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Platelet Regulation of Angiogenesis, Tumor Growth and Metastasis

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1. Introduction

Angiogenesis - formation of new capillary blood vessels - is essential during development and physiological conditions, such as wound healing and the reproductive cycle. Prolonged and excessive angiogenesis has been implicated in a number of pathological processes, for instance rheumatoid arthritis, retinopathy and tumor growth. The normal vasculature is tightly regulated by a balance between pro- and anti-angiogenic factors. The most well studied pro-angiogenic factor - vascular endothelial growth factor-A(VEGF-A) – is required for development of a vascular system during embryogenesis and is also a central regulator of adult neovascularization (Olsson et al., 2006).

Angiogenesis is a multistep process involving oxygen sensing, growth factor signaling, matrix degradation, endothelial cell proliferation, migration and differentiation into a functional blood vessel. This process - formation of new blood vessels from pre-existing ones - must take place without compromising blood flow.

Platelets are central players in maintaining hemostasis of the blood. At sites of blood vessel injury, platelets are activated to induce blood coagulation and form aggregates at the site of the damaged endothelium to prevent hemorrhage and thereby protects us from fatal bleedings. Besides their role in hemostasis, platelets have been shown to contribute to non-hemostatic processes such as wound healing, immunity, angiogenesis, cardiovascular disease and tumor metastasis (Felding-Habermann et al., 1996; Jurk and Kehrel, 2005).

A connection between platelets and malignant disease has been recognized since the end of the 19th century, when Armand Trousseau observed increased thrombotic events in patients that were later diagnosed with cancer (Trousseau, 1865). This enhanced tendency to form blood clots, or hypercoagulability, is named Trousseau's syndrome (Varki, 2007) and is especially pronounced in certain forms of cancer such as pancreatic and lung cancer.

Growth of solid tumors, like all expanding tissues, is dependent on angiogenesis for oxygen and nutrient supply, as well as for removal of waste products. The hypotheses that platelets contribute to tumor-induced angiogenesis was put forward by Pinedo and collegues in 1998 (Pinedo et al., 1998). During the last decade, this hypotheses has been experimentally supported by several independent research groups, demonstrating that platelets can regulate endothelial cell behavior and angiogenesis. Platelets are now recognized as the major source of VEGF-A in the body (Holmes et al., 2008; Peterson et al., 2010; Verheul et al., 1997). In addition to stimulation of tumor growth and angiogenesis, platelets have also been found to regulate metastasis. Possible explanations involve protection of the tumor cells

from immune recognition and shear stress in the circulation. Platelets may also enable easier tumor cell adherence and subsequent extravasation through the vessel wall.
The current chapter will review the role of platelets in regulation of angiogenesis, tumor growth and metastasis. We do not claim a full coverage of the existing literature, but wish to highlight certain aspects of these processes.

2. Platelet regulation of hemostasis and thrombosis

Platelets are anuclear cellular fragments, derived from megakaryocytes in the bone marrow (Lecine et al., 1998). These cell fragments play a crucial role in regulating blood hemostasis and thrombosis. At sites of blood vessel injury, platelets are activated and aggregate at the site of the damaged endothelium to prevent hemorrhage. Although platelets lack nuclei, they are highly organized and contain different organelles such as granula, mitochondria and the cytoskeletal components microtubules and actin filaments. Platelets contain three different types of granules; dense granules, lysosomes, α-granules (Rendu and Brohard-Bohn, 2001). Dense granules are involved in recruitment of other platelets by release of small, non-protein molecules, lysosomes play a role in eliminating circulating platelet aggregates by secretion of hydrolases and α-granules contain proteins involved in the healing reaction (Rendu and Brohard-Bohn, 2001).
Upon blood vessel injury, exposure of sub-endothelial molecules such as von Willebrand factor (vWF) and collagen trigger adhesion, activation, aggregation and degranulation of platelets (Jackson, 2007; Varga-Szabo et al., 2008). Released adenosine diphosphate (ADP) reinforce the activation by binding to P2Y receptors on the platelet, which in turn induce activation of the fibrinogen receptor GPIIb/IIIa. Fibrinogen can bridge between platelets, forming a temporary plug. This is converted to a more stable fibrin clot by cleavage of fibrinogen by the serine protease thrombin, which is activated in the coagulation cascade (Jackson, 2007; Varga-Szabo et al., 2008). The fibrin clot will be enzymatically degraded by plasminogen when the damaged vessel is repaired (Cesarman-Maus and Hajjar, 2005).
In addition to prevent bleeding, platelets can also promote wound healing. Gastric ulcer healing, a process known to be dependent on VEGF-A and angiogenesis, can be regulated by platelets (Ma et al., 2001; Ma et al., 2005). Similarly, healing of diabetic wounds can be enhanced by platelet rich plasma (Pietramaggiori et al., 2008).

3. The role of platelets in tumor angiogenesis and growth

It is well recognized that cancer patients commonly suffer from problems with thrombotic occlusion of vessels as well as other abnormalities of their coagulation system such as high platelet turnover, elevated platelet counts and bleeding disorders (Dvorak, 1994; Sun et al., 1979). The prothrombotic environment of tumors can induce platelets to form microthrombi and to release endothelial stimulating factors from their granules, such as VEGF-A (Mohle et al., 1997; Pinedo et al., 1998). As will be described below, several lines of evidence suggest that platelets promote tumor growth by stimulating its vasculature by different mechanisms.
As mentioned in section 2. above, platelets play a role during wound healing. Indeed, tumors have been described as “wounds that do not heal”, due to the chronic inflammation of the cancer tissue (Dvorak, 1986). In a normal wound, platelets provide important growth factors for recruitment of myofibroblasts and stimulation of tissue regeneration. In this

physiological situation platelet activation is terminated when the wound is healed. In cancer however, platelets are continuously activated via for instance tumor cell expression of tissue factor (TF), which activates thrombin, a potent platelet activator. This can be parallelled to the capacity of tumor cells to attract various cell types to their stroma, such as fibroblasts and macrophages, and to stimulate these to secrete factors that maintain survival and proliferation of the tumor cells. Similarly, malignant cells may take advantage of the physiological function of platelets during wound healing to support the continued expansion of the tumor mass.

Platelets contain a variety of both pro- and anti-angiogenic molecules, which can be released upon activation. Examples of positive regulators of angiogenesis found in platelets are vascular endothelial growth factor (Mohle et al., 1997), platelet-derived growth factor (Heldin et al., 1981) and basic fibroblast growth factor (Brunner et al., 1993), while negative regulators include thrombospondin, platelet factor-4 (PF-4), endostatin and plasminogen activator inhibitor type-1 (PAI-1) (Browder et al., 2000; Staton and Lewis, 2005). Despite their content of both positive and negative regulators of blood vessel formation, platelets have in several different experimental settings been shown to stimulate angiogenesis. Early studies identified platelets as a source of endothelial stimulating factors (Busch et al., 1977). Rafii and collegues showed that megakaryocytes contain high levels of VEGF-A and that bone marrow microvascular endothelial cells can be maintained in serum-free medium if cocultured with megakaryocytes (Mohle et al., 1997). This effect was attributed to the release of VEGF-A from the megakaryocytes. In another *in vitro* study, purified human platelets were shown to promote tube formation of human umbilical vein endothelial cells (HUVECs) in Matrigel (Pipili-Synetos et al., 1998). The platelet stimulating effect on endothelial cell differentiation was not dependent on activation and granule release from the platelets, since unstimulated platelets were equally potent in this respect. Instead, direct adhesion of platelets to the endothelial cells was suggested as the event promoting tube formation in this assay (Pipili-Synetos et al., 1998). Along the same lines, Verheul et al showed that activation of cultured endothelial cells with VEGF-A promoted adhesion of unstimulated platelets (Verheul et al., 2000). This adhesion was dependent on VEGF-A-induced expression of TF by the endothelial cells, which in turn generated thrombin and subsequent activation and adhesion of the previously non-activated platelets. These three *in vitro* studies together nicely illustrate the reciprocal relationship between activated platelets and endothelial cells.

The concept of platelet-regulated angiogenesis is further supported by a number of *in vivo* studies. In a mouse model of hypoxia-induced retinal angiogenesis, inhibition of platelet aggregation as well as thrombocytopenia was demonstrated to reduce vascularization (Rhee et al., 2004). Using two other *in vivo* models of angiogenesis; the cornea micropocket assay and the Matrigel plug assay, Kisucka et al showed that platelets contribute significantly to angiogenesis (Kisucka et al., 2006). In addition, platelet-derived CD40L has been suggested to play a central role in stimulating tumor angiogenes via CD40-expressing endothelial cells in a transgenic mouse model for breast cancer (Chiodoni et al., 2006).

What is the reason for this primarily stimulating effect on vascularization by platelets, considering that they contain both anti-angiogenic as well as pro-angiogenic factors in their granules? This apparent contradiction may be explained by recent data showing that pro- and anti-angiogenic factors are stored in separate α-granules in the same platelet and that these granules can release their content in a regulated manner by selective stimulation of the thrombin protease activated receptors PAR-1 and PAR-4 (Italiano et al., 2008; Ma et al., 2005). These data demonstrate a more fine-tuned regulation of platelet degranulation than

was previously known. Similarly, ADP stimulation of platelets resulted in release of VEGF-A, but not of endostatin (Bambace et al., 2010; Battinelli et al., 2011), while thromboxane A2 released endostatin but not VEGF-A (Battinelli et al., 2011). Furthermore, Batinelli et al could show that the breast cancer cell line MCF-7 stimulated platelets to release pro-angiogenic factors (Battinelli et al., 2011). However, it remains to be addressed under which *in vivo* conditions a selective stimulation of different platelet receptors such as PAR-1 and PAR-4 could occur. The concept of functionally co-clustering of proteins in distinct granules was also recently challenged. Using different quantitative immunofluorescence microscopy techniques, Kamykowski et al reported that they did not obtain data in support of this model (Kamykowski et al., 2011).

Another less complex explanation for the stimulating effect on endothelial cells could be that the platelet-derived pro-angiogenic factors simply predominate among the released factors, resulting in a net effect of platelet activation on angiogenesis. In support of this hypotheses are data from Brill et al showing that platelet relesate is able to support angiogenesis *in vitro* and *in vivo*, despite the presence of anti-angiogenic factors such as platelet factor-4. If however the action of PF-4 was blocked by an antibody, the angiogenic response to the platelet relesate was potentiated (Brill et al., 2004). Thrombospondins (TSPs), well-studied inhibitors of angiogenesis, are the most abundant proteins in the platelet α-granules (Baenziger et al., 1972). Although TSPs are expressed in several cell types, Rafii and co-workers have elegantly shown that TSP-1 and TSP-2 derived from megakaryocytes and platelets function as a major anti-angiogenic switch (Kopp et al., 2006). The proposed mechanism involves binding and sequestration of stromal cell-derived factor 1 (SDF-1), a potent pro-angiogenic factor. Platelets from TSP-1 and TSP-2 double knock-out mice were also more efficient stimulators of angiogenes in Matrigel plugs compared to platelets from wild-type mice (Kopp et al., 2006), supporting the concept that pro- and anti-angiogenic factors released from platelets balance each other.

Factors that regulate angiogenesis can also be generated during platelet activation. One example is the angiogenesis inhibitor angiostatin, which is generated from proteolytic cleavage of plasminogen. Platelets release functional angiostatin, but the inhibitor is also generated during platelet activation and aggregation. Platelets contain plasminogen and enzymes like matrix metalloproteinases (MMPs) and urokinase plasminogen activator (uPA), that are capable of generating angiostatin by cleavage of plasminogen (Jurasz et al., 2003). In addition to release and generation, a third mechanism to locally increase the concentration of an angiogenesis inhibitor during platelet activation has been described. This mechanism involves creation of a microenvironment that favors retention of the anti-angiogenic molecule at sites of platelet degranulation (Thulin et al., 2009). This finding may reflect a host response to counteract angiogenesis during pathological conditions where platelets are activated.

In addition to various growth factors, platelets can release proteases such as MMPs (Jain et al., 2010) as well as phospholipids (English et al., 2001) that have the capacity to promote neovascularization by different mechanisms. Moreover, recent data point to a novel role for platelets in hypoxia-induced angiogenesis. Using three different tumor models and the hindlimb ischemia assay, platelet secretion from α-granules were shown to recruit bone marrow-derived cells into the growing neovasculature (Feng et al., 2011). These findings suggest a role for platelets as a way for communication between hypoxic tissue and the bone marrow during angiogenesis.

Platelets can stimulate endothelial cells by other mechanisms than release of pro-angiogenic factors from their granules. Fibrin, a product of coagulation and platelet activation, is

commonly found deposited in tumors (Dvorak, 1994). This provisional matrix can support endothelial cell adhesion, survival and migration and hence promote angiogenesis (Dvorak et al., 1995; Qi et al., 1997). Also, platelets are also able to stimulate angiogenesis via shedding of microparticles (reviewed below in a separate section).

At which stage of angiogenesis do platelets have an influence? Studies by Kisucka et al using the cornea micropocket assay in thrombocytopenic mice indicate that it is primarily the early stages in neovascularization that are affected by platelets (Kisucka et al., 2006). Moreover, mice lacking the endogenous angiogenesis inhibitor histidine-rich glycoprotein (HRG) have a coagulation defect and enhanced activation of platelets (Tsuchida-Straeten et al., 2005; Ringvall et al., 2011). HRG-deficient Rip1-Tag2 mice, a transgenic model of insulinoma, have a significantly increased number of angiogenic islets of Langerhans in their pancreas (Thulin et al., 2009). This elevated angiogenic switch can be suppressed by induction of thrombocytopenia, i.e. reduced platelet count, two weeks before onset of the switch. However, thrombocytopenia had no effect on angiogenesis at a later stage when the angiogenic islets had developed into invasive carcinomas (Ringvall et al., 2011). In addition, negative regulation of angiogenesis by platelet-derived TSP was demonstrated to play a role during early stages of tumor vascularization (Zaslavsky et al., 2010). Together these data support a role for platelets early in the angiogenic process. Later, when the tumor cell mass has expanded and a new microenvironment been created, with for example recruited inflammatory cells, the contribution of platelets may be of less significance.

Platelet-derived factors have not only been shown to regulate angiogenesis, but also to play a critical role in preventing hemorrhage from angiogenic (Kisucka et al., 2006) and inflamed microvessels (Goerge et al., 2008). Interestingly, this ability to support the integrity of blood vessels is not dependent on the capacity of platelets to form thrombi. Instead it seems to rely on the secretion of their granule content (Ho-Tin-Noe et al., 2008).

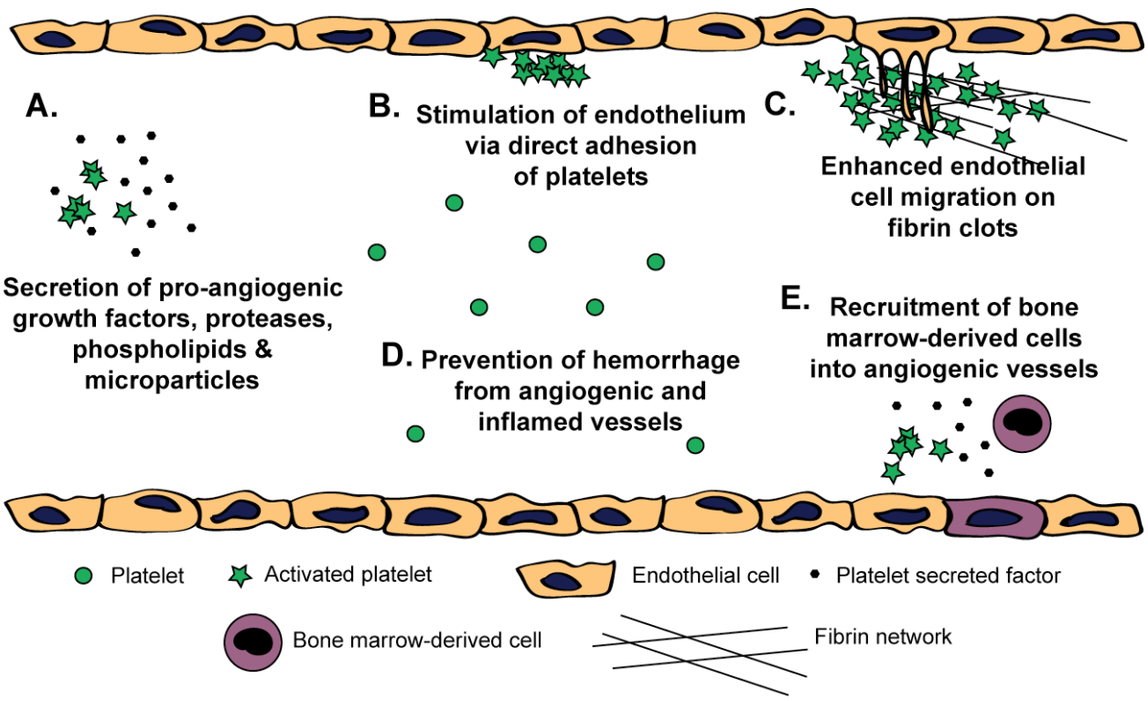

Fig. 1. Schematic illustration showing how platelets can stimulate angiogenesis.

Another interesting feature of platelets is their reported ability to selectively take up and sequester angiogenic regulators (Klement et al., 2009). Even a very small amount of VEGF-A secreted by microscopic subcutaneous tumors was reported to result in elevated levels of platelet VEGF-A. The "angiogenic profile" of platelets was therefore suggested as a possible early marker of malignant disease. It was recently shown that the TSP-1 protein present in platelets is derived from megakaryocytes (Zaslavsky et al., 2010), highlighting that not only platelet uptake but also endocytosis or increased production in the bone marrow precursor cells may account for the protein content of platelets.

Besides being a potent activator of platelets, thrombin can also have direct effects on tumor cells. Thrombin-treated tumor cells show an enhanced adhesion to endothelial cells and secrete endothelial growth factors such as VEGF-A and growth regulated oncogene alpha (GRO-α). In addition, thrombin can stimulate chemokinesis and possibly proliferation via the receptor PAR-1 on tumor cells (Nierodzik and Karpatkin, 2006). TF expression by tumor cells – which may be a thousand-fold higher than the amount expressed by their normal counterpart - generates thrombin, as described in a previous section. However, TF may also support tumor progression by formation of a cancer stem cell niche. In tumors a small subset of CD133-positive cells can be found, which are known as cancer stem cells or tumor-initiating cells. These cells have the ability to generate new tumors, either by relapse or metastasis. Interestingly, CD133-positive tumor cells have been found to express sinificantly higher levels of TF than the corresponding CD133-negative cells (Milsom and Rak, 2008).

In addition to the platelet-endothelial cell interactions that have been the focus of this section, platelet-tumor cell interactions significantly affect the capacity of tumor cells to form distant metastases, as discussed below.

4. The role of platelets in tumor metastasis

In addition to regulating tumor angiogenesis and growth, there is also experimental evidence that platelets are involved in the metastatic process. This has been shown by several independent research groups using various approaches. Experimental studies revealed that tumor cells with the ability to activate platelets *in vitro*, form more metastases upon xenografting in mice, than tumor cells lacking that capacity (Gasic et al., 1973; Pearlstein et al., 1980). Furthermore, it has been shown that thrombocytopenia is closely associated with reduced metastasis in various mouse models (Gasic et al., 1968). In yet another study, addition of platelets to thrombocytopenic mice restored the capacity for metastases formation to levels corresponding to non-thrombocytopenic control mice (Karpatkin et al., 1988). However, by interfering with platelet adhesion via inhibition of vWF or GpIIb/IIIa, the rescuing effect was lost. Intervening with the production of platelets by disturbing the maturation of megakaryocytes resulted in an almost complete inhibition of metastasis in an experimental mouse model (Camerer et al., 2004). Another study has revealed that intravenous injection of tumor cells in mice may lead to thrombocytopenia, giving further proof for the important tumor-platelet interplay (Karpatkin et al., 1981). Today, it is known that platelets can affect the metastatic process in several ways; by physical coverage of the tumor cells in the blood stream, by aiding in tethering and adhesion to the vessel wall, and by activation-induced secretion of various factors involved in invasion and migration.

Survival of tumor cells in the blood stream is essential for metastasis. Experimental mouse models of metastasis show that the metastatic process is highly inefficient, and that the

majority of tumor cells do not survive in the hostile microenvironment after intravasation. Natural Killer (NK) cells, cytotoxic lymphocytes capable of inducing tumor cell lysis, exert the major threat to tumor cells in the blood stream (Gorelik et al., 1982; Hanna, 1985). Elimination of NK-cells has repeatedly been shown to increase metastases formation in experimental mouse models. Platelets are suggested to serve as a physical guard for the tumor cells in the blood circulation, allowing protection against immune elimination. A study published by Nieswandt et al. showed that xenografts derived from several NK-sensitive tumor cell lines exhibited decreased metastatic potential after platelet depletion (Nieswandt et al., 1999) . The same study did also show that aggregation of platelets on the tumor surface protected the tumor cells from NK-cell induced cytotoxicity *in vitro.* This observation was confirmed a few years later by another research group, showing that mice with platelets unable to undergo activation, have decreased numbers of experimental as well as spontaneous metastases (Palumbo et al., 2005). Further investigation revealed that the effect was due to lower NK-cell cytotoxicity. However, there are studies indicating that platelets may inhibit NK-cell cytotoxic activity independent of direct contact, e.g. via soluble factors. For example, a study performed by Skov Madsen et al. suggested that supernatant from activated platelets are able to reduce NK-cell dependent lysis of human leukemia cells *in vitro* (Skov Madsen et al., 1986). More recently, a possible mechanism for this effect was presented, suggesting that platelet-derived TGFβ may down-regulate the cytokine NKG2D (Natural Killer Group 2, member D) on the NK-cell surface, resulting in decreased NK-cell cytotoxicity (Kopp et al., 2009).

Intervening directly with the platelet-tumor cell interaction affects metastasis in a similar way. Several lines of evidence confirm that intervening with receptors or ligands of importance for this interplay have significant impact on the tumors capacity to disseminate. Activated platelets bind to tumors via fibrinogen, using the fibrinogen receptor GpIIb/IIIa. Studies performed in mouse models reveal that inhibition of the fibrinogen receptor on platelets result in reduced numbers of metastases in the lungs (Amirkhosravi et al., 2003). Results from fibrinogen-deficient mice reveal diminished experimental metastasis, spontaneous hematogenous metastasis and lymphatic metastasis (Camerer et al., 2004; Palumbo et al., 2000; Palumbo et al., 2002). Interacting with the function of thrombin also affects the binding of platelets to the tumor cells. It has been shown *in vitro* that treating platelets with thrombin can enhance their binding to tumor cells by several times (Nierodzik et al., 1991). In line with this, thrombin-activated tumor cells have been shown to generate up to 150 times higher incidence of experimental metastasis, as detected using cell lines derived from murine melanoma and colon carcinoma (Nierodzik et al., 1996). P-selectin is expressed on the surface of activated platelets and is involved in platelet aggregation as well as platelet-tumor cell complex-formation. It has been shown that P-selectin deficient mice have decreased metastasis, a result obtained using both human and mouse carcinoma cells in immunodeficient and immunocompetent mice, respectively (Borsig et al., 2002; Kim et al., 1998). P-selectin dependent binding between platelets and tumor cells can be inhibited by heparin and results from syngenic mouse models reveal that heparin-dependent inhibition of P-selectin results in impaired experimental metastasis (Borsig et al., 2001).

Tissue factor expressed by platelets or tumor cells can induce formation of platelet-tumor cell aggregates. Furthermore, it is capable of enhancing fibrin-binding of tumor cells, facilitating tumor cell adhesion to the endothelium. This indicates that TF, due to its pro-coagulant function, might be of importance for tumor progression. Several studies do indeed show that TF is involved in the metastatic process. For example, inhibition of TF

resulted in decreased metastasis in a mouse model for pulmonary metastasis (Amirkhosravi et al., 2002; Mueller et al., 1992; Mueller and Ruf, 1998). Results from clinical studies are also in line with the results obtained using animal models. Increased levels of TF correlates to an increased risk for metastasis and decreased overall survival in patients with colorectal cancer (Seto et al., 2000). However, it has been suggested that TF affects the metastatic potential of tumor cells, independent of its role as an initiator of the coagulation cascade. One study showed that its cytoplasmic domain, a part that is not involved in the coagulation process, could exert the pro-metastatic function of TF. Modulation of the pro-coagulant part of TF in melanoma cells did not show any effect on the metastatic potential, further suggesting that TF might affect metastasis also via coagulation-independent mechanisms (Bromberg et al., 1995; Paborsky et al., 1991).

Another group of molecules that have the capacity to affect hematogenous metastasis are the PARs. In mice, *in vivo* grafting of B16 mouse melanoma cells overexpressing PAR-1 was shown to result in significantly increased numbers of experimental metastases compared to cells with normal levels of PAR-1 (Nierodzik et al., 1998). Similarly, PAR-2 expression has been shown to affect spontaneous metastasis in a mouse model for mammary adenocarcinoma (Versteeg et al., 2008). In the clinic, a direct correlation between PAR-1 expression and breast cancer invasiveness has been detected (Even-Ram et al., 1998). PAR-4 knock-out mice, lacking the capacity for platelet aggregation in response to thrombin, have reduced metastasis compared to wild type PAR-4 positive mice (Camerer et al., 2004). GPVI, an adhesion-receptor on platelets responsible for the binding of collagen, has been shown to be of importance for metastatic spread in a study using GPVI knock out-mice (Jain et al., 2009). Intravenous injection of metastatic tumor cells of both melanoma and lung cancer origin in GPVI-deficient mice resulted in a 50% reduction of observable tumor formation, as compared to injection of the same cell lines in wild-type mice with normal GPVI function.

Platelets can also affect the metastatic capacity of tumor cells by secretion of pro-metastatic as well as metastasis-preventing factors. These factors are released upon activation, for example during tumor cell induced platelet aggregation, facilitating or inhibiting further tumor progression. For example, matrix-degrading proteases, such as matrix metalloproteinase -1, -2 and -9, are stored in α-granules and released upon platelet activation, facilitating invasion and migration (Fernandez-Patron et al., 1999; Sawicki et al., 1997). Another factor stored in platelet α-granules is the protein vWF, which participates in platelet aggregation after binding to the GP1b-V-IX complex, suggesting it to have metastasis-preventing effects. This has also been confirmed in a study where melanoma and lung tumor cells were grafted in mice lacking vWF (Terraube et al., 2006). Indeed, these mice had significantly higher numbers of pulmonary metastases than vWF-expressing mice. This phenotype could be corrected by restoring the vWF levels in the knock-out mice. The difference in metastatic burden was suggested to depend on increased survival of the metastatic tumor cells in the lungs during the first 24 hours. Fibronectin, another extracellular matrix-protein involved in platelet aggregation and stored in platelet α-granules, seem to have pro-metastatic effects, reverting the metastasis-preventing effects of clot formation (Malik et al., 2010). Fibronectin binds to αvβ3 integrin and forms complex with fibrin during clot formation. Mice lacking fibronectin have reduced numbers of experimental melanoma metastases spreading to the lungs.

Platelets are able to facilitate the metastatic process by affecting the vascular permeability. For example, secretion of growth factors such as PDGF, TGFβ, EGF or VEGF-A, influences the integrity of the endothelium. Release of serotonin and histamin, stored in the dense-

granules in platelets, might also affect the metastatic process by enhancing the vascular permeability and thereby facilitate transport over the vascular endothelium. It has been shown that introduction of tumor cells into the circulation of mice results in significant increase of serotonin in the blood, and inhibition of serotonin receptors or calcium channels results in diminished metastasis to the liver (Skolnik et al., 1989; Skolnik et al., 1984). The vascular permeability may also be regulated by the signalling molecules sphingosine-1 phosphate (S1P) and lysophosphatidic acid (LPA), which are stored in platelet α-granules. Both S1P and LPA are capable of regulating the vascular integrity, S1P as an inhibitor of vascular leakage and LPA as a stabilizer for certain endothelial cell types (Sarker et al.; 2010 Schaphorst et al., 2003; Yin and Watsky, 2005).

Finally, platelets are suggested to support rolling and tethering of tumor cells on the vessel wall. This is a prerequisite for subsequent firm adhesion to the vessel wall, which is necessary for extravasation from the circulatory system into tissues for establishment of metastatic foci. Activated platelets secrete several factors that can activate endothelial cells in the vasculature and enable binding of platelets and tumor cells. Several studies indicate that platelets can support a transient tumor cell interaction with the vascular endothelium and that this is partly mediated via selectins (Laubli and Borsig, 2010). Selectins are expressed by endothelial cells and leukocytes and enables migration of leukocytes during inflammation by promoting their adhesion to the vessels wall. In a similar manner, selectins on the platelet-surface seem to be able to support transient tumor cell adhesion to the vessel wall (Laubli and Borsig, 2010). The adhesion formed between platelets and tumor cells has been suggested to depend on CD44 expressed on the tumor cell surface, interacting with fibrin (Alves et al., 2008). Selectin-dependent interactions between tumor cells, platelets and leukocytes might also have indirect metastasis-promoting effects, by inducing CCL5-release from endothelial cells (Laubli et al., 2009). This results in recruitment of monocytes leading to further increase in metastatic capacity. The low affinity binding supplied by selectins has to be replaced by high affinity adhesion, to enable extravasation. The platelet integrin that mainly contributes to firm arrest of tumor cells within the vasculature is integrin αIIbβ3 (Shattil et al., 2010). In addition, interaction between endothelial vWF and the Gp1bα receptor on platelets is important for platelet tethering to the endothelium during thrombus formation. Several studies confirm the involvement of both vWF and Gp1bα in the metastatic process, suggesting that this mechanism might be of importance also during cancer progression (Jain et al., 2007; Kitagawa et al., 1989). Prevailing adhesion, invasion and migration needed for extravasation, are further supported by tumor cell expression of integrin αvβ3, in interaction with platelets (Desgrosellier and Cheresh, 2010; Felding-Habermann et al., 1996).

5. Platelet-derived microparticles

Platelets also affect tumor progression indirectly by shedding of microparticles, containing fragments of the platelet plasma membrane and α-granules. Microparticles are small vesicles, sized between 0,1-1 μm and derived from a variety of healthy as well as malignant cells upon activation or apoptosis. They are present in the blood of healthy individuals, and are suggested to be involved in thrombosis, inflammation and angiogenesis (Burnier et al., 2009; Morel et al., 2006; Nieuwland and Sturk, 2010). Microparticles facilitates communication between neighbouring cells via several different mechanisms; by affecting direct cell-cell contacts, by their function as transport vesicles carrying and transferring

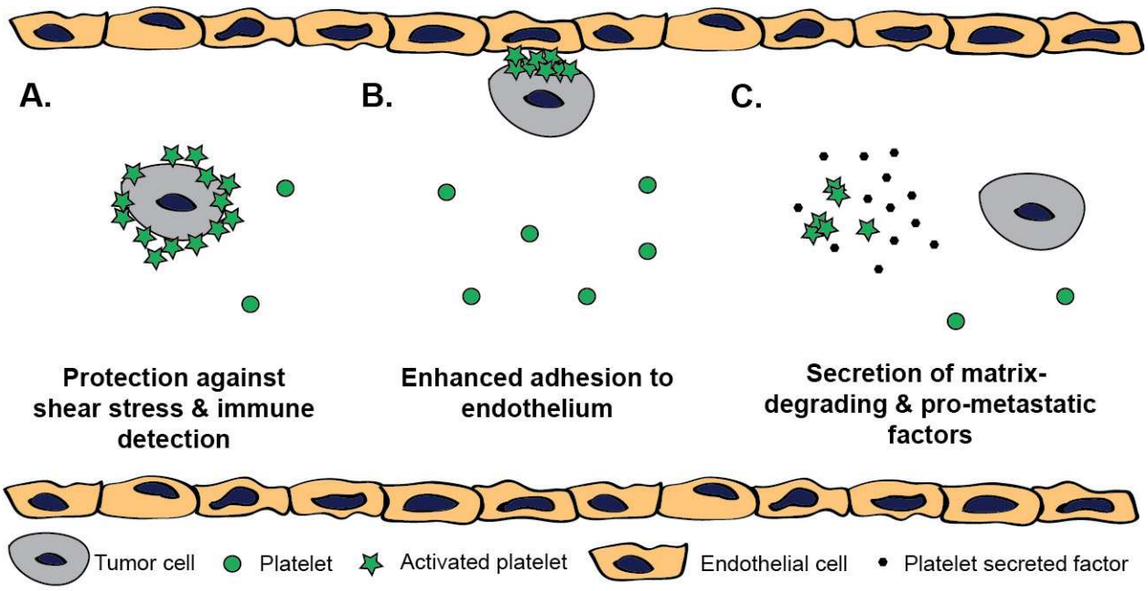

Fig. 2. Schematic illustration showing how platelets can affect tumor metastasis.

proteins and mRNA between cells and by direct regulation of cell signalling (Baj-Krzyworzeka et al., 2006; Essayagh et al., 2007; Mack et al., 2000; Simak and Gelderman, 2006). The levels of microparticles in the blood are increased in several diseased states, including cardiovascular disease, inflammation but also cancer (Piccin et al., 2007). Increased numbers of microparticles in the circulation of cancer patients also correlates with the risk for thrombosis (Khorana et al., 2008; Zwicker, 2010).

The majority of the microparticles in the blood stream are derived from megakaryocytes or platelets (Diamant et al., 2004). Platelet-derived microparticles have a negatively charged surface allowing binding of factors involved in clotting, and contain a specific set of proteins reflecting their platelet origin (Zwicker, 2008). The size and the major components of the platelet-derived microvesicles differ; large microparticles derived from the platelet plasma membrane contain platelet surface protein such as integrin αIIbβIII, Gp1b, TF, PECAM and P-selectin, while others are of subcellular-origin containing α-granules or platelet organelles (Denzer et al., 2000; Gracia Ballarin, 2005; Jin et al., 2005; Perez-Pujol et al., 2007). Pro-coagulant microparticles containing TF may not only be derived from platelets, but also from tumor cells. TF-bearing microparticles have a central role in regulating the coagulation cascade and they tend to accumulate during clot formation induced by cellular injury, resulting in increased risk for thrombosis (Diamant et al., 2004).

As described, platelet-derived microparticles affect blood coagulation, but they are also involved in processes of importance for tumor progression, such as angiogenesis (Kim et al., 2004). Platelet-derived microparticles carry adhesion-molecules as well as growth factors and proteases, which are needed for angiogenesis. *In vitro* studies indicate that microparticles from platelets stimulate mRNA-expression of pro-angiogenic factors, such as MMP-9, VEGF-A and HGF, in tumor cells (Janowska-Wieczorek et al., 2005). It has also been shown, both *in vitro* and *in vivo*, that platelet-derived microparticles are capable of inducing angiogenic sprouting to a similar extent as whole platelets (Brill et al., 2005). Furthermore, another study shows that platelet-derived microparticles can stimulate angiogenic tube formation from endothelial progenitor cells (Prokopi et al., 2009).

Several studies have shown associations between platelet-derived microparticles and tumor progression. Higher levels of platelet-derived microparticles in blood from prostate cancer patients have been correlated to aggressive disease and poor clinical outcome (Helley et al., 2009). Similarly, platelet-derived microparticle levels were suggested to be good predictors for tumor metastasis in patients with gastric cancer (Kim et al., 2003). The mechanisms behind these effects are not fully understood and still under investigation. However, it has been suggested that platelet-derived microparticles might induce tumor secretion of various matrix-metalloproteinases, facilitating the metastatic process (Dashevsky et al., 2009) (Janowska-Wieczorek et al., 2005). A study on the role of platelet-derived microparticles for metastatic capacity in lung cancer revealed that there might as well be direct effects on both tumor cell proliferation and adhesion to fibrinogen and endothelial cells (Janowska-Wieczorek et al., 2005).

As mentioned above, microparticles derived from other types of cells than platelets also have pro-coagulant functions. This means that microparticles can affect tumor progression via platelets in an indirect manner. It has been shown that TF-bearing microparticles originating from the tumor cells *per se* are present in the circulation of cancer patients (Tesselaar and Osanto, 2007; Zwicker, 2008). In general the levels of TF-bearing microparticles, independent of origin, have been associated with a more progressed cancer. For example, breast- and pancreatic cancer patients with metastatic disease have significantly higher levels of TF-bearing microparticles, as compared to healthy individuals and patients with non-metastatic cancer (Tesselaar and Osanto, 2007). In the same study, a negative correlation was found between microparticle-associated TF-activity and overall survival, further indicating the importance of TF-bearing microparticles in cancer. Results obtained from clinical studies are supported by studies performed in experimental mouse models. It has been shown that the amount of tumor-derived TF-bearing microparticles in the circulation correlates with tumor burden (Davila et al., 2008). Resection of tumors of several different sorts, including glioblastoma, pancreatic carcinoma and prostate cancer, has also resulted in decreased levels of TF-positive microparticles in several independent studies (Haubold et al., 2009; Sartori et al.; Zwicker et al., 2009). As expected, higher levels of TF-bearing microparticles have been associated with higher risk for cancer-associated venous thromboembolism (Tesselaar et al., 2009).

6. Therapeutic implications

Targeting the platelet-tumor interplay to inhibit tumor progression and metastasis is not used as a cancer therapy in the clinic today, but is an interesting strategy. Growing evidence indicate that the platelet-tumor cell interplay has an important role for tumor progression in several different ways. Hence, therapeutic approaches striking against this interaction might be an option for future development of cancer drugs. Such treatment would need further studies and potential targets identified in animal trials, such as P-selectin, GpVI or modulation of NK-cell reactivity, have to be validated in the clinical situation. Moreover, targeting platelet-endothelial interactions may be of equal importance in preventing malignant growth, since a significant part of the tumor-promoting effect of platelets seem to be via stimulation of tumor angiogenesis. The challenge in developing drugs that inhibit platelet function is to avoid the risk of bleedings. However, the recent findings that platelet-mediated protection of tumor vessel integrity requires platelet degranulation, but not plug formation, may allow for specific targeting of the two processes (Ho-Tin-Noe et al., 2009).

Species-specific differences concerning platelet biology and function makes it difficult to predict how well results obtained in mice would reflect the situation in humans (Schmitt et al., 2001). However, results from studies on clinical material confirm a strong connection between platelets and tumor growth and progression, suggesting platelets to be promising targets also in human cancer.

7. Conclusion

The literature describing tumor-promoting effects of platelets is significant and rapidly growing. Despite the extent of our current knowledge of these processes, many questions remain to be answered. How is differential release of separate platelet α-granules regulated in a mechanistic manner and which are the underlying signal transduction pathways? Do platelets contribute at a specific stage of tumor development, or are they equally important throughout cancer progression? Could targeting of platelet interactions with the vessel wall inhibit angiogenesis? Is it possible to target a distinct platelet process, such as degranulation, without affecting others, like clot formation? Considering the rapid development of this field of research and the continued efforts of several laboratories around the world, answers - as well as new questions - will surely come in a near future.

8. References

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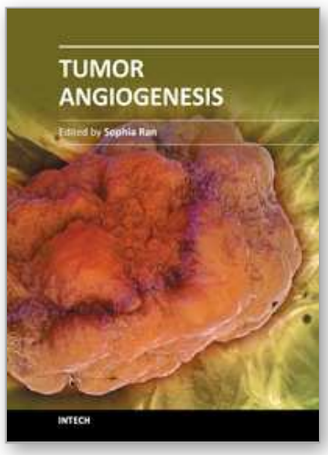

Tumor Angiogenesis

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Tumor angiogenesis is the main process responsible for the formation of new blood vessels that promote tumor growth and metastasis. This process is driven by potent pro-angiogenic factors that are predominant in the tumor environment and are produced by both malignant cells and the host cells recruited to the tumor site. Tumor environment is characterized by the imbalance between pro-angiogenic and anti-angiogenic factors, which drives the construction of numerous but structurally defective vessels. These poorly perfused and abnormal vessels significantly contribute to the tumor pathology not only by supporting the expansion of the tumor mass but also by promoting chronic inflammation, enhancing thrombosis, impeding drug delivery, and disseminating tumor cells. These problems associated with tumor vasculature continue to attract great attention of scientists and clinicians interested in advancing the understanding of tumor biology and development of new drugs. This book complies a series of reviews that cover a broad spectrum of current topics related to the pathology of tumor blood vessels including mechanisms inducing new vessels, identification of new targets for inhibition of tumor angiogenesis, and potential clinical use of known and novel anti-angiogenic therapies. The book provides an update on tumor angiogenesis that could be useful for oncologists, cancer researchers and biologists with interests in vascular and endothelial cell behavior in the context of cancer.

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