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Review

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Biomarkers in Medicine

The aegis: platelets as biomarkers of tumor progression

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There is increasing awareness that platelets play a significant role in creating a hypercoagulable environment that mediates tumor progression, beyond their classical hemostatic function. Platelets have heterogenic responses to agonists, and differential release and uptake of bioactive molecules may be manipulated via reciprocal cross-talk with cells of the tumor microenvironment. Platelets thus promote tumor progression by enhancing tumor growth, promoting the development of tumor-associated vasculature and encouraging invasion. In the metastatic process, platelets form the shield that protects tumor cells from high-velocity forces and immunosurveillance, while ensuring the establishment of the pre-metastatic niche. This review presents the complexity of these concepts, considering platelets as biomarkers for diagnosis, prognosis and potentially as therapeutic targets in cancer.

First draft submitted: 8 November 2019; Accepted for publication: 6 February 2020; Published online: 28 May 2020

Keywords: angiogenesis • biomarker • cancer • epithelial–mesenchymal transition • metastasis • platelet • thrombosis • tumor progression

Cancer is a global concern, with approximately 18 million new cases and 9.6 million cancer deaths predicted worldwide for 2018 [1]. Following metastasis, venous thromboembolic complications are a leading cause of cancer-related mortality and morbidity [2,3]. The association between cancer and vascular events was identified as early as 1000 BC by the Indian surgeon Sushruta in the Sanskrit treatise 'Sushruta Samrita' [4]. In 1865, the French physician Armand Trousseau described migratory thrombophlebitis as an associative pre-warning system of occult malignancy, confirming his observations by self diagnosing his own thrombotic complications before succumbing to gastric cancer [5]. In later years, the term 'Trousseau's syndrome' was consolidated to an extent and presented as a range of signs and symptoms associated with thrombosis and various stages of tumor presentation, from the occult to the overt [4,5].

The pathophysiology of thrombosis considers parameters of abnormal blood flow, dysregulated cellular constituents and aberrations of the vascular wall, all of which are typically presented by cancer patients [6–8]. Thrombotic disorders are discriminated as either venous or arterial disorders based on clinical presentation and anatomical features. Arterial thrombosis, predominantly linked with platelet activation and endothelial cell wall damage, is described as a platelet-rich clot generated under high shear stress that, clinically, can present as myocardial infarction and ischemic stroke [6,8]. Venous thrombosis, described as associated with activation of coagulation processes and downstream engagement of platelets, are essentially fibrin-rich clots generated under low shear stress independent of vascular wall damage [6,7,9], which, clinically, presents as deep vein thrombosis, splanchnic vein thrombosis and pulmonary embolism [3,6]. Cancer therapies including radiotherapy, selected chemotherapies and treatment with monoclonal antibodies and tyrosine kinase inhibitors are associated with increased risk for thrombosis [8,10]. A closer look at the cellular contents of blood to understand the pathophysiology of thrombosis reveals that platelets are a principal cell type involved in creating a hypercoagulable state in cancer patients [3,11–13]. While the mechanisms and manifestations of 'Trousseau's syndrome' are diverse, that platelets also play a significant role in tumor progression is no longer in dispute, with clear evidence from both preclinical and clinical studies; however, the precise interaction between tumor cells and platelets in the life history of a tumor require elucidation to determine

Future Medicine

whether platelets can indeed be biomarkers that hold diagnostic or prognostic significance or whether they can be targeted for therapy.

Platelet structure & function

Platelets were identified in 1882 as blood elements contributing to thrombosis by the Italian physician Giulio Bizzozero. Mature discoid platelets are anucleate, typically 2–3 μm in diameter with an average lifespan of 7 days [14]. In healthy human circulation the platelet count can range between 150,000 and 400,000/μl; however, cancer is typically associated with thrombocytosis defined as a platelet count greater than 400,000 or 450,000/μl [15].

Hemostatic and nonhemostatic functions of platelets are mediated by a range of biologically active compounds stored within granules and released during activation. Three types of granules identified at an ultrastructural level include alpha (α)-granules, dense (δ) granules and lysosomes. In resting platelets, these granules together with organelles, typically but not exclusively, occupy a central position known as the granulomere [4,16].

Presenting with an electron dense core, α-granules are the largest (200–400 nm) and most numerous granules (40–80 per platelet) [17]. The vast majority of factors are contained within α granules and include several membrane proteins involved in adhesion (e.g., GPIb complex, GPIIb/IIIa, P-selectin/CD62p); coagulation factors (e.g., Factor's V, XI, XIII; fibrinogen and vWF); growth factors involved in inflammation, wound healing and angiogenesis (e.g., PDGF, VEGF, TGF-β, HGF), an array of chemokines (e.g., CCL2, CCL5, CXCL4, CXCL5); and matrix metalloproteinases (e.g., MMP-1, MMP-2, MMP-9) for activation of proteins via cleavage and tissue remodeling [4,18,19].

Dense δ granules, termed as such for their electron dense core, are approximately 250 nm in size with three–eight granules present per platelet. The contents of these granules include high concentrations of nucleotides, ADP and ATP; catecholamines, serotonin and lysosome membrane proteins (LAMP2 and LAMP3 [CD63]) that are associated primarily with recruitment of additional platelets following engagement of the hemostatic response [14,20]. A third category of platelet granules and the least numerous (zero–three lysosomes per platelet), are electron dense lysosomes that range between 175–250 nm in size [16]. Lysosomes contain hydrolytic enzymes and CD63 thereby contributing to the latter stages of clot formation, fibrinolysis and extracellular matrix remodeling [20,21].

In their classical hemostatic role, platelet surface glycoprotein receptors respond to vascular damage by interacting with subendothelial matrix proteins including vWF, collagen and fibronectin [22]. This enables secure platelet attachment to the vascular wall and facilitates platelet aggregation via binding of amino acid sequences, arginine–glycine–asparagine with or without serine, to GPIIb/IIIa [23]. Local activation of platelets within the vasculature can also be induced by endothelial cell expression of tissue factor (TF) – a major initiator of the coagulation cascade which ultimately results in conversion of prothrombin to thrombin [24]. Thrombin, regarded as the prime agonist of platelet activation, binds to protease-activated receptors one or four (high affinity PAR1, low affinity PAR4), which are part of the G-protein coupled receptor class. In addition to engagement of other cognate agonist receptors, this results in a cascade of intracellular events activating PI3K and PLC pathways, thereby mediating the release of stored calcium ions, platelet shape changes and ultimately secretion of granular contents [14,25].

Sorting and docking of α granules is first facilitated by N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes, which include vesicle-associated membrane proteins (e.g., VAMP-8) isoforms, plasma membrane syntaxins (e.g., syntaxin 11) and synaptomsomal-associated proteins (e.g., SNAP-23) [26,27]. These molecules mediate fusion of α granules (singular or compound fusion) either directly with the plasma membrane or with the open canalicular system (OCS) – a unique series of plasma membrane invaginations that serves as an entry point for signaling molecules and an exit point for the release of granular cargo to the exterior [26–28]. As a result, CD62p translocates from the α granules to be exposed on the noncytosolic side of the platelet plasma membrane where ligation with PSGL-1 mediates interaction with leukocytes and other cell types expressing the ligand [29]. CD62p expression is regarded as a critical, irreversible point in platelet activation and is thus a gold standard in defining platelet activation status [29]. During thrombus formation CD62p expression is associated with a highly active platelet core mediated by thrombin activity, surrounded by less active CD62p-negative platelets mediated by the secondary agonists, ADP and thromboxane A_2 (TXA_2) [30,31]. However, loss of CD62p has also been implicated as reflecting highly active platelets, potentially associated with microvesicle release [28]. Identifying markers less likely to be affected by the heterogeneity of the platelet response is thus essential. Recently, triggering receptor present on myeloid cells (TREM)-like transcript 1 (TLT-1) has been suggested as a more sensitive and stable marker of platelet activation [29].

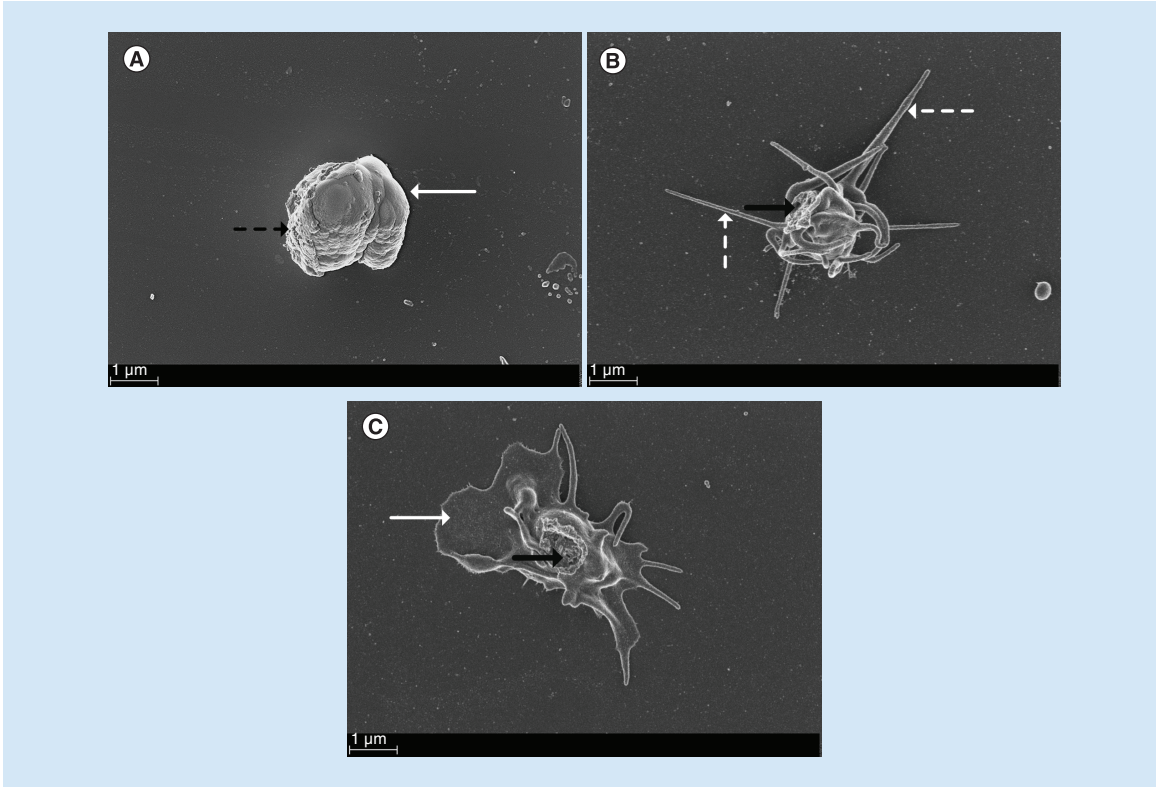


Figure 1. Scanning electron micrographs showing changes in morphology during platelet activation. (A) Resting, discoid platelet with most of the plasma membrane, smooth (white arrow) and some membrane ruffles noted (dashed black arrow). **(B)** Platelet in early stage of activation showing extension of pseudopodia (dashed white arrow) reflecting cytoskeletal alterations. Putative pores to the open canalicular system shown (black arrow). **(C)** Platelet in late stage of activation showing spreading of the plasma membrane (white arrow) with pores to the open canalicular system evident.

Platelets possess another system of channels, the dense tubular system (DTS) derived from megakaryocyte smooth endoplasmic reticulum, a closed system linked to calcium ion storage [17]. During engagement of cognate agonist receptors, downstream activation of phospholipase C isoforms facilitates a sharp and fast increase in cytosolic calcium ion concentration, coupled with secondary influx mediated by P2X1 calcium ion channels [4,32,33]. This increase in the availability of calcium ions is regarded as essential for platelet activation, promoting granular exocytosis [26,33] and facilitating actin-myosin contraction to induce the extension of pseudopodia and ultimately cell body (hyalomere) spreading for which the OCS provides an additional membranous component [17,21]. Late-stage platelet activation is achieved by the subsequent release of agonists from α and δ granules, including thromboxane, ADP and prothrombin in a positive feedback loop (Figure 1). Ligand binding with the GPIIb/IIIa receptor then facilitates cross-linking of proteins, ultimately facilitating platelet aggregation [25]. Recent data are however, highlighting that platelet function may be more dynamic than revealed by gross morphological changes and aggregation alone [34]. Activation of GPVI via exposure to low collagen concentration, while insufficient to induce the hallmarks of activation including externalization of CD62p and phosphatidylserine (PS), morphological changes and aggregation, are nevertheless able to induce the release of soluble factors from α and δ granules [34]. A nonclassical approach may thus indeed better explain platelet interaction with tumor cells in the tumor bed itself.

Proteomic analysis reveals that normal, healthy platelets contain approximately 4000 proteins [33], a testament to their function. Granular content, which is postulated to be heterogenous, is related to packaging of proteins during platelet development and may reflect the differential contents of demarcation compartments in megakaryocyte proplatelet extensions [20,35]. Platelets also possess an extensive transcriptome, translational machinery and the ability to conduct post-translational modifications that allow them to behave as a responsive cell type despite being anucleate [36,37]. The established platelet transcriptome and proteome can be further altered by receptor-mediated endocytosis, for example, Factor V; or receptor-independent endocytosis, for example, immunoglobulin

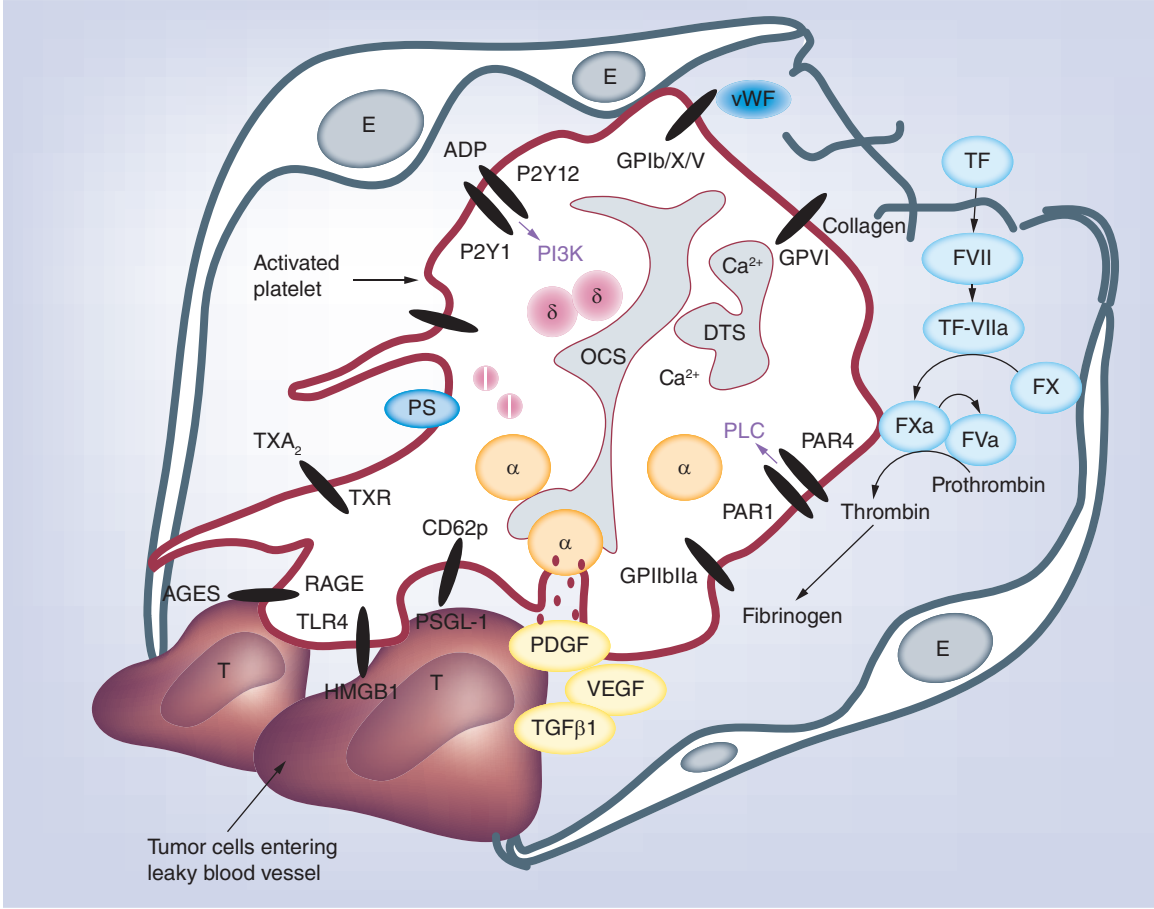


Figure 2. Schematic (not drawn to scale) showing tumor cells (T) entering a leaky blood vessel lined by endothelium (E) and interacting with an activated platelet (note spread cell body and extending pseudopodia). Several processes have been shown to highlight the complexity of this interaction. Exposed collagen and vWF is indicated binding to cognate platelet receptors (GPIb/IX/V, GPVI respectively). The impact of TF on generation of thrombin is shown. Thrombin-mediated generation of fibrin is shown, as well as binding to PAR (1/4) receptors. Agonists ADP and TXA_2 ligation on P2Y1 and P2Y12, and the TXR receptor respectively, is shown. Tumor expression of PSGL-1 (binding to platelet CD62p), AGEs (binding to platelet RAGE) and HMGB1 (binding to TLR4) are also able to activate platelets. Following platelet activation, as above, engagement of the PI3K and PLC pathways are indicated within the platelet. Impact on Ca_{2+} stores in the dense tubular system (DTS), and secretion of stored components of α granules (e.g., PDGF, TGFβ1 and VEGF) and δ dense granules via the plasma membrane and open canalicular (OCS) are highlighted. Presence of lysosomes (l) are shown as well as phosphatidylserine (PS) expression on the outer leaflet of the plasma membrane.

G [20,33,38,39]. Granular heterogeneity is also reportedly reflected by differential secretion in response to different agonists, at varying concentrations [34,40,41]. In addition to granular secretion, platelets shed microvesicles including exosomes (40–100 nm) and microparticles (100 nm–1 μM) that contain packaged proteins, mRNA and miRNA that functions in post-translational modification of mRNA and RNA silencing [21,42,43]. All these factors have potential for far-ranging effects and provide a platform that may be manipulated by reciprocal cross-talk with tumor cells or immune cells to mediate nonhemostatic functions (Figure 2).

Disease mechanisms: platelets in tumor progression

Platelets have an integral function during wound healing, regulating the balance between anti-angiogenic and pro-angiogenic factors, recruiting immune cells and forming and remodeling the hemostatic plug. This capacity is exploited to facilitate tumor progression [44].

Angiogenesis & growth

The selectin, CD62p is implicated in recruiting platelets into a range of solid tumors [45]. Platelets may thus interact with tumor cells via their large repertoire of secreted factors and microparticles or by forming heterotypic adhesions that facilitate their activation [43,44,46,47]. Tumor growth, while enabled by hypoxic and necrotic hot spots, is limited by the same and depends on vascularization to facilitate further growth by supplying oxygen and nutrients and removing wastes [18]. The critical role of platelets in tumor-associated angiogenesis is emphasized by platelet-depletion studies in ovarian carcinoma [47], melanoma and mammary carcinoma [44] mouse models, which show a corresponding reduction in blood vessel density and pericyte association [44,47]. Platelet-derived factors implicated in controlling the development and integrity of the vasculature include VEGF and TGF, which, along with other pro-angiogenic proteins such as PDGF and MMPs, also promote tumor growth and invasion [19,48].

Platelets from cancer-bearing patients contain a host of angiogenic regulators including ANGPT-1 and 5-HT, which prevent intra-tumoral hemorrhage [19], a risk of the modified vasculature in the tumor microenvironment. High plasma concentrations of VEGF have been identified in both pancreatic and lung cancer [49]. In advanced lung cancer, sustained VEGF signaling coupled with raised PDGF and a significant decrease in anti-angiogenic proteins TSP-1 and PF4 has been identified [49]. This reflects a change to a more aggressive phenotype in response to growth factors critical to facilitating tumor escape from cell cycle controls [19].

This range of growth factors can be released from platelet α granules in response to endothelial cell expression of TF and the resulting activation of the coagulation cascade. Importantly, tumor cells also directly secrete TF, thrombin and ADP [18,24,50]. Additionally, vWF previously thought to be exclusively associated with endothelium and megakaryocytes, has been identified in glioma and osteosarcoma and is postulated to contribute to the metastatic process [51]. Heightened TF expression and thus corresponding thrombin activity are associated with a multitude of solid tumors including malignant glioma, breast cancer, prostate cancer, bladder cancer and small-cell carcinoma [4,24,52,53].

Experimentally, platelet enhancement of tumor growth has been demonstrated both in *in vitro* and *in vivo* murine models of ovarian cancer, where tumor cell proliferation was significantly linked to the presence of platelets [47] and ADP ligation of platelet-P2Y12 receptors [50]. However, contradictory studies have demonstrated platelet-mediated inhibition of proliferation in murine lymphoma, melanoma and prostate cancer cell lines [54]. Similarly, platelet-derived microparticles, which by their capacity to transfer bioactive molecules to tumor cells are associated with promoting angiogenesis and invasion [19,43,53,55], have recently been found to inhibit tumor growth in a murine model via miRNA-24-transfer [43]. While such studies point to a far more complex role for platelets in tumor progression than previously thought, this does bring to the fore the need to assess the variable response of platelets to different activating factors that would govern their cross-talk with tumor cells and impact tumor progression.

Epithelial-mesenchymal transition

Platelets are proposed to facilitate epithelial-mesenchymal transition (EMT), a complex series of molecular changes where neoplastic epithelial cells lose apico-basal polarity, overcome anoikis and assume a migratory, invasive phenotype [14,44,56,57]. In the classic model of EMT, upregulation of transcription factors including *Snail1*, *Slug, Zeb1* and *TWIST*; and downregulation of adhesion molecules, such as *E-cadherin* and *occludins* facilitate acquisition of a mesenchymal-like phenotype [19,58–60]. Additionally, upregulation of *MMP*s that facilitate degradation of the basement membrane and underlying extracellular matrix aid tumor cell movement through the tissue [19]. The traditional concept of EMT has however, been revised to be inclusive of hybrid or partial EMT states where tumor cells display characteristics of both epithelial and mesenchymal cells thus reflecting variance in migratory and invasive behavior [58,61].

Direct tumor cell-platelet interaction is proposed to enable reciprocal cross-talk that induces release of platelet-secreted factors thereby influencing EMT in tumor cells [60]. Such interaction has been identified in pancreatic ductal carcinoma where extravascular CD42+ platelets at the invasive front associate primarily with tumor cells expressing Snail1, reflecting the first steps of EMT [56]. In addition to secretion of platelets agonists, tumor cells physically interact with platelets by expressing ligands for the platelet receptors GPIb-IX-V, GPIIb/IIIa and CD62p [19]. Tumor cell expression of HMGB1, Mac-1 and AGEs, which engage with platelet pattern-recognition receptors TLR4 and RAGE have also been identified [62,63]. Ensuing secretion of platelet-sequestered growth factors thus enable engagement of the TGF-β/Smad and NF-κB signaling pathways in tumor cells, ultimately culminating in transendothelial migration and metastasis [58,61]. Recently, tumor necrosis factor receptor-associated factor (TRAF)

family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1) has been identified as a major player in mediation of platelet-induced EMT in mammary carcinoma, further directing invasion and metastasis in a mouse model [64].

Metastasis

The seminal work by Gasic and colleagues in 1968 demonstrating that tumors in thrombocytopenic mice were less likely to metastasize, brought to the fore the significance of platelets in the establishment of secondary sites [65]. Implicated in this mechanism is the reduction of platelet-derived TGFβ bioavailability and subsequent downregulation of MMP2 and MMP9, all of which are fundamental factors for enhancing tumor invasion [44]. Platelets promote tumor cell entry into the vascular system by releasing vascular disruptors including sphingosine-1-phosphate that induces the loss of tight junction proteins ZO-1 and Claudin-18; LPA that induces heightened permeability [66,67]; and ATP, which mediates endothelial cell retraction [68]. This latter phenomenon is highly dependent on Munc13-4-facilitated exocytosis from δ granules, as opposed to α-granules, and the presence of cognate endothelial $P2Y_2$ receptors [68]. Recent data suggests that coordinated transendothelial tumor cell migration and vascular disruption is heavily dependent on tumor-expressed CD97. CD97, a GPCR that is overexpressed in colorectal, thyroid, breast and prostate cancers [67,69], independently initiates platelet activation via engagement with GPIIb/IIIa, amplifying the resulting LPA and ATP-induced vascular dysfunction, and simultaneously activating Rho signaling thereby inducing a motile, invasive phenotype [67]. Correspondingly, inhibition or blockade of GPIIb/IIIa is associated with a reduction in LPA and metastasis [53].

In addition to the secretion of platelet agonists, tumor cells express a host of ligands that mediate direct, physical interaction with platelets. Tumor cell-expressed PSGL-1 and podoplanin bind to their cognate receptors, CD62p and CLEC-2 respectively, enabling tumor cell-induced platelet aggregation (TCIPA) [70]. TCIPA not only can occur in the absence of clinically detectable tumors, but also correlates with metastatic potential [21,71]. This interaction allows platelets to form a shield or cloak that protects tumor cells from high-velocity shear forces in the vasculature, and from natural killer (NK) cell antitumor activity [62]. The expression of TLR4 [62], and transfer of major histocompatibility complex 1 molecules onto tumor cells prevent NK cell recognition of tumor cells [72]. Platelets additionally express GITRL, which functions as an inhibitory NK cell ligand [19] and further secrete TGF-β and sheddases implicated in downregulating the NK cell activating immunoreceptor, NKG2D [73]. Should these efforts fail, by expressing RANKL, platelets are then able to decrease NK cell-mediated interferon (IFN)-γ secretion [74] thereby preventing tumor clearance.

The majority of tumor cells in circulation are postulated to succumb to the harsh environment; however, those that survive remain dependent on platelet-facilitated endothelial cell dysfunction [44,66–68] and tumor cell expression of high affinity $\alpha_v\beta_3$ for extravasation and subsequent colonization of secondary sites [66]. Moreover, the efficacy of the metastatic process is also dependent on the generation of a suitable premetastatic niche, a process in which platelets play a fundamental role. Abrogation of platelet-derived TGF-β1 prevents metastasis and seeding of tumor cells [75]; while inhibition of the recruitment of granulocytes by preventing platelet secretion of the chemokines CXCL5 and CXCL7, prevents metastatic seeding [76].

Platelets as biomarkers

Thrombocytosis is regarded as a useful indicator of occult disease and is linked with poor overall survival and progression of disease in ovarian cancer, gastrointestinal tract cancers, breast cancer, lung cancer and others [14,15,47,50,77–79]. Thrombocytosis, at least in the UK, at primary care level remains unused as an indicator for early stage cancers [15]; however, the value of thrombocytosis as a predictive marker does vary between studies and may not be applicable to all tumor types or grades. In lung cancer for instance, platelet counts are reported to hold predictive value [15], whereas it is also reported that platelet counts in early tumor stages are similar to that of age and gender matched controls, thus bringing into question the utility of platelet counts as a predictor [49]. Moreover, contrastingly in patients presenting with pancreatic tumors, high platelet counts are already apparent in early stages [49]. Thrombocytosis, thus while a useful indicator of disease may be insufficient to explain platelet function in early disease and may further be dependent on the underlying tumor.

Assessment of platelet omics data have shown value in the hunt for potential biomarkers not only as a putative diagnostic tool but to explain disease progression. Additionally, in the experimental setting such data highlight that due cognizance must be given to variance between human and model animals, particularly with regard to PAR1, for example, which is absent in mice [80]. Platelets from cancer patients present a transcriptome and proteome dis-

tinguishable from healthy individuals [37,81,82]. RNA-sequencing analysis is able to accurately discriminate tumor type (including non-small-cell lung carcinoma (NSCLC), breast cancer, pancreatic cancer, hepatobiliary cancer and glioblastoma, although further studies are required to distinguish nonmetastatic from their metastatic counterparts [37]. In NSCLC patients, RNA-sequencing of platelets was able to discriminate early and late stage cancer [79], distinguish lung adenocarcinoma from lung squamous cell carcinoma, and identify metastatic signatures [82]. These studies identified the upregulation of several genes associated with vesicular transport, cytoskeletal elements, immune response, platelet activation and cell–cell communication [37,79,82,83], which echo the role of platelets in tumor progression identified by a host of other studies. Additionally, genes associated with adhesion, including integrin-encoded ITG genes, have been found to be upregulated and are postulated to play a significant role in the interaction between tumor cells and platelets during hematogenous spread [82]. Recently, CD41 (integrin αIIb) part of the GPIIbIIIa complex, has shown efficacy as a biomarker for identifying NSCLC, surpassing the utility of CEA, particularly in cases where patients are CEA negative [84]. RNA sequencing thus holds considerable potential in identifying transcriptional alterations that may be specifically targeted; however, additional research in terms of utility across tumors and feasibility in the clinical environment is required. Moreover, since the transcriptome and proteome are not fully correlated, functionally the role of these transcripts requires further assessment [81].

Platelets derived from lung and pancreatic cancers present a proteome with significant increases in proteins associated with inflammation, immunity and cytoskeletal elements [49,81]. Four critical proteins were identified in these cancers that were substantially upregulated from their levels in healthy platelets. These included RELN, LRSAM1, RPS27L and ENY2, all of which are implicated in promoting tumor aggression and invasion [81]. Notably, following surgical resection, the platelet proteome was shown to normalize [81], highlighting reciprocal cross-talk between tumor cells and platelets. Platelet-derived VEGF and P4, implicated in angiogenesis and thus reflective of advancing tumor stage, may also hold value as prognostic markers [21,49], coupled with other clinical indicators.

Such data require validation in larger cohorts that are amenable to data stratification and the exploration of additional, noninvasive and easily accessible biomarkers to generate multivariate approaches that have utility as diagnostic markers for early cancer detection and tumor progression. Assessment of the platelet–lymphocyte ratio has been shown to have prognostic value [21] in predicting the course of cervical cancer [85], colorectal cancer [86] and others [87]. In breast cancer, a combination of low platelet–lymphocyte ratio and low neutrophil–lymphocyte ratio reflects pathological complete response to neoadjuvant chemotherapy [88], highlighting not only the impact platelet function has in disease, but the utility of such markers in monitoring treatment strategies.

Assessing hypercoagulation as a manifestation or proxy of cancer has twofold applications in that it may allow for assessment of tumor progression and for assessment of thromboembolic disease, thereby impacting management. In addition to high platelet count, mean platelet volume, high levels of soluble CD62p and presence of platelet-derived microparticles are regarded as risk factors for cancer-associated thromboembolic events [48,49,89]. However, contrary results have also been reported where low platelet counts [90] and low mean platelet volume [89,91] were associated with high VTE risk or poor outcomes. Studies assessing other factors released during α granule secretion, including soluble CD40L implicated in activating the adaptive immune system, TSP-1 associated with anti-angiogenic and pro-coagulant functions, and the procoagulant PF4 were not able to predict the risk of VTE in brain, breast, lung, gastrointestinal tract, pancreas, kidney, reproductive, sarcoma and hematological malignancies [89].

The Khorana score aimed at identifying cancer-associated VTE risk, is based on assessing hemoglobin levels, white blood cell and platelet counts, tumor site and body mass index and was initially derived from a large sample of 2701 patients and validated in an independent sample [3]. Despite its efficacy [3], there remain cases in which the predictive value is unclear particularly in patients undergoing anticancer treatment [92]. Other assessment models for cancer-associated VTE are thus being developed [93]. Additionally, studies indicate that anti-cancer treatments pose significant risk for arterial thromboembolic events [13,92]; highlighting that both types of thromboembolic events require consideration in studying the impact of platelets on tumor progression. Moreover, while considerable progress has been made in identifying putative platelet biomarkers further validation across cohorts and tumor types is necessary to unravel contradictory findings and ascertain the feasibility of incorporating such in the clinical environment.

Antiplatelet strategies in cancer treatment

Targeting platelet activation pathways is a route by which it may be possible to limit platelet-mediated tumor progression while simultaneously managing thrombosis. Aspirin irreversibly inhibits the activity of COX-1 and

thus the ultimate synthesis of prostaglandins and TXA_2 [94]. Aspirin administration can reduce metastasis of mammary carcinoma and melanoma [70] and inhibit tumor growth and EMT [95] in animal models. In clinical studies, aspirin has shown a moderate effect in lowering incidence of gastrointestinal tumors and improving overall survival in breast cancer patients, but further, direct evidence is required to establish its efficacy and mechanism of action [70,94]. Co-administration of Aspirin with clopidogrel, an inhibitor of P2Y12, has had inconclusive effects in reducing circulating breast tumor cells in a Phase II clinical trial [96]; however, in an orthotopic model of 4T1 breast cancer, co-administration of these drugs induced vascular mimicry, malignancy and ultimately mortality [97]. Concerns have also been found in clinical trials evaluating the utility of Prasugel, another P2Y12 inhibitor, and clopidogrel in cardiovascular patients where a risk of developing malignant tumors was identified [94,98].

The drugs abciximab, tirofiban and eptifibatide are GPIIb/IIIa inhibitors that block platelet crosslinking and fibrinogen binding [14]. In preclinical cancer models, GPIIb//IIIa inhibitors reduce angiogenesis and metastasis in murine models; however, such inhibition is associated with bleeding risk [21,53]. This adverse outcome can be mitigated by specific targeting of the GPIIIa subunit β3 [53]. Crizanlizumab is a monoclonal antibody directed at CD62p directed for use in sickle cell anemia [14]. However, while preventing CD62p-mediated tumor cell engagement has the potential to reduce metastasis, the effect of this drug in cancer treatment is not known. Targeting PARs has also been identified as a putative anti-tumor method; however, development of drugs, for example, Atopaxar, pose challenges in terms of adverse bleeding, liver enzyme modifications and cardiac effects [14].

In repurposing drugs designed for cardiovascular disease or designing anti-platelet therapeutics for cancer management, clinical factors including tumor type and grade, comorbidities, treatment, presence of bleeding and treatment-associated thrombocytopenia among others would have to be considered [70]. The challenge remains that with the countless functions of platelets, inhibition or blockade of any receptor may result in compensatory mechanisms nevertheless allowing platelet function. Such drugs or combinations thereof may also have actions on cells in the premalignant and malignant microenvironment that may be protumorigenic. The complexity of such a microenvironment thus makes predicting the effect of treatment strategies difficult and serves a cautionary function.

Future perspective & conclusion

Fluid-phase biopsies allowing for identification of blood-based biomarkers is proposed as a minimally invasive, accessible and adaptable method with which to survey disease. Liquid biopsies have focused on evaluating cell-free DNA or circulating tumor cells; however, challenges remain with regard to specificity, accuracy and analysis of low-frequency events [14]. Platelets show potential not only for use as a diagnostic indicator of cancer, but as a tool to define molecular subphenotypes. Platelets provide a repository of cancer-associated information, and with their ability to sequester RNA transcripts and bioactive molecules, monitoring platelet activation status, proteome and transcriptome would give insight to not only disease state but progression and response to treatment. Narrowing down datasets to find easily identifiable markers, coupled with applying laboratory findings to clinical practice, may provide a platform for accessible individualized treatment protocols. However, in translating bench to bedside, validation of putative biomarkers is essential, and scaling of diagnostic and prognostic techniques must also be considered. Much remains to be explored in the realm of anti-platelet therapies, but certainly more preclinical and clinical studies are required to truly comprehend the role of platelets in tumor progression.

Acknowledgments

The author thanks the blood donors and nurses from Charlotte Maxeke Johannesburg Academic Hospital for sample collection. The author also thanks W van der Spuy for assistance in acquiring scanning electron photomicrographs at the Unit for Microscopy and Microanalysis, University of Pretoria.

Financial & competing interests disclosure

Gratitude is extended to the National Research Foundation of South Africa for their grant (NRF 106926). The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

Ethics clearance was obtained from the University of the Witwatersrand, Human Research Ethics Committee (#M140155).

Executive summary

Background

- As early as 1865, it was known that thrombotic complications have the potential to serve as a warning system for occult malignancy prior to the development of clinically detectable tumors. Platelets have now been identified as a critical cell type in creating not only a hypercoaguable state but in actively promoting tumor progression. Understanding platelet nonhemostatic functions is key to gaining insight to their critical role in the life history of tumors.

Platelet structure in relation to their function

- Although anucleate, platelets actively respond to their microenvironment by engagement of a range of receptors mediating intracellular signaling pathways. Proteomic analysis reveals platelets contain over 4000 proteins. Additionally, platelets possess an extensive transcriptome, translational machinery and the capacity to conduct post-translational modifications. With further ability to conduct differential secretion of molecules and microparticles, and to uptake and transfer molecules, the concept of platelet function has fundamentally changed.

Platelets in tumor progression

- The identification of platelets within the tumor bed has removed the limitations of trying to comprehend tumor cell–platelet interaction strictly across the vascular wall. Tumor cells actively recruit platelets into the tumor bed, manipulating their secretions to induce angiogenesis for sustained tumor growth, to facilitate epithelial–mesenchymal transition, to provide a route for metastasis and prepare the pre-metastatic niche. Within the circulation, this relationship is maintained with tumor cells protected by platelets from the harsh vascular microenvironment and immune cell recognition.

Platelets as biomarkers

- In this review the utility of different aspects of platelets as potential biomarkers is presented. This ranges from thrombocytosis to omics data that describe vast underlying molecular processes. Given their consistent presence in the pre-malignant to the metastatic environment, we assess their value in diagnosis, tracking of disease progression and in response to treatment. We further present assessment of hypercoagulation as a proxy for those parameters.

Anti-platelet therapeutics

- Inhibiting platelet activation theoretically has potential two-fold benefit in preventing thromboembolic complications and in limiting tumor progression. In this review, selected anti-platelet therapeutics are presented in brief showing those with good potential for impact, and others where the side-effects may include malignancy itself.

Future perspective & conclusion

- This review highlights the impact that platelets have on tumor progression. As a noninvasive, accessible cell type, platelets hold much information that could be used as diagnostic and prognostic tools. We further highlight that so much more remains to be elucidated and highlight the complexity of platelets in their functions and interaction with other cell types.

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