REVIEW

Nuclear Factor—Erythroid-2—Related Factor 2 in Aging and Lung Fibrosis

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Age-related diseases are increasing at a rapid rate worldwide in part related to increasing aging populations in developed countries. Fibrotic diseases, including those of the lung, are associated with aging. The mechanisms that predispose the aging lung to fibrosis are not known. Recent evidence suggests that the activation of nuclear factor-erythroid-2—related factor 2 (encoded by NFE2L2 gene; Nrf2) may be dysregulated during the aging process and that this dysregulation may contribute to the development and progression of fibrosis. Nrf2 is a basic leucine zipper stress-responsive transcription factor that maintains cellular reduction-oxidation (redox) homeostasis. It regulates the transcription of >200 cytoprotective genes through antioxidant/electrophile response elements (ARE) in target gene promoters. The Nrf2 response pathway is an evolutionarily conserved adaptive mechanism to limit oxidative stress and maintain cellular/tissue homeostasis. The pathway may confer unique protection to the lungs because of the exposure of this organ to a variety of pro-oxidant environmental insults, including airborne toxins and infectious agents. In this article, we review the evidence for impaired Nrf2 activation in aging and the mechanisms for this deficiency. Improved understanding of altered Nrf2 activation in aging will pave the way for the design of novel therapeutic approaches for age-related fibrotic diseases.

Nrf2-Keap 1 System

Nrf2 belongs to a cap’n’collar family of proteins with conserved basic leucine zipper structure and seven functional Nrf2-ECH homology (Neh) domains, Neh1–7. The Neh2 regulatory domain contains ETGE and DLG binding motifs separated by seven lysine residues. The ETGE and DLG binding motifs in Neh2 interact with Kelch-like ECH-associated protein 1 (Keap1), whereas the lysine residues are ubiquitinated leading to proteasomal degradation to regulate levels of Nrf2 at the post-translational level. The Neh4, Neh5, and Neh3 domains interact with coactivators for transactivation of Nrf2 target genes. The Neh1 domain facilitates dimerization with other transcription factors and DNA binding. The serine-rich Neh6 domain regulates the stability of Nrf2. A recent report reveals that retinoic X receptor α binds to the Neh7 domain and down-regulates Nrf2, suggesting a mechanism for Nrf2 repression independent of Neh2-Keap.

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Nrf2 is ubiquitously expressed in most eukaryotic cells. Its activation, under homeostatic conditions, is tightly restrained by the cytosolic repressor Keap. Keap1 consists of five domains (Figure 1): the N-terminal broad complex, tramtrack and bric-à-brac domain, intervening region, a double glycine repeat, and a C-terminal region. The bric-à-brac domain contains the cysteine residue (Cys-151), and the intervening region contains an additional two reactive cysteine residues (Cys-273 and Cys-278) that are involved in stress sensing. Apart from these two domains, a double glycine repeat and C-terminal region together form a β-propeller structure where Keap1 interacts with Nrf2.

Various models have been proposed to explain the activation and stabilization of Nrf2. Of these, oxidation of Keap1 leading to the release of Nrf2 is the most widely studied mechanism (Figure 2). Under basal physiological conditions, Keap1 binds to Nrf2 in the cytoplasm and promotes ubiquitination by the Cullin E3 ligase, leading to subsequent degradation by the 26S proteasome. In response to electrophilic or oxidative stress, oxidation of key cysteine residues (Cys-151, Cys-273, and Cys-278) in Keap1 alters its conformation and, in turn, facilitates ubiquitination by Cullin E3 ligase and subsequent proteosomal degradation by the 26S proteasome. In aging or cellular senescence, alterations in post-translational modifications (PTMs) involving oxidation of sulfhydryl groups (SH) in cysteine residues in Keap1 and phosphorylation (P) and/or acetylation (Ac) of Nrf2 modulates release/activation of Nrf2. In addition, impaired autophagy (e.g., p62 aggregation), p21 and cytoskeletal alterations may impair cytoplasmic-nuclear trafficking. In the nucleus, dysregulation in binding of cofactors (Maf and BTB domain and CNC homolog 1), epigenetics, sumoylation, miRNAs, and BMAL/CLOCK may contribute to age-related impairment in Nrf2 activation. BMAL/CLOCK, brain and muscle arnt-like protein-1/circadian locomotor output cycles kaput; Maf, musculoaponeurotic fibrosarcoma.
thereby, destabilizes the Keap1-Nrf2 complex, causing it to dissociate. Release of Nrf2 is followed by protein kinase mediated phosphorylation at Ser-40 and translocation to the nucleus. Once in the nucleus, Nrf2 binds to ARE/electrophile response elements in the promoter regions of target cytoprotective genes and regulates their transcription.

Nrf2 in Cellular Redox Homeostasis

The protective effect of Nrf2 is largely attributed to its ability to transactivate genes that are responsible for antioxidative defense, anti-inflammatory actions, repair functions, and metabolic regulation (Tables 1 and 2). Stress-dependent induction of Nrf2 signaling directly regulates the transcription of several antioxidant enzymes, including glutamate-cysteine ligase (GCL; both GCLC and GLCM subunits),

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
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<tbody>
<tr>
<td>GSTA1</td>
<td>Glutathione S-transferase A1</td>
<td>Detoxifies and metabolizes electrophilic compounds; metabolizes bilirubin and certain anticancer drugs; also displays glutathione peroxidase activity.</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Glutathione S-transferase M1</td>
<td>Detoxifies and metabolizes electrophilic compounds.</td>
</tr>
<tr>
<td>NQO1</td>
<td>NAD(P)H quinone oxidoreductase 1</td>
<td>Reduces quinones to hydroquinones and prevents one-electron reduction of quinones that would generate free radicals.</td>
</tr>
<tr>
<td>HMOX1</td>
<td>Heme oxygenase 1</td>
<td>Catalyzes the hydrolysis of palmitoyl-CoA and other long-chain fatty acids to form free fatty acid and CoA.</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
<td>Transcription factor that orchestrates lipid metabolism; key regulator of adipocyte differentiation and glucose homeostasis.</td>
</tr>
<tr>
<td>ACOT7</td>
<td>Acyl-CoA thioesterase 7</td>
<td></td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>Generates NADPH in the pentose phosphate pathway; maintains cellular glutathione reduction-oxidation status.</td>
</tr>
<tr>
<td>IDH1</td>
<td>Isocitrate dehydrogenase 1</td>
<td>Catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, using NADP⁺ as a cofactor.</td>
</tr>
<tr>
<td>p62/SQSTM1</td>
<td>Sequestosome 1</td>
<td>Required for formation and autophagic degradation of polyubiquitin-containing proteins; used as a scaffold protein.</td>
</tr>
<tr>
<td>TKT</td>
<td>Transketolase</td>
<td>Channels excess sugars from the pentose phosphate pathway to glycolysis by forming glyceraldehyde 3-phosphate.</td>
</tr>
<tr>
<td>GCLC</td>
<td>Glutamate-cysteine ligase catalytic subunit</td>
<td>Catalytic subunit of the enzyme responsible for the rate-limiting step in synthesis of cellular glutathione.</td>
</tr>
<tr>
<td>GLCM</td>
<td>Glutamate-cysteine ligase modifier subunit</td>
<td>Modifuer subunit of the enzyme responsible for the rate-limiting step in synthesis of the cellular glutathione.</td>
</tr>
<tr>
<td>GPX</td>
<td>Glutathione peroxidase</td>
<td>Detoxification of hydrogen peroxide and an important cellular antioxidant.</td>
</tr>
<tr>
<td>PRDX1</td>
<td>Peroxiredoxin 1</td>
<td>Reduces peroxides, regulates cellular concentrations of hydrogen peroxide.</td>
</tr>
<tr>
<td>TRX1</td>
<td>Thioredoxin 1</td>
<td>Reversible oxidation of active center allows participation in dithiol-disulfide exchange reactions; reduces sulfenic acid in proteins.</td>
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<tr>
<th>Table 1</th>
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Nrf2, nuclear factor-erythroid-2—related factor 2.
serves as source of reducing equivalents to maintain cellular redox balance. Nrf2-induced GSH biosynthesis has been implicated in development of cellular resistance to drug-induced apoptosis; in contrast, depletion of GSH by cytotoxic electrophiles increases oxidative stress. Circadian regulation of Nrf2 is controlled by BMAL/CLOCK transcription factors and mediates rhythmic control of GSH levels as well as diurnal variation in drug-induced toxicity. It has been reported that age-dependent alterations in the reduced versus oxidized ratio of glutathione (GSH/GSSG) significantly affects the redox state of the tissues. These studies, although not definitive, suggest that decreases in total GSH and/or reduced/oxidized ratio of GSH may be mediated, in large part, by the impaired activation of Nrf2 in aging.

Heme oxygenase 1 (HO-1; encoded by HMOX1 gene) catalyzes the breakdown of heme to carbon monoxide, free iron, and biliverdin. Biliverdin in the presence of bilirubin, which possesses antioxidative properties. In addition, innate immunity and wound healing processes may be modulated by the up-regulation of HO-1 by Nrf2. NQO1 catalyzes the two-electron reduction of highly reactive quinones to hydroquinones. In response to genotoxic stress, Nrf2-mediated up-regulation of NQO1 reduces the formation of free radicals by redox cycling of quinones and may mediate tumor suppressor activities, in part by stabilizing p53. Activated Nrf2 modulates the transcription of other antioxidant genes such as superoxide dismutase, catalase, thioredoxin reductase, and peroxiredoxin. Recently, it has been recognized that Nrf2 regulates inflammation and promotes resolution via cross talk with NF-κB. In endothelial cells, modulation of HO-1 activity by Nrf2 inhibited NF-κB-mediated transcription of vascular cell adhesion molecule-1 and E-selectin. Induction of Nrf2 signaling in mouse peritoneal macrophages reduces lipopolysaccharide-mediated induction of cyclooxygenase 2, tumor necrosis factor-α, and IL-1β expression. In response to acute lung injury, deletion of Nrf2 was associated with impaired resolution of inflammation.

In addition to its antioxidant defense anti-inflammatory actions, Nrf2 contributes to cytoprotection by repair and removal of damaged macromolecules. Activation of Nrf2 mediates the clearance of inclusion bodies formed by damaged and misfolded proteins through proteosomal degradation. As an adaptation to hydrogen peroxide and menadione-induced oxidative stress, up-regulation of Nrf2 induces the expression of 26S proteosome and Ps28αβ regulator. Nrf2-induced proteosomal activity and expression of 20S and 19S proteosome subunits have been demonstrated in murine liver. Induction of the DNA glycosylase, 8-oxoguanine glycosylase, expression via Nrf2 signaling has been shown to inhibit reactive oxygen species (ROS)-mediated DNA damage in breast cancer.

Nrf2 signaling regulates several metabolic pathways and mitochondrial function. Activation of Nrf2 induces the expression of various genes and enzymes catalyzing the rate-limiting steps in carbohydrate, lipid, and protein biosynthesis. Constitutive activation of Nrf2 in mouse embryonic fibroblast enhances the efficiency of oxidative phosphorylation, ATP levels, and mitochondrial membrane potential. Deficient expression of Nrf2 has been reported to alter respiration and ATP production through decreased fatty acid oxidation in mitochondria of liver and heart.

Although Nrf2-mediated protection against inflammation and/or oxidative stress by up-regulation of cytoprotective genes is well documented, the precise role of Nrf2 in the context of aging and age-related diseases remains elusive. Thus, understanding the molecular mechanisms of Nrf2 signaling in the context of aging is crucial in the development of Nrf2-based therapeutics.

Nrf2 in Aging

Increased ROS/reactive nitrogen species and the resultant oxidative stress-induced macromolecular damage is a hallmark of aging and age-related diseases. Although ROS/reactive nitrogen species mediate redox signaling in normal physiology, sustained and chronic oxidative stress likely contributes to the acceleration of aging and/or age-related diseases. Recent reports in mouse transgenic models suggest that oxidative stress may contribute to age-related pathology, while not altering longevity. Thus, a better understanding of the redox imbalances in specific age-related diseases will aid in the development of more targeted therapies for these diseases.

A few studies report functional Nrf2 signaling in promoting healthy aging with improved life span. The activation of Nrf2 signaling by a Keap1 loss-of-function mutation augments tolerance to oxidative stress and confers increased lifespan in Drosophila. Nrf2-dependent signaling has been reported to restore the loss of proteostasis and prevent premature aging in Drosophila. Furthermore, Nrf2 signaling has been documented to confer resistance to oxidative stress and promote healthy aging in Caenorhabditis elegans.

Reduced activation of Nrf2 in aging has been reported to be associated with various chronic diseases, such as progressive respiratory disease, neurodegeneration, and inflammatory disorders. Age-dependent decline in the levels and/or activation of Nrf2 is accompanied by reduced expression of cytoprotective genes and increased susceptibility to oxidative injury. Age-associated decreases in total and nuclear Nrf2 protein levels have been shown to reduce the expression of antioxidant genes HMOXI, NQO1, and GCLC in the liver and heart of aging animals. We have demonstrated a marked reduction in the capacity for Nrf2 activation and ARE-regulated gene expression in cellular models of senescence, although senescence-associated mechanisms for the diminished activation of Nrf2 are unclear. Current working models of how aging and senescence may alter the stabilization and/or activation of Nrf2 suggest
that this impairment may occur at various levels, including Keap1-dependent and independent pathways (Figure 2).

**Keap1-Dependent Mechanisms**

Keap1 is a well-known cytoplasmic repressor of Nrf2 under basal physiological conditions. Oxidative stress or electrophile-mediated conformational changes in Keap1 because of oxidation of reactive thiols on cysteine residues (Cys-151, Cys-273, and C-278) weaken its interaction with Nrf2, thereby promoting subsequent translocation of Nrf2 into the nucleus. It is possible that other post-translational modifications might stabilize the interactions between Keap1 and Nrf2 during aging, thus decreasing redox sensing by Keap1. In addition to the known mechanism involving oxidation of cysteine residues, a noncanonical regulatory mechanism involving Keap1 also regulates Nrf2 activity. Scaffolding of Keap1 through its double glycine repeat domain to the actin cytoskeleton sequesters Nrf2 and promotes proteasomal degradation, whereas disruption of the actin cytoskeleton—Keap1 interaction releases Nrf2 and promotes its nuclear translocation. Alterations in actin cytoskeletal regulation with aging would be expected to contribute to dysregulated Nrf2 responses. Further studies of Nrf2 activation and cytoskeletal regulation in aging are warranted.

The cyclin-dependent kinase inhibitor, p21, has been shown to activate Nrf2. KRR motifs of p21 directly interact with the DLG and ETGF motifs of the Neh1 functional domain of Nrf2, thereby competing with Keap1 for Nrf2 binding and inhibiting Keap1-mediated ubiquitination of Nrf2. The reasons for a deficiency in Nrf2 activation with cellular senescence associated with high levels of p21 are currently unknown.

Another noncanonical, Keap1-dependent mechanism for Nrf2 activation may involve impaired autophagy, which has been associated with aging. Decreased autophagy resulting in accumulation of p62, an autophagy cargo protein, would be predicted to activate Nrf2 by binding/sequestering Keap1, thus limiting its inhibitory interaction with Nrf2. However, although age-dependent increases in p62 levels may occur, it has been reported to self-aggregate in aging, leading to decreased activity. This represents one potential mechanism for the paradoxical impairment of Nrf2 signaling with aging.

**Keap1-Independent Mechanisms**

Keap1-independent mechanisms of Nrf2 activation involve several reported post-translational modifications, including phosphorylation, acetylation, and interactions with cofactors, such as Maf, ATF4, and CREB. Kinase-mediated phosphorylation of Ser-40 residue on Nrf2 is reported as a requisite for the activation and nuclear translocation of Nrf2. Activation of protein kinase C (PKC) phosphorolates Ser-40 residue on the Neh2 domain of Nrf2, resulting in the release and nuclear translocation of Nrf2. Various isoforms of PKC and atypical PKC have been documented to be involved in phosphorylation of Nrf2. Phosphatidylinositol 3-kinase-mediated phosphorylation of Nrf2 and its dissociation, nuclear translocation, and subsequent induction of antioxidants have been shown in human neuroblastoma cells. In addition to the involvement of PKC and phosphatidylinositol 3-kinase in the activation of Nrf2, accumulating evidence suggests a role for mitogen-activated protein kinases; however, some reports suggest negative regulation of Nrf2 by these protein kinase pathways. For example, extracellular signal regulated kinase 1/2 signaling cascades have been shown to increase the activation/stabilization of Nrf2, leading to induction of cytoprotective genes. Activation of p38 mitogen-activated protein kinase and Janus kinase has also been implicated in the activation and nuclear translocation of Nrf2. In contrast, phosphorylation of tyrosine residues (Tyr-568) on Nrf2 by glycosyn synthetase kinase 3β activated Fyn has been shown to promote nuclear export and degradation of Nrf2.

Acetylation and deacetylation of Nrf2 promote nuclear-cytoplasmic shuttling and regulation of its transcriptional activity. Acetylation of lysine residues in Nrf2 enhances Nrf2-DNA binding and transcription of target genes. The acetylation of lysine residues in the Neh1 domain of Nrf2 by p300/CBP augments promoter-specific DNA binding in response to arsenite-mediated oxidative stress. Mutating Lys-588 and Lys-591 of Nrf2 impairs Nrf2-dependent gene transcription by distorting the transcription-activating effect of CREB-binding protein. Age-related changes in activities of protein kinases or acetyltransferases/deacetylases may lead to impaired Nrf2 regulation. Age-related changes in the expression and activity of PKC, and phosphatidylinositol 3-kinase, have been reported in both aging animal models and humans. Therefore, further studies on these altered phosphorylation and acetylation signaling mechanisms in the context of aging may provide additional insights into dysregulated Nrf2 responses in age-associated disorders.

Several Nrf2 cofactors and transcriptional regulators that control its activity both positively and negatively have been described. Bach1 is reported to be a negative regulator of Nrf2 signaling within the nuclear compartment. Transient or stable binding of Bach1 to ARE in specific promoters has been shown to repress tBHQ-induced Nrf2-ARE activation. An age-related increase in Bach1 binding to the ARE of the GCLC promoter competed with Nrf2-ARE binding in the liver of old mice. On the other hand, dimerization of transcriptional coactivator Mafs with Nrf2 facilitates stable Nrf2-ARE interaction and enhances the transcription of cytoprotective genes. Although no age-related changes in the basal expression of Mafs have been reported, reduced Nrf2 responses have been attributed to a failure in the activation of Mafs during aging in Drosophila; however, overexpression of Mafs is capable of restoring this aging-associated decline of Nrf2-responsive
The role of these E3 ubiquitin ligases in context of Nrf2 by aging is well appreciated. Decline in the function of the ubiquitin-proteosomal system with aging and reported to regulate Nrf2 gene expression. In addition, proteins have been reported, the mechanisms of how sumoylation, and miRNAs are also known to regulate Nrf2 signaling.92,95 Up-regulation of Hrd1 suppresses Nrf2 expression and Nrf2-mediated antioxidant defense in human cirrhotic hepatocytes isolated from lipopolysaccharide-treated mice showed decreased Nrf2 sumoylation, which reduced Nrf2-ARE signaling through altered Nrf2/MafG interaction.94 Deﬁning the precise role of AMPK in age-related regulation of Nrf2 signaling has the potential for clinical translation. Sumoylation has been reported to be involved in regulating Nrf2 signaling.91 Murine macrophages (RAW cells) and hepatocytes isolated from lipopolysaccharide-treated mice showed decreased Nrf2 sumoylation, which reduced Nrf2-ARE signaling through altered Nrf2/MafG interaction.92 Although age-related changes in the sumoylation of proteins have been reported, the mechanisms of how sumoylation of Nrf2 regulates its activity remain unclear.

Recent studies also suggest a role for epigenetics in the regulation of Nrf2. The increased expression of Keap1 protein because of demethylation of the Keap1 promoter region leads to decreased stability of Nrf2 and results in a failure of the antioxidant response in age-related cataracts.96 The miRNAs, miR-144, miR-27a, and miR-153, have been reported to regulate Nrf2 gene expression. In addition, miRNAs are also known to target other Nrf2 regulators and alter Nrf2 activation.98 Although age-related changes in the expression of miRNAs have been reported, investigating their role in regulating Nrf2 activation and downstream transcriptional responses in cellular/animal models of aging requires further investigation.

Additional epigenetic mechanisms involving DNA methylation and histone modifications may regulate Nrf2 activity. Nrf2 gene promoter methylation associated with methyl-CpG-binding protein 2 and histone modifications epigenetically down-regulated the expression of Nrf2 in murine tumor models.90 Genetic disruption of molecular clocks alters the rhythmic activation of Nrf2 and potentiates fibrosis in a lung injury model. Although age-related declines in circadian rhythms are known to occur, it is not known whether changes in the expression of clock genes and altered circadian rhythms lead to increased susceptibility to age-related lung fibrosis through impaired activation of Nrf2.

**Fibrosis as a Disease of Aging**

Fibrosis is a reparative response to injury culminating with the excessive deposition of extracellular matrix (ECM) proteins in tissues/organs. The process of fibrogenesis has been proposed to occur in four phases: primary injury driven response, activation of effector cells, and formation and remodeling of extracellular matrix components. Although fibrosis is a transient event in the normal wound healing process, persistent and/or progressive fibrosis with excess deposition of ECM leads eventually to organ failure and death.

Fibrotic disease has been estimated to cause 45% of total mortality in the United States. Although pathological fibrosis can occur regardless of age, aging is known as one of the major risk factors in its development. The mechanism of age-associated fibrotic response is uncertain and could be attributed to genomic instability, telomere shortening, epigenetic changes, reduced autophagy, and mitochondrial dysfunction. Oxidative stress is known to be associated with age-related diseases, such as idiopathic pulmonary fibrosis (IPF). IPF affects approximately five million people worldwide, with 200,000 in the United States. IPF is the most fatal of the interstitial lung diseases, with a median survival of <3 years.

The role of aging in IPF is incompletely understood. Exposure of lungs to various endogenous and exogenous oxidants may elicit inflammatory and fibrotic responses, leading to impaired antioxidant capacity. This may perpetuate the injury response and impede tissue regeneration with sustained fibrosis. This oxidant-antioxidant imbalance may be accentuated in aging.

After tissue injury, the up-regulation and activation of transforming growth factor-β1 mediates differentiation of myofibroblasts, the main cells involved in connective tissue remodeling. Persistent activation of myofibroblasts with excess secretion of ECM proteins and enhanced contractility is associated with progressive fibrotic disorders, including IPF. Research from our group has revealed that activation of hydrogen peroxide—generating flavoenzyme NADPH oxidase (NOX4) mediates myofibroblast differentiation in response...
to transforming growth factor-β. Since this report, several other studies have demonstrated a critical role of NOX4 in fibrosis involving the liver, kidney, and heart.

More recent studies demonstrate that the up-regulation of NOX4 is coupled to Nrf2 induction, and this adaptive response is critical to apoptosis susceptibility of myofibroblasts and resolution of fibrosis in young mice. In contrast, in aged mice, there is impaired Nrf2 activation leading to an altered NOX4-Nrf2 redox balance and acquisition of an apoptosis-resistant myofibroblast phenotype resulting in persistent fibrosis. This supports a mechanism for the antagonistic pleotropic action of NOX4 that is determined by the activation.

**Nrf2 in Age-Related Lung Fibrosis**

The reduced expression/activation of Nrf2 has been implicated in human and animal models of pulmonary fibrosis. Targeted deletion of Nrf2 in murine models of bleomycin-induced lung injury shows increased susceptibility to fibrosis with decreased ARE-mediated antioxidant gene expression. Bleomycin, cigarette smoke, and environmental oxidants augment oxidative injury in lungs deficient in Nrf2. Genetic ablation of Nrf2 decreases mRNA and protein expression of antioxidants, such as GST, NQO1, and HO-1, whereas the expression of genes encoding ECM and cytoskeletal proteins is induced in lungs of mice exposed to hyperoxia. Fibroblasts isolated from the lungs of injured aged mice and IPF reveal reduced expression of Nrf2 that promotes an apoptosis-resistant phenotype, and impaired capacity for myofibroblast dedifferentiation. Although the mechanism(s) for the reduced expression/activation of Nrf2 in aging is incompletely understood, alterations in redox sensing with aging may contribute to the aberrant cell phenotypes seen in progressive age-related fibrosis.

**Nrf2 as a Therapeutic Target**

The emergence of Nrf2 as a master regulator of multiple antioxidant and anti-inflammatory pathways in pathophysiological conditions has stimulated interest in Nrf2 as a therapeutic target. This is based on the concept that Nrf2 may mediate greater protection than an antioxidant strategy involving a single pathway (eg, augmenting glutathione levels with N-acetylcysteine supplementation, which has shown to be ineffective in IPF). Various dietary phytochemicals, such as polyphenols, isothiocyanate, flavonoids, terpenoids, and synthetic chemical inducers, are known to induce Nrf2. Activation of Nrf2 by dietary phytochemicals and chemical inducer has been reported to mediate protective effects in certain cellular and animal models of disease. SFN, an organosulfur compound derived from cruciferous vegetables, is well-recognized for its robust action on induction of Nrf2. Activation of Nrf2 by SFN in IPF lung fibroblasts inhibited transforming growth factor-β-induced profibrotic effects and induced myofibroblasts to dedifferentiate, an effect that may be important in fibrosis resolution. SN-mediated activation of Nrf2 is also reported to reduce fibrosis in bleomycin mice model of lung fibrosis.

Resveratrol, a naturally occurring phenolic phytochemical, modulates cellular homeostasis through the activation of the Nrf2 pathway. Activation of Nrf2 by resveratrol reduced paraquat-induced ROS-mediated inflammation and fibrotic reactions by up-regulation of cytoprotective genes in human bronchial epithelial cells. The mechanisms by which resveratrol activates Nrf2 are not well defined, but are unlikely to involve activation of sirtuins. Curcumin, a polyphenol obtained from rizome of *Curcuma Longa*, has been shown to be an effective Nrf2 activator and inducer of Nrf2-dependent antioxidant and anti-inflammatory genes.

In human monocytes, curcumin mediates the up-regulation of antioxidant protective genes through Nrf2-ARE signaling via PKC and p38 mitogen-activated protein kinase. Curcumin ameliorated radiation-induced pulmonary fibrosis and enhanced the survival of mice in association with up-regulation of antioxidant defenses in lungs. Quercetin, a dietary flavonoid found in citrus fruits, is another potential activator of Nrf2. Using ARE-luciferase reporter gene assays, quercetin has been shown to activate Nrf2 and up-regulate NQO1. Quercetin-mediated Nrf2 nuclear translocation through p38 and extracellular signal regulated kinase signaling and induction of cytoprotective genes protects human hepatocytes against ethanol-derived oxidative stress. Exposure of NIH3T3 cells and human lung fibroblasts to quercetin induces the nuclear translocation of Nrf2, induces HO-1, and suppresses transforming growth factor-β–simulated collagen synthesis.

In addition to dietary compounds that are known to activate Nrf2, several chemical activators are being developed or are currently in clinical practice. An oral formulation of the chemical inducer dimethyl fumarate (commonly known as BG-132), US Food and Drug Administration approved for the treatment of multiple sclerosis, exerts anti-inflammatory effects through the activation of the Nrf2 pathway. Dibenzoylmethane, an analog of curcumin, has been shown to activate the Nrf2-mediated detoxification pathway and inhibits benzo(a)pyrene-induced DNA adduct formation in lungs. Induction of Nrf2 with the triterpenoid CDDO-imidazolide (bardoxolone) and its analogs attenuates cigarette smoke–induced emphysema and cardiac dysfunction in mice, and diabetic nephropathy in preclinical models. However, in a recent phase II clinical trial of bardoxolone in chronic kidney disease, the study was terminated because of increased rate of heart-related adverse events, including heart failure, hospitalizations, and deaths. Whether this is because of off-target effects of this drug or because of unexpected potential detrimental effects of Nrf2 activation is not known. In recent years, efforts by pharmaceutical companies have focused on the development of novel Nrf2 activators to treat various chronic diseases, including lung fibrosis. It is anticipated...
that Nrf2 activators with greater specificity and efficacy will undergo clinical trials in the near future.

A potential dark side of Nrf2 has been highlighted in the progression of carcinogenesis. Although Nrf2 activation may be protective against cancer development in early stages, sustained or hyperactivation of Nrf2 with increased expression of cytoprotective genes enhanced cancer cell survival and conferred resistance to chemotherapy. Targeted deletion of Nrf2 reduced urethane-induced tumorogenesis in lungs of mice by minimizing the expression of ARE-responsive genes. In addition, the tumor stroma has been reported to express high levels of Nrf2 and Nrf2 inhibitors proposed as an anticancer strategy. Hence, Nrf2 may represent double-edged sword, and this must be considered when administering Nrf2 activators as interventional drugs. Whether sustained or hyperactivation of Nrf2 in the context of fibrosis may predispose to cancer is unknown.

Conclusions

Accumulating evidence supports a role for deficient Nrf2 responses in several chronic age-related diseases, in particular fibrotic lung diseases. Although augmentation of Nrf2 may prove to be beneficial in lung fibrosis, it is important to recognize that such a strategy may mediate untoward effects because of the pleiotropic effects of Nrf2 and redox state on cellular signaling. Thus, it is important to define the context in which Nrf2 function is deficient or dysregulated before treatment of a particular disease can be targeted with Nrf2 activators. In addition, a more in-depth understanding of the mechanisms that lead to altered Nrf2 responses in aging and age-related fibrotic diseases is required; this will lead to more precise targeting approaches that are also likely to be safer and better tolerated.

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