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Radiat Res. Author manuscript; available in PMC 2018 August 15.

Published in final edited form as:

Radiat Res. 2018 August ; 190(2): 99–106. doi:10.1667/RR15059.1.

The Role of Nrf2 in the Response to Normal Tissue Radiation Injury

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Abstract

The transcription factor Nrf2 is an important modulator of antioxidant and drug metabolism, carbohydrate and lipid metabolism, as well as heme and iron metabolism. Regulation of Nrf2 expression occurs transcriptionally and post-transcriptionally. Post-transcriptional regulation entails ubiquitination followed by proteasome-dependent degradation. Additionally, Nrf2-mediated gene expression is subject to negative regulation by ATF3, Bach1 and cMyc. Nrf2-mediated gene expression is an important regulator of a cell's response to radiation. Although a majority of studies have shown that Nrf2 deficient cells are radiosensitized and Nrf2 over expression confers radioresistance, Nrf2's role in mediating the radiation response of crypt cells is controversial. The Nrf2 activator CDDO attenuates radiation-mediated crypt injury, whereas intestinal crypts in Nrf2 null mice are radiation resistant. Further investigation is needed in order to define the relationship between Nrf2 and radiation sensitivity in Lgr5+ and Bmi1+ cells that regulate regeneration of crypt stem cells. In hematopoietic compartments Nrf2 promotes the survival of irradiated osteoblasts that support long-term hematopoietic stem cell (LT-HSC) niches. Loss of Nrf2 in LT-HSCs increases stem cell intrinsic radiosensitivity, with the consequence of lowering the $LD50_{30}$. An Nrf2 deficiency drives LT-HSCs from a quiescent to a proliferative state. This results in hematopoietic exhaustion and reduced engraftment after myoablative irradiation. The question of whether induction of Nrf2 in LT-HSC enhances hematopoietic reconstitution after bone marrow transplantation is not yet resolved. Irradiation of the lung induces pulmonary pneumonitis and fibrosis. Loss of Nrf2 promotes TGF-β/Smad signaling that induces ATF3 suppression of Nrf2-mediated target gene expression. This, in turn, results in elevated reactive oxygen species (ROS) and isolevuglandin adduction of protein that impairs collagen degradation, and may contribute to radiation-induced chronic cell injury. Loss of Nrf2 impairs ΔNp63 stem/progenitor cell mobilization after irradiation, while promoting alveolar type 2 cell epithelial-mesenchymal transitions into myofibroblasts. These studies identify Nrf2 as an important factor in the radiation response of normal tissue.

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INTRODUCTION

Nuclear factor, erythroid 2-like 2 (Nrf2), encoded by the gene *NFE2L2* (HGNC:7782), is a member of the cap'n'-collar (CnC) family of basic leucine zipper transcription factors (1) and is conserved in mammals (2), birds (3), fish (4), insects (5) and worms (6). The CnC family is composed of SKN-1, Nrf1, Nrf2, Nrf2, Nrf3, CncC, Bach1 and Bach2 (2). Nrf2 was originally identified as a key regulator of canonical antioxidant and drug-metabolizing gene expression (7, 8). Nrf2 heterodimerization with MAF-G (9) or the JUN CnC-bZIP factor (10) permits binding to *cis* antioxidant response elements (AREs) located in proximal promoters of Nrf2 target genes that now number over 400 (9), including those involved in carbohydrate and lipid metabolism, NADPH regeneration, heme and iron metabolism, as well as proteasome metabolism (8, 11). The functional ARE has been defined as TMANNRTGACT-CAGCRWWWW, where M = A or C, R = A or G and W = A or T (9). It is important to recognize that Nrf2 not only induces gene expression but also functions as a suppressor of gene expression (e.g., pro-inflammatory gene expression) (12).

Nrf2 is transcriptionally, post-transcriptionally [reviewed in ref. (13)] and post-translationally regulated (14). Under nonstress conditions, Nrf2 undergoes rapid ubiquitination and proteasome degradation (14). Several Cullin- and Cull-in4/DDB1/WDR23 (18). Post-translational regulation by the Cullin3/Keap1/Rbx1 E-3 ligase complex is the most studied. Oxidative and electrophilic stresses that modify critical cysteine residues cause a conformational change in Keap1 that abrogates Keap1's ability to direct Nrf2's ubiquitination [reviewed in refs. (12,19, 20)]. In addition to stress-related activation, a number of proteins [e.g., p21 (21), p62, PALB2, IKKB, DPP3 (22) and iASPP (23)] can competitively bind to Keap1 and inhibit Nrf2 ubiquitination. Inactivation of Keap1 allows *de novo* synthesized Nrf2 to translocate to the nucleus and either transduce or suppress target genes [Supplementary Fig. S1 (http://dx.doi.org/10.1667/RR15059.1.S1) and (12)].

Adaptive homeostasis is defined as ''the transient expansion or contraction of the homeostatic range for any given physiological parameter in response to internal and external stimuli'' (24). Adaptive homeostasis required of Nrf2 can be impaired as a result of circadian rhythm, aging or expression of specific hormones and cytokines. Pekovic-Vaughan *et al. (25)* found that Nrf2 was regulated by circadian rhythm and that stress-related tissue injury was enhanced during the circadian nadir of Nrf2 expression. Suh et *al. (26)* showed that expression of Nrf2 declines with age resulting in impairment of glutamate-cysteine ligase, catalytic subunit (GCLC) and glutamate-cysteine ligase, modifier subunit (GCLM) expression and age-related loss of glutathione synthesis. This initial observation has been validated and expanded in other investigations that have demonstrated an age-related loss of Nrf2 and Nrf2 target gene expression in mice, rats and humans, a consequence, in part, of negative regulation by Bach1 or cMyc (27–29). Angiotensin II, a hormone that is elevated in a number of diseases, is a potent inhibitor of Nrf2 expression, a consequence of angiotensin II-mediated ATF3/Nrf2 heterodimerization (30). TGF-β1 is another cytokine that is elevated in disease (31), blunt injury (32) and radiation injury (33, 34). TGF-β/Smad signaling induces ATF3, which in turn heterodimerizes with Nrf2 at proximal *cis* antioxidant response elements and displaces CBP from the ARE (35). The result is suppression of Nrf2 target

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gene expression (36). Thus, the ability of Nrf2 to respond to stress can be compromised by a number of factors.

Nrf2 AND RADIATION-INDUCED INTESTINAL INJURY

Stem Cell Niche and the Radiation Response

Epithelial cells of the intestine undergo continuous turnover every four to five days. Renewal requires intestinal base columnar stem cells (CBC) located within the crypt basement membrane to cycle daily with an outcome of generating transit amplifying (TA) cells that migrate up along the villus and differentiate into enterocytes, goblet and enteroendocrine cells (37) (Supplementary Fig. S2; http://dx.doi.org/10.1667/RR15059.1.S1). TA progenitor cells can also generate Paneth cells that migrate down into the base and may provide stem cell niche signals to self-renewing CBC cells (37). In the absence of Paneth cells, mesenchymal cells may provide compensatory niche signaling (37). Tian et *al. (38)* identified two stem cell pools in the small intestine. Proliferating CBC cells that express Lgr5, Ascl2 and Olfm4 provide constitutive intestinal renewal. The second pool consists of quiescent Bmi1+ cells located at positions +3 to +6. Diphtheria toxin-specific ablation of Lgr5+ CBCs revealed that Bmi1-expressing cells function as a reserve stem cell pool for regeneration of the crypt (38).

CBC cells undergo radiation-induced p53-dependent apoptosis in a dose-dependent manner. The intestinal villus-crypt can tolerate doses of up to 10 to 12 Gy, although radiation-induced apoptosis can be observed at doses as low as ~0.02 Gy (39). For doses less than 10–12 Gy there is a dose-dependent/strain-dependent reduction of villus-crypt size that is subsequently followed by proliferation of remaining stem cells that regenerate the crypt (40). The identity of the cells that regenerate crypts after irradiation remains a critical question.

Barker *et al.* (41) found that crypt cells located at position +4 are significantly more radiosensitive than CBC et *al. (42)* examined the proliferation status, location and radiation response of Bmi1-expressing cells using Bmi1-CreER;Rosa26-YFP mice exposed to tamoxifen (42). Nonirradiated YFP-labeled cells were quiescent and located at position +4 (ranging from +1 to +6) (42). A 12 Gy whole-body dose induced a proliferative response in the YFP-labeled cells that subsequently resulted in expansion of the Bmi1 + lineage to repopulate crypts and villi. This work has been expanded on by Kuruvilla et *al.* (43), who found that Bim1-mediated stem cell expansion after irradiation requires KLF4 (43).

The response of Lrg5+ CBC cells to radiation was investigated using Lgr5-eGFP-IRES-CreERT2 mice (42). These mice harbor a knock-in allele within the first ATG codon of Lgr5 that is composed of an eGFP-IRES-CreERT2 cassette. The knocked-in cassette results in loss of Lgr5 gene function and expression of eGFP (42). In contrast to the radiation response of the +4 Bmi1 CreER;Rosa26 YFP cells, CBC Lgr5-eGFP-IRES-CreERT2 cell numbers were significantly reduced 48 h after 12 Gy irradiation, although sporadic regeneration was observed 7 days postirradiation. Yan *et al.* (44) then expanded on these studies using RNA-seq to characterize stem cell inter-relatedness. They found that the CBC signature genes (e.g., Lgr5, Ascl2 and Olfm4) were also expressed at low levels in Bmi1-GFP+ cells while

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Lgr5+ cells express 4+ markers Bmi1, mTert and Hopx, but at low levels. These results suggest that crypt position and individual cell markers may be insufficient for cell identification.

Metcalfe *et al.* (40) approached the question of stem cell regeneration after irradiation using $Lgr5^{DTR}$ mice in which the diphtheria toxin receptor is knocked into the endogenous Lgr5 locus. Administration of diphtheria toxin results in Lrg5+-specific cell death (40). A regenerating phenotype was produced after exposure to 10 Gy of radiation. In contrast, diphtheria toxin-mediated ablation combined with 10 Gy irradiation of Lgr5+ cells resulted in attenuation of the regenerative response (40). Metcalfe *et al.* (40) reconciles his results with that of Yan *et al.* (42) by citing the work of Buczackie *et al.* (45), who identified a distinct, nondividing radiation-resistant population of Lgr5+ cells that are also positive for Bmi1+/4+ markers. These cells reside in positions 1–6 and have the potential to regenerate crypts after exposure to 6 Gy or less (40).

The Role of Nrf2

Yang *et al.* (46) addressed the question of whether Nrf2 protected intestinal stem cells from radiation injury. Their approach was to administer 13 Gy to the abdomen of C57BL/6J mice rather than to the whole body. This targeting spares the majority of the bone marrow. All (100%) Nrf2-null mice survived for 30 days postirradiation, whereas only 80% of the wild-type survived. Abdominal irradiation resulted in apoptosis and a significant loss of crypt-villus anatomy in wild-type mice. Abdominal irradiation-induced death day 14 and is a consequence of loss of intestinal stem cells. Strikingly, the crypt-villus structure was spared in the Nrf2-null irradiated mouse. At the stem cell level, the number of Lgr5+ cells was greater in the Nrf2-null crypt compared to crypts in wild-type mice. These investigators found that loss of Nrf2 resulted in greater radiation-induced activation of NF**k**B compared to wildtype intestine. A control cohort of C57BL/6J mice received 7.5 Gy whole-body irradiation. The $LD50_{30}$ for a C57BL/6 mouse is in the range of 8 to 9 Gy (47) and lethality is a consequence of bone marrow stem cell ablation. Of the wild-type mice that received 7.5 Gy whole-body irradiation, 43% survived to day 30. In contrast, less than 10% of Nrf2-null mice survived a 7.5 Gy whole-body dose. This result is consistent with the results reported by McDonald *et al.* (47). The observation that the Nrf2-null mouse was to resistant to radiation after abdominal exposure is unexpected but intriguing and bears further investigation.

Triterpenoids are electrophiles (48) that undergo Michael reaction and inactivate Keap1 (19), thus allowing Nrf2 to avoid ubiquitination and proteasome degradation, and thereby producing elevated Nrf2 signaling. CDDO (1-(2-cyano-3,12-dioxooleana-1,9-dien-28-oyl)) is a triterpenoid that results in robust Nrf2 activation. Kim *et al.* (49) fed mice a diet supplemented with 400 mg/kg CDDO for 3 days prior to whole-body irradiation. CDDO induced Nrf2 and the Nrf2 DNA repair target gene 53BP1. CDDO reduced radiation-induced apoptosis, and attenuated radiation-induced loss of crypt length and cell density in the colon and small intestine. Mechanistically the results appeared to be due to enhanced signaling of the DNA damage response pathway (49).

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Nrf2 AND RADIATION-INDUCED HEMATOPOIETIC INJURY

Stem Cell Niche and the Radiation Response

Long-term hematopoietic stem cells [LT-HSCs: Lin^-, $Sca1^+$, c-Kit^+, $CD150^+$, $CD48^-$, $CD41^-$ (50)] in bone marrow can be found adjacent to sinusoid vessel niches (50) that are supported by endothelial and nestin-expressing mesenchymal cells that produce SCF and CXCL12, as well as other factors (50, 51). Approximately 20% of LT-HSCs are also found adjacent to osteoblasts in bone endosteum niches (50,52). Endosteal trabecular niches gain importance after ablative doses of ionizing radiation. Forty-eight hours after exposing mice to 1.125 Gy irradiation, Dominici *et al.* (53) observed proliferation and expansion of osteoblasts located proximal to the endosteal surface of trabecular bone while noting extensive loss of endothelial niches. These investigators then identified osteoblast niches as the sites of engraftment after transplantation of GFP-expressing donor HSCs into the lethally irradiated mice (53). The Sp7/Osx transcription factor is a Runx2 target gene required for osteoblast differentiation (54). Recently, Himburg *et al.* (51) demonstrated that Sp7-expressing cells secreting dickkopf-1 (Dkk1) promoted hematopoietic stem cell regeneration after ablative irradiation.

The Role of Nrf2

Rana *et al.* (55) found that Nrf2 was critical to osteoblast survival after ablative irradiation. Significantly fewer osteoblasts survived irradiation in Nrf2-null compared to wild-type mice, suggesting that the niche's ability to recover from radiation injury is dependent, in part, on Nrf2. Within the niche, LT-HSCs and hematopoietic stem progenitor cells (HSPCs) exhibit a hypersensitivity to low-dose radiation (0.02 Gy), a consequence of elevated reactive oxygen species (ROS) that induces autophagy and mitophagy (56). Radiation-induced ROS stabilizes Nrf2, resulting in nuclear translocation and induction of Nrf2-mediated antioxidant gene expression. Failure to induce Nrf2 in $Nrf2^{-/-}$ mice increased HSC radiohypersensitivity, whereas inducing Nrf2 prior to irradiation in Mx-Cre-$Keap1^{flox/flox}$ mice treated with poly (I:C) abolished hypersensitivity (56). Additionally, $Nrf2^{-/-}$ LSK were sorted, irradiated at 0.02 Gy and engrafted in recipient mice. Analysis performed 6 months after engraftment showed a dramatic decrease in LT-HSC (56). Nrf2 expression also influences the effectiveness of myoablative doses of radiation: loss of Nrf2 lowers the $LD50_{30}$ (47, 57). Conversely, pharmacological induction of Nrf2 increases the $LD50_{30}$ (49, 57).

Nrf2 Modulates Hematopoiesis

Short-term HSCs (ST-HSCs; Lin^-, $Sca\text{-}1^+$, c-Kit^+, $CD34^+$ $Flt3^-$), multipotent progenitor cells (MPP; Lin^-, $Sca\text{-}1^+$, c-Kit^+, $CD34^+$ $Flt3^+$), committed myeloid and lymphoid lineages, but not LT HSCs are increased in Nrf2-null mice compared to wild-type mice (58). While LT-HSCs cell numbers were independent of Nrf2, fewer of these cells were quiescent compared to wild-type controls, as measured by expression of proliferation and differentiation markers, a consequence of elevated cyclin D1 activity and loss of CXCR4-mediated signaling (58). These defects in Nrf2-deficient HSCs resulted in accelerated hematopoietic exhaustion, as assessed in serial competitive transplantation experiments

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using lethally irradiated mice (58, 59). Thus, an Nrf2 deficiency has the potential to reduce engraftment after myoablative irradiation.

Kim *et al.* (57) used Mx-Cre;Keap1$^{flox/flox}$ mice to study the effects of Nrf2 overexpression on HSC function. In LSK cells (Lin^{-} Sca1^{+} c-Kit^{+}; enriched for LT and ST HSCs, as well as MPP cells) loss of Keapl resulted in elevated Nrf2 target gene expression (57). Under steady-state conditions there was an increase in LSK cell numbers in Mx-Cre;Keap1$^{flox/flox}$ mice compared to control. Use of colonyforming unit assays revealed an increase in committed progenitors (GM-CFU and G-CFU). Competitive transplantation of LSK cells into lethally irradiated mice revealed increased engraftment of Mx-Cre;Keap$^{flox/flox}$ poly (I:C) treated donor cells compared to donor cells from Keapl$^{flox/flox}$ mice (57). These results were interpreted to indicate that prolonged Nrf2 signaling enhances hematopoietic reconstitution after myelosuppressive irradiation due to activation of Notch signaling. Use of tamoxifen-treated CMV-Cre:Keap1$^{flox/flox}$ mice and pharmacological activation of Nrf2 confirmed the results obtained with of Mx-Cre;Keap$^{flox/flox}$ poly (I:C) treated mice (57).

Vav1-Cre:Keap1$^{flox/flox}$ mice were used to test the hypothesis that Nrf2 promotes HSC proliferation (60). Disruption of Keap1 exons 4 through 6 in LSK cells and LT HSCs elevated expression of Nrf2 and Nrf2 target genes. Similar to LSK cells in Mx-Cre;Keap1$^{flox/flox}$ mice, there was a 1.4-fold enrichment of LSK cells in Vav1-Cre:Keap1$^{flox/flox}$ mice (60). LT HSC numbers in Vav1-Cre:Keap1$^{flox/flox}$ did not increase; rather, the cells moved from a G_0 quiescent state into G1. This exit from quiescence was not observed in Vav1-Cre:Keap1$^{flox/flox}$::Nrf2$^{-/-}$ mice, demonstrating that the loss of quiescence was Nrf2 driven (60). Mechanistically, Nrf2-mediated loss of quiescence was a consequence of JAK-STAT signaling. These results suggested that the inability to increase cell number coupled with loss of quiescence would impair self-renewal, thereby limiting the ability of LT HSCs to reconstitute lethally irradiated bone marrow. This hypothesis was tested using a competitive transplantation assay. Indeed, LT HSCs obtained from Vav1-Cre:Keap1$^{flox/flox}$ mice exhibited a deficiency in reconstitution capacity compared to control (60). These results were confirmed using Mx-Cre;Keap1$^{flox/flox}$ mice treated with poly (I:C) or administered the Nrf2 activator 1-(2-cyano-3,12-dioxooleana-1,9-dien-28-oyl) imidazole (CDDO-Im). In these model systems it appears that prolonged activation of Nrf2 reduces LT HSC self-renewal and quiescence while driving differentiation, thereby diminishing the ability of LT HSCs to reconstitute irradiated bone marrow (60). The differences between the study of Kim *et al.* (57) and that of Murakami *et al.* (60) are not currently understood and require further investigation.

Nrf2 AND RADIATION-ASSOCIATED PULMONARY INJURY

The Radiation Response

There are several excellent reviews with in-depth discussions on the subject of radiation-induced pneumonitis and pulmonary fibrotic injury (61–63). In brief, radiation pneumopathy is composed of two pathophysiologies: pneumonitis and fibrosis. Pneumonitis is a mostly resolvable inflammatory reaction that develops within weeks postirradiation, whereas fibrosis takes months to years to develop and is progressive (61). Fibrosis is considered a disease induced by recurrent and/or nonresolving injury to lung epithelium that results in

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cell death and/or phenotypic alterations to surviving cells, which leads to generation of myofibroblasts/fibrocytes and deposition of extracellular matrix (64–71). Radiation-induced pulmonary injury can be characterized by endothelium and pneumocyte apoptosis and senescence (71, 72) that are followed by recruitment of NADPH oxidase-expressing inflammatory cells and myofibroblasts to sites of injury, which in turn contribute to oxidative and cytokine stress. In published studies, it has been shown that suppression of TGF-β/Smad signaling, or overexpression of superoxide dismutase and/or overexpression of catalase abrogates development of radiation-induced pulmonary fibrosis (73–76). Such research supports the hypothesis that TGF-βl and oxidative stress are two drivers of fibrosis. Interestingly, as a side bar, Favaudon *et al.* (77) found that short pulses (less than 500 ms) of radiation administered at dose rates in excess of 40 Gy/s did not induce pulmonary fibrosis if the total dose delivered did not exceed 20 Gy.

The Role of Nrf2

The severity of radiation-induced pulmonary fibrosis is enhanced in the Nrf2-null mouse. Loss of Nrf2 increases radiation-mediated pulmonary tissue remodeling and collagen deposition (78). Loss of Nrf2 in irradiated lung impairs ΔNp63 stem/progenitor cell mobilization and proliferation, regeneration of alveolar type 2 cells (78), with an outcome of elevated FSP1 expression (79) and enhanced Masson's trichrome staining of collagen deposition (78), two markers of fibrosis. In injured lung Δp63+/Krt5+ stem cells can be mobilized to proliferate significantly and differentiate into alveolar type 1 and 2 epithelial cells *in vitro* and *in vivo.* Diphtheria toxin targeting of these stem cells impairs regeneration of injured lung and pulmonary oxygenation while promoting fibrosis, as measured by FSP-1, Masson's trichrome, and α-smooth muscle staining (80, 81). Thus, it has been hypothesized that ΔNp63 + stem/progenitor cell mobilization contributes to alveolar type 2 cell regeneration after radiation injury (78). However, it is important to note that if there are hypoxic regions in injured lung then HIFα/ Notch signaling reprograms Δp63+/Krt5 + cells to form basal-like metaplasia (82). Additionally, an Nrf2 deficiency promotes radiation-induced alveolar type 2 cell epithelial-mesenchymal transitions into myofibroblasts (78). At a molecular level it has been shown that Nrf2 can form a nuclear complex with nuclear pSmad3 at CAGA sites located in the proximal promoters of TGF-β target genes (79), suppressing gene expression (79, 83). Thus, loss of Nrf2 promotes TGF-β/RSmad signaling, a key factor for promoting epithelial-mesenchymal transitions (84). Conversely, TGF-β/RSmad signaling has been shown to suppress Nrf2-dependent gene expression. TGF-β/RSmad signaling induces ATF3, which in turn complexes with Nrf2. The ATF3/Nrf2 complex binds to ARE sites in the promoters of Nrf2 target genes, suppressing recruitment of CBP to AREs, with an outcome of suppressing Nrf2 target gene expression (37, 38, 85). Loss of Nrf2-dependent gene expression has been shown to promote myofibroblast development and increased oxidative stress (35, 86).

The molecular identity of radiation-induced pulmonary oxidant injury has been an enigma until recently. Mont *et al.* (87) provided direct biochemical evidence that isolevuglandin (IsoLG) adduction of proteins represents a radiation-induced, oxidant-mediated injury in lung. Genetic evidence linking oxidant challenge to IsoLG adduction has been provided in studies using genetically engineered mice, where it was shown that oxidative stress,

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generated by either loss of Nrf2 or NADPH oxidase activity, promoted IsoLG adduction (87). Isolevuglandins (IsoLGs), also known as isoketals, are formed by free radical oxidation of arachidonic acid (88). IsoLGs rapidly adduct the ε-amino group of protein lysines. Mont *et al.* (87) identified over 100 proteins susceptible to IsoLG adduction, including lysines on histones H3 and H4. Adduction of these histones would be expected to negatively affect epigenetic regulation and may represent a mechanism that contributes to fibrotic reprogramming (64). Extracellular collagen present in fibrotic lesions has been identified as another target for adduction (87). IsoLG adduction of collagen impairs the ability of MMP1 to degrade it, thus contributing to collagen accumulation. Chronic oxidative stress results in accumulation of IsoLG-adducted protein that can evolve into proteotoxic/apoptotic events (78), i.e., recurrent injury.

CONCLUSION

Research performed in mouse models has shown that Nrf2 expression affects recovery from radiation injury in a tissue-dependent manner. Whereas in the intestine an Nrf2 deficiency protects irradiated Lg5+ CBC cells, loss of Nrf2 in LT-HSCs and progenitor hematopoietic cells increases the radiation sensitivity, while promoting accelerated hematopoietic exhaustion. In the lung an Nrf2 deficiency promotes fibrotic TGF-β signaling and oxidative injury, and inhibits ΔNp63 stem cell mobilization and alveolar type 2 cell regeneration, thereby enhancing pulmonary tissue remodeling and fibrosis. Importantly, the Nrf2 haploinsufficient mouse is as susceptible to radiation-induced lung injury as a homozygous knock-out mouse (79). While loss of Nrf2 can be radiosensitizing, inducing Nrf2 overexpression has been shown to protect both intestine and hematopoietic tissue from acute radiation injury. However, conflicting results have been reported concerning engraftment of cells that overexpress Nrf2 after myoablative irradiation.

There can be significant variability in the expression of human *NFE2L2* that can affect Nrf2-target gene transcription (87). This variability can be a consequence of several mechanisms. As reviewed by Cho (89), there are 14 transcript variants reported for the human NFE2L2 gene and three major protein isoforms (and nine minor). The frequency of NFE2L2 SNPs and other mutations is 1 per 73 bps. Functional polymorphisms in *NFE2L2* that diminish expression of Nrf2 have been linked with several diseases [reviewed in ref. (90)]. Nrf2 transactivation is subject to epigenetic regulation that can be suppressed, with a consequence of inhibiting Nrf2 target gene expression (1,91–93). An important unanswered question is whether Nrf2 expression affects normal tissue radiation sensitivity in humans as it does in mice. There are a number of questions that need to be resolved before a detailed molecular understanding of Nrf2, stem cell function and radiation response is obtained.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This work was supported in part by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH), grant no. R01 HL112286.

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REFERENCES

1. 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. [PubMed: 7937919]
2. Sykiotis GP , Bohmann D , Stress-activated cap'n'collar transcription factors in aging and human disease. Science signaling 2010; 3: re3. [PubMed: 20215646]
3. 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 & development 1999; 13:76–86. [PubMed: 9887101]
4. 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 to cells: devoted to molecular & cellular mechanisms 2002; 7:807–20. [PubMed: 12167159]
5. Sykiotis GP , Bohmann D , Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Developmental cell 2008; 14:76–85. [PubMed: 18194654]
6. Kahn NW , Rea SL , Moyle S , Kell A , Johnson TE , Proteasomal dysfunction activates the transcription factor SKN-1 and produces a selective oxidative-stress response in Caenorhabditis elegans. The Biochemical journal 2008; 409:205–13. [PubMed: 17714076]
7. 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. Biochemical and biophysical research communications 1997; 236:313–22. [PubMed: 9240432]
8. Hayes JD , Dinkova-Kostova AT , The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends in biochemical sciences 2014; 39:199–218. [PubMed: 24647116]
9. Hirotsu Y , Katsuoka F , Funayama R , Nagashima T , Nishida Y , Nakayama K , et al. Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic acids research 2012; 40:10228–39. [PubMed: 22965115]
10. Venugopal R , Jaiswal AK , Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response elementmediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A 1996; 93:14960–5. [PubMed: 8962164]
11. Kwak MK , Wakabayashi N , Greenlaw JL , Yamamoto M , Kensler TW , Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. Molecular and cellular biology 2003; 23:8786–94. [PubMed: 14612418]
12. Suzuki T , Yamamoto M , Stress-sensing mechanisms and the physiological roles of the Keap1-Nrf2 system during cellular stress. J Biol Chem 2017; 292:16817–24. [PubMed: 28842501]
13. Tonelli C , Chio IIC , Tuveson DA , Transcriptional regulation by Nrf2. Antioxid Redox Signal 2017.
14. Sekhar KR , Yan XX , Freeman ML , Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. Oncogene 2002; 21:6829–34. [PubMed: 12360409]
15. McMahon M , Itoh K , Yamamoto M , Hayes JD , Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. J Biol Chem 2003; 278:21592–600. [PubMed: 12682069]
16. Cullinan SB , Gordan JD , Jin J , Harper JW , Diehl JA , The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol Cell Biol 2004; 24:8477–86. [PubMed: 15367669]
17. 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. [PubMed: 21245377]
18. Lo JY , Spatola BN , Curran SP , WDR23 regulates NRF2 independently of KEAP1. PLoS Genet 2017; 13:e1006762. [PubMed: 28453520]
19. Sekhar KR , Rachakonda G , Freeman ML , Cysteine-based regulation of the CUL3 adaptor protein Keap1. Toxicol Appl Pharmacol 2010; 244:21–6. [PubMed: 19560482]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

20. Dinkova-Kostova AT , Kostov RV , Canning P , Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. Arch Biochem Biophys 2017; 617:84–93. [PubMed: 27497696]
21. Chen W , Sun Z , Wang XJ , Jiang T , Huang Z , Fang D , et al. Direct interaction between Nrf2 and p21(Cip1/WAF1) upregulates the Nrf2-mediated antioxidant response. Mol Cell 2009; 34:663–73. [PubMed: 19560419]
22. Lu K , Alcivar AL , Ma J , Foo TK , Zywea S , Mahdi A , et al. NRF2 Induction Supporting Breast Cancer Cell Survival Is Enabled by Oxidative Stress-Induced DPP3-KEAP1 Interaction. Cancer Res 2017; 77:2881–92. [PubMed: 28416489]
23. Ge W , Zhao K , Wang X , Li H , Yu M , He M , et al. iASPP Is an Antioxidative Factor and Drives Cancer Growth and Drug Resistance by Competing with Nrf2 for Keap1 Binding. Cancer Cell 2017.
24. Pomatto LCD , Davies KJA , The role of declining adaptive homeostasis in ageing. J Physiol 2017.
25. Pekovic-Vaughan V , Gibbs J , Yoshitane H , Yang N , Pathiranage D , Guo B , et al. The circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant defense pathway to modulate pulmonary fibrosis. Genes Dev 2014; 28:548–60. [PubMed: 24637114]
26. Suh JH , Shenvi SV , Dixon BM , Liu H , Jaiswal AK , Liu RM , et al. 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. [PubMed: 14985508]
27. Zhang H , Davies KJA , Forman HJ , Oxidative stress response and Nrf2 signaling in aging. Free Radic Biol Med 2015; 88:314–36. [PubMed: 26066302]
28. Zhou L , Zhang H , Davies KJA , Forman HJ , Aging-related decline in the induction of Nrf2-regulated antioxidant genes in human bronchial epithelial cells. Redox Biol 2017; 14:35–40. [PubMed: 28863281]
29. Shih PH , Yen GC , Differential expressions of antioxidant status in aging rats: the role of transcriptional factor Nrf2 and MAPK signaling pathway. Biogerontology 2007; 8:71–80. [PubMed: 16850181]
30. Kang SJ , You A , Kwak MK , Suppression of Nrf2 signaling by angiotensin II in murine renal epithelial cells. Arch Pharm Res 2011; 34:829–36. [PubMed: 21656369]
31. Blobe GC , Schiemann WP , Lodish HF , Role of transforming growth factor beta in human disease. N Engl J Med 2000; 342:1350–8. [PubMed: 10793168]
32. Majetschak M , Borgermann J , Waydhas C , Obertacke U , Nast-Kolb D , Schade FU , Whole blood tumor necrosis factor-alpha production and its relation to systemic concentrations of interleukin 4, interleukin 10, and transforming growth factor-beta1 in multiply injured blunt trauma victims. Crit Care Med 2000; 28:1847–53. [PubMed: 10890631]
33. Biswas S , Guix M , Rinehart C , Dugger TC , Chytil A , Moses HL , et al. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. J Clin Invest 2007; 117:1305–13. [PubMed: 17415413]
34. Barcellos-Hoff MH , Derynck R , Tsang ML , Weatherbee JA , Transforming growth factor-beta activation in irradiated murine mammary gland. J Clin Invest 1994; 93:892–9. [PubMed: 8113421]
35. 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. [PubMed: 18199529]
36. Bakin AV , Stourman NV , Sekhar KR , Rinehart C , Yan X , Meredith MJ , et al. Smad3-ATF3 signaling mediates TGF-beta suppression of genes encoding Phase II detoxifying proteins. Free Radic Biol Med 2005; 38:375–87. [PubMed: 15629866]
37. Sancho R , Cremona CA , Behrens A , Stem cell and progenitor fate in the mammalian intestine: Notch and lateral inhibition in homeostasis and disease. EMBO Rep 2015; 16:571–81. [PubMed: 25855643]
38. Tian H , Biehs B , Warming S , Leong KG , Rangell L , Klein OD , et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 2011; 478:255–9. [PubMed: 21927002]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

39. Ishizuka S , Martin K , Booth C , Potten CS , de Murcia G , Burkle A , et al. Poly(ADP-ribose) polymerase-1 is a survival factor for radiation-exposed intestinal epithelial stem cells in vivo. Nucleic Acids Res 2003; 31:6198–205. [PubMed: 14576306]
40. Metcalfe C , Kljavin NM , Ybarra R , de Sauvage FJ , Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. Cell Stem Cell 2014; 14:149–59. [PubMed: 24332836]
41. Barker N , van Es JH , Kuipers J , Kujala P , van den Born M , Cozijnsen M , et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007; 449:1003–7. [PubMed: 17934449]
42. Yan KS , Chia LA , Li X , Ootani A , Su J , Lee JY , et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci U S A 2012; 109:466–71. [PubMed: 22190486]
43. Kuruvilla JG , Kim CK , Ghaleb AM , Bialkowska AB , Kuo CJ , Yang VW , Kruppel-like Factor 4 Modulates Development of BMI1(+) Intestinal Stem Cell-Derived Lineage Following gamma-Radiation-Induced Gut Injury in Mice. Stem Cell Reports 2016; 6:815–24. [PubMed: 27237377]
44. Yan KS , Gevaert O , Zheng GXY , Anchang B , Probert CS , Larkin KA , et al. Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. Cell Stem Cell 2017; 21:78–90 e6. [PubMed: 28686870]
45. Buczacki SJ , Zecchini HI , Nicholson AM , Russell R , Vermeulen L , Kemp R , et al. Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature 2013; 495:65–9. [PubMed: 23446353]
46. Yang W , Sun Z , Yang B , Wang Q , Nrf2-Knockout Protects from Intestinal Injuries in C57BL/6J Mice Following Abdominal Irradiation with gamma Rays. Int J Mol Sci 2017; 18.
47. McDonald JT , Kim K , Norris AJ , Vlashi E , Phillips TM , Lagadec C , et al. Ionizing radiation activates the Nrf2 antioxidant response. Cancer Res 2010; 70:8886–95. [PubMed: 20940400]
48. Haridas V , Hanausek M , Nishimura G , Soehnge H , Gaikwad A , Narog M , et al. Triterpenoid electrophiles (avicins) activate the innate stress response by redox regulation of a gene battery. J Clin Invest 2004; 113:65–73. [PubMed: 14702110]
49. Kim SB , Pandita RK , Eskiocak U , Ly P , Kaisani A , Kumar R , et al. Targeting of Nrf2 induces DNA damage signaling and protects colonic epithelial cells from ionizing radiation. Proc Natl Acad Sci U S A 2012; 109: E2949–55. [PubMed: 23045680]
50. Morrison SJ , Scadden DT . The bone marrow niche for haematopoietic stem cells. Nature 2014; 505:327–34. [PubMed: 24429631]
51. Himburg HA , Doan PL , Quarmyne M , Yan X , Sasine J , Zhao L , et al. Dickkopf-1 promotes hematopoietic regeneration via direct and niche-mediated mechanisms. Nat Med 2017; 23:91–9. [PubMed: 27918563]
52. Zhang J , Niu C , Ye L , Huang H , He X , Tong WG , et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature 2003; 425:836–41. [PubMed: 14574412]
53. Dominici M , Rasini V , Bussolari R , Chen X , Hofmann TJ , Spano C , et al. Restoration and reversible expansion of the osteoblastic hematopoietic stem cell niche after marrow radioablation. Blood 2009; 114:2333–43. [PubMed: 19433859]
54. Nakashima K , Zhou X , Kunkel G , Zhang Z , Deng JM , Behringer RR , et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108:17–29. [PubMed: 11792318]
55. Rana T , Schultz MA , Freeman ML , Biswas S . Loss of Nrf2 accelerates ionizing radiation-induced bone loss by upregulating RANKL. Free Radic Biol Med 2012; 53:2298–307. [PubMed: 23085426]
56. Rodrigues-Moreira S , Moreno SG , Ghinatti G , Lewandowski D , Hoffschir F , Ferri F , et al. Low-dose irradiation promotes persistent oxidative stress and decreases self-renewal in hematopoietic stem cells. Cell Rep 2017; 20:3199–211. [PubMed: 28954235]
57. Kim JH , Thimmulappa RK , Kumar V , Cui W , Kumar S , Kombairaju P , et al. NRF2-mediated Notch pathway activation enhances hematopoietic reconstitution following myelosuppressive radiation. J Clin Invest 2014; 124:730–41. [PubMed: 24463449]
58. Tsai JJ , Dudakov JA , Takahashi K , Shieh JH , Velardi E , Holland AM , et al. Nrf2 regulates haematopoietic stem cell function. Nat Cell Biol 2013; 15:309–16. [PubMed: 23434824]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

59. Merchant AA , Singh A , Matsui W , Biswal S . The redox-sensitive transcription factor Nrf2 regulates murine hematopoietic stem cell survival independently of ROS levels. Blood 2011; 118:6572–9. [PubMed: 22039262]
60. Murakami S , Suzuki T , Harigae H , Romeo PH , Yamamoto M , Motohashi H . NRF2 activation impairs quiescence and bone marrow reconstitution capacity of hematopoietic stem cells. Mol Cell Biol 2017; 37.
61. Tsoutsou PG , Koukourakis MI . Radiation pneumonitis and fibrosis: mechanisms underlying its pathogenesis and implications for future research. Int J Radiat Oncol Biol Phys 2006; 66:1281–93. [PubMed: 17126203]
62. Ding NH , Li JJ , Sun LQ . Molecular mechanisms and treatment of radiation-induced lung fibrosis. Curr Drug Targets 2013; 14:1347–56. [PubMed: 23909719]
63. Simone CB . Thoracic radiation normal tissue injury. Semin Radiat Oncol 2017; 27:370–77. [PubMed: 28865520]
64. Blackwell TS , Tager AM , Borok Z , Moore BB , Schwartz DA , Anstrom KJ , et al. Future directions in idiopathic pulmonary fibrosis research. An NHLBI workshop report. Am J Respir Crit Care Med 2014; 189:214–22. [PubMed: 24160862]
65. Selman M , Pardo A . Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. Proc Am Thorac Soc 2006; 3:364–72. [PubMed: 16738202]
66. King TE ., Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011; 378:1949–61. [PubMed: 21719092]
67. Romero F , Shah D , Duong M , Penn RB , Fessler MB , Madenspacher J , et al. A pneumocyte-macrophage paracrine lipid axis drives the lung toward fibrosis. Am J Respir Cell Mol Biol 2015; 53:74–86. [PubMed: 25409201]
68. Lemay AM , Haston CK . Radiation-induced lung response of AcB/ BcA recombinant congenic mice. Radiat Res 2008; 170:299–306. [PubMed: 18763862]
69. Paun A , Kunwar A , Haston CK . Acute adaptive immune response correlates with late radiation-induced pulmonary fibrosis in mice. Radiat Oncol 2015; 10:45. [PubMed: 25889053]
70. Mancini ML , Sonis ST . Mechanisms of cellular fibrosis associated with cancer regimen-related toxicities. Front Pharmacol 2014; 5:51. [PubMed: 24734020]
71. Zhang Y , Zhang X , Rabbani ZN , Jackson IL , Vujaskovic Z . Oxidative stress mediates radiation lung injury by inducing apoptosis. Int J Radiat Oncol Biol Phys 2012; 83:740–8. [PubMed: 22270165]
72. Citrin DE , Shankavaram U , Horton JA , Shield W , Zhao S , Asano H , et al. Role of type II pneumocyte senescence in radiation-induced lung fibrosis. J Natl Cancer Inst 2013; 105:1474–84. [PubMed: 24052614]
73. Rabender C , Mezzaroma E , Mauro AG , Mullangi R , Abbate A , Anscher M , et al. IPW-5371 proves effective as a radiation countermeasure by mitigating radiation-induced late effects. Radiat Res 2016; 186:478–88. [PubMed: 27841740]
74. Flechsig P , Dadrich M , Bickelhaupt S , Jenne J , Hauser K , Timke C , et al. LY2109761 attenuates radiation-induced pulmonary murine fibrosis via reversal of TGF-beta and BMP-associated proinflammatory and proangiogenic signals. Clin Cancer Res 2012; 18:3616–27. [PubMed: 22547771]
75. Vujaskovic Z , Batinic-Haberle I , Rabbani ZN , Feng QF , Kang SK , Spasojevic I , et al. A small molecular weight catalytic metallo-porphyrin antioxidant with superoxide dismutase (SOD) mimetic properties protects lungs from radiation-induced injury. Free Radic Biol Med 2002; 33:857–63. [PubMed: 12208373]
76. Machtay M , Scherpereel A , Santiago J , Lee J , McDonough J , Kinniry P , et al. Systemic polyethylene glycol-modified (PEGy-lated) superoxide dismutase and catalase mixture attenuates radiation pulmonary fibrosis in the C57/bl6 mouse. Radiother Oncol 2006; 81:196–205. [PubMed: 17069914]
77. Favaudon V , Caplier L , Monceau V , Pouzoulet F , Sayarath M , Fouillade C , et al. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. Sci Transl Med 20l4; 6:245ra93.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

78. Traver G , Mont S , Gius D , Lawson WE , Ding GX , Sekhar KR , et al. Loss of Nrf2 promotes alveolar type 2 cell loss in irradiated, fibrotic lung. Free Radic Biol Med 2017; 112:578–86. [PubMed: 28870520]

79. Travis EL , Rachakonda G , Zhou X , Korhonen K , Sekhar KR , Biswas S , et al. NRF2 deficiency reduces life span of mice administered thoracic irradiation. Free Radic Biol Med 2011; 51:1175–83. [PubMed: 21712086]

80. Kumar PA , Hu Y , Yamamoto Y , Hoe NB , Wei TS , Mu D , et al. Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. Cell 2011; 147:525–38. [PubMed: 22036562]

81. Zuo W , Zhang T , Wu DZ , Guan SP , Liew AA , Yamamoto Y , et al. p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. Nature 2015; 517:616–20. [PubMed: 25383540]

82. Xi Y , Kim T , Brumwell AN , Driver IH , Wei Y , Tan V , et al. Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. Nat Cell Biol 2017; 19:904–14. [PubMed: 28737769]

83. Oh CJ , Kim JY , Min AK , Park KG , Harris RA , Kim HJ , et al. Sulforaphane attenuates hepatic fibrosis via NF-E2-related factor 2-mediated inhibition of transforming growth factor-beta/Smad signaling. Free Radic Biol Med 2012; 52:671–82. [PubMed: 22155056]

84. Li M , Luan F , Zhao Y , Hao H , Zhou Y , Han W , et al. Epithelial-mesenchymal transition: An emerging target in tissue fibrosis. Exp Biol Med (Maywood) 2016; 241:1–13. [PubMed: 26361988]

85. Michaeloudes C , Chang PJ , Petrou M , Chung KF . Transforming growth factor-beta and nuclear factor E2-related factor 2 regulate antioxidant responses in airway smooth muscle cells: role in asthma. Am J Respir Crit Care Med 2011; 184:894–903. [PubMed: 21799075]

86. Artaud-Macari E , Goven D , Brayer S , Hamimi A , Besnard V , Marchal-Somme J , et al. Nuclear factor erythroid 2-related factor 2 nuclear translocation induces myofibroblastic dedifferentiation in idiopathic pulmonary fibrosis. Antioxid Redox Signal 2013; 18:66–79. [PubMed: 22703534]

87. Mont S , Davies SS , Roberts Second LJ , Mernaugh RL , McDonald WH , Segal BH , et al. Accumulation of isolevuglandin-modified protein in normal and fibrotic lung. Sci Rep 2016; 6:24919. [PubMed: 27118599]

88. Brame CJ , Salomon RG , Morrow JD , Roberts LJ . Identification of extremely reactive gamma-ketoaldehydes (isolevuglan-dins) as products of the isoprostane pathway and characterization of their lysyl protein adducts. J Biol Chem 1999; 274:13139–46. [PubMed: 10224068]

89. Cho HY . Genomic structure and variation of nuclear factor (erythroid-derived 2)-like 2. Oxid Med Cell Longev 2013; 2013:286524. [PubMed: 23936606]

90. Cho HY , Marzec J , Kleeberger SR . Functional polymorphisms in Nrf2: implications for human disease. Free Radic Biol Med 2015; 88:362–72. [PubMed: 26117318]

91. Chen D , Tavana O , Chu B , Erber L , Chen Y , Baer R , et al. NRF2 is a major target of ARF in p53-independent tumor suppression. Mol Cell 2017; 68:224–32.e4. [PubMed: 28985506]

92. 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. [PubMed: 19273602]

93. Kageyama S , Saito T , Obata M , Koide RH , Ichimura Y , Komatsu M . Negative regulation of the Keap1-Nrf2 pathway by a p62/ Sqstm1 splicing variant. Mol Cell Biol 2018.