The Vascular Endothelial Growth Factor Receptor Flt-1 Mediates
Biological Activities

IMPLICATIONS FOR A FUNCTIONAL ROLE OF PLACENTA GROWTH FACTOR IN MONOCYTE ACTIVATION
AND CHEMOTAXIS*

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Two distinct receptors for vascular endothelial growth factor (VEGF)1, the tyrosine kinase receptors Flt-1 and FIK-1/KDR, have been described. In this study we show that monocytes, in contrast to endothelium, express only the VEGF receptor Flt-1, and that this receptor specifically binds also the VEGF homolog placenta growth factor (PIGF). Both VEGF and PIGF stimulate tissue factor production and chemotaxis in monocytes at equivalent doses. In contrast, endothelial cells expressing both the Flt-1 and the Flk-1/KDR receptors produce more tissue factor upon stimulation with VEGF than after stimulation with PIGF. Neutralizing antibodies to the KDR receptor reduce the VEGF-stimulated tissue factor induction in endothelial cells to levels obtained by stimulation with PIGF alone, but do not affect PIGF-induced tissue factor induction in endothelial cells nor the VEGF-dependent tissue factor production in monocytes. These findings strongly suggest Flt-1 as a functional receptor for VEGF and PIGF in monocytes and endothelial cells and identify this receptor as a mediator of monocyte recruitment and procoagulant activity.

Vascular endothelial growth factor (VEGF),2 also known as vascular permeability factor, was described as an inducer of angiogenesis in a variety of physiological and pathological processes including embryogenesis (1), corpus luteum formation (2), tumor growth (3), wound healing (4), and compensatory angiogenesis in the heart (5). In addition, VEGF induces vascular permeability in vivo (6) and exerts procoagulant activity via its ability to stimulate the production of the potent initiator of coagulation tissue factor (7) in endothelial cells and monocytes (8).

There are two known phosphotyrosine kinase receptors for VEGF: the fms-like tyrosine kinase Flt-1, and the fetal liver kinase, Flk-1 (derived from the mouse), or its human homolog KDR (kinase insert domain-containing receptor), with apparent Kd values of 16–114 pm for Flt-1 (9–12) and 400-1000 pm for Fik-1/KDR (11-14). The Kd values derived from studies using transfected cell lines correspond closely to the Kd values of the two binding sites which have been observed in endothelial cell types tested in vitro (11, 15, 16). So far, biological activities observed in both in vitro and in vivo studies appear to be mediated exclusively by the Flk-1/KDR receptor. Transfection of either the human Flt-1 or the KDR receptor cDNA in porcine aortic endothelial (PAE) cells, which do not express either VEGF receptor, demonstrated that, at least in PAE cells, the ability of VEGF to stimulate chemotaxis and proliferation occurs in KDR-transfected (KDR-PAE) but not in Flt-transfected (Flt-PAE) PAE cells (11). In addition, using a dominant negative approach, the Flk-1 receptor was shown to be essential for tumor angiogenesis in a glioblastoma model in vivo (17).

In addition to its endothelial cell-specific activities, VEGF also attracts peripheral blood monocytes, raises their intracellular Ca2+ levels (18), and induces tissue factor production (8). In contrast to the expression of both VEGF receptors in endothelial cells, only one specific binding site has been found on cells of the monocyte/macrophage lineage. Upon activation with VEGF, a high molecular weight band corresponding to a tyrosine-phosphorylated protein can be observed following immune precipitation with an anti-phosphotyrosine antibody (18).

Placenta growth factor (PIGF) has been described recently as a secreted growth factor with strong homology to VEGF based on amino acid and cDNA sequences. PIGF is expressed in human umbilical vein endothelial (HUVE) cells and placenta (19, 20). PIGF is a very weak stimulator of endothelial chemotaxis and proliferation (20), a finding which can be explained by the hypothesis that PIGF displays lower affinity to one or both of the two VEGF receptors. Indeed, when binding competition studies are performed with extracellular domains from either Flk-1/KDR or Flt-1, PIGF appears to be able to bind to Flt-1 but not to KDR/Flk-1 (12, 21). In this study we compared the human heparin binding isoform of PIGF, also known as PIGF-2 (20), with the corresponding human VEGF165 isoform. Using PAE cells, stably transfected with either KDR or human Flt-1, we identified Flt-1 as a specific PIGF receptor. In addition, we demonstrate by assessing tissue factor production and chemotaxis that Flt-1-expressing human monocytes react similarly to PIGF as to VEGF, while Flt-1 and KDR coexpressing human endothelial cells are primarily responsive to VEGF. Furthermore, neutralizing polyclonal antibodies to the KDR receptor inhibit VEGF- but not PIGF-mediated induction of endothelial tissue factor, whereas in monocytes these antibodies do not affect VEGF-mediated

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§ The abbreviations used are: VEGF, vascular endothelial growth factor; PIGF, placenta growth factor; RT-PCR, reverse transcriptasepolymerase chain reaction; PAE cells, porcine aortic endothelial cells; HUVE, human umbilical vein endothelial cells.
tissue factor induction. These data identify Flt-1 as a functional receptor for both VEGF and PlGF in cells of the endothelial and monocyte/macrophage lineage.

EXPERIMENTAL PROCEDURES

Materials—Laboratory reagents not listed otherwise were purchased from Sigma (München, FRG). Media and other cell culture reagents were obtained from Life Technologies, Inc. (Eggenstein, FRG). Heparin, concanavalin A- and phenyl-Sepharose and Superdex S200 were obtained from Pharmacia (Freiburg, FRG). Human umbilical cords were kindly donated from hospitals in the "Wetterau." Citrated pooled plasma was obtained from volunteers. HUVE cells were prepared by the method of Jaffe et al. (22) as modified by Thornton et al. (23). HUVE cells were cultured in medium 199 supplemented with 10 mM Hepes (pH = 7.4), 10% fetal calf serum, 10% human serum (Sigma, München, FRG), 100 μg/ml endothelial cell growth factor (cpro, Neustadt, FRG), heparin (20 μg/ml), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 2.5 μg/ml fungizone. Experiments were carried out within 48 h of the cells achieving confluency. Expression of tissue factor in endothelial cells was assessed by incubating cultures with purified recombinant VEGF or PlGF in medium 199 containing 10 μM Hepes (pH = 7.4), 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, in the presence or absence of antibodies. For studies with neutralizing antibodies against the VEGF receptor KDR (2-12), cells were submitted to a 15-min incubation period before addition of the cytokines. Cells were further incubated for 6 h at 37°C. Assays were carried out with whole cells obtained in suspension following scraping, and tissue factor activity equivalents were determined as described previously (8). Briefly, endothelial cells were removed from the dish by scraping with a rubber policeman. 100 μl of these resuspended cells were mixed with 100 μl of citrated plasma, and clotting times were measured after recalcification with 100 μl of a 25 mM CaCl2 solution. Tissue factor equivalents were determined by using a standard curve of purified human tissue factor. In certain experiments, cells were incubated for 15 min at 37°C with a monoclonal tissue factor neutralizing antibody (Laxo, Dassenheim, FRG) prior to the clotting assay.

Peripheral human blood monocytes were purified from buffy coats employing gradient centrifugation over Ficoll (histopaque 1077) and subsequent adhesion to tissue culture plastic as described previously (8). Chemotaxis of monocytes was investigated using the method of Quinn et al. (24) as modified recently (25). Briefly, cells were placed in the upper chamber, and a test substance was placed in the lower chamber. Chemotactic assays were performed after 3 h of incubation, and cells in at least 4 power fields were counted for each condition assessed.

RESULTS

PIGF-VEGF Binding Studies with Endothelial Cells Expressing Either One of the VEGF Receptors and Isolated Human Monocytes—In radioligand binding studies, PIGF has previously been shown to be binding to the KDR receptor in endothelial cells. We isolated human blood monocytes and studied the competitive binding of PIGF or VEGF with [125I]-VEGF in PAE cells which were transfected with either the KDR or the Flt-1 receptor (11). PIGF was found to compete with [125I]-VEGF for binding to Flt-1/PAE cells at similar concentrations as that observed with unlabeled VEGF (Fig. 1A). In contrast, no competition of PIGF with [125I]-VEGF occurred at any of the concentrations tested in KDR-PAE cells, although unlabeled VEGF showed the expected concentration-dependent competition (Fig. 1B). When primary endothelial cells were used, which endogenously express both VEGF receptors (FK-1/KDR and the Flt-1), PIGF only competed up to a maximum of 50% of that achieved with unlabeled VEGF (data not shown). Employing isolated human monocytes, PIGF competed for binding with [125I]-VEGF in a similar dose-response as noniodinated VEGF. Similar to the results obtained with Flt-1/PAE cells, VEGF competed at a 2-4-fold lower dose than PIGF for [125I]-VEGF binding to monocytes (Fig. 1C).

Expression of Flt-1 mRNA in Peripheral Human Blood Monocytes—Previous studies with iodinated [125I]-labeled VEGF have revealed one specific binding site on monocytes (18). The results described above suggest that Flt-1 is the monocyte VEGF receptor. To further investigate this hypothesis, we analyzed
monocyte mRNA for the presence of Flt-1 transcripts. RNA isolated from monocytes and HUVE cells share a transcript of 7.5 kilobases in size (Fig. 2A) when probed with a specific Flt-1 cDNA as described (3). The presence of transcripts coding for the Flt-1 receptor in monocytes was also confirmed by RT-PCR analysis with specific primers for human Flt-1 cDNA. Using the same PCR approach, transcripts for KDR could not be detected in cDNA derived from the same monocyte preparation (Fig. 2B).

Comparison of PlGF- and VEGF-mediated Tissue Factor Induction on Monocytes and Endothelial Cells—VEGF has been shown to induce procoagulant activity on the surface of human monocytes and HUVE cells by causing the de novo synthesis of tissue factor (8). As assessed by binding studies, HUVE cells express lower numbers of Flt-1 than KDR receptors (11, 16), in contrast to monocytes which exclusively express Flt-1 receptors. Therefore, we decided to compare the actions of PlGF and VEGF, in both monocytes and HUVE cells, in terms of their potential to induce tissue factor production. When isolated human monocytes were exposed to increasing concentrations of human recombinant PlGF and VEGF, an equivalent, dose-dependent induction of tissue factor was observed with both agents. The half-maximal response occurred at a concentration of 0.3 nM with VEGF and 0.5 nM with PlGF. Incubation of the cells with a monoclonal antibody specific to human tissue factor prior to the assessment of tissue factor abolished all of the tissue factor-inducing activity of PlGF and VEGF (Fig. 3A). When HUVE cells were incubated with PlGF, a dose-dependent induction of tissue factor, with a half-maximal response at 0.5 nM, was observed. VEGF-stimulated tissue factor expression appeared to be half-maximal at 1 nM, and maximal tissue factor production by VEGF is induced to a greater extent as compared to similar concentrations of PlGF (Fig. 3B).

Chemotactic Response of Peripheral Human Blood Monocytes to PlGF and VEGF—Using a modified Boyden chamber assay, VEGF has been shown to attract monocytes in a dose-dependent manner. Checkerboard analysis revealed that the response of monocytes to VEGF was a result of chemotaxis and not of chemokinesis (8). Due to the selective affinity of PlGF to Flt-PAE cells and our finding that Flt-1, but not KDR, is expressed on freshly isolated human peripheral blood monocytes, we hypothesized that PlGF may specifically induce monocyte migration. When recombinant PlGF was added to the lower well of a modified Boyden chamber, the number of migrating monocytes was similar to that induced by the addition of VEGF as a chemotactic stimulus (Fig. 4). Migration of monocytes was observed with a PlGF concentration of 100 pM, the response was maximal at 300 pM. The extent of chemotaxis in response to PlGF and VEGF was equivalent to that observed with the potent formylated chemotactic tripeptide fMet-Leu-Phe (29) which was used as a positive control. The chemotactic activity of PlGF was abolished by heating the peptide at 95 °C for 10 min, prior to the addition to the lower well of the Boyden chamber (Fig. 4).

Inhibition Studies with a Neutralizing Serum against KDR—In order to further distinguish between the two VEGF receptors, a polyclonal serum against the KDR receptor was developed. Rabbits were immunized with a 110-kDa protein coding for the extracellular seven IgG-like domains of the KDR receptor. In binding competition assays, serum (r-212) from one of these rabbits inhibited binding of 125I-VEGF to KDR-PAE
cells (Fig. 5). A 40-fold dilution neutralized binding of labeled VEGF to the same extent as a 50-fold excess of nonradioactive VEGF, whereas the same dilution of the preimmune serum did not affect binding of $^{125}$I-VEGF to KDR-PAE cells. In contrast, $^{125}$I-VEGF binding to human monocytes (which do not express KDR) was not affected by a 40-fold dilution of serum r-212 (Fig. 5).

Serum r-212 was assessed for its ability to inhibit the production of tissue factor induced by either VEGF or PIGF in HUVE cells. Although the PIGF-induced tissue factor production is much less than the one induced by VEGF at the same concentration, it is clearly above the values obtained with media alone. KDR neutralizing serum r-212 had no effect on this PIGF-mediated tissue factor induction, which is in concordance with the assumption that the effect of PIGF is solely dependent on Flt-1. However, when a 2 nM VEGF concentration was applied in the presence of serum r-212, tissue factor production was reduced to values obtained with a 2 nM concentration of PIGF alone. A control rabbit serum did not affect the tissue factor production achieved with 2 nM VEGF or PIGF (Fig. 6).
dependent binding competition for 125I-VEGF (similar to that observed in Flt-PAE) was observed with both PI GF and VEGF. This strongly supports the assumption that monocytes express only one VEGF receptor, which is Flt-1, and that PI GF is also a ligand for this receptor. Further support for this hypothesis came from expression studies probing mRNA isolated from monocytes or HUVE cells for either KDR or Flt-1 transcripts. Again, only expression of Flt-1 could be detected in freshly isolated human blood monocytes. These findings concur with those of an earlier study demonstrating only one specific binding site on monocytes/macrophages for VEGF (18), in contrast to binding studies on endothelial cells which revealed two distinct receptor binding sites (11, 15, 16). In addition, cross-linking studies with 125I-VEGF on isolated human monocytes clearly demonstrated only one specific band of a molecular mass greater than 200 kDa (18). These data can only be reconciled by a model in which the receptor-mediating biological activity in monocytes is promiscuous, i.e., it is activated by both VEGF and PI GF, which is the situation in monocytes for the Flt-1 receptor.

A more complex situation, however, emerges in respect to HUVE cells. By means of cross-linking studies with 125I-VEGF, two novel receptors for VEGF165 were identified in a breast cancer cell line by Soker et al. (31). These two novel receptors, which are also found in HUVE cells, can be distinguished from Flk-1/KDR and Flt-1 on the basis of cross-linking and immunoprecipitation experiments. The relative molecular masses of the novel receptor complexes are 165 and 175 kDa in comparison with molecular masses greater than 200 kDa for the complexed forms of the Flk-1/KDR and Flt-1 receptors (31). The binding affinities of these receptors for VEGF are similar to the binding affinity of VEGF for Flk-1/KDR (1963), making the distinction of these receptor types difficult by means of binding studies. While the two novel receptors may mediate an as yet undefined biological activity, it is highly unlikely that they contribute to tissue factor induction by PI GF in HUVE cells and monocytes or chemotaxis in monocytes alone as they do not bind soluble PI GF (31).

Although it has been shown that Flt-1-transfected fibroblasts respond to VEGF in terms of intracellular signaling, VEGF-dependent mitogenic activity was not observed (32). Similarly, VEGF-induced [3H]thymidine incorporation and chemotaxis could not be shown in the endothelial Flt-PAE transfectants in comparison with KDR-PAE cells (11). The Flt-1 receptor, however, appeared to be active, since intracellular signaling was observed with Flt-PAE cells after stimulation with VEGF. One possible explanation for this phenomenon was that PAE cells lack an important intracellular pathway required for Flt-1 signaling. One other possibility is that Flt-1 functions as a decoy receptor, analogous to the interleukin-1 receptor type I as suggested recently (21). We believe this report is the first which confirms that the Flt-1 receptor mediates signaling leading to distinct biological responses (which are tissue factor induction and chemotaxis in monocytes) and does not act as a decoy receptor.

In order to exclude the possibility that Flt-1 can only transduce signaling in monocytes, we determined whether PI GF was also able to activate endothelial cells known to express Flt-1. After stimulation of HUVE cells with PI GF, a dose-dependent tissue factor induction could be observed, although this response was less prominent when compared to VEGF. The half-maximal induction of tissue factor production occurred at a 2-fold lower concentration with PI GF as compared with VEGF. This is consistent with the lower K_d values described for Flt-1 in comparison with KDR (11). In contrast to monocytes (which can be stimulated to a similar maximal production of tissue factor by both PI GF and VEGF), maximal tissue factor produc-
tion with VEGF in HUVE cells is greater than with PI GF. There are at least two possible explanations for the difference in tissue factor production induced by equivalent concentrations of VEGF and PI GF in HUVE cells. The KDR receptor could either evoke a stronger biological response or may be present in higher amounts than Flt-1. The latter possibility is favored by binding studies which show significantly more binding sites on HUVE cells for the receptor with the lower affinity for PI GF (16), that is, the KDR receptor (11).

The hypothesis that Flt-1 is also a functional receptor in HUVE cells is further supported by studies using neutralizing antibodies (serum r-212) to the KDR receptor. These antibodies reduce tissue factor induction by VEGF to the same values as observed with Flk-1 heterodimers. In contrast, this serum cannot reduce endothelial tissue factor production by PI GF, which supports the hypothesis that PI GF-mediated tissue factor production is dependent on Flt-1 signaling. This signaling may be expected to occur upon Flt-1 homodimerization, since the effect of PI GF is independent of the anti-KDR serum. However, these results cannot exclude the possibility that PI GF also employs KDR-Flt-1 receptor heterodimerization, as the antibodies should not distinguish between KDR homodimers and KDR-Flt-1 heterodimers.

PI GF has been hypothesized to play a role in placental development and angiogenesis (19). PI GF- and Flt-1 expression, as determined by in situ hybridization, co-localizes with the spongiotrophoblast region of the placenta, whereas VEGF and Flk-1/KDR are coexpressed predominantly at the embryonic sites. It has been reported that Flt-1 "knockout" mouse embryos lack functional vascular tubes, suggesting that Flt-1 is important for endothelium lumenal differentiation or interactions of endothelium with the extracellular matrix (33). Whether this defect in Flt-1 knockout mice is linked to endothelial functions or to enzymatic or other functions of monocytes/macrophages is unknown. Monocytes/macrophages have been proposed as critical players in the process of angiogenesis and wound healing (34). The strong placental expression of PI GF could contribute to the increased demand for angiogenesis in the growing placenta which may be partially mediated by chemoattraction of peripheral blood monocytes.

We show here a new feature of PI GF, the induction of procoagulant tissue factor production in endothelial cells and monocytes. Expression of tissue factor in the endothelium of tumor vessels and within the tumor cells correlates with the switch to the malignant and angiogenic phenotype of breast carcinomas (35). Tissue factor expression can also regulate VEGF expression as shown by tissue factor antisense transfection studies. Tumors derived from tissue factor antisense expressing cells exhibit reduced angiogenesis (36). In addition, tissue factor knockdowns in mice reveal an impaired pattern of extraembryonic angiogenesis during early embryogenesis.

Furthermore, tissue factor production by PI GF- and/or VEGF-stimulated monocytes/macrophages may contribute to a common pathological complication of pregnancy, the increased risk of thrombosis. Further studies should reveal whether production and secretion of PI GF in placenta contributes to the consistently observed placental thrombosis during pregnancy.

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REFERENCES

1. Breier, G., Albrecht, U., Sterrer, S., and Risau, W. (1992) Development 114, 521–532.
2. Ravindranath, N., Littlehig, L., Phillips, H. S., Ferrara, N., and Zeleznik, A. J. (1992) Endocrinology 131, 254–260.
3. Phillips, L. K., Breier, G., Weich, H. A., and Risau, W. (1992) Nature 359, 845–848.
4. Brown, L. F., Yeoh, K. T., Berse, B., Yeo, T. K., Serger, D. R., Dvorak, H. F., and Vandewater, L. (1992) J. Exp. Med. 175, 1375–1379.
5. Banai, S., Jaklitsch, M. T., Shou, M., Lazarus, D. F., Scheinowitz, M., Biro, S., Epstein, S., and Unger, E. F. (1994) Circulation 90, 2183–2189.
6. Serger, D., Gali, S., Dvorak, A., Peruzzi, C., Harvey, V., and Dvorak, H. F. (1993) Science 258, 582–584.
7. Neumeyer. Y. (1988) Blood 11, 1–8.
8. Wieder, G., Gerlic, M., Gerlich, H., Blett, J., Wang, F., Familietti, P. C., Pan, Y.-C., Glander, J. V., Connolly, D. T., and Stern, D. M. (1990) J. Exp. Med. 172, 1539–1545.
9. Devidas, C., Escobedo, J. A., Ueno, H., Houck, K., Ferrara, N., and Williams, L. T. (1992) Science 255, 899–901.
10. Breier, G., Claus, M., and Risau, W. (1995) Dev. Dyn. 204, 228–239.
11. Waltenberger, J., Claesson-Welsh, L., Siewbahn, A., Shibuya, M., and Heldin, C.-H. (1994) J. Biol. Chem. 269, 26988–26995.
12. Kopsilai, R. L., Wang, G., Dalsko, J., and Thomas, K. (1994) Biochem. Biophys. Res. Commun. 201, 326–330.
13. Millauer, B., Wizigmann-Voos, S., Schürch, H., Martinez, R., Moller, N. P. H., Glander, J. V., Connolly, D. T., and Sterr, D. M. (1994) Biochem. Biophys. Res. Commun. 201, 52, 68–76.
14. Millauer, B., Shawver, L., Plate, K. H., Risau, W., and Ullrich, A. (1994) Cell 72, 833–846.
15. Quinn, M. T., Peters, K. G., Deviere, C., Ferrara, N., and Williams, L. T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7533–7537.
16. Plouet, J., and Mokdad, L. (1990) J. Biol. Chem. 265, 22071–22074.
17. Glander, J. V., Connolly, D. T., and DaLorc, J. E. (1991) Biochem. Biophys. Res. Commun. 175, 68–76.
18. Hauser, S., and Weich, H. (1993) Growth Factors 19, 259–268.
19. Park, J. E., Chen, H. H., Winer, H., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25846–25854.
20. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745–2753.
21. Thornton, S., Mueller, S., and Levine, E. (1983) Science 222, 623–626.
22. Quinn, M. T., Parthasarathy, S., and Steinberg, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2805–2808.
23. Hauser, S., and Weich, H. (1993) Growth Factors 19, 259–268.
24. Marme, D., Hug, H., and Weich, H. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2119–2123.
25. Kopsilai, R. L., Wang, G., Dalsko, J., and Thomas, K. (1994) Biochem. Biophys. Res. Commun. 201, 52, 68–76.
26. Schifft, B. L., Jager, B., Schollmann, C., Weidner, K., Wilting, J., Kochs, G., Marme, D., Hug, H., and Weich, H. A. (1993) Eur. J. Biochem. 219, 21–26.
27. Dallaric, G. S., Hug, H., Zachary, I. C., Breier, G., Baskerville, P. A., Yla-Herttuala, S., Risau, W., Martin, J. F., and Erusalimsky, J. D. (1995) FASEB J. 9, 2119–2127.
28. Baggio, M., and Clark-Lewis, I. (1992) FEBS Lett. 307, 97–101.
29. Magri, S., Guerriere, V., Viglietto, G., Benecke, P., and Persico, M. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10671–10675.
30. Hofman, D., Guevremont, V., Ronsini, G., Risau, W., and Yla-Herttuala, S. (1995) J. Exp. Med. 181, 955–962.
31. Soker, S., Fidder, H., Neufeld, G., and Klagsbrun, M. (1996) J. Biol. Chem. 271, 5761–5767.
32. Seetharam, L., Gotoh, N., Maru, Y., Ueno, N., Sambagawa, S., and Shibuya, M. (1995) Oncogene 10, 353–359.
33. Fong, G. H., Rossant, J., Gertsenstein, M., and Breitman, M. L. (1996) Nature 376, 66–70.
34. Polverini, P. J. (1989) in Cytokines (Sorg, C., ed) Vol. 1, pp. 54–60, S. Karger, Basel.
35. Contra, J., Hair, G., Kretzler, D. L., and Rickles, F. R. (1996) Nat. Med. 2, 209–215.
36. Zhang, T., Deng, Y., Luter, T., Muller, M., Ziegler, R., Waldherr, R., Stern, D. M., and Nawroth, P. P. (1994) J. Clin. Invest. 94, 1320–1327.