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Brief Review

Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: implications for atherosclerosis and preeclampsia

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Abstract: Increased generation of reactive oxygen species (ROS) in vascular diseases such as atherosclerosis, diabetes, chronic renal failure and preeclampsia readily leads to impaired endothelium-dependent relaxation and vascular injury. To counteract ROS- and electrophile-mediated injury, cells can induce a number of genes encoding phase II detoxifying enzymes and antioxidant proteins. A *cis*-acting transcriptional regulatory element, designated as antioxidant response element (ARE) or electrophile response element (EpRE), mediates the transcriptional activation of genes such as heme oxygenase-1, γ-glutamylcysteine synthethase, thioredoxin reductase, glutathione-*S*-transferase and NAD(P)H:quinone oxidoreductase. Other antioxidant enzymes such as superoxide dismutase and catalase and non-enzymatic scavengers such as glutathione are also involved in scavenging ROS. Nuclear factor-erythroid 2-related factor 2 (Nrf2), a member of the Cap 'n' Collar family of basic region-leucine zipper (bZIP) transcription factors, plays an important role in ARE-mediated antioxidant gene expression. Kelch-like ECH-associated protein-1 (Keap1) normally sequesters Nrf2 in the cytoplasm in association with the actin cytoskeleton, but upon oxidation of cysteine residues Nrf2 dissociates from Keap1, translocates to the nucleus and binds to ARE sequences leading to transcriptional activation of antioxidant and phase II detoxifying genes. Protein kinase C (PKC), mitogen-activated protein kinases (MAPKs) and phosphotidylinositol 3-kinase (PI3K) have been implicated in the regulation of Nrf2/ARE signaling. We here review the evidence that the Nrf2/ARE signaling pathway plays an important role in vascular homeostasis and the defense of endothelial and smooth muscle cells against sustained oxidative stress associated with diseases such as atherosclerosis and preeclampsia.

Key words: nuclear factor-erythroid 2-related factor 2; antioxidant response element; oxidative stress; endothelial cells; vascular smooth muscle cells; heme oxygenase; cystine transporter; nitric oxide synthase; antioxidant genes; phase II detoxifying enzymes; atherosclerosis; diabetes; preeclampsia

内皮细胞和平滑肌细胞氧化应激时 Nrf2/ARE 信号通路对抗氧化基因表达的调控：与动脉粥样硬化和先兆子痫的关系

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摘 要：动脉粥样硬化、糖尿病、慢性肾功能衰竭和先兆子痫等血管疾病时活性氧(reactive oxygen species, ROS) 生成增加，容易导致内皮依赖性血管舒张功能的损害和血管损伤，而细胞可以诱导多种编码 II 相解毒酶和抗氧化蛋白的基因表达，从而减轻 ROS 和亲电子物质介导的细胞损伤。一个被称为抗氧化反应元件(antioxidant response element, ARE)或亲电子反应元件(electrophile response element, EpRE)的顺式转录调控元件，可以介导诸如亚铁血红素加氧酶 1、γ- 谷氨酰半胱氨酸合成酶、硫氧还蛋白还原酶、谷胱甘肽 -*S* 转移酶和 NAD(P)H:苯醌氧化还原酶等基因的转录。其他抗氧化酶，如超氧化物歧化酶、过氧化氢酶和非酶清除剂(如谷胱甘肽)等也参与 ROS 的清除。 转录因子 NF-E2 相关因子 2 (nuclear factor-erythroid 2-related factor 2, Nrf2)是属于 Cap 'n' Collar 家族的转录因子，具有碱性亮氨酸拉链(basic region-leucine zipper, bZIP)，它在 ARE 介导的抗氧化基因表达中起重要的作用。在正常情况下，Kelch 样环氧氯丙烷相关蛋白 -1 (Kelch-like ECH-associated protein-1, Keap1)与 Nrf2 耦联，并与肌动蛋白细胞骨架结合被锚定于胞浆，但是在半胱氨酸残基发生氧化的情况下，Nrf2 和 Keap1 解耦联，进入细胞核并与 ARE 结合，从而激活多种抗氧化基因和 II 相解毒酶基因的转录。蛋白激酶 C、丝裂原活化蛋白激酶和磷脂酰肌醇 -3 激酶参与 Nrf2/ARE 信号转导的调控。本文综述了有关 Nrf2/ARE 信号转导通路在血管稳态和动脉硬化、先兆子痫等疾病情况下内皮及平滑肌细胞对抗持续性氧化应激中起的作用。

关键词：转录因子 NF-E2 相关因子 2；抗氧化反应元件；氧化应激；内皮细胞；血管平滑肌细胞；亚铁血红素加氧酶；胱氨酸转运体；一氧化氮合酶；抗氧化基因； II 相解毒酶；动脉粥样硬化；糖尿病；先兆子痫
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Nitric oxide (NO)——a mediator of endothelium-dependent relaxation

In 1980, Furchgott and Zawadzki demonstrated the dependence of acetylcholine-induced vasodilation on an intact endothelium and attributed smooth muscle relaxation to endothelium-derived relaxing factor (EDRF)[1]. Subsequent studies established that the properties and short half-life of EDRF were identical to those of NO, a labile, gaseous vasodilator synthesized from the semi-essential cationic amino acid *L*-arginine by endothelial nitric oxide synthase (eNOS)[2-4] . The discovery that *L*-arginine is the physiological precursor for NO biosynthesis precipitated extensive research into the role of circulating and intracellular *L*-arginine in the function of vascular cells in health and disease[4-8]. As summarized in the schematic model in Fig.1, classical vasoactive agonists such as histamine, bradykinin and thrombin stimulate endothelial NO synthesis via an elevation in intracellular calcium ($[Ca^{2+}]_i$) and Ca^{2+}/calmodulin-dependent activation of eNOS[2,3,9]. In contrast, fluid shear stress, adenosine, β_2-adrenoceptor agonists, 17β-estradiol and soy isoflavones stimulate phosphorylation of eNOS, dissociation of the enzyme from the membrane protein caveolin-1 and association with the chaperone heat shock protein 90 (Hsp90), leading to increased NO production independent of cytosolic Ca^{2+} mobilization[10-14].

We recently reported that feeding aged male rats a soy protein diet, rich in isoflavones genistein and daidzein, increases mRNA expressions of eNOS and antioxidant enzymes, improves endothelium-dependent relaxation and lowers blood pressure *in vivo*[15]. Moreover, feeding a soy isoflavone-rich diet improved agonist-stimulated release of endothelium-derived hyperpolarizing factor (EDHF) and reduced contractile force in isolated resistance vessels[16], most likely as a consequence of elevated basal NO synthesis[15].

Endothelial dysfunction in preeclampsia (PE), diabetes and intrauterine growth retardation (IUGR)

Vascular diseases such as PE, diabetes, chronic renal failure and atherosclerosis are all characterized by increased oxidative stress[8,17-22], and increased production of reactive oxygen species (ROS), such as superoxide anions ($O_2^{\cdot-}$) which scavenge NO, leading to the formation of peroxynitirite, another damaging ROS[23]. Reduced availability of the eNOS cofactor tetrahydrobiopterin and/or substrate *L*-arginine leads to uncoupling of eNOS, resulting in $O_2^{\cdot-}$ rather than NO generation[24,25].

It is well recognized that impaired endothelium-dependent relaxation in the maternal circulation is a hallmark of PE[26,27], with endothelial dysfunction most likely the consequence of elevated plasma lipid peroxides and generation of ROS in the vasculature[19,28-30] with the under-perfused placenta, a likely source of pro-inflammatory mediators[29,30]. PE affects 3%-5% of all pregnancies and is a leading cause of maternal and fetal morbidity and mortality. It is normally defined as the onset of hypertension and proteinuria after 20 weeks of gestation in previously normotensive and non-proteinuric pregnant women[29,30]. ROS-induced damage to lipids and proteins in PE results in increased hemolysis, liver damage and low platelet count (HELLP syndrome). Endothelial dysfunction in PE may be the consequence of diminished NO bioavailability (secondary to oxidative degradation) and an excess of peroxynitrite[31]. Elevated

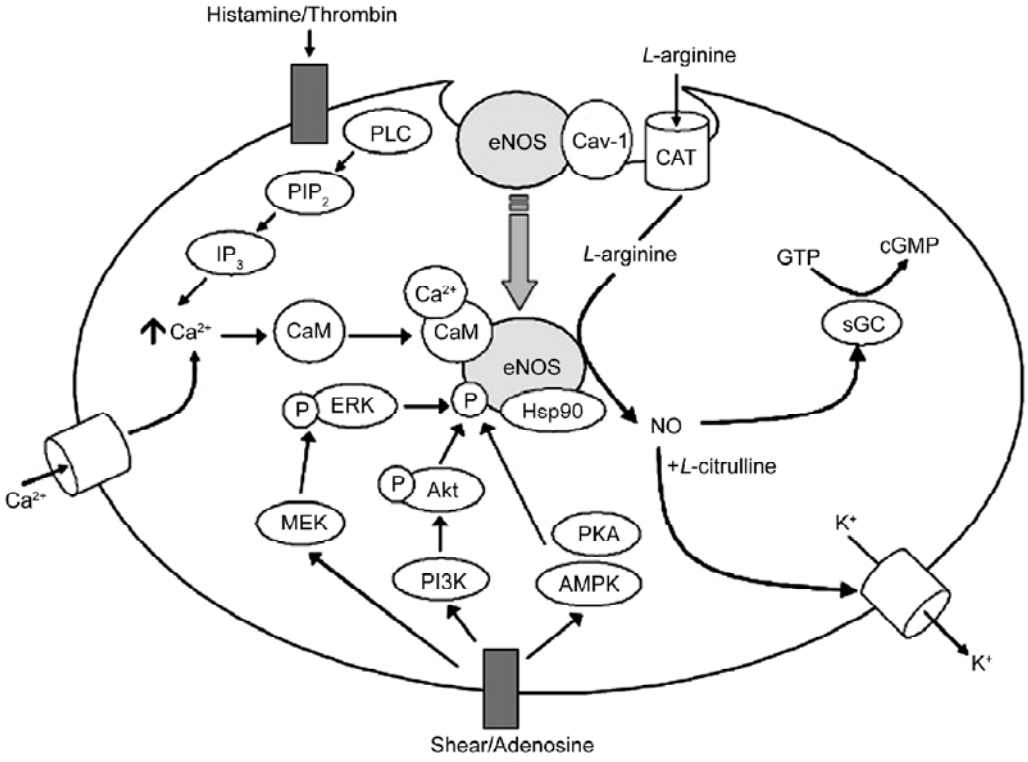

Fig. 1. Regulation of endothelial nitric oxide synthase (eNOS) via Ca^{2+}-dependent and Ca^{2+}-insensitive pathways. Association of eNOS with caveolin-1 (Cav-1) in plasma membrane caveolae maintains the enzyme in an inactive state. Ca^{2+} mobilizing agonists such as histamine, bradykinin or thrombin result in a Ca^{2+}-calmodulin (CaM)-dependent dissociation of eNOS from Cav-1 and association with the chaperone heat shock protein 90 (Hsp90), leading to post-translational phosphorylation of eNOS and synthesis of NO and *L*-citrulline from the cationic amino acid *L*-arginine. Laminar shear stress and Ca^{2+}-independent agonists (e.g. adenosine, 17β-estradiol, isoflavones)[5,12,13] activate the phosphoinositiol 3-kinase (PI3K)/protein kinase B (Akt) and extracellular-regulated kinase 1/2 (ERK1/2) and/or AMP-activated protein kinase (AMPK)/protein kinase A (PKA) signaling pathways, leading to eNOS phosphorylation and association with Hsp90 at basal cytosolic Ca^{2+} levels. We have shown that generation of NO in response to adenosine activates outward K^+ currents, leading to a membrane hyperpolarisation which in turn stimulates uptake of *L*-arginine via cationic amino acid transporters (CAT)[4] expressed in plasma membrane caveolae[12]. NO activates soluble guanylyl cyclase (sGC) in endothelial cells to increase cGMP levels, which we have used to assay NO production (inhibitable by NOS inhibitors such as *L*-NAME or *L*-NMMA)[5,9,12,13,38]. Diffusion of NO to smooth muscle cells will also increase cGMP levels, modulating protein kinase G (PKG), ion channels, cGMP-activated phosphodisterases (PDE) and vascular tone[2,3].

nitrite/nitrate concentrations have been detected in umbilical vein blood in PE[32], and these authors hypothesized that an increase in feto-placental NO production may compensate for diminished utero-placental blood flow. However, in PE-affected pregnancies, eNOS expression/activity is either unchanged, decreased or increased in placental villous tissue[31,33,34] and regulation of eNOS by tetrahydrobiopterin is impaired[35]. Expression of eNOS protein in placental villous tissue decreases during normal pregnancy. Although eNOS protein levels appear to be diminished in umbilical artery endothelium in pregnancies affected by PE or IUGR[34], we have not detected differences in eNOS expression in fetal endothelial cells isolated from normal, preterm and PE pregnancies[36].

We have reported that PE is associated with alterations in Ca^{2+} regulation, cation permeability and NO production in human umbilical vein endothelial cells (HUVECs)[36]. Ca^{2+} influx was markedly inhibited in HUVECs derived from PE pregnancies (Fig.2*A*), yet paradoxically both basal and histamine-stimulated cGMP production (as an index of NO synthesis) were elevated in PE endothelial cells (Fig.2*B*). Increased cGMP levels in HUVECs from PE pregnancies may reflect activation of soluble guanylyl cyclase (sGC) by NO and/or lipid hydroperoxides, known to be elevated in PE[19,28-30]. As changes in cation permeability and cGMP accumulation persisted in culture, this implies that PE induces phenotypic alterations in the fetal vasculature[36]. Our studies with fetal vascular smooth muscle cells (SMCs) *in vitro* provide further evidence of vascular dysfunction in PE[37]. Arachidonic acid-stimulated Ca^{2+} signaling was

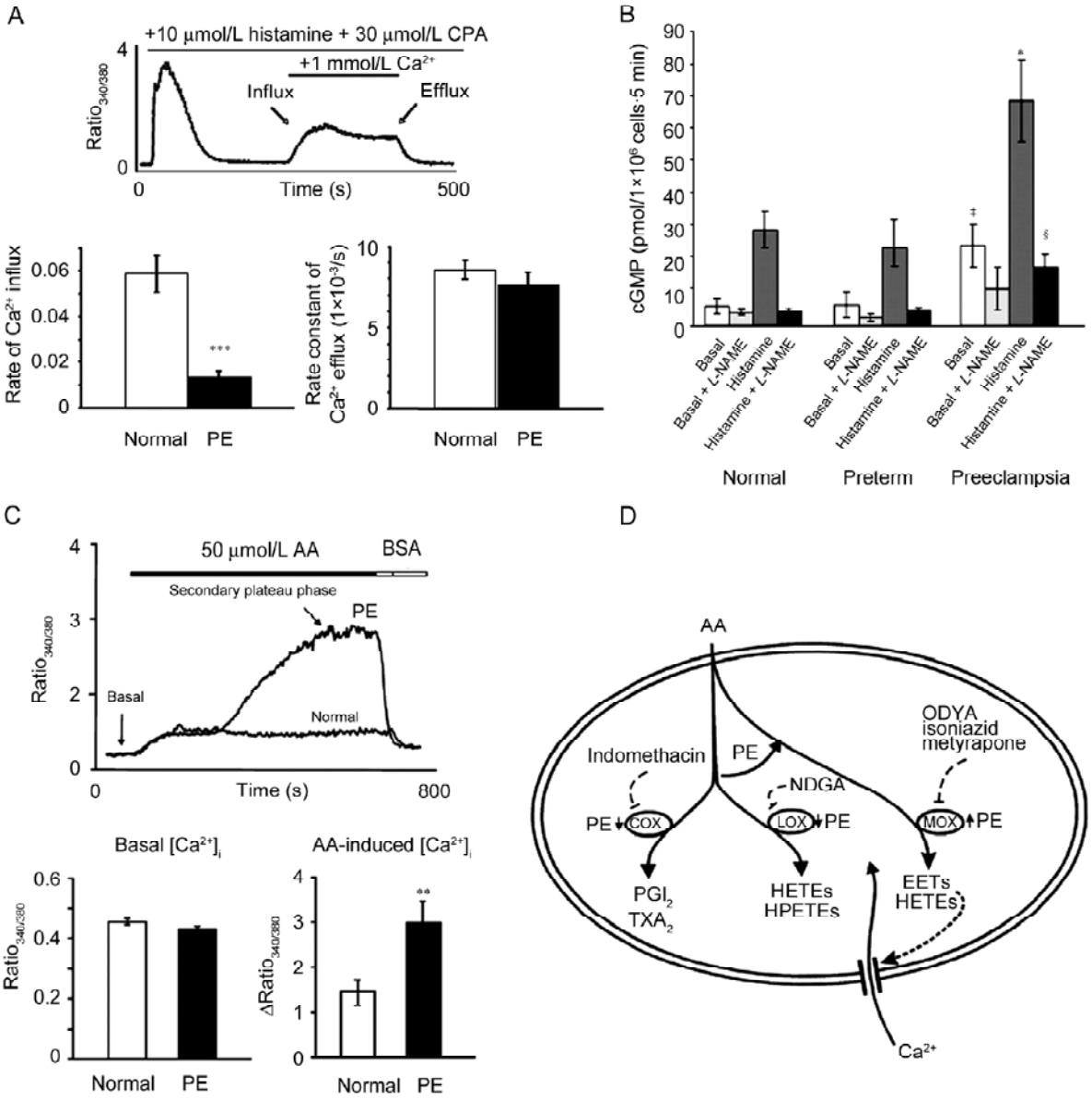

Fig.2. Oxidative stress associated with preeclampsia (PE) modulates Ca^{2+} mobilization and NO production in fetal endothelial and smooth muscle cells isolated from human umbilical cords at term. *A*: Human umbilical vein endothelial cells (HUVECs) from normal pregnancies were store-depleted in Ca^{2+}-free solution with 10 μmol/L histamine in the presence of 30 μmol/L cyclopiazonic acid (CPA, Ca^{2+}-ATPase inhibitor) to avoid internal Ca^{2+} store refilling. After a brief pulse of extracellular 1 mmol/L Ca^{2+} was applied, the rate of rise of Ca^{2+}, Ca^{2+} peak and plateau levels, and rate constant for Ca^{2+} removal were measured. Influx rates and efflux rate constants in normal and PE endothelial cells were compared. means±SEM of 9 normal and 6 PE cultures. *B*: Basal and histamine-stimulated cGMP accumulation in HUVECs (100 μmol/L *L*-arginine, 0.5 mmol/L 3-isobutyl-1-methyl-xanthine in the absence or presence of *L*-NAME (100 μmol/L). means±SEM of 3 normal, 4 preterm and 3 PE cell cultures. $^{‡}P<0.02$ *vs* normal basal, $^{*}P<0.02$ *vs* normal histamine, $^{§}P<0.02$ *vs* normal histamine + *L*-NAME. Data replotted from Steinert *et al* [36]. *C*: Arachidonic acid (AA) induced increases in intracellular Ca^{2+} in normal and PE umbilical artery smooth muscle cells. In the presence of extracellular Ca^{2+}, AA (50 μmol/L)-evoked increases in intracellular Ca^{2+} were significantly augmented in PE cells whereas basal Ca^{2+} levels were not significantly different. means±SEM of 3 replicate measurements from 16 normal and 19 PE cultures. $^{**}P<0.009$ *vs* normal cells. *D*: Schematic model summarizing AA-induced Ca^{2+} mobilization in fetal smooth muscle cells from PE pregnancies. AA is metabolized through the cyclooxygenase (COX), lipoxygenase (LOX) and monooxygenase (MOX) pathways. In PE, the balance swings from AA metabolism via the COX and LOX pathways to the MOX pathway, either because COX and LOX are down-regulated or because MOX is up-regulated. Increased production of MOX metabolites in turn stimulates Ca^{2+} entry, which can be reversed by inhibiting the MOX pathway. Data replotted from Steinert *et al*[37].

modulated differentially in fetal vascular SMCs derived from PE pregnancies, with arachidonic acid-induced Ca^{2+} influx increased significantly compared to that in normal SMCs (Fig.2*C*). The enhanced influx of Ca^{2+} in PE cells was mimicked in normal umbilical artery SMCs by inhibition of arachidonic acid metabolism via cyclooxygenase and/or lipoxygenase pathways (Fig.2*D*). Based on these findings, we concluded that the potentiation of arachidonic acid-induced Ca^{2+} influx was due to a monooxygenase (MOX) metabolite, since inhibition of the MOX signaling pathway by structurally dissimilar compounds inhibited the enhanced Ca^{2+} influx in indomethacin-treated normal SMCs and in arachidonic acid-challenged PE SMCs[37].

We and colleagues have also described phenotypic changes in fetal endothelial cells isolated from pregnancies affected by either gestational diabetes or IUGR[6,38-40]. Endothelial cells isolated from gestational diabetic pregnancies exhibit a membrane hyperpolarization and enhanced *L*-arginine transport and NO synthesis which persist in culture *in vitro*[6,38]. However, it is worth noting that eNOS activity is reduced in fetal endothelial cells cultured from IUGR pregnancies[40]. Increased oxidative stress in pregnancy-related diseases may thus have important implications for long-term 'programming' of the fetal cardiovascular system[41-43], as implied by a study in which men whose mothers suffered from PE were at risk of developing hypertension in adulthood[44].

ROS as intracellular signaling molecules and activators of antioxidant gene expression

Under physiological conditions, ROS are short-lived molecules generated as by-products of normal aerobic metabolism and can modulate the intracellular signaling pathways involved in the control of vascular function[45-52]. Excessive ROS generation causes damage to membrane lipids, proteins and DNA, and impairs endothelium-dependent relaxation[53]. Endothelial and smooth muscle cells can generate O_2^- and hydrogen peroxide (H_2O_2) from xanthine oxidase, peroxidases, lipoxygenase, cyclooxygenases, NOS and NAD(P)H oxidases[45], with membrane associated NAD(P)H oxidase(s) serving as a primary source of ROS in vascular diseases[46-48,50-52,54].

Redox systems located in the vicinity of the plasma membrane provide protection against damage induced by environmental oxidants. Cells have evolved antioxidant defenses including phase II detoxifying and antioxidant enzymes, as well as, non-enzymatic scavengers of ROS and metal ions[45]. Dismutation of O_2^- by cytosolic copper-zinc superoxide dismutase (CuZnSOD), mitochondrial MnSOD and extracellular CuZnSOD generates H_2O_2, which is converted to H_2O and O_2 by catalase and glutathione peroxidase. Enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione-*S*-transferases (GST), γ-glutamylcysteine synthethase (GCS), thioredoxin reductases and heme oxygenases (HO) metabolize ROS and toxic compounds to readily exportable forms. Peroxiredoxins (Prxs) are a family of antioxidant proteins that use thioredoxin as an electron donor, scavenge H_2O_2 and thereby also play a key role in cellular antioxidant defenses[55,56]. NQO1, a two electron quinone reductase, maintains the reduced state of ubiquinones to enhance antioxidant defenses[57]. HO-1 is a microsomal enzyme induced in oxidative stress to metabolise heme to biliverdin, carbon monoxide and iron[58,59]. Biliverdin is subsequently converted by biliverdin reductase to bilirubin, an antioxidant which can scavenge lipid peroxyl radicals while iron is sequestered by ferritin. Carbon monoxide has both anti-apoptotic and anti-inflammatory properties and may act as a vasodilator in atherogenesis when bioavailability of NO is diminished due to inactivation by ROS[59]. Moreover, HO-1 has been identified in human atherosclerotic lesions, and adenoviral overexpression of HO-1 in rodent models of vascular disease protects against both atherogenesis and restenosis[60-62].

Transcriptional activation of antioxidant genes by the nuclear factor-erythroid 2-related factor 2 (Nrf2)/ antioxidant response element (ARE) signaling pathway

Nrf2, a member of the Cap 'n' Collar family of basic region-leucine zipper (bZIP) transcription factors, is an important regulator of ARE-activated gene expression (Fig.3)[63-68]. Under normal physiological conditions, Nrf2 is bound to Kelch-like ECH-associated protein-1 (Keap1) and thereby sequestered in the cytoplasm in association with the actin cytoskeleton[64-68]. In oxidative or xenobiotic stress, Nrf2 is released from Keap1 and rapidly translocates to the nucleus, where it binds to ARE sequences leading to transcriptional activation of antioxidant genes such as HO-1, Prx I, thioredoxin-1 and the cystine-glutamate anionic amino acid transporter (xCT)[63]. Nuclear translocation and export of Nrf2 have been reported to be modulated by phosphorylation via mitogen-activated protein kinases (e.g. ERK1/2, p38 MAPK) and/or protein kinase C[69-74]. The importance of Nrf2 in the defense against oxidative stress is highlighted by the increased sensitivity

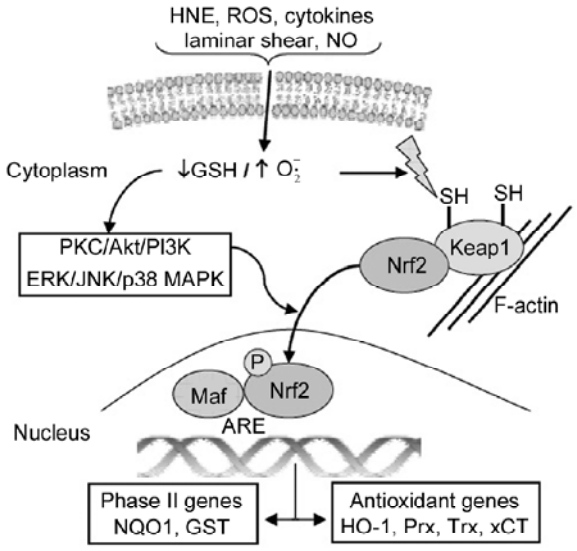

Fig.3. Activation of the Nrf2/ARE pathway by oxidized low density lipoproteins, 4-hydroxy-2-nonenal (HNE), reactive oxygen species (ROS) and pro-inflammatory cytokines. Increased generation of ROS depletes intracellular glutathione (GSH) levels, leading to increased oxidation of thiol groups on cellular proteins. Keap1 retains Nrf2 in the vicinity of the proteosome, thereby enhancing its degradation in the cytoplasm of quiescent cells. Under conditions of increased oxidative or xenobiotic stress, Nrf2 dissociates from Keap1, translocates to the nucleus, binds to antioxidant responsive element (ARE) sequences in association with other members of the basic leucine zinc zipper transcription factor family, such as Maf G or Maf K, resulting in transcriptional activation of phase II detoxifying enzymes and antioxidant genes such as heme oxygenase-1 (HO-1), peroxiredoxin-1 (Prx I), thioredoxin (Trx) and the cystine-glutamate anionic amino acid transporter (xCT). In some cell types, Nrf2 translocation and nuclear export have been found to be modulated by phosphorylation via mitogen-activated protein kinases (e.g. ERK1/2, p38 MAPK) or protein kinase C (PKC)[69-74]. Schematic model adapted from references[63,64,70].

in Nrf2-deficient mice to carcinogenesis due to the loss of induction of ARE-regulated drug metabolising enzymes and antioxidant genes[75,76].

Induction of antioxidant genes in atherosclerosis

Enhanced oxidation of low density lipoproteins (LDL) within the vascular wall induces the formation of foam cells that accumulate cholestrol[77]. Oxidatively modified LDL contains high levels of lipid peroxides, lysophosphatidylcholine, oxysterols and aldehydes, which cause vascular inflammation, fibrosis and induction of anti-inflammatory genes in the vessel wall. We previously reported that moderately oxidized LDL, rich in lipid hydroperoxides, induces antioxidant responses in vascular SMCs, e.g. up-regulation of *L*-cystine transport, glutathione biosynthesis and HO-1 expression[78-81]. Pretreatment of SMCs with physiological concentrations of vitamin C afforded protection against glutathione depletion in response to moderately oxidized LDL, electrophilic agents and hypochlorous acid[78,80,82,83]. We subsequently established that moderately oxidized LDL rapidly stimulates phosphorylation of p38 MAPK, ERK1/2 and c-jun-N-terminal kinase (JNK), leading to transcriptional activation of HO-1 expression via the Nrf2/ARE signaling pathway[81].

Components of oxidized LDL activate the peroxisome proliferator-activated receptor-γ (PPAR-γ) signaling pathway, leading to an up-regulation of the major LDL scavenger receptor CD36[84,85]. Our recent studies in oxidized LDL-treated murine macrophages provided the first evidence that, in addition to PPAR-γ, ROS-mediated activation of Nrf2 also up-regulates CD36 expression[86]. Moreover, treatment of aortic SMCs from Nrf2 heterozygous mice with 4-hydroxynonenal, a major end product of lipid oxidation involved in the rapid activation of intracellular kinase cascades[87], enhanced Nrf2 nuclear translocation and expression of HO-1 and Prx I, which was abolished in SMCs derived from Nrf2-deficient mice[86]. Thus, Nrf2 serves as a second transcription factor to regulate CD36 and antioxidant defense gene expression in atherosclerosis[86]. The potential cross-talk between the Nrf2 and PPAR-γ signaling pathways merits further study and may provide a basis for therapeutic strategies to treat atherosclerosis.

Laminar shear stress- and NO-mediated regulation of the Nrf2/ARE signaling pathway

Activation of the Nrf2/ARE signaling pathway by NO and peroxynitrite is associated with an up-regulation of HO-1[88-92]. Laminar shear stress and mechanical stimuli enhance endothelium-derived NO production[93-97], and recent studies in endothelial cell monolayers subjected to steady laminar or oscillatory flow have shown that laminar shear stress is associated with an activation of the Nrf2/ARE signaling pathway[89,98,99]. In view of the ability of NO to activate Nrf2-regulated HO-1 expression, it is intriguing that inhibition of eNOS activity had no effect on laminar flow-mediated induction of Nrf2-dependent genes, although inhibition of xanthine oxidase, NAD(P)H oxidase and mitochondrial respiration diminished Nrf2/ARE-mediated gene expression[99]. Under conditions of oscillatory flow, activation of Nrf2 and induction of atheroprotective genes such as HO-1 and NQO1 are suppressed[89,98] and may thereby

contribute to the progression of atherosclerotic lesion formation *in vivo*. Sustained superoxide generation in endothelial cells exposed to oscillatory flow has been implicated in the inactivation of NO and subsequent ROS-dependent up-regulation of eNOS mRNA levels[100]. It is generally accepted that disturbed or oscillatory flow near arterial bifurcations is associated with enhanced atheroma formation[93-97], and hence limited bioavailability of NO may in part account for reduced Nrf2-mediated atheroprotective gene expression in blood vessels exposed to oscillatory flow *in vivo*.

Concluding remarks

The Nrf2/ARE pathway plays an essential role in mediating atheroprotective gene expression in vascular endothelial and smooth muscle cells, and studies *in vitro* have provided valuable insights into potential abnormalities underlying ROS-mediated vascular dysfunction in atherosclerosis and PE[101]. Laminar shear stress modulates NO production and Nrf2/ARE-regulated gene expression in endothelial cells *in vitro*, however, further studies are required to establish whether laminar flow activates Nrf2 *in vivo* to provide protection against inflammation and the progression of atherosclerosis.

Interestingly, impaired uteroplacental blood flow in PE has been attributed in part to an absence of shear stress-induced NO release from myometrial resistance arteries[27]. Moreover, decreased expression of the constitutive HO-2 isoform in endothelial cells derived from PE and IUGR-affected pregnancies has been implicated as a causal factor in reduced placental blood flow under these conditions[102]. These observations are also consistent with reports of diminished eNOS expression and/or activity in placental villous tissue and fetal umbilical blood vessels. It has recently been hypothesized that women at risk of PE may be deficient in glucose-6-phosphate dehydrogenase (G6PD)[103]. In this context, decreased activity of G6PD in endothelial cells is associated with oxidative stress due to a lack of NADPH and reduced NO bioavailability[104], and overexpression of G6PD protects endothelial cells by maintaining NADPH and glutathione levels, decreasing ROS generation and increasing NO bioavailability[105]. Our ongoing studies of Nrf2-regulated antioxidant gene expression in fetal umbilical endothelial and smooth muscle cells suggest that sustained oxidative stress during the course of PE leads to impaired nuclear translocation of Nrf2 and transcriptional activation of antioxidant defense genes such as HO-1 and Prx I[106,107]. We recently reported the first evidence that G6PD activity is decreased in umbilical vein endothelial cells from PE pregnancies. Moreover, diminished G6PD activity was associated with impaired redox regulation in both maternal erythrocytes and fetal endothelial cells[108]. Impaired G6PD activity in PE would affect redox regulation in endothelial cells and could potentially account for reduced Nrf2/ARE-mediated gene transcription in pregnancies affected by sustained oxidative stress.

The impact of an 'oxidative' intrauterine environment on fetal and long-term adult cardiovascular health remains to be investigated. In order to validate the hypothesis that sustained oxidative stress *in utero* leads to programming of cardiovascular disease in adulthood, it would be important to obtain long-term measurements of endothelial function and the redox status of circulating blood cells in infants born to women with PE, IUGR or gestational diabetes. Further studies are warranted to evaluate the therapeutic potential of modulating the Nrf2/ARE signaling pathway by dietary compounds to ameliorate the progression of atherosclerosis and other diseases associated with oxidative stress.

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