Platelets: "First Responders" in Cancer Progression and Metastasis

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Abstract

A long-standing postulate in oncology is that platelets facilitate cancer metastasis (Menter et al. Cancer Metastasis Rev 33:231–269, 2014; Menter et al. Invasion Metastasis 7:109–128, 1987; Gasic et al. Proc Natl Acad Sci USA 61:46–52, 1968; Woods Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften 20:92–121, 1964). As their most critical biological response, platelets serve as "first responders" during the wounding process and hemostasis. As a part of the metastatic process, platelet receptors recognize complexes of tumor cell receptors and surface-bound matrix proteins or cellular products as they invade blood vessels. This recognition triggers platelet activation and platelet-tumor cell interactions. Once activated by tumor cells, platelets change shape, degranulate, and release proteins, growth factors, bioactive lipids, and other factors that recruit additional platelets and immune cells along with initiating thrombogenesis. Extensive membrane changes occur at bilayer interfaces between platelets and tumor cells. Tumor cells form extensive membrane/cytoskeletal processes that heavily interdigitate with a central platelet aggregate and involves the uptake of platelet fragments and mitochondria. These interactions are thought to result in the suppression of immune recognition/cytotoxicity or the promotion of cell arrest at the endothelium or entrapment in the microvasculature. These responses all support survival and

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spread of cancer cells and the establishment of secondary lesions. Additional mechanisms of the platelet-metastasis relationship may include the production of platelet exosomes or extravascular migratory behavior of platelets helping to drive cancer progression or preconditioning of secondary metastatic sites. In contrast to the many mechanisms involved in platelet-metastasis relationships, little is known about the role of platelets in precancerous lesion development. This paucity of knowledge exists despite numerous large randomized clinical trials illustrating the cancer preventive effects of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin in reducing the cancer incidence, mortality, and metastasis. Aspirin covalently acetylates and inactivates platelet cyclooxygenase 1 and thereby eliminates all downstream prostaglandin production from arachidonic acid (AA) by platelets. This includes the key bioactive lipid involved in platelet activation, thromboxane A_2 (TxA_2). Another prostaglandin, prostacyclin (PGI_2), counterbalances and inhibits platelet activation (Honn et al. Science 212:1270–1272, 1981). Metabolically, the genesis of TxA_2 and other bioactive lipids are also impacted by ω-3 polyunsaturated fatty acid substrate substitution for AA. Although not well studied, this places platelets not only at the center of the metastasis discussion but also the progression of premalignancies. Since neoangiogenesis produces leaky blood vessels during early cancer progression, it stands to reason that platelets are the "first responders" to extravasate, activate, and release their stroma-stimulating, proangiogenic, chemoattractive, and immunomodulatory contents. These normal platelet functions and products undoubtedly promote precancerous lesion progression as a series of cyclic amplification events. Platelets are suspected to have a key role within the full spectrum of the cancer progression continuum, which makes limiting their first response an important target for both prevention and therapy.

Platelets in Cancer Metastasis

Platelets as "First Responders"

"First responders" are a perfect moniker for platelets as active participants in the hemostasis, wounding, immune, and metastatic processes (Menter et al. 2014; Gasic et al. 1968; Woods 1964). Platelets are often neglected or overlooked during in vivo experimental or pathologic observations. This is partly due to their small size/volume (mean platelet volumes range is 9.7–12.8 femtoliter or spheres 2.6–2.9 μm in diameter) and the requirement for ultrastructural analysis to effectively observe morphologic or activational structural changes in individual platelets (Fig. 1). Aggregates of activated platelets can be detected by immunohistochemistry at the microscopic level, but this is not routinely done (Qi et al. 2015). As a part of their functional biophysical dynamics, the platelet discoid shape, small size/volume, and physical characteristics cause them to segregate toward the outer fluid shear fields of flowing blood (Fedosov et al. 2014; Kumar and Graham 2012; Tokarev et al. 2011a, b; Lee et al. 2009). Normal human numbers range between 150,000 and 400,000 platelets per microliter (μl), and the concentration of platelets near the vessel wall in vivo is two to three times greater than at the vessel core. Thus, their overall biophysical properties in circulation facilitate platelet distribution toward the endothelial surfaces of vessel walls. Platelet flow patterns, near-wall excess, and proximity enhance their ability to encounter and recognize any lesions in the vascular wall due to a laceration or wound. These platelet recognition properties include the exposure of the subendothelial basement membrane or underlying matrix induced by wounding or endothelial retraction (Menter et al. 1987d; Crissman et al. 1988; Walsh et al. 2015; Kim et al. 2013; Spectre et al. 2012). Platelets can also actively migrate across the inflamed vascular wall in response to stromal cell-derived factor 1 (SDF1 or CXC chemokine ligand 12:CXCL12) and into tumor extravascular spaces (Goubran et al. 2014; Unwith et al. 2015; Schmidt et al. 2012; Kraemer et al. 2010; Brandt et al. 2000; Stone et al. 2012). Platelets can also release SDF1: CXCL12, CXCL1 (GRO-α), CXCL4 (PF4), CXCL5 (ENA-78), CXCL7 (PBP; βTG), and CXCL8 (IL8) among numerous other cytokines and chemokines from storage granules that can potentially initiate the migration and invasion of additional platelets, immune cells (macrophages and leukocytes), endothelial progenitor cells, and tumor cells (Kraemer et al. 2011; Chatterjee et al. 2011; Shenkman et al. 2004; Gleissner et al. 2008). A case for first respondership is further strengthened by the discovery of CXC chemokine receptor 4 and 7 (CXCR4, CXCR7), the cognate receptors for SDF1: CXCL12 on platelets (Rath et al. 2014, 2015; Rafii et al. 2015; Chatterjee et al. 2014b). Expanding upon the concept
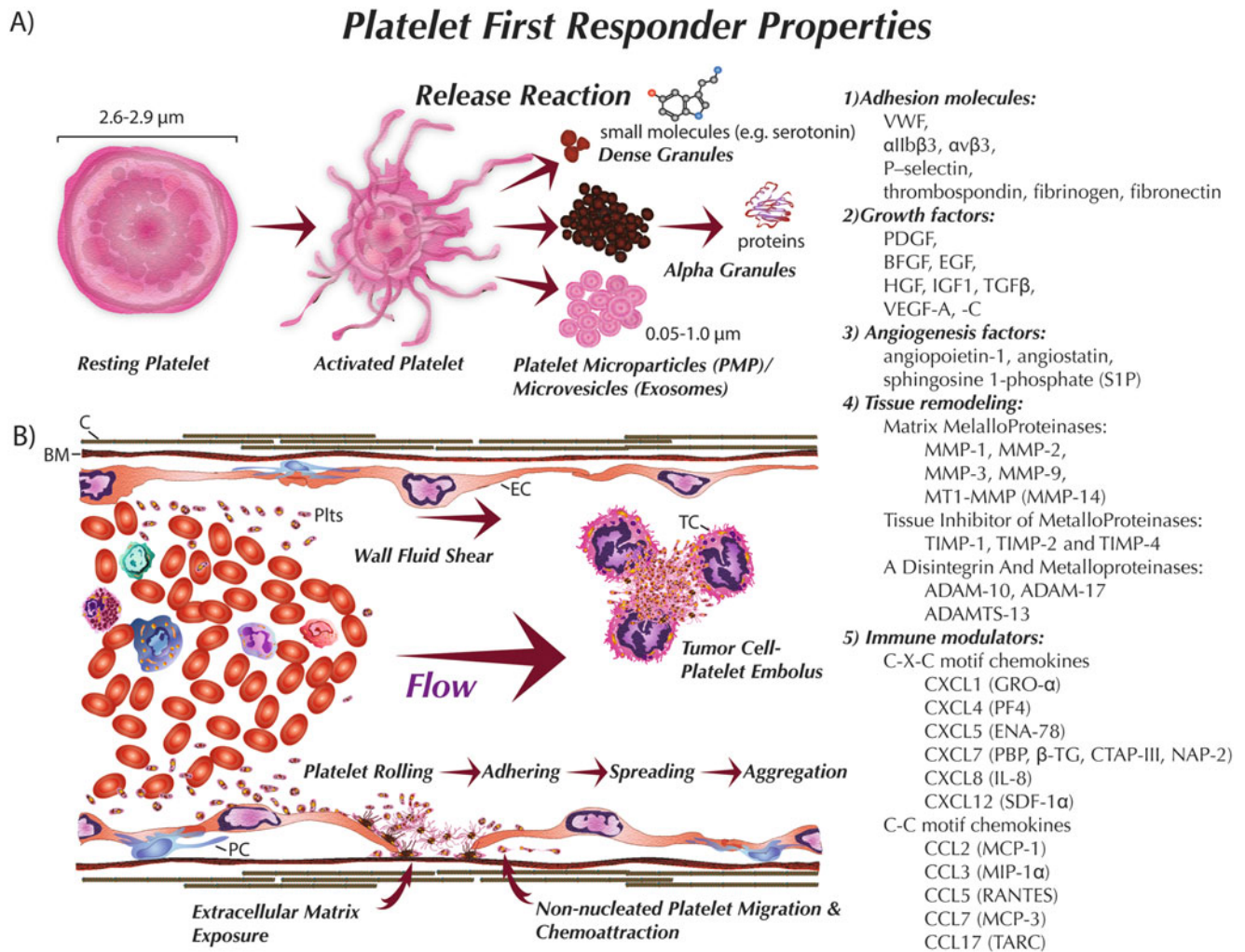
Fig. 1 Platelet first responder properties. (**a**) The activation of platelets is triggered by tumor cells and microenvironmental factors and constitutes an integral part of their first responder properties. This involves shape change and the release reaction. Multiple components that are released into the microenvironment include dense granules, alpha granules, and platelet microparticles (PMP) or microvesicles. The factors released include (1) adhesion molecules, (2) growth factors, (3) angiogenesis factors, (4) tissue remodeling factors, and (5) immune modulators, among others. (**b**) Due to their biophysical and biological properties, platelets accumulate near blood vessel walls within the fluid shear parameters of flowing blood. This enables them to elicit first responses to lesions in the vasculature and the exposure of extracellular matrix. This can lead to the migration of nonnucleated platelets into the perivascular space. As tumor cells enter the blood stream, they can interact with platelets to form heterotypic emboli. *EC* endothelial cell, *PC* pericyte, *BM* basement membrane, *C* collagen, *TC* tumor cell, *PLT* platelet

of migration during potential first responses, platelets are ideally suited for movement because of the highly active cytoskeletal responses linked to activation, adhesion, and aggregation (Menter et al. 1987d, e, 2014; Chopra et al. 1992; Bennett et al. 1999; Jackson et al. 2000; Machlus and Italiano 2013; Qi et al. 2015). Together with an active cytoskeleton, platelets are more streamlined, not only because they are small in size and have minimal displacement volume, but they are also unencumbered by the presence of nuclei, which limits the migration of other immune cells (Breckenridge et al. 2010; Ellingsen et al. 2000; Friedl et al. 2011). The lack of nuclei may also limit the distance that platelets can move into tissue; because of limited protein synthesis capabilities, they cannot indefinitely sustain the

replacement of proteins (Bruce and Kerry 1987; Borisova and Markosian 1977). In addition as part of their immune surveillance properties, platelet can also recognize foreign bodies or invading pathogens (Menter et al. 2014; Rossaint and Zarbock 2015; Garraud et al. 2011; von Hundelshausen and Weber 2007). Due to these combined properties, during metastasis, circulating platelets can also elicit a first response to the exposure, sloughing, or active invasion of tumor cells into the blood stream at primary tumor sites. Obstruction of blood flow and angiogenesis associated with primary tumor growth are likely to further enhance the probability of platelet-tumor cell encounters through membrane interactions (Horejsova et al. 1995; Benazzi et al. 2014; Fein and Egeblad 2013). The net result is likely to be platelet activation either to

subdue cells at the primary site or generate tumor cell-platelet emboli in circulation (Menter et al. 1987b, 2014; Crissman et al. 1988). Based on such significant numbers in circulation, small size, biophysical shear properties, adhesion, aggregation, and streamline migration properties, platelets are well suited to serve as "first responders" to a variety of pathologic stimuli, including metastasis.

Tumor Cell: Migration, Invasion, and Intravasation

The proliferation and migration of cancer cells within primary tumors drives a number of events that can impact metastasis (Starke et al. 2014; Gritsenko et al. 2012; Friedl et al. 2012, 2014; Haeger et al. 2014). Direct impact can occur by shedding, sloughing, or active entry of tumor cells into the blood vessels. Based on single cell profiling of circulating tumor cells (CTCs), there is a large diversity of cells found in the circulation that reflect tumor heterogeneity (Deng et al. 2014; Powell et al. 2012). Within the diversity spectrum, CTCs also frequently exhibit stem cell properties (Tang et al. 2015). A variety of triggers can initiate entry of CTCs into the circulation. For example, decreased availability of blood vessels can increase the induction of hypoxia as tumors outgrow their blood supply and release of angiogenesis or wounding related factors (Pasula et al. 2012; Hellberg et al. 2010; Carmeliet 2005). These factors stimulate the formation of new blood vessels that are typically abnormal and leaky, enabling entry of tumor cells into the blood stream (Keskin et al. 2015; Nagy et al. 2012; Fukumura and Jain 2008). Although not extensively studied, there is also potential for leakage or migration of platelets into the tumor that may further enhance the angiogenesis/leaky blood vessel genesis cycle (Kisucka et al. 2006; Goubran et al. 2014; Schumacher et al. 2013; O'Byrne and Steward 2001). More aggressive tumor cells that enter the circulation often undergo epithelial-mesenchymal transition (EMT) (Satelli et al. 2015). In fact, direct signaling between platelets and cancer cells induces an EMT and promotes metastasis in vitro and in vivo (Labelle and Hynes 2012; Labelle et al. 2011; van Es et al. 2014). Cells with EMT characteristics are more fibroblastic in morphology and are typically much more motile and invasive as a result (Labelle et al. 2011; Labelle and Hynes 2012). These EMT cells are prone to actively invade blood vessels by using matrix metalloproteinase (MMP) to digest the extracellular matrix and basement membrane of blood vessels (Nistico et al. 2012). As part of the invasion process, interactions between platelets and tumor cells increase the production of MMP-9 (Labelle et al. 2011). This vascular tumor cell invasive process is termed intravasation and is considered an early dissemination step of the hematogenous metastatic cascade (Fidler 1978).

Tumor Cell-Platelet Recognition/Interaction: A Two-Way Street

Surface receptors abound on both platelets and tumor cells that trigger the primary response mechanisms which drive biologic function (Goubran et al. 2014; Menter et al. 2014). In the case of a nucleated platelets, the lack of nuclei and restricted protein synthesis capabilities limit the adaptation dynamics of platelet surface receptor expression, which is generally established during platelet genesis by megakaryocytes in the bone marrow (Menter et al. 2014). The genesis of heterogeneous populations of platelets that contain carbon copy surface receptor subsets from specific megakaryocytes capable of adapting to disease has not been well established but could have a key impact on cancer if this were true (Bakchoul and Sachs 2015; Penington et al. 1976). In contrast to platelets, nucleated tumor cells have both surface and nuclear receptors that respond to a variety of molecules. Tumor cells also retain the capacity to replace or increase receptor expression by upregulating gene expression and new protein synthesis as they adapt to their microenvironment (Menter et al. 2014).

Integrin and Collagen Receptors

Platelet-tumor cell encounters can be initiated by surface contacts. Integrins are one class of surface receptor molecules that interact with certain extracellular matrix proteins or by direct interactions and consist of α-subunit and β-subunit heterodimer complexes (Fig. 2a) (Menter and Dubois 2012). From the platelet side (pink text), integrin heterodimer receptor complexes include those for vitronectin (α_v/β_3:ITGAV/CD61), fibrinogen (α_{IIb}/β_3:CD41/CD61), laminin (α_2/β_1:CD49b/CD29), and fibronectin (α_5/β_1:ITGA5/CD29) (Menter et al. 2014; Grossi et al. 1987). On the tumor cell side (purple text), many of the same integrin heterodimer complexes can be involved in binding these matrix proteins along with other classes of integrins (Menter and Dubois 2012).

Additional matrix protein receptors include those for collagens. On the platelet side, in this case, collagen receptors are relatively unique to platelets that include GP_{Ib} complexes (consisting of GP_{IX}, $GP_{Ib\alpha}$, $GP_{Ib\beta}$, and GP_V subunits) as well as GP_{VI} (Menter et al. 2014). Tumor cells by contrast can express the entire range of collagen receptors including α_2/β_1 (Menter and Dubois 2012).

Cell Adhesion Molecules, Selectins, and Mucins

Cell adhesion molecules (CAMs) are transmembrane proteins that bind to extracellular matrix and cell-cell adhesion (Fig. 2b). The primary CAM expressed on platelets that
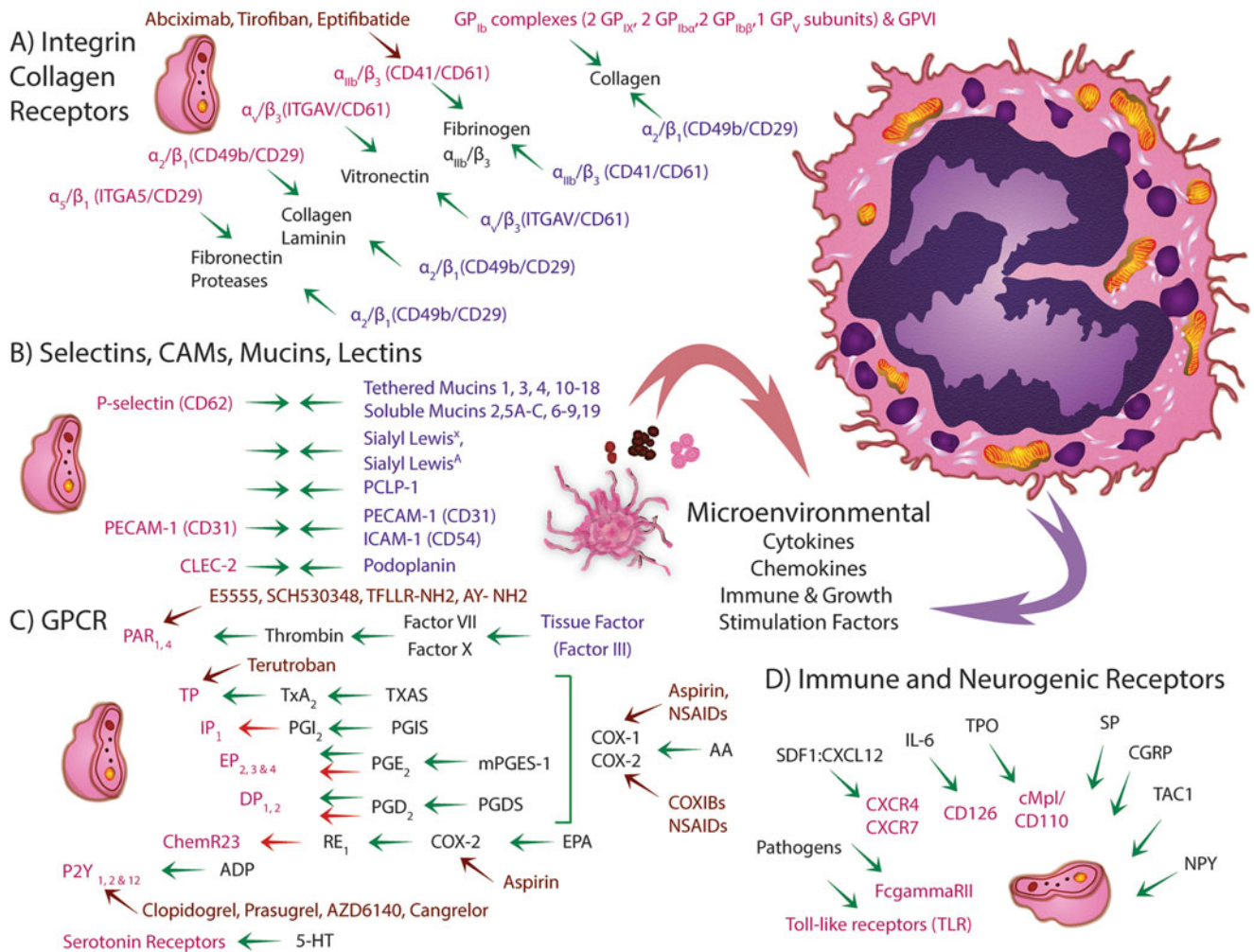
Fig. 2 Receptor and factors that mediate platelet-tumor cell interactions. Integrin and collagen receptors mediate cell-cell interactions between platelets and tumor cells along with collagen and extracellular matrix (ECM) proteins. (**a**) Platelet GPIb complexes recognize vWF tethers, along with thrombospondin, thrombin, $\alpha_M\beta_2$ integrin, kininogen, and clotting factors XI and XII. The GPIb complex consists of two sets of GPIX, GPIbβ, and GPIbα along with a centrally situated GPV protein. Adhesive contacts with collagen exposed by tumor cells are stabilized by GPVI. The $\alpha_{IIb}\beta_3$ integrin has the widest range of interactions and binds to fibrinogen, fibrin, fibronectin, vitronectin, thrombospondin, and vWF. Platelet $\alpha_{IIb}\beta_3$ integrin inhibitors include abciximab, tirofiban, and eptifibatide. Additional platelet and tumor cell integrins are: α_v/β_3(ITGAV/CD61; vitronectin receptors), α_2/β_1(CD49b/CD29; collagen, laminin receptors), and α_5/β_1 (ITGA5/CD29; fibronectin, protease receptors). (**b**) Selectins, cellular adhesion molecules (CAMs), mucins, and lectins can profoundly influence platelet-tumor cell interactions. P-selectin binds to a variety of carbohydrate-rich molecules including: Sialyl Lewis^x or Sialyl Lewis^A as well as tethered (1,3,4,10-18) or soluble (2,5A-C, 6-9,19) mucins and podocalyxin-like protein 1 (PCLP1). Podoplanin on tumor cells can interact with CLEC-2 on platelets. CAMs such as PCAM-1 (CD31) and ICAM-1 (CD54) also regulate platelet tumor cell interactions. (**c**) G protein-coupled receptors (GPCRs) mediate a wide variety of biological interactions. Tissue factor (thromboplastin or factor III) can form complexes with factor VII and factor X to generate thrombin that in turn binds to and activates protease-activated receptors (PAR) 1 or 4 on platelets, which can be inhibited by PAR-specific inhibitors, E5555, and SCH530348. Bioactive lipids also alter platelet-tumor call interactions. Arachidonic acid (AA) is converted by cyclooxygenase 1 and 2 to

prostaglandins (PG) and are inhibited by aspirin, NSAIDs, and COXIBs. COX inhibition inhibits multiple downstream steps in the PG cascade. The key target includes the primary platelet-activating pathway: thromboxane A_2 synthase (TXAS) > TxA_2 > TxA_2 receptor (TP) that can be inhibited by a receptor antagonist, terutroban. This activation is countered by the endogenous prostaglandin I_2 synthase (PGIS) > PGI_2 > PGI_2 receptor (IP) inhibition. Other arms of the PG pathway also influence these heterotypic interactions. Another endogenous inhibitor includes resolvin-E1, a product of eicosapentaenoic acid (EPA). Nucleotide receptors can also activate platelets ADP/purinergic G protein-coupled receptors 1 and 12 (P2Y 1, 2 and 12) that are inhibited by clinically relevant receptor antagonists: clopidogrel, prasugrel, AZD6140, and cangrelor. Serotonin (5HT) receptors are also influenced by the uptake and release of 5-HT by platelets. (**d**) Immune receptors are involved in responses to pathogens, including FcgammaRIIB and toll-like receptors (TLR). Platelet genesis is influenced by IL-6 receptors CD126 and thrombopoietin (TPO) receptors cMpl/CD110. Platelet chemotaxis and migration are influenced by stromal cell-derived factor 1 (SDF1 or CXC chemokine ligand 12:CXCL12) through CXCR4 and CXCR7 receptors. Neuropeptides that include substance P (SP), calcitonin gene-related peptide (CGRP), and neurokinin A (NPY) stimulate inflammation when released into the microcirculation, where platelets are key responders that react by modulating arachidonate metabolic pathways. In terms of angiogenic response, the platelet-derived form of the sympathetic neurotransmitter neuropeptide Y (NPY) induces vasoconstriction and ischemic angiogenesis. *Pink text* platelet receptors and factors, *purple text* tumor cell receptors and factors, *maroon text and arrows* inhibitors, *green arrows* stimulation or interaction, *red arrows* inhibition

regulates binding to tumor cells is relatively uniformly expressed (PECAM-1:CD31) (Pang et al. 2015a; Coupland and Parish 2014; Aceto et al. 2014). On the tumor cell side, the expression of surface receptors is not as uniform and can vary extensively with tumor heterogeneity and adaptability (Gunning et al. 2008; Moins-Teisserenc et al. 2015; Raeisossadati et al. 2011). These tumor cell receptors include PCAM-1:CD31, intercellular (ICAM-1:CD54), and epithelial (EpCAM: CD326). Selectins are another family of CAMs consisting of single-chain transmembrane glycoproteins with lectin and cell adhesion properties that bind carbohydrate. Platelets express P-selectin (CD62), which interacts with a variety of carbohydrate-rich molecules produced by tumor cells that include numerically identified mucins that are either tethered (1, 3, 4, 10–18) or free (2, 5A–C, 6–9, 19). Some reports also show evidence of platelets actively recruiting ovarian cancer cells and upregulating tissue factor (Orellana et al. 2015). P-selectin also interacts with Sialyl Lewis^x or Sialyl Lewis^A as well as podocalyxin-like protein 1 (PCLP1) expressed by tumor cells. Platelets can also express C-type lectin receptor 2 (CLEC-2), which interacts with podoplanin on tumor cells (Menter et al. 2014; Goubran et al. 2015).

G Protein-Coupled Receptors (GPCR)

A series of cell-signaling G protein-coupled receptors (GPCRs) can help mediate interactions between platelets and tumor cells (Fig. 2c). Once again on the platelet side, the receptor/signaling networks are very complex (Boyanova et al. 2012; Menter et al. 2014). Tissue factor (thromboplastin or factor III) can form complexes with factor VII and factor X to generate thrombin, which binds to and activates protease-activated receptors (PAR) 1 or 4 on platelets (Menter et al. 2014; Goubran et al. 2014). Tissue factor-dependent thrombin is also enhanced by phospholipid-esterified eicosanoids in agonist-activated platelets (Thomas et al. 2010). The 12-lipoxygenase arachidonate metabolite, 12(S)-HETE, is a bioactive lipid that contributes to endothelial cell retraction as a free acid (Honn et al. 1994). However in its esterified form, after platelet activation, it appears to contribute to coagulation as a result of its translocation to the platelet outer surface. This is supported by the observation that in Scott syndrome, platelets that are defective in cell surface coagulation, the esterified HETE is not presented to the cell surface despite being made to the same degree as in thrombin-stimulated platelets from normal patients. Both the PAR1 and PAR4 agonists (TFLLR-NH$_2$ and AY-NH$_2$, respectively) stimulate esterification of 12(S)-HETE with PC and PE to a similar degree (Thomas et al. 2010).

Prostaglandin (PG) receptors are prominent GPCRs expressed by platelets that respond to PGs, which are also important bioactive lipids that profoundly alter platelet function. The PG receptor for thromboxane A$_2$ (TxA$_2$), for example, is among the strongest agonists to stimulate platelet activation via thromboxane receptors (TP) (Fontana et al. 2014; Gleim et al. 2013). A differently structured PG is prostaglandin I$_2$ (prostacyclin) that binds to a separate prostaglandin I$_2$ receptor (IP$_1$) and exhibits the strongest inhibition of platelets (Honn et al. 1981; Hubertus et al. 2014). Platelets also express PGE$_2$ receptors (EP 2, 3 and 4) and PGD$_2$ receptors that can activate or inhibit platelet function (Petrucci et al. 2011; Cooper and Ahern 1979; Hubertus et al. 2014). Platelets also express additional bioactive lipid receptors. One orphan receptor, *chem*erin *r*eceptor *23* (ChemR23; chemokine-like receptor1: CMKLR1) binds resolvin E$_1$, a bioactive lipid synthesized from ω3-polyunsaturated fatty acid eicosapentaenoic acid (EPA) found in fish oil that inhibits ADP-induced platelet activation (Fredman et al. 2010; Petrucci et al. 2011). This receptor also serves as a chemokine (C-C motif) receptor-like 2 (CCRL2) that binds chemerin contained in platelets, an adipokine/chemoattractant that has reported roles in inflammation, adipogenesis, and insulin resistance (Du et al. 2009). Tumor cells by contrast express a wide variety of PG receptors depending on tissue origin. Both cyclooxygenase 2 (COX-2) and microsomal PGE$_2$ synthase1 (mPGES1) are commonly upregulated in cancer, and the production of PGE$_2$ activates EP receptors (Menter et al. 2010).

Platelets express a number of additional GPCRs. These include thrombin/protease-activated receptors 1 and 4 (PAR 1 and 4) along with ADP/purinergic G protein-coupled receptors 1, 2, and 12 (P2Y 1, 2, and 12) (Franchi and Angiolillo 2015). Both sets of receptors typically trigger platelet activation, and a variety of clinically relevant inhibitors exist for target intervention (Raju et al. 2008). PAR1 and PAR4 are activated following thrombin cleavage that exposes a tethered ligand, which binds to PAR and causes a conformational change and interactions with G proteins (Fu et al. 2015; Wallace and Smyth 2013). Platelet PAR activation can lead to platelet aggregation and thrombosis as well as neutrophil recruitment (Fu et al. 2015; Wallace and Smyth 2013). In contrast, the binding of ADP to P2Y receptors mobilizes intracellular calcium ions via activation of phospholipase C that activates platelets (Cattaneo 2015; Liverani et al. 2014) along with stimulating tumor-cell transendothelial migration and metastasis (Schumacher et al. 2013).

Neurogenic Inflammation and Platelets

The connection between receptor-mediated neurogenic inflammation and cancer is being actively investigated for its role both in potentiating local tumor growth and perineural cancer invasion (Stopczynski et al. 2014; Liebig et al. 2009; Black 2002). Substance P (SP), calcitonin gene-

related peptide (CGRP), and neurokinin A (TAC1) are neuropeptides that stimulate inflammation when released into the microcirculation, where platelets are key responders that react by modulating arachidonate metabolic pathways (Gecse et al. 1999). In terms of angiogenic response, the platelet-derived form of the sympathetic neurotransmitter neuropeptide Y (NPY) is required for capillary angiogenesis during late-phase recovery from ischemic injury (Tilan et al. 2013) and is also produced in animals with hypertension (Ogawa et al. 1992), a condition whose association with cancer has long been suspected (Hamet 1996; Stocks et al. 2012). As a number of cells upregulate NPY to promote metastasis and NPY receptor has been described in the context of its role in cancer, it is conceivable that platelet-derived NPY provided through platelet-tumor cell interactions may also contribute to metastasis (Hong et al. 2015; Medeiros et al. 2012; Magni and Motta 2001).

Serotonin Receptors

Platelet dense granules contain serotonin (5-HT) that is taken up through serotonin transporter (SERT) activity to regulate 5-HT circulating levels (Mauler et al. 2015; Jedlitschky et al. 2012; Linder et al. 2007). More than 95 % of total body 5-HT is synthesized in the enterochromaffin cells of the intestine. Once released from platelets following stimulation, 5-HT binds to cognate receptors and induces vasoconstriction (Mauler et al. 2015; Jedlitschky et al. 2012; Linder et al. 2007). The role of platelet serotonin in malignancy has been pondered for decades (Crawford et al. 1967). Platelet-derived serotonin has been linked to tissue fibrosis (Dees et al. 2011) [considered a form of EMT, reviewed in Tucker and Honn (2013), Crooks et al. (2014), Lopez-Novoa and Nieto (2009)], where platelet aggregation has also been described (Kahaleh et al. 1982). Furthermore, serotonin may be a marker for hepatocellular carcinoma (Pang et al. 2015b; Pai et al. 2009) and has been proposed as a contributing factor to breast cancer (Pai et al. 2009). Platelet serotonergic mechanisms have also been invoked in bladder cancer (Pawlak et al. 2000).

Immune Response Receptors

Additional critical receptors that can impact interactions between platelets and tumor cells include those influencing immunomodulation (Fig. 2d). Platelets express a wide range of receptors that interact with pathogens (Hamzeh-Cognasse et al. 2015) including complement. These receptors include: FcγRII, toll-like receptors (TLR), and also integrins conventionally described in the hemostatic response, such as $\alpha_{IIb}\beta_3$ or GP_{Ib} (Hamzeh-Cognasse et al. 2015). Some of these receptors such as Fcγ receptor IIa can mediate platelet-

tumor cell cross talk and initiate tumor cell-induced platelet secretion (TCIPS) (Mitrugno et al. 2014). Interactions with histocompatibility antigen also influence platelet behavior in cancer patients, but this has not been extensively studied (Messerschmidt et al. 1988). Also as previously mentioned, chemokine/cytokine release and subsequent binding to immune receptors on platelets or other cells in the local or systemic environment can stimulate the migration and growth of a variety of cells (Kraemer et al. 2011; Chatterjee et al. 2011, 2014a, b; Shenkman et al. 2004; Gleissner et al. 2008; Rath et al. 2014, 2015; Rafii et al. 2015).

Cancer-Induced Thrombocytosis

Finally, cancer-induced thrombocytosis significantly increases the number of circulating platelets and is linked to tumor cell proliferation (Bouvenot et al. 1977; Honn et al. 1992; Levin and Conley 1964; Cho et al. 2012). This constitutes an important link to Trousseau's syndrome, but the mechanisms responsible remained poorly understood until recently (Stone et al. 2012) when a multicenter study involving 619 ovarian cancer patients examined the associations between platelet counts and disease outcome (Stone et al. 2012; Davis et al. 2014). During this study, thrombocytosis was elevated in association with circulating levels of thrombopoietin (TPO) and interleukin-6 (IL-6). This elevation was associated with advanced disease and shortened survival in ovarian cancer patients and was verified in orthotopic mouse models (Stone et al. 2012). In pancreatic cancer studies as well, paraneoplastic thrombocytosis predicts poor prognosis in patients with locally advanced disease (Chadha et al. 2015). These tumor-liver-bone marrow-platelet feedback mechanisms are thought to occur through IL-6 receptors: CD126 (Marino et al. 2013) and TPO receptors: cMpl/CD110 (Hitchcock and Kaushansky 2014).

Anticoagulant Therapy for VTE Prevention in Cancer Patients

It is estimated that individuals with cancer have a fivefold higher incidence of venous thromboembolic events (VTEs) than the general population (Chao et al. 2011). Chemotherapy can also contribute to elevated levels of thrombosis. In healthy individuals, venous thrombosis can be the first manifestation of malignancy. Since the 1970s when the Veterans Administration Cooperative Study Program first tested warfarin in cancer patients, many other studies have examined agents that interrupt the coagulation pathway as a means to attenuate VTEs and impede cancer progression (Chao et al. 2011). Unfortunately, the doses of warfarin required to achieve therapeutic results were not without side effects

(Zacharski et al. 2005), and heparin required continuous intravenous administration (Hull et al. 1986). As a result of numerous clinical trials, low molecular weight heparin (LMWH or nadroparin) replaced unfractionated heparin (UFH) nearly 20 years ago as the preferred treatment and prophylaxis agent for several reasons (Chao et al. 2011; Salzman et al. 1980). Its chemical structural properties allow it to specifically inhibit factor Xa without all of the off-target serine protease inhibition seen with UFH. Compared to UFH, LMWH is easier to administer and significantly lowers recurrent thrombosis, major bleeding, and mortality (van Dongen et al. 2004). There is some suggestion that the beneficial effects of LMWH in treating cancer patients may be direct against the tumor cells in addition to the anticoagulant effect (Barni et al. 2014). Even with these advances and new reagents, a major concern to consider is the potential to induce heparin-induced thrombocytopenia (Wharin and Tagalakis 2014; Greinacher 2015).

To Decorate, Cloak, or Hold Tumor Cells: That Is the Question

As part of their function in immune surveillance, platelets interact with viral particles, bacteria, and parasites as well as virally transformed tumor cells (Chabert et al. 2015; Hamzeh-Cognasse et al. 2015; Kofman et al. 2013; Ahmed et al. 2014). Many of these interactions essentially help to decorate pathogens and trigger platelet aggregation and the release of factors that stimulate leukocyte activation and neutrophil extracellular trap (NET) formation (Hamzeh-Cognasse et al. 2015). In contrast, tumor cell-platelet interactions are postulated to suppress immune recognition/cytotoxicity and promote arrest at the endothelium or entrapment in the microvasculature. These responses support survival and spread of cancer cells and the establishment of secondary lesions. The current dogma suggests that once tumor cells enter the circulation, they can become enveloped or decorated by a coating of platelets that shields them from recognition by cytotoxic immune cells. While this notion has received support from numerous investigators, it may not be exactly what occurs. When examining heterotypic aggregates that formed during tumor cell-induced platelet aggregation (TCIPA), the platelets tend to cluster at the center, not the external surfaces (Menter et al. 1987a, c) (Fig. 3). In most cases the tumor cells are not completely enveloped enough following TCIPA to fully encase and hide the tumor cells from immune cells (Menter et al. 1987a, c, e). Furthermore, from a logistical standpoint, the feasibility of completely covering the large tumor cell surface area with much smaller platelets is in doubt.

Platelet Changes in Cancer Patients

Recent studies have demonstrated that there are profound ultrastructural changes in platelets from cancer patients as compared to normal controls or individuals with benign disease. Electron cryotomography (cryo-ET) was used to examine platelets from patients with invasive ovarian cancer and controls (either benign adnexal mass or free from disease). Significant morphological differences were detected between the cancer and control platelets including disruption of microtubules as well as altered numbers of mitochondria (Wang et al. 2015). Furthermore, RNA-seq studies have revealed that molecular pathway changes in tumor-educated blood platelets (TEPs), thus connecting them to systemic and local responses to tumor growth resulting in altered RNA profiles (Best et al. 2015).

Heterotypic Emboli, Platelet Interactions, and Extensive Membrane/Structural Changes

The heterotypic contacts between platelets and tumor cell lead to dynamic focal interactions during TCIPA (Menter et al. 1987a, c, e). As platelets cluster centrally during heterotypic aggregate formation, they change shape and degranulate and release proteins, growth factors, bioactive lipids, and other factors that recruit additional platelets toward the central core of the interaction (Menter et al. 1987a, c, e). Extensive membrane changes occur at the membrane interfaces between platelets and tumor cells. There is very little data on whether the tumor cells or platelets drive these membrane interactions, but they appear to be bidirectional. Extensive membrane/cytoskeletal tumor cell processes heavily interdigitate with a central platelet aggregate, demonstrating a very active process. As these interactions progress, extensive uptake of platelet fragments and mitochondria occurs into the tumor cells in vitro (Menter et al. 1987a, c, e). This interdigitation and uptake of platelet fragments by tumor cells are also observed in vivo following tail vein injections of syngeneic tumor cells into mice (Crissman et al. 1985, 1988; Menter et al. 1987b).

The engulfment of platelets or platelet particles such as mitochondria may be a form of emperipolesis or entosis. These are processes whereby cells take up other cells without degradation with varied outcomes (Xia et al. 2008; Rastogi et al. 2014). This is reminiscent of frustrated phagocytosis where organisms ride along the plasma membrane or become engulfed only to be subsequently released. Some metastatic tumor cells even take on behaviors of M2 polarized macrophages (Caruso et al. 2012; Djaldetti and Strauss 1982).
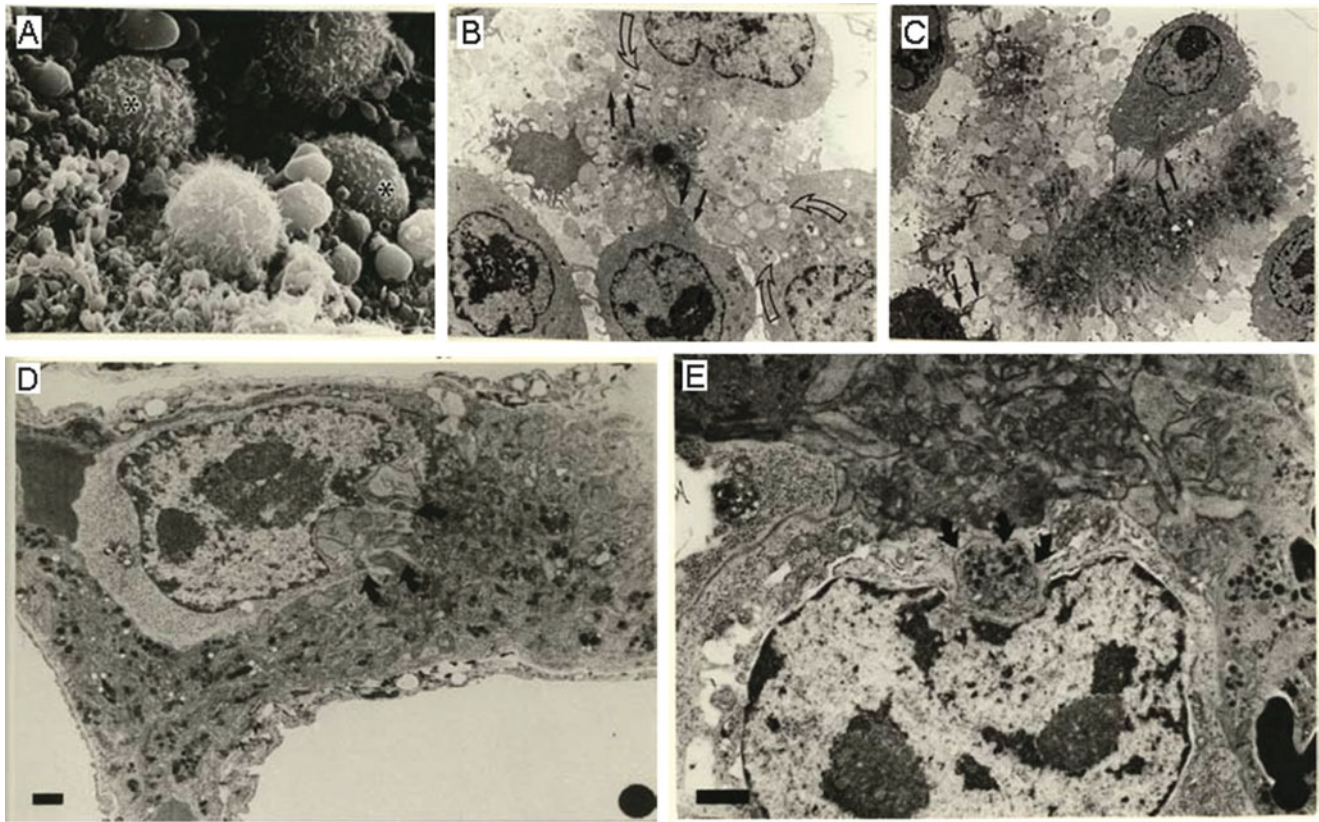
Fig. 3 Early events in platelet-tumor cell interactions. (**a**) Elutriated W256 tumor cells are shown interacting with platelets in an aggregometer cuvette at the plateau phase (10 min). A reduction in tumor cell surface microvilli occurs by this time (*asterisks*, scanning electron micrograph; magnification x3900). (**b**) Elutriated W256 tumor cells project small processes (*arrows*) into platelet aggregates by midphase of aggregation that become more (**c**) prominent and protrude deeper into forming platelet emboli as these interactions plateau (*arrows*) that associates with tumor cell engulfment of platelet fragments (*open arrows*) (transmission electron micrographs; magnifications—(**b**) x3000, (**c**) x4300). (**d**) Elutriated Lewis lung tumor cells form intravascular aggregates in the mouse lung that involves process penetration (*arrows*) into syngeneic C57Bl/6 platelet emboli, which occurs 2 min after tail vein injection and (**e**) engulfing platelet fragments (*arrows*; transmission electron micrographs; magnifications—(**d**) x6800, (**e**) x12,000, *scale bars* 1 μm). (**a–c**) Reprinted from Honn et al. (1983) with permission from S. Karger AG, Basel; (**d, e**) courtesy of Menter

While genomic and epigenetic elements are fundamental to the cancer continuum, the contribution of exosomes/microvesicles, free nucleic acid, and cytoplasmic elements is just as intriguing. It has been suggested that the reaction of the mitochondrial genetic system to genomic (nuclear DNA) stress response genes leads to mitochondrial dysfunction and could account for the marked increase in metabolic diseases and cancer (Wallace 2005; Brahimi-Horn et al. 2015). Studies have demonstrated that tumorigenic phenotypes can be down-modulated when mitochondrial DNA is ablated and can be complemented by reintroduction of mitochondria (Cavalli et al. 1997). Therefore, hyperpolarization of mitochondria in activated platelets and the observation that platelets release mitochondria to potentiate immune response (Zharikov and Shiva 2013; Boudreau et al. 2014) raise the specter that platelet mitochondria potentially make a direct contribution to malignancy by regulating tumor cells. Whether platelet mitochondria compensate for tumor cell mitochondrial deficits or augment some tumor cell function through genetic cross programming remains to be determined. With respect to the latter idea, mitochondrial transcription factor binding sites are seen throughout the nuclear genome. Coordinated regulation of nuclear genes in response to mitochondrial stress or mitochondrial uptake has not yet been established, but it is a topic being scrutinized (Haynes et al. 2013).

Secondary Sites, Exosomes, and Extravasation: Differences or Similarities?

Tumor cell exosomes play key roles in cancer progression and metastasis (Melo et al. 2014, 2015; Kahlert and Kalluri 2013; Milane et al. 2015; An et al. 2015; Robbins and Morelli 2014; Colombo et al. 2014). Functionally, these exosomes can influence the biology of both the local tumoral

and systemic environments (Kahlert and Kalluri 2013). Tumor cell exosome biogenesis often begins with endocytosis, then endosome genesis, followed by the formation of multivesicular bodies (MVBs) or by microvesicle formation (Huber and Holvoet 2015; Zhang et al. 2015). Exosomes formed within MVBs are released following membrane fusion into the surrounding microenvironment or circulation. Locally, release of tumor cell exosomes into the microenvironment can influence proliferation and chemoresistance and initiate fibroblast or immune cell activation and recruitment along with stimulating angiogenesis. Systemically, tumor cell exosomes can stimulate a hypercoagulable state, initiate preneoplastic niches at secondary metastatic sites, recruit bone marrow-derived hematopoietic and immune cells, or elicit immunosuppression (Lima et al. 2013). Classically by contrast, platelets contain numerous electron-dense subcellular organelles called alpha granules, electron-dense and very electron-dense granules, lysosomes, and dense bodies (Fukami and Salganicoff 1977). The many factors contained in these granules constitute what is commonly found in serum used to grow cancer cells in tissue culture and in regenerative medicine (Dhillon et al. 2014; Burnouf et al. 2013; Ross et al. 1978). These organelles are sometimes called secretory granules, since the appropriate stimuli causes degranulation that deposits granule contents in the extracellular surroundings. These organelles were identified and visualized by electron microscopy, microprobe analysis, secretion experiments, and subcellular fractionation studies from patients with platelet storage pool deficiency diseases. In many respects, this degranulation process can be equated with the formation of platelet microparticles (PMP), which are essentially very exosome-like (Goubran et al. 2015; Mezouar et al. 2014). Platelet extravascular migratory behavior and exosome deposition is likely to drive cancer progression or preconditioning of secondary metastatic sites (Goubran et al. 2015). Preventing platelet activation inhibits the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo (Mezouar et al. 2015).

Blood-borne CTCs are commonly found in the blood stream of patients with metastases (Aceto et al. 2014; Li et al. 2015; Joosse et al. 2015). In a patient study of CTCs isolated from breast and prostate cancer patients, their presence correlated with poor prognosis (Aceto et al. 2014). This study focused on comparing single CTCs to CTC clusters and found that markers for platelets were present in both populations (Aceto et al. 2014). The presence of these clusters was also associated with high plakoglobin expression and poor prognosis (Aceto et al. 2014). Since plakoglobin plays an important role in the regulation of CAM interactions (Ilan et al. 2001; Rival et al. 1996;

Reynolds et al. 1992, 1994), its upregulation may influence platelet/tumor cell heterotypic CTC cluster formation. In a separate randomized phase II trial, therapeutically targeting platelet function did not significantly change the number of CTCs in circulation in patients with metastatic breast cancer. Unfortunately, the number of circulating cells was lower than expected, so a more definitive study is needed (Roop et al. 2013). In early mouse studies, although large numbers of CTCs may be present in circulation, only those with specific characteristics survive to establish metastasis (Fidler and Nicolson 1978; Fidler 1975; Hart and Fidler 1980). In other mouse hematogenous metastasis studies, cell arrest at secondary sites precedes the establishment of lesions. This may occur by a variety of mechanisms. Tumor cell interactions directly with the vascular endothelium can initiate the process. In this case, the engagement of platelets can occur during or after tumor cell arrest has occurred, and platelets can fill in around arrested tumor cells. Tumor cell-platelet heterotypic interactions can also occur in suspension in flowing blood to initiate TCIPA. The resulting heterotypic platelet-tumor cell emboli can then undergo attachment or entrapment in secondary organ sites. Platelet-tumor cell emboli initially interact with vascular surfaces at cellular junctions or gaps in the endothelial cell monolayer (Menter et al. 1987e). Platelets are also capable of forming chains that tether tumor cells to these attachment sites (Menter et al. 1987e). As these cellular interactions progress, platelets engage both endothelial cell surfaces and gaps in the endothelial cell monolayer facilitating the linkage to tumor cell membranes (Menter et al. 1987e). In other studies, agent-based, computational, theoretical modeling simulated the basic dynamics of circulating tumor cell adhesion involving endothelial, neutrophil, and platelet interactions and proposed multiple therapeutic targets involving platelets (Uppal et al. 2014). Other studies have utilized intravital microscopy to visualize human red fluorescent protein (RFP) expressing HT29 (RFP-HT29) colon cancer cells injected into the spleens of GFP expressing nude mice (Tanaka et al. 2014). These studies revealed extensive interactions between GFP platelets and RFP-HT29 in liver sinusoids (Tanaka et al. 2012, 2014). Intravital imaging has also been done using a syngeneic C57/Bl6 mouse model, and interactions were shown to involve $\alpha_v\beta_3$ integrins on B16 melanoma cells to $\alpha_{IIb}\beta_3$ on platelets using blocking antibodies (Lonsdorf et al. 2012).

Tumor cells can exit the vasculature at secondary sites (Crissman et al. 1985), and platelets can facilitate this process known as extravasation (Coupland et al. 2012; Crissman et al. 1985; Fein and Egeblad 2013; Pang et al. 2015a; Schumacher et al. 2013). Thrombin activation involving platelet membranes can facilitate the activation of ECM degradation by tumor cells in a P-selectin and

$\alpha_{IIb}\beta_3$-dependent manner (Pang et al. 2015a). Taken together these studies support the notion of platelet involvement in the establishment of hematogenous metastasis.

New Thoughts: Platelets in Premalignancy

So What About Aspirin, Arachidonic Acid, and Omega-3 PUFA?

Meta-analyses of large randomized clinical trials (Bousser et al. 2011; Chan et al. 2012; Chan and Cook 2012; Cuzick et al. 2015; Goldstein and Rothwell 2012; Langley and Rothwell 2013, 2014; Rothwell 2013a) show that aspirin reduces the incidence (Algra and Rothwell 2012), metastasis (Rothwell et al. 2012), and mortality (Rothwell et al. 2012) of gastrointestinal and other cancers, especially adenocarcinomas (Algra and Rothwell 2012; Chan et al. 2012; Cuzick et al. 2015; Langley and Rothwell 2013; Rothwell 2013a, b; Rothwell et al. 2012). Despite substantial cumulative knowledge regarding cancer prevention by aspirin (Umar et al. 2016), little is known about the role of the primary target found in platelets during precancerous lesion development. Aspirin covalently acetylates Ser-530 and irreversibly inactivates platelet cyclooxygenase 1 (DeWitt et al. 1990; Rowlinson et al. 2000), thereby eliminating all downstream prostaglandin production from arachidonic acid by platelets. This includes the key bioactive lipid involved in platelet activation, thromboxane A_2 (TxA_2) (Fontana et al. 2014; Fitzpatrick and Gorman 1977; Hammarstrom and Falardeau 1977). Pronounced elevation of circulating TxA_2 levels accompanies CRC progression in familial adenomatous polyposis (FAP) patients (Li et al. 2015). As a strong platelet TP receptor agonist, TxA_2 produced by TxA_2 synthase in platelets is counterbalanced by an equally strong platelet inhibitory response by IP_1 receptors. IP_1 receptors are activated by PGI_2 that is produced by PGI_2 synthase in endothelial cells lining the vasculature. Both TxA_2 and PGI_2 have epoxide bonds that contain considerable molecular strain, which limits their half-life in circulation. Activation by TCIPA shifts this delicate eicosanoid balance triggering multiple pro-carcinogenic responses centered on platelets.

Diets rich in ω-3 PUFA, EPA, and docosahexaenoic acid (DHA) inhibit carcinogenesis (Holla et al. 2008; Schroeder et al. 2007; Yang et al. 2006, 2014a, b). EPA feeds into the COX-1 and COX-2 pathways to produce TXA_3 and PGE_3 lipid metabolites that are ineffective at PG receptor activation and protect against CVD and lung or colon tumorigenesis (Algra and Rothwell 2012; Chan et al. 2012; Chan and Cook 2012; Cuzick et al. 2015; Goldstein and Rothwell 2012; Holla et al. 2008; Langley and Rothwell 2013, 2014; Liyasova et al. 2010; Macdonald et al. 1999;

Pinckard et al. 1968; Rothwell 2013a; Rothwell et al. 2012; Schroeder et al. 2007; Yang et al. 2006, 2014a). Taking aspirin in combination with EPA and DHA leads to the formation of resolvins, maresins, and protectins (Serhan et al. 2006, 2009; Spite et al. 2014; Spite and Serhan 2010; Krishnamoorthy et al. 2015; Serhan 2014). Resolvins were originally named for their ability to resolve inflammation. More specifically, aspirin-inactivated COX-2 generates a longer half-life R-epimer of resolvin-D_1 (Arita et al. 2005; Serhan et al. 2004; Sun et al. 2007) from DHA and resolvin-E_1 from EPA (Dona et al. 2008; Pirman et al. 2013). Although not well studied, this places platelets not only at the center of the metastasis discussion but also the progression of premalignancies.

Leaky Vessels, Extravasation, and "First Response"

The role of angiogenesis in premalignancy remains an important question, particularly how early this change occurs (Menakuru et al. 2008) (Fig. 4). Biomarkers of this process include factor VIII, CD31, and CD34. Increased microvascular density can occur in ductal carcinoma in situ (DCIS) (Menakuru et al. 2008; Sapino et al. 2001). These premalignant lesions range in size from 1 to 1.5 cm (Allegra et al. 2009). Newly formed vessels can be identified by CD105 (Fox and Harris 2004; Smith et al. 2012; Ratajczak et al. 2012; Dubinski et al. 2012; Marioni et al. 2011; Sapino et al. 2001). Platelet-derived thrombospondin 1 helps regulate angiogenesis and serves as a potential biomarker (Zaslavsky et al. 2010). The presence of highly permeable, disorganized, poorly formed, and leaky blood vessels contribute to progression in advanced cancers (Fang et al. 2011). These leaky vessels provide ample opportunity for platelet first responders to extravasate and enter the precancerous microenvironment. Once in the perivascular space, platelet activation triggers the release of growth-promoting factors found in serum. These serum factors are highly likely to provide a permissive and growth-promoting microenvironment for transformation.

Angiogenic and immune stimulation often switch on during premalignancy, before cells achieve the invasive state (Ferrati et al. 2012; Folkman et al. 1989). Many factors influence this switching, including microRNAs (Bergers and Benjamin 2003; Grandis and Argiris 2009). Switching has been elegantly demonstrated in the adenoma-adenocarcinoma transition of colorectal cancer and the transition from DCIS to invasive ductal carcinoma of the breast. Specifically, the initiation of angiogenesis accompanies dysplasia during adenoma formation in colorectal cancer progression (Staton et al. 2007) and hyperplasia during DCIS
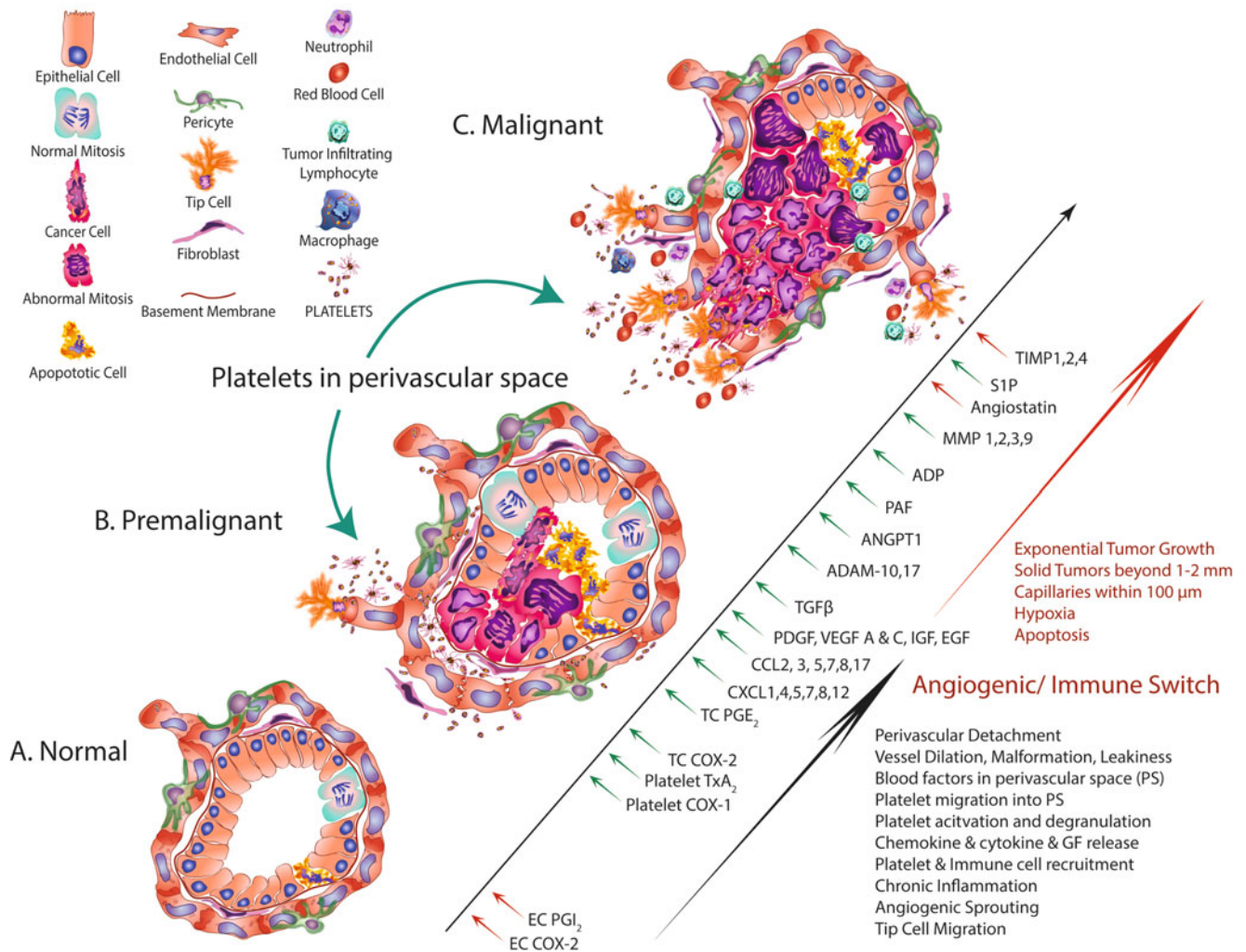
Fig. 4 Platelet influences on angiogenic and immune switches. Angiogenic and immune switches can turn on at different stages of carcinogenesis. (**a**) Biological maintenance of normal tissue involves a delicate balance between cell growth and apoptosis and homeostasis that is regulated by immune surveillance, tissue repair, and immune clearance. (**b**) Premalignant disease may involve the earliest stages of angiogenic and inflammatory switch activation. Premalignancy-associated inflammation triggers immune cell infiltration in response to cytokines and prostaglandins that promotes vessel dilation and the detachment of pericytes from blood vessels. During these early stages of premalignancy, blood factors accumulate in the perivascular space. This provides the opportunity for platelets to migrate into the perivascular space along with activation and the release of platelet granules containing vascular endothelial cell growth factor (VGEF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), and angiopoietin-2 (Ang2) that enhances both angiogenic and immune switch activation. (**c**) The onset of malignancy, heightened hypoxia, and apoptosis further increases vessel leakiness, platelet activation, platelet migration, and granule release. The cycle accelerates as angiogenic sprouting and tip cell-mediated migration into the growing tumor lead to further recruitment of additional platelets, immune cells, and tumor cell migration and proliferation as cancer progression continues

progression to invasive breast cancer (Bluff et al. 2009). Mechanistically, mice that lack histidine-rich glycoprotein (HRG) exhibit an accelerated angiogenic switch and enhanced platelet activation, inducing molecular changes in the pre-tumorigenic environment accompanied by increased survival, angiogenesis, and EMT (Cedervall et al. 2013). Although more studies are needed, these effects suggest an early, vital role for platelets early in the premalignancy to malignancy transition. Abnormal or leaky blood vessels that arise during tumor-initiated angiogenesis provide adequate opportunity for the leakage or migration of platelets into the precancerous microenvironment. Subsequent activation and release of their stroma-stimulating, proangiogenic, chemoattractive, and immuno-modulatory contents can then promote precancerous lesion progression as a series of cyclic amplification events. In support of this hypothesis, release of VEGF and other platelet factors occurs during early breast cancer (McDowell et al.

2005), and platelet GP$_{Ib\alpha}$ also increases in invasive intraductal breast carcinoma tissues (Oleksowicz et al. 1997). These early angiogenic and immunogenic switching events during premalignancy suggest that platelets are likely contributors to early cancer progression along with potential targets for early diagnosis (Han et al. 2014).

Immunotherapy?

Platelets are derived from megakaryocytes (Menter et al. 2014; Machlus and Italiano 2013), arising during the process of megakaryopoiesis from hematopoietic stem cell lineage that share commonalities with both lymphoid and granulocytic cells prior to differentiating toward hematopoietic progenitors. Specifically, in the bone marrow, hematopoiesis produces hemangioblasts that give rise to *hematopoietic stem* cells (HSCs) and *multipotent progenitor* cells (MPP) (Deutsch and Tomer 2013; Meng et al. 2012; Thon and Italiano 2010; Akbar et al. 2011). Genesis from MPP forms *common myeloid progenitor* cells (CMP) (Deutsch and Tomer 2013; Akbar et al. 2011; Thon and Italiano 2010; Meng et al. 2012) that give rise to *megakaryocyte-erythroid progenitor* (MEP) cells followed by *megakaryopoietic progenitors* (MKP) (Kanz et al. 1987). MKP then generate immature—followed by mature—megakaryocytes before finally generating platelets (Deutsch and Tomer 2013; Geddis 2010; Machlus and Italiano 2013; Tijssen and Ghevaert 2013; Meng et al. 2012). Immune thrombocytopenia (ITP) involves cellular checkpoints during the normal regulation of immunological self-reactivity and the development of B and T cells (Cines et al. 2009). This may occur through cell deletion, gene editing, induction of anergy, and extrinsic cellular suppression. When immune checkpoints fail, tolerance to self-antigens may be downregulated (Toltl et al. 2011; Cines et al. 2009). Checkpoint failure is a key target of immune checkpoint blockers that are being used in combination with interferon to overcome immune tolerance (Rafique et al. 2015). Importantly, platelet genesis and lineage development are also influenced by interferon (Rivadeneyra et al. 2015; D'Atri et al. 2015). With the advent of immune checkpoint cancer therapy, the influence of these agents may cross over to impact platelet biology and function (Topalian et al. 2015; Miller and Sadelain 2015; Gubin et al. 2014), which may also influence immune-related adverse events that are autoinflammatory in nature (Weber et al. 2015). Most notably, preclinical data demonstrate that pharmacologic inhibition or genetic deletion of COX-1 and COX-2 synergizes with anti-PD-1 blockade to induce the eradication of tumors more effectively than PD-1

treatment alone (Zelenay et al. 2015). Aspirin treatment was highly effective in synergizing with anti-PD1, but platelet function was not followed in these studies and deserves additional study (Zelenay et al. 2015). In view of their "first responder" properties, it is critical to develop a better understanding of this role or potentially harness platelets in some fashion to alter checkpoint blockade. One could envision taking bone marrow aspirates from cancer patients and engineering in vitro cultures of megakaryocytes (Nishikii et al. 2015; Hatami et al. 2015; Meinders et al. 2015; Panuganti et al. 2013; Thiele et al. 2012; Emond et al. 2012; Pallotta et al. 2009) to alter tumor recognition and inflammatory responses and reinfuse these reengineered platelets though adoptive bone marrow transfer.

Platelets: "First Responders" in the Complete Cancer Continuum

Platelets as vital contributors to the early stages of cancer formation are a new concept. Support for this notion comes from a variety of sources. One line of evidence is the long-standing link between chronic inflammation and the risk of developing colon cancer. Patients with inflammatory bowel disease or Crohn's disease, for example, are at high risk for developing colon cancer (Herszenyi et al. 2015; Burisch and Munkholm 2015; Sanduleanu and Rutter 2014). A potential association with platelet biology may be found in mean platelet volume changes that accompany Crohn's inflammatory disease states, among others (Kilincalp et al. 2015; Tang et al. 2015; Huang et al. 2013; Harris et al. 2011). Similarly, changes in mean platelet volume are found during adenoma formation (Kilincalp et al. 2015) along with colorectal cancer (Li et al. 2014; Kemal et al. 2014).

Additional support for this notion comes from $Apc^{min\backslash+}$ mouse crossbreeding with $P\text{-}selectin^{-\backslash-}$ (CD62P) knockout mice, which lack expression on activated platelets and reduced VEGF expression (Qi et al. 2015). These crosses resulted in reduced formation of low- and high-grade adenomas accompanied by decreased vessel density (Qi et al. 2015). This study also examined human adenomas and stages I–IV of CRC progression by IHC for CD41 (integrin GP$_{IIb}$ subunit) positivity. Similar levels of platelet staining were observed in the polyp and stage I CRC tissues (Qi et al. 2015), suggesting that the platelets were already involved at the polyp stage. This CD41 staining increased in intensity with each progressive stage II–IV of CRC tumor development. Our group has also recently observed extravascular platelet structures in $Apc^{min\backslash+}$ polyps (Fig. 5).
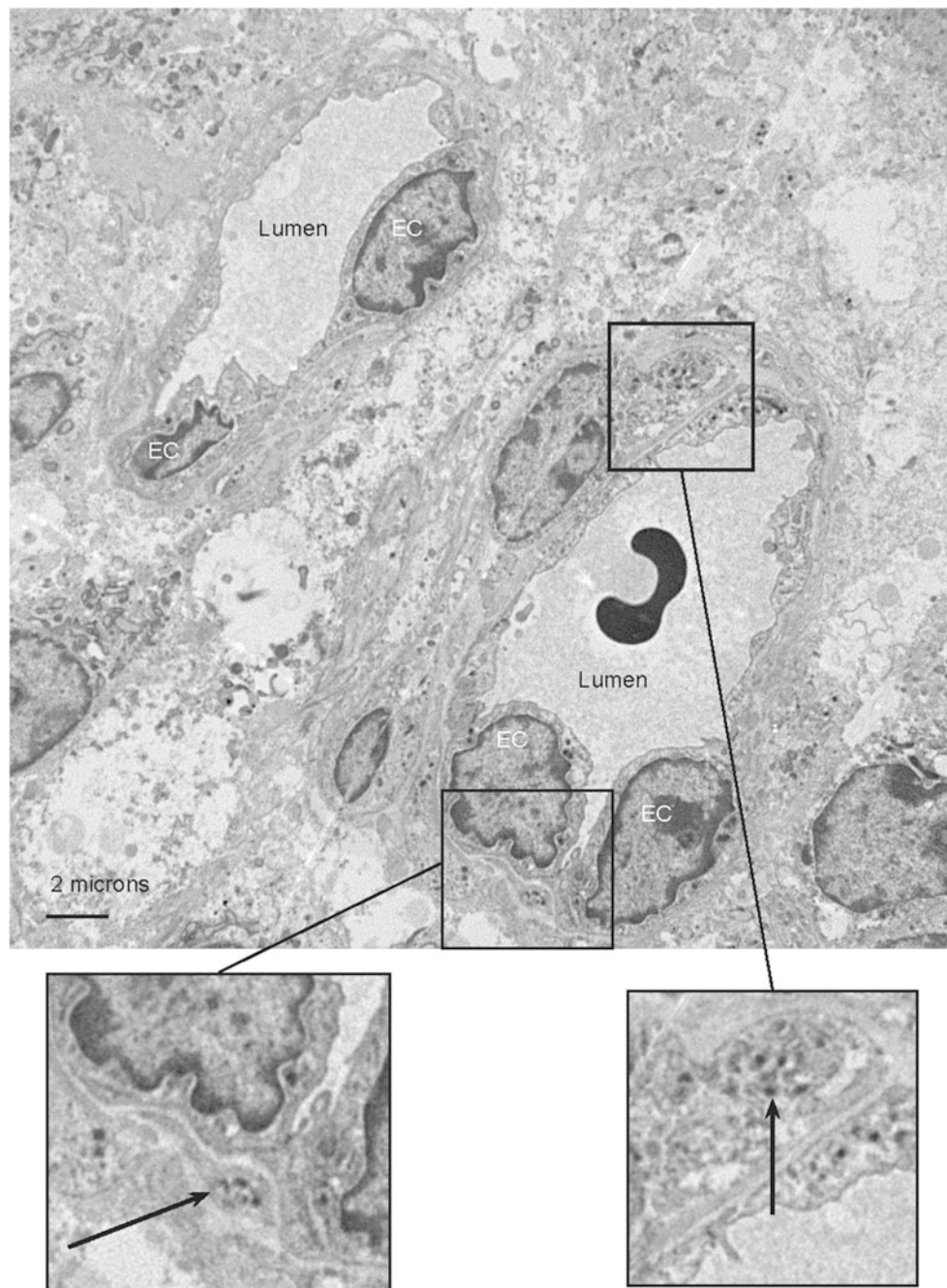
Fig. 5 Platelet migration into adenoma perivascular spaces. Ten-week-old adenomatous polyposis coli $Apc^{min\backslash+}$ mice were euthanized and necropsied, and the intestinal tract was excised and fixed. Adenoma polyps were dissected and processed for transmission electron microscopy. The examination of polyp tissue revealed platelet structures in the perivascular space of blood vessels (*arrows*; *insets*). These platelets were underlying endothelial cells (EC) and had exited the vascular lumen (x4000, *scale bars* 2 μm)

These data, along with our previous studies illustrating the dynamic activation of platelets in heterotypic platelet-tumor cell aggregates and syngeneic intravascular metastasis, highlight a role for platelets throughout the cancer continuum. Collectively, these data strongly suggest that altering the "first responder" capabilities of platelets is an important target for cancer prevention and therapy.

Take Home Messages

- Platelets are "First Responders" as active participants in the hemostasis, wounding, immune, and metastatic processes.
- Platelets are often neglected or overlooked during in vivo experimental or pathologic observations due to their small size and lack of defining nuclei.
- Surface receptors abound on both platelets and tumor cells trigger the primary response mechanisms that drive biologic function.
- When examining heterotypic aggregates that form during tumor cell-induced platelet aggregation (TCIPA), the platelets tend to cluster at the center.
- Platelets can also actively migrate across the inflamed vascular wall in response to a variety of microenvironmental and tumor factors.
- Additional critical receptors that can impact interactions between platelets and tumor include those influencing immunomodulation.
- Platelets serve as "First Responders" in the complete cancer continuum.

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