



Review

The role of blood platelets in tumor angiogenesis

Siamack Sabrkhanly^{a,b}, Arjan W. Griffioen^{b,*}, Mirjam G.A. oude Egbrink^a^a Laboratory for Microcirculation, Cardiovascular Research Institute Maastricht (CARIM), Dept. of Physiology, Maastricht, The Netherlands^b Angiogenesis Laboratory, Dept of Medical Oncology, VU university Medical Center, Amsterdam, The Netherlands

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ABSTRACT

Coagulation abnormalities occur frequently in cancer patients. It is becoming evident that blood platelets have an important function in this process. However, understanding of the underlying mechanisms is still very modest. In this review, we discuss the role of platelets in tumor angiogenesis and growth and suggest their potential significance in malignancies. Platelets contain various pro- and antiangiogenic molecules, which seem to be endocytosed and sequestered in different populations of α -granules. Furthermore, tumor endothelial cells are phenotypically and functionally different from endothelial cells in healthy tissue, stimulating local platelet adhesion and subsequent activation. As a consequence, platelets are able to secrete their angiogenic and angiostatic content, most likely in a regulated manner. The overall effect of these platelet–endothelium interactions appears to be proangiogenic, stimulating tumor angiogenesis. We favor the view that local adhesion and activation of blood platelets and dysregulation of coagulation represent underestimated pathways in the progression of cancer.

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1. Introduction

Platelet functioning in hemostasis and coagulation has been widely studied for a long time, resulting in our current understanding of platelet behavior in physiological and pathological conditions. In addition to their role in hemostasis, platelets have also been implicated in malignant diseases. It is not uncommon for cancer patients to develop coagulation abnormalities, in which platelet functioning may be markedly disturbed as well. These abnormalities were unexplained for a long time, but over the last few years numerous data have been obtained suggesting the involvement of platelets in tumor development and growth.

Angiogenesis, which is the process of new blood vessel growth from pre-existing vessels, is imperative in malignant tumor growth. It is regulated by a balance of proangiogenic and angiostatic factors, which, upon the switch of tumor cells to an angiogenic phenotype,

leads to tumor growth and progression [1]. Since angiogenesis research got an enormous boost after the early 1990s, different angiogenesis regulators have been discovered. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were identified as positive regulators of angiogenesis. Interferon- α , angiostatin, endostatin and TNP-470 are examples of the first generation of angiogenesis inhibitors, while compounds such as bevacizumab, sunitinib and erlotinib are examples of current clinically used compounds [2]. During the past years several of these angiogenesis inhibitors have found their way into mainstream oncology treatment [3]. Very often angiogenesis inhibitors are used in combination with chemotherapy to extend progression free survival in various malignant diseases such as colorectal, lung, and breast cancer [4–6]. Effective use of angiogenesis inhibitors as monotherapy for cancer has thus far been mainly limited to renal cancer [7]. Fundamental research as well as these clinical applications demonstrated that tumor endothelial cells are a particularly suitable target for cancer therapy, as they play an essential role in angiogenesis. The fact that they are easily accessible for intravenously

* Corresponding author. Tel.: +31 20 4443374; fax: +31 20 4443844.

E-mail address: aw.griffioen@vumc.nl (A.W. Griffioen).

administered drugs, genetically more stable than cancer cells, and less susceptible to drug-induced resistance after prolonged treatment [8], illustrates the attractiveness of angiogenesis inhibition as a treatment modality against cancer.

Platelets have been associated with malignant diseases since the late 19th century, as different abnormalities were observed in the coagulation and hemostasis systems of cancer patients [9]. These patients are at increased risk of different thromboembolic events such as venous thromboembolism, including deep vein thrombosis and pulmonary embolism [10]. Laboratory tests also indicate a variety of irregular platelet related values in the blood of cancer patients, e.g. raised platelet counts, increased platelet turnover and elevated concentrations of fibrinogen breakdown products [11–14]. A relation between platelets and tumor angiogenesis is suggested by the fact that platelets appear to be the main physiologic transporters of VEGF [15]. This hypothesized link is supported by experimental data showing that platelets release their VEGF content upon activation [16,17] and that they are activated in tumor vasculature, enabling them to secrete their content locally within the malignant tissue [18]. Actual involvement of platelets in angiogenesis is illustrated by their modulating function in gastric ulcer healing, a process known to be dependent on VEGF and angiogenesis [19,20], and also by the finding that they stimulate capillary growth in matrigel, which is indicative of their capability to stimulate endothelial cells to transform into vessel like structures [21]. The functional relevance of platelets in tumor angiogenesis *in vivo* has been demonstrated in a cornea assay in which lack of platelets led to haemorrhage during angiogenesis, while platelet depleted mice showed an inhibition of early angiogenesis and intratumor haemorrhage [22]. In addition, thrombocytopenic tumor bearing mice show reduced tumor cell proliferation and growth, and increased tumor necrosis [23]. Several reports, showing that the inhibition of platelet function in cancer patients not only reduces the number of thromboembolic events, but also results in reduction of tumor growth and risk of relapse [24–29], underline the notion of a possible role of platelets in tumor angiogenesis.

The data mentioned above support the idea that platelets are positively involved in tumor angiogenesis and growth. The question of which mechanisms underlie platelet mediated angiogenesis remains. This review will first present a short overview of current knowledge on platelet functioning in hemostasis and thrombosis, then summarize data that support the hypothesized role of platelets in tumor angiogenesis, and end with the potential meaning of the involvement of platelets for cancer therapy.

2. Platelet functioning during hemostasis and thrombosis

Platelets play a central role in hemostasis, limiting blood loss after injury, but also in thrombosis, inflammation, and restenosis. Under physiological conditions, endothelial cells prevent platelets from binding to the vessel wall; platelets flow in the blood stream in close proximity to the endothelium, but rarely adhere to it [30,31]. Endothelial cell damage or alteration may lead to exposure of the subendothelial extracellular matrix (ECM), whereafter platelets can come into contact with thrombogenic components of the ECM. The ECM contains various macromolecules that are appropriate ligands for platelet adhesion. These molecules include different types of collagen, von Willebrand factor (vWF), laminin, vitronectin, proteoglycans, thrombospondin, and fibronectin [32].

The hemostatic response to endothelial damage or alteration depends on the extent of the injury, the local fluid dynamic conditions and the specific ECM components that are exposed [32]. Of these components, especially collagen and vWF are important for platelet adhesion and also for platelet activation [33]. Under high shear conditions, platelet adhesion to the vessel wall is strictly dependent on vWF that is bound to collagen. Soluble vWF does not bind to circulating platelets, but when it is immobilized on a collagen surface

high shear stress induces conformational changes, resulting in a fast, reversible interaction with platelet glycoprotein (GP) Ib-V-IX [34]. This interaction reduces platelet flow velocity and results in rolling of the platelets over the collagen surface. In addition, vWF can also bind to GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$) on the platelet membrane. As a result, vWF can function as a bridge between platelets and collagen [35]. Platelet rolling may enable direct interactions between platelets and collagen, resulting in firm adhesion. Platelets adhere to collagen through two receptors, GPVI and GPIa-IIa (integrin $\alpha_2\beta_1$) [31,36]. Direct adhesion of platelets to collagen induces their activation, increasing their cytosolic free calcium concentration [37]. This is accompanied by shape change and by exposure of phosphatidylserine (PS) on the outer platelet membrane, which turns the platelets procoagulant [38].

Activated platelets that adhere to collagen can recruit other platelets from the blood stream to form an aggregate. Activation of these newly recruited platelets is caused by the release of autocrine agents, partly from secretory granules. The α -granule, which is the most abundant granule in platelets, contains a multifunctional array of proteins such as vWF, fibrinogen, thrombospondin and P-selectin. Another type of secretory vesicles in platelets are the dense granules. They serve as storage pools for serotonin, adenosine diphosphate (ADP), adenosine triphosphate (ATP) and the divalent cations Ca^{2+} and Mg^{2+} [39–42]. Two of the molecules that are especially important during the formation of a platelet aggregate are ADP and thromboxane A₂ (TXA₂). TXA₂ is formed from arachidonic acid, which is released from membrane phospholipids by phospholipase A₂ upon platelet activation. TXA₂ diffuses through the cell membrane and activates other platelets by binding to their thromboxane-prostanoid (TP) receptors, TP α and TP β [43]. ADP, secreted from the dense granules, activates platelets via the receptors P2Y₁ and P2Y₁₂; P2Y₁ activation mediates shape change and initiates aggregation, while P2Y₁₂ is needed to complete and amplify the aggregation process [31,44,45].

Procoagulant platelets accelerate the formation of thrombin from prothrombin. Thrombin is a crucial protein linking coagulation, platelet activation and thrombus formation [46]. It is one of the strongest platelet agonists known, and binds to the protease-activated receptors PAR-1 and PAR-4. PAR-1 is responsible for the majority of the thrombin response and responds to low concentrations of thrombin, while PAR-4 only responds to higher concentrations of thrombin [47]. Thrombin also catalyzes the formation of fibrin from fibrinogen. Fibrinogen, which is found both in plasma and in α -granules, is involved in the aggregation process as well. It has binding sites for integrin $\alpha_{IIb}\beta_3$ on both ends, and acts as a bridging molecule between aggregating platelets [48]. Fibrin stabilizes the aggregate and consolidates the resulting thrombus or haemostatic plug.

3. Platelets are involved in the regulation of the angiogenic balance in tumors

Beside molecules involved in hemostasis and thrombosis, platelet secretory granules also contain various proteins that regulate angiogenesis (Table 1). Platelets are for example reported to be the main physiologic transporters of VEGF, which is one of the most potent proangiogenic factors known [15]. They take up such angiogenesis regulatory factors in a dose-dependent manner by active endocytosis and sequester these proteins in their α -granules [49]. Beside the selective uptake of endogenous angiogenesis regulatory proteins, platelets also endocytose intravenously administered monoclonal antibodies, like bevacizumab. When this anti-VEGF antibody is taken up in the α -granules, it neutralizes VEGF [50]. This results in reduced platelet angiogenic activity, which may explain the clinical side effects of bevacizumab treatment [50].

The fact that platelets contain so many proteins that regulate angiogenesis underlines their potential significance in tumor development and growth. Yet, in order for these substances to be released

Table 1
Platelet-derived angiogenesis stimulators and inhibitors.

| Platelet-derived angiogenesis stimulators | | Platelet-derived angiogenesis inhibitors | |
|---|-----------|--|-------|
| VEGF | [16] | TGF- β | [130] |
| PDGF | [131] | Endostatin | [19] |
| EGF | [132] | Angiostatin | [133] |
| BFGF | [134] | PF4 | [135] |
| IGF I | [136] | PF4 fragments | [137] |
| IGF II | [136] | CXCL4L1 | [138] |
| HGF | [130] | Thrombospondin | [139] |
| PD-ECGF | [140] | PAI-1 | [141] |
| Ang-1 | [142] | HGF domain NK1 | [56] |
| MMPs | [143–145] | HGF domain NK2 | [56] |
| S1P | [146] | | |
| SDF-1 | [147] | | |

inside the tumor tissue, platelets need to be activated in tumor vasculature. This has been shown to be the case in different cancer types. Verheul et al. have shown that platelets are activated in tumor vasculature of soft tissue sarcomas by applying immunofluorescence microscopy of tumor tissue and by measuring coagulation markers in tumor aspirates [18]. Tissue factor (TF) concentration was approximately twice as high, and thrombin concentration showed a 63-fold increase when compared to normal plasma values. Tumor tissue showed dense vascularization with intense VEGF expression and the presence of activated platelets. These platelets were associated with fibrinogen in aggregates as was shown by double staining for $\alpha_{IIb}\beta_3$ integrin and fibrin(ogen). Other groups have demonstrated platelet activation in different malignancies such as breast and prostate cancer, where increased plasma levels of soluble P-selectin and fibrinogen were found when compared to controls [51,52].

Increased expression of TF by tumor endothelial cells may contribute to the localized activation of platelets in the tumor vessels. TF is an integral membrane protein that initiates the coagulation cascade after binding to factor VII/VIIa, ultimately leading to the formation of platelet activating thrombin [47,53]. A significant relation between TF expression and microvessel density, and between TF and VEGF has been found in breast cancer [54,55]. Increased expression of TF has also been observed in many other tumor types, including small cell carcinoma, bronchoalveolar carcinoma, head and neck cancer, malignant glioma, bladder cancer, and prostate cancer [56–58]. Solid tumors have been shown to express TF on their endothelial cells as well as on the tumor cells themselves [58,59]. Furthermore, stimulation of human umbilical vein endothelial cells (HUVECs) with bFGF leads to TF expression by these cells [54]. The same holds for VEGF; there is increasing evidence suggesting that VEGF induces endothelial cells in tumor vasculature to express TF [60–64]. Moreover, transfection of VEGF in different tumor cell lines results in an upregulation of TF in proangiogenic and antiangiogenic properties of tumor cells [62,65]. There is also evidence of high concentrations of thrombin within tumors, most likely as a result of an increased TF expression in tumors [66]. TF and thrombin are not the only platelet activating factors in tumors; B16F1 and B16F10 murine melanoma cell lines, and 253J cells have been shown to activate platelets by an ADP dependent mechanism [67]. Therefore, one could hypothesize that tumors promote platelet activation and aggregation in their vasculature by the expression and/or release of platelet activating factors such as TF, thrombin and ADP, resulting in the release of angiogenesis regulatory factors from platelet granules, which in turn affects tumor growth [68].

Although platelets contain both angiogenic and angiostatic compounds, their overall effect on angiogenesis seems to be stimulating [41,69]. Several recent studies have provided evidence that suggest that the secretion of the angiogenic and angiostatic proteins by platelets is a regulated process. The group of Folkman showed that angiogenic and angiostatic proteins are organized into separate populations of α -

granules [40]. They and others also provided data that suggest that these proteins can be secreted differentially by selective stimulation of the thrombin receptors PAR-1 and PAR-4 [40] or by ADP dependent platelet activation through the P2Y receptors [70]. Ma et al. were the first to demonstrate a link between PAR-1 and PAR-4 stimulation and release of either VEGF or endostatin from platelet granules, leading to evidence of a counter-regulatory modulation of angiogenic compound release by stimulation of these thrombin receptors [20]. Selective PAR-1 stimulation leads to VEGF release, while at the same time the secretion of endostatin from platelets is inhibited. By contrast, PAR-4 activation results in the secretion of endostatin, while VEGF secretion is suppressed [40]. Moreover, PAR-1 inhibition in rats decreases gastric ulcer healing, which is known to be dependent on VEGF [20]. Together these findings suggest a regulatory role for platelet PAR receptors in angiogenesis and, hence, in tumor growth.

ADP-mediated platelet activation seems to have a regulatory effect on VEGF and endostatin release as well [70]. After stimulation with ADP via either or both P2Y receptors, platelets secrete significant amounts of VEGF, but almost no endostatin. As compared to thrombin receptor activation, ADP activation appeared to be a weaker stimulus for VEGF release. This is consistent with the role of ADP as a relatively weak platelet agonist when compared to thrombin [70]. Overall, these results suggest the involvement of platelets and their PAR and P2Y receptors in regulation of the angiogenic balance in tumors by selective secretion of pro- and antiangiogenic factors (Fig. 1).

Platelets are also able to influence angiogenesis by shedding of microparticles. Microparticles are small vesicles originating from plasma membranes and can be produced by various cells, e.g. endothelial cells, leukocytes, erythrocytes, megakaryocytes and platelets. Microparticles are different from exosomes, which are released from endocytic multivesicular bodies [71]. The cellular origin of microparticles can be established on the basis of their surface antigens; platelet microparticles ($\sim 0.5 \mu\text{m}$) express CD41 and/or CD42b antigens [72,73]. There is increasing evidence showing the potential of these submicron fragments of platelets and their role in normal physiology

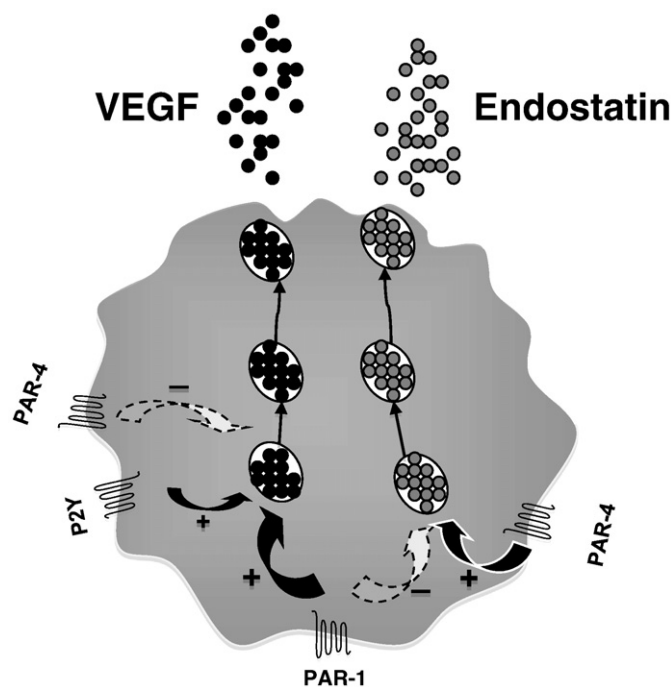


Fig. 1. Activation of specific platelet receptors stimulates the selective release of either VEGF or endostatin from specific α -granules. PAR-1 stimulation results in the release of VEGF while inhibiting endostatin secretion, whereas PAR-4 stimulation induces endostatin release and inhibits secretion of VEGF. VEGF release in response to P2Y stimulation is significantly less when compared to PAR-1 stimulation.

and pathological conditions [74]. The role of platelet microparticles (PMPs) in normal thrombosis and homeostasis is well defined, as platelets shed their particles upon activation at the site of injury. However, their importance in most other diseases is unknown and mostly based on *in vitro* studies. There are indications suggesting that after attachment or fusion with target cells, PMPs deliver cytoplasmic proteins and genetic material (RNA) to recipient cells, e.g. tumor cells. This way they function as a transport delivery system of bioactive molecules involved in homeostasis and thrombosis, immunity, inflammation and tumor growth and angiogenesis [74,75]. This transfer of bioactive material can affect the recipient cell function. Hematopoietic stem-progenitor cells that are covered with PMPs are able to express numerous platelet membrane receptors such as CD41, CD62, CXCR4 and PAR-1 [76].

Knowledge about the role of PMPs in malignant diseases is only beginning to develop [74]. It is known that some cancer cell types are able to trigger platelets at tumor sites, inducing activation and aggregation, which results in increased local and systemic concentrations of PMPs. The circulating PMP concentration has been shown to be a prognostic marker for gastric cancer and metastatic disease, even more than plasma levels of interleukin-6 and VEGF [77]. In addition, high PMP concentrations are strongly correlated with aggressive tumors and appear to be linked with reduced overall survival [78]. PMPs are also able to stimulate the secretion of matrix metalloproteinase-2 (MMP-2) from prostate cancer cells *in vitro*, a secretion which was independent of other platelet-derived angiogenic factors, like VEGF, PF-4 and bFGF [79]. The role of PMPs in angiogenesis is becoming more evident as several *in vitro* studies have shown that PMPs are able to induce angiogenesis, by stimulating the proliferation, migration, survival and tube formation of human umbilical vein endothelial cells (HUVECs) [80]. Additionally, PMPs also promote sprouting of blood vessels *in vitro* and *in vivo*, an effect mediated by intra-particle FGF and VEGF [81]. Altogether, these data suggest a role of platelet-derived microparticles in the involvement of platelets in tumor angiogenesis.

4. Enhanced platelet adhesion in tumor blood vessels affects tumor angiogenesis

A prerequisite for local platelet activation and secretion of angiogenesis regulatory proteins is that they have to adhere to endothelial cells or exposed ECM components inside the tumor. Several studies have provided data suggesting an increase in platelet tethering and adhesion in tumor vasculature or in a tumor-like environment [18,82–88]. For instance, platelet adhesion to HUVECs increased 2.5-fold after stimulation with VEGF [18], while a >3-fold increase was observed in angiogenic vessels in a mouse skin chamber with implanted matrigel, when compared with mature skin vessels [22]. These data suggest that a pro-angiogenic environment changes the antithrombotic surface of endothelial cells into a prothrombotic state, which may lead to platelet adhesion and activation in tumor vasculature. Beside adhering to endothelial cells, platelets can also interact with tumor cells, which initiates the process of tumor cell-induced platelet aggregation (TCIPA); this process is implicated in hematogenous tumor metastasis and growth [89,90]. The process of TCIPA appears to involve a number of platelet adhesion molecules, and results in platelet activation and aggregation around the tumor cells, with release of their pro- and antiangiogenic content [91]. Taken together, platelet receptors may provide attractive targets to reduce tumor angiogenesis, growth and metastasis, for example by selective blockade of such adhesion molecules on platelets or their endothelial and/or subendothelial ligands (Table 2) [18].

One family of adhesion molecules involved in platelet–endothelial interactions are the integrins. Integrins are heterodimeric transmembrane receptors and consist of non-covalently associated α and β subunits. Endothelial cells express several integrins that bind to components of the subendothelial ECM; several of these integrins are

involved in the regulation of endothelial cell growth and migration during angiogenesis [88]. Inhibition of these integrins by antibodies or low molecular weight antagonists leads to prevention or inhibition of vessel formation in different angiogenesis models [88,92–100]. Platelets express 5 integrins on their cell surface (Table 2) [101]. Both β_3 -integrins and $\alpha_5\beta_1$ engage ECM ligands that contain the canonical Arg–Gly–Asp (RGD) motif. Binding of integrins to their ligands requires a conformational change from a low-affinity to a high-affinity state, which occurs during platelet activation. The coincidence of platelet adhesion and activation in tumor vessels may lead to local secretion of angiogenesis regulatory proteins and, hence, regulation of tumor angiogenesis. In various studies, the antiangiogenic potential of several inhibitors of integrins is tested [93,99,102–104]. One should realize, however, that such treatment may not only affect interactions between platelets and vessel wall components, but also the binding of endothelial cells to ECM proteins. Therefore, one should be aware that the role of platelets and their integrins in tumor angiogenesis cannot be simply deduced from data obtained in such studies.

vWF is one of the endothelial adhesion molecules potentially involved in platelet–endothelial interactions; it can bind to platelet integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, and to GPIb-IX-V. vWF is upregulated on activated endothelial cells [87]. Interaction between vWF and platelet GPIb-IX-V results in platelet rolling over the endothelium and may lead to firm adhesion and activation of the platelets [32]. However, Kisucka and colleagues did not find any angiogenesis defect in mice deficient in vWF using the cornea angiogenesis assay [22]. This finding is in line with previous reports of normal angiogenesis in vWF^{-/-} mice [105]. Platelet GPIb α , however, may have a critical role, because replacement of its extracellular domain did result in abnormal angiogenesis in the mouse cornea model [22]. Beside vWF, this platelet receptor also binds to endothelial P-selectin [106].

P-selectin, one of the three members of the selectin family of adhesion molecules is of potential interest in platelet-dependent angiogenesis. P-selectin is synthesized and stored in Weibel–Palade bodies of endothelial cells and in α -granules of platelets [107]. Upon stimulation, P-selectin is exocytosed to the outer surface of activated endothelial cells and platelets, where it interacts with its ligands [107]. Like vWF, endothelial P-selectin can support rolling of platelets through interactions with either GPIb α or PSGL-1 on platelets [106]. In a study focusing on ischemia, P-selectin mediated platelet adhesion to the vessel wall appeared to be a key event to enhance local angiogenesis [108]. P-selectin seems to be involved in cancer as well, as P-selectin knockout mice showed a significant reduction in tumor growth and metastasis, while at the same time platelets failed to adhere to tumor cell surface [109]. Furthermore, inhibition of P-selectin by heparin treatment reducing platelet interactions with tumor cells and possibly endothelial cells as well appeared to attenuate metastasis in tumor bearing mice [110].

Fibrin(ogen) may also play an important role in platelet adhesion during tumor growth and angiogenesis as tumors have leaky vessels

Table 2

Selected platelet adhesion molecules and their (sub)endothelial ligands, possibly involved in tumor angiogenesis.

| Platelet adhesion molecules | Major (sub)endothelial ligands | |
|----------------------------------|---|------------------|
| Integrin $\alpha_2\beta_1$ | Collagen, laminin | [88,101] |
| Integrin $\alpha_5\beta_1$ | Fibronectin | [88,101] |
| Integrin $\alpha_6\beta_1$ | Laminin | [88,101] |
| Integrin $\alpha_v\beta_3$ | Fibronectin, vitronectin, vWF, osteopontin, thrombospondin | [88,101,115,148] |
| Integrin $\alpha_{IIb}\beta_3$ | Fibrin(ogen), fibronectin, vWF, vitronectin, thrombospondin | [101,115,148] |
| GPIb-IX-V (incl. GPIb α) | vWF, P-selectin | [101,106,148] |
| GPVI | Collagen, laminin | [36,101,149] |
| PSGL-1 | P-selectin | [106] |

that expose subendothelial fibrin(ogen), while fibrin(ogen) can also be deposited on the luminal endothelial cell surface. Furthermore, tumor cells are able to express different factors on their surface that are involved in the regulation of fibrin(ogen) [83–86,111]. This provides platelets with an ideal substrate to which they can adhere. Integrin $\alpha_{IIb}\beta_3$ is the main platelet receptor for fibrinogen and has an essential role in platelet aggregation and activation in malignancies [112–114]. Inhibition of $\alpha_{IIb}\beta_3$ by a humanized monoclonal antibody c7E3 Fab (abciximab; ReoPro) has been shown to reduce platelet stimulated capillary formation in different angiogenesis assays [115]. In a model of hypoxia-induced retinal angiogenesis inhibition of $\alpha_{IIb}\beta_3$ by another specific antagonist reduced retinal neovascularization by 35% [105].

In case of endothelial denudation, components of the ECM may play a role in platelet–vessel wall interactions. The most important thrombogenic subendothelial ECM component is collagen, as it not only supports platelet adhesion but also acts as an important platelet activator [116–118]. A large number of collagen receptors have been identified on platelets [118–120], but GPVI appears to be the most potent and important one [36,121]. In tumors, collagen exposure to blood may be the result of ECM and endothelial cell lining degradation by matrix metalloproteinases during the angiogenic process [122]. Moreover, procollagen molecules collagen 1 α 1 and collagen IV- α are overexpressed on activated endothelial cells in different tumor types [123]. To our knowledge, it is not yet known whether GPVI, collagen and/or the procollagen molecules are involved in tumor angiogenesis.

The above data suggest an upregulation of endothelial (adhesion) molecules such as TF, vWF and maybe also P-selectin in a proangiogenic or malignant environment, which may contribute to local platelet adhesion. At the same time, it is already known that tumor-derived

angiogenic growth factors downregulate other endothelial adhesion molecules that are involved in interactions between leukocytes and endothelial cells [124,125]. Downregulation of adhesion molecules like VCAM-1, ICAM-1 and E-selectin enables tumors to escape from immune surveillance [95,124]. This endothelial cell energy can be reversed by administration of angiostatic factors such as anginex or endostatin, resulting in normalization of leukocyte adhesion and infiltration in tumors [8,126].

Clearly, tumor-derived endothelial cells are phenotypically and functionally different from endothelial cells in healthy tissues [127]. These differences may provide attractive targets for specific cancer therapy, but also offer possibilities for platelets to adhere to the vessel wall with subsequent activation and secretion of growth factors. Although platelets contain both pro- and antiangiogenic factors their overall effect appears to be proangiogenic [22,41,69,128]. Therefore, one could hypothesize that selective blockade of platelet–endothelium interactions in tumor vessels inhibits platelet activation and secretion and, hence, platelet-dependent angiogenesis, which may lead to reduced tumor growth. The fact that platelets have been shown to be able to selectively release pro- and antiangiogenic proteins suggests that they can contribute to fine-tuning of the angiogenesis process. Future studies have to elucidate the signals that control the balance and timing of the release of pro- and antiangiogenic factors within tumors.

5. Concluding remarks

Our current understanding regarding the role of platelets in tumor angiogenesis is still modest, but a number of important facts illustrate the potential significance of platelets in malignancies (Fig. 2).

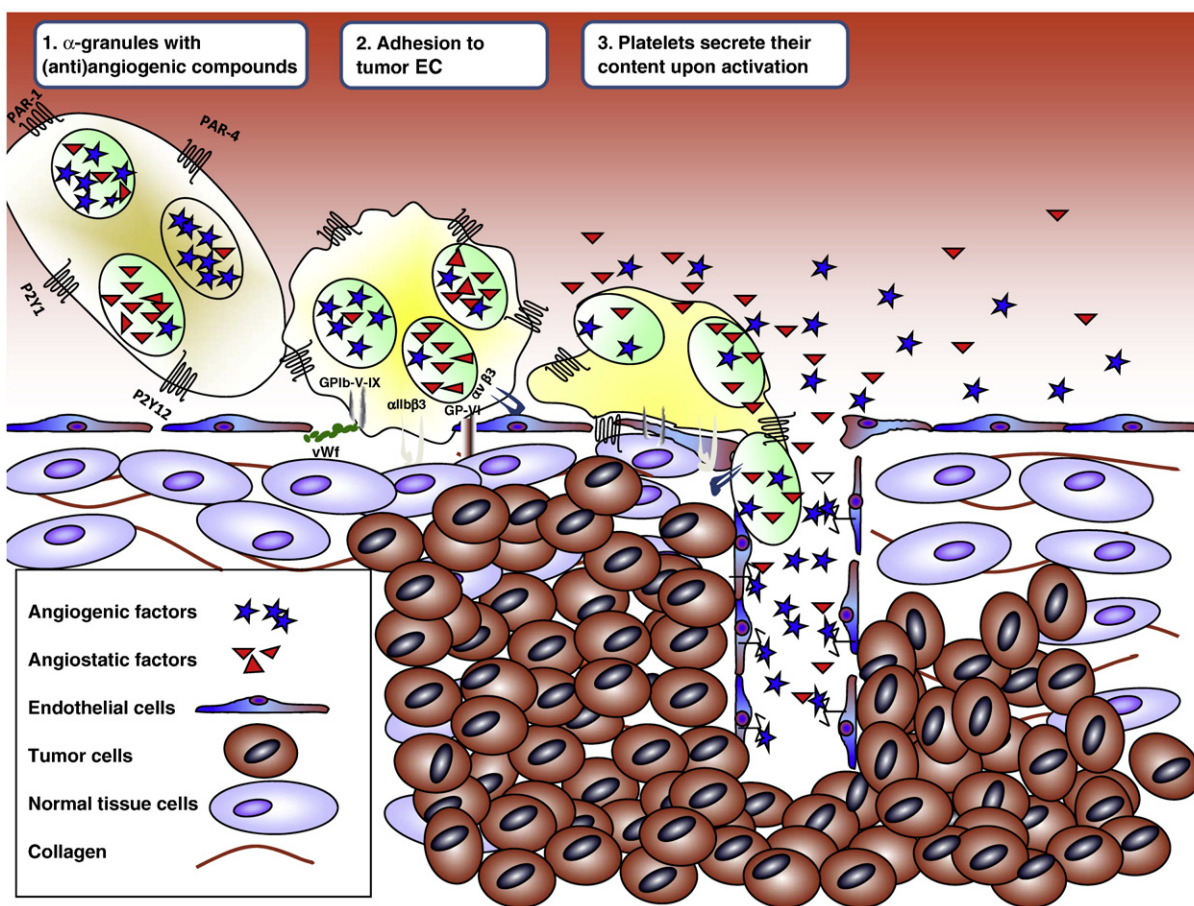


Fig. 2. 1) Platelets contain a whole range of pro- and antiangiogenic compounds which are endocytosed and sequestered in different α -granules. 2) Platelets adhere to and are subsequently activated in tumor vasculature. 3) Platelets secrete their pro- and antiangiogenic content in tumor vasculature. (For reasons of clarity the scale of cells and granules is not correct).

(i) Platelets contain a whole range of pro- and antiangiogenic compounds, which are endocytosed and sequestered in different populations of α -granules. (ii) Tumor vasculature and endothelial cells are phenotypically and functionally different from endothelial cells in healthy tissue, causing adhesion and subsequent activation of platelets. (iii) Platelets secrete their angiogenic and angiostatic content upon activation, possibly in a regulated way. The overall effect of platelet activation on endothelial cells appears to be proangiogenic, stimulating tumor angiogenesis.

Selective inhibition of platelet–endothelium interactions, using specific phenotypic characteristics of tumor endothelial cells as target, may therefore be a potentially interesting therapeutic tool to reduce tumor angiogenesis and, hence, tumor growth. One could also use the proposed link between the platelet PAR receptors and their secretion of pro- or antiangiogenic agents to modulate tumor angiogenesis. Selective stimulation of the platelet PAR-4 receptor may for example inhibit tumor angiogenesis by suppression of VEGF secretion and simultaneous enhancement of the release of endostatin; in this way, the body's own angiostatic ability is utilized to inhibit tumor vascularisation and growth.

Reduction of drug-induced resistance has been suggested to be one of the advantages of angiogenesis inhibitors. However, effects of current angiogenesis inhibitors like bevacizumab (Avastin) and sunitinib (Sutent) do not support this; they increase life expectancy by only a few months as tumors do become resistant to these drugs and produce other growth factors as a form of compensation [129]. Specific blockade of platelet–endothelial interactions in cancer therapy may decrease drug-induced resistance as it does not counteract one growth factor like VEGF as Avastin does. Moreover, inhibition of platelet–endothelial interactions at the level of the endothelial cells may offer additional advantages, as endothelial cells are genetically more stable than tumor cells.

Combination of our knowledge about the role of platelets in hemostasis with that of platelet behavior in tumor angiogenesis may lead to useful hypotheses about new forms of cancer therapy. By unravelling the underlying mechanisms of platelet–endothelial cell interactions in tumors we will not only be able to reduce the coagulation abnormalities in cancer patients, but we may also be able to specifically target these interactions in the treatment of angiogenesis dependent diseases such as cancer.

References

- [1] R.K.J. Folkman, Cancer without disease, *Nature* 427 (2004) 787.
- [2] R. Kerbel, J. Folkman, Clinical translation of angiogenesis inhibitors, *Nat. Rev. Cancer* 2 (2002) 727–739.
- [3] J. Folkman, Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug Discov.* 6 (2007) 273–286.
- [4] H. Hurwitz, L. Fehrenbacher, W. Novotny, T. Cartwright, J. Hainsworth, W. Heim, J. Berlin, A. Baron, S. Griffing, E. Holmgren, N. Ferrara, G. Fyfe, B. Rogers, R. Ross, F. Kabbinavar, Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer, *N. Engl. J. Med.* 350 (2004) 2335–2342.
- [5] A. Sandler, R. Gray, M.C. Perry, J. Brahmer, J.H. Schiller, A. Dowlati, R. Lilienbaum, D.H. Johnson, Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer, *N. Engl. J. Med.* 355 (2006) 2542–2550.
- [6] K. Miller, M. Wang, J. Gralow, M. Dickler, M. Cobleigh, E.A. Perez, T. Shenker, D. Cella, N.E. Davidson, Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer, *N. Engl. J. Med.* 357 (2007) 2666–2676.
- [7] R.J. Motzer, T.E. Hutson, P. Tomczak, M.D. Michaelson, R.M. Bukowski, O. Rixe, S. Oudard, S. Negrier, C. Szczylik, S.T. Kim, I. Chen, P.W. Bycott, C.M. Baum, R.A. Figlin, Sunitinib versus interferon alfa in metastatic renal-cell carcinoma, *N. Engl. J. Med.* 356 (2007) 115–124.
- [8] A.W. Griffioen, G. Molema, Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation, *Pharmacol. Rev.* 52 (2000) 237–268.
- [9] A. Trouseau, Phlegmasia alba dolens, *Clinique Medicale de L'Hotel-Dieu Paris, New Sydenham Society*, 1865, pp. 94–96.
- [10] M.E. Tesselaa, F.P. Romijn, I.K. Van Der Linden, F.A. Prins, R.M. Bertina, S. Osanto, Microparticle-associated tissue factor activity: a link between cancer and thrombosis? *J. Thromb. Haemost.* 5 (2007) 520–527.
- [11] N.C. Sun, W.M. McAfee, G.J. Hum, J.M. Weiner, Hemostatic abnormalities in malignancy, a prospective study of one hundred eight patients. Part I. Coagulation studies, *Am. J. Clin. Pathol.* 71 (1979) 10–16.
- [12] R.E. Tilley, T. Holscher, R. Belani, J. Nieva, N. Mackman, Tissue factor activity is increased in a combined platelet and microparticle sample from cancer patients, *Thromb. Res.* 112 (2008) 604–609.
- [13] P.D. Stein, A. Beemath, F.A. Meyers, E. Skaf, J. Sanchez, R.E. Olson, Incidence of venous thromboembolism in patients hospitalized with cancer, *Am. J. Med.* 119 (2006) 60–68.
- [14] J.W. Blom, J.P. Vanderschoot, M.J. Oostindier, S. Osanto, F.J. van der Meer, F.R. Rosendaal, Incidence of venous thrombosis in a large cohort of 66, 329 cancer patients: results of a record linkage study, *J. Thromb. Haemost.* 4 (2006) 529–535.
- [15] H.M. Verheul, K. Hoekman, S. Luyckx-de Bakker, C.A. Eekman, C.C. Folman, H.J. Broxterman, H.M. Pinedo, Platelet: transporter of vascular endothelial growth factor, *Clin. Cancer Res.* 3 (1997) 2187–2190.
- [16] R. Mohle, D. Green, M.A. Moore, R.L. Nachman, S. Rafii, Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets, *Proc. Natl Acad. Sci. USA* 94 (1997) 663–668.
- [17] U. Wartiovaara, P. Salven, H. Mikkola, R. Lassila, J. Kaukonen, V. Joukov, A. Orpana, A. Ristimaki, M. Heikinheimo, H. Joensuu, K. Alitalo, A. Palotie, Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation, *Thromb. Haemost.* 80 (1998) 171–175.
- [18] H.M. Verheul, K. Hoekman, F. Lupu, H.J. Broxterman, P. van der Valk, A.K. Kakkar, H.M. Pinedo, Platelet and coagulation activation with vascular endothelial growth factor generation in soft tissue sarcomas, *Clin. Cancer Res.* 6 (2000) 166–171.
- [19] L. Ma, S.N. Elliott, G. Cirino, A. Buret, L.J. Ignarro, J.L. Wallace, Platelets modulate gastric ulcer healing: role of endostatin and vascular endothelial growth factor release, *Proc. Natl Acad. Sci. USA* 98 (2001) 6470–6475.
- [20] L. Ma, R. Perini, W. McKnight, M. Dickey, A. Klein, M.D. Hollenberg, J.L. Wallace, Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets, *Proc. Natl Acad. Sci. USA* 102 (2005) 216–220.
- [21] E. Pipili-Synetos, E. Papadimitriou, M.E. Maragoudakis, Evidence that platelets promote tube formation by endothelial cells on matrigel, *Br. J. Pharmacol.* 125 (1998) 1252–1257.
- [22] J. Kisucka, C.E. Butterfield, D.G. Duda, S.C. Eichenberger, S. Saffaripour, J. Ware, Z.M. Ruggeri, R.K. Jain, J. Folkman, D.D. Wagner, Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage, *Proc. Natl Acad. Sci. USA* 103 (2006) 855–860.
- [23] B. Ho-Tin-Noe, T. Goerge, S.M. Cifuni, D. Duerschmied, D.D. Wagner, Platelet granule secretion continuously prevents intratumor hemorrhage, *Cancer Res.* 68 (2008) 6851–6858.
- [24] R.J. Hettiarachchi, S.M. Smorenburg, J. Ginsberg, M. Levine, M.H. Prins, H.R. Buller, Do heparins do more than just treat thrombosis? The influence of heparins on cancer spread, *Thromb. Haemost.* 82 (1999) 947–952.
- [25] M.H. Einstein, E.A. Pritts, E.M. Hartenbach, Venous thromboembolism prevention in gynecologic cancer surgery: a systematic review, *Gynecol. Oncol.* 105 (2007) 813–819.
- [26] L. Borly, P. Wille-Jørgensen, M.S. Rasmussen, Systematic review of thromboprophylaxis in colorectal surgery—an update, *Colorectal Dis.* 7 (2005) 122–127.
- [27] S.M. Smorenburg, R.J. Hettiarachchi, R. Vink, H.R. Buller, The effects of unfractionated heparin on survival in patients with malignancy—a systematic review, *Thromb. Haemost.* 82 (1999) 1600–1604.
- [28] E.A. Akl, F.F. van Doormaal, M. Barba, G. Kamath, S.Y. Kim, S. Kuipers, S. Middeldorp, V. Yosucio, H.O. Dickinson, H.J. Schunemann, Parenteral anticoagulation may prolong the survival of patients with limited small cell lung cancer: a Cochrane systematic review, *J. Exp. Clin. Cancer Res.* 27 (2008) 4.
- [29] E.A. Akl, G. Kamath, S.Y. Kim, V. Yosucio, M. Barba, I. Terrenato, F. Sperati, H.J. Schunemann, Oral anticoagulation may prolong survival of a subgroup of patients with cancer: a cochrane systematic review, *J. Exp. Clin. Cancer Res.* 26 (2007) 175–184.
- [30] G.J. Tangelder, H.C. Teirlinck, D.W. Slaaf, R.S. Reneman, Distribution of blood platelets flowing in arterioles, *Am. J. Physiol.* 248 (1985) H318–H323.
- [31] M.G. Egbrink, M.A. Van Gestel, M.A. Broeders, G.J. Tangelder, J.M. Heemskerk, R.S. Reneman, D.W. Slaaf, Regulation of microvascular thromboembolism in vivo, *Microcirculation* 12 (2005) 287–300.
- [32] Z.M. Ruggeri, G.L. Mendolicchio, Adhesion mechanisms in platelet function, *Circ. Res.* 100 (2007) 1673–1685.
- [33] R.W. Farndale, Collagen-induced platelet activation, *Blood Cells Mol. Dis.* 36 (2006) 162–165.
- [34] Z.M. Ruggeri, Structure and function of von Willebrand factor, *Thromb. Haemost.* 82 (1999) 576–584.
- [35] B.J. Fredrickson, J.F. Dong, L.V. McIntire, J.A. Lopez, Shear-dependent rolling on von Willebrand factor of mammalian cells expressing the platelet glycoprotein Ib-IX-V complex, *Blood* 92 (1998) 3684–3693.
- [36] B. Nieswandt, S.P. Watson, Platelet-collagen interaction: is GPVI the central receptor? *Blood* 102 (2003) 449–461.
- [37] M.A. van Gestel, J.W. Heemskerk, D.W. Slaaf, V.V. Heijnen, S.O. Sage, R.S. Reneman, M.G. oude Egbrink, Real-time detection of activation patterns in individual platelets during thromboembolism in vivo: differences between thrombus growth and embolus formation, *J. Vasc. Res.* 39 (2002) 534–543.
- [38] B.R. Lentz, Exposure of platelet membrane phosphatidylserine regulates blood coagulation, *Prog. Lipid Res.* 42 (2003) 423–438.
- [39] S.P. Jackson, The growing complexity of platelet aggregation, *Blood* 109 (2007) 5087–5095.
- [40] J.E. Italiano Jr., J.L. Richardson, S. Patel-Hett, E. Battinelli, A. Zaslavsky, S. Short, S. Ryeom, J. Folkman, G.L. Klement, Angiogenesis is regulated by a novel

- mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released, *Blood* 111 (2008) 1227–1233.
- [41] A. Brill, H. Elinav, D. Varon, Differential role of platelet granular mediators in angiogenesis, *Cardiovasc. Res.* 63 (2004) 226–235.
- [42] T. Browder, J. Folkman, S. Pirie-Shepherd, The hemostatic system as a regulator of angiogenesis, *J. Biol. Chem.* 275 (2000) 1521–1524.
- [43] Z. Li, G. Zhang, G.C. Le Breton, X. Gao, A.B. Malik, X. Du, Two waves of platelet secretion induced by thromboxane A2 receptor and a critical role for phosphoinositide 3-kinases, *J. Biol. Chem.* 278 (2003) 30725–30731.
- [44] C. Gachet, ADP receptors of platelets and their inhibition, *Thromb. Haemost.* 86 (2001) 222–232.
- [45] M.A. van Gestel, J.W. Heemskerck, D.W. Slaaf, V.V. Heijnen, R.S. Reneman, M.G. oude Egbrink, In vivo blockade of platelet ADP receptor P2Y12 reduces embolus and thrombus formation but not thrombus stability, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 518–523.
- [46] M.A. van Gestel, S. Reitsma, D.W. Slaaf, V.V. Heijnen, M.A. Feijge, T. Lindhout, M.A. van Zandvoort, M. Elg, R.S. Reneman, J.W. Heemskerck, M.G. Egbrink, Both ADP and thrombin regulate arteriolar thrombus stabilization and embolization, but are not involved in initial hemostasis as induced by micropuncture, *Microcirculation* 14 (2007) 193–205.
- [47] L.F. Brass, Thrombin and platelet activation, *Chest* 124 (2003) 185–255.
- [48] S. Kulkarni, S.M. Dopheide, C.L. Yap, C. Ravanat, M. Freund, P. Mangin, K.A. Heel, A. Street, I.S. Harper, F. Lanza, S.P. Jackson, A revised model of platelet aggregation, *J. Clin. Invest.* 105 (2000) 783–791.
- [49] G.L. Klement, T.T. Yip, F. Cassiola, L. Kikuchi, D. Cervi, V. Podust, J.E. Italiano, E. Wheatley, A. Abou-Slaybi, E. Bender, N. Almog, M.W. Kieran, J. Folkman, Platelets actively sequester angiogenesis regulators, *Blood* 113 (2009) 2835–2842.
- [50] H.M. Verheul, M.P. Lolkema, D.Z. Qian, Y.H. Hilkes, E. Liapi, J.W. Akkerman, R. Pili, E.E. Voest, Platelets take up the monoclonal antibody bevacizumab, *Clin. Cancer Res.* 13 (2007) 5341–5347.
- [51] G.J. Caine, G.Y. Lip, P.S. Stonelake, P. Ryan, A.D. Blann, Platelet activation, coagulation and angiogenesis in breast and prostate carcinoma, *Thromb. Haemost.* 92 (2004) 185–190.
- [52] G.J. Caine, A.L. Harris, K. Christodoulos, G.Y. Lip, A.D. Blann, Analysis of combination anti-angiogenesis therapy on markers of coagulation, platelet activation and angiogenesis in patients with advanced cancer, *Cancer Lett.* 219 (2005) 163–167.
- [53] D.M. Martin, C.W. Boys, W. Ruf, Tissue factor: molecular recognition and cofactor function, *FASEB J.* 9 (1995) 852–859.
- [54] T. Kaneko, S. Fujii, A. Matsumoto, D. Goto, N. Makita, J. Hamada, T. Moriuchi, A. Kitabatake, Induction of tissue factor expression in endothelial cells by basic fibroblast growth factor and its modulation by fenofibric acid, *Thromb. J.* 1 (2003) 6.
- [55] J.E. Bluff, S.R. Menakuru, S.S. Cross, S.E. Higham, S.P. Balasubramanian, N.J. Brown, M.W. Reed, C.A. Staton, Angiogenesis is associated with the onset of hyperplasia in human ductal breast disease, *Br. J. Cancer* 101 (2009) 666–672.
- [56] M.Z. Wojtukiewicz, E. Sierko, P. Klement, J. Rak, The hemostatic system and angiogenesis in malignancy, *Neoplasia* 3 (2001) 371–384.
- [57] J.E. Bluff, N.J. Brown, M.W. Reed, C.A. Staton, Tissue factor, angiogenesis and tumour progression, *Breast Cancer Res.* 10 (2008) 10.
- [58] N.S. Callander, N. Varki, L.V. Rao, Immunohistochemical identification of tissue factor in solid tumors, *Cancer* 70 (1992) 1194–1201.
- [59] J. Contrino, G. Hair, D.L. Kreutzer, F.R. Rickles, In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease, *Nat. Med.* 2 (1996) 209–215.
- [60] M. Shoji, W.W. Hancock, K. Abe, C. Micko, K.A. Casper, R.M. Baine, J.N. Wilcox, I. Danave, D.L. Dillehay, E. Matthews, J. Contrino, J.H. Morrissey, S. Gordon, T.S. Edgington, B. Kudryk, D.L. Kreutzer, F.R. Rickles, Activation of coagulation and angiogenesis in cancer: immunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer, *Am. J. Pathol.* 152 (1998) 399–411.
- [61] T.E. Lans, R. Van Harsen, A.M. Eggermont, T.L. Ten Hagen, Involvement of endothelial monocyte activating polypeptide II in tumor necrosis factor- α -based anti-cancer therapy, *Anticancer Res.* 24 (2004) 2243–2248.
- [62] S. Zucker, H. Mirza, C.E. Conner, A.F. Lorenz, M.H. Drews, W.F. Bahou, J. Jesty, Vascular endothelial growth factor induces tissue factor and matrix metalloproteinase production in endothelial cells: conversion of prothrombin to thrombin results in progelatinase A activation and cell proliferation, *Int. J. Cancer* 75 (1998) 780–786.
- [63] A.L. Armesilla, E. Lorenzo, P. Gomez del Arco, S. Martinez-Martinez, A. Alfranca, J.M. Redondo, Vascular endothelial growth factor activates nuclear factor of activated T cells in human endothelial cells: a role for tissue factor gene expression, *Mol. Cell. Biol.* 19 (1999) 2032–2043.
- [64] D. Mechtcheriakova, A. Wlachos, H. Holzmuller, B.R. Binder, E. Hofer, Vascular endothelial cell growth factor-induced tissue factor expression in endothelial cells is mediated by EGR-1, *Blood* 93 (1999) 3811–3823.
- [65] Y. Zhang, Y. Deng, T. Luther, M. Muller, R. Ziegler, R. Waldherr, D.M. Stern, P.P. Nawroth, Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice, *J. Clin. Invest.* 94 (1994) 1320–1327.
- [66] A.M. Norfleet, J.S. Bergmann, D.H. Carney, Thrombin peptide, TP508, stimulates angiogenic responses in animal models of dermal wound healing, in chick chorioallantoic membranes, and in cultured human aortic and microvascular endothelial cells, *Gen. Pharmacol.* 35 (2000) 249–254.
- [67] G. Grignani, G.A. Jamieson, Platelets in tumor metastasis: generation of adenosine diphosphate by tumor cells is specific but unrelated to metastatic potential, *Blood* 71 (1988) 844–849.
- [68] M.L. Nierodzick, S. Karpatkin, Thrombin induces tumor growth, metastasis, and angiogenesis: evidence for a thrombin-regulated dormant tumor phenotype, *Cancer Cell* 10 (2006) 355–362.
- [69] H.M. Verheul, A.S. Jorna, K. Hoekman, H.J. Broxterman, M.F. Gebbink, H.M. Pinedo, Vascular endothelial growth factor-stimulated endothelial cells promote adhesion and activation of platelets, *Blood* 96 (2000) 4216–4221.
- [70] N.M. Bambace, J.E. Levis, C.E. Holmes, The effect of P2Y-mediated platelet activation on the release of VEGF and endostatin from platelets, *Platelets* 21 (2010) 85–93.
- [71] H.F. Heijnen, A.E. Schiel, R. Fijnheer, H.J. Geuze, J.J. Sixma, Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules, *Blood* 94 (1999) 3791–3799.
- [72] L.L. Horstman, Y.S. Ahn, Platelet microparticles: a wide-angle perspective, *Crit. Rev. Oncol. Hematol.* 30 (1999) 111–142.
- [73] J.N. George, E.B. Pickett, R. Heinz, Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate, *Blood* 68 (1986) 307–309.
- [74] J.E. Italiano Jr., A.T. Mairuhu, R. Flaumenhaft, Clinical relevance of microparticles from platelets and megakaryocytes, *Curr. Opin. Hematol.* 17 (2010) 578–584.
- [75] J. Ratajczak, M. Wyszczynski, F. Hayek, A. Janowska-Wieczorek, M.Z. Ratajczak, Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication, *Leukemia* 20 (2006) 1487–1495.
- [76] A. Janowska-Wieczorek, M. Majka, J. Kijowski, M. Baj-Krzyworzeka, R. Reza, A.R. Turner, J. Ratajczak, S.G. Emerson, M.A. Kowalska, M.Z. Ratajczak, Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment, *Blood* 98 (2001) 3143–3149.
- [77] H.K. Kim, K.S. Song, Y.S. Park, Y.H. Kang, Y.J. Lee, K.R. Lee, K.W. Ryu, J.M. Bae, S. Kim, Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor, *Eur. J. Cancer* 39 (2003) 184–191.
- [78] D. Helley, E. Banu, A. Bouziane, A. Banu, F. Scotte, A.M. Fischer, S. Oudard, Platelet microparticles: a potential predictive factor of survival in hormone-refractory prostate cancer patients treated with docetaxel-based chemotherapy, *Eur. Urol.* 56 (2009) 479–484.
- [79] O. Dashevsky, D. Varon, A. Brill, Platelet-derived microparticles promote invasiveness of prostate cancer cells via upregulation of MMP-2 production, *Int. J. Cancer* 124 (2009) 1773–1777.
- [80] H.K. Kim, K.S. Song, J.H. Chung, K.R. Lee, S.N. Lee, Platelet microparticles induce angiogenesis in vitro, *Br. J. Haematol.* 124 (2004) 376–384.
- [81] A. Brill, O. Dashevsky, J. Rivo, Y. Gozal, D. Varon, Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization, *Cardiovasc. Res.* 67 (2005) 30–38.
- [82] L. Oleksowicz, Z. Mrowiec, E. Schwartz, M. Khorshidi, J.P. Dutcher, E. Puszkun, Characterization of tumor-induced platelet aggregation: the role of immunorelated GPIb and GPIIb/IIIa expression by MCF-7 breast cancer cells, *Thromb. Res.* 79 (1995) 261–274.
- [83] L. Oleksowicz, J.P. Dutcher, Adhesive receptors expressed by tumor cells and platelets: novel targets for therapeutic anti-metastatic strategies, *Med. Oncol.* 12 (1995) 95–102.
- [84] P. Castellani, G. Viale, A. Dorcaratto, G. Nicolo, J. Kaczmarek, G. Querze, L. Zardi, The fibronectin isoform containing the ED-B oncofetal domain: a marker of angiogenesis, *Int. J. Cancer* 59 (1994) 612–618.
- [85] J. Kaczmarek, P. Castellani, G. Nicolo, B. Spina, G. Allemanni, L. Zardi, Distribution of oncofetal fibronectin isoforms in normal, hyperplastic and neoplastic human breast tissues, *Int. J. Cancer* 59 (1994) 11–16.
- [86] H.C. Kwaan, H.N. Keer, Fibrinolysis and cancer, *Semin. Thromb. Hemost.* 16 (1990) 230–235.
- [87] L. Zanetta, S.G. Marcus, J. Vasile, M. Dobryansky, H. Cohen, K. Eng, P. Shamamian, P. Mignatti, Expression of Von Willebrand factor, an endothelial cell marker, is up-regulated by angiogenesis factors: a potential method for objective assessment of tumor angiogenesis, *Int. J. Cancer* 85 (2000) 281–288.
- [88] C.J. Avraamides, B. Garmy-Susini, J.A. Varner, Integrins in angiogenesis and lymphangiogenesis, *Nat. Rev. Cancer* 8 (2008) 604–617.
- [89] S. Karpatkin, C. Ambrogio, E. Pearlstein, The role of tumor-induced platelet aggregation, platelet adhesion and adhesive proteins in tumor metastasis, *Prog. Clin. Biol. Res.* 283 (1988) 585–606.
- [90] S. Karpatkin, E. Pearlstein, C. Ambrogio, B.S. Collier, Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo, *J. Clin. Invest.* 81 (1988) 1012–1019.
- [91] L. Erpenbeck, M.P. Schon, Deadly allies: the fatal interplay between platelets and metastasizing cancer cells, *Blood* 115 (2010) 3427–3436.
- [92] S. Kim, K. Bell, S.A. Mousa, J.A. Varner, Regulation of angiogenesis in vivo by ligation of integrin $\alpha 5 \beta 1$ with the central cell-binding domain of fibronectin, *Am. J. Pathol.* 156 (2000) 1345–1362.
- [93] P.C. Brooks, R.A. Clark, D.A. Cheresch, Requirement of vascular integrin $\alpha v \beta 3$ for angiogenesis, *Science* 264 (1994) 569–571.
- [94] M. Friedlander, P.C. Brooks, R.W. Shaffer, C.M. Kincaid, J.A. Varner, D.A. Cheresch, Definition of two angiogenic pathways by distinct αv integrins, *Science* 270 (1995) 1500–1502.
- [95] W.J. Mulder, K. Castermans, J.R. van Beijnum, M.G. Oude Egbrink, P.T. Chin, Z.A. Fayad, C.W. Lowik, E.L. Kaijzel, I. Que, G. Storm, G.J. Strijkers, A.W. Griffioen, K. Nicolay, Molecular imaging of tumor angiogenesis using $\alpha v \beta 3$ -integrin targeted multimodal quantum dots, *Angiogenesis* (2008).
- [96] M. Friedlander, C.L. Theesfeld, M. Sugita, M. Fruttiger, M.A. Thomas, S. Chang, D.A. Cheresch, Involvement of integrins $\alpha v \beta 3$ and $\alpha v \beta 5$ in ocular neovascular diseases, *Proc. Natl. Acad. Sci. USA* 93 (1996) 9764–9769.

- [97] E. Ruoslahti, RGD and other recognition sequences for integrins, *Annu. Rev. Cell Dev. Biol.* 12 (1996) 697–715.
- [98] J.D. Hood, D.A. Cheresh, Role of integrins in cell invasion and migration, *Nat. Rev. Cancer* 2 (2002) 91–100.
- [99] W. Cai, X. Chen, Anti-angiogenic cancer therapy based on integrin alphavbeta3 antagonism, *Anticancer Agents Med. Chem.* 6 (2006) 407–428.
- [100] P.C. Brooks, A.M. Montgomery, M. Rosenfeld, R.A. Reisfeld, T. Hu, G. Klier, D.A. Cheresh, Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels, *Cell* 79 (1994) 1157–1164.
- [101] A. Kasirer-Friede, M.L. Kahn, S.J. Shattil, Platelet integrins and immunoreceptors, *Immunol. Rev.* 218 (2007) 247–264.
- [102] G.C. Tucker, Alpha v integrin inhibitors and cancer therapy, *Curr. Opin. Investig. Drugs* 4 (2003) 722–731.
- [103] J.A. Nemeth, M.T. Nakada, M. Trikha, Z. Lang, M.S. Gordon, G.C. Jayson, R. Corringham, U. Prabhakar, H.M. Davis, R.A. Beckman, Alpha-v integrins as therapeutic targets in oncology, *Cancer Investig.* 25 (2007) 632–646.
- [104] G.C. Alghisi, C. Ruegg, Vascular integrins in tumor angiogenesis: mediators and therapeutic targets, *Endothelium* 13 (2006) 113–135.
- [105] J.S. Rhee, M. Black, U. Schubert, S. Fischer, E. Morgenstern, H.P. Hammes, K.T. Preissner, The functional role of blood platelet components in angiogenesis, *Thromb. Haemost.* 92 (2004) 394–402.
- [106] D.D. Wagner, P.S. Frenette, The vessel wall and its interactions, *Blood* 111 (2008) 5271–5281.
- [107] M. Chen, J.G. Geng, P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis, *Arch. Immunol. Ther. Exp. (Warsz)* 54 (2006) 75–84.
- [108] S. Kato, H. Amano, Y. Ito, K. Eshima, N. Aoyama, H. Tamaki, H. Sakagami, Y. Satoh, T. Izumi, M. Majima, Effect of erythropoietin on angiogenesis with the increased adhesion of platelets to the microvessels in the hind-limb ischemia model in mice, *J. Pharmacol. Sci.* 112 (2010) 167–175.
- [109] Y.J. Kim, L. Borsig, N.M. Varki, A. Varki, P-selectin deficiency attenuates tumor growth and metastasis, *Proc. Natl. Acad. Sci. USA* 95 (1998) 9325–9330.
- [110] L. Borsig, R. Wong, J. Feramisco, D.R. Nadeau, N.M. Varki, A. Varki, Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis, *Proc. Natl. Acad. Sci. USA* 98 (2001) 3352–3357.
- [111] F.R. Rickles, A. Falanga, Molecular basis for the relationship between thrombosis and cancer, *Thromb. Res.* 102 (2001) V215–V224.
- [112] S.J. Shattil, H. Kashiwagi, N. Pampori, Integrin signaling: the platelet paradigm, *Blood* 91 (1998) 2645–2657.
- [113] J.M. Grossi, J.S. Hatfield, L.A. Fitzgerald, M. Newcombe, J.D. Taylor, K.V. Honn, Role of tumor cell glycoproteins immunologically related to glycoproteins Ib and IIb/IIIa in tumor cell-platelet and tumor cell-matrix interactions, *FASEB J.* 2 (1988) 2385–2395.
- [114] M. Trikha, J. Timar, A. Zacharek, J.A. Nemeth, Y. Cai, B. Dome, B. Somlai, E. Raso, A. Ladanyi, K.V. Honn, Role for beta3 integrins in human melanoma growth and survival, *Int. J. Cancer* 101 (2002) 156–167.
- [115] M. Trikha, Z. Zhou, J. Timar, E. Raso, M. Kennel, E. Emmell, M.T. Nakada, Multiple roles for platelet GPIIb/IIIa and alphavbeta3 integrins in tumor growth, angiogenesis, and metastasis, *Cancer Res.* 62 (2002) 2824–2833.
- [116] M.J. Kuijpers, I.C. Munnix, J.M. Cosemann, B.V. Vlijmen, C.P. Reutelingsperger, M.O. Egbrink, J.W. Heemsker, Key role of platelet procoagulant activity in tissue factor- and collagen-dependent thrombus formation in arterioles and venules in vivo differential sensitivity to thrombin inhibition, *Microcirculation* 15 (2008) 269–282.
- [117] K.J. Clemetson, J.M. Clemetson, Platelet collagen receptors, *Thromb. Haemost.* 86 (2001) 189–197.
- [118] B. Savage, F. Almus-Jacobs, Z.M. Ruggeri, Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow, *Cell* 94 (1998) 657–666.
- [119] S.A. Santoro, Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen, *Cell* 46 (1986) 913–920.
- [120] S. Moog, P. Mangin, N. Lenain, C. Strassel, C. Ravanat, S. Schuhler, M. Freund, M. Santer, M. Kahn, B. Nieswandt, C. Gachet, J.P. Cazenave, F. Lanza, Platelet glycoprotein V binds to collagen and participates in platelet adhesion and aggregation, *Blood* 98 (2001) 1038–1046.
- [121] D. Varga-Szabo, I. Pleines, B. Nieswandt, Cell adhesion mechanisms in platelets, *Arterioscler. Thromb. Vasc. Biol.* 28 (2008) 403–412.
- [122] E.I. Deryugina, J.P. Quigley, Matrix metalloproteinases and tumor metastasis, *Cancer Metastasis Rev.* 25 (2006) 9–34.
- [123] J.R. van Beijnum, R.P. Dings, E. van der Linden, B.M. Zwaans, F.C. Ramaekers, K.H. Mayo, A.W. Griffioen, Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature, *Blood* 108 (2006) 2339–2348.
- [124] A.E. Dirix, M.G. Oude Egbrink, M.J. Kuijpers, S.T. van der Niet, V.V. Heijnen, J.C. Bouma-ter Steege, J. Wagstaff, A.W. Griffioen, Tumor angiogenesis modulates leukocyte-vessel wall interactions in vivo by reducing endothelial adhesion molecule expression, *Cancer Res.* 63 (2003) 2322–2329.
- [125] S.C. Tromp, M.G. oude Egbrink, R.P. Dings, S. van Velzen, D.W. Slaaf, H.F. Hillen, G.J. Tangelder, R.S. Reneman, A.W. Griffioen, Tumor angiogenesis factors reduce leukocyte adhesion in vivo, *Int. Immunol.* 12 (2000) 671–676.
- [126] A.E. Dirix, M.G. oude Egbrink, K. Castermans, D.W. van der Schaft, V.L. Thijssen, R.P. Dings, L. Kwee, K.H. Mayo, J. Wagstaff, J.C. Bouma-ter Steege, A.W. Griffioen, Anti-angiogenesis therapy can overcome endothelial cell anergy and promote leukocyte-endothelium interactions and infiltration in tumors, *FASEB J.* 20 (2006) 621–630.
- [127] L.Q. Wu, W.J. Zhang, J.X. Niu, L.Y. Ye, Z.H. Yang, G.E. Grau, J.N. Lou, Phenotypic and functional differences between human liver cancer endothelial cells and liver sinusoidal endothelial cells, *J. Vasc. Res.* 45 (2008) 78–86.
- [128] G. Pintucci, S. Froum, J. Pinnell, P. Mignatti, S. Rafii, D. Green, Trophic effects of platelets on cultured endothelial cells are mediated by platelet-associated fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF), *Thromb. Haemost.* 88 (2002) 834–842.
- [129] G. Bergers, D. Hanahan, Modes of resistance to anti-angiogenic therapy, *Nat. Rev. Cancer* 8 (2008) 592–603.
- [130] T. Nakamura, Y. Tomita, R. Hirai, K. Yamaoka, K. Kaji, A. Ichihara, Inhibitory effect of transforming growth factor-beta on DNA synthesis of adult rat hepatocytes in primary culture, *Biochem. Biophys. Res. Commun.* 133 (1985) 1042–1050.
- [131] C.H. Heldin, B. Westermark, A. Wasteson, Platelet-derived growth factor. Isolation by a large-scale procedure and analysis of subunit composition, *Biochem. J.* 193 (1981) 907–913.
- [132] J. Ben-Ezra, K. Sheibani, D.L. Hwang, A. Lev-Ran, Megakaryocyte synthesis is the source of epidermal growth factor in human platelets, *Am. J. Pathol.* 137 (1990) 755–759.
- [133] P. Jurasz, D. Alonso, S. Castro-Blanco, F. Murad, M.W. Radomski, Generation and role of angiostatin in human platelets, *Blood* 102 (2003) 3217–3223.
- [134] D.R. Kaplan, F.C. Chao, C.D. Stiles, H.N. Antoniades, C.D. Scher, Platelet alpha granules contain a growth factor for fibroblasts, *Blood* 53 (1979) 1043–1052.
- [135] T.E. Maione, G.S. Gray, J. Petro, A.J. Hunt, A.L. Donner, S.I. Bauer, H.F. Carson, R.J. Sharpe, Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides, *Science* 247 (1990) 77–79.
- [136] K.P. Karey, H. Marquardt, D.A. Sirbasku, Human platelet-derived mitogens. I. Identification of insulinlike growth factors I and II by purification and N alpha amino acid sequence analysis, *Blood* 74 (1989) 1084–1092.
- [137] S.K. Gupta, T. Hassel, J.P. Singh, A potent inhibitor of endothelial cell proliferation is generated by proteolytic cleavage of the chemokine platelet factor 4, *Proc. Natl. Acad. Sci. USA* 92 (1995) 7799–7803.
- [138] S. Struyf, M.D. Burdick, P. Proost, J. Van Damme, R.M. Strieter, Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis, *Circ. Res.* 95 (2004) 855–857.
- [139] K.M. McLaren, Immunohistochemical localisation of thrombospondin in human megakaryocytes and platelets, *J. Clin. Pathol.* 36 (1983) 197–199.
- [140] S. Liekens, A. Bronckaers, M.J. Perez-Perez, J. Balzarini, Targeting platelet-derived endothelial cell growth factor/thymidine phosphorylase for cancer therapy, *Biochem. Pharmacol.* 74 (2007) 1555–1567.
- [141] P.J. Declerck, M.C. Alessi, M. Verstreken, E.K. Kruithof, I. Juhan-Vague, D. Collen, Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay, *Blood* 71 (1988) 220–225.
- [142] J.J. Li, Y.Q. Huang, R. Basch, S. Karpatkin, Thrombin induces the release of angiopoietin-1 from platelets, *Thromb. Haemost.* 85 (2001) 204–206.
- [143] S.W. Galt, S. Lindemann, L. Allen, D.J. Medd, J.M. Falk, T.M. McIntyre, S.M. Prescott, L.W. Kraiss, G.A. Zimmerman, A.S. Weyrich, Outside-in signals delivered by matrix metalloproteinase-1 regulate platelet function, *Circ. Res.* 90 (2002) 1093–1099.
- [144] C. Fernandez-Patron, M.A. Martinez-Cuesta, E. Salas, G. Sawicki, M. Wozniak, M.W. Radomski, S.T. Davidge, Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2, *Thromb. Haemost.* 82 (1999) 1730–1735.
- [145] G. Sawicki, E. Salas, J. Murat, H. Miszta-Lane, M.W. Radomski, Release of gelatinase A during platelet activation mediates aggregation, *Nature* 386 (1997) 616–619.
- [146] D. English, Z. Welch, A.T. Kovala, K. Harvey, O.V. Volpert, D.N. Brindley, J.G. Garcia, Sphingosine 1-phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis, *FASEB J.* 14 (2000) 2255–2265.
- [147] H.G. Kopp, S. Rafii, Thrombopoietic cells and the bone marrow vascular niche, *Ann. NY Acad. Sci.* 1106 (2007) 175–179.
- [148] C.H. Yeh, W.C. Wang, T.T. Hsieh, T.F. Huang, Agkistina, a snake venom-derived glycoprotein Ib antagonist, disrupts von Willebrand factor-endothelial cell interaction and inhibits angiogenesis, *J. Biol. Chem.* 275 (2000) 18615–18618.
- [149] Y. Ozaki, K. Suzuki-Inoue, O. Inoue, Novel interactions in platelet biology: CLEC-2/podoplanin and laminin/GPVI, *J. Thromb. Haemost.* 7 (Suppl 1) (2009) 191–194.