

Nrf2 in the Regulation of Endothelial Cell Homeostasis During Inflammation



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

1 Function of Endothelial Cells

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

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77

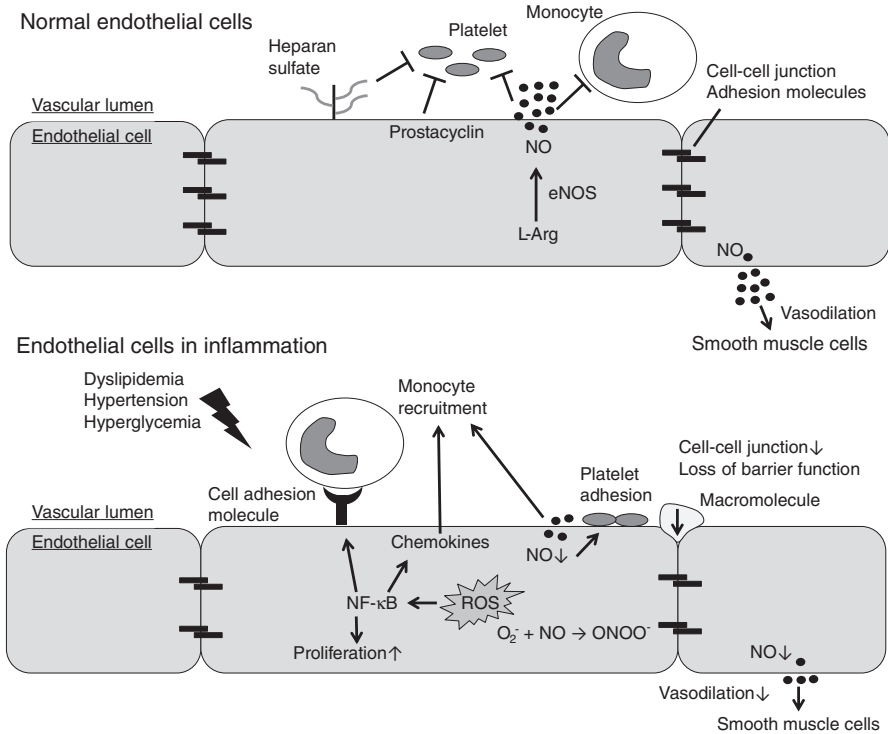


Fig. 1 Endothelial functions. The functions of normal endothelial cells and cells involved in inflammation are shown. See text for details

1.1 Barrier Functions

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

1.2 Control of Blood Pressure

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

1.3 Thrombosis and Fibrinolysis

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

1.4 Angiogenesis

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

1.5 Leukocyte Recruitment

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

2 Inflammation in Endothelial Cells

2.1 Triggers of Inflammation in Endothelial Cells

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

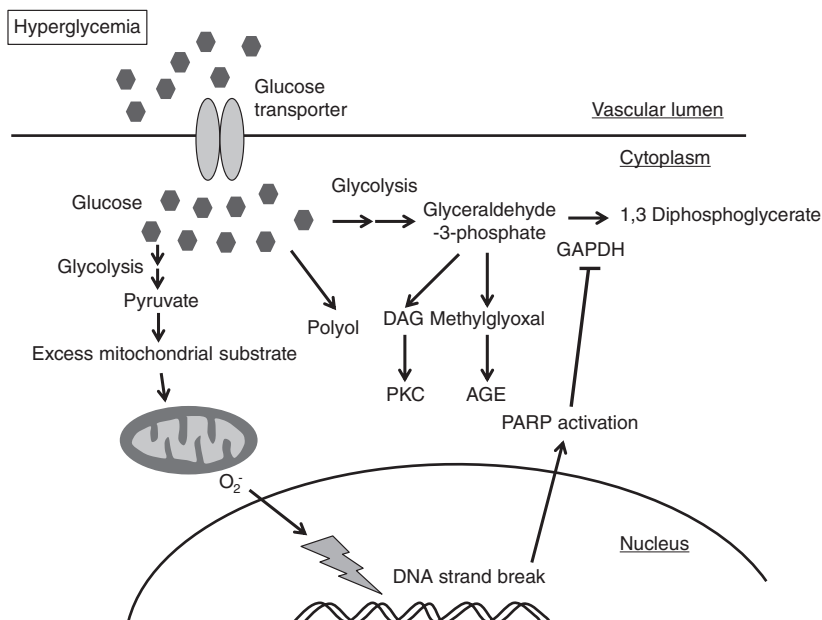


Fig. 2 The molecular mechanisms for ROS production by hyperglycemia. See text for details

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

2.2 Consequences of Inflammation in Endothelial Cells

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

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

3 Shear Stress

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

4 Nrf2 Activation in Endothelial Cells

4.1 Transcription Factor Nrf2

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

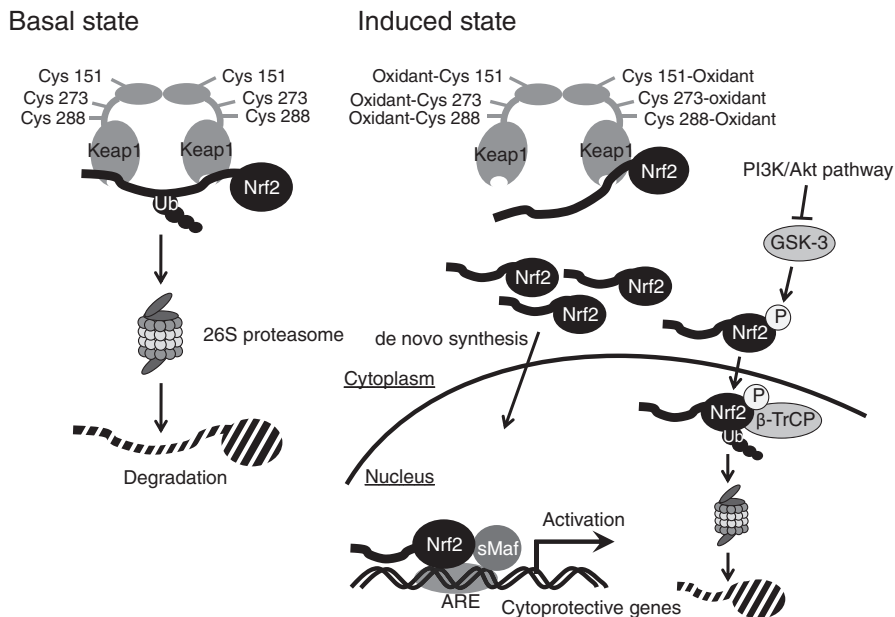


Fig. 3 Regulation of Nrf2. The molecular mechanisms for Nrf2 activation are shown. Nrf2 is repressed by Keap1 via ubiquitin-mediated proteasomal degradation in basal states. Upon oxidative stress, the reactive cysteine residues of Keap1 are modified by oxidants. Subsequently, Nrf2 degradation is attenuated,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

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

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

4.2 Shear Stress and Nrf2

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

Several possible mechanisms have been suggested for Nrf2 activation by L-flow. We showed that the cyclooxygenase-2 (COX-2)-dependent generation of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), which belongs to the cyclopentenone-type prostaglandins with electrophilic character, is essential for the induction of Nrf2 target genes in endothelial cells in response to L-flow (Fig. 4a, b). L-flow induces Nrf2 accumulation, the increased binding of it to the ARE of NQO1, and increases target gene expression, such as NQO1, HO-1, and ferritin heavy chain (Fig. 4b–e). Exposure of endothelial cells to L-flow enhances the production of prostaglandin D₂ (PGD₂) and 15d-PGJ₂ through the upregulation of cytosolic phospholipase A₂, COX-2, and lipocalin-type PGD synthase [64–66], and specific COX-2 inhibitors (Nimesulide and NS-398) repress the production of PGD₂ and Nrf2 activation. Of note, it was reported that 15d-PGJ₂ covalently binds to Cys273 of Keap1 and suppresses Nrf2 degradation [34–36, 58, 67, 68]. Thus, although it is well known that 15d-PGJ₂ possesses anti-inflammation roles by activating PPAR γ and repressing NF- κ B, the novel function involving Nrf2 adds to the anti-inflammatory function of 15d-PGJ₂.

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

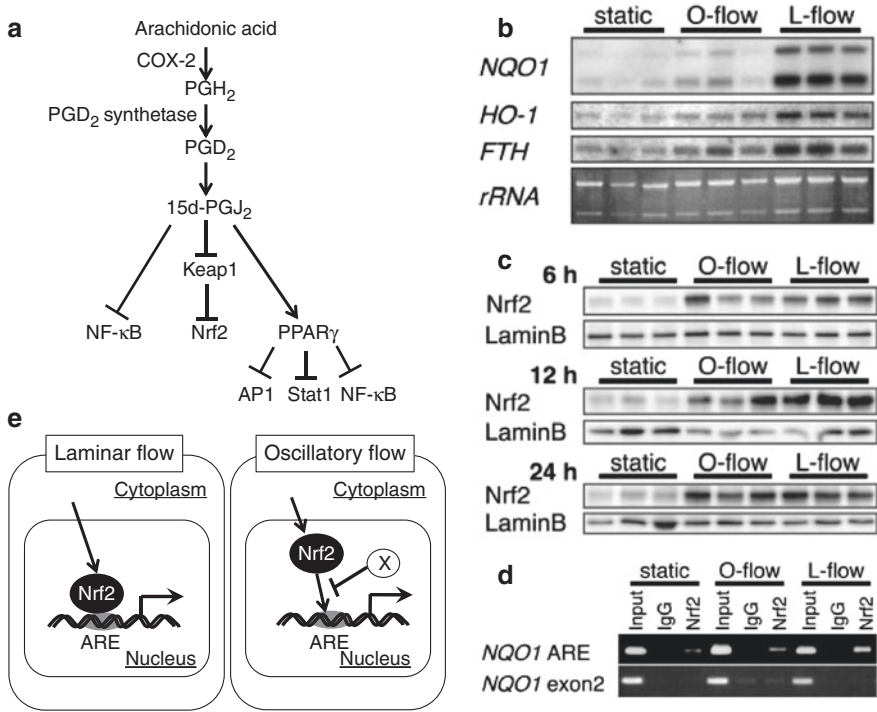


Fig. 4 L-flow activates Nrf2 by producing 15d-PGJ₂. **(a)** The metabolic pathway of arachidonic acid is shown. 15d-PGG₂ exerts anti-inflammatory roles through the activation of PPAR γ and the repression of Nrf2 and NF- κ B. **(b)** The mRNA levels of Nrf2 target genes of HAECs exposed to L-flow, O-flow, or kept under a static condition for 24 h. *FTH*; ferritin heavy chain. **(c)** HAECs were exposed to respective flows, and their nuclear extracts were analyzed by Western blot analysis using the anti-Nrf2 antibody. **(d)** HAECs were exposed to respective flows for 24 h. A chromatin immunoprecipitation (ChIP) assay was carried out with the anti-Nrf2 antibody or normal rabbit IgG. **(b–d)** were reprinted from an already published report [58]. **(e)** Mechanism of Nrf2 activation by blood flow. Although both L-flow and O-flow induce Nrf2 accumulation in the nucleus, only L-flow, but not O-flow activates Nrf2 binding to ARE of target genes and activates their expression. One plausible explanation is the presence of factor X that is inducible by O-flow and inhibits Nrf2 binding to ARE

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

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

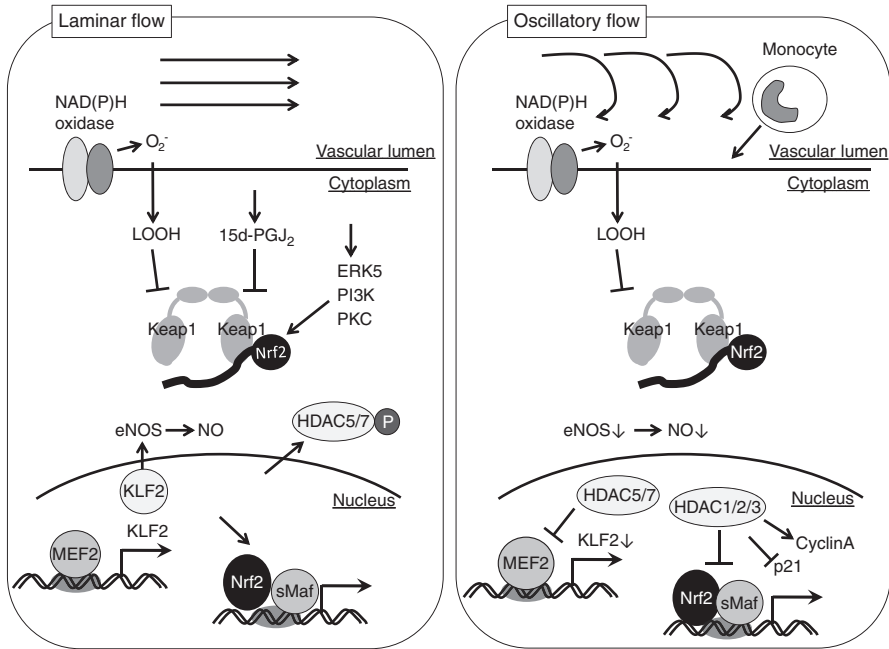


Fig. 5 Differential regulation of Nrf2 activity by blood flows. The left and right schemes show the several mechanisms for Nrf2 regulation in endothelial cells exposed to L-flow and O-flow, respectively. Erk5 also regulates KLF2 expression via MEF2 although it is not shown in the fig [71]

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

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

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

4.3 Aging and Nrf2

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

significant oxidative stress, which was indicated by the increased 8-iso-PGF2 α and 4-hydroxynonenal (HNE) content and decreased glutathione and ascorbate levels, compared with vessels of young macaques [84]. Oxidative stress in aged rhesus macaques induced inflammation phenotype characterized by NF- κ B, IL-6, inducible NOS (iNOS), and intercellular adhesion molecule-1 (ICAM-1) activation but did not activate Nrf2 target genes, such as glutamate–cysteine ligase, catalytic subunit (GCLC), NQO1, and HO-1. Although these results were examined in carotid arteries that contain a variety of cells (e.g., endothelial cells, smooth muscle cells and migrated leukocytes), endothelial cells might be one of the responsible cells.

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

5 The Effect of Nrf2 Activation in Endothelial Cells

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

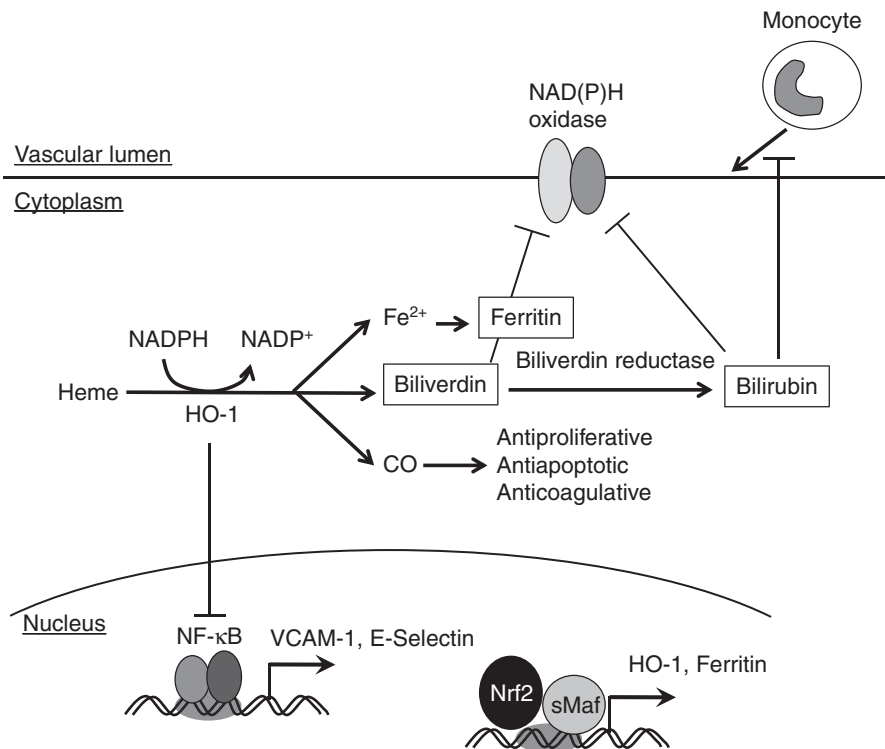


Fig. 6 Anti-inflammatory function of HO-1 in endothelial cells. HO-1 exerts anti-inflammatory functions through the depicted pathways. Enclosed factors act as antioxidants by themselves

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

ex vivo models. The overexpression of HO-1 by adenovirus infection of the vessels inhibited phenylephrine-dependent vasoconstriction in isolated aortic rings, also in a fashion dependent on heme degradation but independent of NO production [97]. Hemin-induced HO-1 activation in microvascular endothelial cells exhibits protection against oxidative stress and leukocyte adhesion by a mechanism that depends on the antioxidant role of bilirubin [98]. In vivo Nrf2 activation in the atherosensitive region of the aorta by sulforaphane induced HO-1 expression and suppressed p38-VCAM-1 signaling in an Nrf2-dependent manner [63]. The inhibition of HO-1 activity using zinc protoporphyrin did not influence the VCAM-1 suppression caused by sulforaphane in HUVECs. These results indicate that HO-1 is not essential for the p38-suppressing effects of sulforaphane and the other Nrf2-induced antioxidants can compensate for the absence of HO-1 activity. It has also been reported that Nrf2 and Oct-1 corporately suppress the transcription of the NAD(P)H oxidase 4 (Nox4) gene under L-flow condition, leading to the suppression of O₂⁻ production [61].

6 Vascular Diseases and Nrf2

Although dyslipidemia and hyperglycemia provokes inflammation in endothelial cells (See Sect. 2.1), Nrf2 activation is protective against these stresses. oxLDL induced the expression of MCP-1, VCAM-1, and ICAM-1 in HUVECs and also provokes cell death. Pretreatment of polyphenol genistein (4',5,7-trihydroxyisoflavone) increased HO-1 expression and simultaneously suppressed MCP-1, VCAM-1, and ICAM-1 expression and restored the cell viability in an Nrf2-dependent manner [99]. Mimicking hyperglycemia in human microvascular endothelial cells (HMEC-1) increased the generation of ROS, which was prevented by Nrf2 inducer sulforaphane [100]. ROS accumulation was increased further by either knockdown of Nrf2 or transketolase, one of the pentose phosphate pathway (PPP) enzymes. PPP generates not only ribose 5-phosphate (R5P), a critical substrate for the nucleotide synthesis, but also NADPH as reducing equivalents. These results suggest that Nrf2-driven PPP activation generates NADPH, which contributes to scavenging of ROS induced by hyperglycemia. Additionally, Nrf2-mediated antioxidant defense has a critical role in fetal endothelial cells that are exposed to hyperglycemia [101]. HUVECs cultured from gestational diabetes mellitus (DM) pregnancies showed reduced rates of cell proliferation to reach confluency [102]. Further proteomic analysis revealed that proteins involved in redox homeostasis were significantly altered in gestational DM and associated with increased mitochondrial superoxide generation, protein oxidation, DNA damage, and diminished GSH synthesis [101]. In accordance, induction of Nrf2 was impaired in response to 4-HNE. Decreased DJ-1, involved in Nrf2 protein stability, and increased GSK3 β phosphorylation in gestational DM cells may account for the deficits in Nrf2 nuclear accumulation. These results suggest that forced expression of Nrf2 might protect fetal endothelial cells from hyperglycemia. Of note, the therapeutic potential of sulforaphane against

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

7 Conclusion

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

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