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REVIEW ARTICLE

Nrf2 dysfunction and impaired cellular resilience to oxidative stressors in the aged vasculature: from increased cellular senescence to the pathogenesis of age-related vascular diseases

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Abstract Aging is associated with increased oxidative stress in vascular endothelial and smooth muscle cells, which contribute to the development of a wide range of diseases affecting the circulatory system in older adults. There is growing evidence that in addition to increased production of reactive oxygen species (ROS), aging critically impairs pathways determining cellular resilience to oxidative stressors. In young organisms, the evolutionarily conserved nuclear factor-erythroid-2-related factor 2 (Nrf2)-mediated antioxidant response pathway maintains cellular reduction-oxidation homeostasis and promotes a youthful cellular phenotype by regulating the transcription of an array of cytoprotective (antioxidant, pro-survival, anti-inflammatory and macromolecular damage repair) genes. A critical mechanism by which increased ROS production and Nrf2 dysfunction promote vascular aging and exacerbate pathogenesis of age-related vascular diseases is

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induction of cellular senescence, an evolutionarily conserved cellular stress response mechanism. Senescent cells cease dividing and undergo distinctive phenotypic alterations, contributing to impairment of angiogenic processes, chronic sterile inflammation, remodeling of the extracellular matrix, and barrier dysfunction. Herein, we review mechanisms contributing to dysregulation of Nrf2-driven cytoprotective responses in the aged vasculature and discuss the multifaceted role of Nrf2 dysfunction in the genesis of age-related pathologies affecting the circulatory system, including its role in induction of cellular senescence. Therapeutic strategies that restore Nrf2 signaling and improve vascular resilience in aging are explored to reduce cardiovascular mortality and morbidity in older adults.

Keywords Senescence · Reactive oxygen species · Oxidative stress · Antioxidant · Stress resistance · Vascular cognitive impairment · Vascular aging · Atherosclerosis · Nrf2 deficiency · Nrf2 dysfunction

Introduction

Epidemiological, clinical, and experimental studies demonstrate that advanced age per se promotes the pathogenesis of a wide range of diseases affecting the circulatory system (Ungvari et al. 2018a, b). Pathophysiological consequences of intrinsic vascular aging are the leading cause of morbidity and mortality among patients over 65 years of age and are major cause for the age-related decline in physical health-related quality of life. It is well established in the literature that aging-induced oxidative macromolecular damage and oxidative stress-mediated proinflammatory signaling pathways in cells within the vascular wall are important determinants of increased disease susceptibility in older adults, contributing to the pathogenesis of hypertension, stroke, coronary heart disease, heart failure, dysregulation of cerebral and peripheral blood flow, vascular stiffening, aneurysm formation, and vascular rupture and atherogenesis (Ungvari et al. 2007, 2010a, 2018b; Csiszar et al. 2002, 2007a, b, 2009; Toth et al. 2014, 2015a).

There is strong evidence that aging is associated with increased production of reactive oxygen species (ROS) in the vascular wall (Ungvari et al. 2018b). Studies on vascular cells isolated from nonhuman primates suggest that mitochondria (Ungvari et al. 2011a) are important sources of increased ROS production in aging. This conclusion is in accord with the findings of studies demonstrating elevated mitochondrial oxidative stress in isolated arteries and primary cells derived from aged rodents (Ungvari et al. 2007, 2008; Springo et al. 2015; Tarantini et al. 2018a). Additional mechanisms of age-related increases in free radical production include activation of NADPH oxidases (Ungvari et al. 2018b; Csiszar et al. 2002; van der Loo et al. 2000; Adler et al. 2003; Donato et al. 2007; Jacobson et al. 2007).

Perhaps more important than ROS production per se is the ability of the vascular cells to protect themselves and scavenge/eliminate excess ROS and repair or otherwise cope with the oxidative damage. The ability of the vascular tissue to respond to oxidative stress and return to homeostasis ("resilience") has been shown to be progressively diminished as a function of age. As a consequence, in the aged vasculature the same pathological stressors elicit exacerbated oxidative stress as compared to young vessels (Toth et al. 2015a; Springo et al. 2015). It is believed that this age-related loss of vascular resilience, which impair redox homeostasis, significantly contributes to the increased propensity of the aged vasculature to pathological alterations ranging from endothelial dysfunction to atherosclerotic diseases and vascular structural damage (e.g., aorta aneurysma, cerebral microhemorrhages).

The Keap1-Nrf2-antioxidant response element pathway

Among the adaptive stress response mechanisms responsible for cellular resilience, an age-related deterioration of Nrf2 (nuclear factor-erythroid-2-related factor 2)/ARE (antioxidant response element)-dependent antioxidative defense pathways is of particular importance (Ungvari et al. 2011a; Ahn et al. 2018; Bailey-Downs et al. 2012; Csiszar et al. 2012, 2014a; Fulop et al. 2018; Tarantini et al. 2018b). Nrf2 is an evolutionarily highly conserved transcription factor regulating the transcription of an array of cytoprotective (antioxidant, pro-survival, anti-inflammatory, and macromolecular damage repair) genes. Under basal conditions, Nrf2 is bound in the cytosol by its negative regulator Keap1 (Fig. 1). Keap1 is a substrate adapter protein for the Cullin3-Rbx1 E3 ligase complex and constitutively targets Nrf2 for proteasomal degradation. Oxidation of critical cysteine residues on Keap1 by increased levels of ROS or electrophiles causes modification in its tertiary structure, leading to dissociation of Nrf2 from Keap1. Keap1 then undergoes degradation, allowing Nrf2 to translocate to the nucleus where it binds to conserved ARE sequences in the promoter region of the Nrf2 target genes. DNA binding of Nrf2 requires small Maf

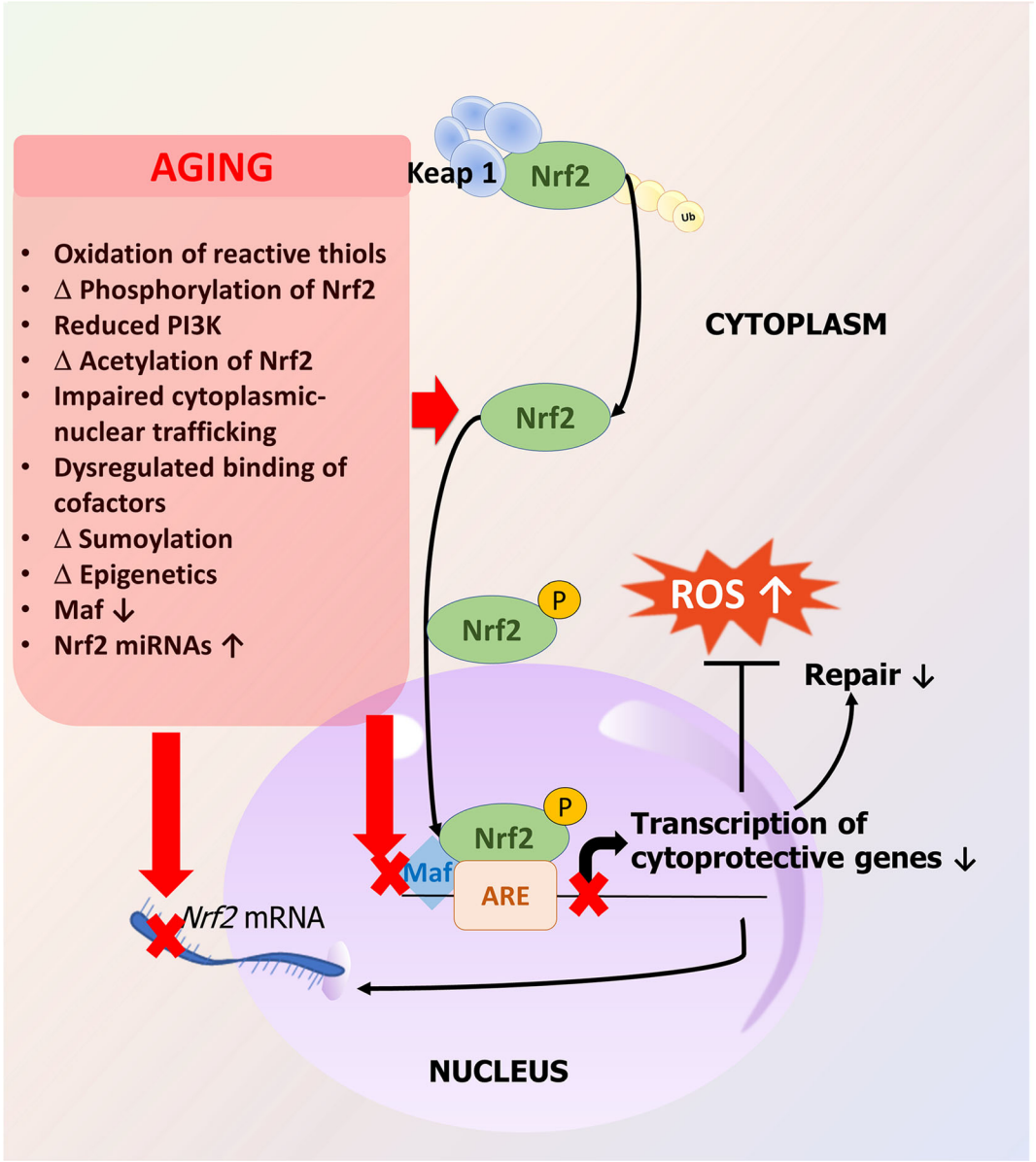

Fig. 1 Mechanisms involved in aging-induced Nrf2 dysfunction. The Nrf2 response pathway is an evolutionarily conserved adaptive mechanism that attenuates oxidative stress, limits the cellular and macromolecular damage caused by the increased free radical production, and maintains cellular homeostasis in the vascular wall. Nrf2 regulates the transcription of over 200 cytoprotective genes involved in antioxidant defenses and repair of macromolecular damage through the antioxidant response elements (ARE) present in target gene promoters. Aging is associated with dysregulation of Nrf2, and as a result, in the aged vasculature oxidative stress fails to activate Nrf2-regulated ROS detoxification systems and repair pathways. The scheme depicts potential mechanisms that contribute to the dysregulation of Nrf2 activation during the vascular aging process

proteins; thus, availability of Maf proteins contributes to the regulation of Nrf2 activity. Negative regulators of Nrf2 activation include Bach1, which competes with Nrf2 for binding to Maf proteins. The Keap1-Nrf2-ARE pathway regulates the expression of hundreds of genes that are involved in the cytoprotective response against oxidative stressors. Classical Nrf2 target genes encode antioxidant proteins (e.g., catalase, heme oxygenase 1, glutathione peroxidases, NAD(P)H quinone dehydrogenase (NQO1), glutathione-S-transferases, peroxiredoxins, thioredoxins, thioredoxin reductases, and glutamate-cysteine ligase, which is the rate-limiting enzyme for glutathione

synthesis). Nrf2 also regulates expression of genes involved in autophagy and the proteasome.

Important for the present discussion, there is increasing evidence that Nrf2 regulates DNA repair pathways, limiting DNA damage induced by genotoxic and oxidative stressors (Jayakumar et al. 2015; Sekhar and Freeman 2015; Singh et al. 2013). The impact of Nrf2 on DNA repair is not dependent on its antioxidant functions. Instead, many repair genes that are involved in DNA repair, including homologous recombination repair and base excision repair mechanisms, are directly regulated by Nrf2 (Jayakumar et al. 2015). Chromatin immunoprecipitation (ChIP) assays also demonstrate that Nrf2 binds to the promoter of key enzymes in the base excision repair pathway and RNAi experiments show that Nrf2 dysfunction associates with impaired DNA repair (Singh et al. 2013). Genetic Nrf2 depletion in mice was shown to result in persistence of residual DNA damage induced by genotoxic stresses.

Mechanisms of aging-induced Nrf2 dysfunction

There is strong evidence that Nrf2 transcriptional activity declines with age in the vasculature (Ungvari et al. 2011a, b), which contributes to the development of a wide range of vascular aging phenotypes. Previous studies in laboratory rodents and nonhuman primates show that aging impairs the ability of vascular endothelial and smooth muscle cells to mount an effective Nrf2-dependent antioxidant defense in response to oxidative stressors, which results in more robust oxidative stress and oxidative damage in aged cells than in young cells (Ungvari et al. 2011a, b; Csiszar et al. 2012, 2014a). Interestingly, the role of Nrf2 dysfunction in the aging process may be evolutionarily conserved. Previous studies show that aging Drosophila progressively lose the ability to activate Nrf2 targets in response to acute stress exposure, which contributes to age-associated functional decline (Rahman et al. 2013). Further, the Nrf2 homolog SKN-1 was shown to regulate longevity in *C. elegans* (Tullet et al. 2008). Recent studies demonstrate that aging in *C. elegans* is also associated with significant impairment of SKN-1-mediated adaptive oxidative stress responses (Raynes et al. 2017).

The mechanisms underlying dysregulation of Nrf2-mediated antioxidant responses in aging are likely multifaceted. Expression of Nrf2 and Nrf2-driven antioxidant genes is modulated by microRNAs (Cheng et al. 2013). Studies on cultured endothelial cells derived from young and aged rats demonstrate that aging-induced dysregulation of miRNA expression contributes to downregulation of Nrf2 (Csiszar et al. 2014a). Aging may also impair the pathways that regulate Nrf2 activation (e.g., dysregulation in binding of cofactors) and nuclear translocation. Age-related alterations in post-translational protein modifications, including oxidation of sulfhydryl groups in cysteine residues in Keap1 and phosphorylation and/or acetylation of Nrf2, can interfere with release/activation of Nrf2. In addition, cytoskeletal alterations may impair cytoplasmic-nuclear trafficking. Aging may also dysregulate binding of cofactors, such as small Maf proteins, impairing ARE-specific DNA binding of Nrf2 and downregulating the transcription of Nrf2 target genes. Interestingly, studies in cells from patients with Hutchinson-Gilford progeria syndrome demonstrate that progerin sequesters Nrf2 and thereby causes its subnuclear mislocalization, resulting in impaired Nrf2 transcriptional activity (Kubben et al. 2016).

It is possible that circulating anti-geronic and pro-geronic factors contribute to the dysregulation of Nrf2 in aged vessels. In humans, aging results in a marked decline in circulating IGF-1, which is thought to promote age-related vascular pathologies, including atherosclerosis (Higashi et al. 2012), cerebral microhemorrhages (Tarantini et al. 2017a), and endothelial dysfunction (Toth et al. 2015b). Importantly, decreased circulating IGF-1 results in marked Nrf2 dysfunction in mouse arteries (Bailey-Downs et al. 2012). There is also evidence that soluble klotho, a putative anti-aging factor, exerts protective effects on vascular cells by inducing Nrf2 (Maltese et al. 2017).

Role of Nrf2 dysfunction in vascular endothelial cells: from vasomotor dysfunction and inflammation to BBB disruption and impaired angiogenesis

Nrf2/ARE-regulated pathways play multifaceted roles in endothelial physiology and pathophysiology, exerting antioxidative, anti-inflammatory, and cytoprotective effects. There is evidence that aging is associated with Nrf2 dysfunction in endothelial cells (Ungvari et al. 2011a, b), which likely has deleterious consequences for cardiovascular health span.

Studies in genetically modified mice demonstrate that increased oxidative stress due to Nrf2 deficiency impairs endothelial function, reducing functional hyperemia in the brain (Tarantini et al. 2018b). Genetic Nrf2 depletion also exacerbates endothelial dysfunction induced by obesity in

the brain, aorta, and the skeletal muscle microcirculation (Tarantini et al. 2018b; Ungvari et al. 2010b, 2011c), mimicking the aging phenotype (Tucsek et al. 2014a). Increased oxidative stress due to Nrf2 dysfunction also promotes proinflammatory phenotypic alteration in endothelial cells, including activation of NF-κB. Adaptive Nrf2 activation has been observed in endothelial cells in response to hyperglycemia (Ungvari et al. 2011c) and high levels of advanced glycation end products (He et al. 2010), which likely protect the vasculature against the deleterious pro-oxidative and proinflammatory effects of diabetes mellitus. It is likely that age-related Nrf2 dysfunction contributes to the exacerbation of vascular dysfunction induced by metabolic diseases in aging (Tucsek et al. 2014a, b; Bailey-Downs et al. 2013). Other pathological conditions in which endothelial Nrf2 activation likely plays critical protective roles include sepsis (Holloway et al. 2016; Kim et al. 2012; Thimmulappa et al. 2006a, b). Studies in endothelial cells suggest that induction of Nrf2-regulated genes (e.g., heme oxygenase-1) in response to treatment with septic sera is impaired in aging (Tucsek et al. 2013) and that the exacerbated endothelial dysfunction at the level of the microcirculation likely contributes to multiple organ failure observed in aged animals (Coletta et al. 2014). There is strong evidence that Nrf2 can be activated by atheroprotective shear stress in endothelial cells, which confers important anti-inflammatory effects (Chen et al. 2003; Dai et al. 2007; Warabi et al. 2007; Zakkar et al. 2009). We posit that age-related impairment of mechanosensitive activation of Nrf2/ARE-mediated pathways promotes atherogenesis.

Cerebromicrovascular endothelial health determines the integrity of the blood-brain barrier (BBB), which is critical for normal brain function. Compromised BBB plays a critical role in the pathogenesis of a number of age-related diseases of the central nervous system, including neurodegeneration. There is evidence that activation of the Nrf2/ARE-dependent cytoprotective mechanisms protect the integrity of the BBB under conditions of pathological stressors, including metabolic diseases (Tarantini et al. 2018b), brain injury (Zhao et al. 2007), and sepsis (Li et al. 2018). Genetic depletion of Nrf2 was reported to exacerbate BBB disruption induced by metabolic stressors (Tarantini et al. 2018b), mimicking the aging phenotype (Tucsek et al. 2014b). Nrf2 is a promising pharmacological target for prevention of BBB disruption. Accordingly, previous studies demonstrate that sulforaphane-mediated activation of Nrf2 in the cerebral vasculature prevents BBB disruption protecting the brain against stroke-related neurological dysfunction (Alfieri et al. 2013).

Angiogenic capacity of endothelial cells is also regulated by Nrf2. There is evidence that in the absence of functional Nrf2 endothelial cells exhibit impaired proliferation, reduced cellular migration, and impaired the ability to form capillary-like structures (Valcarcel-Ares et al. 2012). In addition to regulating angiogenic processes, the known anti-apoptotic action of Nrf2/ARE-mediated pathways likely contributes importantly to the preservation of the structural integrity of newly formed capillaries. It is likely that age-related Nrf2 deficiency contributes to increased rate of endothelial apoptosis (Csiszar et al. 2004, 2007b; Ungvari et al. 2011b) and impaired angiogenesis, promoting microvascular rarefaction (Anversa et al. 1994; Riddle et al. 2003; Sonntag et al. 1997).

Role of Nrf2 dysfunction in vascular smooth muscle cells: from atherosclerosis to structural damage

In vascular smooth muscle cells (VSMCs), adaptive activation of Nrf2 was demonstrated in response to a wide range of stressors, including exposure to oxidized LDL (Anwar et al. 2005) and inflammatory cytokines (Churchman et al. 2009). Preclinical studies suggest that Nrf2 activation in VSMCs mediates potent antiatherosclerotic effects (Bozaykut et al. 2014).

Previous studies demonstrated age-associated alterations in the homeostatic role of Nrf2-driven free radical detoxification mechanisms in the VSMCs (Ungvari et al. 2011a), which likely promote the pathogenesis of multiple age-related diseases. The available data suggest that Nrf2 dysfunction in VSMCs is causally linked to NF-κB activation and chronic low-grade vascular inflammation in aging (Ungvari et al. 2011a). Age-related Nrf2 dysfunction may also contribute to the age-related exacerbation of hypertension-induced vascular oxidative stress (Toth et al. 2015a; Springo et al. 2015) and its pathophysiological consequences, including the increased propensity for microvascular injury in aging (Toth et al. 2015a).

Recent studies demonstrate that age-related exacerbation of the pro-oxidative effects of hypertension leads to activation of matrix metalloproteinases and pathological remodeling of the extracellular matrix in the vascular wall, promoting the pathogenesis of intracerebral hemorrhages (Toth et al. 2015a; Ungvari et al. 2017a, 2018c). It can be speculated that Nrf2 dysfunction may contribute to increased oxidative stress-related vascular

pathologies in aging, including the genesis of cerebral microhemorrhages and aorta aneurysms. Further preclinical studies are evidently needed to experimentally test these ideas. Interestingly, recent evidence shows that treatment with the Nrf2 activator dimethyl fumarate reduces the formation and rupture of intracranial aneurysms (Pascale et al. 2019).

Role of Nrf2 dysfunction promoting cellular senescence: a novel mechanism contributing to vascular aging

Among the cellular and molecular mechanisms contributing to organismal aging in recent years, cellular senescence has emerged as a fundamental aging process (Baker et al. 2016). There is also growing evidence that increased DNA damage (telomeric, non-telomeric, and/or mitochondrial DNA damage) plays a role in vascular aging by promoting cellular senescence (Fig. 2) (Bautista-Nino et al. 2016). Upon induction of cellular senescence, vascular cells undergo cell cycle arrest and therefore can no longer replicate, diminishing the repair and remodeling capacity of the vasculature. Senescent cells also acquire a highly inflammatory senescence-associated secretory phenotype, which consists of increased secretion of proinflammatory cytokines and chemokines, matrix metalloproteinases, and altered release of gaseotransmitters and eicosanoid mediators (Freund et al. 2010). There is growing evidence that increased presence of senescent vascular cells in the tissues contributes to the pathogenesis of various age-related diseases. Recent studies demonstrate that removal of senescent cells expressing the senescent marker cyclin-dependent kinase inhibitor p16INK4A in genetically modified mice (INK-ATTAC and p16-3MR mice) leads to a prolonged lifespan and general health span (Baker et al. 2011, 2016; Jeon et al. 2017; Abdul-Aziz et al. 2019; Kim et al. 2019; Patil et al. 2019; Farr et al. 2017; Xu et al. 2015) as well as improved cardiovascular health (Tarantini and Ungvari 2019, unpublished observation) (Roos et al. 2016), supporting a key role for cellular senescence in the process of aging.

Nrf2 activation has been shown to inhibit the induction of cellular senescence (Romero et al. 2019). Recent

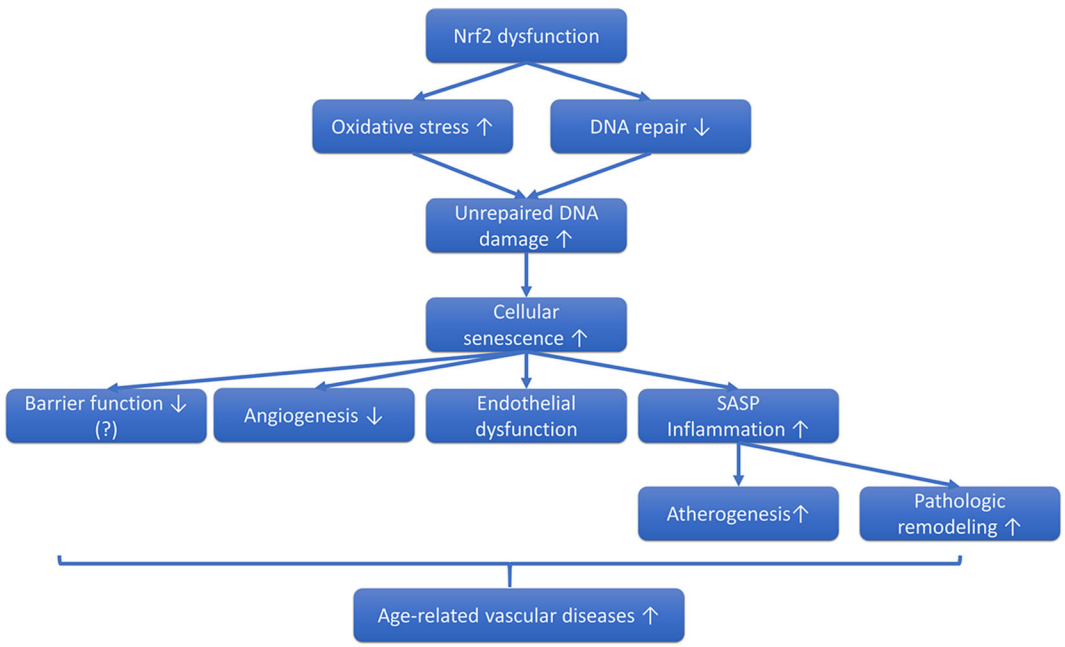

Fig. 2 Role of Nrf2 dysfunction in vascular senescence. The proposed model predicts that Nrf2 dysfunction exacerbates oxidative stress-induced DNA damage, by impairing both antioxidant defenses and DNA repair pathways in the vascular endothelial and smooth muscle cells. The unrepaired DNA damage triggers cellular senescence, which contributes to dysregulation of blood flow, atherogenesis, impaired angiogenesis, disruption of the microvascular barrier function, and pathological remodeling of both large arteries and the microcirculation. All of the aforementioned pathological processes contribute to the deterioration of vascular health in aging

studies demonstrate that genetic depletion of the Nrf2 exacerbates age-related induction of cellular senescence in cerebral arteries, which contributes to upregulated vascular expression of inflammatory cytokines and a heightened inflammatory status of the hippocampus (Fulop et al. 2018). In addition, the effects of increased oxidative stress induced by cardiovascular risk factors (e.g., obesity) are also exacerbated in genetically Nrf2-deficient mice, at least in part, due to accelerated cellular senescence (Tarantini et al. 2018b; Ungvari et al. 2010b, 2011c).

Vasoprotection mediated through activation of Nrf2 by dietary interventions and pharmacological agents in aging

On the basis of the available experimental evidence, it has been proposed that the Nrf2/ARE-mediated antioxidative defense pathway may serve as a therapeutic target for neurovascular protection in stroke and other human disease conditions (Alfieri et al. 2011).

Previous studies show that age-related increase in oxidative stress in microvascular endothelial cells is prevented by the anti-aging dietary regimen caloric restriction (Csiszar et al. 2009, 2014a). Importantly, caloric restriction was shown to restore expression and transcriptional activity of Nrf2 to youthful levels in aged endothelial cells (Csiszar et al. 2014a), which likely significantly contribute to its antioxidative vasoprotective effects. Nrf2 activation was also shown to critically contribute to the anti-cancer effects of caloric restriction (Pearson et al. 2008a). The mechanisms by which caloric restriction upregulates Nrf2 likely includes downregulation of miRNAs that decrease mRNA expression in endothelial cells (Csiszar et al. 2014a).

To mimic the beneficial effects of caloric restriction and to develop novel interventions for vasoprotection in aging several pharmacological activators of Nrf2 have been identified and tested in preclinical studies. Resveratrol (3,4,5-trihydroxystilbene), a plant-derived polyphenolic compound, is a potent activator of Nrf2 in endothelial cells and vascular smooth muscle cells (Csiszar et al. 2012, 2014b; Ungvari et al. 2010b). Recent studies provide strong evidence that the treatment of laboratory rodents with resveratrol exerts significant vasoprotective effects both during aging and in pathological conditions associated with accelerated vascular aging (Toth et al. 2014, 2015a; Mattison et al. 2014; Oomen et al. 2009; Pearson et al. 2008b; Csiszar et al. 2008). In particular, resveratrol was shown to confer microvascular protection, increasing capillarization (Oomen et al. 2009), rescuing endothelium-mediated neurovascular coupling responses (Toth et al. 2014), and preventing hypertension-induced microvascular damage (Toth et al. 2015a) in the aged mouse brain. Previous studies suggest that long-term treatment with resveratrol also confers protection against cerebral vascular dysfunction during nutrient stress in nonhuman primates (Bernier et al. 2016). Resveratrol-induced endothelial protection is also manifested in the large vessels of rodent (Pearson et al. 2008b) and nonhuman primate models of aging (Mattison et al. 2014). It should be noted, however, that the effects of resveratrol are likely not specific to Nrf2 and involve activation of SIRT-1 as well.

Sulforaphane, which is an organosulfur compound found in cruciferous vegetables such as broccoli, cauliflower, and Brussels sprouts, is also a potent inducer of Nrf2 (Alfieri et al. 2013; Santin-Marquez et al. 2019). Importantly, sulforaphane was shown to inhibit vascular inflammation (Zakkar et al. 2009), prevent atherogenesis (Shehatou and Suddek 2016), and improve endothelial function (Pereira et al. 2017) in pathological conditions associated with accelerated vascular aging. Nrf2 activation also plays a critical role in endothelial protection mediated by estrogen and various phytoestrogens (Siow et al. 2007). Sulforaphane was shown to delay cellular senescence in vitro by attenuating cellular oxidative damage (Hariton et al. 2018). Other canonical Nrf2 activators, which have entered clinical trials in the USA with diverse indications, include the synthetic oleanane triterpenoid compound bardoxolone methyl (CDDO-Me), RTA 408, and dimethyl fumarate (Tecfidera). These compounds have shown to exert endothelial protection in preclinical studies. However, there is a paucity of data regarding their efficacy to improve vascular function in aging.

It should be noted that many electrophilic Nrf2 activators are present in natural products. In older adults, dietary supplementation with spices such as turmeric, as well as the inclusion of whole foods (e.g., broccoli sprouts and other cruciferous vegetables, green tea) that contain Nrf2 activator phytochemicals in their diet, is likely to exert important vasoprotective effects.

Conclusions

Nrf2 may provide a therapeutic target for countering vascular oxidative stress and inflammation associated

with aging and pathological conditions characterized by accelerated vascular aging (Ungvari et al. 2017b, 2018c; Ashpole et al. 2017; Csiszar et al. 2017; Deepa et al. 2017; Grant et al. 2017; Tarantini et al. 2017b; Tucsek et al. 2017; Carlson et al. 2018; Cervellati et al. 2018; Chao et al. 2018; Csipo et al. 2018; Cunningham et al. 2018; Logan et al. 2018; Reglodi et al. 2018). In order to develop novel therapeutic interventions to promote cardiovascular and cerebrovascular health in older persons, it is essential to better understand the interconnected cellular pathways through which aging impairs Nrf2-dependent homeostatic mechanisms, reduces vascular resilience to stressors, and promotes cellular senescence. In addition to the Keap1-dependent mechanisms, future studies should also better characterize the role of age-related alterations in the autophagy lysosomal pathway in dysregulation of non-canonical activation of Nrf2 in the aging vasculature. There is strong evidence suggesting that cellular senescence plays an important role in vascular aging and that Nrf2 activation protects against induction of cellular senescence. Thus, future studies should also test the protective effects of long-term treatment with Nrf2 activators on senescence-related endpoints. Studies combining Nrf2 activators with senolytic drugs will be also very informative.

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