitive repressors of the NF-E2 transcription factor. Mol Cell Biol. 1995;15:2180–90.
42. Takagi Y, Kobayashi M, Li L, Suzuki T, Nishikawa K, Yamamoto M. MafT, a new member of the small Maf protein family in zebrafish. Biochem Biophys Res Commun. 2004;320:62–9.
43. Motohashi H, Katsuoka F, Engel JD, Yamamoto M. Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1-Nrf2 regulatory pathway. Proc Natl Acad Sci U S A. 2004;101:6379–84.
44. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, et al. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. EMBO J. 2002;21:5216–24.
45. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13:76–86.
46. Kobayashi A, Ito E, Toki T, Kogame K, Takahashi S, Igarashi K, et al. Molecular cloning and functional characterization of a new Cap'n' collar family transcription factor Nrf3. J Biol Chem. 1999;274:6443–52.
47. Kobayashi M, Itoh K, Suzuki T, Osanai H, Nishikawa K, Katoh Y, et al. Identification of the interactive interface and phylogenic conservation of the Nrf2-Keap1 system. Genes Cells. 2002;7:807–20.
48. Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. Proc Natl Acad Sci U S A. 2001;98:3404–9.

49. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A. 2002;99:11908–13.
50. Itoh K, Mochizuki M, Ishii Y, Ishii T, Shibata T, Kawamoto Y, et al. Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2). Mol Cell Biol. 2004;24:36–45.
51. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, et al. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc Natl Acad Sci U S A. 2004;101:2040–5.
52. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol. 2003;23:8137–51.
53. Buckley BJ, Marshall ZM, Whorton AR. Nitric oxide stimulates Nrf2 nuclear translocation in vascular endothelium. Biochem Biophys Res Commun. 2003;307:973–9.
54. Zipper LM, Mulcahy RT. Inhibition of ERK and p38 MAP kinases inhibits binding of Nrf2 and induction of GCS genes. Biochem Biophys Res Commun. 2000;278:484–92.
55. Zipper LM, Mulcahy RT. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. Toxicol Sci. 2003;73:124–34.
56. Stacy DR, Ely K, Massion PP, Yarbrough WG, Hallahan DE, Sekhar KR, et al. Increased expression of nuclear factor E2 p45-related factor 2 (NRF2) in head and neck squamous cell carcinomas. Head Neck. 2006;28:813–8.
57. Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, et al. Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. J Biol Chem. 2000;275:39907–13.
58. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol. 2003;23:7198–209.
59. Kang KW, Choi SH, Kim SG. Peroxynitrite activates NF-E2-related factor 2/antioxidant response element through the pathway of phosphatidylinositol 3-kinase: the role of nitric oxide synthase in rat glutathione S-transferase A2 induction. Nitric Oxide. 2002;7:244–53.
60. Nakaso K, Yano H, Fukuhara Y, Takeshima T, Wada-Isoe K, Nakashima K. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. FEBS Lett. 2003;546:181–4.
61. Bloom DA, Jaiswal AK. Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. J Biol Chem. 2003;278:44675–82.
62. Huang HC, Nguyen T, Pickett CB. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. Proc Natl Acad Sci U S A. 2000;97:12475–80.
63. Iida K, Itoh K, Kumagai Y, Oyasu R, Hattori K, Kawai K, et al. Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. Cancer Res. 2004;64:6424–31.
64. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci U S A. 2001;98:3410–5.
65. Rachakonda G, Sekhar KR, Jowhar D, Samson PC, Wikswo JP, Beauchamp RD, et al. Increased cell migration and plasticity in Nrf2-deficient cancer cell lines. Oncogene. 2010;29:3703–14.

66. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, Suzuki T, et al. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. Carcinogenesis. 2010;31:1833–43.
67. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013;27:2179–91.
68. Menegon S, Columbano A, Giordano S. The dual roles of NRF2 in Cancer. Trends Mol Med. 2016;22:578–93.
69. Okada F. Inflammation-related carcinogenesis: current findings in epidemiological trends, causes and mechanisms. Yonago Acta Med. 2014;57:65–72.
70. Wang Y, Mandal AK, Son YO, Pratheeshkumar P, Wise JTF, Wang L, et al. Roles of ROS, Nrf2, and autophagy in cadmium-carcinogenesis and its prevention by sulforaphane. Toxicol Appl Pharmacol. 2018;353:23–30.
71. Ma JQ, Tuersun H, Jiao SJ, Zheng JH, Xiao JB, Hasim A. Functional role of NRF2 in cervical carcinogenesis. PLoS One. 2015;10:e0133876.
72. Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, et al. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Med. 2006;3:e420.
73. auf dem Keller U, Huber M, Beyer TA, Kumin A, Siemes C, Braun S, et al. Nrf transcription factors in keratinocytes are essential for skin tumor prevention but not for wound healing. Mol Cell Biol. 2006;26:3773–84.
74. Son YO, Pratheeshkumar P, Roy RV, Hitron JA, Wang L, Divya SP, et al. Antioncogenic and oncogenic properties of Nrf2 in arsenic-induced carcinogenesis. J Biol Chem. 2015;290:27090–100.
75. Cheung KL, Lee JH, Khor TO, Wu TY, Li GX, Chan J, et al. Nrf2 knockout enhances intestinal tumorigenesis in Apc(min/+) mice due to attenuation of anti-oxidative stress pathway while potentiates inflammation. Mol Carcinog. 2014;53:77–84.
76. Ikeda H, Nishi S, Sakai M. Transcription factor Nrf2/MafK regulates rat placental glutathione S-transferase gene during hepatocarcinogenesis. Biochem J. 2004;380:515–21.
77. Basak P, Sadhukhan P, Sarkar P, Sil PC. Perspectives of the Nrf-2 signaling pathway in cancer progression and therapy. Toxicol Rep. 2017;4:306–18.
78. Zhang HS, Du GY, Zhang ZG, Zhou Z, Sun HL, Yu XY, et al. NRF2 facilitates breast cancer cell growth via HIF1a-mediated metabolic reprogramming. Int J Biochem Cell Biol. 2017;95:85–92.
79. Zhang C, Wang HJ, Bao QC, Wang L, Guo TK, Chen WL, et al. NRF2 promotes breast cancer cell proliferation and metastasis by increasing RhoA/ROCK pathway signal transduction. Oncotarget. 2016;7:73593–606.
80. Martinez VD, Vucic EA, Thu KL, Pikor LA, Lam S, Lam WL. Disruption of KEAP1/CUL3/RBX1 E3-ubiquitin ligase complex components by multiple genetic mechanisms: association with poor prognosis in head and neck cancer. Head Neck. 2014;37:727–34.
81. Huang CF, Zhang L, Ma SR, Zhao ZL, Wang WM, He KF, et al. Clinical significance of Keap1 and Nrf2 in oral squamous cell carcinoma. PLoS One. 2013;8:e83479.
82. Singh B, Chatterjee A, Ronghe AM, Bhat NK, Bhat HK. Antioxidant-mediated up-regulation of OGG1 via NRF2 induction is associated with inhibition of oxidative DNA damage in estrogen-induced breast cancer. BMC Cancer. 2013;13:253.
83. Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. Nat Rev. 2014;15:7–18.
84. Jayakumar S, Pal D, Sandur SK. Nrf2 facilitates repair of radiation induced DNA damage through homologous recombination repair pathway in a ROS independent manner in cancer cells. Mutat Res. 2015;779:33–45.
85. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer. 2011;12:68–78.
86. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol. 2010;221:3–12.
87. Galluzzi L, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, Baehrecke EH, Cecconi F, et al. Autophagy in malignant transformation and cancer progression. EMBO J. 2015;34:856–80.

88. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell. 2013;51: 618–31.
89. Ichimura Y, Komatsu M. Activation of p62/SQSTM1-Keap1-nuclear factor Erythroid 2-related factor 2 pathway in Cancer. Front Oncol. 2018;8:210.
90. Mizunoe Y, Kobayashi M, Sudo Y, Watanabe S, Yasukawa H, Natori D, et al. Trehalose protects against oxidative stress by regulating the Keap1-Nrf2 and autophagy pathways. Redox Biol. 2018;15:115–24.
91. Muhammad I, Wang X, Li S, Li R, Zhang X. Curcumin confers hepatoprotection against AFB1-induced toxicity via activating autophagy and ameliorating inflammation involving Nrf2/HO-1 signaling pathway. Mol Biol Rep 2018
92. Walker A, Singh A, Tully E, Woo J, Le A, Nguyen T, et al. Nrf2 signaling and autophagy are complementary in protecting breast cancer cells during glucose deprivation. Free Radic Biol Med. 2018;120:407–13.
93. Fujita K, Maeda D, Xiao Q, Srinivasula SM. Nrf2-mediated induction of p62 controls toll-like receptor-4-driven aggresome-like induced structure formation and autophagic degradation. Proc Natl Acad Sci U S A. 2011;108:1427–32.
94. Jena KK, Kolapalli SP, Mehto S, Nath P, Das B, Sahoo PK, et al. TRIM16 controls assembly and degradation of protein aggregates by modulating the p62-NRF2 axis and autophagy. EMBO J. 2018;37
95. Cheng KW, Cheng SC, Chen WY, Lin MH, Chuang SJ, Cheng IH, et al. Thiopurine analogs and mycophenolic acid synergistically inhibit the papain-like protease of Middle East respiratory syndrome coronavirus. Antivir Res. 2018;115:9–16.
96. Hennig P, Garstkiewicz M, Grossi S, Di Filippo M, French LE, Beer HD. The crosstalk between Nrf2 and Inflammasomes. Int J Mol Sci. 2018;19
97. Yan J, Li J, Zhang L, Sun Y, Jiang J, Huang Y, et al. Nrf2 protects against acute lung injury and inflammation by modulating TLR4 and Akt signaling. Free Radic Biol Med. 2018;121: 78–85.
98. Ryoo IG, Choi BH, Ku SK, Kwak MK. High CD44 expression mediates p62-associated NFE2L2/NRF2 activation in breast cancer stem cell-like cells: implications for cancer stem cell resistance. Redox Biol. 2018;17:246–58.
99. Jeddi F, Soozangar N, Sadeghi MR, Somi MH, Shirmohamadi M, Eftekhar-Sadat AT, et al. Nrf2 overexpression is associated with P-glycoprotein upregulation in gastric cancer. Biomed Pharmacother. 2018;97:286–92.
100. Shao J, Glorieux C, Liao J, Chen P, Lu W, Liang Z, et al. Impact of Nrf2 on tumour growth and drug sensitivity in oncogenic K-ras-transformed cells in vitro and in vivo. Free Radic Res. 2018;52:661–71.
101. Pirpour Tazehkand A, Akbarzadeh M, Velaie K, Sadeghi MR, Samadi N. The role of Her2-Nrf2 axis in induction of oxaliplatin resistance in colon cancer cells. Biomed Pharmacother. 2018;103:755–66.
102. Wu LL, Cai WP, Lei X, Shi KQ, Lin XY, Shi L. NRAL mediates cisplatin resistance in hepatocellular carcinoma via miR-340-5p/Nrf2 axis. J Cell Commun Signal 2018
103. Ciamporcero E, Daga M, Pizzimenti S, Roetto A, Dianzani C, Compagnone A, et al. Crosstalk between Nrf2 and YAP contributes to maintaining the antioxidant potential and chemoresistance in bladder cancer. Free Radic Biol Med. 2018;115:447–57.
104. Kawasaki Y, Okumura H, Uchikado Y, Kita Y, Sasaki K, Owaki T, et al. Nrf2 is useful for predicting the effect of chemoradiation therapy on esophageal squamous cell carcinoma. Ann Surg Oncol. 2014;21:2347–52.
105. Onodera Y, Motohashi H, Takagi K, Miki Y, Shibahara Y, Watanabe M, et al. NRF2 immunolocalization in human breast cancer patients as a prognostic factor. Endocr Relat Cancer. 2014;21:241–52.
106. Shibata T, Saito S, Kokubu A, Suzuki T, Yamamoto M, Hirohashi S. Global downstream pathway analysis reveals a dependence of oncogenic NF-E2-related factor 2 mutation on the mTOR growth signaling pathway. Cancer Res. 2010;70:9095–105.

107. Solis LM, Behrens C, Dong W, Suraokar M, Ozburn NC, Moran CA, et al. Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. Clin Cancer Res. 2010;16:3743–53.
108. Zhao M, Xu H, Zhang B, Hong B, Yan W, Zhang J. Impact of nuclear factor erythroid-derived 2-like 2 and p62/sequestosome expression on prognosis of patients with gliomas. Hum Pathol. 2015;46:843–9.
109. Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M. Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol Cell Biol. 2014;34:832–46.
110. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell. 2006;21:689–700.
111. Goldstein LD, Lee J, Gnad F, Klijn C, Schaub A, Reeder J, et al. Recurrent loss of NFE2L2 exon 2 is a mechanism for Nrf2 pathway activation in human cancers. Cell Rep. 2016;16:2605–17.
112. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature. 2011;475:106–9.
113. Kensler TW, Curphey TJ, Maxiutenko Y, Roebuck BD. Chemoprotection by organosulfur inducers of phase 2 enzymes: dithiolethiones and dithiins. Drug Metabol Drug Interact. 2000;17:3–22.
114. Kuroiwa Y, Nishikawa A, Kitamura Y, Kanki K, Ishii Y, Umemura T, et al. Protective effects of benzyl isothiocyanate and sulforaphane but not resveratrol against initiation of pancreatic carcinogenesis in hamsters. Cancer Lett. 2006;241:275–80.
115. Garg R, Gupta S, Maru GB. Dietary curcumin modulates transcriptional regulators of phase I and phase II enzymes in benzo[a]pyrene-treated mice: mechanism of its anti-initiating action. Carcinogenesis. 2008;29:1022–32.
116. Phillips JT, Fox RJ. BG-12 in multiple sclerosis. Semin Neurol. 2013;33:56–65.
117. Chen AF, Kirsner RS. Mechanisms of drug action: the potential of dimethylfumarate for the treatment of neoplasms. J Invest Dermatol. 2011;131:1181.
118. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. Med Res Rev. 2012;32:687–726.
119. Wang XJ, Hayes JD, Henderson CJ, Wolf CR. Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha. Proc Natl Acad Sci U S A. 2007;104:19589–94.

Role of Nrf2 in Oxidative and Inflammatory Processes in Obesity and Metabolic Diseases

Maria M. Bayliak and Oleksandra B. Abrat

Abstract Excessive fat accumulation in adipocytes leads to obesity, which is a major contributing risk factor for many metabolic diseases such as metabolic syndrome, type 2 diabetes, and cardiovascular diseases. A number of studies showed that overnutrition causes oxidative stress and chronic low-grade inflammation, which both play a crucial role both in obesity prevention and in the development of obesity-related complications. Adipose tissue, especially in the visceral compartment, is considered not only as an energy depository tissue, but also as an active endocrine organ releasing a variety of biologically active molecules known as adipokines, with many of them having pro-inflammatory properties. Here, we summarize current data on the relationship between oxidative stress and inflammation in obesity, with emphasis on metabolic switches and the involvement of redox-responsive signaling pathways such as NF-κB and Nfr2. Experimental data suggest the dual role of Nrf2 signaling in prevention and aggravation of obesity and obesity-related inflammation; the potential mechanisms of Nrf2 duality are discussed.

Abbreviations

AGEs	Advanced glycation end products
BAT	Brown adipose tissue
BMI	Body mass index
CEBPβ	CCAAT/enhancer-binding protein β
ETC	Electron transport chain
FFAs	Free fatty acids
FoxO	Forkhead box O family of proteins
GSK3	Glycogen synthase kinase 3
HO-1	Heme oxygenase-1
IL	Interleukin

M. M. Bayliak (✉) · O. B. Abrat (✉)
Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine

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Keap1	Kelch-like ECH-associated protein (Nrf2 repressor protein)
Keap1-KO	Keap1 knock-out
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein
MetS	Metabolic syndrome
Neh	Nrf2-ECH homology functional domains of nuclear-related factor 2
NF-κB	Nuclear factor-κB
NOX	NADPH oxidase
Nrf2	Nuclear-related factor 2
Nrf2-KO	Nrf2 knock-out
PAI-1	Plasminogen activator inhibitor
PPARγ	Peroxisome proliferator-activated receptor γ
RCS	Reactive carbonyl species
ROS	Reactive oxygen species
TAG	Triacylglycerides
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
WAT	White adipose tissue
β-TrCP	β-transducin repeat-containing protein

1 Introduction

Obesity is a chronic metabolic condition that has become a global problem of twenty-first century, and medical conditions associated with obesity are grouped in the metabolic syndrome [1, 2]. Excessive fat accumulation, especially of visceral fat, increases the risk for various chronic metabolic complications, including hypertension, hyperglycemia, hyperlipidemia, nonalcoholic fatty liver disease, insulin resistance, atherosclerosis, type 2 diabetes, and obesity-related cancer [1, 3, 4].

Overconsumption of high caloric food is a main reason for world growth of obesity. When nutritional supplies exceed the energy needs, excess nutrients may be stored in the form fat reserves in adipose tissue [5]. Adipose tissue, in addition to its function of storing energy reserves, has important functions as an endocrine organ, which consists of adipocytes and many immune cells and produces a variety of biologically active compounds, including adipokines, such as adiponectin and leptin, chemokines, such as monocyte chemoattractant protein (MCP-1), and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), plasminogen activator inhibitor (PAI-1), interleukins 1β (IL-1β), and 6 (IL-6) [6–9]. Upon the accumulation of excessive fat, the adipocyte size/number is increased and the profile of adipokine secretion is altered, leading to a low-grade chronic systemic inflammation in adipose tissue and other peripheral tissues [1, 8–11]. Adipocytes secrete various chemokines, leading to the recruitment of pro-inflammatory M1 macrophages and other types of immune cells into adipose tissue. Enlarged adipocytes and infiltrated

immune cells increase the production of pro-inflammatory cytokines and chemokines, resulting in systemic inflammatory status [11]. Inflammation increases production of reactive oxygen species (ROS) by the immune cells as a part of the immune response [12]. In addition, overloading mitochondria with energy substrates and activation of adipose NADPH oxidase contribute to enhanced ROS production in enlarged adipocytes [3, 7, 10–14]. Moreover, pro-inflammatory and nutrient-induced oxidative stress may also promote further production of pro-inflammatory cytokines, which, in turn, aggravate ROS production in a "vicious cycle" [5, 10]. Thus, chronic oxidative stress and chronic inflammation should be considered as two main interconnecting players in obesity-related metabolic complications.

Increased ROS production is followed by activation of a number of redox-responsive transcription factors, including nuclear factor-κB (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2) [7, 10, 15–17]. Whereas NF-κB is considered as a major regulator of immune response [1], Nrf2 activates cellular defense systems against the cytotoxic effects of oxidative stress [18]. Several studies have demonstrated that Nrf2 also contributes to the anti-inflammatory processes [19, 20]. In addition, pharmacological activation of Nrf2 inhibits inflammation and impairs degenerative diseases providing an interface between redox and anti-inflammatory responses [21, 22]. Given that the inflammation together with oxidative stress act as interacting inductors of obesity-associated complications, antioxidant and anti-inflammatory activities of Nrf2 received much attention in studies on obesity and obesity-related complications. Experimental data found that Nrf2 pathway could have both anti-obesity and obesity-promoting effects, suggesting the complicated regulatory role of Nrf2 in energy metabolism.

2 Obesity and Metabolic Syndrome and as a Worldwide Health Problem

With the successful conquest of many old infectious diseases in the world, non-communicable diseases have become the major cause of morbidity and mortality [23]. One of the most common non-infectious diseases is obesity. Obesity is recognized as a chronic metabolic condition that has become a global problem twenty-first century. This is a medical, social, and economic issue. This pathology affects a significant portion of the human population in both developed and developing countries. According to the World Health Organization, at least 2.8 million people die each year from complications of obesity. The United States holds first place in the world in the number of people who have a high degree of obesity. In Central Europe, 20 to 24% of the adult population is obese. In particular, in Ukraine, 21.3% of people suffer from obesity, and 53.5% are overweight [2, 5, 24].

Overconsumption of high caloric food is thought to be a main reason for obesity. When nutritional supplies exceed the energy needs, they may be stored in the form of carbohydrate or fat reserves for use later under conditions as starvation, stress, or infection [5]. However, chronic overeating, combined with a sedentary lifestyle,

leads to an increasing accumulation of storage fats in the body of both adults and children. Importantly, obese children and adolescents have a high probability of remaining obese as adults. In the development of obesity, especial attention is paid to the periods of pre-school and adolescence regarded as times of risk for the development and maintenance of obesity [24, 25]. There is, however, consistent experimental and epidemiological data evidencing that the risk for developing obesity may largely depend on conditions of early life. Accumulating research findings indicate that epigenetic regulation of gene expression also plays a role in linking prenatal malnutrition to the risk of later-life metabolic disorders [26]. Accordingly, obesity is a condition with genetic and acquired etiology.

There are many ways in which a person's health can be classified in relation to the weight, but the most widely used method is a calculating body mass index (BMI). BMI is not used to definitively diagnose obesity because people who are very muscular sometimes have a high BMI without excess fat.

Overweight and obesity are caused by the increase in the size and the number of fat cells in the body. The latter leads to a number of metabolic complications collectively called as metabolic syndrome. The concept of the metabolic syndrome (MetS) has existed for at least 100 years [27]. This metabolic disturbance was first described in the 1920s by Kylin, a Swedish physician, as the clustering of signs such as hypertension, hyperglycemia, and gout [27, 28]. Later, in 1947, Vague drew attention to upper body adiposity as the obesity phenotype that was commonly associated with metabolic abnormalities related to type 2 diabetes and cardiovascular diseases [27, 29]. Today, MetS is recognized a multifaceted disorder, including dyslipidemia, hyperglycemia and hypertension, insulin resistance, and increased incidence of oxidative stress [4, 5, 30–33].

Nonetheless, there is debate surrounding the etiology and pathogenesis of MetS because a single unifying mechanism is still unknown [32, 33]. Obesity is not always synonymous with MetS. Not all obese people develop MetS and not all people with MetS are obese. For example, Asian Americans have greater prevalence of metabolic syndrome despite lower body mass index [34]. There are the so-called metabolically healthy obese individuals who have high level of insulin sensitivity and no hypertension and hyperlipidemia and other features of MetS [23]. Experimental data suggest that MetS itself has a multifactorial etiology, involving complex interactions between genetic background, hormones, and nutrition [33]. That is why some aspects that make it possible to find relationships between obesity and MetS should be further analyzed.

3 Obesity, Metabolic Complications, and Inflammation

3.1 Adipose Tissue: Functions and Distribution

In mammals, adipose tissue is heterogeneous and can be divided into brown and white adipose tissue (BAT and WAT, respectively) (Fig. 1). BAT is specialized in dissipating energy in the form of heat. This adipose tissue is localized in the

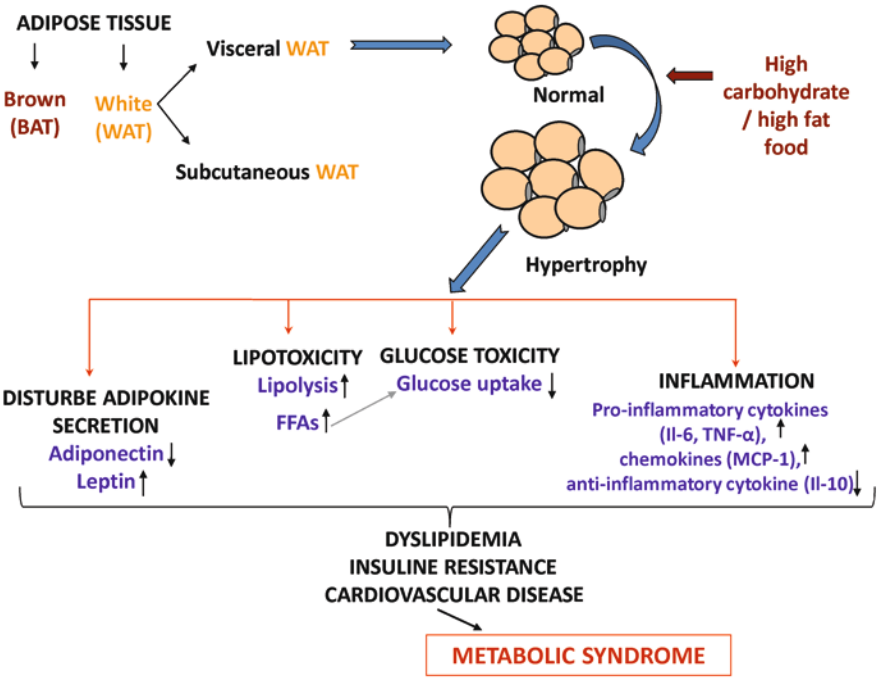

Fig. 1 Potential mechanisms of obesity-related inflammation and metabolic disturbances. With a chronic excessive energy intake and low physical activity, a positive energy balance causes body weight gain and higher nutrient flux into visceral white adipose tissue (WAT). Visceral WAT adipocytes primarily respond to the higher demand for energy storage by increasing their size (adipocyte hypertrophy). Adipocyte hypertrophy is typically associated with increased cellular and tissue stress, disturbed adipokine secretion, increased production of pro-inflammatory cytokines, including interleukins (IL-6, IL-1β), TNF-α, PAI-1, and MCP-1, and anti-inflammatory molecules such as IL-10. These signal molecules can then act through a number of cell signaling pathways to induce dyslipidemia, insulin resistance, vascular dysfunction, and atherosclerosis

interscapular, cervical, and paravertebral regions around large arteries, where it is able to take up glucose and trigacylglycerides from the blood. During lipogenesis, these nutrients are temporarily stored in intracellular lipid droplets of BAT [35]. WAT is not homogenous and includes subcutaneous and visceral compartments. Subcutaneous adipose tissue stores fats and is divided further into upper and lower body adipose tissue [4, 36]. Visceral adipose tissue supplies inner organs with energy and is divided into omental (subcutaneous) and mesenteric adipose [4, 37]. During long time, WAT was defined as an inactive organ, only capable of storing energy in the form of trigacylglycerides (TAGs). However, at present, its role as an endocrine organ playing important functions in whole-body metabolism has been well recognized [9, 35]. WAT was found to be responsible for the synthesis of various hormones, which are crucial in regulation of satiety and insulin sensitivity [6, 9, 11, 35]. WAT also participates in regulation of energy homeostasis because it is capable of releasing TAG-derived fatty acids into the bloodstream, which can

subsequently be used by other organs as an energy substrate or be packaged in TAG-rich lipoproteins in the liver [9, 35]. In the case of the accumulation of excess fats in WAT, this may cause many metabolic dysfunctions (insulin resistance, diabetes mellitus, heart dysfunction, etc.) that are combined in the term "metabolic syndrome."

3.2 Adipose Tissue and Insulin Resistance

A major determinant of metabolic health is the ability of subcutaneous adipose tissue to store excess fat rather than allowing it to accumulate in ectopic depots including liver (i.e., in nonalcoholic fatty liver disease), muscle and heart, or in epicardial/pericardial and visceral fat depots, which cause the metabolic complications of obesity [38]. The ability to recruit and differentiate precursor cells into adipose cells (adipogenesis) in subcutaneous adipose tissue is under genetic regulation. Dysregulation of these signaling pathways is associated with impaired adipogenesis. This leads to hypertrophic, dysfunctional, and insulin-resistant adipose cells with a reduced content of GLUT4, the major insulin-regulated glucose transporter. This, in turn, reduces adipose tissue glucose uptake and de novo lipogenesis [38]. The described above events are the inductors of genetically induced insulin resistance. At the same time, under any circumstances, adipocyte cell is a critical mediator between insulin and liver glucose output. Insulin acts on adipose tissue (1) by stimulation of glucose uptake and TAG synthesis and (2) by suppression of TAG hydrolysis and release of free fatty acids (FFAs) and glycerol into the circulation [39]. It has been hypothesized that FFAs, released during lipolysis in visceral adipose tissue, are important inductors of acquired insulin resistance [4, 40]. Excessive abdominal fat mass is associated with increased concentration of FFAs in the blood plasma. Surplus of circulating FFAs may ectopically be accumulated in insulin-sensitive tissues and impair insulin action. Increased basal lipolysis may also modify the secretory profile of adipose tissue, influencing whole-body insulin sensitivity [4, 36, 40, 41]. Finally, excessive FFA release may also worsen adipose tissue inflammation, a well-known process contributing to insulin resistance [41].

It is possible that insulin resistance of adipocyte itself can be a major cause of the dysregulation of carbohydrate metabolism in the prediabetic state. The inability of insulin to perform its numerous roles leads to an impaired glucose metabolism and an increase in blood glucose levels [42, 43]. In the latter case, subjects undergo hyperglycemia and insulin deficiency. Approximately one quarter of insulin-resistant patients has a normal glucose tolerance test, but this condition increases significantly the risk of development of type 2 diabetes mellitus. If pancreatic β cells function normally, the compensatory hyperinsulinemia will be raised to maintain normal fasting and postprandial glucose concentrations [4, 42].

3.3 Adipose Tissue and Cardiovascular Diseases

People with a central deposition of adipose tissue can experience elevated cardiovascular diseases and mortality, including stroke, congestive heart failure, myocardial infarction, and cardiovascular death [44, 45]. It has long been recognized that an extensive capillary network surrounds adipose tissue. Adipocytes are located close to vessels with the highest permeability, the lowest hydrostatic pressure, and the shortest distance for transport of molecules to and from the adipocytes [27, 45]. Classical cardiovascular risk factors include high low-density lipoprotein (LDL), high cholesterol, hypertension, and dysfunctions in glucose metabolism [27, 44]. The adipose tissue is not simply a passive storehouse for fat but an endocrine organ that synthesizes and releases into the bloodstream a variety of important peptides and non-peptide compounds involved in cardiovascular homeostasis and in its disturbance [4, 27, 45]. Existing data allowing assume that excess fat provoking cardiovascular diseases via the modulation of pro-inflammatory processes.

3.4 Bioactive Substances of Adipose Tissue

Adipose tissue comprises ~50% adipocytes and ~50% other cells including preadipocytes, vascular, neural, and immune cells [46]. Under normal conditions, adipose tissue is involved not only in lipid synthesis, but also in storage and secretion of anti-inflammatory molecules. Provoking factors such as increased fat content can also induce adipose tissue to secrete a number of pro-inflammatory factors [4, 36, 46]. By acting as transmitters of endocrine or paracrine signals, the secreted pro-/anti-inflammatory factors can induce either inflammation or altered insulin sensitivity of the adipocyte [36]. Generally, the adipose tissue derivatives known as adipokines include, besides inflammatory cytokines, other molecules acting as appetite regulators (leptin), insulin sensitizers, and atheroprotectors (adiponectin) [4, 9, 36, 47]. Adipocytes, pre-adipocytes, and macrophages within adipose tissue secrete pro-inflammatory cytokines, including interleukins (IL-6, IL-1β), TNF-α, PAI-1, and MCP-1, and anti-inflammatory molecules such as IL-10 [4, 9, 47] (Fig. 1).

3.4.1 Leptin

Leptin is a small peptide (16 kDa), belonging to a group of anti-inflammatory cytokines [48, 49]. It is an anorexigenic peptide, which increases energy expenditure [9]. Leptin is expressed mainly by adipose tissue although low levels have been detected in the placenta, skeletal muscle, gastric and mammary epithelium, and the brain [9, 49]. Levels of leptin are increased by glucocorticoids, acute infection, and pro-inflammatory cytokines. In addition, its levels are higher in females than males, partly because of inhibition by androgens and stimulation by estrogen [9].

The functional leptin receptor is in the hypothalamus where it functions to increase energy expenditure and to reduce appetite. The leptin receptor is also found in other organs such as the heart, liver, kidneys, and pancreas; it is also present in the smooth muscle and endothelium of heart, brain vasculature, and myometrium [47, 49]. Adipose tissue and plasma leptin concentrations depend on the amount of stored fats as well as the status of energy balance. Therefore, leptin levels are higher in obese individuals and increase with overeating. Conversely, lean individuals have lower leptin levels, and fasting reduces circulating leptin levels. Many studies have shown a positive correlation between leptin levels and metabolic syndrome [47, 50, 51]. Nutritional regulation of leptin is mediated at least partially by insulin, as leptin decreases in response to low insulin levels [9, 52].

3.4.2 Adiponectin

Structurally, adiponectin is related to the complement 1q family [9] and circulates in three isoforms: a trimer of low-molecular mass, a hexamer of medium molecular mass, and a multimeric high molecular mass isoform [9, 47, 53]. Adiponectin, like leptin, is an adipose-derived plasma protein with broad range of effects. However, unlike leptin, it is secreted exclusively by adipocytes. The receptors for adiponectin were found in adipocytes, brain, muscle, liver, artery, etc. [54].

Adiponectin levels display no great fluctuations in the blood; thus, its release is not acute but regulated by long-term metabolic changes [9]. Adiponectin plays an important role in protection against insulin resistance/diabetes and atherosclerosis. It has insulin-sensitizing activity, lipid oxidation enhancement, and vasodilatation activities [9, 47, 53]. Levels of adiponectin are low in subjects with essential hypertension and in the obese, but can increase with a loss of weight [9, 47]. Adiponectin suppresses almost all processes involved in atherosclerotic vascular changes: the expression of adhesion molecules in vascular endothelial cells, adhesion of monocytes to endothelial cells (via TNF-α inhibition), vascular smooth muscle cell proliferation and migration [9, 47]. Levels of adiponectin, unlike that of leptin, are inversely correlated with metabolic syndrome [39, 41, 47].

3.4.3 Interleukin 6

Interleukin 6 (IL-6) is a multifunctional cytokine produced by various cells. Its effects are usually aimed at the development of inflammation, immune response, and hematopoiesis [55]. At the same time, IL-6 is an interleukin, which acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine [56]. IL-6 is often secreted by M1 macrophages as part of the normal inflammatory response against infection and injury [9, 47, 55]. Role of IL-6 as an anti-inflammatory myokine is mediated through inhibition of TNF-alpha and IL-10 [56].

In metabolic syndrome, adipocyte dysfunction is frequently present and is associated with an increase in M1 macrophage population within adipose tissue. This

can result in the increased secretion of IL-6 and other pro-inflammatory cytokines from adipose tissue. These pro-inflammatory cytokines can then act through a number of cellular signaling pathways, including mTOR and protein kinase C (PKC), to induce insulin resistance. Due to its inflammatory properties, IL-6 has been implicated in the endothelial cell damages within blood vessels leading to vascular dysfunction and atherosclerosis [47].

3.4.4 Tumor Necrosis Factor Alpha

Tumor necrosis factor alpha (TNF-α) is synthesized as a 26 kDa transmembrane protein that undergoes cleavage by a metalloproteinase to be released into the circulation as a 17 kDa soluble TNF-α molecule [9, 52]. TNF-α is a cell signaling cytokine involved in systemic inflammation and is one of the cytokines of acute phase inflammation. It is produced preferentially by activated macrophages, and less by other cell types such as $CD4^+$ lymphocytes, neutral killers, neutrophils, mast cells, eosinophils, and neurons. Adipocytes are also able to produce TNF-α [9, 47–49].

For some years, it was assumed that adipocytes are the principal source of elevated TNF-α level in obesity. Nevertheless, it has been recognized more recently that not adipocytes but macrophages from the stromal vascular fraction are the primary source of adipose-derived TNF-α [9, 52]. Macrophages, which constitute about 10% of the stromal vascular fraction, are present in visceral adipose tissue in larger quantities than in the subcutaneous one [9]. Recent studies also postulate that the increased infiltration of adipose tissue with M1 macrophages contributes to the increased TNF-α in obesity [9, 47–49]. Since metabolic syndrome is often characterized by adipocyte dysregulation, and these dysregulated adipocytes tend to secrete TNF-α, IL-6, and other pro-inflammatory adipokines at higher levels, the central obesity often encountered in metabolic syndrome could be a risk factor for elevated TNF-α levels [9, 47].

3.4.5 Plasminogen Activator Inhibitor 1

Plasminogen activator inhibitor 1 (PAI-1) is a single chain 45-kDa glycoprotein involved in microvascular events. Endothelial and vascular smooth muscle cells are presumably the main sources of PAI-1 but other cells, such as platelets, hepatocytes, mesangial cells, fibroblasts, monocytes, macrophages, adipocytes, and stromal cells, have also been shown to secrete PAI-1 [9, 57]. Increased size of adipocytes and adipose tissue mass contribute to higher adipose production to circulating PAI-1. Experimental data show that visceral adipose tissue has a higher capacity to produce PAI-1 than subcutaneous adipose tissue. Studies in human adipocytes indicate that PAI-1 synthesis is up-regulated by insulin, glucocorticoids, angiotensin II, some fatty acids, and potently by cytokines such as TNF-α and transforming growth factor-β, whereas catecholamines reduce PAI-1 production [9, 57].

PAI-1 is involved in fibrinolysis and its levels are altered in obesity [57]. Plasma PAI-1 levels are increased proportionally to increase in visceral adiposity, indicating the possible role of PAI-1 as the link between abdominal/central obesity and cardiovascular diseases. PAI-1 protein can change the balance between fibrinolysis and fibrinogenesis, contributing to the remodeling of vascular architecture and the atherosclerotic process [9]. An altered function of the endocrine system and an impaired auto-/paracrine function at adipocyte levels may mediate this disturbance in fibrinolytic system and thereby increase the risk for cardiovascular diseases [9, 47, 57].

3.4.6 Monocyte Chemoattractant Protein-1

Monocyte chemoattractant protein-1 (MCP-1) is produced predominantly by macrophages and endothelial cells and is a potent chemotactic factor for monocytes [58, 59]. Expression of this pro-inflammatory chemokine is increased in atherosclerotic lesions, and inhibition of its expression reduces the extent of atheroma. These observations indicate that MCP-1 plays an important role in atherogenesis. In mammal models, the increased MCP-1 expression in adipose tissue was shown to contribute to the macrophage infiltration into this tissue, insulin resistance, and hepatic steatosis associated with obesity [58, 59].

3.4.7 Interleukin 10

Interleukin 10 (IL-10) is a predominantly anti-inflammatory cytokine, which is secreted by monocytes or M2 macrophages and functions as a modulation of systemic inflammation; in particular, it helps to promote normal tissue remodeling following an inflammatory response. One of the ways by which IL-10 moderates the inflammatory response is the inhibition of NADPH oxidase, and therefore the decrease in oxidative stress resulting from this enzyme [47]. Anti-inflammatory cytokine IL-10 is secreted by human WAT [60]. Furthermore, one study found that IL-10 levels inversely correlated with levels of total cholesterol, LDL, TAGs, blood glucose, and positively correlated with HDL levels [47, 61]. IL-10 antagonizes pro-inflammatory actions of IL-6, TNF-α, and IL-10 and appears to confer a protective effect against increase in these cytokines, which both are associated with metabolic syndrome and its comorbidities [47].

3.5 Inflammation and Macrophage Infiltration in Adipose Tissue

Macrophage count is increased in adipose tissue during obesity. Increased levels of FFAs, cholesterol, and bacterial lipopolysaccharide (LPS) are well-known inductors of macrophage recruitment. The inductors bind to and activate toll-like receptor 4

(TLR4) and its downstream signaling pathways in adipose resident cells. The activated macrophages secrete cytokines and chemokines, such as MCP-1, and express C–C motif chemokine receptor-2 (CCR2) and CCR5, which, in turn, augment the recruitment of more monocytes and other leukocytes into adipose tissue [62, 63]. Macrophages share the same differentiation and recruitment molecules with other myeloid cells in many inflammatory conditions [63, 64].

M1 macrophages are associated with a pro-inflammatory profile. These macrophages are generally stimulated by T-helper 1 (Th1) type of cytokines, such as interferon γ (IFN-γ), or by pathogen-associated molecular patterns (PAMPs), such as LPS [63]. In turn, M1 macrophages secrete cytokines, including IL-6, TNF-α, IL-1β, IL-12, and IL-23 [9, 47, 48, 49, 63]. M1 macrophages can also induce Th1 responses [63, 65, 66]. On the whole, these cells express high levels of major histocompatibility complex class II (MHC-II), CD80 and CD86 co-stimulatory molecules and CD68 [63]. Moreover, M1 macrophages express Th1 cell-attracting chemokines, including CXCL9 and CXCL10 [63, 67].

M2 macrophages are associated with tissue remodeling and inflammation resolution [63, 68]. M2 macrophages have immunosuppressive properties, have high phagocytic capacity, and secrete extracellular matrix components, angiogenic and chemotactic factors, anti-inflammatory cytokines, and growth factors, such as IL-10 and transforming growth factor-β (TGF-β) [63, 68, 69].

Macrophages are central mediators of obesity-induced inflammation and insulin resistance. They also are key cells for maintenance of adipose tissue homeostasis. Recently, several reports described the importance of these cells as regulators of insulin sensitivity, which requires the activation of innate immune receptors, transcription factors, and intracellular metabolism to support either pro- or anti-inflammatory adipose tissue phenotype. Thus, macrophages have a dual role, changing their status in obesity to reinforce immune responses, obesity progression, and development of related diseases [63].

3.6 Adipocyte Dysfunction and Inflammation

Inflammation is a process of activation of innate immune system in response to exogenous and endogenous factors, such as infection by microorganisms, tissue stress, and injury. The inflammation associated with obesity is triggered by the excessive intake of high caloric food rich in carbohydrates and fats (Fig. 1). Obesity causes the enlargement of adipose tissue that is accompanied by the release of a number of adipokines [47]. The release of chemokines, which induce recruitment of macrophages from the bloodstream, increases macrophage infiltration and inflammation with enhanced production of pro-inflammatory cytokines such as TNF-α, IL-6, and MCP-1 [70]. The macrophage and adipose tissue-derived adipokines act in a paracrine or autocrine way that exacerbates adipose tissue inflammation [47, 70]. This is accomplished by increased release of FFAs and dysregulated secretion of leptin and adiponectin. At the systemic level, altered adipokine secretion, can

lead to decreased muscle and liver insulin sensitivity through enhanced ectopic lipid deposition [47]. The overproduction of adipokines leads to pathological conditions, such as obesity and adipose tissue inflammation that can develop insulin resistance and favor the pathogenesis of type 2 diabetes [70].

4 Role of Oxidative Stress in Obesity

4.1 Oxidative Stress as a Mechanism of Obesity-Associated Metabolic Complications

Excessive fat accumulation, especially of visceral fat, increases risk of metabolic syndrome and various chronic diseases, including type 2 diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer [12]. A number of mechanisms linking obesity to these associated diseases have been proposed, and oxidative stress together with inflammation response was supposed to have a crucial role among them. Oxidative stress has been implicated in vascular complications of diabetes and in pancreatic β cell destruction. Meanwhile, obese people without diabetes also display elevated intensity of oxidative stress. In addition, levels of oxidative stress markers were found to be increased in the adipose tissue of obese mouse models [3, 7, 12, 71].

The typical redox alterations in obese people and models of animal obesity include: (1) activation of cellular systems involved in the production of reactive oxygen species (ROS) and reactive carbonyl species (RCS), (2) increased levels of oxidatively damaged biomolecules (lipid peroxides, malondialdehyde, oxidized LDL, protein carbonyls, 3,5-dinitrotyrosine, advanced glycation end products (AGEs), 8-hydroxy-2′-deoxyguanosine, etc.), (3) an increase or a decrease in antioxidant defense capacity (superoxide dismutase, catalase, glutathione and thioredoxin, and associated enzymes [glutathione S-transferase, glutathione reductase, thioredoxin reductase], peroxiredoxins, NAD(P)H:ubiquinone oxidoreductase (NQO1), paraoxonase, etc.) [3, 5, 12, 14, 71–79]. It was shown that ROS/RCS may contribute to the development of obesity-associated insulin resistance and type 2 diabetes [75, 76, 77, 79], cause unfavorable changes in in the brain and arterial walls [80–82], as well as they are implicated in the pathogenesis associated with hypertension, atherosclerosis, and cancer [12, 82].

A clear relationship between obesity and oxidative stress is not yet fully defined. Many studies report that oxidative stress can be as a result, but also a trigger of obesity. Furthermore, oxidative stress can have both obesity-promoting and anti-obesity effects [14, 17, 76, 83]. It seems that fat content and its distribution in adipose tissue can affect the intensity of oxidative stress determining its downstream effects in obesity [5]. To support it, levels of oxidative damages were found to be higher in obese individuals and correlate directly with BMI and the percentage of body fat and TAG levels [73, 84]; in contrast, antioxidant defense capacity was

lower if the amount of body fat and central obesity were higher [85]. However, there are no clear correlations between body fat accumulation and the protective capacity of antioxidant systems. An increase, decrease, or no change in the activity of antioxidant enzymes was reported in different tissues of obese subjects [12, 72, 73, 74, 79, 86–88]. These controversial data may indicate both tissue-specific responses and time-dependent effects [89].

4.2 Sources of ROS and RCS in Adipose Tissue

The generation of reactive oxygen/carbonyl species (ROS/RCS) is an inevitable aspect of aerobic life [3, 90–93]. Energy resources, mainly glucose and fatty acids, are oxidized via the Krebs cycle with generation of reducing equivalents in the form NADH and $FADH_2$ (Fig. 2). These equivalents are subsequently re-oxidized in the electron transport chain (ETC) of mitochondria and their energy released is used for synthesis of ATP. Transport of protons from NADH and $FADH_2$ across the inner mitochondrial membrane into the intermembrane space is coupled with transfer of electrons through various complexes of ETC. The end acceptor of electrons is oxygen, which undergoes four-electron reduction to water. A consecutive one-electron reduction of oxygen is also possible due to electron escaping from mitochondrial ETC. As a result, reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$) are formed. Mitochondria are supposed to be responsible for over 90% of ROS production [91]. Under physiological conditions, ROS are maintained at low steady-state levels by functioning of complex antioxidant defense systems [91, 92].

Overconsumption of food rich in fats and carbohydrates leads to excessive supply of energy substrates in adipose and non-adipose cells (Fig. 2). Oxidation of free fatty acids and monosaccharides (glucose or fructose) increases levels of acetyl-CoA, which enter the Krebs cycle and may intensify the latter. In turn, the Krebs cycle intensification enhances generation of NADH and $FADH_2$, which overload mitochondrial ETC and promote an increase in ROS production by ETC [90, 91]. Increased ROS levels are first dangerous for mitochondria since many Krebs cycle and ETC proteins and mitochondrial DNA are very sensitive to ROS attack. Mitochondrial ETC dysfunction together with the inhibition of the Krebs cycle, are involved in TAG accumulation via the redirection of acetyl-KoA for synthesis of fatty acids. The latter are further converted to storage lipids in adipose tissue or in the case of overloading of adipose tissue can be appear in the bloodstream and other organs provoking lipotoxicity [94].

Oxidation of free fatty acids in peroxisomes is another source of superoxide anion radial and hydrogen peroxide in adipose tissue [7]. Furthermore, the plasma membrane NADPH oxidase (NOX), especially adipose-specific NOX4 isoform, which converts molecular oxygen to its superoxide anion radical, can be involved in ROS formation in adipocytes under excessive supply of energy resources [12, 76]. This is confirmed by increased ROS levels in cultured adipocytes loaded with free

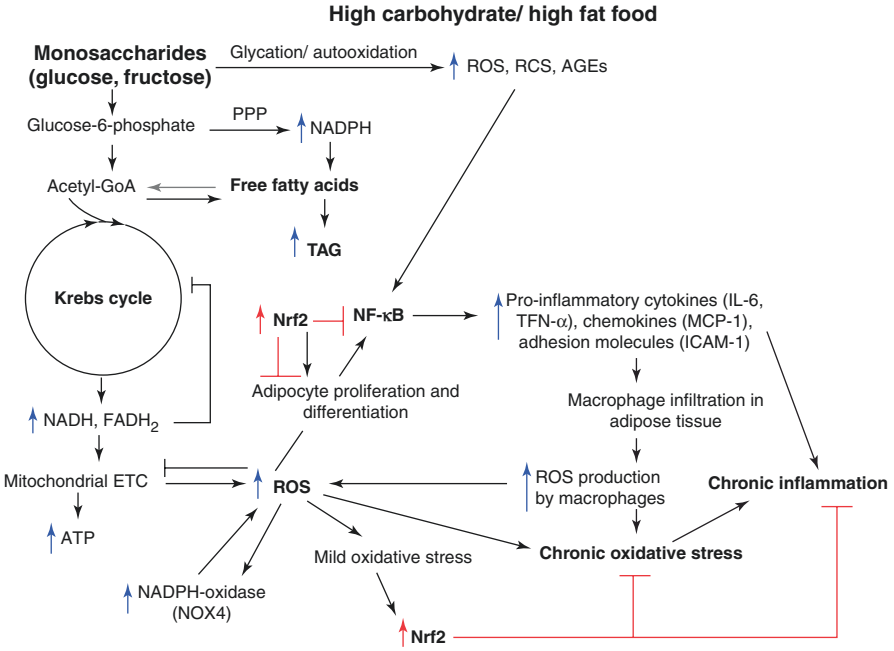

Fig. 2 Relationship between metabolic processes, ROS/RCS production, and inflammation at obesity development. Overnutrition causes intensification mitochondrial respiration followed by ROS increase. Increased ROS levels stimulate adipocyte proliferation and differentiation and promote activation of adipose NF-kB transcriptional factor, which triggers acute inflammation response followed by synthesis and releasing pro-inflammatory mediators. These mediators aggravate macrophage M2 recruitment and infiltration in adipose tissue. Executing their protective function, macrophage produces high levels of ROS resulting in chronic oxidative stress. In turn, chronic oxidative stress causes further increase in pro-inflammatory cytokine synthesis and macrophage infiltration leading inflammation to become chronic. ROS-activated NADPH oxidase (NOX4) and reactions of non-enzymatic glycosylation and monosaccharide oxidation also contribute to an increase in ROS levels under overnutrition. Low increase in ROS levels promotes activation of Nrf2 that triggers adaptive response to combat with raised oxidative stress and inflammation and to inhibit excessive adipocyte proliferation. At the same time, under chronic oxidative stress, Nrf2-mediated induction of protective mechanisms seems to be not able to cope with increased oxidative and inflammatory challenges. In addition, constantly enhanced Nrf2 activity may stimulate adipogenesis

fatty acids or glucose [14, 95, 96]. Excess nutrients activate NADPH oxidase and the pentose phosphate pathway (PPP), which is a major source of cellular NADPH using by NOX [96]. In obese mice, mRNA *NOX4* levels were increased in adipose tissue but not in the liver or muscles [14]. Furthermore, ROS production can be inhibited by treatment with apocynin, an NADPH oxidase inhibitor [14] or the PPP inhibitor dehydroepiandrosterone [97] or via deletion of *NOX4* gene [95]. These data suggest the involvement of plasma membrane NOX4 in ROS generation by excess of both glucose and free fatty acids. High glucose and FFAs, especially palmitate, was shown to stimulate ROS generation through protein kinase C-dependent activation of NOX [98].

Non-enzymatic glycosylation of proteins and nucleic acids, polyol (for glucose) and hexosamine (for fructose) pathways, glyceraldehyde, and monosaccharide autoxidation can be also important sources of ROS on high carbohydrate diets [77, 78, 90, 93, 99–102]. In addition to ROS, reactive carbonyl species (RCS), which include various compounds with one or more carbonyl groups, can be formed in many enzymatic and non-enzymatic processes such as lipid peroxidation, amino acid oxidation, monosaccharide autoxidation, and glycation [78, 100–102]. For example, methylglyoxal, the most common RCS found in biological systems, is produced by the triosephosphate isomerase reaction of glycolysis [78]. Both ROS and RCS as well as the so-called advanced glycation end products (AGEs) are thought to be involved in obesity progression and development of associated metabolic disorders [12, 99].

4.3 ROS as a Regulators Adipocyte Proliferation and Differentiation

Obesity is characterized by adipocyte hypertrophy which resulting from an increase in size and/or number of adipocytes. Adipocyte differentiation and function have been shown to be affected by cellular redox status. Enhanced ROS levels may alter food intake [103] and stimulate proliferation of pre-adipocytes and enlargement of differentiated adipocytes (Fig. 2) [13, 104, 105]. Exposure to low doses of H_2O_2 inhibited adipose differentiation of adipocyte 3T3L1 cells, but higher concentration of this oxidant markedly induced differentiation [106]. Moreover, ROS generated by both NOX4 and mitochondrial ETC are involved in adipocyte differentiation in adipogenic stem cells [13, 105]. Moreover, down-regulation of *NOX4* gene inhibits production of ROS and differentiation of pre-adipocytes, while *NOX4* overexpression was found to have the opposite effect [107]. In obese mice, treatment with NOX inhibitors reduces ROS levels in adipose tissue and improves symptoms of diabetes, hyperlipidemia, and hepatic steatosis [14]. In contrast, Li et al. [108] reported that NOX4 deficiency accelerated obesity in mice fed high-fat diet. It appears that the role of NOXs in obesity is complicated and should be elucidated further. Adipocyte differentiation is also characterized by an increase in mitochondrial metabolism [109], but whether this is essential for differentiation or a by-product of the differentiation process is not clearly understood [13].

4.4 Relationship Between Inflammatory and Oxidative Processes in Obesity

The main function of adipose tissue is to synthesize and to store fats in the form of TAGs and to release free fatty acids during fasting. Under conditions of chronic overnutrition, the excess energy nutrients lead to an increase in adipose tissue stimulating adipocyte proliferation and enlargement. Together with adipocyte size

increment, free radical and inflammatory processes are intensified in adipose tissue (Fig. 2). These processes seem to be developed in parallel but reinforce each other over obesity progression: inflammation leads to the intensification of ROS production, and enhanced ROS levels stimulate inflammatory signaling cascades, thus forming a vicious circle [5].

In addition to metabolic functions, adipose tissue is an important endocrine organ. As described above, adipose tissue consists of adipocytes and various immune cells that produce a variety of biologically active molecules, including adipokines, chemokines, and pro-inflammatory cytokines such as TNF-α and interleukins IL-1β and IL-6 [6, 7, 8, 49, 55, 110]. Production of these proteins by adipose tissue, especially by that forming visceral fat, is increased in obesity; the obese people are, therefore, characterized by a state of chronic low-grade inflammation, which has a functional link with insulin resistance and metabolic syndrome [36, 53, 61, 66, 111]. However, the primary events triggering this inflammation are still unclear since a complex of endocrine and immune factors act to regulate this adipose tissue microenvironment [110].

In visceral adipose tissue, the induction of inflammatory responses can be connected with the involvement of toll-like receptors (TLRs), which were found to be present in plasma membrane of adipocytes [112]. Normally, these TLRs (TLR2 and TLR4) are activated by bacterial lipoproteins and lipopolysaccharides [113]. The activation of TLRs triggers a signaling cascade leading to translocation of nuclear factor-κB (NF-κB) to the nucleus. NF-κB protein is a main transcriptional regulator of adaptive immune response and its activation triggers a synthesis and release by adipose tissue of several pro-inflammatory cytokines, chemokines, and adhesion molecules [7, 10, 15]. Recent studies have showed that non-esterified fatty acids can be modulators of adipokine secretion by adipocytes. It has been shown that TLRs receptors may be activated by specific types of lipids, in particular saturated fatty acids was fond to activate both TLR2 and TLR4, whereas unsaturated fatty acids inhibit TLR-mediated signaling and gene expression [114]. Enlarged adipocytes are characterized by releasing free fatty acids with increasing their levels in the blood and adipose tissue microenvironment. Therefore, FFAs can contribute to a low-grade inflammation in adipose tissue. Besides hyperlipidemia, high glucose levels can also activate inflammatory pathways. Hyperglycemia may lead to the non-enzymatic glycation of proteins and lipids forming advanced glycation end products (AGEs), stimulating activation of the pattern recognition receptor RAGE, and eliciting an immune response through the activation of NF-kB [1, 90]. In obesity-related pro-inflammatory states, the increased size of adipocytes plays a decisive role because, to some extent, it increases adipose tissue and production of adipocytokines, and this triggers a number of inflammation-related pathophysiological processes [11].

Nutrient-induced oxidative stress can also contribute to the activation of inflammation responses in adipose tissue. For example, several pro-inflammatory transcription factors, including NF-κB and activator protein-1 (AP-1), are redox-sensitive and undergo the activation when ROS levels are increased [7, 10, 15]. Thus, mitochondria-derived or/and NOX4-stimulated ROS can mediate the production

pro-inflammatory proteins during overnutrition. The production of adipokines by enlarged adipocytes increases migration of macrophages in adipose tissue, which worsens inflammation. Among chemokines produced by adipocytes, MCP-1, which attracts and triggers monocyte and macrophage migration, plays essential roles in this process [12, 96]. In turn, macrophages are known to produce ROS as a part of the immune response; therefore, macrophages may promote overproduction of ROS in adipose tissue [7, 12, 14]. Enhanced ROS levels, in turn, lead to the development of chronic oxidative stress with a gradual increase in oxidative damages of macromolecules and depletion of protective mechanisms [71]. Moreover, ROS have been shown to increase expression of MCP-1 and NADPH oxidase subunits in adipocytes [14], thus leading to more permanent state of inflammation. Further, enhanced secretion of pro-inflammatory factors by enlarged white adipose tissue contributes to constantly increasing ROS production. Thus, in obesity, oxidative stress may contribute to the establishment of a vicious cycle that promotes increased inflammation in the adipose tissue [10, 12]. The products of the adipocytes, in turn, may modify the metabolic and inflammatory processes in surrounding and other tissues (kidney, liver, brain, pancreatic gland, heart, etc.) causing various obesity-related complications [3, 12].

In addition, to the activation of inflammation signaling cascades, the increased intensity of oxidative stress in adipose tissue results in the induction of protective mechanisms against oxidative damages. Current data suggest that the induction of adaptive and inflammatory responses can have both anti-obesity and obesity-promoting effects [17, 83], that, probably, is a result of time- and intensity-dependent effects of oxidative stress. On the short-term scale, excess nutrients may induce low/moderate increase in ROS production followed by activation of a number of redox-responsive transcription factors such as NF-κB and AP-1, which were mentioned above, as well as nuclear factor erythroid 2 = related factor 2 (Nrf2) and FoxO1, a member of the forkhead box O family of proteins [7, 10, 15, 16, 17, 115]. The activation of NF-κB and AP-1 pathways triggers acute inflammation response, which includes a synthesis and release by adipose tissue of pro-inflammatory cytokines, e.g., TFN-α and IL-6. The increased level of IL-6 stimulates the liver to synthesize and secrete acute phase proteins (C-reactive protein) [1, 7, 10, 15]. The normal acute inflammatory response involves the delivery of plasma components and leucocytes to the site of insult and is initiated by tissue-resident macrophages leading to a production of different types of inflammatory mediators. If successful, the injurious agent is eliminated followed by inflammation cease and tissue repair. This is achieved by switching the lipid mediators from pro-inflammatory to anti-inflammatory and by the action of tissue-resident and newly recruited macrophages [113]. The primary function of Nrf2 is to maintain redox homeostasis by activating expression of antioxidant and xenobiotic-detoxifying genes under exposure to different toxic electrophilic compounds [17, 83]. More detailed functions of Nrf2 in obesity will be discussed below. FoxO proteins, in particular FoxO1, are also transcriptional regulators of adaptive stress responses and they induce the expression of genes coding both intra- and extracellular antioxidant proteins [115]. In addition, FoxO 1 regulates of adipocyte size and adipose tissue-specific gene expression in

response to excessive calorie intake [116]. Thus, on short-term scale, complex adaptive responses are induced in a cell against damaging effects of obesity-associated oxidative stress to prevent obesity progression.

When accumulation of storage lipids in adipose tissue progresses further, hypertrophied adipocytes and other cells present in the adipose tissue produce large amounts of pro-inflammatory adipokines, which stimulate infiltration of macrophages and T-lymphocytes. In the inflammatory process, macrophages in adipose tissue release chemoattractants for macrophages that induces inflammation to become chronic [11]. Chronic inflammation aggravates ROS production that, in turn, leads to chronic activation of stress-responsive transcription factors that finally causes depletion of protective antioxidant mechanisms and increase in oxidation of macromolecules.

5 Nrf2, Oxidative Stress, Obesity, and Inflammation

Recent data suggest that redox-sensitive transcriptional factors, which participate in maintenance of a balanced redox state, play important roles in obesity. It is not surprising because of the developing oxidative stress, which accompanies fat accumulation and obesity progression. Hence, the manipulations with molecular pathways that produce or eliminate ROS are becoming a popular tool for potential intervention for obesity and related metabolic syndrome treatment. In this context, Nrf2 transcriptional factor came under the spotlight in obesity research because it regulates the adaptive response to endogenous and exogenous oxidative or electrophilic stresses [14, 16,-21].

5.1 Regulation of Nrf2 Activity: General Concepts

Nrf2 belongs to the family of the cap ‘n’ collar transcription (CNC) factors with a basic leucine zipper (bZIP). The homologs of Nrf2 found in the invertebrates, nematode *Caenorhabditis elegans* (SKN-1 protein) and *Drosophila melanogaster* (CncC protein), were found to share the same protective functions [17, 117]. In mammals, Nrf2 protein consists of seven functional domains called Nrf2-ECH homology (Neh) 1–7 domains. Neh1 domain is a CNC-bZIP dimerization domain that allows Nrf2 to form heterodimer with small Maf protein, DNA, and other transcription partners [18, 118]. The highly conserved Neh2 domain at N-terminal region contains two important motifs known as DLG and ETGE, which are involved in the interaction between Nrf2 and its negative regulator Keap1 protein [119, 120]. The Neh3 domain is located at the C-terminus of the protein and is essential for the transactivation of responsive genes by Nrf2. The Neh4 and Neh5 domains are considered as transactivation domains that cooperatively bind to cAMP response element binding (CREB) protein. Finally, Neh6 and Ne7domains, which are located in

the middle of Nrf2, have been reported to be associated with redox-insensitive suppression and degradation of Nrf2 protein [18, 121].

The activity of Nrf2 is controlled through a complex transcriptional/epigenetic and post-translational network that ensures an increase in its activity during redox perturbation, inflammation, growth factor stimulation, and nutrient/energy fluxes, thereby allowing the factor to orchestrate adaptive responses to diverse forms of stress [17, 18, 91, 121–123].

Nrf2 is regulated at the transcriptional level by itself [124] and other transcription factors including PPARγ [125] and NF-κB [126]. Epigenetic mechanisms such as methylation of the Nrf2 promoter in CpG islands, H3 histone methylation and H4 histone acetylation, and inhibition of Nrf2 synthesis at the post-transcriptional level due to interference with miRNAs [127] were found to be involved in the regulation of Nrf2 synthesis [118, 122].

The main regulation of Nrf2 activity occurs at post-translational level and can be Keap1-dependent and Keap1-independent (Fig. 3) [20, 118, 122]. Keap1-dependent Nrf2 regulation is the most studied.

Keap1 (Kelch-like ECH-associated protein 1) is a main repressor of Nrf2 activity and is a cysteine-rich protein which exists in the form of homodimer. Under physiological conditions, the KELCH domains of the Keap1 homodimer bind to the DLG and ETGE motifs of the Neh2 domain of Nrf2 in the cytosol, where ETGE acts as a hinge with higher affinity and DLG acts as a latch [20, 120]. Keap1 serves a substrate adaptor for cullin-based E3 (Cul3) ubiquitin ligase, which performs ubiquitination of lysine residues in the Neh2 domain of Nrf2 protein [128–130]. Ubiquitinated Nrf2 becomes a target for 26S proteasome complex and undergoes proteolytic degradation with a $t_{1/2}$ of less than 20 min [129, 130]. Therefore, Keap1 protein is an inhibitor of Nrf2 by blocking Nrf2 translocation to the nucleus and promoting its degradation. The rapid turnover of Nrf2 prevents the unnecessary expression of Nrf2 target genes [18, 118].

Under oxidative stress or upon exposure to Nrf2 activators, Nrf2 dissociates from Keap1 due to oxidation of SH-groups of cysteine residues in Keap1 protein. Because of disturbance of the Keap1-Nrf2-Cul3 complex, Nrf2 is stabilized ($t_{1/2}$ of up to 200 min) and can be translocated to the nucleus. In the nucleus, Nrf2 protein forms a heterodimer with Maf proteins and binds to promoters of genes containing the so-called ARE (Antioxidant Response Element) sequence followed by activating their transcription [120, 121, 131]. In Nrf2, Neh4 and Neh5 domains also act as transactivation domains, but bind to another transcriptional co-activator known as CBP (cAMP response element-binding protein-binding protein) [132]. Neh5 domain has a redox-sensitive nuclear-export signal, which is crucial for the regulation and cellular localization of Nrf2 [133].

Keap1-independent regulation occurs via repression of Nrf2 by β-transducin repeat-containing protein (β-TrCP), which binds to two motifs in the serine-rich Neh6 domain of Nrf2 [20, 134]. β-TrCP is a substrate receptor for the Skp1-Cul1-Rbx1/Roc1 ubiquitin ligase complex that targets Nrf2 for ubiquitination and proteasomal degradation [135]. Notably, the repression of Nrf2 by β-TrCP is increased when serine/threonine kinase glycogen synthase kinase (GSK)-3 is activated.

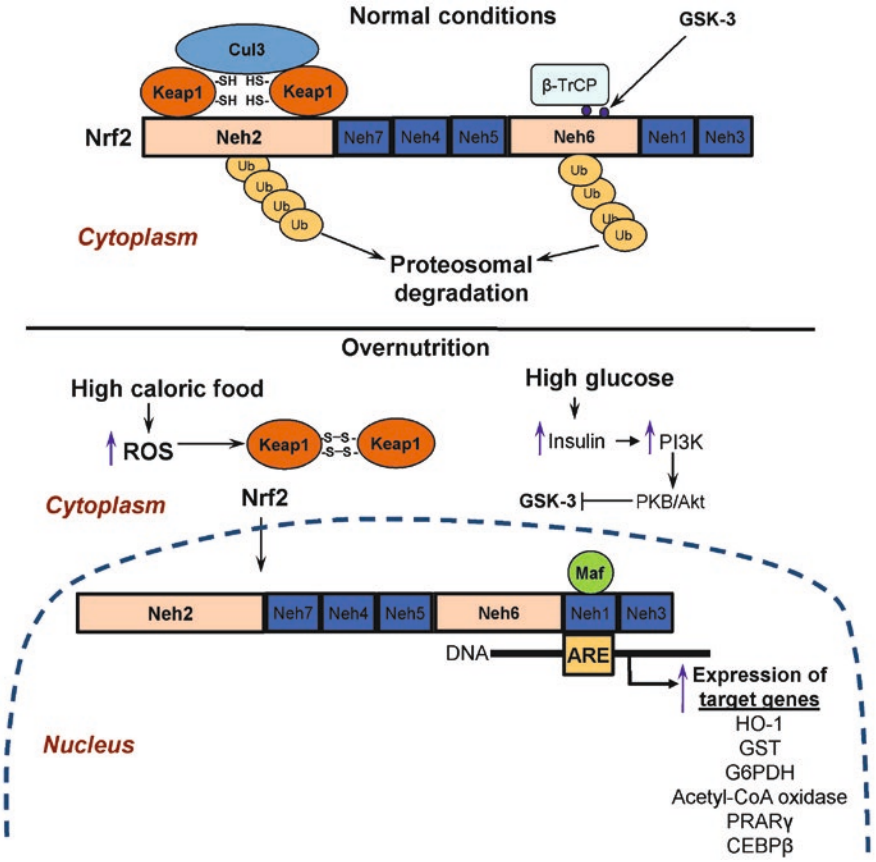

Fig. 3 Regulation of Nrf2 activity under physiological conditions and overnutrition. Under physiological conditions, Nrf2 is sequestered with Keap1 in cytoplasm that leads to its CUL3-mediated ubiquitination followed by proteasome degradation. In addition, glycogen kinase 3 (GSK3) phosphorylates Nrf2, and this facilitates the recognition of Nrf2 by β-TrCP protein for CUL1-mediated ubiquitination and subsequent proteasome degradation. Overnutrition causes intensification mitochondrial respiration followed by ROS increase. Enhanced ROS levels induce oxidation of SH-groups in Keap1 protein, allowing Nrf2 to dissociate from Keap1 and to translocate to the nucleus, where it forms the complex with Maf protein and activates a number of ARE-genes. High glucose also stimulates insulin signaling resulting in activation of kinase PKB/Akt, which, in turn, phosphorylates and inactivates GSK-3

GSK-3 was found to phosphorylate Nrf2 in the Neh6 domain to facilitate the recognition of Nrf2 by β-TrCP and subsequent protein degradation [134, 135]. Inhibition of Nrf2 by GSK-3 is antagonized by protein kinase B (PKB)/Akt, which is activated by phosphoinositide-dependent kinase (PDK)1. In turn, PDK1 is activated by phosphatidylinositol-(3,4,5)-triphosphate (PIP3) produced by phosphatidylinositide 3-kinase (PI3K) by phosphorylation of phosphatidylinositol (4,5)-diphosphate (PIP2) [18]. Elevated insulin signaling, in response to feeding, activates PI3K followed by PKB/Akt activation. In turn, PKB/Akt inhibits GSK-3β through

phosphorylation of Ser9 on GSK-3β [136]. Several other non-canonical regulators of Nrf2 activity were also revealed, and they are described in detail elsewhere [18, 118, 122].

5.2 *Role of Nrf2 in Redox Homeostasis and Energy Metabolism*

The transcriptional factor Nrf2 is considered as the master regulator of the redox cellular state [117, 121, 123, 137]. Nrf2 regulates the expression of over 1000 genes [138], whose products are involved in cell protection against oxidants, electrophiles, and genotoxic compounds, as well in cellular proliferation, intermediary metabolism, and immune responses [18, 118]. Besides regulation of the expression of drug-metabolizing enzymes (e.g., NADPH quinone oxidoreductase (NQO1), glutathione S-transferase, ABC-binding cassette proteins, and P_{450} subunits), Nrf2 simultaneously controls key components of endogenous antioxidant system. In particular, it controls the expression of enzymes of glutathione synthesis, glutathione peroxidase (GPX)2, which reduces of peroxides with forming oxidized glutathione (GSSG), and glutathione reductase (GSR)1, which reduces GSSG [18, 139]. Thus, Nrf2 ensures maintenance of intracellular pool of reduced glutathione (GSH). Besides that, Nrf2 also activates the expression of cytosolic thioredoxin, thioredoxin reductase, and sulfiredoxin, which all reduce oxidized protein thiols [18, 138]. Many drug-metabolizing enzymes and antioxidant systems require NADPH as a cofactor. The enzymes, which generate NADPH, such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, and malic enzyme, are all regulated by Nrf2 [137, 140]. Ntr2 also regulates expression of proteasome subunits and enzymes of lipid metabolism (e.g., acetyl-Coa oxidase, lipases); in particular, Nrf2 inhibits lipogenesis and supports β-oxidation of fatty acids [18, 141]. Moreover, NRf2 induces synthesis of proteins with anti-inflammatory functions (e.g., heme-oxygenase) and inhibits the expression of pro-inflammatory cytokines [18, 20]. Two transcriptional factors involved in adipogenesis are regulated by Nrf2, CCAAT/enhancer-binding protein β (CEBPβ) and peroxisome proliferator-activated receptor γ (PPARγ) [18]. Thus, in addition of stress-stimulated induction of antioxidant and detoxification genes, Nrf2 contributes to adaptation by modulating cell proliferation and metabolism of proteins, carbohydrates, and lipids and by modulating immune responses.

5.3 *Effects of Nrf2 Deficiency and Activation on Obesity*

Many studies had been performed to investigate the detailed role of Nrf2 under physiological and pathological conditions using various experimental approaches such as Nrf2 gene deletion, Nrf2 pharmacological activators, and Nrf2 gene

overexpression. In addition to liver, intestine, lung, and kidney, where detoxification reactions routinely occur, Nrf2 was found to be abundantly expressed or highly inducible in human and mouse adipocytes. Data suggest that in the adipose tissue Nrf2 functions include not only detoxification and antioxidant defense, but also regulation of metabolism, immune response, and cell proliferation [16, 17, 81, 142].

Obesity and its related metabolic complications had already been associated with the increased oxidative stress; researchers made, therefore, the plausible hypothesis that Nrf2, being a central coordinator of antioxidant gene expression, may participate in the pathophysiological mechanisms of these diseases. Many cellular models and Nrf2 knock-out (Nrf2-KO, Nrf2-null) or Keap1 knock-out mice were used to testing this hypothesis. In support of the hypothesis, Nrf2-KO mice have been shown to have increased susceptibility to chemicals inducing hepatotoxicity [143, 144] and inflammation [145]. However, experimental data regarding role of Nrf2 in adipogenesis and adipocyte function are conflicting. In study of Tanaka et al. [146], wild-type and Nrf2-KO mice fed a high-fat diet for 4 weeks did not differ in weight gain and hepatic TAG levels, but Nrf2-KO mice had higher levels of hepatic free fatty acids and malondialdehyde. Hence, that study did not reveal clearly if Nrf2 signaling is implicated in obesity. The study by Pi et al. [147] showed Nrf2-KO mice gain less weight than the wide type mice when were fed a high-fat diet for a long-term period (3 months). To identify the relationship between Nrf2 and adipogenesis, the authors compared the adipocyte differentiation capacity of mouse fibroblasts isolated from wild-type embryos with those from Nrf2-deficient embryos. In the absence of Nrf2, adipocyte differentiation was suppressed, resulting from the down-regulation of PPARγ (peroxisome proliferator-activated receptor gamma), a transcription factor with a central role in the expression of the adipogenic program. The authors also found, that Nrf2 deficiency suppressed adipogenesis in 3T3L1 pre-adipocytes and human subcutaneous pre-adipocytes, whereas of Keap1-KO in 3 T3-L1 cells resulted in the activation of Nrf2 and an enhanced adipogenesis [147]. These findings are also supported by other studies demonstrating that Nrf2 deletion leads to the inhibition of adipogenesis and the protection of mice from high-fat diet-induced obesity and its complications. In particular, Nrf2-KO mice had a higher sensitivity to insulin and altered metabolic profile with lower circulating glucose, HDL, and leptin concentrations [148, 149]. Moreover, the constant activation of Nrf2 due to Keap1 knockdown increased markers of metabolic syndrome [17, 150, 151] and Nrf2 deficiency improved glucose tolerance [151] in mice fed a high-fat diet.

Conflicting results have also been reported showing that the loss of Nrf2 is associated with increased differentiation capacity of adipocytes [152, 153]. In the study of Shin et al. [152], Nrf2-deficient mouse embryonic fibroblast cells had markedly accelerated adipogenic differentiation compared with wild-type cell lines. This phenotype was reversed in Keap1-KO cells, which constitutively express higher levels of Nrf2, resulting in a more delayed differentiation [152]. The enhanced activity of Nrf2 was shown to repress hepatic gluconeogenic and lipogenic program to prevent adipogenesis and onset of diabetes mellitus in mice on a high-fat diet [153, 154,

155, 156]. Other studies showed that the deletion of Nrf2 from adipocytes results in severe systemic metabolic dysfunction, including enhanced oxidative stress, inflammation, and insulin resistance, in mice with high-fat diet-induced obesity [81, 142, 157].

In addition to genetic manipulations, the effects of Nrf2 on obesity and metabolic syndrome were explored using its pharmacological activators, e.g., oltipraz, sulforaphane, curcumin, and 1-[2-cyano-3, 12-dioxooleana-1,9(11)-dien-28-oyl] imidazole. These compounds have been identified as the specific activators of Nrf2 expression in vitro and in vivo [17]. A number of studies [156, 158–160] suggest that the Nrf2 pharmacological activators could protect mice against obesity, improve insulin-resistant phenotype, and suppress oxidative stress, supposedly through the Nrf2 pathway [16, 17].

These discrepancies of Nrf2 effects on obesity may be due to differences in cell lines, animals used, diet composition, feeding period, etc. Nonetheless, it is clear that Nrf2 plays critical roles in adipose tissue. We can suppose that intensity of oxidative stress play an important role in manifestation of effects of Nrf2. At short-term scale, low intensity of oxidative stress can lead to activation of Nrf2 resulting in the induction of adaptive response and the inhibition of proliferation/differentiation of adipocytes. When the intensity of oxidative stress is enhanced, the capacity of protective systems reduces and adipogenic effects of Nrf2 stimulation are observed [5]. Pharmacological activators of Nrf2 can moderately decrease the levels of ROS that ameliorates obesity phenotypes. However, the chronic Nrf2 activation may not be sufficient to cope with increased ROS levels that stimulates adipocyte differentiation, adipose tissue enlargement, and decreases insulin sensitivity.

5.4 Anti-Inflammatory Effects of Nrf2 in Obesity

5.4.1 Nrf2 and Keap1 Deficiency Effects

Activation of Nrf2 transcription factor has been linked to cytoprotection via improving redox sate [18]. In addition, pharmacological activation of Nrf2 inhibits inflammation and impairs degenerative disease providing an interface between redox and anti-inflammatory responses [22]. In consistent with this, Nrf2-KO mice are more susceptible to the pro-inflammatory effects of allergens, LPS, and a high-fat diet [145, 161, 162]. The increased sensitivity of Nrf2-KO mice to inflammation may be connected with a loss of PRARγ protein activity because it is known that Nrf2 positively regulates PRARγ [125] and that PRARγ exerts strong anti-inflammatory effects [163]. Nrf2 deficiency can exacerbate inflammation and promote atherosclerosis and liver injury in a variety of murine models [164–167]. At the same time, it was reported that Nrf2 deficiency prevents the early onset and development of atherosclerosis and promotes lipid metabolism in ApoE knock-out mice [168] and prevents diet-induced obesity and obesity-associated chronic inflammation in mouse adipose tissue [142, 147, 149, 151, 169].

Controversial functions of Nrf2 in the regulation of inflammation and metabolism have also been observed in numerous Nrf2-activated cellular and mouse models. For example, genetic activation of Nrf2 via Keap1 knockdown [154, 155] or using pharmacological inducers of Nrf2 [21, 158, 159, 160, 170] represses inflammation and prevents the onset and development of diabetes mellitus in mice. However, constant enhanced activity Nrf2 has been reported to aggravate inflammation, worsen insulin resistance and promote hepatic steatosis and lipid accumulation in mice [153, 171]. Several studies suggest that regulatory effects of Nrf2 in inflammation may occur independent of its classic function in redox regulation [165, 167]. One may suppose that the intensity of oxidative stress is a contributing factor to the effects of Nrf2 in obesity. Both Nrf2 deficiency and its constitutive activation was found to inhibit mitochondrial respiration and ROS production in mitochondria [123]. Increased ROS levels can result in the overproduction of cytokines, which induces oxidative stress in target cells. Pro-inflammatory oxidative stress may cause further activation of NF-κB and the overproduction of cytokines aggravating inflammation. At the same time, modest activation of Nrf2 by pharmacological compounds or at obesity onset may have beneficial effects, since it allowing maintain stable-state low ROS levels which are sufficient to activation of effective adaptive responses and to control proliferation and differentiation. This can explain the increased sensitivity of both Nrf2-KO mice and Keap1-KO to inflammation.

5.4.2 Protective Effects of Nrf2: Inhibition of Synthesis of Pro-Inflammatory Mediators and Stimulation of Anti-Inflammatory Proteins

The protective mechanisms of Nrf2 in inflammation could be connected with the regulation of expression of proteins participating in immune responses. Heme oxygenase-1 (HO-1) is a well-known target of Nrf2. Nrf2 induces the HO-1 gene expression and it is one of the classic Nrf2 regulated genes, which is widely used in numerous in vitro and in vivo studies. HO-1 is rate-limiting enzyme that catalyzes the degradation of heme into carbon monoxide (CO) and free iron, and biliverdin to bilirubin. Enzymatic degradation of pro-inflammatory free heme as well as the production of anti-inflammatory compounds such as CO and bilirubin play major roles in maintaining the protective effects of HO-1 [20, 172]. Several studies have demonstrated that HO-1 and its metabolites have anti-inflammatory effects in obesity [21, 160, 173,]. Flavonoid quercetin and triterpene celastrol were shown recently to reduce obesity-induced hepatic inflammation by inducing HO-1, which promotes macrophage phenotype switching in adipose tissue from pro-inflammatory M1 to anti-inflammatory M2 macrophages [21, 173].

In addition to induction of HO-1, Nrf2 may inhibit many inflammatory mediators and enzymes [20]. The activation of Nrf2 was reported to prevent LPS-induced transcriptional up-regulation of pro-inflammatory cytokines, including IL-6 and IL-1β [165]. IL-1β and IL-6 production is also increased in Nrf2-KO mice with dextran sulfate-induced colitis [174]. Many plant-derived compounds such as

quercetin, celastrol, butein, curcumin, and broccoli extract display anti-inflammatory effects in high-fat diet-induced obesity. These effects were supposed to be mediated by NRf2 and include reduction of M1 macrophage infiltration and release of chemoattractant protein MCP-1, inhibition of synthesis of intercellular adhesion molecule-1(ICAM-1) and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), as well as increase of anti-inflammatory cytokines (IL-10) in liver [173], adipose tissue, and adipocyte 3 T3-L1 cell cultures [21, 142].

5.4.3 Crosstalk Between Nrf2 and NF-κB Pathways

In the inflammation process, a key player is NF-κB-mediated signaling pathway. NF-κB is normally an inactive cytoplasmic complex, linked to an inhibitory protein, Iκ-B, which masks its nuclear localization signal. External pro-inflammatory stimuli and oxidative stress cause rapid Iκ-B phosphorylation. This causes dissociation of I-κB from NF-κB and subsequent nuclear translocation. In the nucleus, NF-κB induces the transcription of a number of inflammatory chemokines and cytokines. These effects of NF-κB were found in most metabolic tissues, including hepatocytes, adipocytes, neurons, and β cells [1, 22]. Production of pro-inflammatory cytokines intensifies oxidative stress causing further activation of NF-κB and the overproduction of cytokines. Activation of the Nrf2 plays an important role in disrupting this cycle [20]. The in vivo studies suggest that Nrf2 negatively regulates the NF-kB signaling pathway [22, 175]. In response to bacterial LPS, Nrf2 knockdown significantly increases NF-κB-dependent gene transcription [162]. In addition, increased expression of Nrf2-dependent downstream HO-1 inhibits NF-κB activity [176]. Moreover, long-term overnutrition leads to inactivation of Nrf2 and activation of NF-κB [21, 177]. Pharmacological inductors of Nrf2 suppress NF-κB activation, but promote an increase in Nrf2 activity [21, 177]. We can suppose that low and moderate increase in ROS levels at obesity onset can promote Nrf2 activation followed by induction of protective adaptive response. However, higher increase in oxidative stress intensity leads to activation of NF-κB, which triggers chronic inflammatory response inducing further oxidative stress. Because of high intensity oxidative stress, Nrf2 can undergo inactivation [91, 92]. This allows us to suppose that redox-sensitive Nrf2 signaling could be a potential target for anti-obesity interventions at early stages of the disease.

6 Conclusions

Collectively, current data suggest that obesity is associated with the development of both oxidative stress and inflammation, which are considered to be main culprits of obesity-related metabolic complications. However, there is no clear correlation between metabolic disturbances, inflammation, and oxidative stress in obese individuals. In particular, enhanced oxidative stress can have both anti-obesity and

obesity-promoting effects, including inflammation progression. Furthermore, an obesity-like phenotype does not always show a reduced life span in invertebrate and mammalian models [72–74, 79,] and there is still a question of whether obesity leads to shortening of life span in humans [178]. Emerging data propose the redox-sensitive regulator of oxidative stress response Nrf2 to be a major molecular player in energy metabolism and a potential target for anti-obesity interventions. However, controversial effects of Nrf2 in obesity progression and prevention were observed, possibly due to using preferentially Nrf2-null mice or Keap-null mice/cells displaying constant Nrf2-activation, which seem to have their own phenotypes distinct from that of wild type. Pharmacological activators Nrf2 seems to be promising for further clarification of the effects of graduated activation Nrf2 in the treatment of obesity and related metabolic disorders. An important gap in current knowledge is the inadequate understanding of the tissue-specific effects of Nrf2, and this is particularly important for metabolic and inflammatory diseases.

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References

1. Catrysse L, Van LG. Inflammation and the metabolic syndrome: the tissue-specific functions of NF-kB. Trends Cell Biol. 2017;27:417–29.
2. WHO Global Health Observatory Data Repository. Geneva, World Health Organization, http://apps.who.int/gho/data/view.main. Accessed 21 May 2015.
3. Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-associated oxidative stress: strategies finalized to improve redox state. Int J Mol Sci. 2013;14:10497–538.
4. Nikolopoulou A, Kadoglou NPE. Obesity and metabolic syndrome as related to cardiovascular disease. Expert Rev Cardiovasc Ther. 2012;10:933–9.
5. Bayliak MM, Abrat OB, Storey JM, Storey KB, Lushchak VI. Interplay between diet-induced obesity and oxidative stress: comparison between Drosophila and mammals. Comp Biochem Physiol Part A Mol Integr Physiol. 2019;228:18–28.
6. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 2004;92:347–55.
7. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González Á, Esquivel-Chirino C, Durante-Montiel I, Sánchez-Rivera G, Valadez-Vega C, Morales-González JA. Inflammation, oxidative stress, and obesity. Int J Mol Sci. 2011;12:3117–32.
8. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. 2013;2013:1–12.
9. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci. 2013;9:191–200.
10. Muñoz A, Costa M. Nutritionally mediated oxidative stress and inflammation. Oxidative Med Cell Longev. 2013;2013:610950.
11. Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. Rev Médica del Hosp Gen México. 2017;80:101–5.

12. Matsuda M, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. Obes Res Clin Pract. 2013;7:1–12.
13. Drehmer DL, de Aguiar AM, Brandt AP, Petiz L, Cadena SMSC, Rebelatto CK, Brofman PRS, Filipak Neto F, Dallagiovanna B, Abud APR. Metabolic switches during the first steps of adipogenic stem cells differentiation. Stem Cell Res. 2016;17:413–21.
14. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004;114:1752–61.
15. Bryan S, Baregzay B, Spicer D, Singal PK, Khaper N. Redox-inflammatory synergy in the metabolic syndrome. Can J Physiol Pharmacol. 2013;91:22–30.
16. Seo H-A, Lee I-K. The role of Nrf2: adipocyte differentiation, obesity, and insulin resistance. Oxidative Med Cell Longev. 2013;2013:184598.
17. Zhang Z, Zhou S, Jiang X, Wang Y-H, Li F, Wang Y-G, Zheng Y, Cai L. The role of the Nrf2/Keap1 pathway in obesity and metabolic syndrome. Rev Endocr Metab Disord. 2015;16:35–45.
18. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci. 2014;39:199–218.
19. Chartoumpekis DV, Kensler TW. New player on an old field; the Keap1/Nrf2 pathway as a target for treatment of type 2 diabetes and metabolic syndrome. Curr Diabetes Rev. 2013;9:137–45.
20. Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta Mol basis Dis. 2017;1863:585–97.
21. Luo D, Guo Y, Cheng Y, Zhao J, Wang Y, Rong J. Natural product celastrol suppressed macrophage M1 polarization against inflammation in diet-induced obese mice via regulating Nrf2/HO-1, MAP kinase and NF-kappaB pathways. Aging (Albany NY). 2017;9:2069–82.
22. Lampiasi N, Montana G. An *in vitro* inflammation model to study the Nrf2 and NF-kappaB crosstalk in presence of ferulic acid as modulator. Immunobiology. 2018;223:349–55.
23. Saklayen MG. Hypertension and obesity the global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;9:1–8.
24. Dereń K, Nyankovskyy S, Nyankovska O, Łu E (2018) The prevalence of underweight, overweight and obesity in children and adolescents from Ukraine. 1–7
25. Heerwagen MJR, Miller MR, Barbour LA, Friedman JE. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol Regul Integr Comp Physiol. 2010;299:R711–22.
26. Vaiserman A, Koliada A, Lushchak O. Developmental programming of aging trajectory. Ageing Res Rev. 2018;47:105–22.
27. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol. 2006;26:968–76.
28. Kylin E. Studien ueber das Hypertonie-Hyperglyka" mie-Hyperurika" miesyndrom. Zentralblatt fuer Inn Medizin. 1923;44:105–27.
29. Vague J. La differentiation sexuelle facteur determinant des formes de l'obesite. Presse Med. 1947;30:339–40.
30. Kopelman PG. Obesity as a medical problem. Nature. 2000;404:635–43.
31. Simon GE, Von Korff M, Saunders K, Miglioretti DL, Crane PK, Van Belle G, Kessler RC. Association between obesity and psychiatric disorders in the US adult population. Arch Gen Psychiatry. 2006;63:824–30.
32. Ferrannini E. Metabolic syndrome: a solution in search of a problem. J Clin Endocrinol Metab. 2007;92:396–8.
33. Bruce KD, Hanson MA. The developmental origins, mechanisms, and implications of metabolic syndrome. J Nutr. 2010;140:648–52.

34. Palaniappan LP, Wong EC, Shin JJ, Fortmann SP, Lauderdale DS. Asian Americans have greater prevalence of metabolic syndrome despite lower body mass index. Int J Obes. 2011;35:393–400.
35. Van Eenige R, van der Stelt M, Rensen PCN, Kooijman S. Regulation of adipose tissue metabolism by the endocannabinoid system. Trends Endocrinol Metab. 2018;29:326–37.
36. Gustafson B. Adipose tissue, inflammation and atherosclerosis. J Atheroscler Thromb. 2010;17:332–41.
37. Baer PC. Adipose-derived stem cells and their potential to differentiate into the epithelial lineage. Stem Cells Dev. 2011;20:1805–16.
38. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med. 2016;280:465–75.
39. Gastaldelli A, Gaggini M, DeFronzo RA. Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the San Antonio metabolism study. Diabetes. 2017;66:815–22.
40. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes. 1997;46:3–10.
41. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259–66.
42. Kahn BB, Flier JS. Obesity and insulin resistance find the latest version: obesity and insulin resistance. J Clin Invest. 2000;106:473–81.
43. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444:840–6.
44. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444:875–80.
45. Bastien M, Poirier P, Lemieux I, Després JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis. 2014;56:369–81.
46. Compher C, Badellino KO. Obesity and inflammation: lessons from bariatric surgery. J Parenter Enter Nutr. 2008;32:645–7.
47. Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic review of metabolic syndrome biomarkers: a panel for early detection, management, and risk stratification in the west virginian population. Int J Med Sci. 2016;13:25.
48. Itoh M, Suganami T, Hachiya R, Ogawa Y. Adipose tissue remodeling as homeostatic inflammation. Int J Inflam. 2011;2011:1–8.
49. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol. 2010;316:129–39.
50. Lee SW, Jo HH, Kim MR, You YO, Kim JH. Association between metabolic syndrome and serum leptin levels in postmenopausal women. J Obstet Gynaecol. 2012;32:73–7.
51. Yun JE, Kimm H, Jo J, Jee SH. Serum leptin is associated with metabolic syndrome in obese and nonobese Korean populations. Metabolism. 2010;59:424–9.
52. Laclaustra M, Corella D, Ordovas JM. Metabolic syndrome pathophysiology: the role of adipose tissue. Nutr Metab Cardiovasc Dis. 2007;17:125–39.
53. Kaser S, Tatarczyk T, Stadlmayr A, Ciardi C, Ress C, Tschoner A, Sandhofer A, Paulweber B, Ebenbichler CF, Patsch JR. Effect of obesity and insulin sensitivity on adiponectin isoform distribution. Eur J Clin Investig. 2008;38:827–34.
54. Yamauchi T, Iwabu M, Okada-Iwabu M, Kadowaki T. Adiponectin receptors: a review of their structure, function and how they work. Best Pract Res Clin Endocrinol Metab. 2014;28:15–23.
55. Tanaka T, Narazaki M, Kishimoto T. IL-6 in immunity, inflammation, disease. Cold Spring Harb Perspect Biol. 2014;6:16295–6.
56. Pal M, Febbraio MA, Whitham M. From cytokine to myokine: the emerging role of interleukin-6 in metabolic regulation. Immunol Cell Biol. 2014;92:331–9.
57. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. Int J Obes. 2004;28:1357–64.

58. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa KI, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest. 2006;116:1494–505.
59. Panee J. Monocyte chemoattractant protein 1 (MCP-1) in obesity and diabetes. Cytokine. 2012;60:1–12.
60. Gotoh K, Inoue M, Masaki T, et al. A novel anti-inflammatory role for spleen-derived interleukin-10 in obesity-induced inflammation in white adipose tissue and liver. Diabetes. 2012;61:1994–2003.
61. Gormez S, Demirkan A, Atalar F, Caynak B, Erdim R, Sozer V, Gunay D, Akpinar B, Ozbek U, Buyukdevrim AS. Adipose tissue gene expression of adiponectin, tumor necrosis factor-α and leptin in metabolic syndrome patients with coronary artery disease. Intern Med. 2011;50:805–10.
62. Kosteli A, Zechner R, Ferrante AW Jr, Sugaru E, Haemmerle G, Martin JF, Lei J. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest. 2010;120:3466–79.
63. Castoldi A, De Souza CN, Saraiva Câmara NO, Moraes-Vieira PM. The macrophage switch in obesity development. Front Immunol. 2016;6:1–11.
64. Pollard JW. Trophic macrophages in development and disease. Nat Rev Immunol. 2009;9:259–70.
65. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25: 677–86.
66. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab. 2014;19:512–26.
67. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2014;177:7303–11.
68. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol. 2013;229:176–85.
69. Surmi BK, Hasty AH. Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. Future Lipidol. 2008;3:545–56.
70. Carrillo JLM, Campo, JOM, Coronado OGC, Gutiérrez PTV, Cordero FC, Juárez JV (2018) Adipose tissue and inflammation IntechOpen. https://doi.org/10.5772/intechopen.74227
71. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. Int J Mol Sci. 2015;16:378–400.
72. Alcalá M, Calderon-Dominguez M, Bustos E, Ramos P, Casals N, Serra D, Viana M, Herrero L. Increased inflammation, oxidative stress and mitochondrial respiration in brown adipose tissue from obese mice. Sci Rep. 2017;7:1–12.
73. Amirkhizi F, Siassi F, Djalali M, Hamedi S. Impaired enzymatic antioxidant defense in erythrocytes of women with general and abdominal obesity. Obes Res Clin Pract. 2014;8:e26–34.
74. Brown LA, Kerr CJ, Whiting P, Finer N, McEneny J, Ashton T. Oxidant stress in healthy normal-weight, overweight, and obese individuals. Obesity (Silver Spring). 2009;17:460–6.
75. D'Archivio M, Annuzzi G, Vari R, Filesi C, Giacco R, Scazzocchio B, Santangelo C, Giovannini C, Rivellese AA, Masella R. Predominant role of obesity/insulin resistance in oxidative stress development. Eur J Clin Investig. 2012;42:70–8.
76. Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, Ota T, Yokoyama M, Honda M, Ichi MK, Kaneko S. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism. 2008;57:1071–7.
77. Masania J, Malczewska-Malec M, Razny U, Goralska J, Zdzienicka A, Kiec-Wilk B, Gruca A, Stancel-Mozwillo J, Dembinska-Kiec A, Rabbani N, Thornalley PJ. Dicarbonyl stress in clinical obesity. Glycoconj J. 2016;33:581–9.

78. Semchyshyn H. Is part of the fructose effects on health related to increased AGE formation? In: Uribarry J, editor. Dietary AGEs and their role in health and disease. Boca Raton: CRC Press; 2017. p. 119–28.
79. Tinahones FJ, Murri-Pierri M, Garrido-Sánchez L, García-Almeida JM, García-Serrano S, García-Arnés J, García-Fuentes E. Oxidative stress in severely obese persons is greater in those with insulin resistance. Obesity. 2009;17:240–6.
80. Tucsek Z, Toth P, Sosnowska D, Gautam T, Mitschelen M, Koller A, Szalai G, Sonntag WE, Ungvari Z, Csiszar A. Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease. J Gerontol A Biol Sci Med Sci. 2014;69:1212–26.
81. Tarantini S, Valcarcel-Ares MN, Yabluchanskiy A, Tucsek Z, Hertelendy P, Kiss T, Gautam T, Zhang XA, Sonntag WE, de Cabo R, Farkas E, Elliott MH, Kinter MT, Deak F, Ungvari Z, Csiszar A. Nrf2 deficiency exacerbates obesity-induced oxidative stress, neurovascular dysfunction, blood-brain barrier disruption, neuroinflammation, amyloidogenic gene expression, and cognitive decline in mice, mimicking the aging phenotype. J Gerontol A Biol Sci Med Sci. 2018;73:853–63.
82. Rani V, Deep G, Singh RK, Palle K, Yadav UCS. Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. Life Sci. 2016;148:183–93.
83. Schneider KS, Chan JY. Emerging role of Nrf2 in adipocytes and adipose biology. Adv Nutr. 2013;4:62–6.
84. Pihl E, Zilmer K, Kullisaar T, Kairane C, Magi A, Zilmer M. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. Int J Obes. 2006;30:141–6.
85. Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas I, Papademetriou L, Economou M, Stefanadis C. The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study. Nutr Metab Cardiovasc Dis. 2007;17:590–7.
86. Rovenko BM, Kubrak OI, Gospodaryov DV, Perkhulyn NV, Yurkevych IS, Sanz A, Lushchak OV, Lushchak VI. High sucrose consumption promotes obesity whereas its low consumption induces oxidative stress in *Drosophila melanogaster*. J Insect Physiol. 2015;79:42–54.
87. Rovenko BM, Kubrak OI, Gospodaryov DV, Yurkevych IS, Sanz A, Lushchak OV, Lushchak VI. Restriction of glucose and fructose causes mild oxidative stress independently of mitochondrial activity and reactive oxygen species in *Drosophila melanogaster*. Comp Biochem Physiol Part A Mol Integr Physiol. 2015;187:27–39.
88. Rovenko BM, Perkhulyn NV, Gospodaryov DV, Sanz A, Lushchak OV, Lushchak VI. High consumption of fructose rather than glucose promotes a diet-induced obese phenotype in *Drosophila melanogaster*. Comp Biochem Physiol Part A Mol Integr Physiol. 2015;180:75–85.
89. Bray GA, Champagne CM. Beyond energy balance: there is more to obesity than kilocalories. J Am Diet Assoc. 2005;105:17–23.
90. Garaschuk O, Semchyshyn HM, Lushchak VI. Healthy brain aging: interplay between reactive species, inflammation and energy supply. Ageing Res Rev. 2018;43:26–45.
91. Lushchak VI. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comp Biochem Physiol Part C Toxicol Pharmacol. 2011;153:175–90.
92. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 2014;224:164–75.
93. Semchyshyn HM. Reactive carbonyl species in vivo: generation and dual biological effects. ScientificWorldJournal. 2014;2014:417842.
94. Vankoningsloo S, Piens M, Lecocq C, Gilson A, De Pauw A, Renard P, Demazy C, Houbion A, Raes M, Arnould T. Mitochondrial dysfunction induces triglyceride accumulation in 3T3-L1 cells: role of fatty acid beta-oxidation and glucose. J Lipid Res. 2005;46:1133–49.
95. Den Hartigh LJ, Omer M, Goodspeed L, Wang S, Wietecha T, O'Brien KD, Han CY. Adipocyte-specific deficiency of NADPH-oxidase 4 delays the onset of insulin resis-

tance and attenuates adipose tissue inflammation in obesity. Arterioscler Thromb Vasc Biol. 2017;37:466–75.
96. Han CY, Umemoto T, Omer M, Den Hartigh LJ, Chiba T, Leboeuf R, Buller CL, Sweet IR, Pennathur S, Abel ED, Chait A. NADPH oxidase-derived reactive oxygen species increases expression of monocyte chemotactic factor genes in cultured adipocytes. J Biol Chem. 2012;287:10379–93.
97. Cleary MP, Zisk JF. Anti-obesity effect of two different levels of dehydroepiandrosterone in lean and obese middle-aged female Zucker rats. Int J Obes. 1986;10:193–204.
98. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes. 2000;49:1939–45.
99. Moraru A, Wiederstein J, Pfaff D, Fleming T, Miller AK, Nawroth P, Teleman AA. Elevated levels of the reactive metabolite methylglyoxal recapitulate progression of type 2 diabetes. Cell Metab. 2018;27:926–934.e8.
100. Semchyshyn HM. Fructation in vivo: detrimental and protective effects of fructose. Biomed Res Int. 2013;2013:343914.
101. Semchyshyn HM, Lozinska LM, Miedzobrodzki J, Lushchak VI. Fructose and glucose differentially affect aging and carbonyl/oxidative stress parameters in Saccharomyces cerevisiae cells. Carbohydr Res. 2011;346:933–8.
102. Semchyshyn HM, Miedzobrodzki J, Bayliak MM, Lozinska LM, Homza BV. Fructose compared with glucose is more a potent glycoxidation agent *in vitro*, but not under carbohydrate-induced stress *in vivo*: potential role of antioxidant and antiglycation enzymes. Carbohydr Res. 2014;384:61–9.
103. Drougard A, Fournel A, Valet P, Knauf C. Impact of hypothalamic reactive oxygen species in the regulation of energy metabolism and food intake. Front Neurosci. 2015;9:56–61.
104. Lee H, Lee YJ, Choi H, Ko EH, Kim J-W. Reactive oxygen species facilitate adipocyte differentiation by accelerating mitotic clonal expansion. J Biol Chem. 2009;284:10601–9.
105. Kanda Y, Hinata T, Kang SW, Watanabe Y. Reactive oxygen species mediate adipocyte differentiation in mesenchymal stem cells. Life Sci. 2011;89:250–8.
106. Turker I, Zhang Y, Zhang Y, Rehman J. Oxidative stress as a regulator of adipogenesis. FASEB J. 2007;21:A1053.
107. Schröder K, Wandzioch K, Helmcke I, Brandes RP. Nox4 acts as a switch between differentiation and proliferation in preadipocytes. Arterioscler Thromb Vasc Biol. 2009;29:239–45.
108. Li Y, Mouche S, Sajic T, Veyrat-Durebex C, Supale R, Pierroz D, Ferrari S, Negro F, Hasler U, Feraille E, Moll S, Meda P, Deffert C, Montet X, Krause K-H, Szanto I. Deficiency in the NADPH oxidase 4 predisposes towards diet-induced obesity. Int J Obes. 2012;36:1503–13.
109. De Pauw A, Tejerina S, Raes M, Keijer J, Arnould T. Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. Am J Pathol. 2009;175:927–39.
110. Stolarczyk E. Adipose tissue inflammation in obesity: a metabolic or immune response? Curr Opin Pharmacol. 2017;37:35–40.
111. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415–45.
112. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, Grangette C. Feeding our immune system: impact on metabolism. Clin Dev Immunol. 2008;2008:639803.
113. Monteiro R, Azevedo I (2010) Chronic inflammation in obesity and the metabolic syndrome. Mediat Inflamm. pii:289645 https://doi.org/10.1155/2010/289645.
114. Qatanani M, Lazar MA. Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. 2007;21:1443–55.
115. Klotz LO, Sánchez-Ramos C, Prieto-Arroyo I, Urbánek P, Steinbrenner H, Monsalve M. Redox regulation of FoxO transcription factors. Redox Biol. 2015;6:51–72.

116. Nakae J, Cao Y, Oki M, Orba Y, Sawa H, Kiyonari H, Iskandar K, Suga K, Lombes M, Hayashi Y. Forkhead transcription factor FoxO1 in adipose tissue regulates energy storage and expenditure. Diabetes. 2008;57:563–76.
117. Pitoniak A, Bohmann D. Mechanisms and functions of Nrf2 signaling in *Drosophila*. Free Radic Biol Med. 2015;88:302–13.
118. Silva-Islas CA, Maldonado PD. Canonical and non-canonical mechanisms of Nrf2 activation. Pharmacol Res. 2018;134:92–9.
119. Canning P, Sorrell FJ, Bullock AN. Structural basis of Keap1 interactions with Nrf2. Free Radic Biol Med. 2015;88:101–7.
120. Abed DA, Goldstein M, Albanyan H, Jin H, Hu L. Discovery of direct inhibitors of Keap1-Nrf2 protein-protein interaction as potential therapeutic and preventive agents. Acta Pharm Sin B. 2015;5:285–99.
121. Tebay LE, Robertson H, Durant ST, Vitale SR, Penning TM, Dinkova-Kostova AT, Hayes JD. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radic Biol Med. 2015;88:108–46.
122. Huang Y, Li W, Su Z, Kong A-NT. The complexity of the Nrf2 pathway: beyond the antioxidant response. J Nutr Biochem. 2015;26:1401–13.
123. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. Free Radic Biol Med. 2015;88:179–88.
124. Kwak M-K, Itoh K, Yamamoto M, Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cell Biol. 2002;22:2883–92.
125. Cho H-Y, Gladwell W, Wang X, Chorley B, Bell D, Reddy SP, Kleeberger SR. Nrf2-regulated PPAR{gamma} expression is critical to protection against acute lung injury in mice. Am J Respir Crit Care Med. 2010;182:170–82.
126. Rushworth SA, Zaitseva L, Murray MY, Shah NM, Bowles KM, MacEwan DJ. The high Nrf2 expression in human acute myeloid leukemia is driven by NF-kappaB and underlies its chemo-resistance. Blood. 2012;120:5188–98.
127. Kurinna S, Werner S. NRF2 and microRNAs: new but awaited relations. Biochem Soc Trans. 2015;43:595–601.
128. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13:76–86.
129. Kobayashi A, Kang M-I, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, Yamamoto M. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004;24:7130–9.
130. Villeneuve NF, Lau A, Zhang DD. Regulation of the Nrf2–Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases. Antioxid Redox Signal. 2010;13:1699–712.
131. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284:13291–5.
132. Katoh Y, Itoh K, Yoshida E, Miyagishi M, Fukamizu A, Yamamoto M. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes Cells. 2001;6:857–68.
133. Li W, Yu S-W, Kong A-NT. Nrf2 possesses a redox-sensitive nuclear exporting signal in the Neh5 transactivation domain. J Biol Chem. 2006;281:27251–63.
134. Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene. 2013;32:3765–81.
135. Rada P, Rojo AI, Chowdhry S, McMahon M, Hayes JD, Cuadrado A. SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol Cell Biol. 2011;31:1121–33.

136. Pearl LH, Barford D. Regulation of protein kinases in insulin, growth factor and Wnt signalling. Curr Opin Struct Biol. 2002;12:761–7.
137. Lee J-M, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J Biol Chem. 2003;278: 12029–38.
138. Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW, Biswal S. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Res. 2010;38:5718–34.
139. Walsh J, Jenkins RE, Wong M, Olayanju A, Powell H, Copple I, O'Neill PM, Goldring CEP, Kitteringham NR, Park BK. Identification and quantification of the basal and inducible Nrf2-dependent proteomes in mouse liver: biochemical, pharmacological and toxicological implications. J Proteome. 2014;108:171–87.
140. Wu KC, Cui JY, Klaassen CD. Beneficial role of Nrf2 in regulating NADPH generation and consumption. Toxicol Sci. 2011;123:590–600.
141. Kitteringham NR, Abdullah A, Walsh J, Randle L, Jenkins RE, Sison R, Goldring CEP, Powell H, Sanderson C, Williams S, Higgins L, Yamamoto M, Hayes J, Park BK. Proteomic analysis of Nrf2 deficient transgenic mice reveals cellular defence and lipid metabolism as primary Nrf2-dependent pathways in the liver. J Proteome. 2010;73:1612–31.
142. Xue P, Hou Y, Chen Y, Yang B, Fu J, Zheng H, Yarborough K, Woods CG, Liu D, Yamamoto M, Zhang Q, Andersen ME, Pi J. Adipose deficiency of Nrf2 in Ob/Ob mice results in severe metabolic syndrome. Diabetes. 2013;62:845–54.
143. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, Yamamoto M. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci. 2001;59:169–77.
144. Randle LE, Goldring CEP, Benson CA, Metcalfe PN, Kitteringham NR, Park BK, Williams DP. Investigation of the effect of a panel of model hepatotoxins on the Nrf2-Keap1 defence response pathway in CD-1 mice. Toxicology. 2008;243:249–60.
145. Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, Yamamoto M, Kensler TW, Tuder RM, Georas SN, Biswal S. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. J Exp Med. 2005;202:47–59.
146. Tanaka Y, Aleksunes LM, Yeager RL, Gyamfi MA, Esterly N, Guo GL, Klaassen CD. NF-E2-related factor 2 inhibits lipid accumulation and oxidative stress in mice fed a high-fat diet. J Pharmacol Exp Ther. 2008;325:655–64.
147. Pi J, Leung L, Xue P, Wang W, Hou Y, Liu D, Yehuda-Shnaidman E, Lee C, Lau J, Kurtz TW, Chan JY. Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity. J Biol Chem. 2010;285:9292–300.
148. Kim B-R, Lee GY, Yu H, Maeng HJ, Oh TJ, Kim KM, Moon JH, Lim S, Jang HC, Choi SH. Suppression of Nrf2 attenuates adipogenesis and decreases FGF21 expression through PPAR gamma in 3T3-L1 cells. Biochem Biophys Res Commun. 2018;497:1149–53.
149. Xu J, Donepudi AC, More VR, Kulkarni SR, Li L, Guo L, Yan B, Chatterjee T, Weintraub N, Slitt AL. Deficiency in Nrf2 transcription factor decreases adipose tissue mass and hepatic lipid accumulation in leptin-deficient mice. Obesity (Silver Spring). 2015;23:335–44.
150. More VR, Xu J, Shimpi PC, Belgrave C, Luyendyk JP, Yamamoto M, Slitt AL. Keap1 knockdown increases markers of metabolic syndrome after long-term high fat diet feeding. Free Radic Biol Med. 2013;61:85–94.
151. Zhang L, Dasuri K, Fernandez-Kim S-O, Bruce-Keller AJ, Keller JN. Adipose-specific ablation of Nrf2 transiently delayed high-fat diet-induced obesity by altering glucose, lipid and energy metabolism of male mice. Am J Transl Res. 2016;8:5309–19.

152. Shin S, Wakabayashi N, Misra V, Biswal S, Lee GH, Agoston ES, Yamamoto M, Kensler TW. NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. Mol Cell Biol. 2007;27:7188–97.
153. Xu J, Kulkarni SR, Donepudi AC, More VR, Slitt AL. Enhanced Nrf2 activity worsens insulin resistance, impairs lipid accumulation in adipose tissue, and increases hepatic steatosis in leptin-deficient mice. Diabetes. 2012;61:3208–18.
154. Uruno A, Furusawa Y, Yagishita Y, Fukutomi T, Muramatsu H, Negishi T, Sugawara A, Kensler TW, Yamamoto M. The Keap1-Nrf2 system prevents onset of diabetes mellitus. Mol Cell Biol. 2013;33:2996–3010.
155. Slocum SL, Skoko JJ, Wakabayashi N, Aja S, Yamamoto M, Kensler TW, Chartoumpekis DV. Keap1/Nrf2 pathway activation leads to a repressed hepatic gluconeogenic and lipogenic program in mice on a high-fat diet. Arch Biochem Biophys. 2016;591:57–65.
156. Sampath C, Rashid MR, Sang S, Ahmedna M. Specific bioactive compounds in ginger and apple alleviate hyperglycemia in mice with high fat diet-induced obesity via Nrf2 mediated pathway. Food Chem. 2017;226:79–88.
157. Chartoumpekis DV, Palliyaguru DL, Wakabayashi N, Fazzari M, Khoo NKH, Schopfer FJ, Sipula I, Yagishita Y, Michalopoulos GK, O'Doherty RM, Kensler TW. Nrf2 deletion from adipocytes, but not hepatocytes, potentiates systemic metabolic dysfunction after long-term high-fat diet-induced obesity in mice. Am J Physiol Endocrinol Metab. 2018;315: E180–95.
158. He H-J, Wang G-Y, Gao Y, Ling W-H, Yu Z-W, Jin T-R. Curcumin attenuates Nrf2 signaling defect, oxidative stress in muscle and glucose intolerance in high fat diet-fed mice. World J Diabetes. 2012;3:94–104.
159. Yu Z, Shao W, Chiang Y, Foltz W, Zhang Z, Ling W, Fantus IG, Jin T. Oltipraz upregulates the nuclear factor (erythroid-derived 2)-like 2 [corrected](NRF2) antioxidant system and prevents insulin resistance and obesity induced by a high-fat diet in C57BL/6J mice. Diabetologia. 2011;54:922–34.
160. Tuzcu Z, Orhan C, Sahin N, Juturu V, Sahin K. Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rats. Oxidative Med Cell Longev. 2017;2017:1583098.
161. Meakin PJ, Chowdhry S, Sharma RS, Ashford FB, Walsh SV, McCrimmon RJ, Dinkova-Kostova AT, Dillon JF, Hayes JD, Ashford MLJ. Susceptibility of Nrf2-null mice to steatohepatitis and cirrhosis upon consumption of a high-fat diet is associated with oxidative stress, perturbation of the unfolded protein response, and disturbance in the expression of metabolic enzymes but not with insulin resistance. Mol Cell Biol. 2014;34:3305–20.
162. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, Biswal S. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest. 2006;116:984–95.
163. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab. 2012;23:351–63.
164. Yuan X, Huang H, Huang Y, Wang J, Yan J, Ding L, Zhang C, Zhang L. Nuclear factor E2-related factor 2 knockdown enhances glucose uptake and alters glucose metabolism in AML12 hepatocytes. Exp Biol Med (Maywood). 2017;242:930–8.
165. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, Tanaka N, Moriguchi T, Motohashi H, Nakayama K, Yamamoto M. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624.
166. Collins AR, Gupte AA, Ji R, Ramirez MR, Minze LJ, Liu JZ, Arredondo M, Ren Y, Deng T, Wang J, Lyon CJ, Hsueh WA. Myeloid deletion of nuclear factor erythroid 2-related factor 2 increases atherosclerosis and liver injury. Arterioscler Thromb Vasc Biol. 2012;32:2839–46.
167. Ding L, Yuan X, Yan J, Huang Y, Xu M, Yang Z, Yang N, Wang M, Zhang C, Zhang L. Nrf2 exerts mixed inflammation and glucose metabolism regulatory effects on murine RAW264.7 macrophages. Int Immunopharmacol. 2019;71:198–204.

168. Sussan TE, Jun J, Thimmulappa R, Bedja D, Antero M, Gabrielson KL, Polotsky VY, Biswal S. Disruption of Nrf2, a key inducer of antioxidant defenses, attenuates ApoE-mediated atherosclerosis in mice. PLoS One. 2008;3:e3791.
169. Chartoumpekis DV, Ziros PG, Psyrogiannis AI, Papavassiliou AG, Kyriazopoulou VE, Sykiotis GP, Habeos IG. Nrf2 represses FGF21 during long-term high-fat diet-induced obesity in mice. Diabetes. 2011;60:2465–73.
170. Xu L, Nagata N, Ota T. Glucoraphanin: a broccoli sprout extract that ameliorates obesity-induced inflammation and insulin resistance. Adipocytes. 2018;7:218–25.
171. Gerstgrasser A, Melhem H, Leonardi I, Atrott K, Schafer M, Werner S, Rogler G, Frey-Wagner I. Cell-specific activation of the Nrf2 antioxidant pathway increases mucosal inflammation in acute but not in chronic colitis. J Crohns Colitis. 2017;11:485–99.
172. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci. 2016;73:3221–47.
173. Kim C-S, Choi H-S, Joe Y, Chung HT, Yu R. Induction of heme oxygenase-1 with dietary quercetin reduces obesity-induced hepatic inflammation through macrophage phenotype switching. Nutr Res Pract. 2016;10:623–8.
174. Osburn WO, Karim B, Dolan PM, Liu G, Yamamoto M, Huso DL, Kensler TW. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. Int J Cancer. 2007;121:1883–91.
175. Jiang T, Tian F, Zheng H, Whitman SA, Lin Y, Zhang Z, Zhang N, Zhang DD. Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-kappaB-mediated inflammatory response. Kidney Int. 2014;85:333–43.
176. Bellezza I, Tucci A, Galli F, Grottelli S, Mierla AL, Pilolli F, Minelli A. Inhibition of NF-kappaB nuclear translocation via HO-1 activation underlies alpha-tocopheryl succinate toxicity. J Nutr Biochem. 2012;23:1583–91.
177. Illesca P, Valenzuela R, Espinosa A, Echeverria F, Soto-Alarcon S, Ortiz M, Videla LA. Hydroxytyrosol supplementation ameliorates the metabolic disturbances in white adipose tissue from mice fed a high-fat diet through recovery of transcription factors Nrf2, SREBP-1c, PPAR-gamma and NF-kappaB. Biomed Pharmacother. 2019;109:2472–81.
178. Walls HL, Backholer K, Proietto J, McNeil JJ. Obesity and trends in life expectancy. J Obes. 2012;2012:107989.

Modulators of Nrf2 Activation During Inflammation

Nady Braidy

Abstract Increased oxidative stress and inflammation are involved in the pathogenesis of several disorders including cancers and neurodegenerative diseases. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway plays an important role in mediating protection against oxidative and xenobiotic stresses. It is well established that the Nrf2 pathway induces the transcription of major phase II and cytoprotective antioxidant genes that may play a beneficial role against cancers and degenerative disorders. However, while activation of Nrf2 can provide some protection against oxidative stress and inflammation, hyperactivation of Nrf2 is associated with multiple diseases and can promote the survival and proliferation of cancer cells. Therefore, modulation of the Nrf2 pathway (inhibitors/activators) may represent a promising therapeutic strategy to counteract oxidative stress and inflammation in cancers and neurodegenerative diseases.

1 Introduction

Increased oxidative stress and inflammation is associated with damage to lipids, proteins, and nucleic acids, which leads to the development and progression of major systemic diseases, notably a wide variety of cancers, neurodegenerative and cardiovascular disorders [1, 2]. Despite extensive investigations worldwide and the advent of new technologies, degenerative diseases and cancer remain the main causes of human morbidity and mortality [3, 4]. As such, there has been growing attention to maintain the cell redox balance using either naturally occurring phytochemicals or synthetic compounds [5, 6].

To mediate protection against oxidative/nitrosative and electrophilic chemicals, mammalian cells are equipped with complex endogenous defence mechanisms, primarily phase II and antioxidant proteins, which detoxify and serve as antioxidant

N. Braidy (✉)
Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia
e-mail: n.braidy@unsw.edu.au

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enzymes [7]. The activities of these important proteins are regulated by the antioxidant or electrophile response element (ARE/EpRE). The expression of ARE plays a major role in mediating protection against cellular damage due to oxidative stress and inflammation [8, 9]. Nuclear factor erythroid 2-related factor 2 (Nrf2) represents one of the main ARE-binding transcription factors, which is thought to serve a pivotal role in protecting against oxidative and xenobiotic stresses [10, 11]. Modulation of Nrf2 signalling has been previously shown to represent a promising therapeutic target in several diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), diabetes and diabetic complications, airway disorders such as chronic obstructive pulmonary disease (COPD), cardiovascular disease, inflammatory bowel diseases, rheumatoid arthritis, and osteoarthritis [12].

Nrf2 belongs to a family of basic leucine zipper (bZip) transcription factors closely related to NF-E2 and is ubiquitously expressed in several tissues including the heart, lung, liver, kidney, and the brain. Nrf2 is comprised of 605 amino acids divided into six highly conserved Nrf2- ECH homology (Neh) domains (Neh1~Neh6). The cap 'n' collar structural domain (CNC domain) in Nrf2 is conserved amongst all bZip [13]. It has been demonstrated that Kelch-like ECH-associated protein 1 (Keap1) is a major inhibitor of Nrf2 [14]. Under physiological conditions, the repressor protein, Keap1 can inhibit Nrf2 signalling by stimulating the breakdown of Nrf2 via the ubiquitin-proteasome pathway [15–17]. More specifically, Nrf2 is composed of two domains on the Neh2, notably the ETGE and the DLG motif which is cooperatively binding to Keap1. Following exposure to oxidative stress, Nrf2 dissociates from Keap1, and translocates from the cytoplasm to the nucleus where it modulates the gene expression of various antioxidant enzymes, including NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), glutamate-cysteine ligase (GCL), glutathione S-transferase (GST), glutamate-cysteine ligase modifier subunit (GCLM), glutamate-cysteine ligase catalytic subunit (GCLC), thioredoxin reductase (TrxR), UDP-glucuronosyltransferase (UGT), glutathione peroxidase (GPX) [18–20].

Given the importance of Nrf2 in mediating protection from cellular damage, there is growing awareness for the development of potent and efficacious and clinically relevant Nrf2 modulators, which are mostly Nrf2 activators [21–24]. Nrf2 activators can be categorised into two groups based on their binding interactions: (1) Electrophilic activators which modify the Keap1 cysteine residues; (2) Activators specific to the protein–protein interaction (PPI) interface of Keap1–Nrf2 [23]. However, recently it has been shown that many patients with malignant cancers may carry bi-allelic mutations of Keap1. This ultimately leads to constitutive hyperactivation of Nrf2 which mediates protection of cancer cells against cellular stressors and chemotherapeutic agents. Therefore, this has led to the identification of Nrf2 inhibitors for use in the clinic [25–29]. In this chapter, we will describe the chemical and pharmacological properties of traditional and recently developed Nrf2 activator and inhibitors, and their clinical application(s).

2 Nrf2 Activators

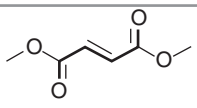
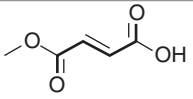
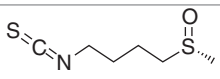
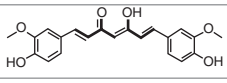
As previously mentioned, there are two main types of Nrf2 activators: electrophilic activators and Keap1–Nrf2 PPI inhibitors. Electrophilic activators covalently alter the cysteines of Keap1 and trigger a conformational change in the Keap1-Nrf2 PPI to activate Nrf2. However, electrophilic activators can interact with other intracellular thiol of proteins, leading to severe adverse events [30]. On the other hand, non-electrophilic Keap1–Nrf2 PPI inhibitors activate Nrf2 without inducing electrophilic damage to cells, and are therefore much safer than traditional electrophilic activators. However, Keap1–Nrf2 PPI inhibitors may be less efficacious than electrophilic activators [28].

2.1 Electrophilic Activators Which Modify the Keap1 Cysteine Residues

2.1.1 Dimethyl Fumarate and its Derivatives

Dimethyl fumarate (DMF, BG-12, Tecfidera) was developed by Biogen Idec and has been approved by Food and Drug Administration (FDA) (Table 1). It represents the new first-line oral drug for the treatment of patients with relapsing-remitting multiple sclerosis (RRMS) [31]. DMF and its primary metabolite, monomethyl fumarate (MMF) has been shown to activate the Nrf2 transcriptional pathway in vitro and in vivo. Although the exact mechanism of action of DMF remains unclear, it is thought to exhibit immune-modulating and neuroprotective effects. Oral DMF treatment also demonstrated beneficial effects in an animal model for experimental autoimmune encephalomyelitis independent of Nrf2 signalling [32].

Table 1 Chemical structure of dimethyl fumarate and its derivatives

Drugs	Chemical structure	Reference
Dimethyl fumarate		[24, 31]
Monomethyl fumarate		[32]
Sulforaphane		[33]
Curcumin		[34]

The main adverse effects reported from DMF in phase III clinical trials are gastrointestinal ailments. A new series of DMF compounds have since been developed. These compounds exhibit greater half-life and retention for the treatment of MS. These modifications can lead to lower dosage compared to DMF, thus reducing the risk of potential adverse effects reported by DMF [24].

Additionally, Sulforaphane (SFN) (Table 1) has been shown to protect against degeneration of dopaminergic neurons in a Drosophila PD model [33]. Curcumin, a well-known phytochemical has also been demonstrated to activate Nrf2 signalling and attenuate cognitive deficits in transgenic AD mice [34].

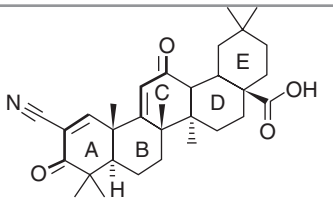
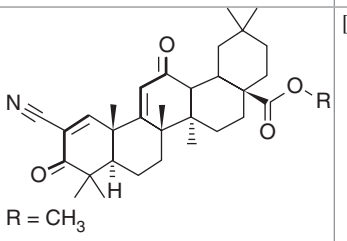
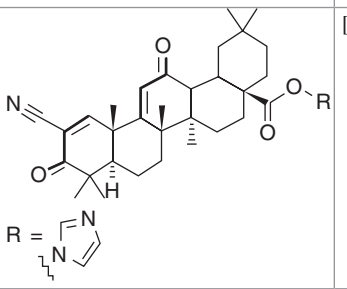
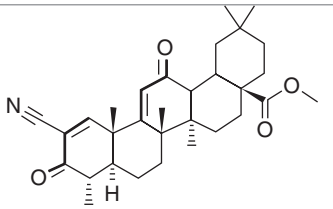
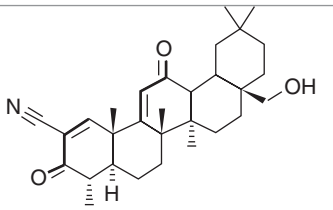
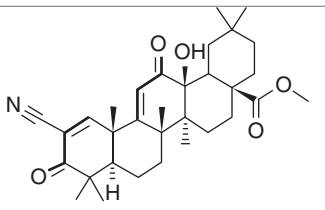
2.1.2 Pentacyclic Triterpenes

Modification of an anti-inflammatory natural product oleanolic acid at the α, β-unsaturated scaffold has been shown to electrophilically activate Nrf2. Examples include three semi-synthetic compounds, CDDO, CDDO-Me, and CDDO-Im (Table 2) [35, 36]. CDDO-Me has been shown to activate Nrf2 and suppress NF-κB signalling [36]. The binding mechanism of CDDO to the cysteine residue sensors of Keap1 has been previously characterised using X-ray crystallography [37]. CDDO-Me demonstrated promising outcomes in a phase III clinical trial for slowing down the progression to end-stage renal disease in patients with chronic kidney disease (CKD) and type 2 diabetes [38]. However, the phase III trial was discontinued due to a higher incidence of unwanted cardiovascular events compared to the placebo.

Nitric oxide production inhibitors and Nrf2 activators have been combined to reduce inflammation and oxidative stress. Two examples (TX-63435 and TX-63545) (Table 2) have demonstrated potent Nrf2-ARE activity, induced Nrf2 transcriptional activity by 7.2-fold at 62.5 nM in human hepatoma HuH7 cancer cells, and significantly suppressed interferon-gamma (IFN-γ)-induced nitric oxide production (IC50 = 1.0 and 0.7 nM, respectively) in mouse macrophage RAW264.7 cells [24].

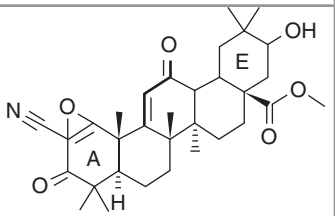
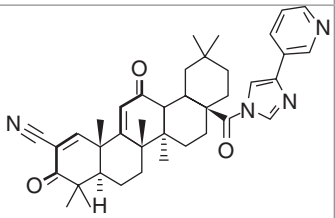
Fluorine-substituted derivatives of CDDO-Me (Table 2) have also been used to activate Nrf2. RTA-408 (omaveloxolone), a synthetic oleanane triterpenoid compound, induced over 16-fold of ARE activity at 62.5 nM in WST1 cells, and dose-dependently upregulated the expression of NQO1, HO-1, GCLM, and TxnR1 at both the transcriptome and protein levels in several cells [24]. A phase II/III clinical trial of omaveloxolone by Reata Pharmaceuticals is currently ongoing for the treatment of Friedreich's ataxia, mitochondrial myopathy, non-small cell lung cancer (NSCLC), and melanoma, and recent findings are not currently available in the literature. More recently, oxidative analogues of CDDO-Me have been developed. These compounds, such as TX-63421 also demonstrated high potency to inhibit NO production [24].

Table 2 Chemical structure of pentacyclic triterpenes

Drugs	Chemical structure	Reference
CDDO		[24, 35–37]
CDDO-me		[35–38]
CDDO-Im		[24, 35, 36]
TX-63435		[24]
TX-63545		[24]
TX-63421		[24]

(continued)

Table 2 (continued)

Drugs	Chemical structure	Reference
RS-9		[24]
CDDO3P-Im		[24]

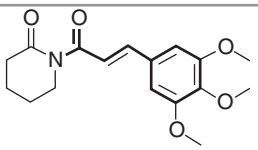
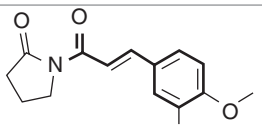
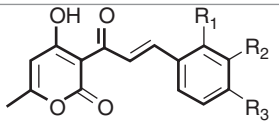
Microbial biotransformation of CDDO-Me has been shown to yield several clinically relevant Nrf2 activators, known as RS compounds (Table 2) [39]. The key structural differences between CDDO-Me and the RS compounds was the epoxidation of the A-ring and hydroxylation or ketonisation at the E-ring. These compounds demonstrated greater Nrf2-ARE activity than CDDO-Me in Hepa1c1c7 cells. RS-9 has been shown to inhibit LPS-induced hydrogen peroxide production in RAW264.7 cells and increase Nrf2-targeted genes in the retina including nqo1, hmox1, and gclm [24].

As well, pyridyl analogues of CDDO have demonstrated greater stability in human plasma and can reach higher concentrations in target tissues such as liver, pancreas, kidney, and lungs [24]. CDDO3P-Im (Table 2) exhibited an inhibitory effect on the production of NO with IC50 = 4.3 nM and induced differentiation of U937 cells with EC50 = 30 nM and apoptosis of the cell death of U937 cells with EC50 100 nM [24].

2.1.3 Chalcone Derivatives

Chalcone derivatives have been chemically synthesised to activate Nrf2 (Table 3). One study showed that the expression of GCLM and NQO1 treated with chalcone derivatives was threefold and fivefold higher respectively compared to sulforaphane in the small intestine [24]. However, these compounds exhibited low aqueous solubility and modest oral bioavailability due to their high lipophilicity. The bioavailability of chalcone analogues can be improved by lowering the lipophilicity through the addition of heteroatoms to the aromatic rings [24]. CVA130031, CVA-130032, and CVA-130035 significantly increased the expression level of HO-1 (49.66-, 98.29- to 115.11-fold upregulation, respectively), NQO1 (2.56-, 2.21- to 3.30-fold upregulation, respectively) in peripheral blood mononuclear cells (PBMCs) [24].

Table 3 Chemical structure of chalcones, piperlongumine analogues, and α-pyrone derivatives

Drugs	Chemical structure	Reference
Chalcones		[24–30]
Piperlongumine analogues		[40]
α-Pyrones	 R_1 = F, R_2 = H, R_3 = H, R_1 = H,R_2 = F, R_3 = F,	[41]

Vinyl sulfones have been shown to activate Nrf2 signalling to reduce the oxidative stress and may be beneficial for the treatment of PD [24]. The sulfone moiety was more active than carbonyl or sulfoxide group. These compounds attenuated PD-associated behavioural deficits in a mouse model for PD [24].

As well, piperlongumine analogues (Table 3) have been shown to protect against hydrogen peroxide and 6-hydroxydopamine (6-OHDA) induced neuronal cell oxidative damage in PC12 cells [40]. Additionally, α-pyrone derivatives (Table 3) of chalcone have been shown to induce Nrf2 activation through MAPK/ ERK pathway [41].

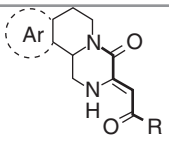
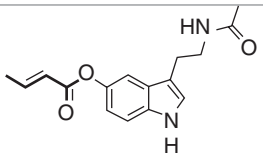
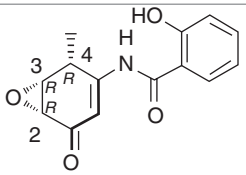
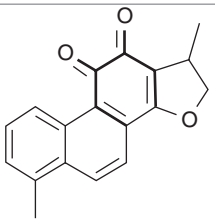
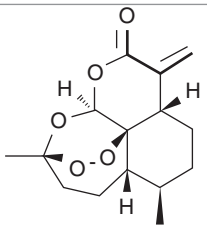
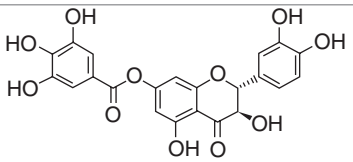
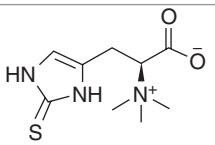
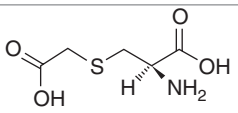
2.1.4 Nrf2 Activators with Pyrazino[2,1-a]Isoquinolin Scaffold

Nrf2 activators with a pyrazino[2,1-a]isoquinolin scaffold (Table 4) have been shown to activate Nrf2-ARE signalling both in vitro and in vivo [42]. The α,β-unsaturated carbonyl scaffold is closely related to the structure of DMF. When ring D was replaced by aliphatic cycles, heterocycles, or aliphatic chains, Nrf2 activity was increased by 13.35- and 38.41-fold at 10 μM, respectively.

2.1.5 3-Alkylamino-1H-Indole Acrylates

3-Alkylamino-1H-indole acrylates (Table 4) containing the α,β-unsaturated scaffold have been developed for the treatment of neurodegenerative diseases [24]. When the methyl group of α,β-unsaturated scaffold was replaced by phenyl ring, Nrf2 activity was significantly reduced (2.54-fold at 60 μM). This suggests that the phenyl ring or substituted phenyl groups are not preferred [24].

Table 4 Other electrophilic activators which modify the Keap1 cysteine residues

Drugs	Chemical structure	Reference
Pyrazino[2,1-a]isoquinolin		[42]
3-Alkylamino-1H-indole acrylates		[24]
2-hydroxybenzamide		[24, 43]
15,16-dihydrotanshinone I		[24]
Artemisitene		[24]
7-O-galloyltaxifolin		[44]
Ergothioneine		[24]
L-carbocysteine		[24]

2.1.6 2-Hydroxybenzamide Core Containing Compounds

Compounds with a 2-hydroxybenzamide core (Table 4) based on the structure of the antibiotic epoxyquinomicin C have demonstrated beneficial effects for the treatment of type II collagen-induced arthritis [43]. These compounds also significantly activated the Nrf2-ARE signalling in human neuroblastoma SK-N-SH cells over fourfold at 10 μM [24].

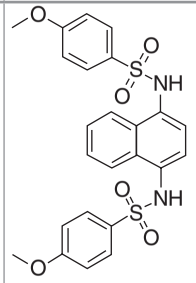
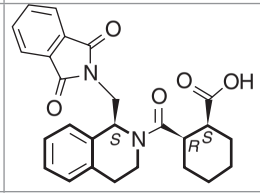
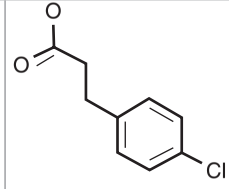
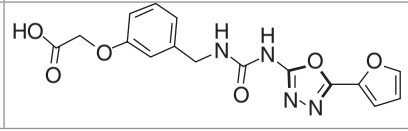
2.1.7 Other nrf2 Activators

15,16-Dihydrotanshinone I (Table 4), a derivative of the natural product tanshinone I, has been shown to protect endothelial cells through a fourfold induction of the Nrf2-ARE pathway at 10 μM activation [24]. As well, artemisitene (Table 4), a derivative of artemisinin, induced a 3.5-fold upregulation of Nrf2-ARE at 2.5 μM. Artemisitene may be beneficial for the treatment of oxidative stress-related diseases such as diabetes, cardiovascular disease, neurodegenerative diseases, chronic kidney disease, and lung disease [24]. Several protolichesterinic acid derivatives containing an α-Methylene-γ-lactone core have been shown to act as dual activators of peroxisome proliferator-activated receptor gamma (PPARγ) and Nrf2 [45]. Several diterpenes isolated from *Salvia officinalis* and containing phenolic carnosol 44 activated Nrf2-ARE signalling, in HT22 cell lines [46]. A compound known as 7-O-galloyltaxifolin containing a mono-galloyl esters of quercetin and taxifolin increased both mRNA and protein levels of HO-1 at concentrations of 50 μM in RAW264.7 cells via activation of the MAPK/Nrf2 signalling pathway [44]. An antioxidant peptide isolated from Sphyrna flesh containing the 6-mer peptide, Ile-Ile-Gly-Leu-Val-Pro, was reported to upregulate the expression level of Nrf2 in HEK293 cells, leading to activation of the Nrf2-ARE pathway [24]. Ergothioneine (Table 4), a naturally occurring amino acid, can exert anti-photooxidative effects in human keratinocytes by increasing the activity of the Nrf2 [24]. Finally, the approved drug, L-carbocysteine (Table 4), can activate Nrf2 signalling and exert anti-inflammatory effects in C57B/6 mice at a concentration of 10 mM [24].

2.2 Keap1–Nrf2 PPI Inhibitors

Apart from electrophilic activators of Nrf2, non-electrophilic activators of the Nrf2-ARE pathway (Table 5) have also been developed. While the X-ray structure of Keap1 is yet to be identified, the three-dimensional co-structure of the Keap1 Kelch domain with Nrf2 has been characterised in mice and humans using X-ray crystallography. These studies identified the E79 and E82 domain of Nrf2 as the main sites for interaction with two highly positively charged pockets of Keap1 interface. This has led to the identification of several direct inhibitors of Keap1–Nrf2 PPI [54–57].

Table 5 Chemical structure of Keap1–Nrf2 PPI inhibitors

Drugs	Chemical structure	Reference
1,4- diaminonaphthalenes		[24, 47–49]
1,2,3,4- tetrahydroisoquinolines		[24, 50]
3-phenylpropanoic acid derivatives		[51]
Five-membered heterocyclic compounds		[24, 52, 53]

2.2.1 Keap1–Nrf2 Inhibitors with a 1,4-Diaminonaphthalene Core

Several Keap1– Nrf2 inhibitors with 1,4-diaminonaphthalene core have been developed to inhibit Keap1–Nrf2 interaction with moderate activity (EC50 = 2.7 μM) [47]. Addition of two acetic acid groups on the amino moieties of these compounds led to a greater binding affinity to Keap1. It has been suggested that the two acetic acid groups mimicked the function of the side chain of Glu79, Glu82 on Nrf2 [48]. A diamide analogue has also been shown to inhibit Keap1–Nrf2 interactions (fluorescence anisotropy IC50 = 63.0 nM. Replacement of the 1,4-diaminonaphthalene core with a 1,4-diaminophenyl core led to a dramatic reduction in activity [49]. Another compound containing a p-acetamide substituent on the side chain phenyl rings was found to lower the levels of circulating proinflammatory in the lipopolysaccharides (LPS)-challenged mouse model and protected against dextran sodium sulphate (DSS) toxicity in NCM460 cells and mouse colon via the activation of Nrf2 signalling [24].

2.2.2 Keap1–Nrf2 Inhibitors with 1,2,3,4- Tetrahydroisoquinoline Core

Small molecules with a 1,2,3,4-tetrahydroisoquinoline core have also been shown to inhibit Keap1–Nrf2 PPI. The carboxylic group is likely to represent major determinant for the activity of the compound [24]. The one-carbon linker between tetrahydroisoquinoline and phthalimido group is necessary and increased length of the linker reduced Keap1–Nrf2 PPI inhibition [24]. Removal of a single carbonyl in the phthalimido group leads to a decline in Keap1 binding. Additionally, interactions between the carbonyl group of the isoxindole and Ser602 and a putative π-π stacking of the aromatic ring with Tyr572 have also been reported. As well, the carboxyl group formed the hydrogen bond with Arg380, which improved binding affinity [50].

2.2.3 Keap1–Nrf2 Inhibitors Originating from 3-Phenylpropanoic Acid

3-Phenylpropanoic acid-based compounds have been shown to interact with the Arg483 of Keap1–Nrf2 PPI, thus mimicking Glu79 of the Nrf2 ETGE motif [51]. While these compounds demonstrated low potency (FP IC50 > 1 mM), attachment of the benzotriazole moiety to the benzylic carbon of the phenylacetic acid, formed a stacking interaction with the side chain of Tyr525 and led to a compound with greater binding affinity (FP IC50 = 61 μM). Addition of sulphonamides to the 3-position of the chlorophenyl ring further improved binding affinity to the Keap1 Kelch domain (ITC Kd = 1.3 nM). Importantly, these compounds have demonstrated limited toxicity in vivo and may represent novel chemical probes to further investigate the molecular pathways of the Keap1–Nrf2 system [51].

2.2.4 Keap1–Nrf2 Inhibitors with Five-Membered Heterocyclic Core

Recently, the co-crystal structures of Keap1 Kelch domain in complex with a compound containing a 1,3,4-oxadiazole core have been recently identified [52]. Basically, the carboxyl group of this compound formed hydrogen bond networks by interacting Ser363, Arg380, and Asn382 of Keap1. The phenyl ring interacted with Tyr334 through π–π contact. The NH of urea moiety formed hydrogen bond with the hydroxyl group of Tyr334. A cation–π interaction was observed between the furan ring of 70 and Arg415 of Keap1 [52]. These compounds have demonstrated marked reduction in the release of proinflammatory cytokines in mouse serum and suppressed LPS-induced inflammation.

As well, novel drugs containing 1,2,3-triazoles have been shown to induce Nrf2-dependent genes. More specifically, the two carboxyl groups interacted with the two polar pockets comprised of Arg415, Arg483, and Arg380, Asn382, respectively [53]. The triazole moiety was also predicted to interact with Ser602. Such compounds inhibited 82.3% of Keap1–Nrf2 interaction at 100 μM. Moreover, new Nrf2 activators with a 1-phenyl-1,3,4-triazole have been identified [53]. These compounds have been shown to occupy the binding pocket, and the nitro group of these compounds formed hydrogen bonds with Arg483 of Keap1, thus enhancing the binding affinity. Apart from lowering inflammation, these compounds can inhibit sirtuins (SIRTs), and cytosolic SIRT2 in particular, in ST14a cells [24].

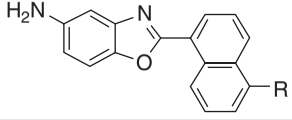
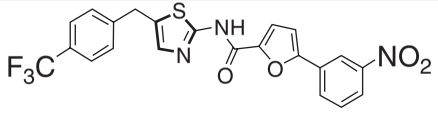
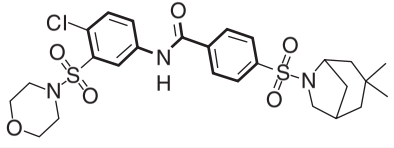
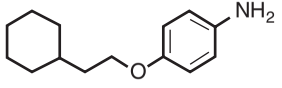
3 Nrf2 Inhibitors

Despite the beneficial effects of Nrf2 activators, activation of Nrf2 pathway has been shown to play a negative role in cancer therapy by mediating protection of cancer cells Hyperactivation of Nrf2 is a major cause of multiple drug resistance (MDR). In cancer cells, upregulation of Nrf2 may be mediated by several reasons, including: (1) mutations in Keap1 and Nrf2 [58–65]; (2) mutations in fumarate hydratase [66–69]; and (3) Keap1 promoter hypermethylation [70]. Therefore, inhibition of Nrf2 activity represents an important therapeutic strategy for increasing the sensitisation of cancer drugs to chemotherapy. Nrf2 inhibitors (Table 6) have been recently combined with chemotherapeutic agents such as cisplatin and doxorubicin to inhibit proliferation of cancer cells and dome animal models [71].

3.1 Nrf2 Inhibitors with Benzo[d]Oxazole Core

Benzo[d]oxazole derivatives with antitumor activity have been shown to inhibit Nrf2-ARE signalling [24]. These compounds not only inhibit Nrf2 activity, but also increased ROS production and accumulation to kill a lung cancer cell line. These

Table 6 Chemical structure of Nrf2 inhibitors

Drugs	Chemical structure	Reference
Benzo[d]oxazoles		[70, 71]
Thiazole core containing compounds		[24]
Benzamide core containing compounds		[24]
4-(2-Cyclohexylethoxy) anilines		[72]

drugs may be useful as chemosensitive or radiosensitive agents to overcome tolerance of the cancer cells to anticancer agent therapy or radiation therapy and enhance cancer cell death [24].

3.2 Nrf2 Inhibitors with Thiazole or Benzamide Core

Nrf2 inhibitors with a thiazole or benzamide core have also been identified and can inhibit Nrf2 at the concentration lower than 5 μM [24]. These drugs have been shown to dose-dependently inhibited the proliferation of different cancer cells lines, including A549, H460, and Panc-1. These inhibitors also sensitised A549 lung tumours to carboplatin therapy [24].

3.3 Nrf2 Inhibitors with 4-(2-Cyclohexylethoxy) Aniline Core

A series of radiosensitisers with 4-(2-Cyclohexylethoxy)aniline core have been shown to inhibit Nrf2 signalling stimulated by tertiary butylhydroquinone (t-BHQ) or radiation in a dose-dependent manner (IC50 = 2.9 μM) with limited effect on cell viability [72]. These compounds also increased ROS accumulation in irradiated cells compared with cells exposed to radiation alone leading to increased apoptosis as evidenced by caspase-3 and poly ADP-ribose polymerase (PARP) cleavage [72].

4 Summary

Within the last 5 years, several compounds have been identified as Nrf2 modulators (activators/inhibitors). The successful application of DMF for the treatment of MS has encouraged the development of novel Nrf2 activators with differing modes of action and improved safety profiles. The role of electrophilic/oxidative stress in the pathobiology of several disorder leading to inflammation, neurodegeneration, autoimmune diseases, and cancer is well established. Therefore, small molecule activators of Nrf2-ARE signalling may be useful for the maintenance of normal cellular homeostasis. However, given the recent discovery that hyperactivation of Nrf2 signalling may mediate self-protection of cancer cells, a new class of Nrf2 inhibitors may provide an additional therapeutic strategy for the treatment of malignant cancers.

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References

1. Birch-Machin MA, Bowman A. Oxidative stress and ageing. Br J Dermatol. 2016;175(Suppl 2):26–9.
2. Martin-Fernandez, B. and R. Gredilla, Mitochondrial oxidative stress and cardiac ageing. Clin Investig Arterioscler, 2018
3. Marabotti C, et al. Mortality for chronic-degenerative diseases in Tuscany: ecological study comparing neighboring areas with substantial differences in environmental pollution. Int J Occup Med Environ Health. 2017;30(4):641–53.
4. Tsuboi M, et al. Do musculoskeletal degenerative diseases affect mortality and cause of death after 10 years in Japan? J Bone Miner Metab. 2011;29(2):217–23.
5. Calabriso, N., et al., Red grape skin polyphenols blunt matrix metalloproteinase-2 and -9 activity and expression in cell models of vascular inflammation: protective role in degenerative and inflammatory diseases. Molecules. 2016;21(9)
6. Hossen MS, et al. Beneficial roles of honey polyphenols against some human degenerative diseases: a review. Pharmacol Rep. 2017;69(6):1194–205.
7. Kwak MK, et al. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. Mutat Res. 2001;480-481:305–15.
8. Beiter T, et al. Exercise, skeletal muscle and inflammation: ARE-binding proteins as key regulators in inflammatory and adaptive networks. Exerc Immunol Rev. 2015;21:42–57.
9. Lee YS, Lee JA, Kaang BK. Regulation of mRNA stability by ARE-binding proteins in synaptic plasticity and memory. Neurobiol Learn Mem. 2015;124:28–33.
10. Zhang L, et al. The effect of nuclear factor erythroid 2-related factor/antioxidant response element signalling pathway in the lanthanum chloride-induced impairment of learning and memory in rats. J Neurochem. 2017;140(3):463–75.
11. Zhao H, et al. The role of nuclear factor-erythroid 2 related factor 2 (Nrf-2) in the protection against lung injury. Am J Physiol Lung Cell Mol Physiol. 2017;312(2):L155–62.
12. Mann GE. Nrf2-mediated redox signalling in vascular health and disease. Free Radic Biol Med. 2014;75(Suppl 1):S1.
13. Malhotra D, et al. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Res. 2010;38(17):5718–34.
14. Stepkowski TM, Kruszewski MK. Molecular cross-talk between the NRF2/KEAP1 signaling pathway, autophagy, and apoptosis. Free Radic Biol Med. 2011;50(9):1186–95.
15. Ding H, et al. Nrf2-ARE signaling provides neuroprotection in traumatic brain injury via modulation of the ubiquitin proteasome system. Neurochem Int. 2017;111:32–44.
16. Sekhar KR, Yan XX, Freeman ML. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. Oncogene. 2002;21(44):6829–34.
17. Villeneuve NF, Lau A, Zhang DD. Regulation of the Nrf2-Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases. Antioxid Redox Signal. 2010;13(11):1699–712.
18. Alfieri A, et al. Targeting the Nrf2-Keap1 antioxidant defence pathway for neurovascular protection in stroke. J Physiol. 2011;589(17):4125–36.
19. Itoh K, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13(1):76–86.
20. Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxid Redox Signal. 2005;7(3–4):385–94.
21. Atilano-Roque A, et al. Nrf2 activators as potential modulators of injury in human kidney cells. Toxicol Rep. 2016;3:153–9.
22. Kumar H, et al. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. Nat Prod Rep. 2014;31(1):109–39.
23. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. Med Res Rev. 2012;32(4):687–726.

24. Sun H, et al. Recent progress in the development of small molecule Nrf2 modulators: a patent review (2012-2016). Expert Opin Ther Pat. 2017;27(7):763–85.
25. Maes M, et al. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates--Nrf2 activators and GSK-3 inhibitors. Inflammopharmacology. 2012;20(3):127–50.
26. McMahon M, et al. HDAC inhibitors increase NRF2-signaling in tumour cells and blunt the efficacy of co-adminstered cytotoxic agents. PLoS One. 2014;9(11):e114055.
27. Rushworth SA, Bowles KM, MacEwan DJ. High basal nuclear levels of Nrf2 in acute myeloid leukemia reduces sensitivity to proteasome inhibitors. Cancer Res. 2011;71(5):1999–2009.
28. Wells G. Peptide and small molecule inhibitors of the Keap1-Nrf2 protein-protein interaction. Biochem Soc Trans. 2015;43(4):674–9.
29. Zhuang C, et al. Updated research and applications of small molecule inhibitors of Keap1-Nrf2 protein-protein interaction: a review. Curr Med Chem. 2014;21(16):1861–70.
30. Wakabayashi N, et al. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc Natl Acad Sci U S A. 2004;101(7):2040–5.
31. Deeks ED. Dimethyl Fumarate: a review in relapsing-remitting MS. Drugs. 2016;76(2):243–54.
32. Schulze-Topphoff U, et al. Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2. Proc Natl Acad Sci U S A. 2016;113(17):4777–82.
33. Morroni F, et al. Neuroprotective effect of sulforaphane in 6-hydroxydopamine-lesioned mouse model of Parkinson's disease. Neurotoxicology. 2013;36:63–71.
34. Patel V, et al. Small molecules and Alzheimer's disease: misfolding, metabolism and imaging. Curr Alzheimer Res. 2015;12(5):445–61.
35. Crowley VM, et al. Synthetic oleanane triterpenoids enhance blood brain barrier integrity and improve survival in experimental cerebral malaria. Malar J. 2017;16(1):463.
36. Liby KT, Sporn MB. Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. Pharmacol Rev. 2012;64(4):972–1003.
37. Cleasby A, et al. Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. PLoS One. 2014;9(6):e98896.
38. Pergola PE, et al. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. N Engl J Med. 2011;365(4):327–36.
39. Nakagami Y, et al. Novel Nrf2 activators from microbial transformation products inhibit blood-retinal barrier permeability in rabbits. Br J Pharmacol. 2015;172(5):1237–49.
40. Peng S, et al. Synthesis of piperlongumine analogues and discovery of nuclear factor erythroid 2-related factor 2 (Nrf2) activators as potential neuroprotective agents. J Med Chem. 2015;58(13):5242–55.
41. Xi MY, et al. Synthesis and bioevaluation of a series of alpha-pyrone derivatives as potent activators of Nrf2/ARE pathway (part I). Eur J Med Chem. 2013;66:364–71.
42. Xi MY, et al. 3-Aroylmethylene-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4(11bH)-ones as potent Nrf2/ARE inducers in human Cancer cells and AOM-DSS treated mice. J Med Chem. 2013;56(20):7925–38.
43. Niitsu Y, et al. Chemoenzymatic synthesis of (2R,3R,4R)-dehydroxymethylepoxyquinomicin (DHMEQ), a new activator of antioxidant transcription factor Nrf2. Org Biomol Chem. 2011;9(12):4635–41.
44. Vrba J, et al. A novel semisynthetic flavonoid 7-O-galloyltaxifolin upregulates heme oxygenase-1 in RAW264.7 cells via MAPK/Nrf2 pathway. J Med Chem. 2013;56(3):856–66.
45. Le Lamer AC, et al. Protolichesterinic acid derivatives: alpha-methylene-gamma-lactones as potent dual activators of PPAR gamma and Nrf2 transcriptional factors. Bioorg Med Chem Lett. 2014;24(16):3819–22.
46. Fischedick JT, et al. Structure activity relationship of phenolic diterpenes from Salvia officinalis as activators of the nuclear factor E2-related factor 2 pathway. Bioorg Med Chem. 2013;21(9):2618–22.

47. Marcotte D, et al. Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism. Bioorg Med Chem. 2013;21(14):4011–9.
48. Jiang ZY, et al. Discovery of potent Keap1-Nrf2 protein-protein interaction inhibitor based on molecular binding determinants analysis. J Med Chem. 2014;57(6):2736–45.
49. Jain AD, et al. Probing the structural requirements of non-electrophilic naphthalene-based Nrf2 activators. Eur J Med Chem. 2015;103:252–68.
50. Jnoff E, et al. Binding mode and structure-activity relationships around direct inhibitors of the Nrf2-Keap1 complex. ChemMedChem. 2014;9(4):699–705.
51. Davies TG, et al. Monoacidic inhibitors of the Kelch-like ECH-associated protein 1: nuclear factor Erythroid 2-related factor 2 (KEAP1:NRF2) protein protein interaction with high cell potency identified by fragment-based discovery. J Med Chem. 2016;59(8):3991–4006.
52. Satoh M, et al. Multiple binding modes of a small molecule to human Keap1 revealed by X-ray crystallography and molecular dynamics simulation. FEBS Open Bio. 2015;5:557–70.
53. Xu LL, et al. Molecular similarity guided optimization of novel Nrf2 activators with 1,2,4-oxadiazole core. Bioorg Med Chem. 2016;24(16):3540–7.
54. Deshmukh P, et al. The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. Biophys Rev. 2017;9(1):41–56.
55. Pandey P, et al. The see-saw of Keap1-Nrf2 pathway in cancer. Crit Rev Oncol Hematol. 2017;116:89–98.
56. Schmoll D, Engel CK, Glombik H. The Keap1-Nrf2 protein-protein interaction: a suitable target for small molecules. Drug Discov Today Technol. 2017;24:11–7.
57. Taguchi K, Yamamoto M. The KEAP1-NRF2 system in Cancer. Front Oncol. 2017;7:85.
58. Goode A, et al. ALS-FTLD associated mutations of SQSTM1 impact on Keap1-Nrf2 signalling. Mol Cell Neurosci. 2016;76:52–8.
59. Hast BE, et al. Cancer-derived mutations in KEAP1 impair NRF2 degradation but not ubiquitination. Cancer Res. 2014;74(3):808–17.
60. Hayes JD, McMahon M. NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. Trends Biochem Sci. 2009;34(4):176–88.
61. Jeong Y, et al. Role of KEAP1/NRF2 and TP53 mutations in lung squamous cell carcinoma development and radiation resistance. Cancer Discov. 2017;7(1):86–101.
62. Konstantinopoulos PA, et al. Keap1 mutations and Nrf2 pathway activation in epithelial ovarian cancer. Cancer Res. 2011;71(15):5081–9.
63. Li QK, et al. KEAP1 gene mutations and NRF2 activation are common in pulmonary papillary adenocarcinoma. J Hum Genet. 2011;56(3):230–4.
64. Shibata T, et al. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proc Natl Acad Sci U S A. 2008;105(36):13568–73.
65. Wong TF, et al. Association of keap1 and nrf2 genetic mutations and polymorphisms with endometrioid endometrial adenocarcinoma survival. Int J Gynecol Cancer. 2011;21(8):1428–35.
66. Badeloe S, et al. Hereditary multiple cutaneous leiomyoma resulting from novel mutations in the fumarate hydratase gene. J Dermatol Sci. 2008;51(2):139–43.
67. Badeloe S, et al. Diffuse and segmental variants of cutaneous leiomyomatosis: novel mutations in the fumarate hydratase gene and review of the literature. Exp Dermatol. 2006;15(9):735–41.
68. Bayley JP, Launonen V, Tomlinson IP. The FH mutation database: an online database of fumarate hydratase mutations involved in the MCUL (HLRCC) tumor syndrome and congenital fumarase deficiency. BMC Med Genet. 2008;9:20.
69. Carvajal-Carmona LG, et al. Adult leydig cell tumors of the testis caused by germline fumarate hydratase mutations. J Clin Endocrinol Metab. 2006;91(8):3071–5.
70. Guo D, et al. A possible gene silencing mechanism: hypermethylation of the Keap1 promoter abrogates binding of the transcription factor Sp1 in lung cancer cells. Biochem Biophys Res Commun. 2012;428(1):80–5.
71. Bollong MJ, et al. A small molecule inhibits deregulated NRF2 transcriptional activity in cancer. ACS Chem Biol. 2015;10(10):2193–8.
72. Lee S, et al. An effective strategy for increasing the radiosensitivity of human lung Cancer cells by blocking Nrf2-dependent antioxidant responses. Free Radic Biol Med. 2012;53(4):807–16.

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