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# **Role of Platelets in Cancer and Cancer-associated Thrombosis: Experimental and Clinical Evidences**

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## **Abstract**

The primary hemostatic function of platelets has been recognized for more than a century, but increasing experimental and clinical evidences suggest that platelets are also important mediators of cancer. Cancer indeed influences platelet physiology, and activated platelets participate in each step of cancer development by promoting tumor growth, angiogenesis, metastasis, and cancer-associated thrombosis. Based on both the results of numerous experimental models addressing the involvement of platelets in cancer progression and the results of epidemiologic studies on the use of anti-platelet drugs to prevent cancer, platelets have been proposed as a potential target to reduce the short-term risk of cancer, cancer dissemination and cancer mortality. However, the cancer-associated thrombosis and the risk of bleeding due to anti-platelet drugs are not enough evaluated in experimental models. Therefore, the interesting contribution of platelets to cancer and cancer-associated thrombosis requires the standardization of preclinical and clinical models.

**Keywords:** Platelet, cancer, immunothrombosis, experimental models, clinical investigations

## Introduction

Platelets are small anucleated blood cell fragments released from bone marrow megakaryocytes that circulate in the bloodstream at concentrations of 150 to  $350 \times 10^9/L$ . Their primary hemostatic function has been recognized for more than a century. In 1882, Bizzozero first observed them microscopically in the circulating blood of living animals and demonstrated their role in promoting thrombosis *in vivo*.

After vascular injury, platelets are able to rapidly adhere to sites of endothelial disruption *via* the interaction of platelet glycoprotein (GP)Ib-V-IX and von Willebrand factor (vWF). This results in platelet translocation on the vessel wall. Firm adhesion activates collagen receptors GPVI and  $\alpha_2\beta_1$ . These receptor-ligand interactions initiate intracellular calcium mobilization, cytoskeletal reorganization, shape change, degranulation, up-regulation of integrins, such as  $\alpha_{IIb}\beta_3$ , and the release of soluble platelet agonists, such as thrombin, adenosine di-phosphate (ADP) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>). These agonists amplify the platelet activation response through specific G-protein-coupled receptors. In addition, local thrombin generation also enhances platelet activation through proteolytic cleavage and the activation of protease activated receptors (PARs). Finally, activated platelets form stable aggregates through the linking of  $\alpha_{IIb}\beta_3$  with fibrinogen. This hemostatic plug is what prevents excess blood loss.

In addition to their central role in hemostasis and thrombosis, platelets contribute to the development of a variety of diseases, including malignancies. Accumulating experimental and clinical evidences support the hypothesis that platelet activation during cancer (in response to endothelial or epithelial injury) promotes disease progression. The concept that platelets may participate in cancer biology is not new since experimental reduction in platelet count or platelet function was found to have anti-metastatic effects in animal models. Gasic et al. first provided in 1968 experimental evidence for the role of platelets in cancer [1]. They demonstrated in a mouse model that intravenous injection of neuramidase leading to thrombocytopenia inhibited metastasis. Several thrombocytopenic mouse models later confirmed that decreased platelet count results in inhibited tumor growth, angiogenesis and metastasis [2]. These models are summarized in the Table 1. Numerous *in vitro* and *in vivo* studies further investigated the platelet-mediated mechanisms of cancer progression including the tumor growth, the angiogenesis and the metastasis development.

In line with experimental data, cancer patients often present thrombocytosis and increased markers of platelet activation including P-selectin and platelet-derived

microparticles (PMPs), reflecting a platelet activation state during the course of the disease [8]. Platelets are additionally thought to contribute to the hypercoagulable state frequently observed in cancer patients, leading to an increase risk of venous thromboembolism (VTE) which is a leading cause during the course of the disease [9]. Finally, the results of numerous randomized clinical trials demonstrated that anti-platelet drugs might reduce the incidence of some cancers [10].

This review summarizes the bidirectional interactions between cancer and platelets. Cancer indeed influences platelet physiology, and activated platelets participate in each step of cancer development by promoting tumor growth, angiogenesis, metastasis, and cancer-associated thrombosis. The potential of platelets as a target in cancer therapy is further discussed.

## **I-Experimental evidences**

### **a) Effects of cancer on platelets**

It has been widely demonstrated that tumor cells have the ability *in vitro* to induce platelet activation and aggregation; this mechanism is referred to as tumor cell-induced platelet aggregation (TCIPA). Different types of tumor cell lines were shown to induce TCIPA, such as pancreatic [11], colorectal [12] and kidney cancer cell lineages [13]. The tumor cells' ability to induce platelet aggregation was found to be dependent on their metastatic potential [14]. Furthermore, several molecular pathways are involved in platelet activation and TCIPA, including the thrombin, ADP, TXA<sub>2</sub>, metalloproteinases (MMPs) and tissue factor (TF) pathways, which seem to be tumor-cell specific and sometimes mutually exclusive [15].

**Thrombin.** Thrombin is a multi-functional serine protease that converts fibrinogen to fibrin. Thrombin also activates other proteins in the coagulation cascade, such as coagulation factors V, VIII, XI, and XIII. It is also arguably the most effective agonist for platelet activation *via* the activation of PARs. It triggers shape change, the release of platelet activators, such as ADP, serotonin and TXA<sub>2</sub>, as well as chemokines and growth factors. It also mobilizes P-selectin and CD40 ligand to the platelet surface and leads to the activation of integrin  $\alpha_{IIb}\beta_3$ . Cancer cells have been shown to directly secrete thrombin which may activate the coagulation cascade and platelets [12]. Pancreatic and lung cancer cell lines in particular were found to induce platelet activation *via* thrombin secretion [12]. These interactions activate PARs on both platelets and tumor cells. In cancer, thrombin signaling was also implicated in tumor cells malignancy and metastasis [16]. Concordantly, the thrombin generation potential is increased in cancer patients and decreased at both prophylactic (40 mg) and therapeutic (80 mg) doses of low molecular weight heparin (LMWH) [17].

**ADP.** ADP is stored in platelet-dense granules and is released upon platelet activation. ADP interacts with platelet receptors P2Y<sub>12</sub> and P2Y<sub>1</sub>, leading to platelet aggregation, shape change and the release of TXA<sub>2</sub>. ADP is expressed by cancer cells and has been shown to contribute to TCIPA [12] [18]. The P2Y<sub>12</sub> receptor was found to be involved in the induction of TCIPA by MCF-7 breast carcinoma cells [18]. The use of the ADP inhibitor apyrase strongly inhibits TCIPA [19].

**Thromboxane A<sub>2</sub>.** TXA<sub>2</sub> is overexpressed in numerous tumor cell lines and can enhance platelet activation and aggregation [20].

**Matrix metalloproteinases.** MMPs are involved in the degradation of the extracellular matrix [21]. Tumor cells express matrix metalloproteinases, such as MMP-2, MT1-MMP and MMP-9 which have been shown to be involved in platelet activation and TCIPA [19] [18] [22]. Breast cancer cells express MMP-1, which upregulates both GPIb- $\alpha$  and GPIIb/IIIa and is also involved in ADP release via a positive feedback pathway that stimulates TCIPA. MMP-2 expressed by HT-1080 human fibrosarcoma cells and MCF7 breast carcinoma cells also induces TCIPA [19] [18]. Generation of MMP-2 has also been correlated with an increase in TCIPA in prostate cancer patients [23].

**Tissue factor.** TF is the main activator of the coagulation cascade in physiologic and pathologic conditions. The coagulation cascade is indeed initiated as soon as TF comes into contact with circulating activated FVIIa. This initial contact creates the TF-FVIIa complex, which directly converts FX to activated FXa. Factor Xa complex, with activated FVa and calcium, catalyzes the conversion of prothrombin to thrombin, leading to fibrin formation, platelet activation and thrombus generation. Numerous cancer cell lines express TF and release TF-bearing microparticles released into the circulation. It should be noted that TF has been demonstrated to have a key role in cancer [24].

Several experimental studies investigate the inhibition of platelet activation and aggregation and show its impact on each steps of the cancer development. These studies focusing on the activating proteins of the platelet receptors are summarized in the **Table 2**.

#### **b) Effects of platelets on cancer**

Cancer promotes platelet activation and activated platelets participate in each step of cancer progression.

- **Tumor growth**

Recently, Stone et al. demonstrated in murine ovarian cancer models that an abundance of platelets were present in the tumor microenvironment outside the vasculature [47]. When activated, platelets recruited to the tumor microenvironment can locally regulate tumor cell behavior and promote proliferation not only through paracrine signaling but also through direct contact with tumor cells [48][49]. Activated platelets and PMPs indeed release numerous mitogenic proteins and growth factors from their  $\alpha$ -granules, including platelet-derived growth factor (PDGF)-A and PDGF-B, insulin-like growth factor (IGF)-I, PF4, vascular endothelial growth factor (VEGF), transforming growth factor- $\beta$  (TGF- $\beta$ 1), stromal cell-derived factor-1 (SDF-1) and angiopoietin, which were found to be involved in tumor

growth in several *in vitro* and *in vivo* studies [50] [51] [52] summarized in Table 3. For example, platelets were found to release SDF-1 [71], which binds primarily to CXCR4 and induces intracellular signaling through several divergent pathways. This triggers responses related chemotaxis, cell survival and/or proliferation, intracellular calcium, and gene transcription [72]. Cho et al. recently demonstrated that platelets enhance the proliferation of ovarian cancer cells *in vitro* via a mechanism involving TGF- $\beta$ ; similarly, they also observed that ovarian tumors from patients with thrombocytosis had an increased proliferative index compared to those with normal platelet counts [73]. Regarding tumor growth, platelets not only secrete growth factors but also release toxic cytokines that inhibit tumor growth. Angiostatic PF4 was shown to inhibit tumor cell proliferation and migration. Because of this, it was proposed as an adjuvant cancer therapy [74]. Co-culture of platelets with melanoma and prostate tumor cells inhibited proliferation of tumor cells because of soluble factors released from platelets as well as direct contact between platelets and tumor cells [74]. This inhibition resulted from a tumor cell cycle arrest in the G0/G1 phase. There conflicting results in the literature concerning this. While some studies found that platelets decrease the cancer cell proliferation through their cytotoxic effects, others demonstrated that platelets increase cancer cell proliferation [75] [74].

Unfortunately, there is a paucity of data concerning the effects of platelet direct interaction with tumor cells on tumor growth. A few studies have investigated the role of  $\alpha_{IIb}\beta_3$  in tumor growth [76] and suggest that a real direct interaction of platelets and cancer cells exists and needs to be further explored *in vivo*.

- **Angiogenesis**

Angiogenesis is defined as the formation of new microvessels from pre-existing vasculature [77]. This process appears to be crucial for primary tumor growth >2 mm [78]. It not only prevents both hypoxia and necrosis but also allows for the delivery of proteases and cytokines that contribute to further intravasation, extravasation and dissemination. Evidence supporting platelet participation in tumor neovascularization have accumulated over the years [77]. During angiogenesis, platelets come into contact with subendothelial prothrombotic structures, such as collagen in new vessels with abnormal configuration. This causes changes in the laminar blood flow, specifically by increasing shear stress, and the VEGF-induced release of vWF by endothelial cells, leading to platelet activation [79]. Activated platelets then release the myriad of angiogenesis-regulating proteins contained in their granules.



Cytokines exerting pro-angiogenic effects include VEGF, PDGF, TGF, epidermal growth factor, angiopoietin-1, IGF-I, sphingosine-1-phosphate, and MMPs; and anti-angiogenic effects include PF4, thrombospondin-1 (TSP-1), endostatin, serotonin, plasminogen activator inhibitor-1 and angiostatin [80]. Therefore, platelets could theoretically stimulate or inhibit angiogenesis; however, in most studies, the pro-angiogenic effects prevail over the anti-angiogenic effects. *In vitro*, activated platelets are able to induce tube formation of human umbilical vein endothelial cells in matrigel tube formation assays by both the secretion of endothelial stimulating factors and direct cellular interactions [81]. In aortic ring assays, platelets, platelet releasates and PMPs all induced a marked increase in angiogenesis [82] [83]. Concordantly, Kisucka et al. used cornea and matrigel assays to observe significantly reduced angiogenic activity in thrombocytopenic mice. This suggests that the presence of platelets stimulates angiogenic vessel growth [84].

VEGF plays a crucial role in regulating angiogenesis. Interestingly, in a meta-analysis, Kut et al. found an increase in platelet VEGF in cancer patients compared to controls [85]. The concentration of VEGF in the platelets of cancer patients was 413 000 pg/ml compared to 216 000 pg/ml in healthy controls [85]. This pro-angiogenic factor may be a potential biomarker in the detection and monitoring of cancer because platelet VEGF was found to predict tumor progression [86]. PDGF has also been associated with tumor growth and angiogenesis in many studies however the involvement of platelet-derived PDGF is not clearly identified *in vivo* [87]. The sources of platelet angiogenic factors are not yet fully understood, but in addition to uptake, it was demonstrated that enhanced production by megakaryocytes could contribute to the platelet angiogenic content [88].

Recently, a study of Italiano et al. aimed to elucidate the regulation of both anti- and pro-angiogenic factors release [89]. The authors demonstrated that the release of different types of angiogenic factors was triggered by different stimuli, specifically PAR-1 and PAR-4 [89]. PAR-4 stimulation led to the secretion of anti-angiogenic proteins, whereas the activation of PAR-1 led to the release of pro-angiogenic factors, such as VEGF.

- **Metastasis**

The highly complex process of metastasis includes the detachment of cancer cells from the primary tumor, intravasation into the lumen of blood vessels, intravascular migration, arrest in distant capillaries, and finally, extravasation into the surrounding tissue. While the lymphatic spread of cancer cells is observed in human tumors and used as an

important prognostic marker for disease progression, dissemination *via* the hematogenous circulation appears to represent the major mechanism of metastasis [90]. However, because of the high shear forces and immune system surveillance, the bloodstream is an unfavorable medium for circulating cancer cells; only 0.1% survive in the circulation [91].

Today there is persuasive evidence that platelets contribute to the metastatic potential of tumor cells at each phase of the metastasis. The first experimental proof-of-concept was provided by Gasic in 1968 [1]. Others discovered tumor cells in thrombi and postulated a relationship between platelets and tumor cells. Numerous studies further demonstrated that platelet blockade or depletion inhibits metastasis [92]. The connection between platelets and circulated cancer cells has since been widely demonstrated [92]. This process is initiated by a direct interaction between platelets and cancer cells. Inhibiting this interaction was demonstrated to impact on the metastatic process (Table 4). Normally, resting platelets circulate within the vasculature and are prevented from binding to the vascular wall by endothelial cells. During cancer progression, platelets present an abnormal activated state that leads to the formation of platelet-cancer cell aggregates, which are crucial in the metastatic process.

### **-Pre-metastatic niche formation**

Ten years ago, two important studies found that tumor primary cells secreted metastasis-related proteins to the target organ [114]. These proteins were shown to stimulate bone-marrow-derived cells to migrate and formed metastatic niches. Kerr et al. demonstrated, in three mouse models of tumor-induced bone formation, that platelets have a role in governing pre-metastatic tumor communication [115]. More recently, Labelle et al. showed that secretion of CXCL5 and CXCL7 chemokines by platelets upon contact with tumor cells induce granulocyte recruitment and the formation of early metastatic niches [116].

### **-Tumor-platelet interaction during the intravascular phase of metastasis**

Tumor cells circulating within the vasculature are exposed to high shear stress that can disrupt their membranes. They also have to evade immune surveillance. Because of this, the activation of platelets by tumor cells (previously detailed in the text) and the adhesion of platelets to tumor cells are crucial mechanisms allowing circulating cancer cells to survive in the bloodstream. Palumbo et al. found that tumor cell survival and metastasis formation was inhibited in mice with deficient platelet activation (*Gαq*, deficient mice) [117]. TCIPA results

in platelets coating the tumor cells, protecting them from neutralization by natural killer cells (NKs) and tumor necrosis factor- $\alpha$  mediated cytotoxicity [92]. NKs are the main anti-tumor cells and directly interact with cancer cells [118]. Impaired activity or depletion of NK cells in mice results in a significant increase in metastasis [119], and immunologic or genetic depletion of NKs was demonstrated to restore metastatic potential in tumor cells. Moreover, platelets that aggregate around tumor cells are able to secrete TGF- $\beta$ , which downregulates NK cytotoxicity and interferon- $\gamma$  production *via* the downregulation of the NKG2DL receptor on NKs [120]. TCIPA also inhibits the maturation of dendritic cells previously implicated in antigen presentation to the immune system [121]. These results are consistent with epidemiological studies demonstrating an increased risk for cancer in patients with decreased NK counts [117].

Furthermore, platelets express high levels of major histocompatibility complex-1 (MHC-1), and platelets attached to tumor cells have been shown to transfer MHC-1 to tumor cells [123]. MHC-1 protects tumor cells from recognition by NK cells [123] and other cells and from the inflammatory response. Several other platelet receptors are also expressed on tumor cells, a phenomenon referred to as platelet mimicry, which allows the tumor cell to escape from immune surveillance [124].

### **-Tumor-platelet interaction during the tumor cell arrest and extravasation**

TCIPA not only permits cancer cells to survive in the bloodstream, but it also facilitates their arrest and extravasation to promote metastasis. TCIPA first shields cancer cells from high shear forces that could damage them. Platelet-cancer cell aggregates resulting from TCIPA can also embolized into the microvasculature, promoting their arrest and adhesion to endothelial cells for transmigration to the metastasis site [125].

The rolling and tethering of tumor cells to the endothelium are primarily mediated by P-selectin [103]. Studies in P-selectin-deficient mice showed a decrease in metastasis in a colon carcinoma model [126]. After this first interaction, platelet GP-Ib $\alpha$ ,  $\alpha_{IIb}\beta_{III}$  integrin and vWF all contribute to firm adhesion [127]. In addition, tumor cells express several “platelet receptors”, such as  $\alpha_{IIb}\beta_{III}$ ,  $\alpha_v\beta_3$  or GPIb- $\alpha$  [128]. This “platelet-mimicry”, previously involved in escaping the immune system, also contributes to the tumor cells’ interaction with leukocytes, platelets and endothelial cells to promote extravasation [124]. Other platelet receptors, such as PAR-1 are expressed by cancer cells and induce the expression of several integrins that contribute to their adhesion to endothelial cells [129]. Platelet  $\alpha_2\beta_1$  was found to

be expressed in prostate cancer cells, suggesting a direct interaction of cancer cells with sub-endothelial collagen [130]. Factors released by activated platelets confer several advantages to tumor cells during the extravasation process. Platelets can both increase and decrease vascular permeability *via* granule release [4]. After TCIPA, activated platelets release their dense granules, such as ATP, which interact with the endothelial receptor P2Y<sub>2</sub>. This interaction promotes the opening of endothelial cell junctions to facilitate cancer cell transmigration [131]. Extravasation is decreased when P2Y<sub>2</sub> receptors are absent or in Munc13-4 deficient mice, in which the release of platelets' dense granules is inhibited [131]. Another important platelet mediator involved in tumor cell aggregation is serotonin, which is also stored in dense granules. Blocking serotonin receptors leads to inhibited experimental metastasis, which is consistent with the fact that cancer patients have increased serotonin levels in the plasma [132]. Activated platelets also release growth factors contained in their  $\alpha$ -granules. These include TGF- $\beta$ , lysophosphatidic acid (LPA), Sphingosine-1 and MMPs. The role of LPA and Sphingosine-1 has been demonstrated as an inhibitor of vascular leakage [133]. LPA, a platelet bioactive lipid, is increased in ascites fluid and plasma from ovarian, endometrial and cervical cancer patients [134] and is strongly involved in the cell proliferation, survival and migration during cancer [135]. The blocking of its receptors suppress tumor metastasis and show the essential contribution of the LPA in the vicious circle of intercellular stimulation between platelets and cancer cells [136]. Activated platelets also secrete MMPs, which contribute to tumor cell invasion by degrading the vessel and remodeling the tissue after transmigration [137]. Platelets are also suggested to induce the epithelial-mesenchymal transition implicated in tumor cell invasion by direct interaction or by TGF-  $\beta$  and VEGF release [138]. Altogether, these mechanisms promote metastasis. Some studies demonstrate that metastasizing tumor cells can also reenter the bloodstream to induce additional metastasis or return to the primary tumor [91].

### c) **Experimental animal models targeting platelets to inhibit cancer progression**

Based on experimental data, targeting platelets to inhibit cancer progression and metastasis appears to be a promising approach. Because of the biological complexity of platelet involvement in these processes, numerous animal models have been developed in addition to *in vitro* approaches.

Numerous studies aimed to target key platelet actors using knockdown mice, antibodies or anti-platelet drugs (**Table 1-2-3 and 4**). The *in vitro* studies were mainly based on the use of washed platelets and cancer cell lines. They focused on tumor cell-platelet

interactions, adhesion, TCIPA, invasion, proliferation and platelet activation (receptor expression or granules components). Protocols to obtain washed platelets strongly varied across studies, particularly in the anticoagulants, anti-platelets and buffers used. Because it is difficult to work with platelets, it would be important to further standardize washed platelets protocols as well as the activation markers used to compare results.

The use of animal models has yielded many important insights into the potential targeting of platelets to treat cancer. In tables of this present review, we summarized the main studies evaluating platelets as a therapeutic target for inhibiting tumor growth and metastasis development. In addition to the type of experimentation and the impact on malignancy, we also quoted the experimental models used. Surprisingly, the majority of the *in vivo* studies is based on the use of the experimental metastasis model, and there were few studies investigating cancer-associated thrombosis. Most of the experimental studies showed that platelet inhibition slows cancer progression, supporting a rationale for clinical studies to evaluate the use of anti-platelet drugs in cancer patients. .

The most-used model of transplantable cancers to study metastasis development is based on the injection of tumor cells directly into the systemic circulation. This model provides several advantages, such as speed, but it has numerous limitations. The tail vein injection of tumor cells first results in microthrombi due to platelet aggregation. The direct injection of a large number of tumor cells does not mimic the process of spontaneous metastasis from a primary site. Injected tumor cells also induce exclusively pulmonary metastases that appear in approximately 15 days. Consequently, this model has previously been described as a model in which multiple primary tumors develop in the lung. This model only allows for a limited examination of the post extravasation step and the presence of a thrombocytopenia due to platelet consumption in microthrombi do not allow to study the involvement of platelets during the progression of the disease. In addition, the mortality of the animals is very high. [139].

Models that more closely mimic human cancers, particularly the metastatic process, are required to evaluate platelets as a therapeutic target. Many studies have demonstrated that orthotopic models reproduce the rapid growth of local tumors and spreading of metastases in humans [140]. With a longer time for metastasis (average time of metastasis become evident at 25 days), the orthotopic animal model induces spontaneous metastasis and appears more relevant in preclinical studies. For example, Yamamoto et al. showed differences in MMP

expression in metastasis, following the experimental metastasis model, in comparison to spontaneous metastasis [141].

## **II- Clinical evidences**

In line with experimental data, clinical data also clearly suggest a role for platelet in cancer and cancer-associated thrombosis.

### **a) Thrombocytosis and platelet activation markers during cancer**

An increased platelet count has been first observed in cancer patients for decades. More than 100 years ago, Tranum and al. indeed described that thrombocytosis was associated with malignancy [142]. Furthermore, in the 1960s Levin et al. found that 40% of patients with inoperable solid malignancies had an increased platelet count (higher than  $400 \times 10^9/L$ ) [10]. High platelet counts were since observed at the time of diagnosis in many patients with solid tumors and may precede the diagnosis [143]. Thrombocytosis is correlated with poor survival and decreased response to surgery and chemotherapy in lung, breast, colorectal, renal and gastric cancers [143]. These data suggest a potential direct role for platelets in the pathogenesis of the disease. Until recently, cancer-associated thrombocytosis was considered to be a paraneoplastic phenomenon, but specific molecular pathways have been identified—specifically the interleukin (IL)-6 pathway—by which tumors can promote platelet production and activation [143].

Platelet activation was also increased in cancer patients. Several clinical studies investigated the levels of biomarkers of platelet activation, such as soluble P-selectin, CD40 ligand, PF4, TSP-1 and  $\beta$ -thromboglobulin ( $\beta$ -TG) in the blood of cancer patients [144] [145]. An increase in soluble P-selectin, CD40 ligand and  $\beta$ -TG levels but no change in PF4 levels was observed in cancer patients but not in healthy controls, suggesting that ongoing platelet activation occurs in cancer patients. Platelet P-selectin expression was also increased in cancer [146], and TSP-1 levels were increased in pancreatic and colorectal cancers but not in lung cancer [147]. Interestingly, platelet activation correlated with metastatic potential in both *in vitro* and *in vivo* studies [14].

Several studies have investigated the role of PMPs in cancer and cancer-associated thrombosis [148]. PMPs levels are dramatically increased in malignancy. An increase in PMPs has been found in gastric cancer [149] or prostate cancer [150]. PMPs levels have been

associated with the presence of metastasis in gastric cancer patients and correlate with the severity of the disease better than vascular endothelial growth factor, IL-6, and RANTES (regulated on activation, normal T cell expressed and secreted) [149]. Furthermore, the overall survival of patients with hormone-resistant was found to be inversely related to the PMPs levels [150]. Interestingly, a flow cytometry study found that circulating PMPs counts decreased while the number of platelet aggregates increased [151].

#### **b) Platelet contribution to cancer-related thrombosis**

Various sources suggest that platelets also participate in the procoagulant state-associated with cancer, which is responsible for VTE, a frequent cause of death in cancer patients [9]. Several platelet laboratory parameters were developed to predict the risk of VTE in cancer patients. First, platelet count was demonstrated to be a biomarker of cancer-associated thrombosis in independent studies. A prospective study of patients starting chemotherapy shows that platelet count, prior to chemotherapy, increases risk of VTE [152]. Numerous other studies indicate an association between thrombocytosis, risk of VTE and survival in cancer patients. Second, several biomarkers of platelet activation were found to be associated with VTE risk. In a prospective study of 687 cancer patients with either recently diagnosed cancer or recently relapsed cancer, increased soluble P-selectin levels ( $>53.1$  ng/mL) were predictive of VTE thromboembolism [153]. A clinical study in pancreatic cancer patients also demonstrated that high levels of PF4 ( $>11$  kU/mL) were associated with an increased risk of VTE. A recent report by the Vienna cancer and thrombosis studies demonstrated in a large cohort of cancer patients that a decreased mean platelet volume was associated with VTE risk [154].

In 2008, Khorana et al. developed a risk assessment model for prediction of VTE in cancer patients at the time of chemotherapy initiation [155]. This score is based on simple clinical variables (type of cancer, body mass index) and complete blood count including platelet counts (pre-chemotherapy platelet count  $> 350 \times 10^9/L$ ). It was later expanded and P-selectin levels (53.1 ng/mL) have been incorporated (Modified Khorana score) [156]. However, P-selectin levels are not used in clinical practice because of their poor standardization, their low availability and their high cost.

Several mechanisms have been proposed for platelet involvement in venous thrombosis. First, activated platelets exhibit a procoagulant surface by exposing negatively charged phospholipids that can induce thrombin generation leading to fibrin formation and

clotting [157]. Second, platelet-leukocyte interactions appear to be crucial for platelet-rich microthrombi generation in adenocarcinoma mice because it was markedly diminished in P- or L-selectin-deficient mice [158]. Third, platelet adhesion to endothelium was demonstrated to be a critical step for deep vein thrombosis in mouse models [82]. Finally, platelets were found to induce formation of Neutrophil Extracellular Traps [159], which may promote the establishment of a pro-thrombotic state during cancer [160]. These findings suggest a crosstalk between inflammation and platelet-dependent thrombosis in cancer (**Figure 1**). Thus, cancer development is composed of pro-thrombotic and pro-inflammatory states, confirming the emerging concept of immunothrombosis.

### **c) Antiplatelet drugs and cancer prevention**

Over the last few decades, numerous clinical studies have emerged suggesting that taking aspirin daily may substantially lower cancer incidence, metastasis and mortality. A 2012 meta-analysis [161] of 5 randomized clinical trials (RCTs) of aspirin versus no aspirin in the prevention of cardiovascular diseases included 17,285 participants. Aspirin treatment lowered the risk of cancer with distant metastasis (all cancers hazard ratio (HR) 0.64, 95% confidence interval (CI) 0.48 to 0.84). When patients were stratified by cancer type, the risk reduction remains statistically significant for adenocarcinoma (HR 0.54, 95% CI 0.38 to 0.77) but not for other solid cancers (HR 0.82, 95% CI 0.53 to 1.28). In adenocarcinoma patients, not only the risk of metastasis upon initial diagnosis (HR 0.69, 95% CI 0.50 to 0.95) but also the risk of future metastasis in patients without initial metastasis (HR 0.45, 95% CI 0.28 to 0.72) and the risk of death by cancer (HR 0.50, 95% CI 0.34 to 0.74) was reduced. In another meta-analysis of 51 RCTs including 77,549 participants [161], daily aspirin treatment significantly reduced cancer deaths (odds ratio (OR) 0.85, 95% CI 0.76 to 0.96). Interestingly, when patients were stratified by follow-up duration, aspirin's benefit in cancer mortality remained significant only for >5 years of follow-up (OR 0.63, 95% CI 0.49 to 0.82). The putative mechanisms explaining aspirin benefits in cancer include inhibition of cyclooxygenase-1 and -2. In contrast, the randomized Physicians' Health Study [162] and the Women's Health Study [163] found no association between the use of aspirin and incidence of total, breast, colorectal, or other site-specific cancers. Several large clinical trials including the Cancer prevention project 3 (CAPP3), the aspirin for dukes C and high risk dukes B colorectal cancer (ASCOLT), the aspirin in reducing events in the elderly (ASPREE) and the aspirin and esomeprazole chemoprevention in barrett's metaplasia (AspECT) studies have been launched to evaluate whether aspirin use can reduce the risk of cancer incidence and



death due to cancer. Several important questions must still be answered before aspirin can be considered as cancer adjuvant therapy. Specifically, what dosage provides the best efficacy and safety, and who may benefit from aspirin use? It is important to note that the United State preventive services task force (USPSTF) recently post a draft recommendation statement (www.uspreventiveservicestaskforce.org) on recommendations for the use of aspirin to prevent cancer and will soon publish its final recommendation statement.

Moreover, aspirin may be used to prevent the risk of cancer-associated thrombosis in high-risk patients. Several studies have investigated the benefits of aspirin for the prevention of secondary VTE in the general population. A meta-analysis of the anti-platelet trialists' collaboration showed that aspirin reduces the risk of both deep vein thrombosis (DVT) and pulmonary embolism (PE) by approximately 40% in high-risk patients [164]. More recently, the PEP study, including more than 17,000 patients undergoing surgery for hip fracture or elective arthroplasty, confirmed that aspirin reduces the risk of VTE by at least one-third [164]. However, the results concerning the effect of aspirin as the primary prevention of VTE are less clear. In a case-control study on determinants and interaction of venous thromboembolism, aspirin use was associated with a significant 50% decreased risk of VTE (OR=0.50, 95% CI 0.34-0.74), whereas neither thienopyridines nor non-steroidal anti-inflammatory drugs had a significant effect on VTE risk [165]. Conversely, The Women's Health Study, a randomized trial of low-dose aspirin for the primary prevention of cardiovascular disease and cancer in initially healthy women, found no significant decrease in VTE risk in patients treated with aspirin compared to the placebo [39]. The warfarin and acetyl salicylic acid trial was the first prospective, randomized, multicenter, double-blind, placebo-controlled study evaluating the use of 100 mg of aspirin daily for the secondary prevention of VTE in patients in whom vitamin K antagonist therapy had been discontinued. This study demonstrated a significant reduction in recurrent VTE among patients treated with 100 mg daily aspirin compared to the placebo. (HR=0.58, 95% CI 0.36-0.93, p=0.02) [166]. The aspirin to prevent recruitment of venous thromboembolism trial only showed a non-significant reduction in the primary outcome of recurrent symptomatic DVT or PE in patients with an unprovoked VTE who had completed at least three months of anticoagulation therapy and received 100 mg of aspirin daily compared to the placebo (HR=0.74, 95% CI 0.52-1.05, p=0.09). A combined analysis demonstrated both a clinically and statistically significant benefit of aspirin with a 32% reduction in rate of recurrent VTE (HR=0.68, 95% CI 0.51-0.90, p=0.007) [167]. The results of an open-label randomized study in multiple myeloma patients treated with thalidomide suggest that aspirin is at least as effective as warfarin, compared to

LMWH, for VTE prevention [168]. Concordantly, the [national comprehensive cancer network](#) guidelines recommend 81 mg to 325 mg of aspirin daily as an option only for multiple myeloma patients with one or no risk factors.

## Conclusion

In the present review, we aimed to summarize the main molecular mechanisms by which cancer cells activate platelets and platelets participate in cancer progression, as well as their role in promoting cancer-associated VTE.

Several markers that reflect platelet activation during cancer have been evaluated as potential biomarkers of cancer diagnosis, prognosis and cancer-associated VTE with promising results. However, most of these biomarkers are expensive and not well standardized and not easily measured in clinical practice.

Targeting platelets has otherwise been proposed as a promising area for the development of new anticancer and antithrombotic strategies that could improve care and survival for cancer patients. Animal models provide convincing results, but these results should be interpreted with caution because some of the animal models used are not representative of the physiopathological steps of cancer clinical history. Even if important meta-analysis studies have suggested that taking aspirin daily may substantially lower cancer incidence, metastasis and mortality, results from clinical trials evaluating the use of aspirin in cancer remain controversial. Additional double-blind, randomized controlled trials evaluating the use of anti-platelets drugs in combination with current chemotherapeutic drugs are required. It is important to note that the use of drugs to target platelets could expose patients to a non-negligible bleeding risk that is often overlooked in experimental studies. Other side effects of using drugs to target platelets should be taken into consideration in future randomized controlled trials evaluating the use of anti-platelets drugs in cancer patients. In the future, a personalized medicine approach, as that proposed by Wistuba et al. [169], will probably be developed to prevent, diagnose, treat and follow-up patients suffering from cancer.

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### **Figure legends:**

**Figure 1: Schematic representation of platelets involvement in cancer-associated immunothrombosis.** Platelets are key actors of thrombosis and inflammation related to cancer progression. Following their activation, platelets overexpressed different proteins at their surface, released their granules contains and secreted microparticles. These different elements participate in tumor progression (tumor growth, metastasis development or angiogenesis; in red), in inflammation (in blue) and in Trousseau syndrome (in green).

### **Table legends:**

**Table 1: Experimental investigations targeting platelets during cancer.**

**Table 2: Experimental investigations targeting platelets activation during cancer.**

**Table 3: Experimental investigations targeting contents of platelet granules during cancer.**

**Table 4: Experimental investigations targeting platelet interactions during cancer.**

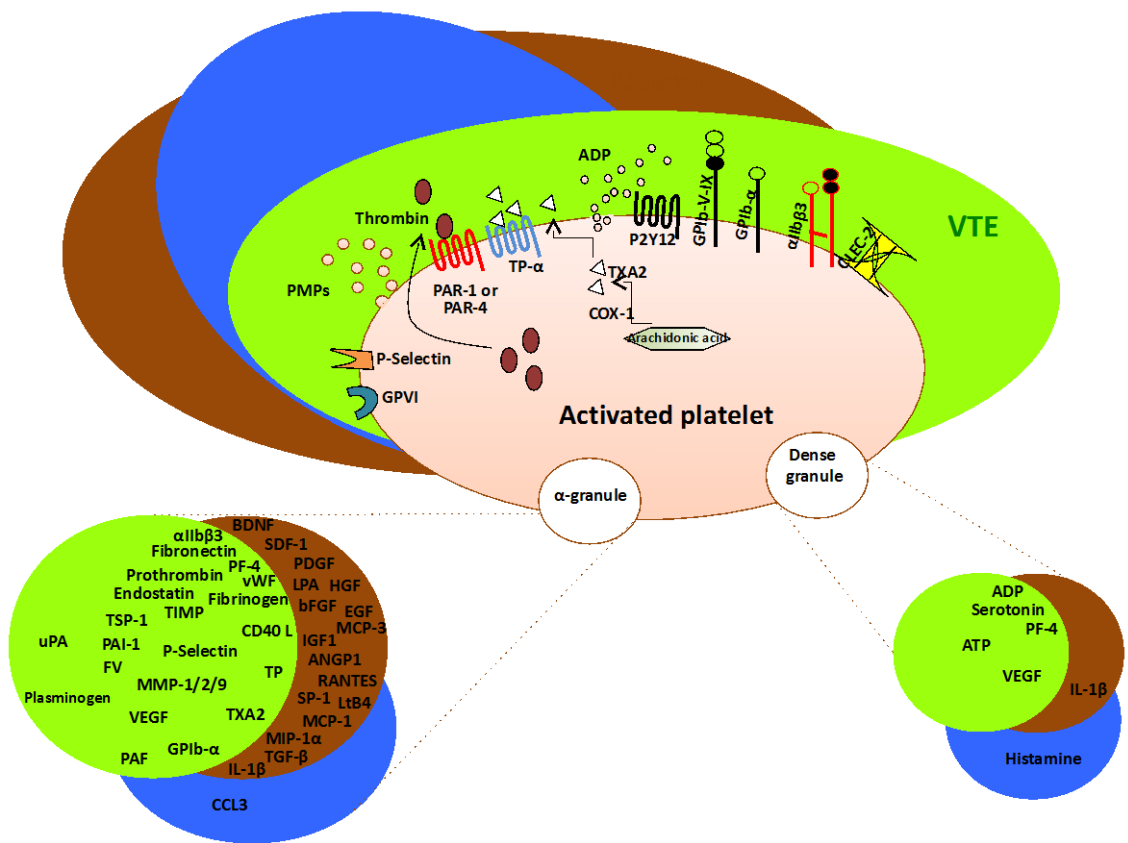


Fig. 1

**Table 1 Experimental investigations targeting platelets in cancer** (Ch:Chemical; Em: Experimental metastasis; Ec: Ectopic; Ge:Genetic, MET: Epithelial-mesenchymal transition; Or:Orthotopic;).

Platelet target (Physiological functions)	Implication in malignancy	Experimentation	Impact on malignancy	Experimental model	References
<b>Platelets (Hemostasis)</b>	Tumor growth	<ul style="list-style-type: none"> <li>• Antibody induced thrombocytopenia</li> <li>• Genetic depletion</li> </ul>	<ul style="list-style-type: none"> <li>• Inhibition of lung metastasis →Rescue by restoration of human platelet count</li> </ul>	Em	[3] [4]
	Angiogenesis	<ul style="list-style-type: none"> <li>• Neuramidase in mice bearing a tumor</li> </ul>	<ul style="list-style-type: none"> <li>• Reduction by 50% of metastases and thrombocytopenia</li> </ul>	Em	[5] [1]
	Metastasis TCIPA, MET Tumor cells invasion	<ul style="list-style-type: none"> <li>• Thrombocytopenia in mice bearing a lung or melanoma cancer</li> </ul>	<ul style="list-style-type: none"> <li>• Intra-tumor hemorrhage involved by an absence of secretion of platelet granules</li> </ul>	Ec	[6]
<b>Nfed-2</b>	-	<ul style="list-style-type: none"> <li>• Nfed-2 (<i>-/-</i>) (induce a thrombocytopenia)</li> </ul>	<ul style="list-style-type: none"> <li>• Decrease of lung metastasis</li> </ul>	Em	[7]

**Table 2 Experimental investigations targeting platelets activation in cancer** (Gnbp: Guanine-nucleotide-binding protein; Em: Experimental metastasis; Ec: Ectopic; Or:Orthotopic; Ch:Chemical; Ge:Genetic).

Platelet target (Physiological functions)	Implication in malignancy	Experimentation	Impact on malignancy	Experimental model	References		
<b>Gnbp (Platelet activation)</b>	Metastasis	<ul style="list-style-type: none"> <li>Mice lacking <i>Gaq</i></li> </ul>	<ul style="list-style-type: none"> <li>Decrease of spontaneous and experimental metastasis →Reversed by immunologic or genetic depletion of NK cells</li> </ul>	Em and Or	[25]		
		<ul style="list-style-type: none"> <li>Pretreatment of cancer cells with thrombin</li> <li>Preinfusion of thrombin into mice</li> </ul>	<ul style="list-style-type: none"> <li>Enhance 4-400 fold of experimental lung metastasis</li> <li>Enhance 10-160 fold of experimental lung metastasis</li> </ul>	Em	[3]		
<b>Thrombin (Platelet activation)</b>	Metastasis TCIPA VTE Tumor growth Angiogenesis	<ul style="list-style-type: none"> <li>Dansylarginine N-(3-ethyl-1,5-pentanediyl) amide a specific thrombin inhibitor</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of TCIPA of human colon adenocarcinomas and anaplastic mouse tumor</li> </ul>	Em	[26]		
		<ul style="list-style-type: none"> <li>Bivalirudin drug</li> <li>FM19 an reversible direct thrombin inhibitor</li> </ul>	<ul style="list-style-type: none"> <li>Decrease prostate cancer cell invasion, tumor growth (size and weight) and angiogenesis</li> </ul>	Ec	[27]		
<b>TXA2 (Platelet activation)</b>		<ul style="list-style-type: none"> <li>OKY-046 (Inhibition of TXA2 synthase)</li> </ul>	<ul style="list-style-type: none"> <li>No effect on osteogenic sarcoma cell induce TCIPA</li> </ul>	<i>In vitro</i>	[28]		
<b>ADP (Platelet activation, adhesion and aggregation)</b>	Metastasis TCIPA Activation of platelets and release of VEGF	<ul style="list-style-type: none"> <li>Ticagrelor treatment (10 mg/Kg)</li> </ul>	<ul style="list-style-type: none"> <li>Reduction of lung and liver metastases</li> <li>Inhibit platelet/cancer cells interaction</li> </ul>	Em and Ec	[29]		
		<ul style="list-style-type: none"> <li>Clopidogrel treatment (8mg/Kg/day)</li> </ul>	<ul style="list-style-type: none"> <li>Decrease tumor growth; metastasis and thrombosis associated with cancer in pancreatic mice models</li> </ul>	Or and Ec	[30]		
		<ul style="list-style-type: none"> <li>APT102 (Apyrase/ADPase; mutated form of CD39) + Aspirin</li> </ul>	<ul style="list-style-type: none"> <li>Decrease breast cancer and melanoma bone metastasis</li> <li>Diminish TCIPA; <b>without bleeding</b></li> </ul>	Em and Ec	[31]		
		<ul style="list-style-type: none"> <li>SR 25989 thienopyridine (enantiomer of Clopidogrel)</li> <li>Ticlopidine (P2Y antagonist)</li> </ul>	<ul style="list-style-type: none"> <li>Inhibit angiogenesis (by thrombospondin-1 release)</li> <li>Inhibit metastasis dissemination and growth</li> </ul>	Ec ,Em and <i>in vitro</i>	[32][33][34]		
		<ul style="list-style-type: none"> <li>Apyrase (250µg/ml)</li> </ul>	<ul style="list-style-type: none"> <li>Reduce TCIPA; Inhibition of platelet function</li> </ul>	<i>In vitro</i>	[19][35]		
		<ul style="list-style-type: none"> <li>MRS2179 (P2Y<sub>1</sub> antagonist)</li> <li>Cangrelor (P2Y<sub>12</sub>)</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of VEGF</li> <li>Minimal increase</li> </ul>	<i>In vitro</i>	[36]		

		antagonist)	of endostatin		
		• 2-methylthio-AMP	• Decrease TCIPA	<i>In vitro</i>	[18]
<b>PARs (Platelet activation by thrombin)</b>	Metastasis TCIPA	• PAR-4 ( / )	• Decrease metastasis	Em	[7]
		• PAR-4 heterozygosity mice	• Some protection to metastasis		
		• PAR-1 and PAR-2 ( / )	• No metastasis protection		
<b>COX-1 COX-2 (Platelet activation by TXA<sub>2</sub>)</b>	TCIPA Tumor growth Angiogenes is Metastasis	• Aspirin	• Inhibition of the release of activated platelets	<i>In vitro</i>	[37]
		• Aspirin (100 mg/Kg twice a day)	• Inhibition of lymph node metastasis	Or	[38]
		• ST0702 (nicotinate aspirin prodrug)	• Inhibition of TCIPA	<i>In vitro</i>	[39]
		• Aspirin low dose (5mg/Kg)	• Prevent cancer and increase the survival in mice models	Ch and Ge	[40]
		• Aspirin	• Suppression of VEGF release from platelets	<i>In vitro</i>	[41]
		• Aspirin (80, 100 and 150 mg/Kg/day)	• Decrease of tumor growth in melanoma mice model	Ec	[42]
		• Aspirin high dose (200 or 400 mg/Kg/day)	• Increase tumor growth, cell proliferation, angiogenesis	Ec	[43]
		• Aspirin treatment (10mg/Kg/day)	• Decrease metastasis in pancreatic mice model	Or	[44]
		• Aspirin (100 μM)	• Reduce TCIPA; Inhibition of platelet function	<i>In vitro</i>	[19][45]
		• Aspirin (50 μg/ml)	• Inhibit TCIPA of human breast cancer cell	<i>In vitro</i>	[46]
		• Aspirin low dose (12,5 or 25 mg/Kg/day)	• Decrease tumor growth	Ec	[43]



**Table 3 Experimental investigations targeting contents of the granules of platelets** (Em: Experimental metastasis; Ec: Ectopic; Or:Orthotopic; Ch:Chemical; Ge:Genetic).

Platelet target (Physiological functions)	Implication in malignancy	Experimentation	Impact on malignancy	Experimental model	References
<b>IGF-1 (Angiogenesis)</b>	Cancer progression Metastasis	• IGF-1 blocking antibody	• Inhibit the breast tumor growth	<i>In vitro</i>	[53][54]
		• Pegvisomant (GH receptor antagonist)	• Inhibit metastatic colon cancer in combination with chemotherapy and breast cancer cell	Em	[55]
<b>VEGF (Angiogenesis)</b>	Angiogenesis Tumor growth	• Bevacizumab	• Decrease of invasiveness of cancer cell	<i>In vitro</i>	[56]
		• Pazopanib (tyrosine kinase inhibitor)	• Antitumor activity in renal cell carcinoma	<i>In vitro</i>	[57]
		• Vatalanib PTK787/ZK 222584 (Tyrosine kinase inhibitor)	• Inhibit growth tumor and metastasis	Ec and Or	[58]
		• Sunitinib SU11248 (tyrosine kinase inhibitor)	• Anti tumor and angiogenic activity	Ec	[59]
<b>TGF-<math>\beta</math> (Cell proliferation, and migration)</b>	Cell proliferation $\rightarrow$ resistance by cancer cells	• 2G7 neutralizing antibody	• Moderate inhibition of tumor growth • Block metastasis	<i>In vitro</i>	[60]
<b>PDGF (Angiogenesis)</b>	Angiogenesis Tumor growth	• STI571 inhibit the phosphorylation of PDGF-R	• Inhibition of human prostate cancer bone metastasis	Ec	[61]
<b>EGF (Angiogenesis)</b>	Angiogenesis Tumor growth	• PKI166 inhibit the phosphorylation of EGF-R	• Inhibition of human prostate cancer bone metastasis	Ec	[61]
<b>MMPs (Matrix degradation Remodeling of tissue)</b>	Tumor cells invasion Angiogenesis Metastasis TCIPA	• Antisense RNA decrease MMP-1	• Decrease the invasiveness	<i>In vitro</i>	[62]
		• MMP-8 (/-)	• Increase of papillomas (carcinogen treatment)	Ch	[63]
		• MMP-2 (/-)	• Inhibit tumor induce angiogenesis	Ec and Or	[64]
		• MMP-9 (/-)	• Inhibit angiogenesis and metastasis	Em and Or	[65]
		• Curcumin (inhibit MMP-9 expression)	• Inhibit cell invasion		[66]
		• DX-2400 (MMP-14 inhibitory antibody)	• Inhibit angiogenesis, tumor growth and metastasis lesion	Or and Ec	[67]
		• Phenanthroline (inhibitor of MMPs) and neutralizing antibodies anti MMP-2	• Reduce TCIPA • Inhibition of platelet function	<i>In vitro</i>	[68]
		• Letrozole (inhibitor of MMP-2 and MMP-9)	• Inhibit cell proliferation, tumor growth and	<i>In vitro</i>	[69]

			invasiveness		
	•	Gebistein (decrease MMP-2)	•	Decrease tumor cell invasion	<i>In vitro</i> [70]

**Table 4 Experimental investigations targeting platelet interactions in cancer** (Em: Experimental metastasis; Ec: Ectopic; Or:Orthotopic; Ch:Chemical; Ge:Genetic).

Platelet target (Physiological functions)	Implication in malignancy	Experimentation	Impact on malignancy	Experimental model	References		
<b>GPVI (Activation, adhesion and aggregation)</b>	Metastasis	• GPVI (✓)	• Decrease metastasis (50% de reduction)	Em and Ec	[93]		
		• P-selectin (✓) • Blocking antibody directed against P-selectin	• Decrease tumor growth, TCIPA and metastasis • Reduction of tumor cells survival	Em and Ec	[94][95]		
<b>P-selectin (Stabilization of aggregation, rolling and adhesion)</b>	Tumor growth Metastasis	• Heparin tetrasaccharides treatment	• Block colon cancer cell adhesion to P-selectin	<i>In vitro</i>	[96]		
		• Heparin treatment	• Inhibition of platelet and cancer cells interactions	Em and Or	[97]		
		• Heparin treatment	• Inhibition of platelet cancer cells interaction and metastasis	Em	[98]		
		• Disaccharide precursor of Sialyl LewisX	• Inhibit metastatic potential of tumor cells	Em	[99]		
		• P- and L-Selectin double (✓) • Heparin treatment	• Inhibition of platelet P-selectin interaction with cancer cells <i>via</i> a sulfated glycolipid	Em	[100]		
		• Blocking antibodies or neuramidase and trypsin treatments	• Inhibition of platelet P-selectin and cancer cells interaction	<i>In vitro</i>	[101]		
		• P-selectin (✓)	• Attenuate both tumor growth and metastasis	Em and Ec	[102]		
		• P-selectin (✓)	• Reduction of lung experimental metastasis in mice	Em and Ec	[103]		
		• O-Sialoglycoprotease treatment (cleave mucin type-O-linked glycoproteins)	• Inhibition of adhesion of colon cancer cells	<i>In vitro</i>	[104]		
		• Dermatan sulfates (interact with P-selectin)	• Attenuation of thrombosis, metastasis and leukocytes in tumor	Em	[105]		
		<b><math>\alpha</math>IIb<math>\beta</math>3/GPIIb/IIIa (Adhesion and aggregation)</b>	Metastasis Interaction platelet/cancer cell (with fibrinogen, fibronectin and vWF) TCIPA	• Preincubation of human platelets with 10E5 antibody	• Inhibition of tumor cells adhesion to platelets	Em	[3][106]
				• RGDS treatment or antibodies directed against fibronectin and	• Inhibition of metastasis after thrombocytopenia		

Release of granules		vWF					
	•	Monoclonal antibody 10E5 in mice melanoma	•	Decrease of angiogenesis, TCIPA and tumor growth	Em and Ec	[76]	
	•	Antibody directed against $\beta 3$ (A11)	•	Decrease of pulmonary nodules; No effect on angiogenesis; Inhibit TCIPA	Em, Or and Ec	[107]	
	•	$\beta 3$ ( $\prime$ ) mice	•	Reduction of bone lesion and metastasis	Em	[108]	
	•	Blocking antibody (TM83)	•	Inhibit TCIPA	<i>In vitro</i>	[109]	
	•	7E3 F(ab') <sub>2</sub> (Targeted activated GPIIb/IIIa)	•	Lysis of activated platelets and protect the lung metastasis	Em, Or and Ec	[107]	
	•	XV454	•	Decrease TCIPA and metastasis and inhibit $\alpha v\beta 3$	Em	[110]	
	•	Integrilin (antagonist of GPIIb/IIIa)	•	Deprivation of LPA, decrease of tumor growth and bone metastasis	Em and Ec	[111]	
<b>GPIIb (Platelet adhesion)</b>	Metastasis	•	Blocking antibody (TM60)	•	Inhibit TCIPA	<i>In vitro</i>	[109]
		•	Inhibition by Fab (pOp/B Fab)	•	Increase metastasis	Em	[112]
		•	7E3 and SZ-1 (monoclonal antibodies)	•	Inhibit TCIPA of human breast cancer cell	<i>In vitro</i>	[35]
		•	GPIIb ( $\prime$ ) (thrombocytopenia and giant platelets)	•	Decrease of metastasis	Em	[113]

## Highlights

- The role of platelets in cancer progression and metastasis is described
- The mechanisms involved in thrombosis associated with cancer are discussed
- The use of anti platelet or anticoagulant drugs in cancer is considered.