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Effects of platelets on cancer progression

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ABSTRACT

Platelets are small (2–4 μm), anucleate, hematopoietic cells released by bone marrow megakaryocytes in the bloodstream. For a long time, platelets were described as the major effectors of hemostasis and thrombosis.

In 1865, Armand Trousseau demonstrated a close relation between thrombosis and cancer. Subsequently, much clinical and experimental evidence supports the idea that platelets play several roles in the progression of malignancies and in cancer-associated thrombosis.

In this review, we will discuss the roles of tumor-educated platelets (TEPs) in the progression of cancer from primary tumors to secondary metastatic outbreaks.

1. Introduction

Platelets are small (2–4 μm), anucleate, hematopoietic cells released by bone marrow megakaryocytes in the bloodstream. In healthy humans, the concentration of circulating platelets is approximately 150 to 350 × 10^9/L. Recently, in a mouse model, it was shown that platelets can also be produced in the lung, by intravascular megakaryocytes originating from extrapulmonary sites [1]. For a long time, platelets were described as the major effectors of hemostasis and thrombosis. The hemostatic functions of platelets were first described in 1873 by Osler, who showed the presence of “blood plaques” in white thrombi.

In 1865, Armand Trousseau demonstrated a close relation between thrombosis and cancer [2]. Subsequently, much clinical and experimental evidence supports the idea that platelets play several roles in the progression of malignancies and in cancer-associated thrombosis [3].

Platelets are a huge reservoir of biomolecules, including platelet-specific and circulating ingested biomolecules. Upon activation, platelets release the biomolecule content on their granules that participates in the progression of malignancy. A real cross-talk exists between platelets and cancer. Indeed, cancer itself can influence platelet count and activation state which is critical for cancer progression. Inhibition of thrombocytosis or induction of thrombocytopenia are related to a decrease in tumor growth and metastasis, indicating that the ability of cancer cells to enhance platelet count is not harmless [7,8]. However, the roles of platelets in tumor growth are subjects of controversy. On one hand, much evidence has demonstrated that platelets enhance cancer cell proliferation, pro-survival signaling, angiogenesis and invasiveness. For example, Egan and collaborators showed that platelet adhesion and degranulation induced pro-survival and pro-angiogenic signaling in ovarian cancer cells [9]. In addition, platelet adhesion and platelet-released factors seem to actively participate in the epithelial to mesenchymal transition (EMT) of cancer cells, promoting their invasiveness and metastatic potential [10]. On the other hand, a few studies demonstrated an anti-proliferative role of platelets or platelet-derived microparticles on cancer cells, with the induction of cell cycle arrest and inhibition of DNA synthesis, and the induction of apoptosis, respectively [11,12].

Cancer cells can also “educate” platelets by modulating their RNA profiles and phenotypes. Platelets RNA profiles changes were report in several cancer types including lung, prostate, glioma and breast carcinoma [4–6]. Using RNA-seq analysis, Best et al. were able to distinguish cancer patients from healthy individuals with 96% of accuracy, suggesting that the emerging concept of tumor educated platelets (TEPs) may provide interesting tools for cancer diagnostic [6]. In this review, we will discuss the roles of platelets and tumor-educated platelets (TEPs) in the progression of cancer from primary tumors to secondary metastatic outbreaks.

2. Growth and angiogenic factors in tumor growth, angiogenesis and metastasis

Platelet alpha granules are enriched in mitogens and growth factors

Abbreviations: TAN, tumor associated neutrophils; TAM, tumor associated macrophages; CAF, cancer associated fibroblast; ECM, extracellular matrix; PMPs, platelets microparticles; TCIPA, tumor cell induced platelet aggregation

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such as TGF-β (transforming growth factor β), EGF (epidermal growth factor) and PDGF (platelet-derived growth factor). Platelet-derived growth factors are peptides that signal through cell-surface receptor tyrosine kinases (PDGFR) and activate various cellular functions such as proliferation, growth and differentiation. Upon activation, platelets release mitogens including PDGF and EGF that are both involved in the regulation of proteolytic enzyme activity and are released by cancer cells, mediating their invasive behavior [13]. PDGF, through its signaling, is also involved in the acceleration of growth of some metastatic breast tumors. Furthermore, the elevated serum level of PDGF in breast cancer patients is associated with a significantly greater degree of metastasis and shortened survival [14]. PDGF overexpression was proposed as prognostic marker in colorectal cancer [15]. Its expression has also been identified in various types of solid tumors, including glioblastomas and prostate carcinomas [16].

Platelet alpha granules also contain vascular endothelial growth factor (VEGF), a growth factor involved in the induction of vasculogenesis and angiogenesis. The notion of tumor angiogenesis was first described by Folkman and collaborators in 1974 [17]. They demonstrated that tumors cannot reach more than 2 mm without forming new blood vessels to support the diffusion of oxygen and nutrients required for tumor growth. VEGF, the strongest angiogenic factor, and its signaling, through its VEGF-R, stimulates endothelial cell migration, proliferation and vessel formation in the tumor microenvironment. Platelets contain a plethora of angiogenesis-regulating proteins in their granules: (i) proteins that positively regulate angiogenesis, including VEGF, PDGF, TGF, EGF, angiopoietin-1, IGF-1, sphingosine-1-phosphate and MMPs and (ii) proteins that negatively regulate angiogenesis, including platelet factor-4 (PF4), thrombospondin-1 (TSP-1), endostatin, serotonin, PAI-1 and angiostatin [18]. In addition, Rong Li and colleagues demonstrated that intratumoral platelets are implicated in the regulation of vessel density and the maturation of blood vessels, mainly by VEGF and TGF-β released by the platelets [19]. The angiogenic role of VEGF has been demonstrated in various type of cancers, including colorectal, breast, lung and ovarian carcinomas, which led to the development of therapeutic VEGF monoclonal antibodies and tyrosine kinase inhibitors [16,20].

TGF-β is another mitogen contained in alpha granules and released upon platelet activation. TGF-β signaling, through its serin/threonine protein kinase receptor, is involved in several biological processes. Overexpression of TGF-β has been demonstrated in various cancer types, including colon, breast, esophageal, gastric, hepatocellular, lung and pancreatic cancer, and has been correlated with tumor progression, metastasis and poor prognostic outcome. However, depending on cancer stages and microenvironment, TGF-β can have the opposite effects. For example, in early stages, TGF-β can act as a tumor suppressor and potently inhibit cancer cell proliferation and tumor growth [21]. However, TGF-β released by platelets into the microenvironment is mostly considered a tumor growth and metastasis promoter [22]. Platelet-derived TGF-β1 has been described as increasing human and mouse ovarian cancer cell proliferation [23]. Furthermore, the inhibition of TGF-β signaling in cancer cells (induced by an overexpression of dominant negative TGF-β receptor II) strongly reduced intravasation and lung metastasis [24,25]. Direct interaction between platelets and cancer cells and the release of platelet-derived TGF-β synergistically activates TGF-β/smad and NF-kB pathways in cancer cells, resulting in the acquisition of an invasive mesenchymal-like phenotype *in vitro* and the enhancement of metastasis *in vivo* [10].

3. Platelet agonists, hemostatic factors and procoagulant proteins in tumor growth, angiogenesis and metastasis

Some cancer cells can release platelet agonists, including ADP, TXA2 and thrombin, that have all been shown to induce platelet activation and to contribute to tumor growth and metastasis [26–28]. Cho et al. demonstrated that platelets enhance ovarian cancer cell proliferation in a TGF-β1-dependent manner [23]. Moreover, this team recently showed that inhibition of the platelet ADP receptor P2Y12 by ticagrelor reduced ovarian tumor growth by 60% in comparison to aspirin and by 75% in comparison with placebo-treated mice [29]. Using an orthotopic pancreatic cancer mouse model, our team demonstrated that inhibition of platelet activation by clopidogrel, another P2Y12 inhibitor, reduces pancreatic tumor growth and metastasis [30]. In addition, Simon Gebremeskel and colleagues showed that reversible inhibition of P2Y12 by ticagrelor inhibits metastasis and improves survival in a mouse model of melanoma [31]. These findings support a role of P2Y12-mediated platelet activation in promoting tumor growth and metastasis and provide proof of concept for the clinical use of P2Y12 inhibitors such as clopidogrel and ticagrelor for the prevention of tumor progression and metastasis. In addition, platelet-tumor cell interaction-dependent release of ATP has been shown to enhance vascular permeability through activation of the endothelial P2Y2 receptor, facilitating tumor cell extravasation and metastatic seeding [32].

Thromboxane released following platelet activation by cancer cells has been highlighted since the 1980s and is positively associated with ovarian cancer advancement. Overexpression of thromboxane receptors and thromboxane synthase has been reported in various types of cancers, including colorectal, prostate, bladder and non-small-cell lung carcinomas. The inhibition of thromboxane synthase activity reduced tumor proliferation, which was rescued by TXA2 addition, and induced apoptosis [33,34].

Thrombin generation following platelet activation that is mediated by cancer cells and thrombin secretion by cancer cells themselves play several roles in tumor growth, angiogenesis and invasion. Thrombin treatment up-regulates cathepsin D (CD) mRNA and protein expression in different cancer cell lines, including human and mouse breast carcinomas and prostate carcinomas and melanomas, and in primary endothelial cells, also called HUVEC (human umbilical vein endothelial cells). In these cell lines, up-regulation of CD expression and secretion was responsible for the enhancement of cancer cell chemotaxis and migration, and HUVEC Matrigel tube formation. In addition, pharmacological inhibition of thrombin by hirudin and the use of CD knocked-down cancer cells strongly reduces tumor growth and metastasis [35,36]. Thrombin also promotes tumor invasion through the induction and association of MMP-9 and β1-integrin on the cell surface *via* a PI3K-dependent pathway [37]. Furthermore, thrombin can induce EMT in SKOV3 ovarian cancer cells, promoting their invasive properties [38]. In addition, thrombin up-regulates Tissue Factor (TF) activity and VEGF released by MDA-231 breast cancer cells that enhanced their metastatic potential [39].

TF is a transmembrane glycoprotein involved in the initiation of the extrinsic pathway of normal blood coagulation protease cascade. TF can bind factor VIIa (TF/fVIIa) to form an active complex responsible for the proteolytic activation of factor IX and X, thrombin generation and fibrin deposition. Extravasation of coagulation factors, mostly due to an enhanced tumor vasculature permeability, were observed in the tumor microenvironment. Liu and colleagues demonstrate that TF expressed by cancer cells and the TF-activated coagulation cascade in the tumor microenvironment play critical roles in tumor growth. Treatment of mice bearing breast tumors with an inhibitor of TF/FVIIa, led to growth retardation, whereas treatment with doxorubicin-based prodrugs that are selectively activated by the protease activity of TF, FVIIa, FXa and thrombin, totally eliminated primary tumors and metastasis [40]. The deposition of the non-coagulant alternatively spliced isoform of TF into the tumor stroma led to the induction of angiogenesis through the ligation of endothelial integrins αvβ3 and α6β1. Moreover, this team showed that inhibition of TF-VIIa-PAR2 signaling but not TF-initiated coagulation suppressed breast tumor growth and angiogenesis [41]. Taken together these studies demonstrate that TF plays critical roles in tumor growth and angiogenesis and that the protease activity of the coagulation cascade in the tumor microenvironment may serve as an enzymatic target to chemotherapeutic pro-drugs.