

Oxidized Phospholipids Regulate Expression of ATF4 and VEGF in Endothelial Cells via NRF2-Dependent Mechanism: Novel Point of Convergence Between Electrophilic and Unfolded Protein Stress Pathways

Taras Afonyushkin, Olga V. Oskolkova, Maria Philippova, Therese J. Resink, Paul Erne, Bernd R. Binder, Valery N. Bochkov

Objective—The ATF4 arm of the unfolded protein response is increasingly recognized for its relevance to pathology, and in particular to angiogenic reactions. Oxidized phospholipids (OxPLs), known to accumulate in atherosclerotic vessels, were shown to upregulate vascular endothelial growth factor (VEGF) and induce angiogenesis via an ATF4-dependent mechanism. In this study, we analyzed the mechanism of ATF4 upregulation by OxPLs and more specifically the involvement of NRF2, the major transcriptional mediator of electrophilic stress response.

Methods and Results—Using reverse transcription/real-time polymerase chain reaction and Western blotting, we found that OxPLs induced upregulation of ATF4 mRNA and protein in several types of endothelial cells and that these effects were suppressed by short interfering RNA (siRNA) against NRF2. Electrophilic (iso)prostaglandins and oxidized low-density lipoprotein, similarly to OxPLs, elevated ATF4 mRNA levels in an NRF2-dependent mode. Chromatin immunoprecipitation revealed OxPL-dependent binding of NRF2 to a putative antioxidant response element site in the *ATF4* gene promoter. Knockdown of NRF2 inhibited OxPL-induced elevation of VEGF mRNA and endothelial cell sprout formation.

Conclusion—Our data characterize NRF2 as a positive regulator of ATF4 and identify a novel cross-talk between electrophilic and unfolded protein responses, which may play a role in stress-induced angiogenesis. (*Arterioscler Thromb Vasc Biol.* 2010;30:1007-1013.)

Key Words: oxidized phospholipids ■ NRF2 ■ ATF4 ■ vascular endothelial growth factor ■ angiogenesis

Oxidation of unsaturated fatty acids esterified in phospholipids generates a group of mediators demonstrating multiple biological effects, including proinflammatory, thrombogenic, antiendotoxin, and immunomodulating activities.¹ Previously, we characterized the angiogenic activity of oxidized phospholipids (OxPLs) and showed that induction of vascular endothelial growth factor (VEGF) is an important, although not exclusive, mechanism of angiogenic switch initiated by OxPLs.² The analysis of signaling pathways activated by OxPLs identified ATF4 as the major regulator of VEGF expression in OxPL-treated cells.³ ATF4 is a key transcriptional effector of cellular stress reaction generally known as unfolded protein response (UPR), which is induced by accumulation of improperly folded proteins.⁴ In addition, ATF4 is upregulated by deficit of amino acids, oxidative stress, and other deleterious conditions, and therefore the ATF4 branch of UPR is often referred to as integrated stress response.⁵ The ATF4 pathway is initiated by stress-activated protein kinases, eg, eukaryotic translation initiation factor 2- α kinase 3 (PERK), which phosphorylates

eukaryotic translation initiation factor 2, subunit 1 α (eIF2 α), leading to general inhibition of protein synthesis but selective translation of ATF4.⁶

A separate stress pathway that protects cells from chemically reactive compounds is the electrophilic stress response (ESR). Canonical activation of ESR is initiated through oxidative modification of KEAP-1 by electrophiles,⁷ leading to dissociation of NRF2/KEAP-1 complexes⁸ and enhanced transcription of genes having NRF2-binding antioxidant response elements (AREs) in their promoter regions,⁹ eg, heme oxygenase-1, known for its antioxidant, antiinflammatory, and proangiogenic properties.^{10,11} In addition to sensing electrophilic stress, NRF2 is thought to be of key importance for antioxidant and antiinflammatory effects of shear stress in atherosclerosis-resistant areas of vessels.¹² However, the general role of NRF2 in atherogenesis may be more complex, because knockout of *Nrf2* inhibits development of atherosclerosis.¹³

OxPLs were shown previously to activate both ATF4- and NRF2-dependent transcription.^{14–16} Activation of NRF2 may

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From the Department of Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Austria (T.A., O.V.O., B.R.B., V.N.B.); Department of Biomedicine, Basel University Hospital, Switzerland (M.P., T.J.R., P.E.); Division of Cardiology, Cantonal Hospital Luzern, Switzerland (P.E.).

Correspondence to Valery N. Bochkov, PhD, Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, Schwarzschanerstrasse 17, 1090 Vienna, Austria. E-mail valery.bochkov@meduniwien.ac.at

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be explained either by direct electrophilic attack of reactive OxPL species or by activation of enzymes producing oxidizing and electrophilic molecules, eg, NADPH oxidase or endothelial NO synthase,^{17,18} the detailed mechanisms of ATF4 upregulation by OxPLs are not known. In this work, we analyzed intracellular signaling mechanisms whereby OxPLs stimulate expression of ATF4, leading to an angiogenic switch. Unexpectedly, we found that induction of ATF4 by OxPLs significantly depends on NRF2 activity. These data point to the existence of a previously unidentified link between UPR and ESR pathways and suggest that NRF2 can promote pathological effects induced via ATF4.

Experimental Procedures

A detailed description of materials and methods is presented in the Supplemental Data, available online at <http://atvb.ahajournals.org>.

Lipids

Arachidonate-containing synthetic lipids were purchased from Avanti Polar Lipids. Dry lipids were oxidized by exposure to air and analyzed by mass spectrometry.¹⁹ The dried lipids were resuspended by vigorous vortexing in medium 199 supplemented with 2% FCS before use in cell culture experiments.

mRNA and Protein Analysis

mRNAs levels were quantified by reverse-transcription/quantitative real-time polymerase chain reaction and were normalized to β_2 -microglobulin mRNA levels.

Western Blotting

Protein samples were separated on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membrane by electroblotting. Membranes were probed with rabbit antibodies against ATF4, NRF2, PERK, phospho-eIF2 α , total eIF2 α , β -actin, and peroxidase-conjugated secondary antibody.

RNA Interference

Cells were transfected with 100 nmol/L of short interfering RNA (siRNA) using polyethylenimine (PEI) reagent.²⁰ Efficiency of silencing was checked at the mRNA and/or protein level.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation analysis was performed using EZ-Chip kit (Upstate Biotechnology). Purified immunoprecipitated and input DNA was analyzed by polymerase chain reaction. Polymerase chain reaction products were resolved on polyacrylamide gels and stained with ethidium bromide.

NRF2 Binding Competition Assay

NRF2-DNA interactions were also characterized using the ELISA-based TransAM NRF2 kit (Active Motif). Nuclear extracts from untreated human umbilical vein endothelial cells (HUVECs) and cells stimulated with oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC) were prepared using the NE-Per kit (Pierce).

Spheroid Assay of Angiogenesis In Vitro

After transfection with siRNA, cell spheroids were formed and embedded in fibrin gels as described.² Polymerized gels were overlaid with medium 199 supplemented with 2% FBS with or without inclusion of OxPAPC (130 μ mol/L). After 24 hours, gels were fixed and actin structures were visualized with tetramethylrhodamine B isothiocyanate-conjugated phalloidin (Sigma-Aldrich).

Statistical Analysis

The data are representative for 2 to 4 independent experiments and are expressed as mean \pm SD of triplicate or quadruplicate measurements. The analysis was performed by the 2-tailed Student *t* test. A probability value below 0.05 was considered as significant.

Results

OxPLs Upregulate ATF4 mRNA Levels

The classic mechanism of ATF4 upregulation during UPR is based on selective translation resulting from phosphorylation of eIF2 α by PERK.²¹ Our previous kinetic measurements showed that the levels of eIF2 α phosphorylation and ATF4 protein in HUVECs started to increase after 1 to 2 hours of treatment with OxPAPC.³ In this study, we characterized an additional mechanism of ATF4 regulation by OxPLs. OxPAPC induced an elevation of ATF4 mRNA that began after 1 hour and was sustained for at least 6 hours after addition of the lipid to HUVECs (Figure 1A). Thus, elevation of ATF4 mRNA coincided with the time course of phosphorylation of eIF2 α ,³ expected to induce selective translation of ATF4. Therefore, elevated levels of ATF4 mRNA are likely to contribute to the increased synthesis of ATF4 protein.

Elevation of ATF4 mRNA induced by OxPAPC in HUVECs (Figure 1B) developed within the range of concentrations previously shown to induce ATF4-dependent elevation of VEGF and stimulation of angiogenic reactions.^{2,3} Human coronary artery and uterine microvascular endothelial cells were also sensitive to OxPAPC, demonstrating statistically significant elevation of ATF4 mRNA at concentrations above 3 μ mol/L (Figure 1C). OxPLs with different head groups induced comparable elevation of ATF4 mRNA (Figure 1B), which agrees with previous findings that different classes of OxPLs can elevate ATF4 protein and its downstream target VEGF.^{2,3} These data further support our hypothesis that oxidized fatty acid residues rather than polar head groups are critical for the angiogenic activity of OxPLs,^{2,3} in turn suggesting that different OxPL classes formed in vivo can induce an angiogenic switch in an additive manner.

Elevation of ATF4 mRNA levels was induced by representative and abundant molecular species of OxPLs containing fragmented and full-length oxidized *sn*-2 residues (Figure 1D), further suggesting that the combined concentration of all active classes and molecular species of OxPLs may be of higher biological relevance than levels of individual species. Because the most active of these species contained electrophilic groups, we tested whether the induction of ATF4 mRNA may be modulated by OxPLs via the NRF2 pathway characteristic of ESR.¹⁵

NRF2 Is Involved in OxPAPC-Induced Upregulation of ATF4 mRNA and Angiogenic Reactions

In support of our hypothesis about involvement of the NRF2 pathway in upregulation of ATF4 mRNA, we found that reduction of electrophilic groups in OxPAPC by sodium borohydride resulted in statistically significant attenuation of ATF4 mRNA elevation (Figure 2A). Thus,

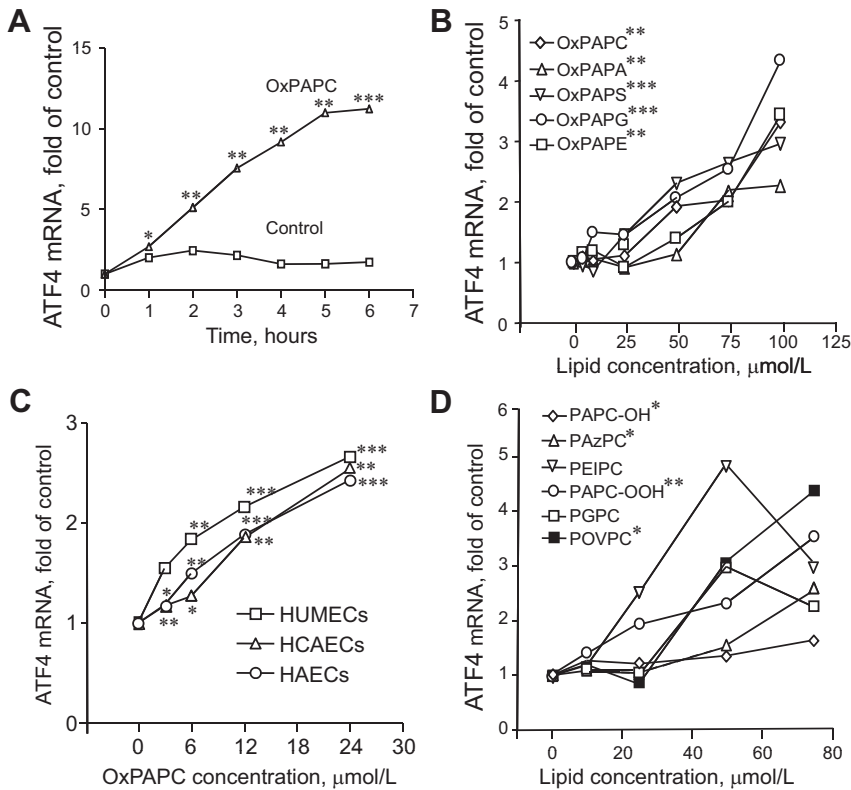


Figure 1. OxPLs upregulate ATF4 mRNA. A, Kinetics of ATF4 mRNA elevation induced by OxPAPC. HUVECs were stimulated in quadruplicate with 130 $\mu\text{mol/L}$ OxPAPC for the indicated time periods. The treatment was stopped by the addition of Trizol. Levels of ATF4 mRNA were measured by quantitative real-time polymerase chain reaction (qRT-PCR) and normalized to the β_2 -microglobulin mRNA levels. The figure shows only mean values. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus time-matched controls. B, Concentration dependence of ATF4 mRNA elevation in HUVECs treated with different classes of OxPLs. OxPLs were generated by oxidation of phospholipid precursors containing *sn*-1-palmitic and *sn*-2-arachidonic acids and the following head groups: phosphocholine (OxPAPC), phosphoserine (OxPAPS), phosphoglycerol (OxPAPG), phosphoethanolamine (OxPAPE) or phosphate (PxPAPA). Cells were treated with OxPLs at the indicated concentrations for 4 hours. Thereafter, mRNA from combined quadruplicates was analyzed as described in A. Asterisks indicate statistically significant positive correlation between OxPL concentrations and ATF4 mRNA levels (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). C, Concentration dependence of ATF4 mRNA elevation in human carotid artery (HCAECs), human aortic (HAECs), and human uterine microvascular (HUVECs) endothelial cells treated with OxPAPC for 4 hours. The analysis was performed in quadruplicate; only mean values are shown. D, Elevation of ATF4 mRNA induced by different molecular species of phosphatidylcholine containing *sn*-1-palmitic and the following oxidized *sn*-2 residues: azelaic acid (PAzPC), 5,6-epoxy isoprostane E2 (PEIPC), glutaric acid (PGPC), (5-oxo)valeric acid (POVPC), monohydroperoxide (PAPC-OOH) and monohydroxide (PAPC-OH). Stimulation and analysis were performed as described in B.

electrophilic properties of OxPLs seem to be important determinants of their ability to increase ATF4 mRNA. Typical inducers of electrophilic stress, such as butylated hydroxyanisole and cyclopentenone (iso)prostaglandins, also elevated levels of ATF4 mRNA (Figure 2A), further supporting the role of NRF2 as a positive modulator of the ATF4 pathway.

Pretreatment of cells with siRNA against NRF2, but not with scrambled siRNA, consistently resulted in inhibition of OxPAPC-induced upregulation of ATF4 mRNA, as well as glutamate-cysteine ligase, modifier subunit (GCLM), which is a classic NRF2-activated gene (Figure 2B; Supplemental Figures I and III). siRNA targeting a nonoverlapping site in NRF2 mRNA produced a comparable decrease in OxPAPC-induced ATF4 mRNA elevation in HUVECs, from 6.53 ± 3.40 -fold to 1.94 ± 1.17 -fold ($P < 0.036$), pointing to the specificity of the effect of NRF2 silencing. The effect did not result from toxic action of lipids or siRNA, because OxPAPC either alone or after the transfection procedure did not significantly influence the levels of housekeeping protein actin in 3 endothelial cell types 48 hours after transfection (Figure 3A and 3B), nor induction by OxPAPC of other genes, eg, COX-2 (Supplemental Figure IV). Furthermore, mRNAs encoding ATF4 and classic electrophilic genes GCLM and GCLC were upregulated by oxidized (but not native) low-density lipoprotein (Supplemental Figure IIA through IIC), and this elevation was suppressed by siRNA NRF2

(Figure 2C). Similarly, induction of ATF4 mRNA by electrophilic isoprostane also depended on NRF2 (Figure 2C).

Expression of ATF4 Protein in Response to OxPAPC Is Mediated by Enhancement of Both Transcription and Translation

In addition to the inhibition of OxPAPC-induced ATF4 mRNA elevation, silencing of NRF2 resulted in decreased levels of ATF4 protein (Figure 3A and 3B). NRF2 knockdown did not influence accumulation of ATF4 protein induced by classic UPR agonist tunicamycin and did not reduce phosphorylation of eIF2 α induced by OxPAPC, further confirming specificity of the inhibitory effect (Figure 3A).

Because OxPLs induced phosphorylation of eIF2 α (Gargalovic et al¹⁴ and Figure 3A), we further tested the role of the PERK-eIF2 α mechanism in OxPAPC-induced elevation of ATF4 protein. Knockdown of PERK using siRNA resulted in a $74\% \pm 17\%$ decrease of PERK mRNA. Silencing of PERK induced no change in ATF4 mRNA (Figure 3C), but it inhibited elevation of ATF4 protein (Figure 3D) and its downstream gene VEGF in response to OxPAPC (Figure 3C). These data support the notion that OxPL-induced elevation of ATF4 protein (and its downstream genes) involves 2 parallel mechanisms: NRF2-dependent elevation of ATF4 mRNA and concomitant enhanced translation of this mRNA because of PERK-dependent phosphorylation of eIF2 α .

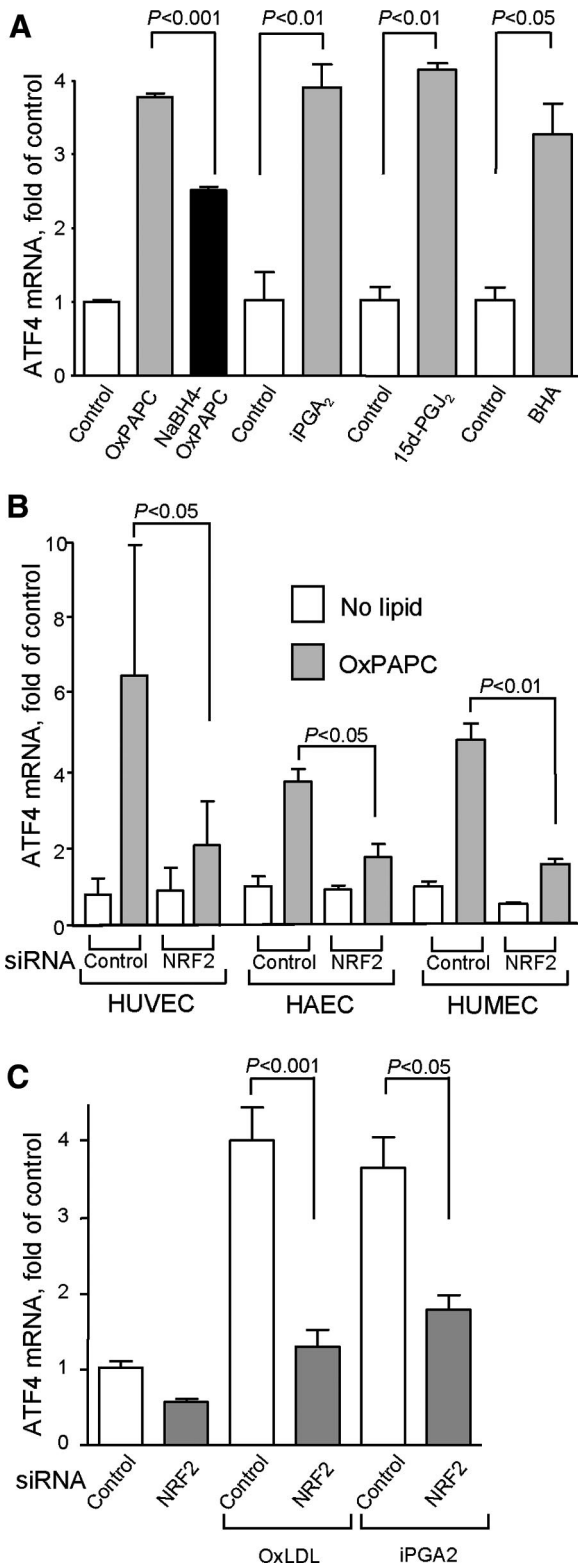


Figure 2. Upregulation of ATF4 mRNA by OxPAPC depends on NRF2. A, Electrophilic properties are important for elevation of ATF4 mRNA. Reduction by sodium borohydride of electrophilic groups in OxPAPC (130 $\mu\text{mol/L}$, 6 hours) inhibited elevation of ATF4 mRNA, whereas the electrophilic compounds 8-iso-PGA2 (50 $\mu\text{mol/L}$, 6 hours), 5-deoxy- Δ 12,14-PGJ2 (50 $\mu\text{mol/L}$, 6 hours), and butylated hydroxyanisole (BHA, 200 $\mu\text{mol/L}$) elevated ATF4 mRNA levels. B, siRNA against NRF2 inhibits elevation of ATF4 and GCLM mRNA in HUVECs treated with OxPAPC (130 $\mu\text{mol/L}$, 6 hours). Efficiency of NRF2 knockdown,

NRF2 Binds to a Site in the ATF4 Promoter Region

To characterize the mechanisms of NRF2-dependent elevation of ATF4 mRNA, we performed in silico analysis²² of the ATF4 gene 5'-upstream region and found a putative ARE site at position -290/-280. Chromatin immunoprecipitation demonstrated OxPAPC-induced binding of NRF2 protein to a DNA region (-363/-183) containing this site (Figure 4A). Lack of NRF2 binding to a neighboring promoter region (-201/-19; data not shown) and absence of DNA amplification in samples precipitated by nonimmune IgG (Figure 4A) validate binding specificity. Amplification of a fragment of GCLM gene immunoprecipitated with anti-NRF2 from HUVECs treated with electrophilic compound sulforaphane (Figure 4A) served as the positive control.

The ELISA-based NRF2 assay demonstrated significantly higher binding of NRF2 to consensus ARE in nuclear extracts prepared from OxPAPC-treated HUVECs compared with unstimulated cells (3.17 ± 0.57 fold, $P=0.0042$). To further characterize binding of NRF2 to the putative ARE within ATF4 promoter, wild-type and mutated oligonucleotides representing this ARE were synthesized. The wild-type oligonucleotide effectively competed with binding of NRF2 to immobilized consensus ARE (Figure 4B and 4C). Single substitution of G to T strongly inhibited the ability of ATF4 ARE to compete with NRF2 binding to consensus ARE (Figure 4B and 4C). Taken together with chromatin immunoprecipitation data, these results allow the hypothesis to be made that NRF2 induced by OxPAPC binds to a regulatory ARE site in the ATF4 promoter and stimulates transcription of this gene.

NRF2 Is Important for Angiogenic Effects of OxPLs

Finally, we obtained evidence that the NRF2 pathway is functionally important for the angiogenic switch induced by OxPLs. NRF2 knockdown inhibited both OxPAPC-induced expression of the ATF4 downstream gene VEGF and HUVEC sprouting (Figure 5A and 5B). Incomplete inhibition of sprouting may be because, apart from VEGF, angiogenic effects of OxPLs are mediated by additional autocrine mediators, such as COX-2-derived prostanoids.² In support of this possibility, we found that induction of COX-2 by OxPAPC is independent of NRF2 (Supplemental Figure IV).

Discussion

The major finding done in this work is a novel mechanism of cross-talk between cellular stress signaling pathways. OxPLs are known to induce both UPR and ESR pathways.¹⁴⁻¹⁶ Previous studies demonstrated elevated phosphorylation of eIF2 α in response to OxPAPC, suggesting that selective translation in-

Figure 2 (Continued). determined by quantitative real-time polymerase chain reaction (qRT-PCR), was $70\% \pm 7\%$. C, siRNA against NRF2 inhibits elevation of ATF4 in HUVECs treated with oxidized low-density lipoprotein (100 $\mu\text{g/mL}$, 6 hours) or 8-iso-PGA2 (50 $\mu\text{mol/L}$, 6 hours). After mRNA isolation, expression of ATF4 mRNA was quantified by qRT-PCR and normalized to the β_2 -microglobulin mRNA. Efficiency of NRF2 knockdown, determined by qRT-PCR, was $85\% \pm 12\%$.

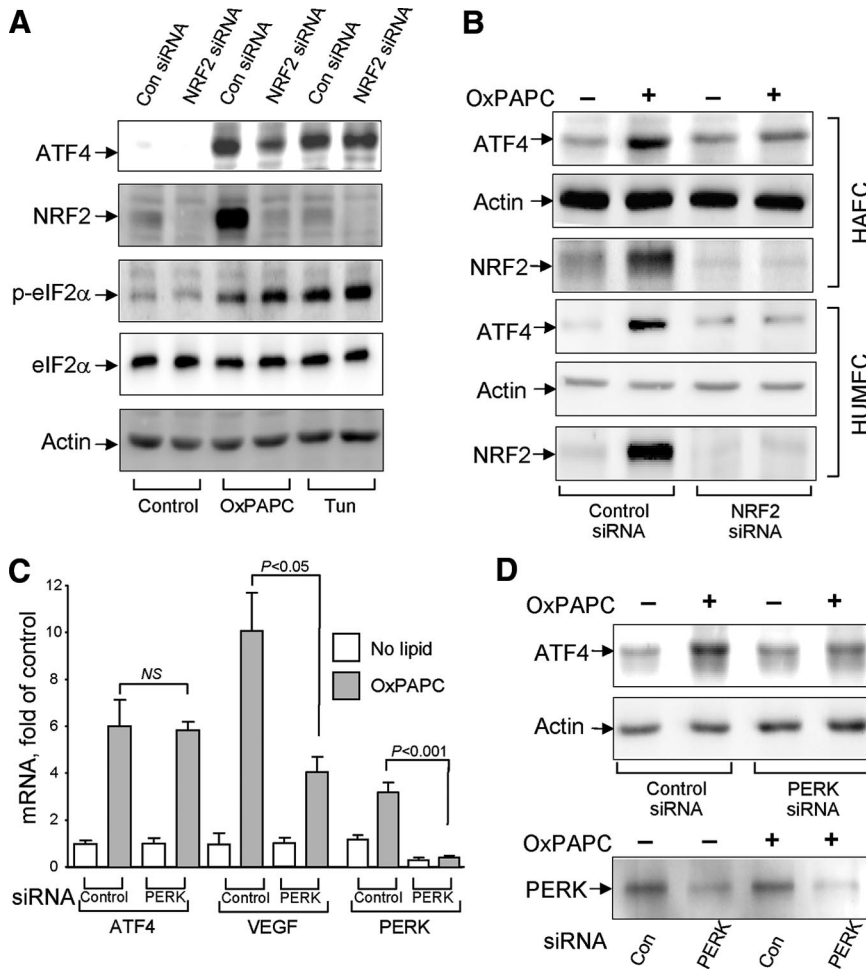


Figure 3. NRF2 and PERK are necessary for maximal induction of ATF4 protein by OxPAPC. A, Western blotting analysis of effects of NRF2 knockdown on OxPAPC- and tunicamycin-induced ATF4, NRF2, phospho-eIF2 α , total eIF2 α , and actin protein levels. siRNA-transfected HUVECs were treated for 2 hours with OxPAPC (130 μ mol/L) or tunicamycin (5 μ g/mL). B, ATF4 mRNA levels in cells treated with PERK siRNA. Efficiency of PERK knockdown, determined by quantitative real-time polymerase chain reaction (qRT-PCR), was 74% \pm 17%. OxPAPC-stimulated values were normalized to their corresponding controls. C, Western blotting analysis of effects of PERK knockdown on OxPAPC-induced elevation of ATF4 protein. HUVECs transfected with siRNA against PERK were treated for 4 hours with OxPAPC (130 μ mol/L). D, HUVECs transfected with siRNA against PERK were treated for 4 hours with OxPAPC (130 μ mol/L), followed by quantification of VEGF mRNA using qRT-PCR. Con, control.

duced by this phosphorylation plays a significant role in upregulation of ATF4 protein in cells treated with OxPLs.^{3,14} Here we show that in parallel with enhanced phosphorylation of eIF2 α , OxPLs upregulate ATF4 mRNA levels via NRF2-dependent mechanisms. Therefore, the convergence of selective translation of ATF4 with NRF2-dependent elevation of ATF4 mRNA may amplify effects of OxPAPC on expression of ATF4 protein and its downstream targets, such as VEGF. Furthermore, ATF4 and NRF2 can act as direct coactivators of the transcription of genes such as heme oxygenase-1,²³ which because of its angiogenic properties may have an additional impact on OxPL-induced angiogenesis.

The existence of points of convergence between electrophilic and unfolded protein stress pathways strongly suggests that optimal adaptation of cells to environmental and endogenous stresses requires tight coordination of ESR and UPR reactions.

Several groups showed that combined concentrations of OxPLs in human and animal hypercholesterolemic plasma, as well as in atherosclerotic vessels, can reach levels of tens of micromoles per liter or kilogram of wet weight.^{24–26} Thus, at least in pathologies such as hypercholesterolemia and atherosclerosis, combined concentrations of active OxPL species achieve the levels that upregulated ATF4 and VEGF in our experiments. Moreover, our data demonstrate that NRF2-dependent upregulation of ATF4 mRNA is not unique for OxPLs but is also induced by other oxidized lipids relevant

for cardiovascular pathology, including oxidized low-density lipoprotein and electrophilic (iso)prostaglandins.

In the context of biological importance, our findings are likely to have implications for vascular biology. The ATF4 branch of UPR is increasingly recognized as a pathogenic mechanism in vascular damage characteristic of diabetes and atherosclerosis. ATF4 has been shown to play a role in induction of VEGF and angiogenic reactions associated with diabetic retinopathy, destabilization of lesions, and response to balloon injury.^{2,27,28} Furthermore, ATF4 is upregulated by oxidative stress and cardiovascular disease risk factors, including cholesterol, homocysteine, oxidized low-density lipoprotein, and OxPLs.^{14,27,29,30} Enhanced levels of ATF4 and its downstream target proteins were documented in atherosclerotic vessels,^{14,31} where ATF4-induced genes can execute apoptosis of macrophages thus increasing vulnerability of atheroma.³⁰ These data characterize ATF4 as an important player in cardiovascular disease. Although NRF2 is usually regarded as an atheroprotective factor,¹² a gene knockout study has demonstrated amelioration of atherosclerosis in *Nrf2*-null mice.¹³ Our data allow it to be hypothesized that in addition to upregulation of scavenger receptors,¹³ proatherogenic action of NRF2 may be explained by its ability to induce ATF4 and its downstream product VEGF, which, among other activities, is capable of recruiting monocytes³² and stimulating plaque formation.³³ Furthermore, it has been shown that regulation of a large proportion of UPR genes,

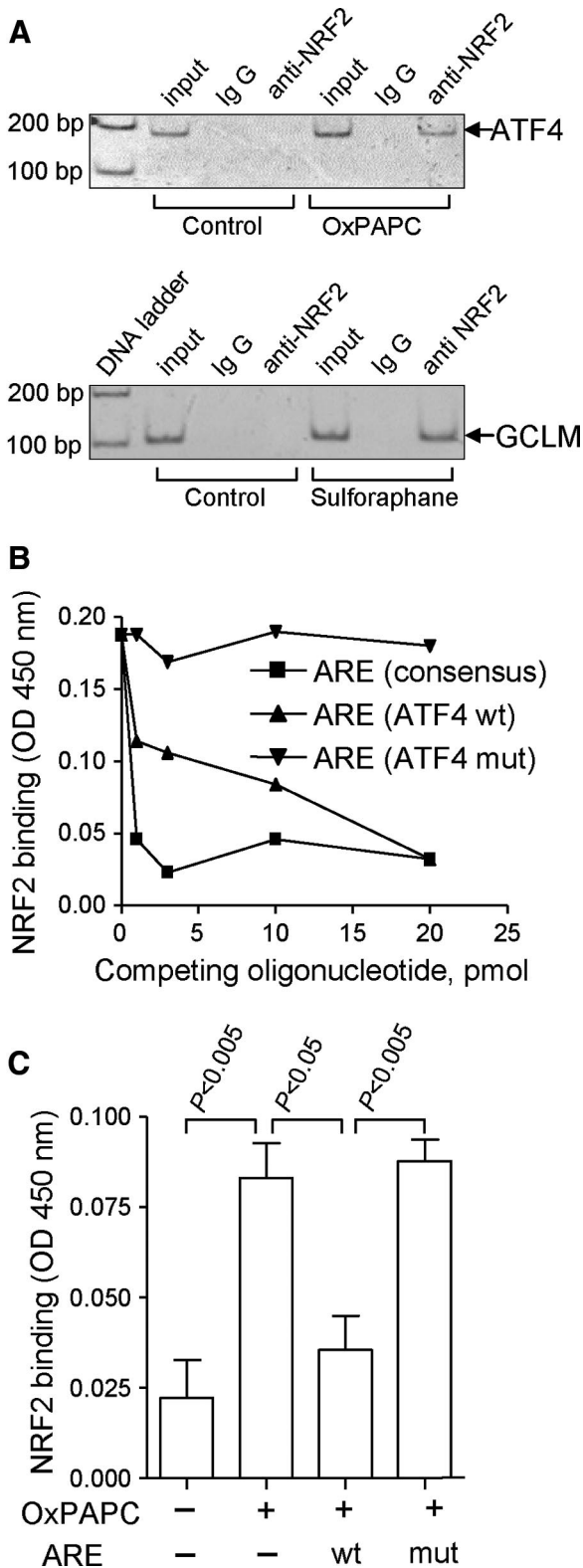


Figure 4. NRF2 binds to an ARE site in *ATF4* promoter. A, Chromatin immunoprecipitation using anti-NRF2 and control IgG. After 2 hours of stimulation with 130 $\mu\text{mol/L}$ OxPAPC or 5 $\mu\text{g/mL}$ sulforaphane or vehicle, DNA-protein crosslinking was performed using 1% formaldehyde. Immunoprecipitated DNA fragments containing ARE sites from the *ATF4* and *GCLM* genes were amplified by polymerase chain reaction, separated in polyacrylamide gel, and visualized by ethidium bromide staining. B and C, NRF2/ARE binding competition assay. Binding of

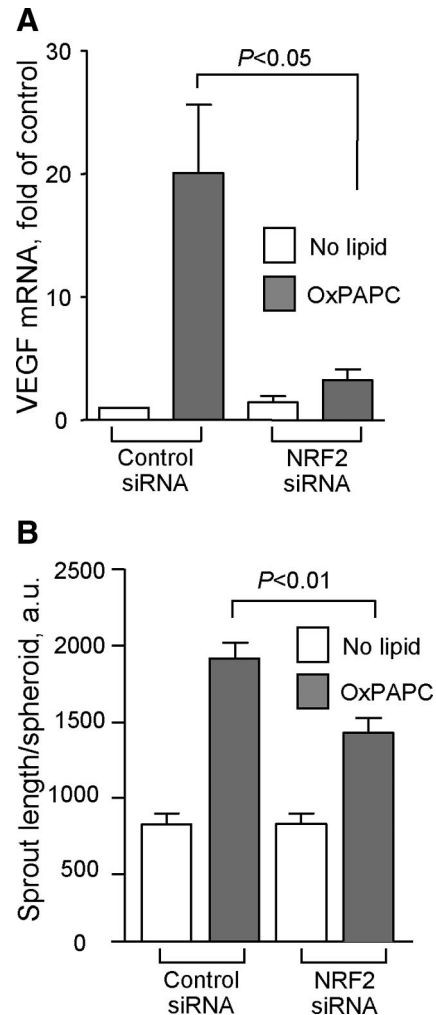


Figure 5. Knockdown of NRF2 inhibits OxPAPC-induced angiogenic switch. A, VEGF mRNA was quantified in control- and NRF2 siRNA-transfected HUVECs treated with OxPAPC (130 $\mu\text{mol/L}$, 6 hours). Stimulation was terminated by the addition of Trizol. VEGF mRNA expression was quantified by quantitative real-time polymerase chain reaction (qRT-PCR) and normalized to β_2 -microglobulin mRNA levels. Efficiency of NRF2 knockdown, determined by qRT-PCR, was 70% \pm 7%. B, Knockdown of NRF2 inhibits formation of sprouts by HUVECs in response to OxPAPC. Forty-eight hours after cell transfection with control or NRF2 siRNA, cell spheroids were formed and embedded in fibrin gels. Thereafter, spheroids were incubated for 24 hours in the presence of medium 199 containing 130 $\mu\text{mol/L}$ OxPAPC and then stained with tetramethyl rhodamine isothiocyanate-conjugated phalloidin for the sprout length measurement.

including ones that are ATF4 regulated, in mouse liver and intestine depends on intact *Nrf2*.³⁴ Our data offer a potential mechanism of this effect and allow the hypothesis that in addition to vascular wall cells, the NRF2-ATF4 positive cross-talk may be important in other tissues, organs, and disease states.

Figure 4 (Continued). NRF2 from control and OxPAPC-stimulated HUVEC nuclear extracts to immobilized consensus ARE site was quantified using the ELISA-based TransAM NRF2 kit and is expressed as optical density at 450 nm. Two micrograms of nuclear extracts was incubated with the indicated amounts of double-stranded nucleotides corresponding to the ARE consensus binding site, putative ARE site from *ATF4* promoter, or its mutated version containing a single-nucleotide mutation.

In summary, the data presented in this report support the notion that OxPLs induce an angiogenic switch in endothelial cells at least partially acting via a cross-talk between NRF2 and ATF4, thus leading to induction of VEGF. The involvement of NRF2 in regulation of ATF4 and VEGF by atherogenic lipids opens new possibilities for specific interventions.

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Disclosures

None.

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