Stem Cell Factor/c-kit Signaling Promotes the Survival, Migration, and Capillary Tube Formation of Human Umbilical Vein Endothelial Cells*

Received for publication, October 23, 2003, and in revised form, February 19, 2004
Published, JBC Papers in Press, February 24, 2004, DOI 10.1074/jbc.M311643200

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Angiogenesis, the formation of new blood vessels from pre-existing vessels, occurs not only in physiological processes but also in several pathological conditions especially the growth and maintenance of tumors (1–4). Recent evidence indicates that, in addition to the sprouting and co-opting of adjacent, pre-existing vessels, the mobilization and incorporation of bone marrow-derived progenitor cells, which are circulating ECPs, and HSCs can contribute to this process, especially to tumor angiogenesis (5–10). These cells are highly proliferative and provide endothelial cells that are required for neo-vessel formation (11). HSCs were originally identified as pluripotent progenitor cells that can differentiate into all hematopoietic lineages. However, it was revealed that the cells also could differentiate into endothelial lineages. ECPs and HSCs are now believed to originate from common progenitor cells, termed hemangioblast cells (12).

Interestingly, these progenitor cells and mature endothelial cells express several common molecular markers, such as von Willebrand factor, c-kit, Tie-2, Fli-1/VEGF-receptor-1, KDR/Flik-1/VEGF receptor-2, Sca-1, CXCR4, CD146, CD31, and CD34 (13). These markers disappear according to the differentiation of the cells into mature blood cells of most hematopoietic lineages, whereas their expression is still highly maintained in mature endothelial cells (14, 15). Among them, c-kit, a receptor tyrosine kinase, is known to play important roles in amplification and recruitment of progenitor cells from bone marrow into the circulation (16–19). In bone marrow, its ligand, SCF, is expressed on stromal cells as a membrane-anchored form and released by MMP9, resulting in the activation of SCF/c-kit signaling in the progenitor cells (17). However, its role in mature endothelial cells is not well understood, although the cells produce both c-kit and SCF (20–22).

Proliferation, migration, and capillary tube formation of endothelial cells are essential processes of angiogenesis and directed by several angiogenic cytokines, such as VEGF (23), bFGF (24), and HGF (25). VEGF is known to be the master stimulus of angiogenesis through binding to two receptor tyrosine kinases, Flt-1 and KDR, which belong to the PDGF receptor superfamily (23). KDR is a major positive signal transducer and has a stronger tyrosine kinase activity compared with Flt-1 (23). VEGF/KDR signaling in endothelial cells is not fully elucidated, but at least protein kinase C-dependent activation of the MAPK pathway involving Erk1/2 (26) and activation of the PI3K-Akt (27) pathway should be important for proliferation and survival of the endothelial cell. Since c-kit is also a member of the PDGF receptor superfamily (28), and SCF/c-kit signaling in hematopoietic cell lines are reported to involve Erk1/2, and Akt (29, 30), we studied whether SCF/c-kit signaling has similar roles to VEGF/KDR signaling in endothelial cells or not. In this report we show that SCF/c-kit signaling can promote the survival, migration, and capillary tube formation of HUVEC. This signaling involves Akt and Erk1/2, as VEGF signaling does, but the overall signaling pathways is different.

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* The abbreviations used are: ECP, endothelial progenitor cell; SCF, stem cell factor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; Ang-1, angioptoinet-1; SDF-1α, stromal derived factor 1α; HUVEC, human umbilical vein endothelial cell; HSC, hematopoietic stem cell; KDR, kinase insert domain receptor; Flk-1, fetal liver kinase; Flt-1, fms-like tyrosine kinase; Erk1/2, extracellular signal-regulated kinase; P38, phosphoinsertisitide 3-kinase; MEK, mitogen-activated protein kinase kinase; FBS, fetal bovine serum; PBS, phosphate-buffered saline; CXCR4, CXC receptor 4; SFM, serum-free medium; TF, tube formation; sTF, sandwich tube formation; RT, reverse transcriptase; MT, T-3(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium; PLCγ, phospholipase Cγ.
Experimental Procedures

Reagents—HMVECs were purchased from Cambrex BioScience Walkersville, Inc. (Walkersville, MD). Type I collagen coated dishes were prepared from Sumitomo Bakelite Co., Ltd. (Tokyo, Japan). Type I collagen gel solution, >5 RPMI 1640, and reconstitution buffer were purchased from Nitta Gelatin Inc. (Osaka, Japan). SCF was purchased from PeproTech EC, Ltd. (London, UK). Recombinant human VEGF was purchased from Genzyme Tech (Minneapolis, MN). Recombinant human hFGF, hEGF, human endothelial SDF basal growth medium, and TR-plating agent were purchased from Invitrogen (Tokyo, Japan). Bullet kit EGM-2 containing 2% heat-inactivated fetal bovine serum, hFGF-B, VEGF, R3-IGF-1, ascorbic acid, heparin, hEGF, gentamicin, and amphotericin-B were purchased from Sanko Junyaku Co., Ltd. (Tokyo, Japan). Anti-human SCF neutralizing antibody and VEGF neutralizing antibody were purchased from R & D systems (Minneapolis, MN). Anti-c-kit, KDR, and anti-goat IgG-horse radish peroxidase antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phospho-c-kit antibody, phospho-KDR antibody, Erk1/2 antibody, phospho-Erk1/2 antibody, Akt antibody, phospho-Akt antibody, p38 antibody, phospho-p38 antibody, and anti-rabbit IgG (H&L) HRP-linked antibody were purchased form Cell Signaling Technology (Beverly, MA). Anti-CD31 antibody was purchased from Sigma. Anti-mouse IgG HRP-linked antibody was purchased from Amersham Biosciences (Tokyo). PD98059, a specific inhibitor of MEK, and wortmanin and LY294002, specific inhibitors of PI 3-kinase, were purchased from Calbiochem. The chemical identity of STI-571 was supported by nuclear magnetic response and mass spectroscopy data.

Cell Culture—HUVECs were isolated from human umbilical cord vessels using collagenase and were cultured as described previously (31). Briefly, the inside of an umbilical vein was rinsed with sterile saline and incubated with trypsin-EDTA at 37 °C for 30 min. Then, endothelial cells that come off the vessel were obtained and cultured on type I collagen-coated plates at 37 °C with 5% CO2 in EGM-2. In this study we used three to six passage cells. The mRNA level of Prox-1, which is a marker of lymphangio endothelial cells, was checked by real time RT-PCR. HMVECs were used as a control, about half of which were reported to be lymphangio endothelial cells (32). Endothelial cell purity was measured as CD31-positive cells by flow cytometer (FACS Calibur) and determined to be more than 99%.

sTF Assay—The sTF assay (a collagen three-dimensional culture of HUVECs) was performed according to Derouanne et al. (33) with a small modification. Briefly, HUVECs were maintained by changing culture medium every day and used in subconfluence. Cells were harvested by trypsin-EDTA and counted. Seven volumes of type I collagen solution were mixed with 2 volumes of 5% RPMI 1640 and 1 volume of reconstitution buffer on ice. An aliquot (0.4 ml) of the collagen gel mixture was added to well of 24-well plates and allowed to polymerize for 15 min at 37 °C. HUVECs were plated onto the gel at 1.5 × 105 cells per well with SFM containing 10 ng/ml EGF (assay medium) and incubated overnight at 37 °C in a 5% CO2 atmosphere. Medium was removed, and 0.4 ml of type I collagen gel mixture was added upon the cells and allowed to gel for 4 h at 37 °C. An aliquot (0.5 ml) of vehicle/test compounds/neutrlizing antibody (three times final concentration) and 1 ml of assay medium plus SCF/VEGF/hFGF/hGF-containing solution (1.5 times final concentration) were added to each well. HUVECs sandwiched in collagen gel were incubated at 37 °C in a 5% CO2 atmosphere for 3 days. The medium was removed, and 0.4 ml of MTT solutions (3.3 mg/ml 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium (Sigma) in PBS) were added to each well and incubated for another 4 h. Photomicrographs of capillaries were taken with a light microscope. Tube length of each capillary was measured using image analysis (Angiogenesis Image Analyzer, version 1.0, Kurabo, Osaka, Japan). Assays were performed in duplicate.

Survival Assay—HUVECs (1 × 105 /50 μl/well) were cultured in collagen type I-coated 96-well plate under EGM-2. After 6 h of culturing, culture medium was exchanged for SFM (assay medium). After overnight culture, assay medium containing several concentrations of growth factor and/or a neutralizing antibody, a compound was added to each well, and cells were cultured at 37 °C for 72 h. After incubation, 0.01 ml/well of the WST-1 reagent was added in each well and incubated for another 4 h. Absorbance of each well was measured in a microplate reader (Spectra Max 250, Molecular Devices Corp., Sunnyvale, CA) at 415 nm (reference wavelength at 660 nm) (34). Assays were performed in duplicate.

Wound Healing Assay—HUVECs were maintained by changing culture medium every day and used in subconfluence. Cells were harvested by trypsin-EDTA and counted. HUVECs were seeded in collagen-coated 12-well plates (2.2 × 105/ml/well) and incubated at 37 °C and 5% CO2 in EGM-2 medium. When cells adhered (2 h), medium was aspirated, and SCF (100 ng/ml), VEGF (20 ng/ml), FGF-2 (20 ng/ml) in SFM was added (1 ml/well). After 6-h incubation, confluent monolayers of HUVECs were wounded with a pipette chip and incubated in the same medium (35). After 24 h, the cells that had migrated across the edge of the wound were observed.

Quantitative RT-PCR Assay—Using TRizol reagent, total RNA was extracted from HUVECs, human microvascular endothelial cells (HMVECs), and H526 cells. Reactions of cDNA (0.0375 ng/ml) were prepared for assay. Real time RT-PCR was performed using TaqMan Gold RT-PCR kit and an ABI PRISM 7900 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer’s instruction. A set of primers and TaqMan probe for each gene were purchased from Applied Biosystems. c-kit and Prox-1 were Assay on Deman® gene expression products: ID numbers Hs00174029 and Hs00174029.

Flow Cytometric Analysis—Cells in assay medium condition were washed with PBS and detached with trypsin-EDTA. After centrifugation the cell pellet was suspended in 1–3 × 105 cells/ml in 50 μl of PBS containing 1% bovine serum albumin and 0.05% Na2CO3 and incubated with 1 μg of primary antibody (anti-CD31 or anti-c-kit antibody) for 30 min at 4 °C. After washing with PBS, cells were incubated in 50 μl of anti-conjugated second antibody dilute 1:50 in PBS at 30 min at 4 °C. The control sample (for background) was incubated in PBS, and cells were analyzed by flow cytometry using FACS Calibur (BD Biosciences) to assess the staining intensity. The expression intensity was calculated using the mean fluorescence intensity of each sample as determined by flow cytometry: relative expression = mean fluorescence intensity of sample/mean fluorescence intensity of background.

Western Blotting Analysis—Confluent-grown HUVECs in 6-well multiculture plates were washed with PBS medium containing 0.5% FBS for 24 h. Cells were treated with the indicated concentrations of compounds for 60 min and then with SFM or VEGF stimulation (20 ng/ml) for the indicated time. Cells were washed with cold PBS twice, collected in 100 μl of lysis buffer (50 mM Hepes, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EDTA, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 50 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 mM Na3VO4), and allowed to lyse for 10 min on ice. Samples were clarified by centrifugation for 30 min at 15,000 rpm at 4 °C. Western blotting was performed as described previously (36). A sample (20 μg) of lysate protein was subjected to SDS-PAGE under reducing conditions and immunoblotting. Briefly, samples were loaded onto an SDS-PAGE gel and run at 30 mA for 2 h. The proteins were then transferred onto polyvinylidene difluoride membrane at 550 mA for 1.5 h and analyzed by Western blot analysis. Blots were probed with a primary antibody to detect a target protein, followed by a secondary biotinylated antibody (three times final concentration) and 1 ml of assay medium plus SCF/VEGF/hFGF/SCF-containing solution (1.5 times final concentration) were added to each well. HUVECs sandwiched in collagen gel were incubated at 37 °C in a 5% CO2 atmosphere for 3 days. The medium was removed, and 0.4 ml of MTT solutions (3.3 mg/ml 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium (Sigma) in PBS) were added to each well and incubated for another 4 h. Photomicrographs of capillaries were taken with a light microscope. Tube length of each capillary was measured using image analysis (Angiogenesis Image Analyzer, version 1.0, Kurabo, Osaka, Japan). Assays were performed in duplicate.

RESULTS

SCF Drives the Capillary Tube Formation of HUVECs—Capillary tube formation is one of the most unique features of mature endothelial cells. We initially examined whether SCF can drive capillary tube formation of HUVEC in collagen three-dimensional culture. As shown in Fig. 1A, HUVEC formed capillary tube networks in collagen gel with an assay medium as described under “Experimental Procedures” containing 100 ng/ml SCF, and the network structure was maintained for at least 3 days. The capillary tube formation did not proceed well in the gel with assay medium only (Fig. 1A), and the total length of tube structure depended on the dose of SCF (Fig. 1B). The expression of c-kit or -c was measured by using an image analyzer (IDS Image Analysis Software, Eastman Kodak Co.).

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detailed morphologies were characteristic of each angiogenic factor.

Effects of Anti-SCF, Anti-VEGF, Anti-bFGF, and Anti-HGF Antibodies—Since the amount of SCF (100 ng/ml) that fully drove the capillary tube formation of HUVECs was relatively high, it is possible that the effects of SCF are indirect, such as stimulation of HUVECs to produce some angiogenic factors. To exclude this possibility, we investigated the effects of neutralizing antibody against known angiogenic factors on capillary tube formation of HUVECs. As shown in Fig. 2, capillary tube formation of HUVECs driven by SCF was not inhibited by neutralizing antibody against either VEGF, bFGF, or HGF, whereas those driven by VEGF, bFGF, or HGF were markedly inhibited by each specific antibody (data not shown). These results indicate that production of VEGF, bFGF, and HGF does not contribute to the effects of SCF.

SCF Promotes Cell Survival and Migration of HUVECs—It is known that promotion of cell mobility and maintenance of cell survival signaling are essential for capillary tube formation. Thus, we next investigated whether HUVECs proliferate and/or survive in SFM containing SCF. Subconfluent HUVECs were starved in SFM containing 0.5% FBS for 24 h, and the medium was changed to SFM containing various amounts of SCF. As shown in Fig. 3A, the number of viable cells after 3 days in culture depended on the concentration of SCF. Generally, the number of viable cells using 100 ng/ml SCF was the same as the initial number of inoculated cells. When the medium had been exchanged to a fresh one containing 100 ng/ml SCF every 3 days, HUVECs survived at least 1 week (data not shown). These results indicate that production of VEGF, bFGF, and HGF does not contribute to the effects of SCF.

Effects Downstream of SCF/c-kit Signaling—To confirm the direct effect of SCF on HUVECs, we first measured the expression of its receptor, c-kit in HUVEC at both mRNA and protein level by real time RT-PCR and flow cytometric analysis.
cells (lung cancer), which are well studied as c-kit-expressing cells (37, 38), were used as an expression control. The expression levels of c-kit mRNA and protein in the assay medium were 25 and 27% of that of H526 cells, respectively, confirming that HUVECs actually express considerable amounts of c-kit. Next we analyzed tyrosine autophosphorylation of the c-kit by SCF. As shown in Fig. 4, c-kit was also expressed in HUVECs by protein level, and the level was comparable with that of H526 cells, which respond well to SCF (data not shown). After adding SCF to HUVECs, c-kit was immediately tyrosine-phosphorylated (within 5 min), showing that SCF directly stimulates its specific receptor. The phosphorylation gave a peak at 5–10 min and persisted for 30 min. Protein levels of c-kit were not changed during this period. Akt and Erk1/2 were also tyrosine-phosphorylated simultaneously with the phosphorylation of c-kit. STI-571, an inhibitor of c-kit (39, 40), clearly inhibited the phosphorylation of Akt and Erk1/2 as well as c-kit, dose-dependently (Fig. 5C). STI-571 also inhibited SCF-driven capillary tube formation and cell survival at the same dose (Fig. 5, A and B), suggesting that Akt and Erk1/2 should promote survival and tube formation downstream of SCF/c-kit signaling.

Comparison of Events Downstream of SCF c-kit and VEGF/KDR Signaling—It is well known that VEGF induces tyrosine phosphorylation of several signal transducers including Erk1/2 and Akt (26, 27). Our preliminary studies showed that their maximum phosphorylation could be observed at about 10 min after treatment of VEGF and SCF. Therefore, we next compared the tyrosine phosphorylation profile of several signal transducers in HUVECs treated with VEGF and SCF for 10 min. As shown in Fig. 6, VEGF induced phosphorylation of Akt, Erk1/2, p38, and PLCγ as well as its receptor, KDR, in a dose-dependent manner. SCF also induced phosphorylation of Akt, Erk1/2, p38, and c-kit dose-dependently, confirming the results shown in Fig. 4, whereas it did not induce the phosphorylation of PLCγ. Furthermore, maximal tyrosine phosphorylation levels of each molecule in HUVECs with SCF and those with VEGF were different. Akt is more strongly phosphorylated, whereas Erk1/2, p38, and PLCγ were more weakly phosphorylated with SCF than with VEGF. Protein levels of all these molecules were not changed in these analyses. These results indicate that their overall signaling pathways are partly different and that although the same molecules such as Erk1/2, Akt, and P38 are involved downstream of SCF/c-kit and VEGF/KDR, their share of signaling in each downstream pathway are different.

Contribution of Akt and Erk1/2 to Capillary Tube Formation in HUVECs Stimulated with SCF or VEGF—Since tyrosine phosphorylation profiles of Akt and Erk1/2 were different in HUVECs treated with SCF and VEGF, we then evaluated the contributions of Akt and Erk1/2 in the cells using two kinds of tyrosine kinase inhibitors. Wortmannin and LY294002, inhibitors of PI3K, inhibit the tyrosine phosphorylation of Akt, and PD98059, an inhibitor of MEK, inhibits the tyrosine phosphorylation of Erk1/2 (41, 42). As shown in Fig. 7D, wortmannin (0.1 μM) and LY294002 (4 μM) inhibited both SCF- and VEGF-dependent tyrosine phosphorylation of Akt, and this dose of
wortmannin and LY294002 completely blocked SCF-driven capillary tube formation (Fig. 7, A and B). However, they could not inhibit VEGF-driven capillary tube formation (Fig. 7, A and B). Higher concentrations of the inhibitors were not studied, since they showed nonspecific cytotoxicity. We have not evaluated above this concentration because of compound originated cytotoxicity. On the other hand, PD98059 (10 μM) inhibited both SCF- and VEGF-dependent tyrosine phosphorylation of Erk1/2, and Akt. Confluent monolayers of HUVECs were cultured in the assay medium containing 0.5% FBS for 24 h on dishes coated with collagen type I. Cells were then treated with increasing amounts of PD98059 for 60 min, followed by SCF stimulation (100 ng/ml) for 10 min. Cells were lysed and analyzed by Western blotting. All of experiments were done at least in duplicate and repeated three times.

**DISCUSSION**

Although it is well known that endothelial cells express c-kit, a receptor tyrosine kinase for SCF (20), the role of SCF/c-kit signaling in endothelial cells themselves has not been well understood to date (43). In this paper, we report that SCF promoted the survival, migration, and capillary tube formation of HUVEC and suggest that Akt and Erk1/2 should have essential roles downstream of SCF/c-kit signaling.
The effects of SCF on HUVECs should be direct, because SCF immediately induced the autophosphorylation of c-kit within 5 min, and inhibition of the kinase activity of c-kit by STI-571 resulted in the inhibition of the output of SCF, which was the promotion of capillary tube formation of HUVECs. Neutralizing antibody against SCF completely blocked the survival and capillary tube formation promoted by SCF, showing that these known factors did not contribute to the effects of SCF. Therefore, SCF might be another angiogenic cytokine, although it cannot support the proliferation of the cells.

It is known that endothelial cells themselves produce SCF (22). Although we did not measure the level of SCF in the conditioned medium of HUVECs, the contribution of endogenous SCF might be small in our experiments, because 1) all the cells died in SFM without exogenous SCF after 3 days in culture, and 2) the level of SCF in the conditioned medium of HUVECs was reported to be 20-40 pg/ml (22), which is not enough for the support of survival, migration, and capillary tube formation of HUVECs as shown in Figs. 1 and 3. However, since production of SCF by endothelial cells is known to be up-regulated by a number of inflammatory mediators, such as IL-1α and bacterial lipopolysaccharide (21), we cannot exclude a possible autocrine loop of SCF and c-kit in some special environments in vivo. Akt and Erk1/2 were found to be tyrosine-phosphorylated in HUVECs simultaneously with the autophosphorylation of c-kit induced by SCF. STI-571, an inhibitor of c-kit (39, 40), markedly inhibited the phosphorylation of Akt and Erk1/2 as well as phosphorylation of c-kit in a dose-dependent manner, indicating that they are downstream of the SCF/c-kit signaling pathway. Both Akt and Erk1/2 were shown to be essential for SCF-dependent survival and TF of HUVECs, since PI3K inhibitors (wortmannin and LY294002) and MEK inhibitor (PD98059) abrogated the SCF-dependent survival and TF at the same doses. Our results are consistent with evidence that Akt is a key regulator of cell survival in a number of systems (44). Transfection of adenovirus expressing a dominant negative Akt mutant decreased endothelial cell viability (45), and transfection of constitutive active Akt conferred endothelial cell survival in the absence of Akt. Erk1/2 is thought to have an important role in proliferation of endothelial cells (46). However, in SCF-treated HUVECs, the role of Erk1/2 might be cell survival rather than proliferation. We noticed that p38 was also phosphorylated by SCF. p38 is known to be involved in the regulation of actin re-arrangement and has a role in cell migration (47). This is analogous to our results that SCF supported the migration of HUVECs. SCF/c-kit signaling in hematopoietic cells, especially in mast cells, has been well studied (29, 30, 48–50). It involves the phosphorylation of Akt, Erk1/2, and p38, similar to our results obtained with HUVECs. However, SCF supports not only survival and migration but also proliferation of the precursor of mast cells (51, 52),
suggesting that the SCF/c-kit signaling in mast cells is more complex than that of HUVECs.

The main difference between the effect of SCF and VEGF on HUVECs is that VEGF can, but SCF cannot, promote the proliferation of HUVECs. We found that the tyrosine phosphorylation profiles in HUVECs induced by SCF and VEGF were also different, yet some molecules were commonly phosphorylated. VEGF induced phosphorylation of Akt, Erk1/2, p38, and PLCγ as well as its receptor, KDR. SCF also induced phosphorylation of Akt, Erk1/2, p38, and c-kit, but it did not induce the phosphorylation of PLCγ. Since PLCγ is known to be important at the downstream locus of most growth-stimulating cytokines (50), this molecule might contribute to the proliferative effect of VEGF. Furthermore, the maximal tyrosine phosphorylation levels of Akt and Erk1/2 in SCF-treated cells and in VEGF-treated cells are different. The level of Akt in SCF-treated cells is much higher than that in VEGF-treated cells, whereas the level of Erk1/2 in SCF-treated cells is lower than that in VEGF-treated cells. PI3K inhibitors and MEK inhibitor clearly inhibited the tyrosine phosphorylation of Ark and Erk1/2 in VEGF-stimulated HUVECs, respectively, just as it did in SCF-treated HUVECs. However, MEK inhibitor completely inhibited capillary tube formation, but PI3K inhibitors inhibited it only partially in VEGF-stimulated HUVECs, suggesting that the role of Akt in VEGF/KDR signaling is relatively weak compared with its role in SCF/c-kit signaling. The results might be consistent with the report (53) that tubular morphogenesis of bovine capillary endothelial cells stimulated with VEGF and bFGF was unaffected by PI3K inhibitors. Since VEGF/KDR signaling involves many pathways, the role of Akt could be compensated by other pathways. Above all it was suggested that the pathway downstream of SCF/c-kit in HUVECs is relatively simple, and this might correlate with the result that SCF cannot promote the proliferation of HUVECs as VEGF does.

Many angiogenic factors have been characterized so far, and they might be categorized into two groups. One involves VEGF, bFGF, and HGF, whose receptors are flt-1/KDR, FGFR-R, and c-met, respectively. They are all receptor tyrosine kinases. The other involves Ang-1, SDF-1α, and as proposed in this report, SCF. Their receptors are Tie-2, CXCR4, and c-kit, respectively (54–56). Tie-2 and c-kit are receptor tyrosine kinases, whereas CXCR4 is a G protein-coupled receptor. The former (category 1) promotes survival, migration, and capillary tube formation but also proliferation of endothelial cells. The latter (category 2) promotes survival, migration, and capillary tube formation but promotes proliferation only minimally. Recent reports show that Ang-1 and SDF-1α support survival via activation of Akt (18, 56). Interestingly, Tie-2, CXCR4, and c-kit are also expressed in hemangioblast cells, which are common progenitor cells of hematopoietic cells and vascular endothelial cells, and Ang-1/Tie-2, SDF-1α/CXCR4, and SCF/c-kit signaling have important roles in amplification and mobilization or recruitment of endothelial precursor cells (17, 58, 59). Therefore, category 2 factors should have multiple roles in the recruitment of precursor cells to the promotion of the angiogenic support of angiogenic performance of mature endothelial cells involving survival, migration, and capillary tube formation. Studies of molecules that are commonly expressed in both hemangioblast cells and endothelial cells should be very fruitful for total understanding of blood vessel formation, and they might be promising therapeutic targets for new anti-angiogenesis drugs.

At this time, how much SCF/c-kit signaling contributes to the angiogenesis in vivo is not well understood. But, evidence is accumulating that some breast cancers and colorectal cancers produce high amounts of SCF and that most small lung cancers express both SCF and c-kit, suggesting that SCF/c-kit signaling could stimulate angiogenesis in some tumors expressing SCF (21, 59–61). In a rat model, microvascular density and the expression level of von Willebrand factor in mammary tumors were found to be correlated to the amounts of SCF expressed by the tumors in that rat model. Although the report discussed a contribution of mast cells, since SCF can stimulate the proliferation and maturation of mast cells, which release mediators including VEGF and bFGF, our findings suggest that SCF could also directly stimulate the angiogenesis in tumors.

In summary, we demonstrated that SCF promotes survival, migration, and capillary tube formation, but not the proliferation of HUVEC, and suggested that Akt and Erk1/2 should have essential roles downstream of SCF/c-kit signaling. SCF, together with Ang-1 and SDF-1α, might be a new category of angiogenic cytokines, which can promote both the recruitment of bone marrow-derived progenitor cells and angiogenic process of mature endothelial cells. Further studies will be required to understand their physiological consequences and their validation as a therapeutic target for anti-angiogenesis.
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J. Biol. Chem. 2004, 279:18600-18607.
doi: 10.1074/jbc.M311643200 originally published online February 24, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M311643200

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