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Role of Platelets in Angiogenesis in Health and Disease

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Abstract: Increasing experimental and clinical research suggests a role for platelets in angiogenesis. These cells are major storage and delivery vehicles of a broad array of growth factors, chemokines, cytokines, proteases and cell adhesion molecules, which are secreted upon activation and determine the local angiogenic stimulus. Although platelets contain both, pro- and antiangiogenic molecules, there is a general consensus that platelets promote angiogenesis by stimulating chemotaxis, proliferation, and differentiation of endothelial cells and recruitment of progenitor cells to sites of vascular injury. However, a growing body of evidence indicates that the angiogenic proteome of platelets can be modified under stressful microenvironmental conditions such as cancer. How platelets regulate angiogenesis in different clinical scenarios is not completely understood. The amplification of angiogenesis by platelets plays a positive and beneficial role in several processes, such as pregnancy and tissue healing, where new vessel development is required. However, in clinical conditions associated with abnormal or excessive angiogenesis including cancer, atherosclerosis, and arthritis, platelets might contribute to the detrimental progression of these diseases. This review represents an integrated summary of the current knowledge regarding the role of platelets in angiogenesis and its consequences in health and disease.

Keywords: Alpha-granules, angiogenesis, cancer, endothelial cells, growth factors, platelets.

INTRODUCTION

Although platelets are widely recognized as having a critical role in primary hemostasis and thrombosis, increasing experimental and clinical evidence identifies these enucleated cells as relevant modulators of other physiopathologic processes, including atherosclerosis, inflammation, immunity and angiogenesis [1].

An established concept in vascular biology is that under normal conditions platelets circulate without interacting with the intimal endothelial lining of the vessel wall, and only after endothelial injury, they firmly adhere to adhesive proteins exposed on the subendothelial matrix allowing thrombus formation [2]. However, during the last decade, substantial experimental and clinical data revealed that during inflammatory states, even in the absence of any apparent morphological damage, platelets can bind to the intact endothelium, partly because the physiological inhibitory mechanisms are impaired, and partly because new adhesion molecules are expressed on the surface of activated endothelial cells [3]. During the sequential steps of the adhesion process, platelets become activated and secrete from their alpha granules an arsenal of potent inflammatory and angiogenic molecules that can be released or exposed on the cell surface after platelet activation by rolling over activated endothelium or subendothelium. In the adjoining endothelial cells, the platelet-secretory mediators alter the chemotactic, adhesive and proteolytic properties of the endothelium further promoting the switch to an angiogenic endothelial phenotype [4-7].

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In the normal adult, angiogenesis, defined as the growth of new blood vessels from preexisting ones, is mostly limited to wound healing, pregnancy, uterine cycling and tissue repair. Angiogenesis also occurs in various pathologies, including neoplastic growth, retinopathies, and atherosclerosis. The formation of new vessels is a systemic process that is not limited to existing vasculature expansion but also involves the recruitment of other cell types, including immune cells, bone marrow-derived cells (BMDCs), and fibroblasts. This process is regulated by a continuous interplay of stimulators and inhibitors that tightly controls the normally quiescent vasculature, and their imbalance contributes to numerous disorders [8]. Here we discuss the most important recent advances in the cross talk between platelets and vascular cells during angiogenesis and the clinical consequences of these interactions.

PLATELET-DERIVED ANGIOGENESIS MODULATING FACTORS

Platelet alpha granules are major storage units of a plethora of growth factors, chemokines, cytokines, proteases, and cell adhesion molecules [1].

Among the proangiogenic substances, platelets contain vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinases (MMPs), which promote and mediate the initial phase of angiogenesis. This phase consists of the dissolution of the basement membrane and surrounding matrix of the parent vessel, followed by endothelial migration, proliferation, and lumen formation [9]. Through the release of platelet-derived growth factor (PDGF) and angiopoietin-1, platelets are crucial in the maturation of newly formed vessels. This stabilization phase whereby endothelial proliferation stops, the

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basement membrane is reconstructed, and the capillaries are surrounded by pericytes [9]. Additionally, platelets release cytokines and chemokines that also promote angiogenesis. These molecules include CD40L which promotes survival, migration and synthesis of VEGF, bFGF, and angiopoietin-1 in endothelial cells [10]. Furthermore, the stromal cell-derived factor-1 alpha (SDF-1α) released from alpha granules promotes chemotaxis, homing, proliferation, and differentiation of $CD34^+$ cells into endothelial progenitor cells [11]. Conversely, platelets also release antiangiogenic molecules including thrombospondin-1 (TSP-1), endostatin, platelet factor-4 (PF-4), angiostatin, tissue inhibitor of metalloproteinases-1 and -4 (TIMP-1 and -4), and plasminogen activator inhibitor-1 (PAI-1) [1]. These molecules act by several mechanisms, including inhibition of endothelial proliferation and migration, interference with mitogenic effects of proangiogenic molecules, or induction of endothelial cell apoptosis [12-14].

Considering that platelets interestingly contain both, pro- and antiangiogenic molecules, the question arises of how platelets regulate angiogenesis? Elegant studies from Raffi's group led to a proposed model in which, upon tissue ischemia or wound healing, activated platelets release SDF-1α and MMP-9, which promote neovascularization. In addition, secretion of TSP-1 and -2 by platelets acts as antiangiogenic switches and controls the extent of vessel formation [15-17]. Although a number of important questions about the roles of pro- and antiangiogenic regulators derived from platelets in angiogenesis have been answered by these studies, other questions remained unanswered. Particularly, again, how do platelets orchestrate the secretion of these proteins in various clinical scenarios (*e.g.*, wound healing, ischemia, cancer, and inflammation)? More recent studies show that this phenomenon involves new concepts about platelet alpha granule secretion. It has been revealed that angiogenic factors are packed into morphologically distinct populations of platelet alpha granules [18-20] and they can be differentially released based on selective engagement of platelet receptors providing a mechanism by which platelets can locally and sequentially modulate angiogenesis [21-24]. Among the pro- and antiangiogenic platelet proteins, VEGF and endostatin have been the most studied respectively, and they both can be differentially released upon platelet activation. While platelet stimulation with ADP and PAR-1 activated peptide (AP) promotes the release of VEGF, activation by thromboxane A_2 (TXA_2) or PAR-4 AP triggers the release of endostatin [21-24]. We found that platelet activation by thrombin, which activates PAR-1 and PAR-4 and induces the release of ADP and TXA_2, results in the secretion of VEGF as well as endostatin [25]. Remarkably, not only platelet agonists but also stress signals, such as extracellular acidosis or hyperthermia, differentially regulate the release of VEGF and endostatin as well as other cargo proteins, such as von Willebrand factor (vWF) and SDF-1α [26, 27]. In contrast with these theories, another study shows that alpha granule content proteins are stochastically packaged into subdomains within single granules and that proteins displayed little, if any, pattern of functional co-clustering [28]. Additional evidence comes from the observation that platelet secretion, rather than having a limited thematic response to specific agonists, appears to be a stochastic process potentially controlled by several factors, such as cargo solubility, granule shape, and/or granule-plasma membrane fusion routes [29]. Interestingly, Batinelli *et al*. demonstrated a correlation between the differential release of VEGF and endostatin with a pro- or antiangiogenic effect mediated by platelet releasates from ADP or TXA_2 stimulated platelets [23]. However, we recently demonstrated that although thrombin, the most potent platelet agonist, promotes the release of both VEGF and endostatin, the overall effect of platelet releasates was proangiogenic and independent of VEGF action [25].

Although the structure and dynamics of alpha granule secretion appear somewhat controversial and still remain to be clarified, the different theories seem to converge on the notion that selective signaling pathways might be involved in the regulation of alpha granule content exocytosis and further studies are required to fully understand the potential time-spatial secretion of angiogenic regulators from platelets.

Platelet dense granules store Ca^{2+}, ADP/ATP, inorganic polyphosphate, pyrophosphate, and serotonin, which are essential contributors and amplifiers of platelet activation. Among these substances, serotonin is the only one that has been shown to play a direct role in angiogenesis by induction of endothelial activation, proliferation, migration, and tube formation [30, 31]. Considering that circulating blood platelets contain the majority of systemic serotonin [32], the release of this molecule could be another mechanism by which platelets modulate angiogenesis.

PLATELET MICROPARTICLES

Microparticles are small plasma membrane vesicles shed from cells upon their activation or apoptosis. Platelet-derived microparticles (PMPs) constitute the majority of the pool of microparticles circulating in the blood and participate in several biological functions, including thrombosis, hemostasis, inflammation and angiogenesis [33]. The role of PMPs in blood vessel development was first demonstrated by Kim *et al*. They reported that PMPs promote the proliferation, survival, migration, and tube formation of human umbilical vein endothelial cells (HUVECs) mainly by the action of lipid components present on PMPs surface [34]. In addition, Brill *et al.* showed both *in vivo* and *in vitro* that, besides lipid components, PMPs induce sprouting and invasion of endothelial cells in a process dependent on VEGF, bFGF, and PDGF presence [33]. Another mechanism through which PMPs induce angiogenesis has recently been demonstrated and involves the interaction between PMPs and endothelial progenitor cells. This phenomenon is mediated by the expression of P-selectin and the glycoprotein (GP) IIb/IIIa and Ib on PMPs surface, promoting the differentiation of progenitors to mature endothelial cells, the processes of adhesion, migration and proangiogenic factor release from endothelial cells, and tissue remodeling that favors vascular regeneration after injury [35, 36]. The angiogenic processes mediated by platelets in angiogenesis are schematized in Fig. (**1**).

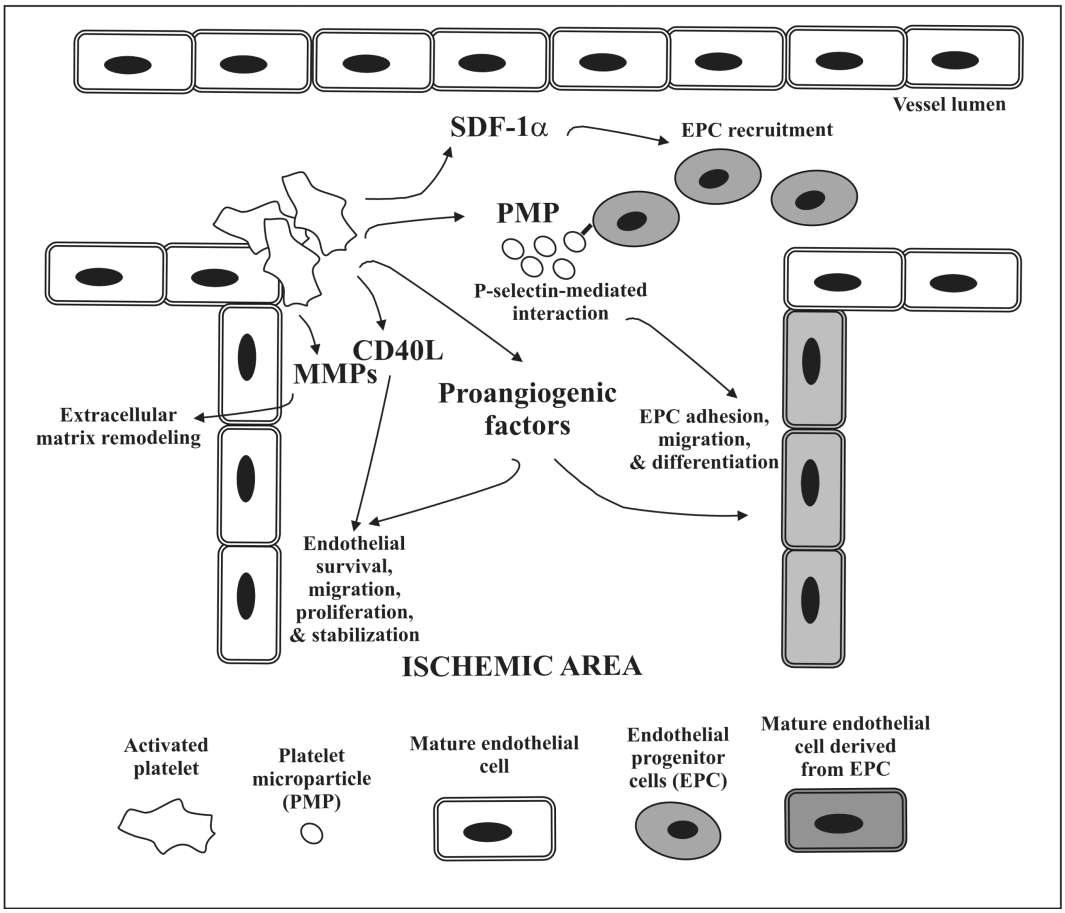

Fig. (1). Platelet-mediated angiogenesis. Platelet adhesion to activated endothelium in the ischemic area promotes the release of several angiogenic modulators including matrix metalloproteinases (MMPs) that induce the tissue remodeling and many proangiogenic factors that promote endothelial cell survival, proliferation, migration and vessel stabilization. This process is synergized by soluble CD40L released from platelets, which also enhances the endothelial paracrine action. In addition, platelet-derived SDF-1α supports the recruitment of endothelial progenitor cells (EPC) from the bone marrow through the interaction of P-selectin expressed on PMP surface. These progenitors along with local tissue resident EPC contribute to the revascularization process. The amplification of angiogenesis by platelets plays a positive and beneficial role in several processes but in clinical conditions associated with abnormal or excessive angiogenesis including cancer, atherosclerosis, and arthritis, platelets might contribute to the detrimental progression of these diseases.

BENEFICIAL ROLES OF PLATELET-MEDIATED ANGIOGENESIS

Early Pregnancy and Fetal Vascular Remodeling

The sequential steps of early pregnancy, including corpus luteum stimulation, endometrial differentiation, embryo invasion, and placentation, require angiogenesis [37]. Platelets act as crucial mediators of pregnancy vascularization by deposition of local chemotactic and angiogenic factors, including RANTES, PDGF, VEGF, and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [37-39]. Furthermore, platelets contribute to nutrients transportation from mother to embryo through the ductus arteriosus (DA), a fetal shunt vessel between the pulmonary artery and the aorta that closes promptly after birth. Platelets induce DA closure by promoting extravillous trophoblast invasion to reconstruct the maternal endometrial artery and to induce vascularization during corpus luteum formation [40]. In addition to contributing to the maternal tissue remodeling, platelets also play an essential role in separating the blood and lymphatic vasculatures during embryonic angiogenesis. The association of platelets with vascular endothelium at zones of contact between lymphatic sacs and veins confirmed a direct role of platelets in the separation of the two vasculatures [41, 42]. The platelet receptor, C-type lectin-like receptor 2 (CLEC-2), facilitates lymph/blood vessel separation in the developmental stage by binding to its ligand, podoplanin, on lymphatic endothelial cells [43]. In the developmental stage, when lymphatic vessels separate from cardinal veins, CLEC-2 in platelets binds to podoplanin in lymphatic endothelial cells. Subsequent platelet activation, results in the release of granule contents, including transforming growth factor 8 family proteins. These platelet contents inhibit migration, proliferation, and tube formation of lymphatic endothelial cells, which facilitates blood/lymphatic vessel separation [44-47].

Considering the principal role of platelets in pregnancy and embryonic angiogenesis, it has recently been postulated that declination of circulating platelets during the first trimester of pregnancy is a useful index for prognosis of

pre-eclampsia or other obstetric complications later in pregnancy [48, 49].

Stroke

Stroke by mechanism of thrombotic cerebral ischemia is one of the leading causes of death and/or disability worldwide [50]. Because activated platelets and their microparticles contain a variety of growth and trophic factors essential to angiogenesis and neurogenesis, they have been proposed to serve as novel therapeutic agents for brain injury [51]. Specifically, *in vitro* studies demonstrated that platelets increase angiogenesis, as well as survival, proliferation, and differentiation of neural stem cells, through the release of their PMPs and several other factors, including VEGF, FGF, PDGF, and brain derived neurotrophic factor (BDNF) [52, 53]. These effects were also shown *in vivo* in a model of cerebral ischemia where PMPs were applied topically on the injured brain using a biodegradable polymer [54]. In addition, a recent study with a different application method (direct intra-cerebro-ventricular injection) demonstrated that exogenous platelet lysates promote angiogenesis, proliferation, survival, and terminal differentiation of endogenous neural stem cells in the cortex. Furthermore, local delivery of platelets promote neuroprotection and reduces behavioral deficits after brain ischemia [55]. These findings indicate that platelets appear to result in improved functional gain after stroke through the promotion of angiogenesis, neurogenesis, and neuroprotection.

Gastric Ulcer

Ulcer formation is a dynamic imbalance between aggressive mucosal factors and defensive/repairing factors. When these defensive and healing factors are less than the aggressive factors, mucosal injuries worsen and ulcers develop [56]. Ulcer healing is a complex and tightly regulated process of filling the mucosal defect with proliferating and migrating epithelial and connective tissue cells. This process includes the re-establishment of the continuous surface epithelial layer, glandular epithelial structures, microvessels, and connective tissue within the scar [57]. Several growth factors and cytokines produced locally contribute to gastric ulcer healing by regenerating cells, controlling re-epithelialization and the reconstruction of glandular structures [57, 58]. Using a model of gastric ulcer in rats Wallace's group demonstrated that platelets modulate gastric ulcer healing through the local release of VEGF [59-61]. Supporting evidence was also found in a recent study that showed a correlation between low platelet count and diminished expression of local growth factors with worse prognosis of gastric ulcers observed in cirrhotic patients [62].

Cardiovascular Disease

Traditionally, it was thought that once an intact vascular system was established further vessel growth occurred only by the sprouting of endothelial cells from existing vessels. However, in 1997, Asahara *et al*. demonstrated that postnatal vascularization does not rely exclusively on sprouting from preexisting vessels; instead BMDCs are released to incorporate into, and thus contribute to, postnatal physiological and pathological neovascularization [63]. Vascular remodeling occurs after ischemia and platelets contribute structurally and instructively to this process. Platelets are integral to vasculogenesis, delivering angio-active growth factors and cytokines that direct the site-specific recruitment and differentiation of BMDCs at vasculogenic sites [64, 65]. Pioneer studies of Raffi's group demonstrated that the secretion of SDF-1α by cytokine-stimulated platelets is a hallmark event in the revascularization of ischemic limbs. They showed that elevation of plasma SDF-1α levels support the mobilization and recruitment of hemangiocytes to the neoangiogenic niche [17]. Latter studies extended these findings, showing that SDF-1α secreted by activated platelets supports $CD34^+$ progenitor cell recruitment to arterial thrombi and differentiation of the cells to endothelial progenitor cells *in vivo* [11, 66]. Moreover, in clinical studies it was observed that in patients with myocardial infarction, platelet-derived SDF-1α correlated with the number of circulating progenitor cells and was associated with restoration of left ventricular function and improved prognosis [67, 68]. In addition, formation of circulating platelet/$CD34^+$ progenitor cell aggregates has been described in patients with acute coronary syndromes, which was associated with a significantly decreased myocardial infarct size and better left ventricular function [69]. The recruitment of progenitor cells to the injured tissues was ascribed not only to SDF-1α but also to platelet-derived bFGF [70].

In addition to secreting chemokines and growth factors, platelets also modulate healing after ischemia *via* the release of PMPs. Intra-myocardial injection of PMPs markedly elevated the amount of novel capillaries developed in the heart muscle in the background of ischemia in a mechanism that was not only dependent on the presence of VEGF, bFGF, and PDGF [33] but also could be associated with the recruitment of progenitor stem cells [35, 36]. In addition, a recent study shows that PMP-secreted RANTES may play a role in augmenting the adhesion and neovascularization capacities of circulating angiogenic cells (CACs), suggesting that the injection of PMP-CACs may be a new strategy to augment the effects of therapeutic angiogenesis for limb ischemia in atherosclerotic patients [71].

NEGATIVE ROLES OF PLATELET-MEDIATED ANGIOGENESIS

Although the amplification of angiogenesis by platelets plays a positive and beneficial role in several processes where new vessel development is required, in clinical conditions associated with abnormal or excessive angiogenesis, platelets might contribute to the deleterious progression of these diseases.

Cancer

The relationship between platelets and cancer has been studied for more than a century, with one of the earliest reports from Armand Trousseau dating back in 1865 [72]. Since then, numerous studies have shown that the interaction between tumor cells and platelets promotes the reciprocal activation of both cell types, favoring tumor progression, tethering and distant spread. Moreover, elevation of platelet counts is associated with poor survival in a variety of cancers [73]. One of the principal mechanisms by which platelets promote tumor progression is through the induction of angi-

ogenesis [74]. In the tumor microenvironment platelets display an improved ability to deliver angiogenesis regulators, which induce endothelial cell proliferation and chemotaxis, capillary tube formation, and neovascularization promoting tumor growth [23, 75-77]. In tumor microcirculation, platelets may be activated by changes in blood flow, contact with subendothelial prothrombotic proteins due to leaky vessel walls or by direct interaction with tumor cells [73]. Tumor cells are known to express mucins that bind to P-selectin on the platelet membrane and trigger platelet granule secretion [78]. In addition, several types of tumor cells overexpress galectins which exert tumoral and angiogenic activity [79]. We have recently described that galectins are potent platelet activators [80-82], therefore, it could be speculated that the overexpression of galectins in tumor cells could represent another trigger for platelet activation allowing the release of alpha granule contents, including growth factors, which can promote tumor progression and angiogenesis. In this sense, we observed that releasates derived from platelets activated by galectin-1, -3, and -8 trigger endothelial cell proliferation and neo-vessel formation [83].

Using an experimental model of Lewis lung carcinoma, it was recently demonstrated that platelet depletion led to a rapid destabilization of tumor vasculature with intratumor hemorrhage and that infusion of resting but not degranulated platelets prevented thrombocytopenia-induced tumor bleeding. These intriguing findings suggested that platelets not only contribute to the formation of new vessels but also continuously support tumor vascular homeostasis by regulating the stability of tumor vessels through the secretion of the content of their granules [77].

In the clinical setting, it has been observed that cancer patients have elevated serum levels of platelet-derived proangiogenic substances, including VEGF, PDGF, and interleukin IL-6 suggesting a correlation between soluble factors from platelets with the proangiogenic phenotype observed in these patients [84-86]. This phenomenon could be associated with the ability of platelets to selectively take up tumor-derived pro-angiogenic factors [87, 88]. It was demonstrated that the platelet concentrations of angiogenesis regulatory proteins, although relatively constant and stable under physiologic conditions, are modified by and reflect the presence of a tumor [87, 89]. Moreover, in mice bearing tumors, the analysis of the platelet angiogenesis proteome reflected the presence of dormant, microscopic-sized tumors, months before these tumors could be detectable by conventional methods, and before the angiogenesis regulatory proteins could be detected in plasma [87]. These data led to the suggestion that evaluation of the platelet angiogenesis proteome, instead of circulating angiogenic factors, could serve as a biomarker to detect a tumor before the patient is symptomatic [12]. In this way, it was shown that changes in platelet-associated PF-4 detect malignant growth across a spectrum of human cancers in mice [89]. Although the functional relevance of the upregulation of this angiostatic molecule expression in platelets was not determined, further studies of Zaslavsky *et al*. revealed that TSP-1, another potent antiangiogenic molecule, was also upregulated in the platelets of tumor bearing mice as a consequence of both, increased levels of TSP-1 mRNA in megakaryocytes and increased numbers of these cells in the bone marrow of tumor bearing mice. In addition, using transplant experiments, they demonstrated that 1) mice inoculated with Lewis lung carcinoma with circulating platelets lacking TSP-1 developed tumors 4 to 6 days earlier, 2) tumors isolated from these mice had significantly higher microvessel density, and 3) increased volume than tumors isolated from mice with wild-type platelets [90]. Collectively, these data suggest that the production and delivery of antiangiogenic molecules by platelets may be a critical early host response to suppress tumor growth and highlight the potential of these molecules as biomarkers of tumor growth and regression. However, the early antiangiogenic response mediated by platelets could be overcome during tumor progression, inducing the switch to a platelet-proangiogenic phenotype and, therefore, it would also be interesting to determine whether the platelet proteome changes after longer periods following the appearance of a tumor.

In addition to the secretion of angiogenic modulators from alpha granules, platelets also promote angiogenesis in cancer through PMPs release [91]. PMPs levels are highly correlated with aggressive tumors, elevated numbers of platelets, and a poor clinical outcome [92, 93]. PMPs can induce the activation of endothelial cells promoting a proangiogenic phenotype, the recruitment and adhesion of cancer cells to endothelium and the remodeling of extracellular matrix *via* increased MMP activity [94, 95].

Together these data indicate that platelets are deeply involved in the angiogenesis of the tumor niche. Inhibition of the release of platelet-derived proangiogenic molecules is currently considered an important potential strategy for cancer treatment and includes 1) the impairment of platelet-tumor interactions, 2) inhibition of platelet-derived angiogenic modulators release, and 3) the use of platelets as carriers of angiogenic inhibitors.

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by the infiltration of immune cells into the subendothelial layers of the arterial wall [96]. During the formation of the atheroma plaque, physiologic angiogenesis is instrumental for restoration of vessel wall normoxia and resolution inflammation, leading to atherosclerosis regression. However, pathological angiogenesis enhances disease progression, increasing macrophage infiltration and vessel wall thickness, perpetuating hypoxia and necrosis. In addition, thin-walled fragile neovessels may rupture, leading to intraplaque hemorrhage [97]. Several studies provide evidence for the crucial role of activated platelets in the pathogenesis of atherosclerosis beyond the acute atherothrombotic events [98]. Platelets influence atherogenesis by adhering to activated endothelial cells and depositing chemotactic mediators on the endothelial surface; promote the pathological angiogenesis observed in atherosclerosis because they are a major source of several angiogenic molecules that contribute to vulnerable atheromatous plaque formation [99, 100].

Systemic Sclerosis

Systemic sclerosis (SSc, scleroderma) is a chronic, multisystem connective tissue disorder affecting the skin and

Table 1. Platelet-derived angiogenesis modulators in physiopathological processes.

PRO-ANGIOGENIC FACTORS		
Factor	**Beneficial angiogenic modulation**	**Negative angiogenic modulation**
RANTES	• Early pregnancy and fetal vascular remodeling	
	• Revascularization of ischemic limbs	
PDGF	• Early pregnancy and fetal vascular remodeling	• Promotes cancer- and arthritis-related angiogenesis
	• Neuroprotection after brain ischemia	
	• Revascularization of ischemic limbs	
VEGF	• Early pregnancy and fetal vascular remodeling	• Promotes cancer-, arthritis-, and systemic sclerosis-related angiogenesis
	• Neuroprotection after brain ischemia	
	• Gastric ulcer healing	
	• Revascularization of ischemic limbs	
HB-EGF	• Early pregnancy and fetal vascular remodeling	
FGF	• Neuroprotection after brain ischemia	• Promotes arthritis-related angiogenesis
	• Revascularization of ischemic limbs	
BDNF	• Neuroprotection after brain ischemia	
SDF-1α	• Revascularization of ischemic limbs	
IL-6		• Promotes cancer- and arthritis-related angiogenesis
P-selectin	• Revascularization after vascular injury	• Promotes cancer- and arthritis-related angiogenesis
MMP		• Promotes cancer-related angiogenesis
EGF		• Promote arthritis-related angiogenesis
IGF		
HGF		
TNF-α		
IL-1, -8, -15, -18		
Angiogenin		
Angiopoietin		
PAF		
CD40L		
ANTI-ANGIOGENIC FACTORS		
Factor	**Beneficial angiogenic modulation**	**Negative angiogenic modulation**
TSP-1	• Inhibits cancer- and arthritis-related angiogenesis	• Inhibits vascularization after ischemia
TSP-2	• Inhibits arthritis-related angiogenesis	• Inhibits vascularization after ischemia
PF-4	• Inhibits cancer-related angiogenesis	
Endostatin	• Inhibits arthritis-related angiogenesis	

BDNF: brain derived neurothophic factor; FGF: fibroblast growth factor; EGF: epidermal growth factor; HB-EGF: heparin-binding epidermal growth factor-like growth factor; HGF: hepatocyte growth factor; IGF: insulin-like growth factor; IL: interleukin; MMP: matrix metalloproteinase; PAF: platelet-activating factor; PDGF: platelet-derived growth factor; PF-4: platelet factor-4; RANTES: regulated on activation, normal T cell expressed and secreted; SDF-1α: stromal cell-derived factor-1 alpha; TNFα: tumor necrosis factor alpha; TSP: thrombospondin; VEGF: vascular endothelial growth factor.

various internal organs. Although the disease is characterized by a triad of widespread microangiopathy, fibrosis and autoimmunity, increasing evidence indicates that vascular damage is a primary event in the pathogenesis of SSc. A severe imbalance between pro-angiogenic and angiostatic factors may also lead to impaired angiogenic response during SSc [101]. As a consistent circulating source of bioactive compounds, platelets contribute to the development of many characteristic phenomena of SSc, such as fibrosis and impaired vascular tone [102]. In the setting of SSc, platelets are detectable in a persistent activated state [103, 104], which is intimately linked to the concomitant presence of an injured endothelium and to the widespread activation of the innate and adaptive immune system [103, 105]. Aberration in endothelial cell proliferation and mitogen secretion is a hallmark of SSc and also presents as "frustrated angiogenesis" [102]. Surprisingly, despite insufficient angiogenesis, VEGF is overexpressed in the skin of patients with SSc. It was suggested that a controlled overexpression of VEGF might help to protect against the manifestation of ischemic conditions. However, a long-term uncontrolled overexpression of VEGF might have paradox effects on the formation of new vessels, leading to capillary changes similar to those observed in SSc [106]. In this regard, it has been demonstrated that in SSc patients, platelets are the most important contributors to the high serum levels of VEGF. Platelet transport and secretion of VEGF in the microvascular beds of the patients underlines the potential importance of platelets in the altered angiogenesis observed in this disease [107].

Arthritis

Rheumatoid arthritis is a chronic inflammatory and autoimmune disorder that typically affects the synovial joints of the hands and feet [108]. Relationships between platelet activation and rheumatoid arthritis have been shown in many studies. Platelets amplify inflammation in rheumatoid arthritis *via* collagen-dependent microparticle production and by promoting angiogenesis [6, 109]. Rheumatoid arthritis is closely linked to angiogenesis [110]. VEGF expressed in response to soluble mediators, such as cytokines and growth factors, and its receptors comprise the best-characterized system in the angiogenesis regulation of rheumatoid joints. Moreover, other angiogenic mediators, such as PDGF, FGF-2, EGF, IGF, HGF, TGF-β, TNF-α, IL-1, IL-6, IL-8, IL-13, IL-15, IL-18, angiogenin, PAF, and angiopoietin play an important role in angiogenesis in rheumatoid arthritis. In contrast, endostatin, and TSP-1 and -2 are angiogenic inhibitors in rheumatoid arthritis. The persistence of inflammation in rheumatoid joints is a consequence of an imbalance between these inducers and inhibitors of angiogenesis [111]. In addition to the secretion of these angiogenic modulators, platelets also express other activation markers, including CD40L and P-selectin, which are elevated on the surface of circulating platelets in rheumatoid arthritis patients [112, 113]. Formation of the CD40L-CD40 bridge may thus allow up-regulation of VEGF, which could further augment the recruitment of inflammatory cells into the synovium by promoting neovascularization. In this context, CD40L could be responsible for establishing a critical amplification loop, which leads to the persistence of synovitis [114].

The role of pro- and antiangiogenic factors derived from platelets in the physiopathological processes is summarized in Table **1**.

CONCLUSIONS

Over the last decade, we have witnessed impressive advances regarding the biology of platelets and their role in angiogenesis. Platelet alpha granules are major storerooms of a plethora of growth factors, chemokines and cell adhesion molecules that are released upon platelet activation, a phenomenon that has been considered the principal mechanism by which platelets participate in vessel formation. New paradigms in platelet-mediated angiogenesis are that platelets contain different populations of alpha granules that differ in their protein cargo and morphology and that platelets can differentially release pro- and antiangiogenic regulators according to the stimulus. However, because the net biological effect of platelet releasates is clearly proangiogenic, the relevance of the differential release of alpha granule content still remains to be elucidated. Moreover, the evaluation of platelet angiogenic proteome is now considered a potential biomarker for early tumor development and its implications for the diagnoses and benefit of early therapeutic intervention may be of great impact in the management of cancer patients. Finally, it is now recognized that platelets not only contribute to the formation of vessels from preexisting ones but also intervene in supporting development de novo of blood and lymphatic vessels.

Gaining a deeper insight into each of these new processes in platelet-mediated angiogenesis is crucial to progress in the development of therapeutic strategies for different angiogenesis-related diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

AP	=	Activated peptide
BDNF	=	Brain derived neurothophic factor
bFGF	=	Basic fibroblast growth factor
BMDCs	=	Bone marrow-derived cells
CAC	=	Circulating angiogenic cells
CLEC-2	=	C-type lectin-like receptor 2
DA	=	Ductus arteriosus
EGF	=	Epidermal growth factor
GP	=	Glycoprotein

HB-EGF	=	Heparin-binding epidermal growth factor-like growth factor
HGF	=	Hepatocyte growth factor
IGF	=	Insulin-like growth factor
IL	=	Interleukin
MMP	=	Matrix metalloproteinase
PAF	=	Platelet-activating factor
PAI-1	=	Plasminogen activator inhibitor-1
PAR	=	Proteinase-activated receptor
PDGF	=	Platelet-derived growth factor
PF-4	=	Platelet factor-4
PMP	=	Platelet-derived microparticle
RANTES	=	Regulated on activation, normal T cell expressed and secreted
SDF-1α	=	Stromal cell-derived factor-1 alpha
SSc	=	Systemic sclerosis
TGF-β	=	Transforming growth factor beta
TIMP	=	Tissue inhibitor of metalloproteinase
TNFα	=	Tumor necrosis factor alpha
TSP-1	=	Thrombospodin-1
VEGF	=	Vascular endothelial growth factor
vWF	=	Von Willebrand factor

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