The Vascular Endothelial Growth Factor Receptor KDR Activates Multiple Signal Transduction Pathways in Porcine Aortic Endothelial Cells*

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Vascular endothelial growth factor A (here referred to as VEGF) is an endothelium-specific growth factor that binds to two distinct receptor tyrosine kinases, designated Flt-1 and KDR/flk-1. VEGF stimulates autophosphorylation of both receptors, but little is known about their signal transduction properties. In this study, we used porcine aortic endothelial (PAE) cells overexpressing KDR (PAE/KDR) to evaluate the interaction of KDR with intracellular proteins and compared them with Flt-1-expressing PAE cells (PAE/Flt-1). VEGF-induced stimulation of KDR results in the association and phosphorylation of the 46-, 52-, and 66-kDa isoforms of Shc and the induction of Shc-Grb2 complex formation. In a similar fashion, KDR associates with Grb2 and Nck in a ligand-dependent fashion, suggesting Shc, Grb2, and Nck as potential candidates involved in the regulation of endothelial function. Another strong candidate is mitogen-activated protein (MAP) kinase, which is strongly activated in response to VEGF stimulation as demonstrated by phosphorylation of the specific substrate myelin basic protein. Inhibition of MAP kinase activation by PD98059, a specific MAP kinase inhibitor, results in inhibition of VEGF-induced proliferation of PAE/KDR cells. In contrast, VEGF-induced stimulation of Flt-1 does not activate MAP kinase in PAE/Flt-1 cells. In this study we provide the first two examples of molecules potentially capable of functionally counteracting the endothelial response to VEGF, namely SHP-1 and SHP-2. These two SH2 protein-tyrosine phosphatases physically associate with KDR secondary to VEGF stimulation, raising the interesting possibility that both molecules participate in the generation and/or modulation of VEGF-induced signals. Taken together, our results substantially broaden the spectrum of KDR-associating molecules, indicating that endothelial function and angiogenesis are regulated by a diverse network of signal transduction cascades.

Vascular endothelial growth factor A is a dimeric endothelium-specific growth factor (1–3). It stimulates mitogenicity (4), chemotaxis (5), and thrombogenicity (6) in endothelial cells in vitro and is up-regulated by hypoxia both in vitro (7) and in vivo (8, 9), indicating that VEGF† is a major angiogenic factor. The VEGF gene encodes four different proteins (VEGF121, VEGF165, VEGF189, and VEGF206) (10) as a result of alternative splicing. Recently, two novel and distinct VEGF-A-related genes were identified, namely VEGF-B (11) and VEGF-C (12). The functional roles of VEGF-B and VEGF-C remain to be elucidated.

VEGF-A binds to two different receptor tyrosine kinases, designated Flt-1 (flas-like tyrosine kinase-1; VEGF receptor-1) (13, 14) and KDR (kinase-insert domain-containing receptor; VEGF receptor-2) (15, 16), the human homologue of mouse Flk-1 (17). Flt-4 is a structurally related receptor that binds VEGF-C (12), but not VEGF-A (18). Each of these receptors consists of an extracellular domain containing seven immunoglobulin-like motifs, a transmembrane domain, a juxtamembrane domain, a carboxyl-terminal tail, and a tyrosine kinase domain, which is interrupted by a long kinase-insert domain consisting of >100 amino acids. Binding of VEGF-A (referred to as VEGF below) induces conformational change in KDR and Flt-1, followed by dimerization and autophosphorylation on tyrosine residues (5, 19). The phosphorylation on tyrosine residues makes them become targets for SH2, SH3, and phosphotyrosine-binding domain-containing molecules, which frequently become phosphorylated themselves. Thereafter, consecutive intracellular signal transduction events are triggered (20, 21). Three classes of SH2 domain-containing molecules are currently known: (i) molecules without any catalytic activity named adapter molecules; (ii) SH2 domain-containing enzymes including cytoplasmic tyrosine kinases, phospholipase Cγ, GAP, and protein-tyrosine phosphatases SHP-1 and SHP-2 (22) as well as nucleotide exchange factors; and (iii) structural proteins (23).

So far, only limited information is available concerning function and signal transduction of VEGF receptors. KDR is able to induce striking changes in cell morphology, actin reorganization, membrane ruffling, mitogenicity, and chemotaxis in porcine aortic endothelial (PAE) cells overexpressing KDR (PAE/KDR) in response to VEGF stimulation (5). In molecular terms, VEGF stimulation of KDR-expressing cells results in the phosphorylation of GAP, members of the Src family of protein kinases, and phospholipase Cγ as well as of p42 MAP kinase as determined by an in vitro kinase assay and Western blot analysis (5, 24, 25). In the case of Flt-1, the information about cellular function and mechanism of action is even further limited. Flt-1 mediates chemotactic activity in monocytes and stimulates tissue factor expression in monocytes and endothelial cells (26). However, VEGF stimulation of Flt-1 does not factor; GAP, Ras GTPase-activating protein; PAE, porcine aortic endothelial; MAP, mitogen-activated protein; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor.

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† The abbreviations used are: VEGF, vascular endothelial growth factor; GAP, Ras GTPase-activating protein; PAE, porcine aortic endothelial; MAP, mitogen-activated protein; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor.
induce mitogenicity and chemotaxis in Flt-1-expressing PAE cells (PAE/Flt-1) (5). VEGF stimulation of Flt-1-expressing cells, however, results in autophosphorylation of Flt-1 and phosphorylation of members of the Src family of protein kinases, GAP, phospholipase C\gamma, and Shc proteins (5, 27). Both VEGF receptors play important roles in developmental angiogenesis. Mouse knockout experiments in KDR/Flk-1 cells and Flt-1 resulted in the generation of distinct developmentally lethal phenotypes. While Flt-1−/− mice showed defects in vessel formation (28), the Flk-1−/− knockout did not produce endothelial cells at all (29).

In this study we used PAE/KDR cells in culture for the analysis of KDR signal transduction. Using this approach, we are able to demonstrate that VEGF stimulation of KDR results in the activation of multiple intracellular signal transduction pathways. We have shown the ligand-induced phosphorylation of all three isoforms of Shc proteins and the phosphorylation of GAP as well as the strong activation of MAP kinase. In addition, KDR is shown to associate with Shc, Shc-Grb2, Nck, Grb2, and GAP. Moreover, we demonstrate that KDR associates with at least two distinct protein-tyrosine phosphatases, namely SHP-1 and SHP-2, which are potentially involved in either negative regulation (i.e. dephosphorylation) or positive regulation (i.e. signal enhancement) of VEGF-induced signals.

MATERIALS AND METHODS

Cells, Antiserum, and Other Reagents—PAE cells transfected with a modified pcDNA vector carrying KDR cDNA or Flt-1 cDNA were used as described previously (5). Nontransfected PAE cells served as controls. Cells were cultivated in Ham's F-12 medium (Biochrom) containing 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂. For starvation of cells, serum-free medium was supplemented with 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂. For starvation of cells, serum-free medium was supplemented with 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂.

Preparation of cell lysates and other reagents has been described previously (5). Nontransfected PAE cells served as controls. Cells were cultivated in Ham's F-12 medium (Biochrom) containing 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂. For starvation of cells, serum-free medium was supplemented with 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂. For starvation of cells, serum-free medium was supplemented with 10% fetal calf serum, glutamine, and antibiotics at 37 °C and 5% CO₂.

RESULTS

Molecules of the Ras Pathway Are Involved in VEGF-induced Signaling, Resulting in a Strong Activation of MAP Kinase and Proliferation of PAE/KDR Cells—VEGF stimulation of PAE/KDR cells resulted in a strong autophosphorylation of the receptor KDR. Using a receptor-specific antibody, a number of phosphorylated proteins could be immunoprecipitated together with KDR. They migrated at approximate sizes of 44, 52, 60, 65, 85, and 140 kDa (Fig. 1). VEGF-activated KDR is able to induce mitogenicity and chemotaxis in PAE/KDR cells in vitro (5), but only limited information is available about the identity and function of molecules that transmit KDR-induced signals.

Phosphorylation of Shc proteins is an early event in intracellular signaling in a number of well characterized receptor tyrosine kinases. The ability of KDR to induce phosphorylation of Shc proteins was investigated in PAE/KDR cells using Western blot analysis and in vitro kinase assays. A strong phosphorylation of the 46- and 52-kDa isoforms of Shc was observed upon VEGF stimulation of PAE/KDR cells (Fig. 2A, upper panel). VEGF-induced tyrosine phosphorylation of the 66-kDa isoform of Shc was demonstrated after a longer exposure of the same membrane (Fig. 2A, lower panel). In addition, this phosphotyrosine blot was sensitive enough to detect the phosphorylation of three as yet unidentified proteins migrating on SDS-PAGE according to approximate sizes of 110, 140, and 200 kDa (Fig. 2A, lower panel). No VEGF-dependent phosphorylation of Shc proteins was observed in nontransfected PAE cells (data not shown). Grb2 associated with Shc molecules in re-
response to VEGF stimulation of PAE/KDR cells as demonstrated by stripping of the phosphotyrosine blot (shown in Fig. 2A) and reprobing with an antiserum recognizing Grb2 (Fig. 2B). Association of KDR with Shc in response to VEGF stimulation was demonstrated using an in vitro kinase assay. KDR was visualized in anti-Shc immunoprecipitates from lysates of VEGF-stimulated PAE/KDR cells on SDS-PAGE as a 210-kDa band following in vitro kinase reaction (Fig. 2C). In a similar experiment, stimulation of nontransfected PAE cells either with VEGF or with 10% fetal calf serum did not result in the generation of a signal of 210 kDa (data not shown). To characterize the 210-kDa signal, we used tyrophostin AG1433, a specific inhibitor of the PDGF β-receptor and KDR/Flik-1 (31). In our hands, tyrophostin AG1433 completely inhibited PDGF-BB-induced autophosphorylation of the PDGF β-receptor at a concentration of 10 μM in PAE cells overexpressing the PDGF β-receptor. Complete inhibition of VEGF-induced autophosphorylation of KDR in PAE/KDR cells was found at a concentration of 30 μM (data not shown). Since nontransfected PAE cells do not express the PDGF β-receptor (32) and because KDR and the PDGF β-receptor can clearly be distinguished by their different electrophoretic mobilities on SDS-PAGE, tyrophostin AG1433 can be regarded as a specific KDR inhibitor in PAE/KDR cells. Preincubation of PAE/KDR cells with 30 μM tyrophostin AG1433, VEGF stimulation, and Shc immunoprecipitation followed by the in vitro kinase assay did not result in the generation of a phosphorylated signal at 210 kDa (data not shown).

KDR associated with the SH2/SH3 domain-containing adapter molecules Grb2 and Nck. KDR associated with Grb2 in response to VEGF stimulation as demonstrated by Grb2 immunoprecipitation followed by the in vitro kinase assay (Fig. 3). Similar results were obtained for the Nck protein (Fig. 4A). Abundant Nck expression was detectable in PAE/KDR cells using Western blot analysis (Fig. 4B). Preincubation of PAE/KDR cells with tyrophostin AG1433 abolished the signal at ~210 kDa in both Grb2 and Nck immunoprecipitates (Figs. 3 and 4A). No VEGF-dependent association of either Grb2 or Nck with KDR was observed in nontransfected PAE cells (Figs. 3 and 4A). We did not, however, observe tyrosine phosphorylation of the Nck or Grb2 protein in response to VEGF stimulation using both the in vitro kinase assay and Western blot analysis (data not shown).

Previous work has shown that KDR is able to induce weak phosphorylation of GAP (5). In addition to these findings, we were able to demonstrate the VEGF-induced association of GAP with KDR by the in vitro kinase assay (Fig. 5).

A functionally important molecule of the Ras pathway is MAP kinase. We therefore tested whether MAP kinase is activated in response to VEGF stimulation of PAE/KDR cells. A strong activation of MAP kinase was found as indicated by the phosphorylation of the MAP kinase-specific substrate myelin basic protein. In contrast, VEGF stimulation of nontransfected PAE cells or of PAE/Flik-1 cells did not induce the activation of MAP kinase (Fig. 6A). Preincubation of PAE/KDR cells with PD98059 at concentrations up to 100 μM, a selective inhibitor of the phosphorylation and activation of MAP kinase (33), resulted in a dose-dependent inhibition of MAP kinase activity in PAE/KDR cells (Fig. 6A). VEGF-induced DNA synthesis of PAE/KDR cells was inhibited by PD98059 in a dose-dependent fashion at concentrations up to 10 μM (Fig. 6B).

KDR Associates with Distinct Protein-tyrosine Phosphatases in a Ligand Stimulation-dependent Fashion—Recent data have provided compelling evidence that not only tyrosine kinases, but also tyrosine phosphatases are involved in the signal transduction of receptor tyrosine kinases (34). Expression of the two
tyrosine phosphatases SHP-1 and SHP-2 was demonstrated in PAE cells using Western blot analysis and antibodies recognizing SHP-1 (Fig. 7A) and SHP-2 (Fig. 7B). VEGF stimulation of PAE/KDR cells induced a physical association of either phosphatase with KDR as determined by protein-tyrosine phosphatase-specific immunoprecipitation followed by the in vitro kinase assay. Its appearance was dependent on VEGF stimulation, and moreover, treatment of PAE/KDR cells with the KDR-specific tyrphostin AG1433 resulted in a complete inhibition of KDR phosphorylation and in the disappearance of the 210-kDa band. VEGF stimulation of nontransfected PAE cells showed no corresponding signal in a similar experiment (data not shown). To further verify the

Fig. 3. Association of Grb2 with KDR. PAE/KDR cells and nontransfected PAE cells were stimulated for 5 min with 50 ng/ml VEGF. Cell lysates were immunoprecipitated (IP) with an antiserum recognizing Grb2, followed by the in vitro kinase assay. Samples were analyzed by SDS-PAGE, followed by KOH treatment and autoradiography. Tyrphostin AG1433 was added to PAE/KDR cells 10 min prior to VEGF stimulation at a final concentration of 30 μM.

Fig. 4. Nck and KDR signaling. A, association of Nck with KDR. PAE/KDR cells and nontransfected PAE cells were stimulated for 5 min with 50 ng/ml VEGF. The cell lysate was immunoprecipitated (IP) with an antiserum recognizing Nck, followed by the in vitro kinase assay. Samples were analyzed by SDS-PAGE, followed by KOH treatment and autoradiography. Tyrphostin AG1433 was added to PAE/KDR cells 10 min prior to VEGF stimulation at a final concentration of 30 μM. B, identification of Nck in PAE/KDR cells by Western blot analysis. The PAE/KDR cell lysate was subjected to SDS-PAGE and blotted onto a nitrocellulose membrane. Nck was identified by incubation with an anti-Nck antiserum, followed by peroxidase-conjugated rabbit antimouse Ig as a secondary antibody and chemiluminescence. The human HeLa cell lysate was used as a positive control (Ctrl) for the Nck protein. IB, immunoblot.

Fig. 5. In vitro phosphorylation and association of GAP in PAE/KDR cells. PAE/KDR cells were stimulated for 5 min with 50 ng/ml VEGF. The cell lysate was immunoprecipitated (IP) with an antiserum recognizing GAP, followed by the in vitro kinase assay. Samples were separated by SDS-PAGE, fixed, treated with KOH, dried, and autoradiographed.

Fig. 6. Role of MAP kinase in KDR signaling. A, inhibition of MAP kinase (MAPK) activation by PD98059. PAE/KDR, PAE, and PAE/Flt-1 cells were stimulated with 50 ng/ml VEGF for 5 min at 37 °C. Cell lysates were immunoprecipitated (IP) using an antiserum recognizing MAP kinase, followed by the in vitro kinase assay in the presence of myelin basic protein (MBP). Samples were separated by SDS-PAGE, fixed, treated with KOH, dried, and autoradiographed. The MAP kinase kinase inhibitor PD98059 was incubated at different concentrations for 30 min prior to VEGF stimulation. B, inhibition of VEGF-induced proliferation of PAE/KDR cells by PD98059. PAE/KDR cells were grown in 12-well culture plates; serum-starved for 48 h; incubated for 30 min with 0.3, 1, 3, and 10 μM PD98059; and then stimulated with 1 ng/ml VEGF for 20 h. The data represent the means of triplicates, and similar results were obtained in at least two different experiments.
shown). In vitro kinase assay or phosphotyrosine blot analysis (data not shown). PAE/KDR cells following VEGF stimulation using either the in vitro kinase assay, no signal of phosphorylation of SHP-1 or SHP-2 was not detected in these cells. In order to test specificity of this coprecipitation, we repeated the same experiment using an antibody to immunoprecipitate the insulin receptor. In this case, in the in vitro kinase assay, no signal of -210 kDa was observed (data not shown). In our hands, tyrosine phosphorylation of SHP-1 or SHP-2 was not detected in PAE/KDR cells following VEGF stimulation using either the in vitro kinase assay or phosphotyrosine blot analysis (data not shown).

**DISCUSSION**

In this study, we were able to significantly extend our knowledge on the signal transduction properties of the VEGF receptor KDR. For our in vitro studies, we used porcine aortic endothelial cells overexpressing KDR. VEGF stimulation results in the autophosphorylation of KDR, the phosphorylation of a number of intracellular proteins, and the association of intracellular proteins with phosphorylated KDR. So far, only little information has been available concerning the identity of signal transduction molecules involved in KDR signaling. In this study, we were able to demonstrate that Shc, Nck, and Grb2 as well as SHP-1 and SHP-2 are involved in these processes.

The phosphorylation of Shc proteins is an early event in the signal transduction cascade of many activated receptor tyrosine kinases leading to the MAP kinase pathway. Shc is able to associate with several growth factor receptors through its C-terminal SH2 domains and N-terminal phosphotyrosine-binding domain (35, 36). Shc mediates the coupling of the Grb2-Sos complex to Ras via its major phosphorylation site, Tyr-317 (37). According to our data, 5 min of VEGF stimulation results in the phosphorylation of all three isoforms of Shc proteins, whereby the 46- and 52-kDa isoforms were more strongly phosphorylated compared with the 66-kDa isoform. Simultaneously, activated KDR triggers the complex formation between Shc molecules and the adapter molecule Grb2. Thus, KDR transmits mitogenic signals via the Shc/Grb2/Sos/Ras cascade, inducing a strong activation of MAP kinase and finally resulting in the proliferation of PAE/KDR cells. These data are strongly supported by the fact that inhibition of MAP kinase using PD98059 leads to inhibition of VEGF-induced proliferation of these cells. The phosphorylation of Shc proteins also plays a role in the signaling of the other VEGF receptor, Flt-1 (27), and the related receptor, Flt-4 (38, 39). VEGF stimulation of NIH3T3 cells overexpressing Flt-1 induced a slight increase in tyrosine phosphorylation of the 52- and 66-kDa isoforms of Shc proteins (27). Despite this weak phosphorylation of Shc proteins by Flt-1, VEGF stimulation of Flt-1 in NIH3T3 fibroblasts does not result in activation of MAP kinase and does not generate any mitotic response. Activation of MAP kinase in sinusoidal endothelial cells by VEGF may be mediated via KDR since these cells express both Flt-1 and KDR (40). Failure of induction of a mitogenic response in PAE/Flt-1 cells secondary to VEGF stimulation has been described recently (5). In accordance with these results, we show that Flt-1 is unable to activate MAP kinase in PAE/Flt-1 cells, which is consistent with our recent observation that Flt-1 is unable to induce DNA synthesis in PAE/Flt-1 cells (5).

Besides Shc, KDR was found to associate with Grb2 and Nck in a ligand-dependent fashion. Nck (41) and Grb2 (42, 43) are small adapter molecules consisting largely of SH2 and SH3 domains. Grb2 links receptor tyrosine kinase activity to Ras activation (44), whereas the downstream signaling pathway of Nck remains to be elucidated. It is important to note, however, that overexpression of Nck leads to transformation of fibroblasts (45), indicating that Nck is a positive mediator of mitogenic signaling. Nck becomes phosphorylated on tyrosine, serine, and threonine in response to epidermal growth factor and PDGF β-receptor activation (46). Our data clearly show that both Grb2 and Nck are involved in VEGF-induced signaling of KDR. Further studies will be required to elucidate the detailed function of Grb2 and Nck in endothelial cells.

A weak phosphorylation of GAP secondary to VEGF stimulation of KDR has previously been reported (5). With this study, we are able to add more data, as we have demonstrated the VEGF-dependent association of GAP protein with activated KDR. Likewise, GAP is phosphorylated by Flt-1 (5, 27) and by stimulation of bovine aortic endothelial cells with VEGF (47). VEGF stimulation of PAE/KDR cells followed by immunoprecipitation with a number of different antibodies (such as the ones recognizing Shc, Grb2, Nck, and GAP) prior to SDS-PAGE leads to the detection of a phosphorylated signal migrating at -210 kDa. This signal is likely to represent KDR because its appearance depends on VEGF stimulation and it shows the electrophoretic mobility of KDR (210 kDa) and is not detectable after precubination of PAE/KDR cells with the KDR inhibitor tyrphostin AG1433. Moreover, stimulation of nontransfected PAE cells with VEGF or with 10% fetal calf serum does not result in the generation of such a signal, indicating that this 210-kDa band truly represents KDR. However, the possibility that KDR phosphorylated another protein of similar size cannot be fully excluded.

The intracellular signaling cascades represent a complex network, which is influenced by a variety of components and conditions. Following stimulation of kinase-dependent pathways leading to proliferation, differentiation, migration, or other cellular events, it seems to be important for cells to switch off these signals to prevent uncontrolled cellular growth.
One such possibility is the activation of phosphatases, which can inactivate phosphoproteins through dephosphorylation. It has been shown that the SH2 domain-containing protein-tyrosine phosphatase SHP-1, predominantly expressed in hematopoietic cells, can associate with activated growth factor receptors such as the stem cell factor receptor/c-Kit (48) and the CSF-1 receptor (49). SHP-1 seems to play a role in the negative regulation of receptor signaling. It associates with the activated insulin receptor and is consecutively phosphorylated itself on Tyr-538, resulting in an increase in phosphatase activity (50). Transient overexpression of SHP-1, together with overexpression of the PDGF α-receptor, the PDGF β-receptor, or the β-subunit of the insulin receptor, has led to partial or complete dephosphorylation of these receptors or receptor domains (51).

Surprisingly, we have found that SHP-1 phosphatase is expressed in PAE cells, suggesting that SHP-1 is potentially involved in endothelial cell function. Stimulation of PAE/KDR cells with VEGF resulted in a physical association of SHP-1 with SHP-1, suggesting that SHP-1 is potentially regulated by VEGF, resulting in a positive or a negative effect on specific signal transduction pathways (53). However, the functional regulation of receptor signaling. It associates with the activated KDR. Similar to the findings observed for SHP-1, no association of SHP-2 with the activated PDGF α-receptor, the PDGF b-receptor, or the insulin receptor.

In contrast, recent work has demonstrated that SHP-1 can associate with the protein-tyrosine kinase c-Src in human platelets (52), where SHP-1 has a positive effect on mitogen-activated signaling in 293 cells (53), and that SHP-1 is potentially involved in the Ras-mediated activation of the MAP kinase pathway (54). Taken together, these results indicate that the action of SHP-1 is dependent on the cellular background, resulting in either a positive or a negative effect on specific signal transduction pathways (53). However, the functional role of SHP-1 in endothelial cells remains to be elucidated in more detailed work.

Finally, we have found that another protein-tyrosine phosphatase, namely SHP-2, physically associates with VEGF-activated KDR. Similar to the findings observed for SHP-1, no tyrosine phosphorylation of SHP-2 was observed after VEGF stimulation of PAE/KDR cells, even though two different methods (in vitro kinase assay and phosphotyrosine blot analysis) and several experimental conditions have been tested. SHP-2 is able to associate with other activated receptors, and it is phosphorylated by these receptors on tyrosine residues (34). In turn, SHP-2 can dephosphorylate the activated PDGF β-receptor preferentially at phosphotyrosines 751 and 771 in vitro (55), whereas it is unable to dephosphorylate several other receptor tyrosine kinases (51). In PAE/KDR cells, however, we have no data suggesting the deactivation of KDR by SHP-2. An interesting recent observation was the association of SHP-2 with Grb2, leading to the activation of the MAP kinase pathway (56, 57). This positive role of SHP-2 in the signal transduction of receptor tyrosine kinases was rather unexpected, and most recent work indicates the possibility that, depending on the specific signaling pathway, SHP-1 and SHP-2 may have either positive or negative effects (58). Future work will reveal the functional role of SHP-1 and SHP-2 in the control of endothelial cell function.

In this paper, we have shown that the VEGF receptor KDR is able to induce multiple signal transduction events in porcine aortic endothelial cells. We have shown that KDR activates proteins of the Ras pathway in a similar fashion to other receptor tyrosine kinases and that inhibition of MAP kinase activation results in abrogation of VEGF-induced proliferation of PAE/KDR cells. In addition, the involvement of SH2 protein-tyrosine phosphatases in VEGF signaling was established.

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