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VEGF Signaling Through NADPH Oxidase-Derived ROS

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ABSTRACT

Angiogenesis is a key process involved in normal development and wound repair, as well as ischemic heart and limb diseases, and atherosclerosis. Vascular endothelial growth factor (VEGF), a potent angiogenesis factor, stimulates proliferation, migration, and tube formation of endothelial cells (ECs), primarily through the VEGF receptor type2 (VEGFR2). Reactive oxygen species (ROS) function as signaling molecules to mediate biological responses. In ECs, NADPH oxidase is one of the major sources of ROS and consists of catalytic subunits (Nox1, Nox2, and Nox4), p22phox, p47phox, p67phox, and the small GTPase Rac1. VEGF stimulates ROS production via activation of gp91phox (Nox2)-based NADPH oxidase, and ROS are involved in VEGFR2-mediated signaling linked to EC migration and proliferation. Moreover, ROS derived from NADPH oxidase are involved in postnatal angiogenesis. Localizing NADPH oxidase and its regulators at the specific subcellular compartment is an important mechanism for activating specific redox signaling events. This review focuses on a role of NADPH oxidase-derived ROS in angiogenesis and critical regulators involved in generation of spatially and temporally restricted ROS-dependent VEGF signaling at leading edge, focal adhesions/complexes, caveolae/lipid rafts, and cell–cell junctions in ECs. Understanding these mechanisms should facilitate the development of new therapeutic strategies to modulate new blood vessel formation. *Antioxid. Redox Signal*. 9, 731–739.

INTRODUCTION

ANGIOGENESIS, the formation of new blood vessels from the pre-existing vasculature, is involved in physiological processes, including embryonic development and wound repair, as well as in various pathologies such as ischemic heart and limb disease, cancer, diabetic retinopathy, or chronic inflammation (33). This tightly regulated process involves the degradation of extracellular matrix combined with migration and sprouting endothelial cells (ECs) from preexisting capillaries. Vascular endothelial growth factor (VEGF) is one of the key angiogenic growth factors and stimulates migration, proliferation, and tube formation of ECs primarily through the VEGF receptor type2 (VEGR2, KDR/Flk1) (76). Excess amounts of reactive oxygen species (ROS) such as O_2.- and H_2O_2 have deleterious effects on cells and contribute to various cardiovascular diseases including hypertension, heart failure, atherosclerosis, and diabetes (41), while physiological concentrations of ROS are involved in signaling to mediate cell migration, growth, and differentiation (32). ECs generate ROS which play a role in physiological and pathophysiological responses, depending on their intracellular concentrations (68).

The major source of ROS in ECs is an NADPH oxidase (38). ROS stimulate induction of VEGF, which in turn increases ROS through activation of NADPH oxidases that are involved in VEGFR2 autophosphorylation, EC migration, and proliferation (49, 100, 111). Moreover, NADPH oxidase-derived ROS are involved in postnatal angiogenesis *in vivo* (97, 100). However, mechanisms by which NADPH oxidase is activated and how ROS participate in redox signaling linked to angiogenesis remain unclear. Evidence suggests that VEGFR2-mediated signaling is temporally and spatially controlled and that NADPH oxidases are localized within discrete subcellular compartments, which is required for localizing ROS production and activation of specific redox

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signaling events (95, 98). The role of NADPH oxidase in angiogenesis has been reviewed recently (99). This review will focus on critical regulators involved in spatial and temporal organization of VEGF signaling through NADPH oxides-derived ROS in ECs.

NADPH OXIDASE IN EC

There are several enzymatic sources of ROS in mammalian cells, including the mitochondrial electron transport system, xanthine oxidase, cytochrome p450, NADPH oxidase, and nitric oxide synthase (41). In ECs, NADPH oxidase is a major source of ROS (7). Endothelial NADPH oxidase is activated by numerous stimuli including growth factors, cytokines, shear stress, hypoxia, and G-protein coupled receptor agonists (41). In phagocytic cells, NADPH oxidases consist of the membrane-bound cytochrome b558 comprising the catalytic gp91phox and the p22phox subunits, as well as cytosolic components including p47phox, p67phox, and the small Rho GTPase Rac1 (6). Upon stimulation, cytosolic components translocate to cytochrome b558 at the membrane to form a multimeric protein complex, leading to oxidative burst (7). In nonphagocytic cells, NADPH oxidases produce ROS at low levels and are activated by cytokines, growth factors, and hormones, suggesting that ROS function as signaling molecules (41). In recent years, several homologues of gp91phox (also termed Nox2)—Nox1, Nox3, Nox4, and Nox5—have been identified in nonphagocytic cells (18). ECs express all the phagocytic NADPH oxidase subunits, including Nox2, p22phox, Rac1, and p47phox, as well as Nox1 and Nox4 (7, 68). Their expression levels are dependent on cell types and environmental context.

Nox2 is a critical component of endothelial NADPH oxidase (38), and its expression and Nox2-dependent ROS production are increased by oxidized LDL, endothelin-1, angiotensin II, VEGF, and angiopoietin-2 in ECs (99). Nox1 is involved in shear stress-induced ROS production which is required for monocyte adhesion (93) and stimulates branching morphogenesis in sinusoidal ECs (55). Nox4 is most abundantly expressed in ECs, and involved in basal- (2) and Ang II-induced (109) O_2- production. Moreover, p47phox is involved in cytokine, growth factor, or shear stress-stimulated ROS production in ECs (48, 67, 107). New homologues of the p47phox and p67phox subunits, NoxO1 and NoxA1 have been described in other cell types (4, 36, 94), but their presence and functional role in ECs remain unknown.

NADPH oxidases are now recognized to have specific subcellular localizations such as lamellar leading edge/membrane ruffles, focal adhesions/complexes, caveolae/lipid rafts, endosomes, and nuclei (95, 98), which may contribute to localized ROS production and activation of specific redox signaling pathways that mediate various cell functions. In unstimulated ECs, Nox2 and its regulatory proteins as well as Nox4 exist in an intracellular perinuclear compartment, especially endoplasmic reticulum that is associated with actin cytoskeleton (4, 66, 73, 85, 102). Nox4 also localizes to the nucleus in human ECs, which may be required for oxidative stress-responsive gene expression (58). After agonist stimulation or during active EC migration, p47phox translocates to the membrane ruffles through association with WAVE1 and Rac1/PAK1 (107), as well as to the focal complexes in lamellar protrusions through binding to adaptor TRAF4 and Hic-5, a focal contact scaffold (108). Nox2 and Rac1 also accumulate at the site of new leading edge in actively migrating ECs (50), which is consistent with the highest activation of Rac1 and endogenous H_2O_2 accumulation at this specific membrane compartment (56, 79). Furthermore, in ECs death receptor activation causes clustering of lipid rafts, where Nox2, p47phox, and Rac1 are recruited to increase ROS and to form redox signaling platforms (113). These various targeting of NADPH oxidase to the specific subcellular compartments are required for localized ROS production, which in turn promotes cytoskeletal reorganization and directed EC migration (50, 79, 107), as well as activation of specific redox signaling events.

ROLE OF NADPH OXIDASE-DERIVED ROS IN ANGIOGENESIS

Exogenous H_2O_2 stimulates induction of HIF1α and VEGF by various cell types including ECs, and promote cell proliferation and migration, cytoskeletal reorganization, and tube formation in ECs (99). Hypoxia/reoxygenation as well as adhesion of activated polymorphonuclear leukocytes to ECs increase ROS production, which results in capillary tube formation (65, 112). VEGF-induced migration of human ECs is suppressed by NADPH oxidase inhibitors (1). VEGF and angiopoietin-1 stimulate ROS production via activation of Nox2-based NADPH oxidase and oxidase-derived ROS are involved in EC migration and/or proliferation (43, 49, 100, 111) (Fig. 1). Thus, many of angiogenic responses in ECs are dependent on ROS.

In vivo, there is strong correlation between neovascularization, ROS production, and VEGF expression in eyes of diabetics (12, 31) and balloon-injured arteries (89), and during reperfusion of the ischemic retina (59). Short ischemia/reperfusion promotes ROS production, thereby stimulating coronary collateral development and angiogenesis in the ischemic noninfarcted heart (77). ROS are also involved in wound repair whose process is dependent on angiogenesis (88). Nox2 or Nox4 expression are upregulated in neovasculature in mice ischemia models (3, 97, 101). Transgenic mice overexpressing p22phox show vascular ROS production, which contributes to VEGF expression and neovascularization in experimental atheroma (53). Inhibition of ROS or NADPH oxidase using chemical inhibitors or knockout mice demonstrates that NADPH oxidase-derived ROS play an important role in postnatal angiogenesis (99). Nox1 overexpression increases VEGF and VEGF receptor expression and matrix metalloproteinase activity through an increase in ROS, thereby promoting tumor angiogenesis (5).

ROLE OF NADPH OXIDASE-DERIVED ROS IN VEGF SIGNALING IN EC

ROS derived from NADPH oxidase function as second messengers to stimulate diverse redox signaling pathways linked to various functions including angiogenesis. VEGF binds to two tyrosine kinase receptors, VEGF receptor-1 (VEGFR1, Flt-1) and VEGFR2 in ECs. The mitogenic and

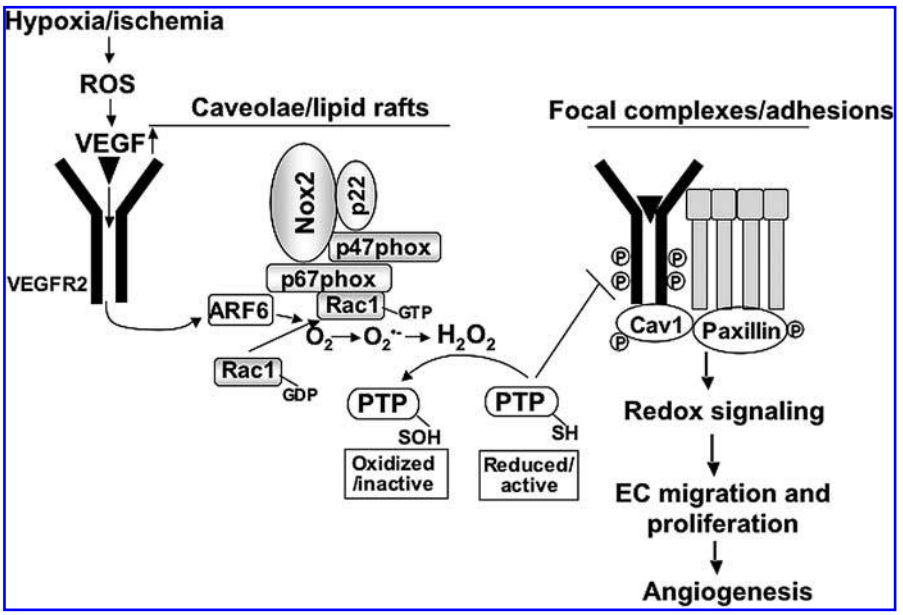

FIG. 1. Temporally and spatially organized ROS-dependent VEGF signaling linked to angiogenesis in ECs. Hypoxia/ischemia stimulates VEGF induction through ROS, and VEGF binding to VEGFR2 leads to the activation and translocation of the small GTPase Rac1 to the plasma membrane, which stimulates the Nox2-based NADPH oxidase in ECs. ROS derived from this oxidase may oxidize and inactivates PTPs which negatively regulate VEGFR2, thereby promoting VEGFR2 autophosphorylation and activation of downstream redox signaling events linked to EC proliferation and migration, which may contribute to angiogenesis. NADPH oxidases have been identified in caveolae/lipid rafts, which could be important for the localized ROS production and specific activation of downstream redox signaling events. ARF6, Rac1, and VEGFR2 are present in caveolae/lipid rafts basally in ECs. VEGF stimulation promotes egress of VEGFR2 from caveolae/lipid rafts, which in turn results in appearance of phospho-Cav1 (Tyr14) and VEGFR2 at focal complexes/adhesions where phospho-paxillin localizes. ARF6-Rac1-ROS pathways are important for VEGFR2 autophosphorylation and phospho-Cav1 seems to be required for proper localization of activated VEGFR2 at focal complexes/adhesions. Thus, ROS-dependent VEGF signaling linked to angiogenesis is termporally and spatially coordinated by various signaling molecules/enzymes such as ARF6, Rac1, NADPH oxidases, Cav1, and PTPs in ECs. ARF6, ADP ribosylation factor 6; PTP, protein tyrosine phosphatase; Cav1, caveolin-1; VEGFR2; VEGF receptor type2.

chemotactic effects of VEGF in ECs are mediated mainly through VEGFR2 (76). VEGFR2 is activated through ligand-stimulated receptor dimerization and transphosphorylation (autophosphorylation) of tyrosine residues in the cytoplasmic kinase domain. At present, tyrosine residues 951 and 996 in the kinase insert domain, and 1054 and 1059 in the kinase catalytic domain, have been identified as autophosphorylation sites for VEGFR2 in a bacterial expression system (30). This event is followed by activation of downstream signaling pathways such as Src, PI3 kinase, MAP kinases, and Akt, which is essential for VEGF-induced actin reorganization, cell migration, and proliferation of ECs. Of note, ROS stimulate VEGF induction, and VEGF activates Nox2-based NADPH oxidase to increase ROS that are involved in VEGFR2 autophosphorylation and Akt activation, thereby promoting EC migration and proliferation (21, 49, 100). This could represent a feed-forward mechanism by which ROS are involved in proangiogenic pathway (Fig. 1).

It is currently believed that H_2O_2 functions as a signaling molecule by reversible oxidative inactivation of protein tyrosine phosphatases (PTPs)(19, 87) and lipid phosphatase PTEN (60, 64) at cysteines in the catalytic site (19, 32, 87). Several PTPs, including SHP-1, SHP-2, and LMW-PTP (HCPTPA), inducibly associate with VEGFR2 after VEGF stimulation (42, 46, 57). TNF-α inhibits VEGF signaling by recruiting SHP-2 to the VEGFR2 in ECs (42). HCPTPA overexpression inhibits VEGF-induced VEGFR2 autophosphorylation, EC migration, and proliferation (46). High cell density-enhanced PTP1 (DEP-1)/CD148 attenuates phosphorylation of VEGFR2 in contact-inhibited confluent ECs (40). A small molecule inhibitor of PTP1B enhances VEGF-induced VEGFR2 autophosphorylation, migration, and proliferation of EC, as well as neovascularization in a mouse matrigel model (92). Angiopoietin-1 stimulates association of SHP-2 to the phosphorylated Tie-2 receptor (47), which in turn inhibits PI3 kinase-dependent signaling pathways leading to EC migration. The SHP-2 is oxidatively inactivated by PDGF-induced ROS when it binds to PDGFR (78). Thus, it is essential to identify the PTPs which are reversibly oxidized and inactivated after VEGF stimulation in ECs (Fig. 1).

CRITICAL REGULATORS IN ROS-DEPENDENT VEGF SIGNALING IN EC

Evidence suggests that VEGF signaling through NADPH oxidase-derived ROS is temporally and spatially organized by various signaling molecules. It has been shown that Rac1 and its regulators play an important role in this process. A partial list and description of the role of these proteins in ROS-dependent VEGF signaling in ECs are discussed below.

Rac1

The small G protein Rac1 is a key regulator for actin cytoskeleton and cell migration (105, 106) and is also a critical component of endothelial NADPH oxidase (7, 100). Endothelial migration is essential for formation of new blood vessels, and is a highly localized event requiring the generation of spatially and temporally restricted signals including PI3 kinase, Rac1, and H_2O_2 at the site of the new leading edge (Fig. 2) (56, 79). In addition to Rac1, p47phox and Nox2

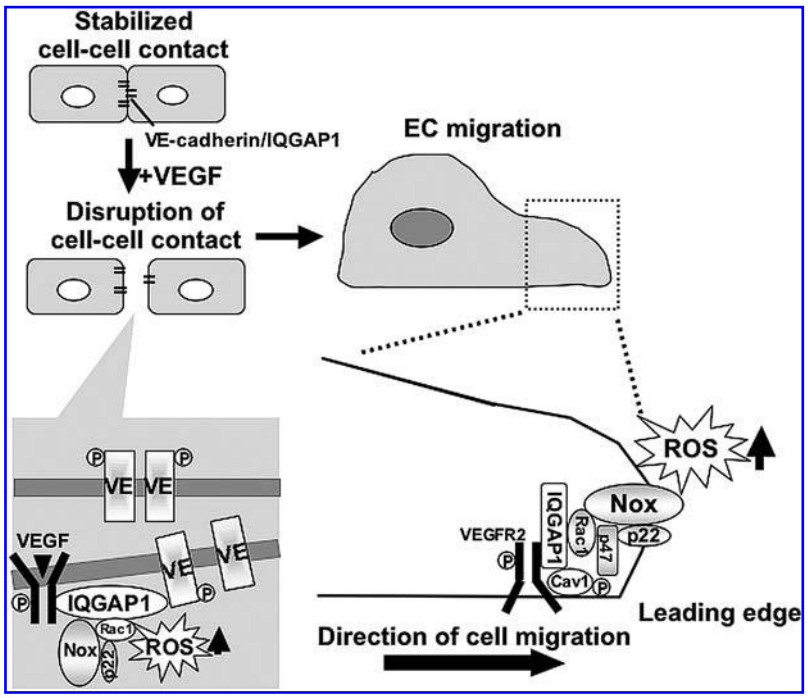

FIG. 2. Role of IQGAP1, an effector of Rac1, in ROS-dependent VEGF signaling linked to EC migration. Endothelial migration is a key event for angiogenesis and is a highly localized process requiring the generation of spatially and temporally restricted signals. One of the first events required for initiating EC migration is the loss of stable cell–cell contacts between ECs. IQGAP1 colocalizes with VE-cadherin at cell–cell junctions to stabilize cell–cell contact in confluent monolayer of ECs. VEGF stimulation promotes IQGAP1 association with activated VEGFR2 to link VEGFR2 to VE-cadherin to stimulate Rac1/ROS-dependent tyrosine phosphorylation of VE-cadherin, thereby promoting disruption of cell–cell contacts to initiates EC migration. During active EC migration, IQGAP1 functions as a scaffold protein to target active VEGFR2, Rac1, and NADPH oxidase (Nox and p47phox) to the focal complexes where phospho-Cav1 is found and lamellipodial leading edge, thereby stimulating localized ROS production and directional endothelial migration. Cav1, caveolin-1; VE, VE-cadherin; VEGFR2, VEGFR receptor type2.

subunits of NADPH oxidase also target to the focal complexes in lamellipodia, membrane ruffles, and the leading edge in actively migrating ECs, thereby stimulating localized ROS production (Fig. 2) (50, 107, 108). This locally increased ROS through activation of Rac1-dependent NADPH oxidase is required for cytoskeletal reorganization and directed EC migration (50, 79, 107), which may contribute to angiogenesis (Fig. 2). Consistent with this, VEGF promotes p47phox association with WAVE1, Rac1, and Rac1 effector PAK1 at the membrane ruffles, thereby stimulating p47phox phosphorylation, ROS production, and membrane ruffle formation in ECs (107). VEGF rapidly activates Rac1 and promotes translocation of Rac1 from the cytosol to the membrane, thereby activating NADPH oxidase in ECs (100). Overexpression of dominant negative N17Rac1 significantly inhibits VEGF-induced ROS production that is involved in VEGFR2 activation, EC migration, and proliferation (100). These findings suggest that Rac1 plays an important role in temporally- and spatially-organized ROS-dependent VEGF signaling linked to angiogenesis in ECs.

Caveolin-1

Caveolae and lipid rafts are cholesterol- and sphingolipid-rich plasma membrane microdomains where multiple signaling molecules, including G protein-coupled receptors, receptor tyrosine kinases, protein kinase C, Src family kinases, and G proteins are localized (20). They serve as platforms for compartmentalization of signaling. Caveolae and its signature protein caveolin-1 (Cav1) are polarized in migrating ECs, indicating their role in cell motility (81). Spatially restricted redistribution of lipid rafts at the leading edge is required for localized activation and coordination of the signaling linked to directed cell migration (37, 71). The polarization of cholesterol-enriched domains changes the plasma membrane microviscosity properties at the front of moving cells, thereby stimulating formation of the actin network required to push the leading-edge membrane forward (72). Furthermore, cell adhesion via integrins regulates targeting of active Rac1 and its coupling of PAK to the plasma membrane by preventing internalization of the lipid rafts (26).

NADPH oxidases have been identified in caveolae and lipid rafts in various cell systems (45, 104, 113), which could be important for the localized ROS production and specific activation of downstream redox signaling events. Zhang *et al*. showed that Fas ligand stimulation promotes recruitment of Nox2, p47phox, and Rac1 into lipid rafts, where there is an increase in NADPH oxidase activity and ROS production in ECs (113). VEGFR2 is present in endothelial caveolae through association with Cav1 which negatively regulates receptor activity in basal state (Fig. 1) (61). Dissociation of VEGFR2 from caveolae/Cav1 seems to be essential for VEGFR2 autophosphorylation and activation of downstream signaling events (61). Cav1 is tyrosine phosphorylated by ROS (14, 17) and by various growth factors (54, 70), including VEGF (61). We have demonstrated that VEGF promotes the release of VEGFR2 from caveolae/lipid rafts, which is contemporaneous with the tyrosine phosphorylation of Cav1 (Tyr14) and VEGFR2 and their colocalization at focal complexes, appearing as small-dot like structures at the edge of lamellipodia (Figs. 1 and 2)(52). Phospho-caveolin interacts with Grb7 (63), low molecular weight protein tyrosine phosphatase (16), and Csk, a negative regulator for Src (13). Thus, phospho-caveolin-1 seems to function as a scaffolding protein for growth factor-mediated signaling by serving as a docking site for phosphotyrosine-binding molecules at focal adhesions/complexes.

Of note, overexpression of mutant Cav1 (Y14F) blocks VEGF-stimulated localization of phospho-VEGFR2 at focal complexes as well as EC migration and proliferation. These findings suggest that pY14-Cav1 is required for proper localization of activated VEGFR2 at focal complexes and activation of ROS-dependent VEGF signaling linked to angiogenesis in ECs (Fig. 1). Given that Rac1 directs the Src to lamellipodia to phosphorylate focal complex proteins to promote formation of focal complexes (62, 96), it is tempting to speculate that Rac1 may play an important role in regulating pY14-Cav1-mediated, ROS-dependent VEGFR signaling at focal complexes in ECs.

ADP ribosylation factor 6

Little is known about the mechanisms by which VEGF activates Rac1 to stimulate ROS production and its downstream redox signaling in ECs. The small GTPase ADP-ribosylation factor 6 (ARF6) is involved in membrane trafficking, Rac1-mediated membrane ruffling, cortical actin remodeling, and cell motility (29). ARF6 also colocalizes with Rac1 in endosomes, and the two are simultaneously transported to the plasma membrane during motility (9). Both Rac1 and ARF6 have nucleotide-dependent interactions with the Arfaptin and Arfophilin proteins (91), which may play a role in their localization and transport linkage. The localization of ARF6 is guanine nucleotide dependent; in its GDP-bound form; it localizes to the cytosol and endosomal compartments, and when bound to GTP, it translocates to the plasma membrane with ARNO (35), its specific nucleotide exchange factor (34). ARNO promotes cell migration in part through activation of Rac1 (90). Bombesin-induced Rac1 activation is ARF6-dependent in CHO cells transfected with Rac1 (9). ARF6 is involved in membrane ruffling through regulating Rac1 translocation to the membrane (9, 86) and via interacting with the Rac1 binding protein POR1 (24). Thus, ARF6 and Rac1 regulate various biological responses in a coordinated manner. In ECs, VEGF stimulates ARF6 association with Rac1, which is cotemporaneous with Rac1 activation. The dominant negative ARF6 (T27N), inhibits VEGF-stimulated GTP-loading of Rac1, ROS production, and VEGFR2 autophosphorylation. These results suggest that ARF6 is an upstream mediator for Rac1, which may contribute to ROS production that mediates VEGFR2 autophosphorylation in ECs (Fig. 1). Although the mechanism by which ARF6 modulates Rac1-ROS pathway remains unclear, it is plausible that ARF6 may activate Rac1 through regulating a Rac-GEF (guanine nucleotide-exchange factor) such as beta-Pix, Vav, Tiam1, or Sos-1 (11).

We demonstrated that ARF6 is localized in caveolae/lipid rafts which contain Rac1 and VEGFR2 basally in ECs (49). ARF6 (T27N) inhibits VEGF-induced egress of VEGFR2 from caveolae/lipid rafts, and subsequent localization of activated VEGFR2 and pY14-Cav1 at focal complexes/adhesion, as well as EC migration and proliferation (49). Given that Cav1 binding to VEGFR2 inhibits receptor activity (61), ARF6 may act a positive regulator for VEGFR2 function by facilitating the egress of VEGFR2 from Cav1 in caveolae/lipid rafts. Because Rac1 promotes formation of focal complexes (82), and VEGF-induced Rac1 activation and tyrosine phosphorylation of Cav1 are inhibited by ARF6 (T27N), ARF6-Rac1 pathway may be involved in the formation of focal complexes to which activated VEGFR2 and pY14-Cav1 are recruited from caveolae/lipid rafts (Fig. 1). Additionally, ARF6 may regulate VEGFR2 phosphorylation through Rac1-ROS pathways, while downstream pY14-Cav1 is required for proper localization of activated VEGFR2 at focal complexes/adhesions (Fig. 1). In a mouse hindlimb ischemia model in which angiogenesis is dependent at least in part on VEGF (23), VEGFR2 (51), and NAD(P)H oxidase-derived ROS (97), ARF6 expression is markedly upregulated in the neovasculature. Thus, ARF6 plays an important role in the temporal-spatial organization of caveolae/lipid rafts- and ROS-dependent VEGF signaling in ECs, as well as in angiogenesis *in vivo* (Fig. 1).

IQGAP1

One of the first events required for initiating EC migration is the loss of stable cell–cell contacts between ECs in the parent vessel and the transition of a quiescent stationary to a dynamic migratory phenotype. The molecule primarily responsible for cell–cell adhesions of ECs is the VE-cadherin (22, 25, 39). Tyrosine phosphorylation of VE-cadherin is required for reducing cell–cell adhesions and is mediated through cSrc which is dependent on ROS (69, 103). The cytoplasmic domain of VE-cadherin binds directly to either β-catenin, plakoglobin, or p120 which couple to the actin cytoskeleton through α-catenin (8). VEGFR2 forms a complex with VE-cadherin, β-catenin, and PI3 kinase that is required for phosphorylation of Akt, which plays an important role in EC survival (15) and migration (27, 28, 80). Thus, VE-cadherin-based endothelial adherens junction is a membrane compartment which is essential for initial activation of VEGFR2-mediated, ROS-dependent signaling linked to angiogenesis.

Another important protein involved in ROS-dependent, VEGF-stimulated loss of cell–cell contact is IQGAP1, an effector of Rac1 (44, 74). Using a yeast two-hybrid system, we identified IQGAP1 as a novel VEGFR2 binding protein (111). IQGAP1 is a scaffold protein that interacts directly with actin, calmodulin, E-cadherin, and β-catenin (10, 75, 84), thereby regulating actin cytoskeleton, cell–cell adhesion, and morphogenesis. We demonstrated that IQGAP1 colocalizes with VE-cadherin at the sites of cell–cell contacts in unstimulated, confluent monolayers of ECs (Fig. 2)(110). VEGF stimulation reduces the junctional staining of VE-cadherin and IQGAP1, which represents the loss of cell–cell contacts. Knockdown of IQGAP1 using siRNA inhibits localization of VE-cadherin at cell–cell junctions, suggesting that IQGAP1 is required for establishment of EC integrity in quiescent ECs. VEGF stimulation promotes IQGAP1 binding to the active VEGFR2 which associates with VE-cadherin/β-catenin complex, which in turn stimulates recruitment of Rac1 to the IQGAP1 containing junctional complex, thereby activating Akt in ECs (15). Moreover, VE-cadherin tyrosine phosphorylation is inhibited by IQGAP1 siRNA, suggesting that IQGAP1 may function as a scaffold protein to link VEGFR2 to the VE-cadherin-containing complex at adherens junctions, thereby promoting Rac/ROS-dependent tyrosine phosphorylation of VE-cadherin, which may contribute to the loss

of cell–cell contacts and activation of downstream redox signaling events (Fig. 2).

In addition to its effect on cell–cell junction, IQGAP1 functions to couple Rac1 to the cytoskeleton and regulate Rac-mediated polarized cell migration (10, 75). Linkage between the microtubule plus-ends and cortical regions is essential for the establishment of cell polarity and directional migration. IQGAP1 captures and stabilizes microtubules by interacting with CLIP-170, a microtubule plus end binding protein, near the cell cortical regions (83). Activated Rac1 promotes capture of CLIP-170-capped microtubules in lamellipodia (75). At the leading edge of cells, Rac1 also links the adenomatous polyposis coli (APC) protein to actin filaments through binding to IQGAP1, thereby regulating polarization and directional migration by forming a complex with APC and CLIP-170. Of note, Nox2 also binds to and colocalizes with IQGAP1 at the leading edge in actively migrating ECs (Fig. 2) (50). IQGAP1 siRNA inhibits Nox2 translocation to the leading edge, ROS production, and cell migration (50); these findings suggest that IQGAP1 also functions as a scaffold protein to target Rac1 and Nox2 to the leading edge to promote localized ROS production, thus achieving specificity of redox signaling involved in EC migration (Fig. 2). We also demonstrated that VEGF-induced capillary tube formation in three-dimensional culture in type I collagen gels is impaired in IQGAP1 knocked down ECs. Moreover, IQGAP1 protein expression is markedly increased in newly formed ECs in a mouse hindlimb ischemia model which is dependent on ROS and VEGF (97). These findings suggest that IQGAP1 may play an important role in postnatal angiogenesis *in vivo*. This point requires further investigation using IQGAP1$^{-/-}$ mice.

CONCLUSIONS AND FUTURE DIRECTIONS

The effects of ROS are tightly regulated and dependent on the amount and site of production. The information presented here is consistent with the notion that ROS derived from NADPH oxidase and their regulators play an important role in VEGF signaling linked to angiogenesis in ECs *in vitro* and *in vivo*. NADPH oxidase appears to be localized within discrete subcellular compartments, thereby activating specific redox-signaling pathways. These events could be regulated by Rac1, Cav1, ARF6, PTPs, and IQGAP1, which may contribute to the temporal and spatial organization of ROS-dependent VEGF signaling linked to angiogenesis in ECs. These findings provide new insights into the NADPH oxidase and regulators of redox signaling as potential therapeutic targets for treatment of angiogenesis-dependent diseases. Development of new molecular tools as well as tissue-specific transgenic and knockout and knockin animals will be an important objective for future investigation.

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ABBREVIATIONS

APC, adenomatous polyposis coli; ARF6, ADP-ribosylation factor 6; Cav1, caveolin-1; DEP-1, high cell density-enhanced PTP1; ECs, endothelial cells; PTPs, protein tyrosine phosphatases; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor type2; VEGFR1, VEGF receptor-1.

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