Sphingosine 1-phosphate (S1P) and vascular endothelial growth factor (VEGF) elicit numerous biological responses including cell survival, growth, migration, and differentiation in endothelial cells mediated by the endothelial differentiation gene (29), a family of G-protein-coupled receptors, and fetal liver kinase-1/kinase-insert domain-containing receptor (Flk-1/KDR), one of VEGF receptors, respectively. Recently, it was reported that S1P or VEGF treatment of endothelial cells leads to phosphorylation at Ser-1179 in bovine endothelial nitric oxide synthase (eNOS), and this phosphorylation is critical for eNOS activation. S1P stimulation of eNOS phosphorylation was shown to involve G_i, protein, phosphoinositide 3-kinase, and Akt. VEGF also activates eNOS through Flk-1/KDR, phosphoinositide 3-kinase, and Akt, which suggested that S1P and VEGF may share upstream signaling mediators. We now report that S1P treatment of bovine aortic endothelial cells acutely increases the tyrosine phosphorylation of Flk-1/KDR, similar to VEGF treatment. S1P-mediated phosphorylation of Flk-1/KDR, Akt, and eNOS were all inhibited by VEGF receptor tyrosine kinase inhibitors and by antisense Flk-1/KDR oligonucleotides. Our study suggests that S1P activation of eNOS involves G_i, calcium, and Src family kinase-dependent transactivation of Flk-1/KDR. These data are the first to establish a critical role of Flk-1/KDR in S1P-stimulated eNOS phosphorylation and activation.

Nitric oxide (NO) produced by endothelial NO synthase (eNOS) has a crucial role in the regulation of vascular tone, vascular remodeling, and angiogenesis (1–3). Recent evidence has established the involvement of NO in vascular endothelial growth factor (VEGF)-induced angiogenesis (4, 5). For example, eNOS inhibitors block VEGF-induced endothelial cell (EC) migration, proliferation, and tube formation in vitro as well as VEGF-induced angiogenesis in vivo. VEGF is known to stimulate phosphoinositide 3-kinase (PI3K) and Akt-dependent phosphorylation of eNOS, resulting in activation of eNOS and increased NO production (6–8). In EC the predominant VEGF receptor that mediates eNOS phosphorylation is Flk-1/KDR (9, 10).

Recently, sphingosine 1-phosphate (S1P), a bioactive lipid released by activated platelets, has emerged as an important mediator of angiogenesis. S1P induces migration, proliferation, and cytoskeletal changes of EC by binding to the endothelial differentiation gene (EDG), a family of G-protein-coupled receptors (GPCR) (11–14). In the EDG family, EDG-1, EDG-3, EDG-5, EDG-6, and EDG-8 work as S1P receptors (15, 16). Among them, EDG-1 is the best studied receptor and plays a major role in EC and smooth muscle cell function. Activation of EDG-1 receptor triggers several signaling pathways via pertussis toxin (PTx)-sensitive G protein. Although the signaling pathways activated by S1P have been extensively studied in several cell types, the precise signaling mechanism by which S1P elicits angiogenesis remains unclear. Recent studies revealed that S1P binding to EDG-1 receptor increases NO synthesis through the PI3K-Akt pathway in EC, similar to VEGF (17–19).

The similarity in S1P and VEGF activation of eNOS through PI3K and Akt suggested shared upstream signaling mediators. Transactivation of receptor tyrosine kinases such as epidermal growth factor (EGF) receptor, platelet derived growth factor (PDGF) receptor and insulin-like growth factor receptor in response to activation of many GPCR has been reported (20–27). Transactivation of the EGF receptor by S1P has also been reported (28). We hypothesized that transactivation of a growth factor receptor may be involved in S1P-stimulated PI3K-Akt-eNOS phosphorylation and investigated the specific role of Flk-1/KDR.

Here we show that S1P signaling increases tyrosine phosphorylation of VEGF receptor Flk-1/KDR. Importantly, we demonstrate that inhibition of tyrosine kinase activity of Flk-1/KDR reduces S1P-stimulated Akt and eNOS phosphorylation. These results demonstrate a critical role of Flk-1/KDR in S1P-induced PI3K-Akt-eNOS activation.

MATERIALS AND METHODS

Reagents—S1P, o-phenanthroline, N-acetylcycteine (NAC), 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron), ebselen, and diphenylene iodonium (DPI) were purchased from Sigma.
from R & D Systems (Minneapolis, MN). VEGF receptor tyrosine kinase inhibitor (VTKI, 4-((4’-chrolo-2’-fluoro)phenylamino)-6,7-dimethoxyquinazoline), SU1498, AG 1478, PTX, 1,2-bis(aminophenoxyl)ethane-N,N,N’,N’-tetraacetic acid (tetracetylmethyl) ester (BAPTAM), 4-amino-5-(4-chlorophenyl)-7-(3-butyryl)pyrazolo[4,3-d]pyrimidine (FP2), chelerythrine chloride, wortmannin, AG1024, and ATP were purchased from Calbiochem (San Diego, CA). Anti-phospho-eNOS antibody (phosphoserine 1177 in the human eNOS sequence, corresponding to Ser–1179 in bovine eNOS), anti-phospho-Akt antibody (Ser-473), and anti-Akt antibody were from Cell Signaling Technologies (Beverly, MA). Anti-eNOS monoclonal antibody was from Transduction Laboratories (Lexington, KY). Anti-flk-1 polyconal antibody and protein A/G PLUS-agarose were from Santa Cruz (Santa Cruz, CA). 577B11 anti-VEGF neutralizing monoclonal antibody is a gift from Dr. Bjercke. Horseradish peroxidase-conjugated anti-rabbit secondary antibody, anti-mouse secondary antibody, and chemiluminescence ECL detection kit were from Amersham Biosciences.

Cell Culture—Bovine aortic endothelial cells (BAEC) were purchased from Clonetics (San Diego, CA) and were cultured in medium 199 supplemented with 10% fetal bovine serum (Invitrogen), basal MEM vitamins, and amino acids (Invitrogen) as described before (29). Cultures grew in 60-mm dishes were serum-starved for 18–24 h and stimulated by 1% VEGF or S1P. For the inhibitor studies, cells were pretreated with 50 ng/ml PTX for 24 h, 30 μM SU1498, 10 μM VTKI, 1 μM AG1478, 10 μg/ml TransIT-L1 (Mirus, Madison, WI) was mixed with 360 nM oligonucleotide as reported by Berne et al. (30) against bovine Flk-1/KDR (AS-KDR: 5’/H11032-GTC TGG CAT GTG CGT TGT-3’/H9262) and stimulated with medium 199 with 10% serum, and cells were cultured for 20 h. The cells changed to medium 199 with 10% serum and cultured for 20 h. The cells were washed with Opti-MEM and incubated at room temperature for 20 min. Then 1.5 μl of 1 μM sense or scramble S-oligonucleotide was added to the solution, mixed gently, and incubated at room temperature for 30 min. This mixture was added to BAEC in 2 ml of Opti-MEM (final concentration of S-oligonucleotide was 0.6 μM) and cultured for 4 h. The medium was changed to medium 199 with 10% serum and cultured for 20 h. The cells were washed with Opti-MEM and treated again with TransIT-LT1/S-oligonucleotide (final concentration 0.3 μM) for 4 h. The medium was changed to medium 199 with 10% serum, and cells were cultured for 20 h. Finally, cells were serum-starved for 24 h and stimulated by S1P or VEGF.

Immunoblot Analysis—Western blot analyses were performed as described previously (24, 31). BAEC were lysed in Triton/Nonidet P-40 lysis buffer (0.5% Triton X100, 0.5% Nonidet P-40, 10 mM Tris, pH 7.5, 2.5 mM KCl, 150 mM NaCl, 30 mM β-glycerophosphate, 50 mM NaF, 1 mM Na3VO4, and 0.1% protease inhibitor mixture (Sigma), scraped off the dish, and centrifuged at 10,000 × g for 10 min, and the supernatant was used as a total cell lysate. The supernatant was analyzed for total protein concentration by the Bradford method (BioRad), and equal amounts of cellular proteins (50 μg/lane) were separated by SDS-PAGE and transferred to a nitrocellulose membrane (HybondTM ECL, Amer sham Biosciences). After incubation in blocking solution (5% bovine serum albumin, phosphate-buffered saline, pH 7.5, 0.1% Tween 20), membranes were incubated with appropriate primary antibodies, such as anti-phospho-Akt (Ser-473), anti-phospho-eNOS (Ser-1177), or anti-phosphothreonine (clone 4G10) antibodies, for 2 h at room temperature. Membrane-bound primary antibodies were visualized by horseradish peroxidase-conjugated secondary antibodies and the ECL system. The membranes were probed with anti-Akt, anti-eNOS, or anti-Flk-1/KDR antibodies. Densitometric analyses of immunoblots were performed by NIH image. Results were normalized by arbitrarily setting the densitometry of control cells to 1.0.

RESULTS

Rapid Activation of the 230-kDa Protein (VEGF Receptor Flk-1/KDR) by S1P—BAEC were treated with 1 μM S1P for 0–60 min, cell lysates prepared, SDS-PAGE performed, and Western blots analyzed for phosphotyrosine. Very rapid and transient phosphorylation of a 230-kDa protein band (peak at 2 min) was observed (Fig. 1A, upper panel, B). Based on its molecular weight and tyrosine phosphorylation we predicted that this band was the VEGF receptor Flk-1/KDR. We reprobed the membrane with anti-Flk-1/KDR antibody and detected the same molecular mass band as a highly glycosylated mature form of Flk-1/KDR. A 200-kDa protein was also detected by anti-Flk-1/KDR antibody (presumably a less glycosylated form of Flk-1/KDR), but this band was not phosphorylated. As reported previously by Igarashi et al. (18) and Morales-Ruiz et al. (19), both Akt and eNOS are time dependently phosphorylated in response to S1P (Fig. 1A, middle and lower panels, B). The peak of Akt phosphorylation was at 3 min, that of eNOS was set at 5 min, and later both phosphorylations returned to baseline by 60 min. As shown in Fig. 1C, the same molecular mass protein, Flk-1/KDR, was time-dependently tyrosine-phosphorylated in BAEC treated with VEGF. The 250-kDa protein, Akt, and eNOS are dose dependently phosphorylated in response to S1P (Fig. 1D). The EC50 values for phosphorylation of the 230-kDa protein, Akt, and eNOS were ~30 nM.

The 230-kDa Protein Tyrosine Phosphorylation in Response to S1P Is VEGF Receptor Flk-1/KDR—To confirm that the 230-kDa protein was Flk-1/KDR, samples were immunoprecipitated either with anti-phosphotyrosine antibody (4G10) or with anti-Flk-1/KDR antibody. Afterward, SDS-PAGE membranes were immunoblotted with anti-Flk-1/KDR antibody or anti-phosphotyrosine antibody, respectively. As shown in Fig. 2, the same 230-kDa protein was immunoprecipitated and immuno- blotted with both anti-phosphotyrosine antibody and anti-Flk-1/ KDR antibody when cells were treated with 1 μM S1P. Thus, the 230-kDa protein tyrosine-phosphorylated by S1P is Flk-1/KDR, which is transactivated by the S1P receptor, EDG-1.

Phosphorylation of Flk-1/KDR, Akt, and eNOS Were Inhibited by VEGF Receptor Tyrosine Kinase Inhibitors—Comparison of the time course for phosphorylation of eNOS, Akt, and Flk-1/KDR indicated that phosphorylation of Flk-1/KDR was faster than eNOS and Akt (Fig. 1B). This finding suggests that transactivation of Flk-1/KDR is “upstream” of the phosphorylation of Akt and eNOS. To access this mechanism, BAEC were pretreated with the VEGF receptor tyrosine kinase inhibitors, SU 1498 and VTKI. Both compounds inhibited phosphorylation of Flk-1/KDR, Akt, and eNOS in response to either S1P or VEGF (Fig. 3). Based on these results it seems likely that transactivation of Flk-1/KDR is required for phosphorylation of Akt and eNOS induced by S1P.

To determine if the transactivation of the EGF receptor family (ErbB family) tyrosine kinase contributes to S1P-mediated phosphorylation of Akt and eNOS, we used AG1478, an inhibitor of ErbB family kinases. AG1478 (1 μM) did not inhibit phosphorylation of eNOS, Akt, or Flk-1/KDR in response to S1P or VEGF stimulation. S1P did not stimulate the release of VEGF since the VEGF neutralization antibody 577B11 inhibited VEGF-stimulated phosphorylation of Flk-1/KDR, Akt, and eNOS, but not S1P-stimulated phosphorylation of these molecules.

Suppression of the Flk-1/KDR Protein Expression by Antisense Oligonucleotides—To verify the results of inhibitor studies described above, BAEC were pretreated with antisense Flk-1/KDR oligonucleotides and stimulated with S1P or VEGF. Because of low transfection efficiency of the antisense oligonucleotides to BAEC, Flk-1/KDR protein expression was de-
creased by about 50% similar to data as reported by Bernatchez et al. (30) (Fig. 4). The expression of Akt and eNOS did not change. After antisense oligonucleotides treatment, both S1P- and VEGF-stimulated phosphorylation of Flk-1/KDR, Akt, and eNOS were inhibited to a similar extent. There was no change in cells treated with scramble oligonucleotides. Thus, transactivation of Flk-1/KDR is required for phosphorylation of Akt and eNOS induced by S1P.

Transactivation of Flk-1/KDR is G i-, Ca2+/H11001- and Src Family Kinase-dependent—To characterize the signal transduction pathway from the EDG-1 receptor to Flk-1/KDR, we examined the effects of several inhibitors on the phosphorylation of Flk-1/KDR, Akt, and eNOS after stimulation by S1P or VEGF (Fig. 5). As reported previously (18, 19), the G i inhibitor PTx blocked phosphorylation of Flk-1/KDR, Akt, and eNOS by S1P but had no effect on phosphorylation of Flk-1/KDR, Akt, and eNOS by VEGF. The Src family kinase inhibitor SU1498, the Src family kinase inhibitor AG1478, and the Src family kinase inhibitor AG1478 inhibited phosphorylation of Flk-1/KDR, Akt, and eNOS by S1P and VEGF. These results suggest that transactivation of Flk-1/KDR is G i-, Ca2+/H11001-, and Src Family Kinase-dependent.

Transactivation of Flk-1/KDR is G i-, Ca2+/H11001- and Src Family Kinase-dependent—To characterize the signal transduction pathway from the EDG-1 receptor to Flk-1/KDR, we examined the effects of several inhibitors on the phosphorylation of Flk-1/KDR, Akt, and eNOS after stimulation by S1P or VEGF (Fig. 5). As reported previously (18, 19), the G i inhibitor PTx blocked phosphorylation of Flk-1/KDR, Akt, and eNOS by S1P but had no effect on phosphorylation of Flk-1/KDR, Akt, and eNOS by VEGF. The Src family kinase inhibitor SU1498, the Src family kinase inhibitor AG1478, and the Src family kinase inhibitor AG1478 inhibited phosphorylation of Flk-1/KDR, Akt, and eNOS by S1P and VEGF. These results suggest that transactivation of Flk-1/KDR is G i-, Ca2+/H11001-, and Src Family Kinase-dependent.
no effect on VEGF-stimulated phosphorylation. BAPTA/AM inhibited Flk-1/KDR phosphorylation by S1P but not by VEGF. This means that Ca\(^{2+}\)/H\(_{11001}\) is required for the transactivation from EDG-1 to Flk-1/KDR. The Src kinase inhibitor, PP2, also blocked S1P-stimulated phosphorylation of Flk-1/KDR, but as expected had no effect on VEGF-stimulated phosphorylation of Flk-1/KDR. Interestingly, BAPTA/AM and PP2 inhibited both S1P- and VEGF-stimulated phosphorylation of Akt and eNOS. The protein kinase C inhibitor, chelerythrine, had no effect on both S1P and VEGF-induced phosphorylation. Wortmannin, an inhibitor of PI3K, did not block the phosphorylation of Flk-1/KDR by S1P or VEGF, but inhibited Akt and eNOS phosphorylation.

Transactivation of Flk-1/KDR by S1P Does Not Depend on the Release of Endogenous Ligand for Flk-1/KDR—EGF receptor transactivation by several GPCR ligands involves activation of metalloproteases and release of heparin binding-EGF, an endogenous membrane binding ligand (32). Because VEGF-neutralizing antibody did not block S1P-stimulated Flk-1/KDR transactivation (Fig. 3), release of an endogenous ligand appeared unlikely. As shown in Fig. 1C VEGF time dependently phosphorylated Flk-1/KDR, but the peak was 5 min, which is slower than S1P-stimulated phosphorylation (Fig. 1A). The rapid activation by S1P is also not consistent with release of an endogenous ligand. To rule out this mechanism, we pretreated BAEC with the metalloprotease inhibitors, o-phenanthroline and GM6001, and stimulated with S1P or VEGF. No effect was observed with these inhibitors (Fig. 6A).

Transactivation of Flk-1/KDR Is Not Reactive Oxygen Species (ROS)-dependent—EGF receptor transactivation by AngII was reported to be ROS-dependent (33). Therefore we examined the effects of various anti-oxidants NAC, Tiron, ebselen, and DPI on tyrosine phosphorylation of Flk-1/KDR by S1P (Fig. 6B). NAC, Tiron, and ebselen had no effect on S1P-stimulated phosphorylation of Flk-1/KDR, while DPI showed a partial inhibition. All four compounds showed no effect on VEGF-stimulated phosphorylation of Flk-1/KDR. Based on these findings, ROS appear not to be involved in S1P stimulated Flk-1/KDR transactivation.

**DISCUSSION**

The major finding of the present study is that S1P transactivates the VEGF receptor Flk-1/KDR leading to phosphorylation and stimulation of the PI3K-Akt-eNOS pathway in endothelial cells. Based on our experiments and previously reported data we summarize that S1P stimulated eNOS activation as follows. VEGF receptor Flk-1/KDR localizes to caveolae (34), while EDG-1 receptor exists in both non-caveolae and caveolae membranes. After stimulation EDG-1 translocates and concentrates in caveolae (35). Upon G\(_3\) protein-mediated activation of phospholipase C, intracellular Ca\(^{2+}\) levels increase and Ca\(^{2+}\)
complexes with calmodulin (CaM). The Ca$^{2+}$/CaM complex then activates eNOS (36). Simultaneously, Ca$^{2+}$ and Src family kinase-dependent transactivation of Flk-1/KDR occurs. Flk-1/KDR then stimulates Src family kinase and PI3K causing Akt and eNOS (Ser-1179) to be phosphorylated and activated. We speculate that different molecules of Src family kinase transduce the signaling before and after Flk-1/KDR. Ca$^{2+}$/CaM and Ser-1179 phosphorylation synergistically activate eNOS (9). In addition, heat shock protein 90 and other molecules are also likely to regulate eNOS activation (37, 38). Further studies are needed to identify individual Src family kinases and other molecules involved in this pathway.

EGF receptor transactivation by GPCR ligands has been well studied. The best elucidated mechanism involves binding of ligand to GPCR, activation of matrix metalloproteases, cleavage of membrane binding heparin binding-EGF, and binding of heparin binding-EGF to the EGF receptor (26, 27, 32). However, age of membrane binding heparin binding-EGF, and binding of ligand to GPCR, activation of matrix metalloproteases, cleavage of molecules involved in this pathway.

needed to identify individual Src family kinases and other likely to regulate eNOS activation (37, 38). Further studies are needed to identify individual Src family kinases and other molecules involved in this pathway.

REFERENCES

1. Huang, P. L., Huang, Z., Mashimo, H., Bloch, K. D., Moskovitz, M. A., Bevan, M. A., and Nature 409, 229–232.
2. Rudie, R. D., Shesely, E. G., Maeda, N., Smithies, O., Segal, S. S., and Sesso, W. C. (1998) J. Clin. Invest. 101, 731–736.
3. Muruhara, T., Ashara, T., Silver, M., Gautier, C., Maeda, H., Kalka, C., Kearney, M., Chen, D., Symes, J. F., Fishman, M. C., Huang, P. L., and Siris, J. (1998) J. Clin. Invest. 101, 2567–2578.
4. Ziche, M., Morbidelli, L., Choudhuri, R., Zhang, H. T., Donnini, S., Granger, H. J., and Bicknell, R. (1997) J. Clin. Invest. 99, 2625–2634.
5. Papapetroupolous, A., Garcia, C. G., Madri, J. A., and Sesso, W. C. (1997) J. Clin. Invest. 100, 3131–3139.
6. Fulton, D., Gratton, J. P., McCabe, T. J., Fontana, J., Fujio, Y., Walsh, K., Franke, T. F., Papapetroupolous, A., and Sesso, W. C. (1999) Nature 399, 597–601.
7. Dimmeler, S., Fleming, I., Fischthalher, B., Herrmann, C., Busse, R., and Zeiher, A. M. (1999) Nature 399, 691–695.
8. Gerber, H. P., McMurtrey, A., Kowalski, J., Yan, M., Keyt, B. A., Dixit, V., and Ferraran, N. (1998) J. Biol. Chem. 273, 30336–30343.
9. Michell, B. J., Griffiths, J. E., Mitchell, K. I., Rodriguez-Crespo, I., Tiganis, T., Bosinovski, S., de Montellano, P. R., Kempen, B. B., and Pearson, R. B. (1999) Curr. Biol. 9, 845–848.
10. Kroll, J., and Waltenberger, J. (1999) Biochem. Biophys. Res. Commun. 263, 536–539.
11. Lee, M. J., Van Brocklyn, J. R., Tangdara, S., Liu, C. H., Hand, A. R., Menzeler, R., Spiegel, S., and Hla, T. (1998) Science 279, 1552–1555.
12. Lee, M. J., Tangdara, S., Claffey, K. P., Ancellin, N., Liu, C. H., Kluk, M., Volpi, M., Sha’ari, R. I., and Hla, T. (1999) Cell 99, 301–312.
13. Wang, F., Van Brocklyn, J. R., Hobson, J. P., Movaifah, S., Zukowski-Grejka, Z., Milstein, S., and Spiegel, S. (1999) J. Biol. Chem. 274, 35343–35350.
14. Lee, O. H., Kim, Y. M., Lee, Y. M., Moon, E. J., Lee, D. J., Kim, J. H., Kim, K. W., and Kwon, Y. G. (1999) Biochem. Biophys. Res. Commun. 264, 743–750.
15. Hla, T. (2001) Prostaglandins 64, 135–142.
16. Pyne, S., and Pyne, N. (2000) Pharmacol. Ther. 88, 115–131.
17. Kwon, Y. G., Min, J. K., Kim, K. M., Lee, D. J., Billiari, T. R., and Kim, Y. M. (2001) J. Biol. Chem. 276, 10627–10633.
18. Igarashi, J., Bernier, S. G., and Michel, T. (2001) J. Biol. Chem. 276, 12420–12426.
19. Morales-Ruiz, M., Lee, M. J., Zolinier, S., Gratton, J. P., Scotland, R., Shiogimi, J., Walsh, K., Hla, T., and Sesso, W. C. (2001) J. Biol. Chem. 276, 19672–19677.
20. Daub, H., Weiss, F. U., Wallasch, C., and Ulrich, A. (1996) Nature 379, 557–560.
21. Daub, H., Wallasch, C., Lankenzau, A., Herrlich, A., and Ulrich, A. (1997) J. Clin. Invest. 100, 3131–3139.
22. Preznel, Z., Ewig, E., Prenzel, N., Leserer, M., and Ulrich, A. (2001) Oncogene 20, 1594–1600.
23. Pierce, K. L., Luttrel, L. M., and Leffklow, R. J. (2001) Oncogene 20, 1532–1539.
24. Kim, J. H., Song, W. K., and Chun, J. S. (2000) J. Clin. Invest. 105, 14705–14711.
25. Fulton, D., Gratton, J. P., and Sesso, W. C. (2001) J. Pharmacol. Exp. Ther. 298, 289–297.
26. Bernatchez, P. N., Sok, S., and Sirios, M. G. (1999) J. Biol. Chem. 274, 31047–31054.
27. Igarashi, J., Haelder, J., Hojo, Y., Yamamoto, K., and Berk, B. C. (2001) Mol. Cell. Biol. 21, 6387–6394.
28. Preznel, Z., Ewig, E., Daub, H., Leserer, M., Aramb, B., and Berk, O. C. (1999) Nature 402, 884–888.
29. Ushio-Fukai, M., Griendling, K. K., Becker, P. L., Hlienski, L., Halleran, S., and Alexander, R. W. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 488–495.
30. Feng, Y., Venema, V. J., Venema, R. C., Tsai, N., and Caldwell, B. R. (1999) Biochem. Biophys. Res. Commun. 256, 192–197.
31. Igarashi, J., and Michel, T. (2000) J. Biol. Chem. 275, 32363–32370.
32. Venema, R. C., Sayegh, H. S., Arnal, J. F., and Harrison, D. G. (1995) J. Biol. Chem. 270, 14765–14771.
33. Garcia-Cardena, G., Fan, R., Shah, V., Sorrentino, R., Cirino, G., Papapetroupolous, A., and Sesso, W. C. (1998) Nature 392, 821–823.
34. Fulton, D., Gratton, J. P., and Sesso, W. C. (2001) J. Pharmacol. Exp. Ther. 298, 289–297.
35. Igarashi, J., and Michel, T. (2001) J. Biol. Chem. 276, 36281–36288.
36. Rikitake, Y., Hira, K., Kawashima, S., Ozaki, M., Takahashi, T., Ogawa, W., Inoue, N., and Yokoyama, M. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 108–114.
37. Takahashi, T., Yamaguchi, S., Chida, K., and Shibuya, M. (2001) EMBO J. 20, 2768–2778.
Transactivation of Vascular Endothelial Growth Factor (VEGF) Receptor Flk-1/KDR Is Involved in Sphingosine 1-Phosphate-stimulated Phosphorylation of Akt and Endothelial Nitric-oxide Synthase (eNOS)

Tatsuo Tanimoto, Zheng-Gen Jin and Bradford C. Berk

J. Biol. Chem. 2002, 277:42997-43001.
doi: 10.1074/jbc.M204764200 originally published online September 10, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M204764200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 41 references, 21 of which can be accessed free at http://www.jbc.org/content/277/45/42997.full.html#ref-list-1