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Forum Review

Activation of Tyrosine Kinases by Reactive Oxygen Species in Vascular Smooth Muscle Cells: Significance and Involvement of EGF Receptor Transactivation by Angiotensin II

GERALD D. FRANK and SATORU EGUCHI

ABSTRACT

Enhanced production of reactive oxygen species (ROS) such as H_2O_2 and a failure in ROS removal by scavenging systems are hallmarks of several cardiovascular diseases such as atherosclerosis and hypertension. ROS act as second messengers that play a prominent role in intracellular signaling and cellular function. In vascular smooth muscle cells (VSMCs), a vascular pathogen, angiotensin II, appears to initiate growth-promoting signal transduction through ROS-sensitive tyrosine kinases. However, the precise mechanisms by which tyrosine kinases are activated by ROS remain unclear. In this review, the current knowledge that suggests how certain tyrosine kinases are activated by ROS, along with their functional significance in VSMCs, will be discussed. Recent findings suggest that transactivation of the epidermal growth factor receptor by ROS requires metalloprotease-dependent heparin-binding epidermal growth factor-like growth factor production, whereas other ROS-sensitive tyrosine kinases such as PYK2, JAK2, and platelet-derived growth factor receptor require activation of protein kinase C-δ. Each of these ROS-sensitive kinases could mediate specific signaling critical for pathophysiological responses. Detailed analysis of the mechanism of cross-talk and the downstream function of these various tyrosine kinases will yield new therapeutic interventions for cardiovascular disease. *Antioxid. Redox Signal.* 5, 771–780.

INTRODUCTION

INCREASING EVIDENCE over the past several years has revealed important clues regarding reactive oxygen species (ROS), in particular, their role in both normal and abnormal cellular function. ROS are reduction/oxidation molecules derived from molecular oxygen that include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). They are generated by a variety of extracellular stimuli such as growth factors, G protein-coupled receptor (GPCR) agonists, cytokines, ultraviolet radiation, increased osmolarity, and other cellular stresses (22, 45). Recently, ROS were widely recognized as prominent players in the pathophysiology of many diseases, including cardiovascular diseases such as hypertension, atherosclerosis, and restenosis after vascular injury (8, 32). In fact, the majority of vascular cell types that construct vessel walls generate ROS, supporting the notion that ROS may function as regulators and inducers of these diseases (32). Currently, ROS are proposed to induce cardiovascular diseases by three major mechanisms: (1) oxidation of lipid (LDL) to produce oxidized LDL, which plays a major role in the development of atherosclerosis; (2) inhibition of nitric oxide function through $O_2^{\bullet -}$ and nitric oxide interaction that forms peroxynitrite; and (3) activation of intracellular signal transduction pathways as second messengers (8, 32, 50).

ROS-mediated signaling pathways are known to promote a variety of cellular events, such as growth, differentiation, survival, apoptosis, and inflammation, as well as gene expression, in vascular cells (32, 38, 50). However, of particular interest is the precise mechanism of how ROS stimulate or modulate

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.
The current address of Dr. Eguchi is Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Room 802 MRB, 3420 North Broad Street, Philadelphia, PA 19140.

extracellular or intracellular targets that leads to pathophysiological responses in vascular cells. Phosphorylation of receptor and non-receptor tyrosine kinases is a critical step in signal transduction that leads to specific cellular functions such as cell growth. Interestingly, both protein tyrosine phosphorylation and ROS production are commonly induced by several growth factors, and ROS can activate many tyrosine kinases. Thus, understanding the mechanism and downstream consequences of ROS-mediated tyrosine kinase activation is a current research focus by many researchers. Researchers have elucidated important roles and mechanisms of ROS in vascular smooth muscle cells (VSMCs), especially in regards to activation of tyrosine kinases, which could be responsible for specific cellular responses leading to cardiovascular diseases.

It is now recognized that events such as hypertrophy, hyperplasia, and migration of VSMCs may be initiated through tyrosine kinase activation induced by ROS production in response to various vascular pathogens such as angiotensin II (AngII) and platelet-derived growth factor (PDGF). AngII, a highly studied vasoactive hormone of the renin–angiotensin system, plays a significant role in the regulation of cardiovascular homeostasis mainly through the AngII type 1 (AT_1) receptor (90). One major undesired consequence of AngII is its role in the development of cardiovascular diseases, including hypertension, atherosclerosis, and heart failure (13, 14, 29, 79). Stimulated AT_1 receptors lead to rapid protein tyrosine phosphorylation of various signaling molecules in AngII target cells, and thereby lead to a prototypical growth promoting signal such as mitogen-activated protein kinase (MAPK/extracellular signal-regulated kinase [ERK]) activation and induction of c-*fos* and c-*jun* expression (7, 15, 31). ROS have been shown to mediate many of the pathological effects of AngII. In VSMCs, AngII is a potent ROS inducer that generates the majority of ROS through NAD(P)H oxidase. Acting via a ROS-dependent mechanism, AngII mediates hypertrophy and/or proliferation of VSMCs (30).

PDGF is produced in a variety of cardiovascular cells and is one of the strongest inducers of VSMC growth and migration (35). Importantly, PDGF is also a potent inducer of ROS in VSMCs and is known to stimulate p42/44 MAPK/ERK and subsequent mitogenic responses through ROS production (86). Pathophysiologically, PDGF has been reported to play a role in cardiovascular diseases such as atherosclerosis and restenosis, which could involve ROS-dependent mechanisms (35, 36).

New accumulating evidence suggests that activation of several ROS-sensitive tyrosine kinases is an important initial signaling trigger that leads to specific signaling events responsible for functional modulation of VSMCs. Therefore, in this review, the discussion will focus on current findings that suggest novel mechanisms of how ROS mediate the activation of several receptor and non-receptor tyrosine kinases in VSMCs, as well as the pathophysiological significance of their activation.

ROS-ACTIVATED TYROSINE KINASES IN VSMCS

Tyrosine kinases (receptor and non-receptor) in VSMCs that are ROS-sensitive or utilize ROS-dependent mechanisms for activation are listed in Table 1 (21, 24, 25, 28, 33, 55, 56, 70, 71, 74, 76, 78, 84, 86, 93). It can be hypothesized that multiple risk factors → ROS → tyrosine kinase activation → abnormal VSMC response → cardiovascular disease. Generally, three mechanisms have been proposed by which ROS activate tyrosine kinases. First, ROS may directly activate kinases by altering protein–protein interactions depending on sulfhydryl groups. Second, protein tyrosine phosphatases that contain a cysteine residue in their activation site may be directly inhibited by ROS, which in turn results in tyrosine phosphorylation of the kinases and may affect their activities. Third, oxidation stimulates proteolysis of regulatory proteins that may inhibit tyrosine kinase activity (8). It has been suggested that at least two or more distinct mechanisms are involved in tyrosine kinase activation by ROS in VSMCs. In the following sections of this review, we will discuss the role of each tyrosine kinase with respect to how ROS induce their activation and the functional and pathophysiological consequence in VSMCs. In addition to the tyrosine kinases listed in Table 1, several other tyrosine kinases expressed in different cell types have also been shown to be activated via a ROS-dependent mechanism. However, the possible roles of these potential ROS-sensitive tyrosine kinases such as insulin-like growth factor 1 receptor and focal adhesion kinase in VSMCs remain to be studied.

Epidermal growth factor (EGF-receptor "trans"-activation by ROS

Recently it has become apparent that the EGF receptor is a significant contributor in a number of signaling networks activated by stimuli that do not directly interact with the receptor ("transactivation") (10). These stimuli include various GPCR

TABLE 1. ROS-SENSITIVE TYROSINE KINASES IN VSMCS

Tyrosine kinase	*Agonists*	*References*
Receptor		
EGF receptor	H_2O_2, AngII, PDGF, oxidized LDL	25, 28, 70, 76, 84, 93
PDGF receptor	H_2O_2, AngII, PDGF, oxidized LDL	21, 33, 74, 86
FGF receptor	H_2O_2	71
Non-receptor		
JAK2	H_2O_2, $O_2^{\bullet -}$, AngII, thrombin	28, 55, 56, 78
PYK2	H_2O_2, AngII	24
Src	H_2O_2, AngII	74, 93

FGF, fibroblast growth factor.

ligands, membrane depolarizing agents, and environmental stressors that rapidly induce EGF receptor tyrosine phosphorylation (some act within 1 min). In particular, studies have shown that not only AngII (16) but also H_2O_2 (70), endothelin (40), thrombin (44), oxidized LDL (84), β-migrating very-LDL (99), and mechanical stretch (41) transactivate the EGF receptor in cultured VSMCs (Fig. 1). Moreover, a rapid transactivation of aortic EGF receptor by acute AngII infusion *in vivo* was reported (46). Since the EGF receptor transactivation mediates several critical mitogenic signal transductions by these stimuli, the EGF receptor could be a point of signal convergence by which several cardiovascular risk factors mediate pathophysiological responses associated with cardiovascular diseases. How is the EGF receptor transactivated by these risk factors in VSMCs?

H_2O_2 has been shown to induce tyrosine phosphorylation of the EGF receptor and to cause the association of Shc–Grb2–Sos complex with the EGF receptor in VSMCs (70). In VSMCs, the EGF receptor is a redox-sensitive tyrosine kinase that is activated by a GPCR agonist, AngII (25, 93). It has been reported that *N*-acetylcysteine, a potent ROS scavenger, is capable of inhibiting AngII-induced EGF receptor transactivation, and that H_2O_2 induced EGF receptor autophosphorylation at Tyr1,068, a Grb2 binding site, strongly suggesting ROS are required for EGF receptor transactivation induced by AngII (25). Furthermore, lysophosphatidic acid-induced transactivation of the EGF receptor requires ROS in HeLa cells (12). Taken together, these data indicate that the EGF receptor utilizes ROS for transactivation by GPCRs, however, the precise mechanisms by which ROS transactivate the EGF receptor are still unknown.

A mechanism by which ROS transactivate the EGF receptor may involve inhibition of tyrosine phosphatases, which in turn results in enhanced phosphorylation of tyrosine kinases (22). In support of this notion, H_2O_2 and various other thiol-oxidizing agents could inhibit dephosphorylation of the EGF receptor in rat-1 cells (47). However, in VSMCs there exists another mechanism by which ROS can transactivate the EGF receptor. Metalloprotease-dependent heparin-binding EGF-like growth factor (HB-EGF) generation has been implicated in EGF receptor transactivation initiated through several GPCRs

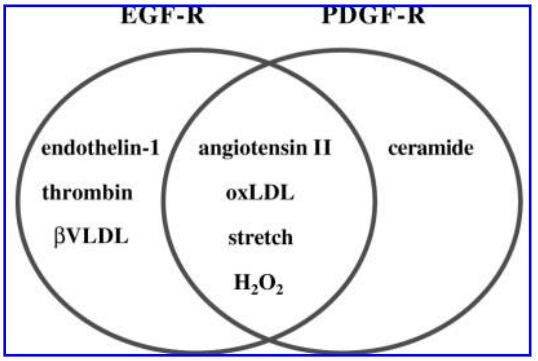

FIG. 1. Multiple risk factors lead to phosphorylation and transactivation of both the EGF receptor (EGF-R) and the PDGF receptor (PDGF-R) in VSMCs. βVLDL, β-migrating very-LDL; oxLDL, oxidized LDL.

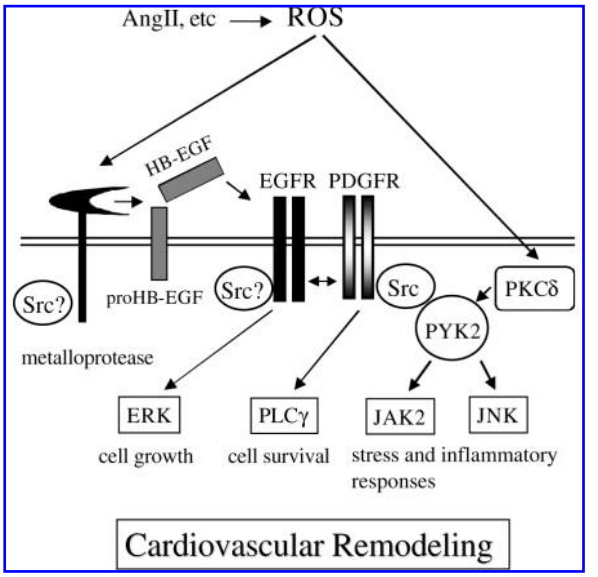

FIG. 2. Hypothetical signaling mechanisms of ROS lead to transactivation of the EGF receptor (EGFR) and PDGF receptor (PDGFR) and activation of non-receptor tyrosine kinases (PYK2, JAK2, and Src) promoting functional responses critical for vascular pathogenesis.

(19, 67). HB-EGF belongs to the EGF family of growth factors and is a strong stimulant of mitogenesis and migration of VSMCs, two critical events involved in the pathogenesis of atherogenic vascular diseases (68). In fact, HB-EGF is present in atherosclerotic plaques, and VSMCs produce HB-EGF in response to various pathogens (68), suggesting a critical role for HB-EGF in mediating vascular remodeling.

In VSMCs, H_2O_2 stimulates EGF receptor transactivation via metalloprotease-dependent HB-EGF generation (Fig. 2) (28). Although the metalloprotease responsible for the HB-EGF generation induced by ROS has not been identified, both matrix metalloproteases (87, 97) and a disintegrin and metalloprotease (ADAM) family of metalloproteases (5, 42, 51) have been implicated in ectodomain shedding of HB-EGF stimulated by various agonists. Interestingly, H_2O_2 was recently shown to enhance ADAM17 activity directly and ADAM17-mediated ectodomain shedding (98). Activation of metalloproteases is thought to occur via a thiol group from a cysteine residue within the inhibitory prodomain of these metalloproteases that interacts with zinc in their catalytic domain. Since ROS are known to interact with thiol groups, they may oxidize these electrophilic thiol groups and disrupt the cysteine–zinc bond, leading to activation of the metalloprotease.

In addition, the mechanism of ROS-induced EGF receptor transactivation may further involve intracellular Ca^{2+} elevation, upstream tyrosine kinases, and/or heterodimer formation with the PDGF receptor. Intracellular Ca^{2+} elevation is required for the EGF receptor transactivation by AngII, endothelin, and mechanical stretch in VSMCs (16, 40, 41). It has been reported that Ca^{2+} exists upstream of HB-EGF generation (19). Since H_2O_2 has no major effect on intracellular Ca^{2+} in VSMCs (24), Ca^{2+} may mediate ROS production, which leads to EGF

receptor transactivation. c-Src has been implicated in EGF receptor transactivation by several GPCRs (4, 54, 93), and activation of Src-mediated cascades could further amplify ROS production in VSMCs (81). Moreover, H_2O_2-induced EGF receptor transactivation was inhibited by a selective Src inhibitor, PP2, in endothelial cells (11). ROS may modulate c-Src, which then activates the metalloprotease responsible for HB-EGF generation through protein–protein interaction. In this regard, c-Src appears to exist upstream of HB-EGF release in fibroblasts (66). Alternatively, c-Src phosphorylates the EGF receptor in response to ROS to induce EGF receptor transactivation. To support this idea, only certain tyrosine sites have been shown to be phosphorylated by AngII in an ROS-dependent manner in VSMCs (93). Also, it has been shown that PDGF stimulation of VSMCs results in the formation of a PDGF-β receptor–EGF receptor heterodimer leading to transactivation of EGF receptor, which involves ROS production and a Src kinase activation (76). Therefore, c-Src may have multiple roles in mediating EGF receptor transactivation by H_2O_2. In contrast to these studies, a recent paper has demonstrated that the direct association of EGF receptor and AngII receptor after AngII stimulation induces EGF receptor transactivation in COS cells, suggesting that an ROS-independent mechanism of EGF receptor transactivation may also exist in certain conditions (82).

Transactivation of the EGF receptor has been implicated in several disease processes (5, 51, 63), making it a current and important topic of signal transduction research. AngII-induced transactivation of the EGF receptor is necessary for the activation of downstream signal transduction molecules, such as ERK, p38 MAPK, Akt, and p70 S6 kinase, that subsequently promote hypertrophy and hyperplasia of VSMCs (15, 18, 19). Among these kinases, p38 MAPK and Akt have been shown to be redox-sensitive in VSMCs, whereas redox sensitivity of ERK in VSMCs remains controversial (23, 25, 91, 92). Interestingly, EGF receptor transactivation through GPCRs is required for cardiac hypertrophy induced by AngII as well as by pressure overload (5, 43). Also, EGF receptor transactivation mediates VSMC migration in response to AngII (75). Therefore, it is now becoming clear that the transactivation of the EGF receptor plays a significant role in the development and progression of cardiovascular diseases and that this signaling cascade may provide for alternative therapeutic targets for prevention of such diseases.

PDGF receptor "trans"-activation by ROS

The PDGF receptor, which exists as an α- or β-isoform, is a transmembrane spanning receptor tyrosine kinase (35). Similar to the EGF receptor, the PDGF receptor can be activated not only by its cognate ligands but also by other stimuli in a ligand-independent manner in VSMCs (Fig. 1). Cyclic mechanical stretch rapidly (witin 4 min) induces tyrosine phosphorylation of PDGF-α receptor and its association with Grb2, an adaptor molecule essential for ERK/MAPK activation in VSMCs (37). Oxidized LDL, ceramide, and AngII transactivate the PDGF-β receptor in cultured VSMCs (21, 53). The transactivation of the PDGF-β receptor appears to involve ROS in VSMCs (21, 33). Since PDGF is a potent mitogen and chemoattractant in VSMCs and has long been implicated in atherosclerosis as well as other cardiovascular diseases (35), these findings indicate the unique activation mechanism of the PDGF receptor induced by ROS and its downstream pathophysiological significance.

In VSMCs, H_2O_2 stimulates phosphorylation of the PDGF-β receptor on tyrosine residues, one of which was identified as Tyr1,021, a phospholipase C (PLC)-γ binding site (74). Both the binding of PLC-γ to phosphorylated Tyr1,021 in the C-terminal tail of the PDGF-β receptor and the activation of PLC-γ are believed to be involved in cell growth and chemotaxis in certain circumstances (34). The fact that PLC-γ is recruited to the PDGF-β receptor after H_2O_2 stimulation is important supportive evidence regarding H_2O_2-induced PDGF-β receptor transactivation (74). Interestingly, in the same study, H_2O_2 induced association of c-Src and protein kinase C (PKC)-δ with the PDGF-β receptor. These non-receptor kinases (c-Src and PKC-δ) are required for H_2O_2-induced PDGF-β receptor transactivation but not for PDGF-BB (ligand)-induced receptor activation. Thus the PDGF receptor transactivation is ligand-independent, and therefore does not require PDGF receptor kinase activity or metalloprotease activation (74). In addition, it has been shown that a non-receptor tyrosine kinase, PYK2, is required for PDGF receptor but not EGF receptor transactivation by ROS in VSMCs by using dominant-negative PYK2 mutants (20) (Fig. 3). Other investigators have demonstrated an involvement of phosphotyrosine phosphatase in ROS-induced PDGF-β receptor transactivation in mesangial cells (9) and in VSMCs (21). Alternatively, ROS-dependent transactivation of PDGF receptor induced by AngII may require a tyrosine kinase distinct from Src, PYK2, or JAK2 in VSMCs (33). It should be noted that extracellular administration of H_2O_2 and receptor stimulation of H_2O_2 production may differentially activate signaling pathways, which may explain some of the disagreement in the literature. Taken together, these findings clearly highlight the unique mechanism of PDGF receptor transactivation that is distinct from a ligand-dependent autophosphorylation of the PDGF receptor as well as

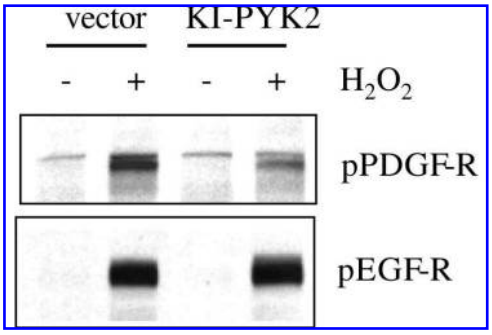

FIG. 3. ROS-dependent phosphorylation of the PDGF receptor requires tyrosine kinase activity of PYK2. VSMCs were transfected with either vector or kinase-inactive (KI)-PYK2 (10 multiplicity of infection) for 48 h and stimulated by H_2O_2 (20 μ*M*) for 10 min. The cell lysates were immunoblotted with antibodies to Tyr1,021-phosphorylated PDGF receptor (pPDGF-R) and Tyr1,068-phosphorylated EGF receptor (pEGF-R) (25, 72). PDGF receptor but not EGF receptor phosphorylation was inhibited by KI-PYK2 transfection.

metalloprotease-dependent transactivation of the EGF receptor by ROS in VSMCs (Fig. 2).

In comparison with the EGF receptor, little is known about the downstream function of PDGF receptor transactivation. Since its transactivation mechanism is not via autophosphorylation, the downstream significance should be different from PDGF-mediated responses in VSMCs. Our finding that PLC-γ as well as c-Src and PKC-δ associates with the PDGF receptor suggests that the receptor acts as a scaffold in ROS signaling. Interestingly, PLC-γ activated by ROS was recently shown to be involved in cell survival against ROS-induced apoptosis (60). In addition, PDGF receptor phosphorylation was enhanced in balloon-injured carotid arteries, which was inhibited by AT_1 receptor antagonists (2). Taken together, these data suggest the possible involvement of PDGF receptor transactivation in the cardiovascular remodeling process.

JAK2 activation by ROS and its signaling

JAK2 is a member of the JAK family of tyrosine kinases, which are critical for signal transduction important for several biological functions. In particular, JAK activation is required for activation of the signal transducers and activators of transcription (STAT) pathway in response to activated cytokine receptors (69). An early study demonstrated that AngII could also stimulate tyrosine phosphorylation and activation of JAK2, which subsequently leads to STAT isoform tyrosine phosphorylation (57). Interestingly, AngII also stimulates the association of JAK2 with the AT_1 receptor in VSMCs (57), cardiac myocytes (48), and renal mesangial cells (58), which may partly explain the cytokine-like actions of AngII in mediating cardiovascular remodeling. In addition to AngII, a variety of other GPCR agonists have recently been shown to activate JAK/STAT pathways (94). Research has revealed that JAK2 activation by AngII requires ROS in VSMCs (28, 78) and that H_2O_2 rapidly and strongly induces JAK2 activation in VSMCs (28, 55). Similarly, H_2O_2 activate the JAK/STAT pathway in cultured fibroblasts (1, 83). These findings suggest that GPCR agonists activate JAK2 through ROS production.

In VSMCs, intracellular Ca^{2+} elevation and PKC-δ activation initiated by PLC-derived second messengers are involved in AngII-induced JAK2 activation. Furthermore, PYK2 is required for Ca^{2+}- and PKC-δ-mediated JAK2 activation (27). These findings provide new information whereby GPCR-dependent JAK2 activation may be mediated by ROS. Recently, it has been reported that the PKC-δ isoform is required for JAK2 activation by H_2O_2 in VSMCs (28), which is in line with the above-mentioned finding. Previously, PKC-δ has been implicated in ROS-dependent activation of other tyrosine kinases such as c-Abl and c-Src (85). In this regard, several reports indicate that H_2O_2 stimulates PKC-δ activity in various cell types (49, 62). In fact, our own group has shown that H_2O_2 stimulates PKC-δ activity in VSMCs (28). Interestingly, H_2O_2-induced activation of PKC-δ is reported to be independent from tyrosine phosphatase inhibition (95). Utilizing dominant-negative PYK2 mutants, it has been shown that PYK2, which is also downstream of PKC-δ in VSMCs, is required for JAK2 activation but not for EGF receptor activation by H_2O_2 (26). In fibroblasts, a Src kinase was shown to exist upstream of JAK2 activation by ROS (1). As illustrated in Fig. 2, these findings indicate that there are at least two major tyrosine kinase activation mechanisms utilized by ROS in VSMCs. One mechanism involves ROS-activated PKC-δ that leads to the activation of the PYK2/JAK2 pathway or the PDGF receptor transactivation. The other mechanism involves activation of a ROS-dependent metalloprotease cleavage of proHB-EGF to generate active HB-EGF that leads to EGF receptor transactivation.

Although the exact cellular function of JAK2 activation via ROS in VSMCs is unknown, JAK2 activation by AngII has been proposed to mediate VSMC proliferation (59). Also, the AT_1 receptor-mediated JAK/STAT pathway was shown to be involved in cardiac hypertrophy (64) and neointima formation after balloon injury (80). Along with the findings that ROS-dependent JAK2 activation is required for AngII-induced cytokine induction (78) and thrombin-induced heat shock protein induction in VSMCs (56), ROS-induced JAK2 activation could mediate cellular remodeling through a cytokine like inflammatory response in cardiovascular diseases.

PYK2 as unique ROS target

PYK2 is a non-receptor tyrosine kinase, also identified as cell adhesion kinase β, related adhesion focal tyrosine kinase, or calcium-dependent tyrosine kinase (6, 52, 77). PYK2 requires Ca^{2+} and/or PKC for its activation, which results from a wide variety of extracellular stimuli such as GPCR agonists, growth factors, cytokines, and environmental stresses (6, 52). In cultured VSMCs, it has been demonstrated that AngII rapidly stimulates PYK2 kinase activity (26, 73) and phosphorylation at Tyr402, a putative autophosphorylation site of PYK2 (24, 26).

In VSMCs, PYK2 is activated by extracellular administration of H_2O_2 (24). Depending on the cell type, PYK2 activates a downstream MAPK family that include ERK, c-Jun N-terminal kinase (JNK), and p38 MAPK (52, 65, 89). In VSMCs and cardiac fibroblasts, PYK2 is involved in AngII-induced JNK activation (26, 61). In addition, AngII stimulates association of PYK2 with c-Src in VSMCs (17), and c-Src was shown to mediate ROS-dependent JNK activation but not p38 MAPK or ERK activation in VSMCs (96). Some researchers have suggested a partial involvement of PYK2 in EGF receptor transactivation in fibroblasts (4), and that it plays a significant role in mediating AngII-induced growth-promoting signals (72). However, based on the above-mentioned findings, it is more likely that a ROS-sensitive kinase, PYK2, plays a major role in mediating JNK and JAK/STAT pathway activation leading to stress and inflammatory responses in VSMCs.

Src family kinase activation by ROS

Src family kinases now include nine members, of which Src, Fyn, and Yes are expressed in most tissues. These kinases can be activated by a variety of receptors, channels, and extracellular stresses, including ROS (88). As mentioned above, Src kinases seem to be critically involved in ROS-mediated activation of other tyrosine kinases such as activation of the EGF receptor, the PDGF receptor, and JAK2 in VSMCs. Importantly, c-Src was previously shown to mediate the phosphorylation of paxillin that is responsible for focal adhesion formation in VSMCs (39). Src kinase activation by ROS likely requires an interaction with PKC-δ. H_2O_2 induces phosphory-

lation of c-Src at Tyr418, a critical site for activation, leading to association of c-Src with PKC-δ in VSMCs. Also, a Src inhibitor blocks tyrosine phosphorylation of PKC-δ in response to H_2O_2 (74). These findings further suggest that interaction of PKC-δ with a non-receptor tyrosine kinase leads to their phosphorylation by each kinase toward the other, initiating ROS-dependent signal transduction (85).

It should be noted that many of the previous findings defined the role of Src kinases by using pharmacological inhibitors and dominant negative mutants, which could not establish the specific role of each isoform in mediating a specific cellular function. The recent development of isoform-specific knock-out cell lines may provide better information to elucidate the previous confusing role of ROS-dependent Src family kinase activation. In this regard, c-Src is proposed to exist upstream of JNK and Big MAP kinase (BMK1/ERK5) (3, 96), whereas Fyn mediates the JAK2 and Ras/MAPK/ERK cascade in mouse fibroblast cell lines (1). However, whether these cascades can be applied in VSMCs need further study.

FUTURE DIRECTION AND PERSPECTIVE

In summary, the findings discussed here clearly support the original hypothesis, which has been expanded to a new theory that multiple risk factors activate tyrosine kinases by ROS through distinct mechanisms in VSMCs and that each ROS-sensitive kinase has a unique role in mediating cardiovascular disease. Even though tremendous progress has been made in defining the role of ROS in tyrosine kinase-dependent signal transduction in cardiovascular systems, there is still a considerable void in our knowledge regarding the detailed mechanism of ROS-initiated signaling. Here, several mechanisms utilized by ROS to activate receptor and non-receptor type tyrosine kinases have been discussed, of which there are two significant contributors, a metalloprotease and PKC-δ. However, further extensive research is required to determine: (1) the identity of the metalloprotease that could be a critical ROS-sensing molecule in the EGF receptor transactivation pathway, (2) its activation mechanism, and (3) what's the downstream consequence *in vivo*. Further study is also needed to determine whether PKC-δ is directly activated by ROS, or whether there another molecule that senses ROS directly.

The long-term goal ideally is to be able to target these ROS-sensitive mechanisms with selective drugs to alleviate the pathophysiological conditions they promote. Based on this idea, you can imagine that we could target, for example, the stress and inflammatory or cell growth pathway independently according to the type of disease. Therefore, further characterization and understanding of the cellular mechanisms involved in ROS signal transduction via tyrosine kinase activation will provide new targets for effective therapies that will better enable us to control cardiovascular diseases.

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ABBREVIATIONS

ADAM, a disintegrin and metalloprotease; AngII, angiotensin II; AT_1, AngII type 1; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; H_2O_2, hydrogen peroxide; HB-EGF, heparin binding EGF-like growth factor; JNK, c-Jun N-terminal kinase; LDL, low density lipoprotein; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PKC, protein kinase C; PLC, phospholipase C; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription; VSMC, vascular smooth muscle cell.

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Address reprint requests to:
Dr. Satoru Eguchi
Cardiovascular Research Center and Department of Physiology
Temple University School of Medicine
Room 802, MRB
3420 North Broad Street
Philadelphia, PA 19140

E-mail: seguchi@temple.edu

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