

Interaction between circulating cancer cells and platelets: clinical implication

Xiao-Liang Lou^{1*}, Jian Sun^{1*}, Shu-Qi Gong^{1*}, Xue-Feng Yu¹, Rui Gong¹, Huan Deng^{1,2,3}

¹Molecular Medicine and Genetics Center, ²Department of Pathology, The Fourth Affiliated Hospital of Nanchang University, Nanchang 330000, China; ³Renmin Institute of Forensic Medicine, Nanchang 330000, China

*These authors contributed equally to this work.

Correspondence to: Huan Deng, MD, PhD. Department of Pathology, The Fourth Affiliated Hospital of Nanchang University, 133 South Guangchang Road, Nanchang 330003, China. Email: beandeng@hotmail.com.

Abstract: Metastasis is the main cause of cancer-associated mortality. During this complicated process, some cancer cells, also called circulating tumor cells (CTCs), detach from primary sites, enter bloodstream and extravasate at metastatic site. Thrombocytosis is frequently observed in patients with metastatic cancers suggesting the important role of platelets in metastasis. Therefore this review focuses on how platelets facilitate the generation of CTCs, protect them from various host attacks, such as immune assaults, apoptosis and shear stress, and regulate CTCs intravasation/extravasation. Platelet-derived cytokines and receptors are involved in this cascade. Identification the mechanisms underlie platelet-CTCs interactions could lead to the development of new platelet-targeted therapeutic strategy to reduce metastasis.

Keywords: Circulating tumor cells (CTCs); platelet; epithelial-mesenchymal transition (EMT); immune surveillance; metastasis

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Introduction

More than 90% of all cancer-associated deaths are caused by metastasis, a cascade of events beginning with the epithelial-mesenchymal transition (EMT) (1). EMT, as well as its reverse process, mesenchymal-epithelial transition (MET), play pivotal roles in organ development, tissue repair and cancer metastasis by endowing epithelial cells with enhanced migratory capacity, elevated resistance to apoptosis and increased production of ECM components (2). A subpopulation of cancer cells, which undergo an EMT/MET stage, can detach from the primary site, invade through the surrounding tissue, enter and survive in the circulation, and proliferate in a foreign microenvironment (3,4). These cells are called circulating tumor cells (CTCs) (5). The presence of CTCs in patients with carcinoma is associated with a poor prognosis because CTCs may reach a secondary organ prior to the appearance of clinical symptoms. Therefore, CTCs may represent not only a prognostic marker but also be a promising target for

anticancer therapies. To exploit the window of opportunity for clinical intervention, a better understanding of the biological behaviors of CTCs is required.

Thrombocytosis is frequently observed in patients with metastatic malignant tumors (6,7). The risk of venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), is increased up to seven-fold in these patients compared with non-cancer patients (8-10). These clinical data suggest that platelets may contribute to metastasis, in addition to their well-known role in hemostasis and coagulation. Platelets are an anucleate, discoid shaped blood cell, which contain three types of secretory granules, α -granules, dense granules, and lysosomes (11). Alpha granules, which are the most abundant granules in platelets, include large proteins contributing to adhesion and aggregation. Dense granules contain small, nonprotein substances, which upon secretion, recruit subsequent platelets. Lysosomes primarily secrete hydrolases involved in the elimination of platelet aggregates (7).

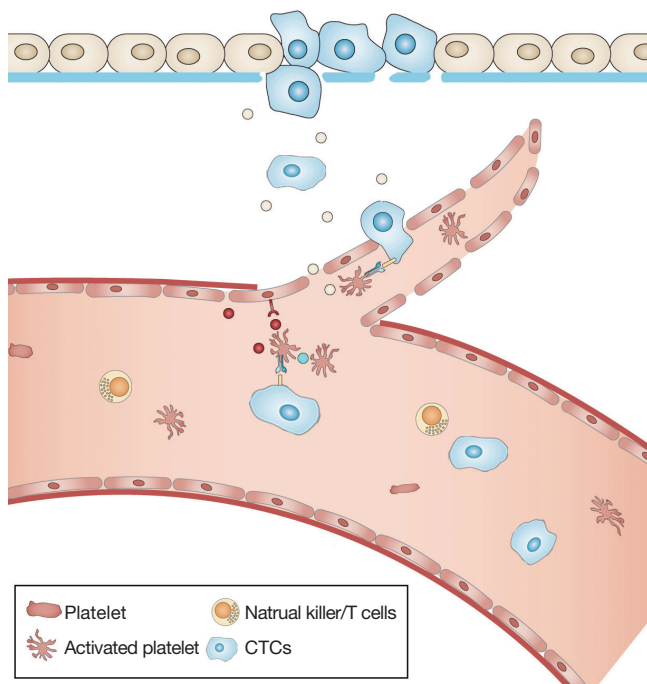


Figure 1 The generation and intravasation of CTCs. Platelets are activated by interactions with CTCs. They then secrete several cytokines, such as LPA. These cytokines up-regulate molecular signaling pathways that facilitate the detachment of cancer cells from the primary site and extravasation into the bloodstream. Activated platelets also promote tumor angiogenesis via platelet-derived VEGF. CTCs, circulating tumor cells; LPA, lysophosphatidic acid; VEGF, vascular endothelial growth factor.

To further demonstrate the contribution of platelets to metastatic processes, several studies using established animal models reported that platelets promote metastasis by protecting tumor cells from host immune surveillance and enhancing CTCs-endotheliocyte adhesion (12-14). Although the underlying molecular mechanisms have not been completely elucidated, advances in our understanding of metastasis have highlighted the importance of direct or indirect interactions between platelets and CTCs.

Role of platelets in angiogenesis

Angiogenesis is a rate-limiting process in cancer metastasis. The formation of CTCs is hampered by tight vascular wall barriers (15). However, the neovasculature of primary tumors typically has weak and leaky endothelial cell junctions, which facilitates transendothelial migration (TEM) (16-20). Platelets contain angiogenic and angiostatic

factors, and the switch to an angiogenesis phenotype can be triggered by metastasizing tumor cells (21,22). Tumor cells can function indirectly via binding to von Willebrand factor (vWF) to initiate platelet aggregation, resulting in the release of vascular endothelial growth factor (VEGF), one of the most powerful positive regulators of angiogenesis (23). Ligation of the protease-activated receptor-1 (PAR-1) can also promote the release of VEGF-containing α -granules (24,25). By contrast, PAR-4 activation up-regulates the secretion of endostatin, a platelet-derived angiogenesis inhibitor (Figure 1) (25).

Platelets also affect angiogenesis by seeding microparticles, which express platelet surface antigens, including CD41 and CD42b (26,27). Metastasizing cancer cells can activate platelets at the primary site, increasing the local concentration of platelet microparticles (PMPs). After fusing with target cancer cells, PMPs may deliver pro-angiogenic factors, such as basic fibroblast growth factor (bFGF) and VEGF (28). Additionally, circulating PMPs may up-regulate the level of matrix metalloproteinase 2 (MMP2) in prostate cancer cells and facilitate the intravasation of metastasizing cancer cells (29). The increased invasive potential of tumor cells induced by PMPs further confirms a robust interaction between platelets and CTCs (30).

Interactions between platelets and primary tumor cells

Although the mechanisms by which platelets act are poorly understood, there is evidence that primary tumor cells express thrombin to promote metastasis through platelets (Table 1). Thrombin enhances tumor cell-induced platelet aggregation (TCIPA) *in vitro* by fully activating specific membrane receptors on platelets (83). Treating mice with established melanoma using r-hirudin, a highly specific antagonist of thrombin, blocks coagulation events and inhibits lung metastasis (84).

Recently, the role of platelets in the progression of malignant tumors has gained attention (85,86). Activated platelets are a primary source of lysophosphatidic acid (LPA), a simple lipid with growth factor-like signaling properties (87,88). Levels of LPA increase in up to 90% of patients with gynecologic cancers (89). LPA is involved in the initiation and progression of several cancers, such as colon, ovarian, prostate, breast, melanoma and thyroid (90,91). The effects of LPA are mediated by at least six different G protein-coupled receptors (LPA₁₋₆) (92). Selective blockage of LPA₁ and LPA₂ inhibit cancer cell proliferation and

Table 1 Agents involved TCIPA

Agents	Origin	References
Adenosine diphosphate (ADP)	Platelet	(31)
Thromboxane A2 (TXA2)	Tumor cell	(32-36)
12-HETE	Tumor cell	(37)
Thrombin	Tumor cell	(38-44)
Cathepsin B	Tumor cell	(45-48)
Matrix metalloproteinase 2 (MMP-2)	Platelet and tumor cell	(49-51)
GPIb-IX-V	Platelet and tumor cell	(52)
von Willebrand factor (vWF)	Tumor cell	(50)
GPIIb/IIIa (α IIb β III)	Tumor cell	(52-61)
avb3	Platelet and tumor cell	(62)
P-selectin	Tumor cell	(63-69)
Podoplanin	Tumor cell	(70-72)
Prostacyclin	Tumor cell	(73-80)
NO	Platelet and tumor cell	(81,82)

TCIPA, tumor cell-induced platelet aggregation.

invasion, which are essential for CTC generation (93-95). LPA up-regulates the activity of MMP2, MMP7 and MMP9 in cancer cells (96-99). MMPs are a family of zinc-dependent endopeptidases that are important mediators of cancer progression. They act via the degradation and remodeling of the ECM (100). The increased expression of MMPs helps tumor cells detach from the primary site and enter into the circulatory system (101). In addition, a tumor bearing mouse model with thrombocytopenia exhibits reduced tumor cell proliferation and increased tumor necrosis (102). Accumulating evidence indicates that the selective inhibition of platelet activity in patients with malignant tumors not only reduces the risk of embolic events but also reduces tumor growth (*Figure 1*) (103-106).

Although considerable progress has occurred in elucidating the interactions between platelets and tumor cells, there is no direct evidence that platelets affect tumor cell intravasation directly (12). Further experimentation is required to identify the mechanisms underlying this key step during metastasis.

Platelets and CTC survival

After leaving the supportive microenvironment, CTCs

face many survival challenges in the circulation, including immunological attack, shear forces and apoptosis. Although the majority of CTCs are destroyed, less than 0.1% of CTCs survives and triggers TCIPA by direct contact or through the release of agonistic mediators, such as ADP, thrombin, TXA2 and tumor-associated proteinases (14,107-109). Platelets are activated in TCIPA and attach to the surface of CTCs by a GPIIb-IIIa-fibrinogen bridge and up-regulated P-selectin (*Figure 2*) (14,110).

However, the molecular mechanism by which platelets promote the survival of CTCs in the blood stream is not fully understood. Several hypotheses propose that the surface coating of platelets may serve as a shield against immune assault because the effect of anti-tumor attacks mediated by NK cells is primarily based on the direct interaction with CTCs (111,112). There is solid experimental evidence that thrombocytopenia caused by either platelet depletion with anti-platelet sera or by defective platelet production significantly enhances the ability of NK cells to lyse CTCs *in vitro* and *in vivo* (113). Furthermore, activated platelets can transfer the major histocompatibility complex (MHC) to CTCs, which in turn mimics host cells and escapes immune surveillance (114). Moreover, platelet-derived TGF- β may reduce the expression of the immunoreceptor NKG2D, thus inhibiting NK cell activity (115). In addition to NK cells, platelet-derived VEGF may inhibit the maturation of dendritic cells, the major antigen-presenting cells in the immune system (116).

EMT, as well as its reverse process, MET, play pivotal roles in cancer metastasis by endowing tumorous cells with migratory, invasive and anti-apoptosis properties (117). CTCs share many phenotypic and functional traits with cells undergoing EMT (118). Recent studies suggest that CTCs in patients with breast cancer or prostate cancer co-express EMT-related markers, including E-cadherin, cytokeratin (CK), vimentin and N-cadherin (119-121). Inhibition of EMT-related signaling elements, such as Twist, Zeb and Snail, can prevent metastatic relapse (122). However, the underlying molecular mechanisms by which CTCs maintain the EMT state have not been elucidated. In addition to their well-established role in protecting CTCs from immune assaults, platelets may also contribute to the EMT of CTCs (123). TCIPA promotes platelets to release α -granules, which contain TGF- β and platelet-derived growth factor (PDGF) at concentrations several-fold higher than most cell types (124). Platelet-derived TGF- β activates the Smad signaling pathway and promotes

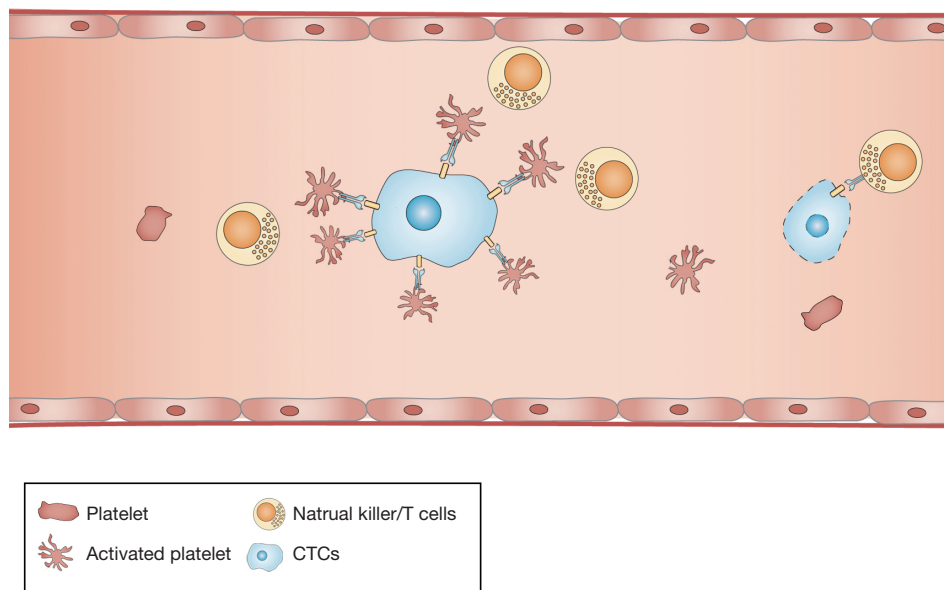


Figure 2 The survival of CTCs in circulation. The majority of CTCs are killed by NK/T cells. Platelets activated in TCIPA can attach to the surface of CTCs by a GPIIb-IIIa-fibrinogen bridge and up-regulated P-selectin to protect CTCs from immune surveillance and physical stress. TCIPA also help CTCs to undergo EMT, which enhances the ability of CTCs to avoid apoptosis. CTCs, circulating tumor cells; TCIPA, tumor cell-induced platelet aggregation; EMT, epithelial-mesenchymal transition.

the transdifferentiation of CTCs into a mesenchymal-like phenotype (123). PDGF is another important EMT driver that contributes to cancer invasion and angiogenesis. Overexpression of PDGF-D promotes the EMT of prostate cancer cells both *in vitro* and *in vivo* via the activation of rapamycin downstream targets, S6K and 4E-BP1 (125). The crosstalk between PDGF and EMT-related signaling pathways, such as the nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) and chemokine (C-X-C motif) receptor 4 (CXCR4), further indicates that PDGF plays an important role in EMT (126,127). Interestingly, in a study of hepatocellular carcinoma (HCC), PDGF was hypothesized to be involved in TGF- β -induced EMT of metastasizing cancer cells (128). Additional studies determined the molecular mechanism underlying this process, by which TGF- β enhances the expression of PDGF and PDGFR via activation of β -catenin and the signal transducer and activator of transcription 3 (STAT3) (129,130).

Platelet-mediated tumor extravasation

After CTCs successfully escape from physical and immune destruction in the circulation, they localize in distant

organs. CTCs must anchor to the luminal side of vascular endothelial cells and then break through the subepithelial extracellular matrix (ECM) (16). Although this process is primarily mediated by the interaction between adhesion receptors on CTCs and ECs, platelets may serve as a potent regulator of this process (*Figure 3*) (14). First, interactions between CTCs and platelets and leukocyte activated vascular ECs induce the expression of C-C chemokine ligand 5 (CCL5), which in turn leads to the increased recruitment of leukocytes to CTCs (131). Indeed, leukocytes are implicated in promoting tumor cell survival and metastasis to the lung (132). Inhibition of CCL5 by a receptor antagonist significantly inhibits this metastatic process (131). Second, by triggering several specific signaling pathways, platelet-derived TGF- β and PDGF induce EMT in CTCs. EMT improves the ability of CTCs to avoid apoptosis and pass through the vessel wall, as described in the previous section. Ablation of platelet-derived TGF- β reduces metastasis, suggesting that they play an important role in this process (123). Third, activated platelets may be involved in the establishment of a prometastatic microenvironment through the recruitment of inflammatory cells. Upregulation of CCL2 expression in CTCs in response to the interaction with platelets promotes

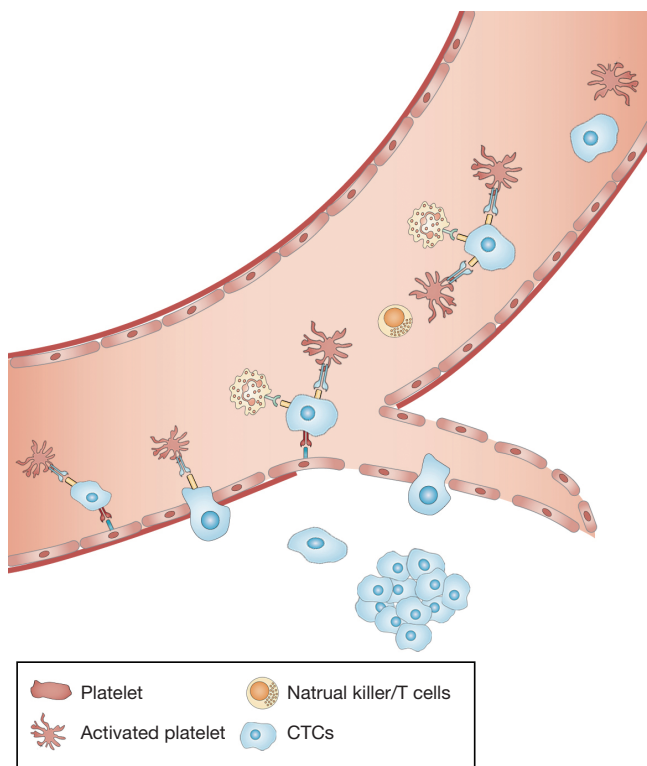


Figure 3 The extravasation of CTCs. At metastatic sites, interactions between CTCs, platelets and leukocytes help CTCs to anchor to the luminal surface of endothelial cells. Platelet-derived TGF- β and PDGF induce EMT in CTCs and endow CTCs with migratory and invasive properties to break through the ECM of blood vessels. CTCs, circulating tumor cells; PDGF, platelet-derived growth factor; EMT, epithelial-mesenchymal transition.

both monocyte recruitment and an increase in vascular permeability (133).

Conclusion and future directions

In conclusion, it is clear that platelets have many roles in tumor metastasis. Platelet-derived cytokines and receptors are important for protecting CTCs from host immune attack and physical stress. Because platelet-tumor cell interactions induce platelet activation and aggregation, it is reasonable to interfere with this process as a therapeutic intervention. Blockade of GPIIb/IIIa with the monoclonal antibody 10E5 reduces lung metastatic events (134). Hirudin, a specific thrombin inhibitor, inhibits metastasis in experimental models (84). Several studies suggest that heparin, a powerful P-selectin inhibitor, can attenuate

tumor metastasis in mice (135). Recently, evidence has shown that the chemotherapeutic effects of aspirin on the metastatic process may depend on the inhibition of platelet function (136). Therefore, platelets are a promising therapeutic target for the attenuation of metastatic events. However, whether patients with cancer will benefit from prophylactic dose of platelet inhibitors has yet to be determined. Although prostacyclin, one of the most potent platelet inhibitors, reduces the metastasis of osteogenic sarcoma, it fails to reduce pulmonary metastasis induced by many types of tumors (137,138). Clinical studies suggest that the daily administration of semuloparin, an ultra-low-molecular-weight heparin, has no significant effect on the mortality of patients with metastatic or locally advanced solid tumors (139,140). These contradictory results suggest that the mechanism underlying platelet-involved metastasis has been only partially elucidated and is likely to be multifactorial, and several issues remain for anti-platelet therapy.

Unfortunately, although many studies have focused on this field during the last decades, significant challenges remain to be overcome before a platelet-targeted therapeutic strategy can be used in humans. Despite technical advances in the detection of CTCs, our ability to explore platelet-CTCs interactions *in vivo* is limited because of the shortage of materials. The majority of cancer patients have fewer than ten CTCs per milliliter of blood, and these CTCs are difficult to purify (141). Therefore, *in vivo* experiments are always performed in established rodent models by injecting human cancer cells into the tail vein. However, it would be ideal to study platelet-CTCs interactions from the initiation of metastasis, rather than after intravascular injection. Moreover, the CTC-associated recruitment of inflammatory cells is not established in these immunocompromised mice (16). The process of TCIPA likely involves several important platelet receptors. Experimental blockage of these receptors results in the inhibition of cancer metastasis (142). However, several studies provided contradictory results, suggesting that the number of metastatic foci increased significantly in vWF-null mice. One hypothesis proposes that the blockade of a given receptor on a platelet may be compensated for by other signaling pathways (142). Physiologically, platelets are best known for maintaining hemostasis. However, several platelet receptors, such as P-selectin, are also expressed on other normal cells. Therefore, the potential side effects of platelet-targeted compounds must be carefully evaluated. Furthermore, a better molecular understanding of

platelet-CTC interactions is needed to identify individual therapeutic strategies for patients in high-risk situations for cancer metastasis.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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