Tyrosine Residues 951 and 1059 of Vascular Endothelial Growth Factor Receptor-2 (KDR) Are Essential for Vascular Permeability Factor/Vascular Endothelial Growth Factor-induced Endothelium Migration and Proliferation, Respectively*

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Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) exerts its multiple functions by activating two receptor tyrosine kinases, Flt-1 (VEGFR-1) and KDR (VEGFR-2), both of which are selectively expressed on primary vascular endothelium. To dissect the respective signaling pathways and biological functions mediated by these receptors in primary endothelial cells with two receptors intact, we, recently developed chimeric receptors (EGDR and EGLT) in which the extracellular domain of the epidermal growth factor receptor was fused to the transmembrane domain and intracellular domain of KDR and Flt-1, respectively. With these fusion receptors, we have shown that KDR is solely responsible for VPF/VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation and migration, whereas Flt-1 showed an inhibitory effect on KDR-mediated proliferation but not migration. To further characterize the VPF/VEGF-stimulated HUVEC proliferation and migration here, we have created several EGDR mutants by site-directed mutagenesis. We show that tyrosine residues 1059 and 951 of KDR are essential for VPF/VEGF-induced HUVEC proliferation and migration, respectively. Furthermore, the mutation of tyrosine 1059 to phenylanaline results in the complete loss of KDR/EGDR-mediated intracellular Ca²⁺ mobilization and MAPK phosphorylation, but the mutation of tyrosine 951 to phenylanaline did not affect these events. Our results suggest that KDR mediates different signaling pathways for HUVEC proliferation and migration and, moreover, intracellular Ca²⁺ mobilization and MAPK phosphorylation are not essential for VPF/VEGF-induced HUVEC migration.

In order to grow beyond minimal size, tumors must generate a new vascular supply for the purpose of gas exchange, cell nutrition, and waste disposal (1–4). They do so by secreting angiogenic cytokines that induce the formation of new blood vessels (3–6). Tumor-secreted angiogenic cytokines include fibroblast growth factor, platelet-derived growth factor-B, and vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) (6–9). VPF/VEGF is likely the most important of these cytokines because it is expressed abundantly by a wide variety of human and animal tumors and because of its potency, selectivity for endothelial cells, and ability to regulate most, if not all, of the steps in the angiogenic cascade (5, 6, 10–13). Moreover, a number of other angiogenic cytokines act, at least in part, by up-regulating VPF/VEGF expression (5, 14). VPF/VEGF extensively reprograms endothelial cell expression of proteases, integrins, and glucose transporters; stimulates endothelial cell migration and division; protects endothelial cells from apoptosis and senescence; and induces angiogenesis in both in vitro and in vivo models (for reviews, see Refs. 5, 6, 13, 15). In addition, VPF/VEGF is the only angiogenic cytokine identified thus far that renders microvessels hyperpermeable to circulating macromolecules, a characteristic feature of angiogenic blood vessels (8, 10–12, 16).

All of the VPF/VEGF activities are thought to be mediated by its interaction with two high affinity receptor tyrosine kinases, vascular endothelial growth factor receptor-2 (KDR, in human and Flk-1 in mice) and vascular endothelial growth factor receptor-1 (Flt-1), which are selectively expressed on the vascular endothelium. A third receptor, neuropilin, has been discovered recently but its function is not clear (17, 18). Both Flt-1 and KDR are essential for normal vascular development (19, 20). Flt-1 null mice do not survive beyond 8.5–9.5 days of gestation because of defective blood vessel formation that may cause increased numbers of endothelial progenitor cells (19). Homozygous Flk-1/KDR knockout mice have different phenotypes that include impaired hematopoiesis and lack of differentiated endothelial cells (20). Because both receptors are expressed on vascular endothelium, it has been difficult to define the respective roles of each in mediating the various signaling events and biological activities induced in the endothelium by VPF/VEGF. Current information, therefore, has been gleaned largely from: (a) studies with a cell line, porcine aortic endothelial cells, which do not express either receptor unless engineered to do so (21, 22); (b) studies with the placenta growth factor (PIGF), a ligand that binds Flt-1 but not KDR (23); (c) the use of a

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1 The abbreviations used are: VPF, vascular permeability factor; VEGF, vascular endothelial growth factor; HUVEC, human umbilical vein endothelial cell; EGF, epidermal growth factor; EC, endothelial cell; MAPK, mitogen-activated protein kinase; BSA, bovine serum albumin; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorter.
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Fli-1-specific antibody (24); (d) studies with antisense oligonucleotides that block Fli-1 expression (25); and (e) characterization of VPF/VEGF mutants that specifically bind Fli-1.

To delineate the respective roles of KDR and Flt-1 in early passage endothelial cells in which both receptors remained intact and functional, we engineered two receptor chimeras (EGDR and EGLT) by fusing the extracellular domain of the EGF receptor (EGFR) with the transmembrane and intracellular domains of either KDR or Flt-1. These two receptor chimeras were overexpressed in early passages of human umbilical vein endothelial cells (HUVEC) with a retroviral vector (27). With this chimeric receptor system, we found results similar to others: KDR is responsible for VPF/VEGF-stimulated HUVEC proliferation and migration (21, 22). Furthermore, our results suggest that the downstream signaling pathway of VPF/VEGF-stimulated HUVEC proliferation was different from that of VPF/VEGF-stimulated HUVEC migration (27). In order to further characterize these different signaling pathways, we conducted the site-directed mutational analysis in the KDR chimera, EGDR.

KDR possesses tyrosine kinase domains, tentative ATP-binding sites, and a long kinase-insert region. This kinase-insert region is known to contain several phosphorylation sites that show binding sites for different signaling molecules. At present, tyrosine residues 951, 996, 1054, and 1059 have been identified as autophosphorylation sites for KDR in a bacterial expression system (28). However, the importance of these tyrosine residues with respect to endothelial cell signaling is not yet well defined. In the present context, we show that the tyrosine residue 1059 of KDR is responsible for ligand-mediated intracellular Ca\(^{2+}\) mobilization, MAPK activation, and EC proliferation, whereas tyrosine residue 951 is required for EC migration.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant VPF/VEGF was obtained from R&D Systems Inc. (Minneapolis, MN). EGM-MV BulletKit, trypsin/EDTA, and trypsin neutralization solution were obtained from Clonetics (San Diego, CA). Vitrogen 100 was purchased from Collagen Biomaterials (Palo Alto, CA). Mouse monoclonal antibodies against the KDR C-terminal domain and against the EGFR N-terminal domain were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phosphotyrosine antibody was obtained from Upstate Biotechnology (Lake Placid, NY). Anti-phospho-p42/p44 MAPK antibodies were obtained from New England Biolabs Inc. (Beverly, MA). \(^{[3]H}\)Thymidine was obtained from PerkinElmer Life Sciences (Boston, MA). Transwell plate inserts were from Fisher. CyQuant, Fura-2 AM, and Pluronic F-127 were obtained from Molecular Probes Inc. (Eugene, Oregon).

**Cell Culture**—Primary HUVEC were obtained from Clonetics. Cells were grown on plates coated with 30 \(\mu\)g/ml vitrogen in EGM-MV BulletKit (5% fetal bovine serum in endothelial basic medium (EBM) with 12 \(\mu\)g/ml bovine brain extract, 1 \(\mu\)g/ml hydrocortisone, 1 \(\mu\)g/ml GA-1000, and hEGF). HUVEC transduced with EGDR or its mutant were grown in the same medium without hEGF. HUVEC were seeded at a density of 0.3 \(\times\) 10⁶ cells per 100-mm plate. After incubation at room temperature for 2 min, 30 \(\mu\)l of effectene was added to the DNA mixture and incubated at room temperature for 5 min. The DNA mixture was added dropwise to 293T cells. The medium was changed after 16 h. The retrovirus was isolated 48 h after transfection and used immediately for infection or frozen at -70 °C.

Twenty-four hours before infection, HUVEC were seeded at a density of 0.3 \(\times\) 10⁶ cells per 100-mm plate. One milliliter of retrovirus solution and 5 ml of fresh medium (2 \(\times\) 10⁶ plate-forming units per milliliter) were added to cells with 10 \(\mu\)g/ml polybrene. Medium was changed after 16 h, and the cells were ready for experiment 48 h after infection.

**Immunoprecipitation and Western Blotting**—Forty-eight hours after infection, HUVEC were serum-starved for 24 h and stimulated with different concentrations of EGF for various lengths of time as indicated. Stimulation was halted by the addition of ice-cold PBS, and the cells were washed three times with ice-cold PBS and lysed with cold radioimmunoprecipitation buffer (20 mM Tris-HCl, pH 7.5, 0.15 \(\mu\)M NaCl, 1 mM Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄, 1 mM EGTA, 1 \(\mu\)g/ml leupeptin, 0.5% aprotinin, and 2 \(\mu\)g/ml pepstatin A). Cell lysates were collected after centrifugation for 15 min at 4 °C. Five-hundred micrograms of lysate protein was incubated with 1 \(\mu\)g of different antibodies (as indicated) for 1 h and protein A-conjugated agarose beads at 4 °C for another hour. Beads were washed with radioimmunoprecipitation buffer three times, and immunoprecipitation buffer was resuspended in 2 \(\times\) SDS sample buffer for Western blot analysis.

**Transwell Migration Assay**—HUVEC were washed with PBS twice and incubated with 4 ml of collagenase solution (0.2 \(\mu\)g/ml collagenase, 0.2 \(\mu\)g/ml soybean trypsin inhibitor, 1 \(\mu\)g/ml BSA, and 2 ml EDTA in PBS) at 37 °C for 30 min. Cells were detached by gentle scraping and centrifuged at 1100 rpm for 3 min. Cell pellets were washed twice with cold PBS containing 0.1% BSA and were resuspended in the same buffer at 0.5 \(\times\) 10⁶ cells/ml. Then aliquots (1 \(\times\) 10⁵ cells in 200 \(\mu\)l) were added to 96-well plates and were pelleted by centrifugation at 1300 rpm for 3 min. Cells were resuspended in 10 \(\mu\)l of the same buffer containing 1 \(\mu\)g of mouse anti-EGFR-N antibody or mouse IgG and incubated at 4 °C for 1 h. Cells were centrifuged at 1300 rpm for 3 min. Cell pellets were washed twice with the same buffer, resuspended in 10 \(\mu\)l of the same buffer containing 2.5 \(\mu\)g/ml fluorescein isothiocyanate-conjugated anti-mouse IgG antibody, and incubated at 4 °C for 0.5 h. Cells were then washed two times and resuspended in 400 \(\mu\)l of the same buffer. FACS analysis was carried out in a FACScalibur instrument (BD Biosciences) with CellQuest software.

**Intracellular Ca\(^{2+}\) Release**—Forty-eight hours after infection, HUVEC were serum-starved for 24 h, washed with PBS twice, and incubated in the same medium without hEGF. Twenty-four hours after infection, HUVEC were seeded at a density of 10⁶ cells per 100-mm plate. One milliliter of retrovirus solution was added to the DNA mixture. After incubation at room temperature for 2 min, 30
of 3 × 10² to 1 × 10³ cells per well were seeded in a 96-well plate for the standard curve. Cells were incubated at 37 °C for 1 h to allow the cells to attach, and then VPF/VEGF or EGF was added at a final concentration of 10 ng/ml. After incubation for an additional 2 h, cells remaining on the upper surface of the transwell filter membrane were wiped off with a cotton tip. The whole transwell membrane was cut out and placed in an individual well of the 96-well plate, which contained the cells for the standard curve. Two-hundred microliters of Cyquant DNA stain was added to each well containing cells or membrane, and the plate was kept at 4 °C overnight. After warming to room temperature, stained cells were counted in a spectrofluorometer (SpectraFluor; TECAN) with Delta Soft 3 software. Data are expressed as the mean ± S.D. of quadruplicate values. All experiments were repeated at least three times.

RESULTS AND DISCUSSION

To further elucidate the different signaling pathways for KDR-mediated endothelial cell proliferation and migration, we created three mutants in the EGFR-KDR chimera (EGDR) by mutating tyrosines 951, 1054, and 1059 to phenylalanine. Tyrosine 951 is located in the receptor’s kinase-insert domain, and the others are located in the catalytic domain. These mutants were designated EGDR(Y951F), EGDR(Y1054F), and EGDR(Y1059F), respectively. EGDR and these mutants were introduced into early passage HUVEC by transduction with a retroviral system, which we developed recently (27). HUVEC transduced with EGDR, EGDR(Y951F), EGDR(Y1054F), or EGDR(Y1059F) were lysed, and equal amounts of total proteins were immunoprecipitated with antibody against the N terminus of EGFR followed by immunoblotting with antibodies against the C terminus of KDR (Fig. 1, panel A). Although similar levels of the N terminus of EGFR followed by immunoblotting with antibody against the C terminus of KDR were observed, similar levels of protein were detected (Fig. 1A). Previously, we showed that no EGF receptor could be detected in HUVEC or HUVEC transduced with LacZ as control (27). In order to confirm that the EGFR-N and EGFR-C terminus antibodies against EGFR-N (dashed line) or mouse IgG (solid line). (If exposed for much longer, a weak band can be

![Fig. 1. Cell surface expression of EGDR mutants. A, extracts of HUVEC that had been transduced with EGDR (lane 2), EGDR(Y951F) (lane 3), EGDR(Y1054F) (lane 4) were immunoprecipitated with antibody against the N terminus of EGFR followed by immunoblotting with antibody against the C terminus of KDR (IB: KDR-C). B, FACS analysis of HUVEC transduced with LacZ, EGDR, or different mutants with fluorescent antibodies against EGFR-N (dashed line) or mouse IgG (solid line).](http://www.jbc.org/content/280/33/32716/F1.large.jpg)

Next, we tested the phosphorylation state of EGDR and these mutants in response to EGF stimulation. HUVEC transduced with LacZ (as control), EGDR, EGDR(Y951F), EGDR(Y1054F), or EGDR(Y1059F) were stimulated with 10 ng/ml EGF for different time intervals as indicated. Cellular lysates were immunoprecipitated with antibody against the N terminus of EGFR followed by immunoblotting with anti-phosphotyrosine antibody. Fig. 2, panel A shows that, in response to EGF, the EGDR receptor chimera is highly phosphorylated and maximized at 1 min, which is correlated well with the KDR phosphorylation in VPF/VEGF-stimulated HUVEC (27). As control, no phosphorylation of receptor can be detected in EGF-stimulated HUVEC transduced with LacZ. Interestingly, the phosphorylation level of EGDR(Y951F) is much lower than that of wild-type EGDR, whereas that of EGDR(Y1059F) is almost undetectable (Fig. 2, panel B, nor caused by the loss of receptor activity (shown below). The results indicate that tyrosine residues 951 and 1059 of KDR are important for receptor phosphorylation in ligand-stimulated HUVEC.

We further examined the effects of these EGDR mutants on HUVEC proliferation. As shown in Fig. 3A, in serum-starved HUVEC transduced with either wild-type EGDR, EGDR(Y951F), or EGDR(Y1054F), EGF stimulation produced similar dose responses in trichloroacetic acid precipitable [³H]thymidine incorporation. On the contrary, HUVEC transduced with EGDR(Y1059F) or LacZ did not show any growth response to EGF (up to 100 ng/ml). However, all HUVEC transduced with different mutant receptors or LacZ show a similar response to VPF/VEGF stimulation, indicating that the lack of proliferative response of EGDR(Y1059F) is not due to the defect of the cells. Moreover, the difference of the maximal proliferation response of HUVEC between VPF/VEGF (endogenous) and EGF (receptor chimera) treat-
ment is due to the anti-proliferative effect of Flt-1 on KDR, whereas EGF does not function through Flt-1 as described previously (27). These data provide evidence that tyrosine residue 1059 is essential for ligand-induced HUVEC proliferation whereas the other two tyrosine residues do not have a significant effect. This is the first report that indicates that only one tyrosine residue of KDR is necessary for VPF/VEGF-induced EC proliferation.

To determine the tyrosine residue(s) responsible for VPF/VEGF-induced HUVEC migration, we have utilized similar strategies to those described previously (27). As shown in Fig. 3B, EGDR-transduced HUVEC show an equivalent maximum extent of migration with either VPF/VEGF or EGF treatment. Both tyrosine residue 1054 and 1059 mutants did not show any difference in the dose-response migratory activity as compared with that of wild-type receptors. Surprisingly, contrary to the other mutants, the single mutation of tyrosine 951 of KDR completely abolished the EGF- (up to 100 ng/ml) induced migratory activity in HUVEC transduced with EGDR(Y951F) but had no effect on VPF/VEGF-stimulated migration. This is the first evidence that different tyrosine residues are required for KDR-mediated HUVEC proliferation and migration.

Next, we further characterized the EGDR-mediated signaling pathways of HUVEC proliferation and migration. As established previously by several groups (29, 30), MAPK phosphorylation is detectable 10 min after stimulation of HUVEC by VPF/VEGF. Furthermore, PD98059, the MAPK kinase inhibitor, inhibited proliferation but not migration in VPF/VEGF-stimulated HUVEC and EGF-stimulated EGDR/HUVEC, suggesting that MAPK phosphorylation is not required for migration (27). In order to investigate the role of the different tyrosine residues of KDR in MAPK activation, cellular extracts from 10 ng/ml EGF-stimulated HUVEC transduced with LacZ, EGDR, and different receptor mutants were utilized to perform immunoblot analysis using an anti-phospho-MAPK antibody. As expected, we detected MAPK phosphorylation 10 min after stimulation of EGDR-transduced HUVEC cells with EGF. In contrast, mutation of tyrosine residue 1059 of KDR (EGDR(Y1059F)) showed no MAPK activation in response to EGF treatment. The other two mutants, EGDR(Y951F) and EGDR(Y1054F), had a similar response of EGF-stimulated MAPK activation to that of the wild-type receptor (Fig. 4). These data indicate that the tyrosine residue 1059 of KDR is essential for increasing intracellular Ca\(^{2+}\) mobilization in response to EGF stimulation whereas the other two mutant receptors operate similarly to those of the wild-type receptor. These results indicate that tyrosine residue 1059 of KDR is also essential for increasing intracellular Ca\(^{2+}\) mobilization and suggest that signaling pathways leading to MAPK activation and intracellular Ca\(^{2+}\) mobilization may initiate from the same tyrosine residue. Moreover, these data also suggest that, similar to MAPK activation, increasing intracellular Ca\(^{2+}\) might be essential for VPF/VEGF-induced EC proliferation but not migration.

**Fig. 2.** Tyrosine phosphorylation of EGDR mutants. Twenty-four hours serum-starved HUVEC transduced with LacZ, EGDR, or different mutants were stimulated with 10 ng/ml EGF for 0, 1, 5, and 10 min. Cell extracts were immunoprecipitated with antibodies against EGFR-N terminus and immunoblotted (IB) with antibody to phosphotyrosine (Anti-Ptyr) (panel A). Blots were then stripped and reprobed with antibodies against KDR-C (Anti-KDR-C) (panel B). Experiments were repeated three times.

**Fig. 3.** Effect of EGDR and different mutants on HUVEC proliferation and migration. Proliferation and migration assays were carried out as described under “Experimental Procedures”. A, proliferation assay. HUVEC transduced with LacZ, EGDR, or different mutants were stimulated with 10 ng/ml VPF/VEGF and different concentrations of EGF (5, 10, 100 ng/ml); n = 4. B, migration assay. HUVEC transduced with LacZ, EGDR, or different mutants were stimulated with 10 ng/ml VPF/VEGF and different concentrations of EGF (5, 10, 100 ng/ml); n = 4.
stimulated HUVEC transduced with EGDR(Y1059F). These results correlate very well with the data obtained from the bacterial overexpression system and from 293 cells expressing these mutants. More importantly, our data also indicate that tyrosines 951 and 1059 are required for EGF-stimulated EGDR/HUVEC migration and proliferation, respectively.

Very recently, Dayanir et al. (35) also reported mutational analysis carried out in the CSF-1/KDR fusion receptor. They demonstrated that mutation of tyrosines 799 and 1173 individually resulted in partial loss of proliferative activity, whereas the double mutant of tyrosines 799 and 1173 completely inhibited cell growth. They also reported that these tyrosine residues were docking sites for the p85 subunit of PI-3 kinase, and these mutants had no effect on ligand-stimulated MAPK phosphorylation. Our data clearly show that the EGDR(Y1059F) mutant loses its ability to mediate not only proliferation but also the MAPK phosphorylation in HUVEC stimulated with ligand. The latter correlates well with the finding that MAPK phosphorylation is required for cell proliferation. Taken together, these results may suggest that tyrosines 799, 1059, and 1173 are all involved in mediating proliferative activity. On the other hand, the tyrosine residues 799 and 1173 might be more involved in cell survival rather than cell proliferation, because neither of those tyrosines are related to MAPK activation and, more importantly, phosphatidylinositol 3’kinase was shown to be required for VPF/VEGF-stimulated EC survival (36). Previously, we also showed that Fli-1/EGLT down-regulates KDR/EGDR-mediated HUVEC proliferation, but not migration, and this inhibitory effect was at or before KDR/EGDR-mediated intracellular Ca$^{2+}$ mobilization (27). Furthermore, the MEK inhibitor, PD98059, inhibited VPF/VEGF-stimulated HUVEC and EGF-stimulated EGDR/HUVEC proliferation, but not migration (27). Here, we show that EGDR(Y1059F) inhibits intracellular Ca$^{2+}$ mobilization and MAPK phosphorylation but not migration. By contrast, EGDR(Y951F), which does not mediate migration, has no effect on ligand-stimulated intracellular Ca$^{2+}$ mobilization and MAPK phosphorylation. Taken together, these results suggest that intracellular Ca$^{2+}$ mobilization and MAPK phosphorylation are not essential for KDR/EGDR-mediated HUVEC cell migration. Moreover, VPF/VEGF-stimulated HUVEC proliferation and migration are mediated by different downstream signaling pathways. This study thus represents the first site-directed mutagenesis analysis of KDR/EGDR function in early passage primary EC and has identified two tyrosine residues responsible for KDR-mediated proliferation and migration, respectively. However, neither of these two signaling pathways is as yet fully defined, and further work is needed to demonstrate the complete complement of signaling steps and pathway regulators.

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A. IB: Anti-p-MAPK

B. IB: Anti-MAPK

**Fig. 4. Phosphorylation of MAPK mediated by EGDR and Mutants.** HUVEC transduced with LacZ, EGDR, and different mutants as indicated were stimulated with 10 ng/ml EGF for 0, 5, and 10 min. Cellular extracts were immunoblotted (IB) with antibodies against phosphorylated MAPK (Anti-p-MAPK) (panel A). The blot was stripped and reprobed with antibody against unphosphorylated MAPK (Anti-MAPK) (panel B).

**Fig. 5. Intracellular Ca$^{2+}$ mobilization mediated by EGDR and mutants.** [Ca$^{2+}$], was measured in HUVEC transduced with LacZ, EGDR, and different mutants as indicated that had been stimulated with EGF (10 ng/ml).

Because KDR has been shown to be specific and crucially important for tumor progression, targeting KDR function is a major goal of current anti-angiogenesis research (32). It is well documented that KDR is responsible for VPF/VEGF-stimulated HUVEC proliferation and migration, but the residues responsible for these activities have not been defined. Previously, it was shown that tyrosines 951, 996, 1054, and 1059 of KDR are autophosphorylated in a bacterial system that overexpressed the catalytic domain of KDR (28). Moreover, it was also demonstrated by Dougher and Terman (33) that tyrosine residues 1054 and 1059 were required for maximum autophosphorylation of KDR in response to VPF/VEGF when the KDR(Y1054F/Y1059F) mutant was overexpressed in 293 cells. However, the physiological role of these residues in endothelial cells was not well described. Furthermore, it was documented that there was no functional response of overexpressed KDR in non-endothelial cells stimulated by the ligand (34). Here, we created mutants with tyrosines 951, 1054, and 1059 to phenylalanine in the EGFR-KDR chimera (EGDR) and expressed them in HUVEC that expressed endogenous KDR. As shown in Fig. 1B, almost 100% of HUVEC transduced with EGDR, EGDR(Y951F), EGDR(Y1054F), or EGDR(Y1059F) expressed the receptors on the cell surface. Our data indicate that the level of receptor phosphorylation is greatly reduced in EGF-stimulated HUVEC transduced with EGDR(Y951F), whereas the receptor phosphorylation is almost undetectable in EGF-stimulated HUVEC transduced with EGDR(Y1059F). These results correlate very well with the data obtained from the bacterial overexpression system and from 293 cells expressing these mutants. More importantly, our data also indicate that tyrosines 951 and 1059 are required for EGF-stimulated EGDR/HUVEC migration and proliferation, respectively.
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