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Forum Review Article

Cell Membrane Redox Systems and Transformation

PIN-JU CHUEH

ABSTRACT

Cell membrane redox systems carry electrons from intracellular donors and transport them to extracellular acceptors. This phenomenon appears to be universal. Numerous reviews have emphasized not only the bioenergetic mechanisms of redox systems but also the antioxidant defense mechanisms in which they participate. Moreover, significant progress has been made in the modulation of the membrane redox systems on cell proliferation. Because membrane redox systems play a key role in the regulation of cell growth, they need to be somehow linked into the signaling pathways resulting in either controlled or unregulated growth by both internal and external signals. Ultimately, these sequential events lead to either normal cell proliferation or cancer cell formation. However, much less is known about the involvement of membrane redox in transformation or tumorgenesis. In this review, the facts and ideas are summarized concerning the redox systems and tumorgenesis in several aspects, such as the regulation of cell growth and the effect on cell differentiation and on signaling pathways. In addition, information on a unique tumor-associated nicotinamide adenine dinucleotide (NADH) oxidase (tNOX) protein is reviewed. Antiox. Redox Signal. 2, 177–187.

INTRODUCTION

NOT ONLY HAVE THE MEMBRANE REDOX SYSTEMS been found to be present in all types of cells but also they play an essential role in cell growth (Morré *et al.*, 1986, 1988a; Brightman *et al.*, 1988) and more specifically in cell enlargement or elongation (Morré *et al.*, 1988b; Hidalgo *et al.*, 1991). Thus far, nicotinamide adenine dinucleotide; (NADH) (or NADPH) is the prime cytosolic electron donor studied by most investigators (Misra, 1991). However, the natural electron acceptors for the membrane redox systems have been a matter of controversy. Identification of electron acceptors has been validated from various laboratories, particularly for molecular oxygen (Crane *et al.*, 1985), cytochrome *c* (Buckhout and Hrubec, 1986; Sandelius *et al.*, 1986), ascorbate free radical (AFR) (Morré *et al.*, 1986; Burón *et al.*, 1987; Luster and Buckhout, 1988), ferricyanide (Craig and Crane, 1981; Sandelius *et al.*, 1986), and protein disulfides (Chueh *et al.*, 1997a). Besides both electron donors and acceptors, the intermediate carriers have also drawn extensive attention. Among those, coenzyme Q has been well investigated. Coenzyme Q is a lipid-soluble redox constituent that functions as an intermediate electron carrier (Sun *et al.*, 1992a; Villalba *et al.*, 1995). Other cofactors, for instance, cytochrome *b* (Crane *et al.*, 1985), α-tocopherylquinone (Sun *et al.*, 1992b), or flavins (Luster and Buckhout, 1989; Møller *et al.*, 1995), also have been investigated.

Redox systems have been related to cell proliferation and cell differentiation (Crane *et al.*, eds., 1990). The presence of the NADH oxidase (NOX) system has been shown to be activated

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907.

by several ligands, including epidermal growth factor (EGF), and platelet derived growth factor (PDGF) (Brightman *et al.*, 1992; Bruno *et al.*, 1992). Kay and Ellem (1986) previously proposed that the activation of an electron transport system was associated with cell proliferation. Similar results were observed in the membrane redox system of a mouse neuroblastoma cell line NB41A3 (Zurbriggen and Dreyer, 1994). In addition, one of the mechanisms proposed for AFR modulation of cell growth was the control of the redox reactions involved in cell elongation mechanisms (Hidalgo *et al.*, 1991; Córdoba and González-Reyes, 1994). Conversely, inhibition of ascorbate oxidation caused a delay of ascorbate-mediated cell elongation.

The phenotype of many cells is altered during differentiation. However, little is known regarding the role of membrane redox in cell differentiation. Inducers or suppressors of cell differentiation have been commonly used to study the changes of redox activities during differentiation. Nonetheless, few papers have focused on the modulation of cell differentiation by membrane redox status compared to intracellular redox status (Esposito *et al.*, 1994; Kamata *et al.*, 1996). Changes in redox may lead to changes of intracellular signals that mediate cell differentiation. These include signals for apoptosis (Wang *et al.*, 1998), activation of NADPH oxidase (Tardif *et al.*, 1998), and the mitogen-activated protein kinase (MAPKs) cascade (Kamata *et al.*, 1996).

Membrane redox systems implicate involvement in cell proliferation, and there are studies demonstrating the direct linkage between membrane redox and growth of tumor cells. It has been found that the redox activities are altered in transformed cells, such as human retinoblastoma (Medina and Schweigerer, 1993), neuroblastoma (Medina *et al.*, 1992), HeLa (Sun *et al.*, 1992c), and HL-60 cells (Alcaín *et al.*, 1991). In SV40-transformed 3T3 cells, membrane redox system activity was lower compared with control cells (Löw *et al.*, 1991). In contrast, the cell-surface NOX activity described by Morré (1998) was found to be elevated in most types of transformed cells when compared to their normal counterpart (Morré *et al.*, 1995a).

Finally, membrane redox systems are significantly involved in cell defense mechanisms and in oxidative stress. Oxidative stress has been shown to induce random cell injury. Additionally, it may elicit a cascade of signaling. Varied signals downstream due to the modulation of membrane redox activity have been elucidated in the past two decades such as the induction of proto-oncogenes c-*fos* and c-*myc* (Wenner *et al.*, 1988) and cytosolic free Ca^{2+} (Hallett and Campbell, 1984; Al-Mohanna and Hallett, 1988). The aberrant signaling subsequently results in the deregulation of cell growth or cell death. In the study of Wolvetang *et al.* (1996), utilization of inhibitors of the membrane redox activity induced apoptosis. There also is evidence indicating an alteration in the intracellular redox equilibrium of cells undergoing apoptosis (Slater *et al.*, 1996). As would be anticipated, the lack of regulation of either cell proliferation, cell differentiation, or apoptosis will eventually lead to cell transformation (cancer) (Vaux *et al.*, 1988; Gregory *et al.*, 1991). Therefore, not only is it important to understand the relationships between membrane redox systems and cell growth but to understand, as well, the basic mechanism of how the changes of membrane redox activity result in uncontrolled cell growth (transformation) and/or cell death.

In this review, the facts and ideas regarding membrane redox systems and cell transformation are examined in different aspects, including the regulation of cell growth and the effect on cell differentiation and on signaling pathways.

MEMBRANE REDOX AND CELL GROWTH

Ascorbate has been shown to be involved in the regulation of plant growth. One of the mechanisms of ascorbate-modulated cell growth was by controlling the redox reactions involved in cell elongation at the plasma membrane (Hidalgo *et al.*, 1991; Córdoba and González-Reyes, 1994). Inhibition of ascorbate regeneration resulted in a retardation of cell elongation. Ascorbate also has been reported to protect HaCaT epithelial cells against UV irradiation-induced apoptosis (Savini *et al.*, 1999).

Coenzyme Q_{10} also plays a critical role in the regulation of cell growth (Crane *et al.*, 1994). The importance of coenzyme Q_{10} was exemplified by the study of Martinus *et al.* (1993). In their report, coenzyme Q_{10} was used to replace pyruvate to provide the growth supplement of rho 0 Namalwa cells through the enhanced activation of membrane NADH oxidase. Another example demonstrating the intimate relationship between membrane redox systems and cell growth is the study of human aortic smooth muscle cells. Glycosphingolipid and its metabolic products have been found to have a positive influence on cell proliferation via activation of NADPH oxidase in human aortic smooth muscle cells (Bhunia *et al.*, 1997).

To elucidate the correlation between membrane redox systems and the growth of tumor cells, various anticancer drugs were used in membrane redox systems. Conjugates of adriamycin linked to transferrin were shown to inhibit membrane oxidoreductase activity of K562 cells (Faulk *et al.*, 1991). This inhibition was correlated with both electron transport and cell growth. Retinoids have been established to inhibit both the membrane NOX activity and the growth of HeLa cells with a close correlation (Dai *et al.*, 1997). Similarly, both NOX activity and the growth of immortalized human keratinocytes by human papillomavirus type 16 DNA (HKc/HPV16) were inhibited by retinoic acid and 1,25-dihydroxy-vitamin D_3 [1,25-$(OH)_2D_3$] (Morré *et al.*, 1992). The NOX activity of the plasma membrane of HeLa cells was also inhibited by antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea (LY181984) as well as the growth of cells (Morré *et al.*, 1997c). Addition of EGF enhanced the inhibition of NOX activity by LY181984. Moreover, a naturally occurring quinone analogue, capsaicin (8-methyl-*N*-vanillyl-6-noneamide), also displayed the inhibitory effect on both the NOX activity and growth of human and mouse melanoma cell lines (Morré *et al.*, 1996a). However, capsaicin had little effect on NOX activity of nontransformed cultured cells or their growth (Morré *et al.*, 1995a).

Redox reactions at the membrane play a vital role in the control of many mechanisms regarding cell growth. Activation of membrane redox systems results in stimulation of growth in various types of cells, whereas the inhibition of membrane redox activity causes retardation of cell proliferation (Morré, 1998). Therefore, the membrane redox system must be tightly regulated to prevent the uncontrolled growth associated with cell transformation.

MEMBRANE REDOX AND CELL DIFFERENTIATION

Because the phenotype of many cells is changed during differentiation, investigators have explored the relationship between membrane redox activity and cell differentiation. To help evaluate the modulation of membrane redox activity during differentiation, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has been used for induction of cell differentiation in HL-60 cells (Burón *et al.*, 1993). Not only did TPA induce cell differentiation, but it also augmented membrane redox activity and caused a shift in the NAD^+/NADH ratio. TPA also has been described to induce differentiation in HL-60 cells via the NADPH oxidase activation (Tardif *et al.*, 1998).

It has been reported that membrane ferric reductase activity of Caco-2 cells declined during rapid growth, while highly differentiated cells had significantly greater redox activity (Ekmekcioglu *et al.*, 1998). Their rationalization was that the major energy sources were used up for cell proliferation in rapidly growing cells. As a result, accumulated electron donors were utilized for bioenergetic mechanisms rather than as the substrates for redox reactions. However, reagents and hormones had little effect on this ferricyanide reductase activity.

The phagocyte-specific NADPH oxidase is a multisubunit complex that generates superoxide ($O_2^{\bullet -}$) during the respiratory burst (Morel *et al.*, 1991; Curnutte, 1993). The NADPH oxidase contains the two subunits of membrane-bound cytochrome *b*-558 (p22-phox and gp91-phox), three cytosolic proteins (p40-phox, p47-phox, and p67-phox), and small G proteins (Rac, Rap) (Dagher *et al.*, 1995). Further evidence suggested that Rac mediated the assembly of the p47-phox and p67-phox on the cytosolic side of the plasma membrane whereas

Rap functioned as the final activation control involving the association with the cytochrome *b* component (Gabig *et al.*, 1995)

When one superoxide is generated, one acid is equivalently produced by the oxidase (Rossi, 1986). Therefore, the stimulation of NADPH oxidase results in the accumulation of metabolic acids. Since cytosolic acidification is rescued by homeostatic H^+ extrusion (H^+ conductance), the expression of NADPH oxidase and that of H^+ conductance were studied during dimethyl sulfoxide-induced differentiation of HL-60 cells (Qu *et al.*, 1994). With TPA as an inducer, expression of NADPH oxidase was increased over time, while the undifferentiated cells exhibited very low oxidase expression. A similar pattern was observed for H^+ conductance. This parallel relationship between oxidase and H^+ conductance suggested an intimate association between these two systems. Furthermore, Henderson *et al.* (1995) reported that the H^+ channel activity involved in H^+ efflux correlated with the expression of the larger component of cytochrome *b* (gp91-phox) of NADPH oxidase but not with the expression of other subunits.

The combination of interferon-γ and retinoic acid or 1,25-$(OH)_2D_3$ also have also been used for the induction of monocyte differentiation (Obermeier *et al.*, 1995). mRNA regulation has been demonstrated to contribute to the induction of NADPH oxidase in differentiating cells. The results coincided with the study by Gupta *et al.* (1992) in human myeloid cell lines induced by tumor necrosis factor and interferon-γ. Furthermore, Miyamoto *et al.* (1994) have indicated that p67-phox protein was the most important cytosolic component during the differentiation of hematopoietic cells because the oxidase activity did not arise until p67-phox was expressed. On the contrary, $p22^{rac}$ is likely to be constantly expressed in HL-60 cells independent of the level of differentiation (Didsbury *et al.*, 1989).

SIGNALING PATHWAY

Cells respond to hormones and growth factors by triggering a series of reactions. Those mechanisms involve several second messengers that mediate the cellular response to growth. As evidence, membrane redox systems respond to hormones and thereafter modulate the growth of cells (Crane *et al.*, 1994). Several intracellular signals have been found to be elicited due to changes in membrane redox systems.

Recent research has been focused on the relationship between the redox systems and oxidative stress because reactive oxygen intermediates (ROI) are constantly generated during electron transfer reactions. The induced oxidative stress not only causes damage to cell structures but also changes intracellular signaling pathways.

The NADH oxidase activated by growth factors and ligands can function in the regulation of second messenger signals as well as on other ligand receptors on the cell membrane (Crane *et al.*, 1994). The putative involvement of membrane redox activities leads to the modulation of signaling, such as on proton release (Sun *et al.*, 1988), Ca^{2+} efflux (Löw *et al.*, 1985), protein phosphorylation (Harrison *et al.*, 1991), or on NAD^+/NADH ratios (Navas *et al.*, 1986).

The Ras family of oncogenes encodes a GTP-binding protein that occupies a key position in the regulation of cell growth. Crowe *et al.* (1993) established the direct linkage between the *Ha-ras* expression and the activation of membrane oxidoreductase. Furthermore, Wilkinson *et al.* (1993) have indicated that enhanced electron flow of plasma membrane by an external acceptor markedly increased GTP binding to $p21^{ras}$. Membrane redox systems also have been elucidated to be associated with the Ras signaling pathway (Mackeller *et al.*, 1994). Recently, the activation of NADPH oxidase has been shown to be stimulated by lactosylceramide, a common glycosphingolipid (Bhunia *et al.*, 1997). The modulation of the redox system then stimulated the loading of GTP to Ras, the activation of p44 MAPK, the expression of the transcription factor c-fos (Bhunia *et al.*, 1996), and cell proliferation.

The activation of the NADPH oxidase has been found to be amplified by the addition of GTP and its nonhydrolyzable analogue and to be repressed by GDP and its analogue (Gabig *et al.*, 1987; Ligeti *et al.*, 1988). Their results suggested a role for a G protein in the membrane redox activation system. Consequent studies have demonstrated that Rac, a small cytosolic

GTP-binding protein, mediated the mechanism by which the activation of membrane redox activity coincided with the modulation of G protein (Abo *et al.*, 1991; Knaus *et al.*, 1991, 1992; Mizuno *et al.*, 1992). The translocation of Rac to the membrane was also shown to be responsible for the activation of membrane redox activity (Sawai *et al.*, 1993). During the differentiation of HL-60 cells, the NADPH oxidase activation was associated with the activation of Rac (Tardif *et al.*, 1998). However, the activation of Rac was in a phosphatidylinositol-3 kinase (PI3K)-and mitogen extracellular signal regulatory kinase (MEK)-independent manner. In addition to Rac, another small GTP-binding protein, Rap, also has been reported to be associated with the regulation of membrane redox activity through affiliation with cytochrome *b* (Quinn *et al.*, 1989, 1992). Therefore, the interaction or assembly of cytochrome *b* with GTP-binding proteins has indicated a role for such proteins in signaling pathways and in the regulation of the membrane redox system.

Another second messenger, cAMP, also was found to be involved in the regulation of membrane redox systems (Rodríguez-Aguilea *et al.*, 1993). cAMP and its related analogues increased the flow of electrons to AFR at the membrane, subsequently stimulating membrane redox activity. Nevertheless, use of 1,25-$(OH)_2D_3$ as a cell differentiation inducer has demonstrated that it also caused a temporary increase in cAMP levels that was mediated by the regulation of membrane redox (López-Lluch *et al.*, 1998). The NADH increase was induced by 1,25-$(OH)_2D_3$ and thereafter inhibited adenylate cyclase, hence the lower cytosolic NADH levels maintained by ascorbate and AFP allowed adenylate cyclase to produce more cAMP. Protein kinase C (PKC) and phosphoprotein phosphatase have also been reported to be involved in the membrane redox activity (del Castillo-Olivares *et al.*, 1996).

Membrane redox activity per se was found to be modulated in tumor cells (Ehrlich cells) through cGMP and Ca^{2+} as second messengers (del Castillo-Olivares *et al.*, 1995). Use of agonists has demonstrated a role for cGMP in the activation of membrane redox activities. To elucidate the potential role of Ca^{2+} in the modulation of membrane redox systems, three methods were used to increase cytosolic Ca^{2+} levels, including the employment of a Ca^{2+} ionophore, a Ca^{2+} transport inhibitor, and a Ca^{2+} channel opener. The data suggested that Ca^{2+} played a role in the control of the membrane redox system. In addition, Wolvetang *et al.* (1996) presented evidence that the membrane redox system altered membrane calcium fluxes and signaled for apoptosis through interaction with calcineurin.

Experiments with inhibitors implied that membrane redox activity was initiated by UV light and coupled to the regulation of calcium release from an intracellular pool. The ensuing calcium efflux via the action of Ca^{2+}-ATPases then triggered gene expression (Long and Jenkins, 1998). However, the authors have not identified the end products of membrane redox activity that served as the messengers to trigger the release of calcium ions.

Medina and colleagues (1992) have described the relationship between membrane redox activity and N-*myc* expression. The Myc oncoprotein plays a pivotal role in cell growth control, transformation, and apoptosis induction (Henriksson and Lüscher, 1996; Ryan and Birnie, 1996). To be functional, Myc is required to form heterodimers with Max (Blackwood and Eisenman, 1991; Blackwood *et al.*, 1992). Besides Myc, Max needs to heterodimerize with the Mad protein, which is also a regulator of cell growth (Henriksson and Lüscher, 1996). Subsequently, the Myc/Max/Mad network triggers intracellular signals leading to transformation and apoptosis. In Medina's study (1992), the increased membrane redox activity correlated with amplification and greater expression of the N-*myc* oncogene in neuroblastoma cells.

TUMOR-ASSOCIATED NADH OXIDASE

Morré (1998) has described a family of hormone-responsive external plasma membrane hydroquinone oxidase that use NADH as an alternative substrate (NADH oxidase) and have protein disulfide-thiol interchange activity. NOX proteins exist in at least two forms one of which, tNOX, is tumor-associated. tNOX activity is anticancer drug-responsive and has

been found to be present in most types of transformed cultured cells as well as the sera of cancer patients (Morré, 1998). The normal counterpart of tNOX is CNOX. CNOX activity is unresponsive to anticancer drugs and appears to be present in all eukaroyotic cells. Bruno *et al.* (1992) first compared NOX activities from isolated plasma membrane of rat liver (CNOX) and that of rat hepatoma cells (tNOX). Their results suggested a biochemical defect in the regulation of NOX activities that resulted in uncontrolled growth specific for neoplastic transformation.

A unique characteristic of tNOX is that a shed form of activity was observed in the culture media conditioned by growth of HeLa cells (Morré *et al.*, 1995b, 1996b; Wilkinson *et al.*, 1996). Apparently, the protein was shed from the unprocessed tNOX via possibly proteolytic cleavage and released into culture media. Similar results were also found for sera of cancer patients (Morré and Reust, 1997; Morré *et al.*, 1997a). The cancer patients were mostly classified as late stage and represented a broad range of cancers including breast, prostate, lung, ovarian, leukemia, and lymphomas. tNOX activity from sera of cancer patients was distinct from CNOX activity in sera of healthy volunteers. The most characteristic difference was that tNOX activity from pooled cancer sera was inhibited by quinone-site inhibitory analogs, whereas the corresponding CNOX activity of sera of healthy volunteers or patients with diseases other than cancer was not.

Because tNOX activity has been reported to be shed into the circulation of all cancer patients tested, pooled human cancer sera were then used as a protein source for tNOX isolation (Chueh *et al.*, 1997b). A monoclonal antibody was generated to the purified tNOX protein. Subsequently, expression cloning was carried out to screen tNOX cDNA from a HeLa library. The identified cDNA encoded a 391-amino-acid sequence with an unprocessed molecular weight of 45.6 kDa that contained several functional motifs (Chueh, 1997). A conserved quinone binding site motif and a conserved adenine nucleotide-binding site were present in the derived amino acids sequence. The protein contained eight cysteines and a putative C-XXXX-C motif that might serve as a potential site for catalyzing the interchange between protein thiols and disulfides.

Several quinone site inhibitors with antitumor activity, such as antitumor sulfonylureas (Morré *et al.*, 1995c, 1995d), adriamycin (Morré *et al.*, 1997b), and capsaicin (Morré *et al.*, 1995a), were used to investigate the relationship between tNOX and cell growth. The results demonstrated that all three antitumor drugs were capable of inhibiting preferentially the tNOX activity and the growth of transformed cells in culture, whereas they exhibited little effect on CNOX activity and the growth of nontransformed cells. The common feature among those drugs is that they are all quinone-site inhibitory analogs. tNOX indeed was found to contain a putative quinone binding site that might account for the drug inhibition (Chueh, 1997). Furthermore, tNOX-transfected COS cells exhibited at least a 10-fold higher drug-susceptibility when compared to COS cells transfected with vector alone (Chueh *et al.*, 1999).

Thiol reagents have been found to inhibit tNOX activity (Morré and Morré, 1995) from plasma membranes of HeLa cells. tNOX activity of rat hepatoma plasma membranes also was modulated by thiol reagents, whereas that of rat liver plasma membranes was unaffected over a wide range of thiol reagents and concentrations. The results further confirmed that a structural modification of tNOX activity involving thiol groups may be present in the plasma membranes of cancer cells but absent in plasma membranes of normal cells. Subsequently, tNOX has been found to exhibit a protein thiol-disulfide interchange activity in HeLa plasma membranes (Morré *et al.*, 1998).

Another unique feature of tNOX is that two activities [hydroquinone (NADH) oxidation and protein disulfide-thiol interchange] oscillate with a period of about 23 min (Morré, 1998). The oscillations in tNOX activity have been examined with HeLa cells, purified HeLa plasma membrane, solubilized and purified tNOX protein, cancer sera, and expressed tNOX protein. Entrainment between two different tNOX preparations with different periods has been observed (Wang, 1997). The mechanism of entrainment has not been exam-

ined. However, Morré (1998) has hypothesized that the entrainment might occur through association and dissociation of subunits. tNOX protein isolated from HeLa cells has been observed to form stable dimers (del Castillo-Olivares *et al.*, 1998). Because tNOX is being shed into the circulation (Morré and Reust, 1997), it could travel from cell to cell throughout the body entraining surface tNOX molecules. This entrainment model might suggest a possibility for a cell-surface tNOX receptor functionally linked to cell enlargement and/or membrane electron transport. Additionally, cell enlargement in HeLa cells has been found to be periodic and the frequencies corresponded to tNOX activity present at the HeLa cell surface (Morré, unpublished results).

tNOX activity present in most of transformed cells and sera of cancer patients is unregulated and responsive to anticancer drugs. Its existence tightly correlates with unregulated growth and loss of differentiated characteristics that are generally linked to the cancer phenotypes. Because tNOX has been found in sera of all cancer patients tested, it might be a common component causative to cancer occurrence. Therefore, tNOX might serve as a diagnostic device or as a therapeutic target.

CONCLUSIONS

In this review, it has been demonstrated that there is a close link between membrane redox systems and the cell transformation in terms of the regulation of cell growth and cell differentiation. During the process, the changes in redox activity trigger a series of signaling pathways that lead to the regulation of many vital mechanisms in cells. All existing evidence provides strong support for an essential function for membrane redox systems in cell growth and the modulation of membrane redox activities in cell transformation. tNOX provides a direct link between membrane electron transport systems and transformation. Its activity correlates closely with the growth of tumor cells, and its external location provides susceptibility to agents delivered through the circulation, such as antitumor drugs and antibodies. Therefore, it is a potential pancancer marker either as a cancer diagnostic device or a therapeutic target. However, the nature of the role for membrane redox systems in cell transformation is still in the early investigative stages, but it is hoped that a clear picture soon will emerge.

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ABBREVIATIONS

1,25-$(OH)_2D_3$, 1,25-dihydroxy-vitamin D_3; AFR, ascorbate free radical; CNOX, normal form of hydroquinone (NADH) oxidase; EGF, epidermal growth factor; NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor κB; IL-6, interleukin 6; MAPK, mitogen-activated protein kinase; MEK, mitogen extracellular signal regulatory kinase; NOX, hydroquinone (NADH) oxidase; PI3K, phosphatidylinositol-3 kinase; PDGF, platelet-derived growth factor; PKC, protein kinase C; PMA, phorbol myristate acetate; ROI, reactive oxygen intermediates; tNOX, tumor-associated hydroquinone (NADH) oxidase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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Address reprint requests to:
Dr. Pinju Chueh
Department of Medicinal Chemistry and Molecular Pharmacology
Hansen Life Sciences Research Building
Purdue University
West Lafayette, IN 47907

E-mail: pinchueh@omni.cc.purdue.edu

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