Vascular endothelial growth factor–stimulated endothelial cells promote adhesion and activation of platelets

Henk M. W. Verheul, Anita S. Jorna, Klaas Hoekman, Henk J. Broxterman, Martijn F. B. G. Gebbink, and Herbert M. Pinedo

Coagulation abnormalities, including an increased platelet turnover, are frequently found in patients with cancer. Because platelets secrete angiogenic factors on activation, this study tested the hypothesis that platelets contribute to angiogenesis. Stimulation with vascular endothelial growth factor (VEGF, 25 ng/mL) of human umbilical vein endothelial cells (HUVECs) promoted adhesion of nonactivated platelets 2.5-fold. In contrast, stimulation of HUVECs with basic fibroblast growth factor (bFGF) did not promote platelet adhesion. By blocking tissue factor (TF) activity, platelet adhesion was prevented and antibodies against fibrin(ogen) and the platelet-specific integrin, $\alpha_{\text{IIb}}\beta_3$, inhibited platelet adhesion for 70% to 90%. These results indicate that VEGF-induced platelet adhesion to endothelial cells is dependent on activation of TF. The involvement of fibrin(ogen) and the $\alpha_{\text{IIb}}\beta_3$ integrin, which exposes a high-affinity binding site for fibrin(ogen) on platelet activation, indicates that these adhering platelets are activated. This was supported by the finding that the activity of thrombin, a product of TF-activated coagulation and a potent platelet activator, was required for platelet adhesion.

Introduction

Coagulation abnormalities occur in more than 80% of patients with disseminated cancer. High concentrations of circulating tissue factor (TF), thrombin-antithrombin complexes, and an increased fibrinogen and platelet turnover have been found as well. An important role for platelets in metastasis formation and subsequent growth has been demonstrated in preclinical studies. In addition, tumor growth and dissemination can be inhibited by anticoagulants. It is not fully clear how and to what extent the coagulation cascade is involved in tumor growth, but growing evidence suggests that several coagulation factors are important for tumor-induced angiogenesis. For example, it has been shown in preclinical vivo models that both TF and thrombin can induce angiogenesis.

Under normal physiologic circumstances, resting endothelial cells (lining the vasculature) generate an active antithrombotic surface. Recent studies indicate that vascular endothelial growth factor (VEGF), the major stimulus of angiogenesis, activates endothelial cells to become prothrombotic. On VEGF stimulation, endothelial cells increase TF expression on their membranes and thereby generate thrombin activity from prothrombin (activation of the coagulation cascade). In addition, TF expression of tumor endothelium was found to correlate with VEGF expression in breast cancer. Tumor-released VEGF may change the antithrombotic surface of the vasculature into a prothrombotic one, causing fibrin formation and platelet adhesion and activation. In a previous study, we indeed found that activated platelets were present in the microvessels of VEGF-producing soft tissue sarcomas. It is well known that on activation, platelets release their contents including angiogenic growth factors, like VEGF and others. For example, platelet-derived sphingosine-1-P has been found to protect endothelial cells from apoptosis and to stimulate their DNA synthesis. Based on these observations, we hypothesized that platelets contribute to angiogenesis.

In this study we investigated whether stimulation of endothelial cells with angiogenic growth factors can cause adhesion and activation of platelets and whether platelets can contribute to endothelial cell proliferation in vitro, as an indication for their role in the angiogenic process in vivo. We developed an adhesion assay for fluorescence-labeled platelets on endothelial cells. In this assay, stimulation with VEGF, but not with basic fibroblast growth factor (bFGF), induced adhesion of nonactivated platelets on human umbilical vein endothelial cells (HUVECs). An essential role for TF and the subsequent activation of the coagulation pathway was found. Finally, we demonstrated that physiologic concentrations of platelets stimulate endothelial cell proliferation in vitro, indicative of a proangiogenic effect in vivo.

Materials and methods

Cell culture

The HUVECs were obtained by a standard procedure as previously described. In brief, after extensive washing with phosphate-buffered...
saline (PBS), cords were incubated for 20 minutes in trypsin/EDTA. Subsequently, cords were washed again with PBS and the obtained cell suspension was centrifuged for 7 minutes at 241g. After removing the supernatant, cells were resuspended in endothelial cell culture medium and plated on fibronectin-coated (2 μg/mL) plates. Endothelial cells were propagated in M199 containing 10% human serum, 10% fetal calf serum (FCS), 5 U/mL heparin, 200 μg/mL penicillin and 200 μg/mL streptomycin, 29.1 g/L glutamine, and 50 μg/mL endothelial cell growth factor derived from bovine hypothalamus. Only cells from passages 1 to 3 were used in all experiments.

Platelet preparation

Blood was drawn by venipuncture from volunteers, who did not take any drugs during the previous 10 days. Platelets were obtained as described by McNicol with only a few minor modifications.15 In brief, after discarding the first 5 mL, blood was drawn in 50-mL syringes containing 0.11 mol/L sodium citrate, acid citrate, and glucose (ACD) and centrifuged at 170g for 20 minutes. After separating the platelet-rich plasma (PRP), PRP was centrifuged for 10 minutes at 1500g and resuspended in M199 to a concentration of 100 × 10^9/mL. Subsequently, platelets were stained with calcine acetoxyethyl ester (calcine-AM, 2.5 μMol/L) (Molecular Probes Europe BV, Leiden, The Netherlands) for 15 minutes at 37°C in the dark under gentle movement to avoid platelet activation.

Leukocyte preparation

Leukocyte separation was performed according to standard procedures as previously described.14 Briefly, blood was drawn by venipuncture into a syringe containing ACD as described for the platelet isolation. Subsequently the blood was centrifuged at 1500g for 5 minutes. The buffy coat was separated, diluted 2 to 3 times in PBS, and carefully added on top of the Ficoll solution (Ficoll-Paque, Pharmacia Biotech AB Uppsala, Sweden). After centrifugation for 20 minutes at 540g, the supernatant was discarded and the interphase containing the leukocytes was selected. In this suspension, the erythrocytes were lysed with lysis buffer (0.15 mol/L NaCl, 1 mmol/L KHCO₃, 0.1 mol/L EDTA) and another centrifugation was performed at 380g for 5 minutes. This last step was repeated until all erythrocytes were lysed. Subsequently, cells were counted and labeled with calcine-AM as described for the platelets, but with a concentration of leukocytes of 2.5 × 10^6/mL.

Adhesion assay of platelets and leukocytes

The HUVECs were grown until confluence on precoated 96-well plates. After confluencc, 29.1 g/L glutamine, and 50 μg/mL endothelial cell growth factor derived from bovine hypothalamus. The HUVECs were grown until confluence on precoated 96-well plates. Adhesion assay of platelets and leukocytes

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**Adhesion assay of platelets and leukocytes**

The HUVECs were grown until confluence on precoated 96-well plates. Fifteen hours before stimulation, cells were gently washed twice with prewarmed M199 and cells were incubated with medium (100 μg/mL sodium citrate, acid citrate, and glucose (ACD) and centrifuged at 170g for 20 minutes. After separating the platelet-rich plasma (PRP), PRP was centrifuged for 10 minutes at 1500g and resuspended in M199 to a concentration of 100 × 10^9/mL. Subsequently, platelets were stained with calcine acetoxyethyl ester (calcine-AM, 2.5 μMol/L) (Molecular Probes Europe BV, Leiden, The Netherlands) for 15 minutes at 37°C in the dark under gentle movement to avoid platelet activation.

**To investigate platelet adhesion on endothelial cells, several blocking antibodies against membrane proteins of platelets or endothelial cells or against plasma proteins were used. The following antibodies were used in a blocking concentration of 40 μg/mL unless otherwise indicated17: C70A (CD31, IgG₁, DAKO, Glostrup, Denmark), A0080 (Fibrinogen, DAKO), E8 (Fibrin, IgG₂, Immunotech, Marseille, France), S2Z (CD42b [glycoprotein-IIIb], IgG₁, Immunotech), tissue factor (American Diagnostica, Greenwich, CT), LM609 (α₁b integrin, Chemicon International, Temecula, CA), P-selectin (Endogen, Woburn, MA), and reopro (αIIb β3 integrin and α₅β₁ integrin in a concentration of 50 μg/mL). E1 Lily Nederland BV, Nieuwegein, The Netherlands). As a negative control IgG was used obtained from DAKO.

In addition, the synthetic and specific thrombin inhibitor I2581 (Chromogenix Instrumentation Laboratory, Milan, Italy) was used in a concentration of 25 μmol/L.

**Determination of thrombin activity**

Determination of the thrombin activity is based on the enzymatic splitting reaction of p-nitroaniline (pNA) from the substrate S-2238 (Chromogenix Instrumentation Laboratory) by thrombin. After a 30-minute incubation of platelets on stimulated or unstimulated endothelial cells, samples of 50 μL were taken. As controls, platelet suspensions (kept for 30 minutes in a well without endothelial cells) and supernatant of stimulated or unstimulated endothelial cells were used. A working solution was composed of Millipore water with 0.5% bovine serum albumin (BSA) and 10% of a buffer (Tris 0.5 mol/L, EDTA 75 mmol/L, and 10% polyethylene glycol 3640). The substrate S2238, dissolved in Millipore water (1 mmol/L) was diluted in this working solution (1:12). In preheated 37°C cuvets, 1 mL of the substrate-working solution and, subsequently, 50 μL of a sample and 50 μL water or 50 μL of the thrombin inhibitor I2581 (450 mmol/L) were added. Cuvets were mixed and the optical density was measured at 406 nm. After 30 minutes the measurement was repeated. Thrombin activity was calculated from a standard activity curve of purified thrombin in a concentration range of 0 to 0.5 U/mL. By measuring the activity of the samples in the presence or absence of the specific thrombin inhibitor, thrombin activity could be calculated.

**Flow cytometry**

To analyze the activation state of the isolated platelets, fluorescence-activated cell sorter (FACS) analysis was performed on the platelets in 3 independent experiments, as described by Simak and coworkers with only minor modifications.18 Platelets were isolated and prepared as if they were used for an adhesion assay and analyzed just before they should have been incubated with endothelial cells. As a positive control of platelet activation, platelets were stimulated with the weak activator adenosine diphosphate (ADP) in a concentration of 25 μmol/L for 15 minutes. In this concentration ADP activates platelets to some extent, but without complete aggregation and fibrin formation, because that interferes with the FACS analysis. After 15 minutes of incubation with ADP or no activator (similar to the incubation of 15 minutes in the adhesion assay with calcine-AM), platelets were fixed in 1% paraformaldehyde for 15 minutes. After washing, the platelets were stained with R-phycoerythrin (PE)-labeled P-selectin-antibody (mouse IgG, CLB, Amsterdam, The Netherlands) in a 1:50 dilution or unspecified IgG in a concentration of 1 μg/mL. Platelet activation was evaluated on the FACScan (FACS Calibur, Becton Dickinson, San Jose, CA) by measuring P-selectin expression. Experiments were performed in duplicate. The state of activation was estimated by the median fluorescence of 10 000 platelets.

**Fluorescence microscopy: localization of platelet adhesion**

The adhesion assay was performed in the same way as described above but now unlabelled platelets were used. At the end of these experiments, instead of lysing the cells and platelets, the wells were fixed with 4% paraformaldehyde for 15 minutes. After preincubation (10% FCS and glycine in PBS), platelets were stained with a monoclonal antibody against the α₅β₁ integrin (M7057, DAKO) and endothelial cells with biotinylated antihuman integrin (α₅β₁, IgG₁, Immunotech), tissue factor (American Diagnostica, Greenwich, CT), LM609 (α₁b integrin, Chemicon International, Temecula, CA), P-selectin (Endogen, Woburn, MA), and reopro (αIIb β3 integrin and α₅β₁ integrin in a concentration of 50 μg/mL). E1 Lily Nederland BV, Nieuwegein, The Netherlands). As a negative control IgG was used obtained from DAKO.
detection of the biotinylated lectin. All antibodies were used in a 1:50 dilution for this double staining. Therefore the wells were mounted with Vectashield mounting medium for fluorescence (H1200, Vector Laboratories). All slides were examined within 1 week after staining on a LEICA confocal microscope. The αIIbβ3 integrin-stained platelets appeared green, the endothelial cells appeared red, and areas of coincident labeling containing colocalized antigens appeared yellow.

**Proliferation assay**

The HUVECs were plated in a density of 3000 cells/well on precoated 96-well plates in endothelial cell medium containing 5% FCS and 5% newborn calf serum, with no endothelial cell growth factor added. Next day, 100 μL containing an increasing number of nonactivated platelets in suspension with or without 0.5 U/mL thrombin in endothelial cell growth medium without serum was added. After 72 hours, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay was performed according to standard procedures. Results were corrected for the platelet activity in the MTT assay. In each experiment, conditions were tested in triplicate and independent experiments have been performed 3 times.

**Results**

**Platelet activation state**

The FACS analysis demonstrated that the isolated platelets stained with fluorescent antibody against P-selectin had a median fluorescence of 3.3 ± 0.15% (n = 3), indicative of a very low activation state. ADP-activated platelets had a significantly higher median fluorescence of 5.6 ± 0.2% (n = 3, P < .0001).

**VEGF induces platelet adhesion**

The occurrence of platelet adhesion after 6 hours of stimulation of confluent endothelial cells with either VEGF or bFGF in a dose range from 0.01 to 50 ng/mL is shown in Figure 1. VEGF (25 ng/mL) induced a significant 2.5-fold increase of platelet adhesion on endothelial cells, whereas bFGF had no stimulatory effect on platelet adhesion. The effect was more pronounced using endothelial cells that were just preconfluent. Prolonged stimulation of the endothelial cells (up to 18 hours) did not (further) increase platelet adhesion (data not shown). VEGF induced platelet adhesion to a similar extent as 1000 U/mL TNF-α. Whereas TNF-α-stimulated endothelial cells promoted leukocyte adhesion, neither VEGF-

![Figure 1. Platelet adhesion on VEGF- or bFGF-stimulated endothelial cells.](image)

**Table 1. Inhibition of platelet adhesion on VEGF-stimulated endothelial cells by blocking antibodies**

<table>
<thead>
<tr>
<th>Incubation with mAb or other inhibitor</th>
<th>With endothelial cells</th>
<th>With platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>17 (16)</td>
<td>20 (15)</td>
</tr>
<tr>
<td>CD31 (PECAM)</td>
<td>0 (8)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Gp-1b</td>
<td>0 (15)</td>
<td>14 (15)</td>
</tr>
<tr>
<td>P-selectin</td>
<td>0 (49)</td>
<td>33 (8)</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>92 (6)†</td>
<td>100 (6)†</td>
</tr>
<tr>
<td>Fibrinogen (LM609)</td>
<td>0 (18)</td>
<td>89 (21)*</td>
</tr>
<tr>
<td>Fibrin</td>
<td>0 (22)</td>
<td>94 (10)†</td>
</tr>
<tr>
<td>αIIbβ3 integrin</td>
<td>0 (9)</td>
<td>61 (12)*</td>
</tr>
<tr>
<td>reopro (αIIbβ3 and αIIbβ3)</td>
<td>0 (20)</td>
<td>72 (22)*</td>
</tr>
<tr>
<td>Thrombin inhibitor (I1285)</td>
<td>ND</td>
<td>100 (3)†</td>
</tr>
<tr>
<td>SUS416 (VEGF-R-blocker)</td>
<td>86 (12)†</td>
<td>ND</td>
</tr>
</tbody>
</table>

VEGF indicates vascular endothelial growth factor; mAb, monoclonal antibody; IgG, immunoglobulin G; PECAM, platelet-endothelial cell adhesion molecule; Gp-1b, glycoprotein-1b; ND, not determined; HUVEC, human umbilical vein endothelial cell.

The fluorescence-labeled platelets were allowed to adhere to the HUVECs for 30 minutes and subsequently the remaining unbound platelets were washed away. Platelet adhesion was determined by a fluorescence plate reader. The mean percentage of inhibition is calculated from 3 independent experiments (± SEM). Significant differences (* = P < .05; † = P < .01) were calculated with the Student t test. Negative inhibition values were set at zero.

**Blocking of VEGF-induced adhesion**

To explore the underlying mechanism, platelet adhesion was studied in the presence of blocking antibodies. Platelets or endothelial cells were incubated with blocking antibodies against TF, fibrinogen, fibrin, glycoprotein-Ib, CD31, αIIbβ3 integrin, P-selectin, or reopro against the αIIbβ3 integrins. In addition to the antibodies that had no effect on platelet adhesion (glycoprotein-Ib, CD31, P-selectin), IgG was used as negative control and did not inhibit platelet adhesion. Blocking of the TF activity inhibited adhesion of platelets for 90% to 100%, independent of whether the antibody against TF was preincubated with the platelets or with the endothelial cells. In addition, incubation of the platelets with antibodies against fibrinogen, fibrin, or αIIbβ3 integrin (LM609 and reopro) prevented the adhesion of platelets for 60% to 90%. These antibodies did not inhibit platelet adhesion when incubated with the endothelial cells. Blocking antibodies against glycoprotein-Ib, CD31, and P-selectin incubated with platelet or endothelial cells had no significant effect on platelet adhesion (Table 1). These data suggest that adhering platelets become activated by thrombin, which is generated by the increased TF expression on VEGF-stimulated endothelial cells. This was further confirmed by the fact that a specific thrombin inhibitor (I1285), prevented platelet adhesion for 100% (Table 1). The selective VEGF receptor blocker SUS416 (obtained from Sugen, San Francisco, CA), used in a concentration of 1 μmol/L (a concentration that inhibits VEGF-stimulated endothelial cell proliferation for 80%19), inhibited the platelet adhesion by 86% ± 7% (n = 5, P < .001), when concomitantly added to the HUVECs with VEGF for 6 hours. SUS416 in the same concentration had no inhibitory effect on TNF-α-stimulated platelet adhesion (1.7% ± 1.7%, n = 3, P = .4).

**Thrombin activity**

Because the thrombin activity appeared to be essential for VEGF-induced platelet adhesion, we determined the thrombin...
activity of the platelet suspension after incubation for 30 minutes on either stimulated or control endothelial cells. A significant 5-fold increase in thrombin activity (0.11 ± 0.03 U/mL, n = 3) was detected in the platelet suspension incubated on stimulated endothelial cells compared with the platelet suspension after incubation on unstimulated endothelial cells (0.02 ± 0.01 U/mL, P = .05). No thrombin activity was detectable in the endothelial supernatant and the nonincubated platelet suspension.

**Fluorescence microscopy**

The localization of the adhering platelets was examined by immunofluorescence microscopy. Staining of platelets (in green) and endothelial cells (in red) reflected that the majority of the platelets adhere to the endothelial cells. Only a few platelets bound to the extracellular matrix. Although some background adhesion of platelets on unstimulated endothelial cells was found, a clear increase in adhesion on stimulated endothelial cells versus unstimulated cells was observed (Figure 2).

**Platelet effect on endothelial cell proliferation**

To study whether platelets may contribute to angiogenesis, in vitro proliferation assays of endothelial cells incubated with platelets and activated platelets were performed. Compared to controls, a 2- to 3-fold increase in endothelial cell number could be reached by increasing the concentration of platelets from 0.5 to 500 × 10^6/mL. In this experiment, resting platelets became activated during the first 10 hours of incubation because they were not being gently moved. A representative experiment is shown in Figure 3.

**Figure 2. Immunofluorescence microscopy of platelet adhesion.** Immunofluorescence microscopy shows adhering platelets (in green) on endothelial cells (in red). (A) Control shows platelet binding to unstimulated endothelial cells, with a few adhering platelets. (B) An increased number of adhering platelets on VEGF-stimulated endothelial cells is depicted compared to the unstimulated control in panel A. Magnification (40 ×) shows that most platelets adhere to the endothelial cells and to a lesser extent to the extracellular matrix.

**Figure 3. Stimulation of endothelial cell proliferation by platelets.** Endothelial cell proliferation in a 72-hour period is shown either stimulated by an increasing number of nonactivated platelets (•) or by thrombin-activated (0.5 U/mL) platelets (∙). Error bars represent the SEM. A 2-fold increase in endothelial cell number, as measured by the MTT assay, was found for both activated and nonactivated platelets.

**Discussion**

This study was designed to investigate whether platelets, as carriers of angiogenic growth factors, are involved in angiogenesis-dependent diseases. We demonstrated that VEGF-stimulated, but not bFGF-stimulated, endothelial cells promote platelet adhesion and activation. This finding indicates that platelet adhesion can occur in the angiogenic microvascular sites of VEGF-producing tumors and other angiogenic diseases in which VEGF is involved.

The importance of platelet adhesion and activation for angiogenesis is reflected by the stimulatory effect of platelets on endothelial cell proliferation in vitro. Taken together, these data support the hypothesis that platelets contribute to tumor-induced angiogenesis.

In 1968, Gasic and coworkers demonstrated that platelets are involved in cancer biology. They found that in thrombocytopenic mice, experimental metastasis formation and tumor growth was reduced compared to normal controls; this inhibition could be recovered by platelet transfusion. In clinical studies, it was found that platelet turnover is increased in cancer patients (3-fold) compared to healthy controls. In addition, thrombocytosis (an increased platelet number) occurs frequently (1%-60%) in cancer patients and it has been described as a negative prognostic factor for survival for some tumor types. In a previous study, we demonstrated that platelets become activated in the microvasculature of soft tissue sarcomas. The results of the current study provide a possible explanation for these observations. Platelet adhesion and subsequent activation in tumor vasculature will lead to an increased platelet turnover in cancer patients.

Angiogenesis is a complex multistep process consisting of the breakdown of the basal membrane, endothelial cell migration and proliferation, subsequent tube formation, and finally the establishment of connections between newly formed tubes and the initiation of blood flow. As is extensively studied, a variety of cells (tumor and stromal) play a role in angiogenesis. Both leukocytes and platelets transport several angiogenic growth factors, including VEGF, but it is unknown what the role of these cells is in angiogenesis-dependent diseases. It has been reported that platelets promote tube formation of endothelial cells in vitro. In addition, in an in vivo corneal neovascularization model a pellet of thrombin-activated platelets induced angiogenesis. As early as 1986, it was hypothesized that tumors are never-healing wounds without the involvement of platelets. However, no data were provided to confirm or to refute the assumption that platelets are
not involved in these never-healing wounds. In contrast, we recently detected intratumoral platelet activation. Here we show that VEGF may stimulate platelet adhesion and activation and, subsequently, that these platelets can stimulate endothelial cell proliferation. These results provide a possible explanation for the clinical observation that thrombocytosis is a negative prognostic factor for survival of cancer patients, because this increased number of platelets may have an extra proangiogenic effect to the tumor-induced angiogenesis in these patients.

The concomitant activation of angiogenesis and coagulation in cancer biology has been extensively described. Transfection of tissue factor enhanced the release of VEGF. VEGF, in turn, induced TF expression on endothelial cells. In breast cancer tissues, TF was highly expressed in the angiogenic tumor microvessels. In addition, it has been reported that thrombin is a proangiogenic factor. Fibrin, the converted form of fibrinogen, can also induce TF expression on endothelial cells. In breast cancer recently detected intratumoral platelet activation. Here we show not involved in these never-healing wounds. In contrast, we

Recently, Bombeli and colleagues reported that activated platelets adhere to endothelial cells through bridging proteins like fibrinogen and von Willebrand factor. In this study we confirmed these results because prevention of fibrinogen bridging inhibited platelet adhesion. Integrins have been shown to play a major role in binding matrix and plasma proteins to cell surfaces, forming bridges between the underlying matrices or for cell-cell interactions directly. Bombeli and coworkers demonstrated that the αβ, integrin and the αβ integrin on endothelial cells and platelets play important roles in the adhesion. We confirmed the inhibiting effect of a cyclic-RGD (arginine–glycine–aspartic acid) peptide (inhibiting for 45% when coincubated with platelets, data not shown). Unexpectedly, reopro, LM609, and the cyclic-RGD peptide failed to inhibit platelet adhesion when coincubated with the endothelial cells. So far, we have no explanation for this finding other than that the expression of αβ integrin on HUVECs is mainly located at the abluminal site and therefore may not interfere with platelet adhesion. These results suggest that the αβ integrin on platelets may play a role in platelet adhesion. The question via what endothelial cell receptor platelet adhesion occurs remains open. One explanation, which is supported by our results, might be that TF on the HUVECs is directly responsible for adhesion, similar to observations for mononuclear phagocytes.

Other stimulators of platelet adhesion to endothelial cells include the proinflammatory cytokine TNF-α, which also induces TF expression on endothelial cells. Therefore, one may expect that VEGF-induced platelet adhesion to HUVECs is caused by a similar mechanism as TNF-α-induced platelet adhesion. Previously, it has been shown that platelet adhesion on TNF-α–stimulated endothelial cells with whole blood is also TF dependent. In that particular study it was found that TNF-α–induced leukocyte adhesion to endothelial cells is platelet and fibrin dependent. The importance of platelets and fibrin for leukocyte adhesion may explain the contrasting results with our assay, in which isolated leukocytes did not adhere to VEGF-stimulated endothelial cells, whereas in the in vivo experiments an increased leukocyte binding was detected on tumor endothelium.

The lack of platelet adhesion on bFGF stimulation of the HUVECs in the platelet adhesion assay might be explained by the fact that bFGF is not able to up-regulate TF expression on endothelial cells. It supports our observation that VEGF promotes adhesion through TF-expression on endothelial cells.

Based on literature data and the results of this study, we conclude that platelet adhesion and activation in the tumor microvasculature might be induced by VEGF stimulation of endothelial cells. Subsequently, the stimulatory effect of platelets on endothelial cell proliferation in vitro is supportive for a proangiogenic activity of adhering and activated platelets in vivo. These findings indicate that intratumoral platelet trapping may be responsible for an increased platelet turnover in cancer patients. In addition, platelets may not only promote tumor-induced angiogenesis, but also other angiogenesis-dependent diseases in which VEGF is involved. Therefore, we believe that prolonged antiplatelet therapy may be beneficial for patients with angiogenesis-dependent diseases.

References

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