Pharmacology & Therapeutics 157 (2016) 112–119

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate Editor: B. Teicher

Involvement of platelets in tumor cell metastasis☆

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ARTICLE INFO

Available online 23 November 2015

Keywords:
Platelet–tumor loop
Epithelial–mesenchymal-like transition
Metastasis
Immune evasion
Angiogenesis
Microparticles

ABSTRACT

Extensive experimental evidence indicates that platelets contribute to tumor cell proliferation and metastasis through direct interactions and secreted bioactive proteins. Activated platelets release secretory factors that promote growth factors, chemokines, proangiogenic regulatory proteins, proteolytic enzymes and microparticles within the microenvironment to promote tumor cell growth and invasion. Furthermore, the formation of platelet–tumor cell heteroaggregates by integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa) bridging plays an important role in tumor survival by forming a physical shield around tumor cells, and thereby protecting circulating tumor cells from immune-mediated lysis by natural killer (NK) cells. Tumor cells directly activate platelets by enhancing expression of surface integrins, selectins and secretion of granules, which amplify platelet aggregation. In addition to the physical coating of tumor cells, platelets release transforming growth factor-β1 (TGF-β1) that induces phenotypic changes of epithelial to mesenchymal-like transition of tumor cells, thereby facilitating their extravasation and dissemination to distant sites during metastasis. Thus, there is a complex interplay between platelet-induced tumor growth and tumor cell-induced platelet activation, with the involvement of multiple components within the tumor microenvironment that enhance metastasis. This review describes the intimate reciprocal cross-talk between platelets and tumor cells, and the various signaling pathways involved in tumor amplification, which may be potential therapeutic targets to disrupt the platelet–tumor loop to reduce metastatic processes.

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1. Introduction

The main function of platelets is to halt hemorrhage after tissue trauma and vascular injury. This is initiated through platelet activation, which results in adhesion and release of a multitude of bioactive factors from platelet granules. Platelets become activated when the continuity of the endothelial layer is disrupted and the underlying subendothelial matrix is exposed, or if inflammation perturbs the endothelium. Platelet

Abbreviations: ADP, adenosine diphosphate; COX, cyclooxygenase; GP, glycoprotein; HMGB1, high-mobility group box1; NO, nitric oxide; PAR, protease activated receptor; TGF-β1, transforming growth factor-β1; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

☆ Disclaimer: This article reflects the views of the author and should not be construed to represent FDA's views or policies.

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http://dx.doi.org/10.1016/j.pharmthera.2015.11.005
0163-7258/Published by Elsevier Inc.

receptors then interact with collagen and von Willebrand factor (vWF), which capture the platelets and induce activation signals (Fig. 1). The process leads to the firm attachment of platelets to the injured vessel wall and to the formation of platelet aggregation to seal the injury.

Platelet activation and adhesion at sites of intimal injury involve the interaction of platelets with the subendothelial matrix, which contains adhesive ligands and membrane receptors (Jackson et al., 2003; Tesfamariam, 2008). Activated platelets initially interact with the subendothelial matrix through interaction with adhesive receptors, such as glycoprotein (GP) Ib-IX-V complex, that mediate rolling and tethering of platelets to vWF at the site of intimal injury (Jackson et al., 2003; Massberg et al., 2003). Next, the platelet collagen receptors GPIa/IIa (integrin $\alpha_2\beta_1$) and GPVI mediate a more firm adhesion, leading to further platelet activation. The recruitment of platelets into a growing platelet thrombus is mediated by a variety of locally generated mediators that are released once platelet adhesion has been initiated (Minuz et al., 2006; Murugappa & Kunapuli, 2006). These include adenine nucleotides, thromboxane A_2 (TXA_2), and serotonin, which are secreted from activated platelets, and thrombin, which is produced on the surface of activated platelets. The activation of G protein-mediated signaling pathways causes a further increase in the secretion of stored granules, thus acting as positive-feedback mediators that amplify the initial signals to the rapid activation and recruitment of platelets into a growing thrombus (Andre et al., 2003; Jackson et al., 2003). These events cause a conformational change in a membrane-bound platelet receptor GPIIb/IIIa ($\alpha_{IIb}\beta_3$), enabling the binding of adhesive ligands to initiate intracellular signaling that further stabilizes the aggregation resulting in thrombus formation (Jackson et al., 2003). In addition to their function in hemostasis, activated platelets also participate in wound healing and tumor cell metastasis (Jain et al., 2010; Labelle & Hynes, 2012).

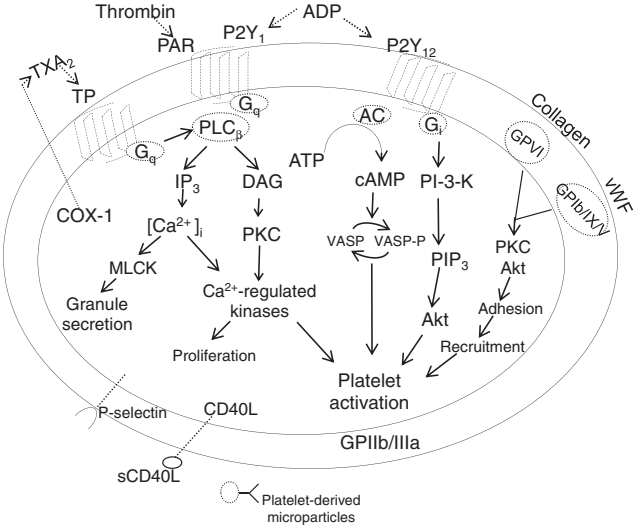

Fig. 1. Platelet signaling pathways: activation of G-protein coupled receptors by ADP, TXA_2 and thrombin activate signaling cascades linked to increase in calcium-regulated kinases, granule secretion and integrin activation. Full secretory responses of platelets to various stimuli require synergistic activation of Ca^{2+}, PKC, and PI-3K-mediated pathways. Thrombin activates protease-activated receptors (PAR), ADP activates $P2Y_1$ and $P2Y_{12}$, and TXA_2 activates TP receptors, which leads to the activation of GPIIb/IIIa ($\alpha_{IIb}\beta_3$), which bridges with fibrinogen to form a link between two platelets and promote platelet aggregation. Collagen and vWF bind to GPVI and GPIb-IX-V receptors to induce stable adhesion and recruitment. AC, adenyl cyclase; ADP, adenosine diphosphate; DAG, diacyl glycerol; MLCK, myocin light chain kinase; PI-3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol-3,4,5-triphosphate; PLC, phospholipase C; PKC, protein kinase C; sCD40L, soluble CD40 ligand; TP, thromboxane/prostanoid receptors; TXA_2, thromboxane A_2; VASP, vasodilator-stimulated phosphoprotein; vWF, von Willebrand factor.

2. Platelet mediators and signaling

Activated platelets secrete several mediators from intracellular dense core granules and α-granules that amplify platelet activation and aggregation. Dense granules contain small molecules such as adenine nucleotides, TXA_2, or serotonin, whereas α-granules contain various proteins including growth factors, chemokines, adhesive molecules, and coagulation factors. Adenosine diphosphate (ADP) induced activation of platelets is mediated through two different G-protein-coupled receptors on the platelet surface, $P2Y_1$ and $P2Y_{12}$. The $P2Y_1$ receptor is linked to phospholipase C and stimulates mobilization of intracellular calcium stores and plays a role in initiating ADP-induced platelet degranulation. The $P2Y_{12}$ receptor is coupled to adenylate cyclase and is necessary for a full activation response to ADP, and is important in potentiating platelet aggregation. Platelet cell surface receptors also include the TP receptors and protease-activated receptors (PAR) that are activated by TXA_2 and thrombin, respectively (Camerer et al., 2004; Ekambaram et al., 2011). Thrombin is generated by the coagulation cascade and acts on platelet surface G-protein coupled protease receptors, PAR-1 and PAR-4 in human platelets, and PAR-3 and PAR-4 in mouse platelets, and cleaves the extracellular terminal domain of the tethered ligand to activate intracellular signaling and mediate platelet activation and aggregation (Camerer et al., 2004).

Platelet membrane contains integrins that participate in adhesion and aggregation processes, which include GPIb-IX-V, GPVI, and GPIIb/IIIa receptors (Bambace et al., 2010). Expression of GPIIb/IIIa complex and its binding to fibrinogen is considered to be the final common pathway for all platelet agonists. The amino acid sequences Arg-Gly-Asp (RGD) or arginine–glycine–asparagine–serine (RGDS) of fibrinogen, vWF and fibronectin bind to the activated GPIIb/IIIa receptor to form molecular bridges between aggregating platelets. Platelet receptors GPVI and integrin α2β1 mediate adhesion to collagen, whereas GPIb-IX-V mediates platelet rolling and adhesion to vWF. Binding of platelet receptors such as GPVI and GPIbα to exposed collagen and vWF, respectively, leads to the activation of platelet integrin GPIIb/IIIa, which enables firm adhesion and platelet aggregation.

P-selectin is an adhesion molecule stored in the α-granules of platelets and is expressed on platelet surface membrane upon activation, and then shed to the external membrane through a process of membrane flipping. P-selectin mediates initial platelet aggregates formed by GPIIb/IIIa-fibrinogen interactions, allowing the formation of large platelet aggregates (Borsig et al., 2001). CD40 ligand (CD40L) is a type II transmembrane protein and a member of the tumor necrosis factor family found on activated platelet surface (Caggiari et al., 2007). The surface-expressed CD40L is cleaved from the platelet membrane to generate a soluble fragment (sCD40L). Platelet factor-4 (PF-4) and β-thromboglobulin are platelet specific CXC chemokines, stored in platelet α-granules, and released extracellularly upon activation. Thrombospondin-1 is a trimeric glycoprotein secreted from platelet α-granules upon activation and is involved in platelet aggregation, by stabilization of fibrinogen binding to platelets.

3. Platelets in tumor cell growth and metastasis

In addition to the regulation of hemostasis and coagulation, platelets play a role in tumor cell growth and metastasis. There is a growing evidence to suggest that there is an interplay between platelets and tumor cells that contribute to the tumor-cell growth and its subsequent role in malignancy progression and survival (Fig. 2). Platelet α-granules are sources of a wide range of growth factors and cytokines that contribute to tumor metastasis. Following activation of platelets, a myriad of angiogenic, mitogenic proteins and growth factors are released from the platelets into the tumor microenvironment to enhance tumor cell growth. In addition, platelets facilitate the adhesion of tumor cells to the vascular endothelium and form a protected tumor cell microenvironment (Nierodzik et al., 1995).