Endothelial Cell Migration in Stable Gradients of Vascular Endothelial Growth Factor A and Fibroblast Growth Factor 2

EFFECTS ON CHEMOTAXIS AND CHEMOKINESIS

Received for publication, June 14, 2007, and in revised form, January 22, 2008 Published, JBC Papers in Press, March 17, 2008, DOI 10.1074/jbc.M704917200

Irmeli Barkefors, Sébastien Le Jan, Lars Jakobsson, Eduar Hejll, Gustav Carlson, Henrik Johansson, Jonas Jarvius, Jeong Won Park, Noo Li Jeon, and Johan Kreuger

From the Department of Medical Biochemistry and Microbiology and the Department of Genetics and Pathology, Uppsala University, SE-751 23 Uppsala, Sweden and the Department of Biomedical Engineering, University of California, Irvine, California 92697

Gradients of secreted signaling proteins guide growing blood vessels during both normal and pathological angiogenesis. However, the mechanisms by which endothelial cells integrate and respond to graded distributions of chemotactic factors are still poorly understood. We have in this study investigated endothelial cell migration in response to hill-shaped gradients of vascular endothelial growth factor A (VEGFA) and fibroblast growth factor 2 (FGF2) using a novel microfluidic chemotaxis chamber (MCC). Cell migration was scored at the level of individual cells using time-lapse microscopy. A stable gradient of VEGFA165 ranging from 0 to 50 ng/ml over a distance of 400 μm was shown to strongly induce chemotaxis of endothelial cells of different vascular origin. VEGFA121, unable to bind proteoglycan and neuropilin coreceptors, was also shown to induce chemotaxis in this setup. Furthermore, a gradient of FGF2 was able to attract venular but not arterial endothelial cells, albeit less efficiently than VEGFA165. Notably, constant levels of VEGFA165, but not of FGF2, were shown to efficiently reduce chemokinesis. Systematic exploration of different gradient shapes led to the identification of a minimal gradient steepness required for efficient cell guidance. Finally, analysis of cell migration in different regions of the applied gradients showed that chemotaxis is reduced when cells reach the high end of the gradient. Our findings suggest that chemotactic growth factor gradients may instruct endothelial cells to shift toward a nonmigratory phenotype when approaching the growth factor source.

Many cells in developing organs and tissues have the capacity to detect extracellular chemical gradients and to respond to these gradients by directed positive or negative migration, a process called chemotaxis. In addition, some factors may also regulate chemokinesis which refers to nondirectional cell migration. Directed cell migration is at the heart of embryonic blood vessel formation, where the growing vessels navigate by a combination of secreted chemoattractants and repellents. The leading front of the embryonic vascular sprout holds a tip cell with numerous filopodia that express receptors for sensing secreted and cell-bound guidance cues provided by surrounding cells (1). One of the most well studied factors that control blood vessel formation and function is vascular endothelial growth factor A (VEGFA) (2–5). The effects of VEGFA on endothelial cells have been intensely studied for many years in an array of different model systems (4, 6–9). However, the ability to generate and maintain stable gradients of soluble factors compatible with cell culture conditions was only recently made possible by the invention of a microfluidic chemotaxis chamber (MCC). Chemotaxis of several cell types, including neutrophils and cancer cells, have been successfully studied in MCCs, but the method has so far not been used to systematically study endothelial cell migration in gradients of chemotactic factors (10, 11).

VEGF receptor 2 (VEGFR2) is a receptor for VEGFA and essential for endothelial cell differentiation and migration (12). VEGFA exists in several isoforms that bind with different affinities to VEGFRs and their coreceptors, neuropilins and heparan sulfate proteoglycans (HSPGs). VEGFA165 is thought to be the most abundantly expressed splice form with the capacity to interact both with neuropilins and HSPGs, whereas the shorter splice form VEGFA121 does not interact with HSPGs or neuropilins (13–16). Interactions between VEGFA165 and HSPG coreceptors are required for proper gradient formation in tissues, and these interactions probably also influence the kinetics and the quality of VEGFR2 signaling (13, 17). However, it has been rather difficult to record long range gradients of VEGFA165 similar to what has been shown for morphogens in various model systems (14, 18, 19). In addition to being presented as a gradient, secreted VEGFA165 sometimes seems to form a “path” over which endothelial cells migrate, as has been convincingly shown in the developing mouse retina (1).

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental S1–S5 and movies 1 and 2.
2 To whom correspondence should be addressed: Husargatan 3, Box 582, SE-751 23, Uppsala, Sweden. Tel.: 46-18-4714366; Fax: 46-18-4714673; E-mail: Johan.Kreuger@imbim.uu.se.
3 This work was supported by Swedish Research Council Project Grants K2006-71X-20223-01-2 and K2006-71P-20224-01-4 (to J. K.), the Swedish Cancer Foundation, the Swedish Foundation for Strategic Research Project A3 05:207g, the Wenner-Gren Foundations, the Magnus Bergvall Foundation, the Jeansson Foundations, the Selander Foundation, and Uppsala University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
4 The abbreviations used are: VEGFA, vascular endothelial growth factor A; FGF2, fibroblast growth factor 2; HSPG, heparan sulfate proteoglycan; HUVEC, human umbilical vein endothelial cell; HUAEC, human umbilical artery endothelial cell; MCC, microfluidic chemotaxis chamber; PDMS, polydimethyl siloxane; VEGFR, vascular endothelial growth factor receptor; FITC, fluorescein isothiocyanate.
Fibroblast growth factor-2 (FGF2) is a wide spectrum angiogenic factor shown to induce survival and proliferation as well as migration of endothelial cells both in vivo and in vitro (20–22). FGF2 is a ligand for FGF receptor 1 (FGFR1) and FGF receptor 2. The literature so far provides diverse reports on the effects of FGF2 on endothelial cell chemotaxis and chemokinesis. Endothelial cells have been reported to respond to FGF2 by increased chemokinesis (6) and/or by chemotaxis (23–25). However, it should be noted that chemotaxis may be difficult to distinguish from chemokinesis depending on the experimental method used to study cell migration.

The aim of this study was to investigate endothelial cell chemotaxis in stable gradients of different VEGFA splice forms and FGF2, as the precise roles of these factors in this context are yet to be fully understood. We hypothesized that a stable gradient of VEGFA would suffice in guiding endothelial cells and that interactions between VEGFA and coreceptors would not be required for the chemotactic response when the ligand was presented as a gradient. We also speculated that FGF2 would be a less potent chemotactic factor than VEGFA and that the shape of the gradient ultimately will dictate the chemotactic response.

By using MCC technology, we could show that the gradient of VEGFA165 efficiently induced chemotaxis but not chemokinesis of endothelial cells. A gradient of VEGFA121 was also shown to guide endothelial cells, suggesting that VEGFA does not need to interact directly with proteoglycan and neuropilin coreceptors to induce chemotaxis. Gradients of FGF2 were shown to weakly attract venular cells but to have no significant effect on arterial cells. Finally, our data provide an approximation of the minimal VEGFA gradient steepness required for induction of endothelial cell chemotaxis, and our data suggest that cells have the capacity to sense changes of the gradient steepness to reduce net cell migration at the high end of the growth factor gradient.

EXPERIMENTAL PROCEDURES

Production of the MCC—The methods and general principles for making different types of MCCs using soft lithography have been described before (10, 26). Briefly, the MCC was manufactured by pouring liquid polydimethyl siloxane (PDMS) (Sylgard 184 Silicone elastomer kit, Dow Corning) into a mold coding for three buffer reservoirs connected via separate channels to the cell migration chamber (800 μm wide, 100 μm deep, and 7 mm long) that ends in a single outlet channel (see Fig. 1A). The mold also encodes a separate micro-channel system running along the perimeter of the PDMS piece (not shown) that when connected to a vacuum source can be used to hold the MCC onto a conventional cell culture dish (27). The PDMS was allowed to solidify at 70 °C over night. Holes for the outlet port at the end of the channel and the liquid reservoirs were made using sharp custom-made punchers. Polyethylene tubing (Intramedic/Clay Adams) with an outer diameter slightly larger than the inner diameter of the outlet port was inserted into the outlet to make the fluidic connection. Tubing from the outlet port was connected to a syringe pump (Pump 11 PicoPlus, Harvard Apparatus) fitted with a gas tight Hamilton syringe (Sigma). Unless otherwise stated, constant perfusion with a flow speed of 200 μm/s (corresponding to shear stress of <0.1 dynes/cm²) was used for maintaining the gradient profile during the migration experiments.

Cell Culture and Recombinant Proteins—Human umbilical vein endothelial cells (HUVECs) and human umbilical artery endothelial cells (HUAECs) were purchased from PromoCell and routinely maintained in endothelial microvascular EBM MV2 growth medium (PromoCell). The endothelial cells were seeded 24 h prior to assembly of the microfluidic chamber (MCC) onto a 3-cm cell culture dish coated with type A gelatin from porcine skin (Sigma). The cells were then starved in EBM growth medium without serum and supplemented with 0.2% bovine serum albumin (denoted starvation media) for 20 h. Growth factors were added to the starvation media and deposited into the middle MCC buffer reservoir (see Fig. 1). Gradient intervals in the text refer to the initial concentrations of factors added to the buffer reservoirs. Stock solutions of recombinant VEGFA165 (PeproTech), VEGFA121 (R & D Systems) and FGF2 (PeproTech) were stored at −20 °C until further use.

Time-lapse Microscopy and Data Acquisition—Endothelial cell migration was observed using a Cell Observer System (Carl Zeiss AB, Stockholm, Sweden) with a Zeiss Axiovert 200 microscope, equipped with an AxioCam MRm camera, a motorized X/Y stage, and an XL incubator with equipment for temperature and CO2 control (Zeiss). During all experiments cells were kept in a humidified atmosphere of 5% CO2 in air at 37 °C. Software for time-lapse imaging and cell tracking was from AxioVision (Zeiss). Phase contrast images of cells and fluorescent images of FITC-dextran (10 kDa, used as an indirect indicator of the VEGF gradient) were taken every 5 min through a ×10 objective (Plan-Neofluar, NA 0.3, Zeiss). Cell migration in response to molecular gradients of chemotactic factors was scored in at least three independent experiments. In each experiment, data were collected from at least five different non-overlapping fields of cells growing in the MCC, as indicated in Fig. 1, during a period of 200 min.

Data Analysis and Gradient Simulation—All angle histograms and polar plots are organized as described in Fig. 2. The statistical significance of the results was tested using the Mann-Whitney nonparametric test. Net migration toward the concentration maxima was calculated for each experiment and ranked. Plots and graphs were created in MATLAB (The MathWorks, Natick, MA). For each analyzed cell, the change in the position of the cell nucleus was determined to calculate the distance and direction of cell migration. Chemotaxis was scored by measuring the net distance of migration (in one dimension) toward higher a concentration of growth factor, i.e. migration perpendicular to the flow direction was scored. Chemokinesis was evaluated by analyzing total cell migration distances (i.e. the full paths of migrating cells in two dimensions) of cells in response to constant levels of VEGFA and FGF2.

Gradient simulations were performed by two-dimensional numerical solution of a convective-diffusive equation with a laminar flow set to either 60, 80, or 200 μm/s in the chamber and zero flux boundary conditions (28–30) (see also supplemental data 1). Figures were assembled in Adobe Photoshop and Adobe Illustrator (Adobe Systems).
RESULTS

A novel MCC was employed to test our hypotheses related to the function of FGF2 and VEGFA in endothelial cell guidance and to determine the influence of gradient shape on the chemotactic response (Fig. 1). The MCCs were manufactured in transparent PDMS compatible with live imaging of cell migration and attached to regular cell culture dishes where endothelial cells were grown (see under “Experimental Procedures” for a detailed description of the MCC). A hill-shaped VEGFA165 gradient was generated by adding VEGFA to the middle reservoir (Fig. 1A). Gradient profiles were assessed by measurement of fluorescence emitted from FITC-dextran added together with VEGFA. As expected, the gradient profile changes along the length axis of the chamber (Fig. 1, B and C). The FITC-dextran employed had a molecular mass of 10 kDa but diffuses with a speed similar to VEGFA165 because of its elongated shape; the diffusion constant for FITC-dextran is 75 μm²/s compared with 133 μm²/s for VEGFA165 (28, 29). Simulations of FITC-dextran and VEGFA165 gradient profiles showed that the measured FITC-dextran profiles serve as a good approximation for the distribution of VEGFA165 in the MCC (Fig. 1, C and D, and supplemental data 1). Control experiments were performed to ensure that the added dextran did not interfere with the activity of added growth factors (supplemental data 2).

Subsequently, cells from different vascular beds were tested for cell migration. Primary cells isolated from either the artery or vein of human umbilical cords (denoted HUAECs and HUVECs, respectively) adhered well to gelatin-coated cell culture dishes, and they remained attached and healthy after application of the MCC and exposure to flow rates between 60 and 2000 μm/s (data not shown). Initial testing of cell migration in response to hill-shaped gradients of VEGFA165 was performed on HUVECs. Cells with a starting position in the middle of the chamber were excluded from analysis as well as cells initially touching the edges of the chamber (as indicated in Fig. 2A). Individual cell migration in each experiment was recorded by time-lapse microscopy. Cell migration was most obvious during the first hour of the experiments, and the length of each experiment was set to 200 min. About 85% of all HUVECs in both the left and right sides of the chamber migrated toward the middle of the MCC and a higher concentration of VEGFA165 (Fig. 2, A–D, and supplemental movies 1 and 2). The relative distance and direction of all HUVECs scored for migration are shown in a polar plot in Fig. 2E. In this type of plot, the starting point of all cells is assigned to the middle of the plot; the relative positions of all individual cells at the end of the migration experiment is shown. Red open circles in Fig. 2E indicate cells migrating in the right side of the chamber, and blue crosses indicate cells migrating on the left side of the MCC. Importantly, HUVEC migration toward the middle of the chamber was not observed without addition of VEGFA165 to the middle reservoir (p < 0.001) (Fig. 2, F–H). Note that in the absence of VEGFA, the cells seemed to be affected by the relatively low flow-induced shear stress (<0.1 dynes/cm²) produced in the device at the flow speed of 200 μm/s, as cells had a tendency to move in the direction of flow (Fig. 2, F–H). Furthermore, arterial endothelial cells were also shown to be attracted toward an increasing concentration of VEGFA165, demonstrating that endothelial cells from different vascular beds respond to the gradient profile present in the MCC (Fig. 2, I–K).

Next, the ability to modulate VEGFA-induced chemotaxis was explored by stepwise lowering of the pump flow speed, creating shallower gradients because of an increased diffusion time of growth factor molecules in the chamber (Fig. 3A). A hill-shaped VEGFA165 gradient (0–50 ng/ml at the inlet) was applied to HUVECs, and flow speeds between 60 and 200 μm/s
corresponding to different gradient shapes were tested. Average chemotaxis (Fig. 3B) corresponds to net migration per cell toward a higher concentration of growth factor. The chemotactic response declined together with decreasing gradient steepness until it resembled random migration at 60 µm/s (Fig. 3, B–D).

A pertinent question related to VEGFA function concerns the role of coreceptors in VEGFR activation and signaling. It has previously been demonstrated that proteoglycan coreceptors are required for proper distribution and activation of several VEGFA splice forms, e.g., VEGFA165, in developing tissues and in a variety of cell signaling assays (13, 14). However, it is unclear if binding of VEGFA to HSPGs is strictly required for the target cell to interpret positional information provided by the gradient. To address this, we generated gradients of VEGFA121, a splice form unable to interact with proteoglycan and neuropilin coreceptors. Presentation of VEGFA121 as a stable hill-shaped gradient (0–50 ng/ml) in the MCC potently induced HUVEC migration (Fig. 4, A–C). Measured positive chemotaxis induced by VEGFA121 was 22/1100610/9262 m as compared with 45/1100610/9262 m for VEGFA165. The diffusion coefficients for VEGFA121 and VEGFA165 are similar, and the gradients formed are comparable (supplemental data 1).

Together with VEGFA and the angiopoietins, FGF2 been considered among the most important regulators of endothelial cell chemotaxis (31). So far, most data on the role of FGF2 in this context are derived from experiments using the Boyden chamber. In our experiments, a hill-shaped FGF2 gradient pro-
The effects of VEGFA and FGF2 on the chemokinetic component of HUVEC migration were evaluated by analyzing total cell migration distances in the absence of growth factor, comparing with growth factor given either at a constant concentration of 50 ng/ml or in the form of a gradient (0–50 ng/ml). Total cell migration was significantly reduced when the cells were treated with a constant level of VEGFA165 as compared with nonstimulated cells (Fig. 6A), whereas a gradient of VEGFA165 promoted migration at a speed comparable with unstimulated cells. A constant concentration of VEGFA165 in a no flow situation was also shown to reduce chemokinesis (Fig. 6B). Similarly, VEGFA121 was found to reduce chemokinesis (data not shown), whereas a constant concentration of FGF2 gave a small increase in chemokinesis at a concentration of 50 ng/ml (Fig. 6C). Next, the effects on chemokinesis of different doses of VEGFA and FGF2 were investigated; VEGFA but not FGF2 was shown to reduce chemokinesis at all tested concentrations (supplemental data 4).

Finally, cell migration was analyzed in different parts of the VEGFA and FGF2 gradients (Fig. 7). Dose-response experiments showed that the maximal chemotactic response toward both VEGFA165 and FGF2 was achieved in the A1 region where the gradient is approximately exponential (Fig. 7, B and C; see supplemental data 5 for data on VEGFA121). Notably, chemotaxis in the A2 region, where the gradient is approximately linear, had a tendency to be stronger in the lower concentration intervals tested (Fig. 7, B and C).

**DISCUSSION**

Gradients of VEGFA most likely exist in many tissues, but with few exceptions these gradients have been difficult to visualize and quantify (1, 14). This study provides direct evidence that a stable gradient of VEGFA is sufficient for induction of endothelial cell chemotaxis. We show the following: 1) gradients of VEGFA are more potent in inducing chemotaxis compared with similar gradients of FGF2; 2) interactions between VEGFA and coreceptors are not absolutely required for cell guidance; 3) constant levels of VEGFA, but not of FGF2, efficiently reduce chemokinesis at all tested concentrations (supplemental data 4). Finally, cell migration was analyzed in different parts of the VEGFA and FGF2 gradients (Fig. 7). Dose-response experiments showed that the maximal chemotactic response toward both VEGFA165 and FGF2 was achieved in the A1 region where the gradient is approximately exponential (Fig. 7, B and C; see supplemental data 5 for data on VEGFA121). Notably, chemotaxis in the A2 region, where the gradient is approximately linear, had a tendency to be stronger in the lower concentration intervals tested (Fig. 7, B and C).

**DISCUSSION**

Gradients of VEGFA most likely exist in many tissues, but with few exceptions these gradients have been difficult to visualize and quantify (1, 14). This study provides direct evidence that a stable gradient of VEGFA is sufficient for induction of endothelial cell chemotaxis. We show the following: 1) gradients of VEGFA are more potent in inducing chemotaxis compared with similar gradients of FGF2; 2) interactions between VEGFA and coreceptors are not absolutely required for cell guidance; 3) constant levels of VEGFA, but not of FGF2, efficiently reduce chemokinesis; and 4) the shape of the VEGFA gradient dictates the migratory response exhibited by endothelial cells.

FGF2 was shown to be a poor chemotactic factor compared with VEGFA165. This result is in agreement with data from Yoshida et al. (6) but at odds with the view that FGF2 is an effective guidance cue for endothelial cells. The effect of FGF2 on chemokinesis was also shown to be small (Fig. 6 and supplemental data 4). Taken together, our findings might in part explain the lack of an obvious vascular phenotype observed in FGF2 knockouts (32, 33), suggesting that FGF2 is not a major guidance cue for endothelial cells. However, it should be noted that different types of endothelial cells may exhibit diverse migratory responses to guidance cues, as demonstrated here by the different responses exhibited by HUVECs and HUAECs to gradients of FGF2 (Fig. 5).
Endothelial Cell Guidance by VEGFA and FGF2

The binding sites for VEGFA165 on cell surfaces include the high affinity receptors VEGFR1 and VEGFR2, together with several coreceptors of neuropilin and proteoglycan type. The interaction between HSPGs and VEGFA165 is lost if the sulfation of heparan sulfate is blocked or severely reduced, or if the heparan sulfate-binding motif in VEGFA (exon 7) is removed to generate the shorter splice form VEGFA121 (4, 13, 17, 34). A gradient of VEGFA165 spanning from 0 to 50 ng/ml over a distance of 400 μm induced the strongest chemotactic response by endothelial cells in our assay. The shorter splice form VEGFA121, unable to interact with neuropilin and proteoglycan coreceptors, was also able to attract endothelial cells, although less efficiently than VEGFA165. These results provide evidence that binding of VEGFA to coreceptors is not absolutely required for cell guidance if the ligand is provided as a gradient. However, one should note that interactions between the longer VEGFA isoforms (e.g. VEGFA165 and VEGFA189) and proteoglycan coreceptors probably are necessary for proper gradient formation in vivo (14, 16, 17).

Varying the flow speed and thus the shape and steepness of the gradients in the MCC allowed us to identify necessary features of growth factor gradients required for an efficient chemotactic response, exemplified by the minimal gradient.

FIGURE 5. Chemotaxis of endothelial cells in response to a gradient of FGF2. A and B, computer-simulated gradient profiles of FGF2 (D = 220 μm²/s (38)) at positions P1 and P2 (see Fig. 1 for comparison). C and D, polar plots showing the migration of HUVECs (C) and HUAECs (D) in response to FGF2. Boxed values indicate net migration distances (μm). E, summary of the effects of FGF2 and VEGFA165 on HUVEC and HUAEC chemotaxis.

FIGURE 6. Effects of VEGFA and FGF2 on chemokinesis. A, full migratory paths of HUVECs were evaluated during flow conditions in response to starvation media with or without the addition of a constant level of VEGFA throughout the MCC, or in response to VEGFA165 presented as a gradient. B and C, chemokinesis in response to constant levels VEGFA165 (B) or FGF2 (C) in the absence of flow.
Notably, the standard deviation for scored chemotaxis was relatively large. This might suggest that there is some variability within the endothelial cell pool with regard to expression levels of the receptors for VEGFA and FGF2.

Based on our findings presented here, we propose a model for how endothelial cells respond to attractive gradients of VEGFA (Fig. 8). In our model, the endothelial cell efficiently responds to the exponential part of the gradient with chemotaxis. However, the steepness of the VEGFA gradient will ultimately be reduced as the cell migrates along the gradient, and as a result the relative difference in VEGFA concentration between the front and the back of the cell will diminish leading to reduced chemotaxis. Furthermore, because a symmetric stimulation with VEGFA was shown to reduce chemokinesis (Fig. 6 and supplemental data 4), we propose that the endothelial cell will shift toward a nonmigratory phenotype at the high end of the gradient.

Earlier in vitro studies of endothelial cell migration in response to various growth factors have mainly been conducted using the Boyden chamber assay (6–9). Unlike the Boyden assay, the MCC assay offers evaluation of stable and thus highly predictable gradients of different shape. Another advantage of the MCC is that the cells are analyzed using time-lapse microscopy, where the migratory paths of cells are directly followed over time. This allows for detailed analysis of cell migration in different regions of growth factor gradients as well the opportunity to capture detailed cell behavior at the level of individual cells. Importantly, flow-induced shear stress has been shown previously to modulate both haptotactic and chemotactic responses by cells in MCC-based assays (35, 36). Although we see an effect of flow-induced shear stress on endothelial cell migration (Fig. 2), the maximum shear stress developed in our experiments was less than 0.1 dynes/cm², and thus below shear stress levels previously reported to impede chemotaxis (35). It should also be noted that the MCCs used in this study were coated with gelatin (isoelectric point 7.0–9.0). Interactions between growth factors and gelatin are weak at physiological pH values (37). Still, there will conceivably be equilibrium between growth factor in solution and factor bound to the floor of the MCC. The distribution of growth factor on the floor of the MCC should, however, faithfully mirror the soluble gradient. Matrix secreted by cells could also influence growth factor binding and retention, but most high affinity binding sites for the growth factors tested in this study should be present at the cell surface.

The ability of endothelial cells to divide and migrate to form new vessels is central to tumor progression and dissemination and is also important in other pathologies where vessels are

**FIGURE 7. Analysis of chemotactic responses in different gradient regions.** A, schematic showing the division of the chamber for detailed analysis of chemotactic responses. The A1 region corresponds to the outmost halves of the right and left sides of the chamber where the gradient is approximately exponential. The A2 region represents the innermost halves of the chamber where the gradient is approximately linear before leveling off. Cells touching the edges of the chamber or lying in the middle region of the chamber were not scored (as indicated in Fig. 2). B and C, dose-dependent effects on HUVEC chemotaxis in the A1 and A2 regions of applied VEGFA165 and FGF2 gradients.

**FIGURE 8. Model for endothelial cell migration in a gradient of VEGFA.** The endothelial cell efficiently responds to the exponential part of the gradient with chemotaxis. The relative difference in VEGFA levels between the front and the back of the cell will ultimately diminish as the cell moves along the VEGFA gradient. Therefore, at the high end of the gradient, chemotaxis will be reduced, and the cell will shift toward a nonmigratory phenotype. Chemokinesis is also reduced by exposure to uniform levels of VEGFA at the high end of the gradient.

steepness required for a VEGFA gradient to impact HUVEC migration (Fig. 3).

Analysis of cell migration in different regions of the applied gradients showed that cells efficiently interpret the positional information provided by the gradients (Fig. 7). The chemotactic responses for both VEGFA and FGF2 were more pronounced in the exponential regions of the gradients (termed A1) compared with the linear regions (A2) at all conditions tested except in the lowest concentration interval (0–1 ng/ml). In this concentration interval, the level of growth factor in the A1 region is probably too low to efficiently stimulate the cells asymmetrically.
affected. The biotechnological platform described here will be an important complement to animal models for the study of the general mechanisms that control cell migration and angiogenesis, and such information will be instrumental in designing improved strategies for the treatment of pathological vessels.

Acknowledgments—We thank Prof. Lena Claesson-Welsh and Dr. Pär Gerwins for stimulating discussions.

REFERENCES

1. Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Altalay, K., Shima, D., and Betsholtz, C. (2003) J. Cell Biol. 161, 1163–1177
2. Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O’Shea, K. S., Powell-Braxton, L., Hillan, K. J., and Moore, M. W. (1996) Nature 380, 439–442
3. Carmeliet, P., Ferreira, V., Breier, G., Pollefeys, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoek, A., Harpal, K., Eberhardt, C., Declercq, C., Pavling, J., Moons, D., Collen, D., Risau, W., and Nagy, A. (1996) Nature 380, 435–439
4. Olsson, A. K., Dimberg, A., Kreuger, J., and Claesson-Welsh, L. (2006) Nat. Rev. Mol. Cell Biol. 7, 359–371
5. Carmeliet, P. (2005) Nature 438, 932–936
6. Yoshida, A., Anand-Apte, B., and Zetter, B. R. (1996) J. Cell Biol. 135, 57–64
7. Senger, D. R., Ledbetter, S. R., Claffey, K. P., Papadopoulos-Sergiou, A., Peruzzi, C. A., and Detmar, M. (1996) Am. J. Pathol. 149, 293–305
8. Vernon, R. B., and Sage, E. H. (1999) Microvasc. Res. 57, 118–133
9. Neufeld, G., Cohen, T., Gengrinovitch, S., and Poltorak, Z. (1999) FASEB J. 13, 9–22
10. Jeon, N. L., Baskaran, H., Dertinger, S. K., Whitesides, G. M., van de Water, L., and Toner, M. (2002) Nat. Biotechnol. 20, 826–830
11. Wang, S. J., Saadi, W., Lin, F., Minh-Canh Nguyen, C., and Li Jeon, N. (2004) Exp. Cell Res. 300, 180–189
12. Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L., and Schuh, A. C. (1995) Nature 376, 62–66
13. Ashikari-Hada, S., Habuchi, H., Kariya, Y., and Kimata, K. (2005) J. Biol. Chem. 280, 31508–31515
14. Ruhrberg, C., Gerhardt, H., Golding, M., Watson, R., Ioannidou, S., Fujisawa, H., Betsholtz, C., and Shima, D. T. (2002) Genes Dev. 16, 2684–2698
15. Soker, S., Takashima, S., Miao, H. Q., Neufeld, G., and Klagsbrun, M. (1998) Cell 92, 735–745
16. Stalmans, I., Ng, Y. S., Rohan, R., Fruttiger, M., Bouche, A., Yuce, A., Fujisawa, H., Hermans, B., Shani, M., Jansen, S., Hicklin, D., Anderson, D. J., Gardiner, T., Hammes, H. P., Moons, L., Dewerchin, M., Collen, D., Carmeliet, P., and D’Amore, P. A. (2002) J. Clin. Investig. 109, 327–336
17. Jakobsson, L., Kreuger, J., Holmborn, K., Lundin, L., Eriksson, I., Kjellen, L., and Claesson-Welsh, L. (2006) Dev. Cell 10, 625–634
18. Hufnagel, L., Kreuger, J., Cohen, S. M., and Shraiman, B. I. (2006) Dev. Biol. 300, 512–522
19. Strigini, M., and Cohen, S. M. (1999) Semin. Cell. Dev. Biol., 10, 335–344
20. Poole, T. J., Finkelstein, E. B., and Cox, C. M. (2001) Dev. Dyn. 220, 1–17
21. Cox, C. M., and Poole, T. J. (2000) Dev. Dyn. 218, 371–382
22. Matsumoto, T., Turesson, I., Book, M., Gerwins, P., and Claesson-Welsh, L. (2002) J. Cell Biol. 156, 149–160
23. Kanda, S., Lerner, E. C., Tsuda, S., Shono, T., Kanetake, H., and Smithgall, T. E. (2000) J. Biol. Chem. 275, 10105–10111
24. Shono, T., Kanetake, H., and Kanda, S. (2001) Exp. Cell Res. 264, 275–283
25. Shono, T., Mochizuki, Y., Kanetake, H., and Kanda, S. (2001) Exp. Cell Res. 268, 169–178
26. Whitesides, G. M., Ostuni, E., Takayama, S., Jiang, X., and Ingber, D. E. (2001) Annu. Rev. Biomed. Eng. 3, 335–373
27. Chung, B. G., Park, J. W., Hu, J. S., Huang, C., Monuki, E. S., and Jeon, N. L. (2007) BMC Biotechnol. 7, 60
28. Arrio-Dupont, M., Cribier, S., Foucault, G., Devaux, P. F., and d’Albis, A. (1996) Biophys. J. 70, 2327–2332
29. MacGabhann, F., Ji, J. W., and Popel, A. S. (2006) PloS Comput. Biol. 2, e127
30. Dertinger, S., Chiu, D., Jeon, N. L., and Whitesides, G. M. (2001) Anal. Chem. 73, 1240–1246
31. Lamalice, L., Le Boeuf, F., and Huot, J. (2007) Circ. Res. 100, 782–794
32. Zhou, M., Sutfiff, R. L., Paul, R. J., Lorenz, J. N., Hoving, J. B., Haudenschild, C. C., Yin, M., Coffin, J. D., Kong, L., Kranias, E. G., Luo, W., Boivin, G. P., Duffy, J. J., Pawlowski, S. A., and Doetschman, T. (1998) Nat. Med. 4, 201–207
33. Pintucci, G., Moscatelli, D., Saponara, F., Biernacki, P. R., Baumann, F. G., Bizekis, C., Galloway, A. C., Baslici, C., and Migliatti, P. (2002) FASEB J. 16, 598–600
34. Kreuger, J., Spillmann, D., Li, J. P., and Lindahl, U. (2006) J. Cell Biol. 174, 323–327
35. Hsu, S., Thakar, R., Liepmann, D., and Li, S. (2005) Biochem. Biophys. Res. Commun. 337, 401–409
36. Walker, G. M., Sai, J., Richmond, A., Stremler, M., Chung, C. Y., and Wilkso, J. P. (2005) Lab Chip 5, 611–618
37. Yamamoto, M., Tabata, Y., and Ikada, Y. (1999) J. Bioact. Compat. Polym. 14, 474–489
38. Filion, R. J., and Popel, A. S. (2004) Ann. Biomed. Eng. 32, 645–663