https://doi.org/10.55415/deep-2023-0030.v1

Exploitation of platelets for antitumor drug delivery and modulation of tumor immune microenvironment

Jie Guo [a, 1], Meng-Fei Wang [a, 1], Yong Zhu [a], Fumio Watari [c], Yong-Hong Xu [b, *], Xiao Chen [a, *]

[a] Department of Pharmacology, School of Basic Medical Sciences, Wuhan University, Donghu Avenue No.185, Wuhan, 430072, China. Hubei Provincial Key Laboratory of Developmentally Originated Disease, Wuhan, 430072, China

[b] Institute of Ophthalmological Research, Department of Ophthalmology, Renmin Hospital of Wuhan University, Wuhan, 430060, China

[c] Department of Biomedical, Dental Materials and Engineering, Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan

*Correspondence: chen-xiao-1976@hotmail.com (Xiao Chen); 2420749884@qq.com (Yong-Hong Xu)

[1] Jie Guo and Meng-Fei Wang contributed equally to this work.

Abstract

Platelets are blood components traditionally believed to play fundamental roles in vascular haemostasis and thrombosis. In recent years, platelets have garnered fresh attention for their roles in tumor genesis and progression. On one hand, platelets are found to be actively recruited by various tumors as a vital part of the tumor microenvironment (TME). This has inspired the idea of employing platelets for tumor targeted drug delivery. To this end, various platelet-based devices have been proposed, such as natural platelets, engineered platelets, platelet membrane, or platelet-derived microparticles. On the other hand, platelets are also involved in tumor immunosuppression mechanisms, directing and/or assisting various tumor-associated immune cells. However, in the context of inflammation and autoimmune diseases, platelets can amplify immune responses by promoting immune cell mobilization and activation, thereby exacerbating tissue damage. Thus, there is emerging interest in tumor-associated platelets as a target for therapeutic modulation of the TME and augmentation of anti-tumor immune responses. In this review, we summarize the latest advances regarding the exploitation of platelets for both antitumor drug delivery and immune modulation of the TME.

1 Introduction

Platelets are small anucleate subcellular fragments derived from the cytoplasm of bone marrow megakaryocytes (MKs).[1] Approximately 200 billion platelets are

produced everyday by an average man at rest.[2] After their release from the bone marrow MKs into the bloodstream, platelets stay in the blood circulation for about 8~10 days. Human platelets are 1~3 μm in diameter and exhibit a plate-like discoid shape, which maximizes planar surface interactions.[3] The outer membrane of the platelet consists of glycoproteins and has abundant integrins that facilitate the adhesive and aggregative processes.[4] Platelets contain messenger RNA (mRNA) but no DNA, thus can only synthesize a limited number of proteins. Platelets have multiple functional organelles, including endoplasmic reticulum, Golgi apparatus, and mitochondria, and three types of functionally-relevant granules i.e. the α-Granules, dense granules and lysosomal granules.[5-7] Each platelet contains about 50–80 α-granules which contain various proteins including membrane-bound adhesive receptors e.g., glycoprotein (GP) VI, GPIIb/IIIa, or the Willebrand factor (vWF) receptor GPIb-IX-V complex. These granules also enclose vWF, fibrinogen, fibronectin, and vitronectin, as well as growth factors e.g. the insulin-like growth factor (IGF), transforming growth factor-beta (TGF-β), and platelet-derived growth factor (PDGF) and numerous immunomodulatory molecules and chemokines e.g., CCL3/MIP-1α, CCL5/RANTES, and CXCL4/PF4.[6] Dense granules contain small non-protein molecules important for hemostasis and innate immunity such as ATP, histamine, ADP, calcium, glutamate, and serotonin.[7] Lysosomal granules contain glycosidases, acid proteases, and cationic proteins that have a bactericidal activity.[8]

Platelets are crucial mediators of hemostasis and thrombosis and their basic

functions are to maintain the integrity of the blood vessels and participate in the coagulation cascade at the site of vascular injury. Upon vascular damage, circulating platelets initially adhere to the subendothelial extracellular matrix (ECM) through a variety of receptors, including the collagen receptors $\alpha_2\beta_1$ and GPVI, and GPIb-IX-V. Once firmly adhered to the subendothelial ECM, platelets experience diffusion, activation, and eventual aggregation to form a thrombus.[9] Activated platelets release granules and signal molecules to recruit and activate nearby platelets as well as other blood cells, such as erythrocytes and leukocytes, to reinforce thrombus formation, thus preventing further bleeding.[10] Activated platelets are eventually cleared by hepatocytes and/or local phagocytes, such as macrophages and neutrophils, activation of GPIb-IX signaling is a key trigger in this process.[11-13] In addition to their primary roles in hemostasis and thrombosis, platelets have distinguished roles in modulating inflammatory reactions and immune responses.[14] It is well recognized that platelets participate in the immune responses through direct interactions with other immune cells or by secreting immunomodulatory and polarizing molecules. Activated platelets highly express the adhesive molecule P-selectin on the surface that is important for their interactions with other immune cells that express P-selectin glycoprotein ligand-1 (PSGL-1), such as lymphocytes, neutrophils, and monocytes. Activated platelets also express CD40L to influence dendritic cells as well as B and T lymphocytes.[15]

Recent studies have shown that tumors actively recruit platelets to promote cancer survival, progression and metastasis. This has inspired the concept of

employing platelets as live carriers for tumor targeted drug delivery. Platelets are also active constituents of the tumor microenvironment (TME) coordinating and regulating the functions of various tumor-associated immune cells. Thus, there is rising interest in tumor-associated platelets as a target for therapeutic modulation of the TME and augmentation of anti-tumor immune response. In this review, we present and discuss the latest advances on the tumor associated platelets and their interactions with the TME. Special emphasis is placed on the exploitation of platelets for both antitumor drug delivery and immune modulation of the TME.

2 Platelets and tumor

2.1 Thrombocytosis and cancer

Clinical studies have shown that patients with late-stage cancer frequently develop thrombotic complications.[16, 17] High platelet counts have been associated with a worse prognosis and survival in many solid cancers, including the lung, breast, kidney, glioblastoma, pancreatic, ovarian, and gastrointestinal cancer.[18] Several molecular mechanisms have been suggested for the progression of cancer-associated thrombocytosis which involves various tumor-related cytokines and humoral factors directly or indirectly affecting megakaryopoiesis and thrombopoiesis during cancer development.[19] Most notable among these factors are the granulocyte-macrophage colony-stimulating factors (GM-CSF), granulocyte colony-stimulating factor (G-CSF), thrombopoietin (TPO), interleukin-6 (IL-6), vascular endothelial growth factor

(VEGF), basic fibroblast growth factor (b-FGF), and interleukin-1 (IL-1).[20] It has been reported that primary tumors can secrete G-CSF and GM-CSF to stimulate megakaryopoiesis in an endocrine manner and induce thrombopoiesis in cancer patients. Tumor-derived G-CSF can also increase the number of neutrophils that can release neutrophil extracellular traps (NETs) to promote thrombosis in lung cancer patients.[21] Many cancer patients also exhibit high serum levels of TPO and a high platelet count. TPO secreted from the liver, kidney, and bone marrow is a crucial cytokine to promote megakaryocyte differentiation and proliferation and resultant platelet generation by binding to its receptor c-MPL, leading to activation of the Janus kinase (JAK)/signal transducer and activator of the transcription (STAT) pathway.[22] In addition, cancer cells can release multiple humoral factors and cytokines to upregulate hepatic TPO biosynthesis, of which the most prominent is the pleiotropic cytokine IL-6, a major mediator of inflammation and activator of STAT3, whose overexpression promotes tumor development.[23] For instance, platelet microparticles (PMPs), released upon platelet activation, could promote the proliferation, survival, adhesion, and chemotaxis of hematopoietic cells through activating a vary of intracellular signaling cascades, such as the MAPK p42/44, and STAT pathways.[24] The activated platelets and excessive thrombin generation facilitate cancer-associated thrombocytosis in patients.

2.2 Platelet activation and aggregation

The high risk of thrombosis in cancer patients arises from the fact that tumor

cells can activate platelets and induce aggregation through direct and indirect mechanisms. The phenomenon of tumor cell-induced platelet aggregation (TCIPA) that is associated with higher invasiveness and metastatic potential of tumors has been demonstrated with a variety of tumors including pancreatic,[25, 26] colorectal,[27] and breast cancer.[28] Tumor cells release soluble platelet agonists on their membrane, including adenosine 5'-diphosphate (ADP), thromboxane A2 (TXA2), thrombin and the Von Willebrand factor (vWF) to directly activate platelets and stimulate thrombus formation.[29] Tissue factor (TF) is often expressed in tumor cells and tumor-derived microparticles and elevated serum levels of TF have been confirmed in multiple tumor types and chemotherapy-induced thrombosis.[30, 31] Thrombin is a serine protease that converts fibrinogen to fibrin, and thrombin is the most potent platelet agonist acting on GPIb-IX-V and the protease-activated receptors (PAR) in platelets. Tumor cells can also release ADP, which activates platelets via the purinergic G protein-coupled P2Y1 and P2Y12 receptors. P2Y1 initiates ADP-induced platelet aggregation and is responsible for platelet shape change and P2Y12 amplifies and stabilizes the aggregation response.[32] On the other hand, tumor cells can directly interact with platelets through receptors and ligands that mediate adhesion and aggregation, such as GPIb-IX-V, GPIIb/IIIa, and P-selectin.[33] Tumor cells can also promote platelet activation through elicitation of coagulation which is mainly triggered by the release of TF from monocytes and provides an active surface for platelet adhesion and thrombus formation.[34, 35]

2.3 Platelet education by tumors

Tumor-educated platelets (TEPs) are functional platelets in the blood circulation expressing tumor-associated molecules.[36] Mechanisms by which tumors educate platelets include direct transfer of tumor-related factors, tumor-induced changes in platelet RNA processing, and abnormal platelet production by MKs. Platelets may be continually stimulated and sequester molecules from tumor cells, resulting in changes in the platelet transcriptome. This can occur via direct contact of membrane proteins with tumor cells or through extracellular molecules released by tumor cells. The platelets then express tumor-specific molecules such as epidermal growth factor receptor (EGFR) vIII, echinoderm microtubule-associated protein-like 4 gene-ALK variant (EML4-ALK), kirsten rat sarcoma viral oncogene homolog (KRAS), EGFR, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) variants, kallikrein-related peptidase (KLK)2, KLK3, and neuropeptide Y (NPY).[37] Besides platelet mRNA content, platelets can selectively uptake plasma proteins, such as fibrinogen (Fg), VEGF and serotonin (5-HT) into their granules and release them upon activation.[38-40] Furthermore, it has been recently shown that platelets also uptake monocyte-derived cytokines, including IL-6, tumor necrosis factor (TNF-α) and IL-10, however, whether they can be stored and released remains to be determined.[41] Platelets also have a complicated endocytic machinery to absorb and store various proteins including those derived from tumor cells. Tumor cell-derived factors including those that promote tumor growth and neovascularization, such as

VEGF, PDGF, CXCL4/PF4, connective tissue-activating peptide III (CTAPIII) and thrombospondin-1 (TSP-1), can influence protein levels in platelets.[42]

Modulation of platelet pre-RNA splicing is another important approach of platelet education by tumors. Despite their small size and lack of nuclei, platelets contain various types of RNA, including unspliced pre-RNA, mRNA, miRNA, ribosomal RNA, small nuclear RNA, transfer RNA, long noncoding RNA (lncRNA), antisense RNA, circular RNA, and a functional spliceosome for pre-RNA processing.[43] Various tumors have been shown to affect platelet mRNA contents. Tumor cells can transfer mRNA to platelets via released microvesicles and may alter the platelet transcriptome by regulating the splicing of pre-platelet mRNA.[44] Intriguingly, tumor-induced alterations in platelet phenotype are considered of significance not only for their possible functional consequences but also for their prognostic potential as biomarkers of tumor presence or its progression.[45, 46] Not only can tumors quantitatively alter platelet production and the number of mature platelets in the blood circulation, there is also evidence that tumor may modulate the platelet transcriptome and proteome by altering the MKs.[47] Platelet content was previously thought to consist of "remnants" of progenitor MKs. However, it is now recognized that MKs specifically sort RNA, proteins, and organelles into developing platelets, which can be altered by various cancers.

2.4 Promotion of tumor invasion and metastasis by platelets

Tumor metastasis results in about 90% of tumor-associated mortality and

platelets participate in multiple steps of metastasis including enhancing the invasive potentials of tumor cells.[48, 49] Platelets have been reported to promote tumor invasion through various mediators and mechanisms. Platelet surface molecules (e.g., P-selectin, GPIbα, αIIbβ3) and secreted factors from α-granules (e.g., TGF-β, lysophosphatidic acid (LPA), MMPs and dense granules (e.g., serotonin, ADP, histamine) all facilitate cancer invasion.[50] Tumor metastasis starts with some tumor cells detaching from the primary tumor, which requires these tumor cells undergoing the epithelial-mesenchymal transition (EMT), a process characterized by disrupted cell-cell interactions, expression of mesenchymal markers, and increased cell plasticity and stemness. Platelets can upregulate mesenchymal markers e.g. the Snail family transcriptional repressor-1, vimentin, N-cadherin, fibronectin, and MMP-2 and downregulate the epithelial markers (E-cadherin, claudin-1) to induce tumor cells into an invasive, mesenchymal-like phenotype.[51] Activated platelets can release TGF-β to change tumor cells into a pro-metastatic EMT phenotype.[52] Labelle et al. demonstrated that platelet-derived TGF-β and direct platelet-tumor cell contact synergize to activate the TGF-β/Smad and nuclear factor-κB (NF-κB) pathways in tumor cells and thereby enhance the EMT.[53] The platelet receptor C-type lectin-like receptor 2 (CLEC2) binds to podoplanin expressed in tumor cells to initiate tumor cell cytoskeletal re-organization via the ezrin/moesin-mediated intracellular signaling, thus enhancing the EMT phenotype and invasiveness of tumor cells.[54] Activated platelets can also release autotaxin (ATX) with lysophospholipase D activity from the

α-granules to catalyze the generation of lysophosphatidic acid (LPA). Platelet-derived LPA stimulates IL-6 and IL-8 production in the tumor cells and eventually enhances osteolytic bone metastasis.[55] Recent study showed that platelet-derived chemokine CCL5 and EGF can stimulate tumor cell secretion of IL-8 by triggering the Akt signaling,[56] and platelet-secreted CCL3 can bind to the CCR5 receptor on tumor cells to upregulate the membrane type I–matrix metalloproteinase (MMP-1) possibly through activation of the NF-κB pathway, consequently promoting the invasion of tumor cells.[57] Platelets also participate in the preparation of the pre-metastatic niche. Primary tumors secrete metastasis-related proteins to instruct target organs to recruit bone marrow-derived cells to generate a pre-metastatic niche and promote angiogenesis. Platelets can regulate pre-metastatic communications.[58] For instance, platelet-derived chemokines (e.g. the CXC motif ligands CXCL5 and CXCL7) can promote early metastatic niche formation by activating granulocyte-derived C-X-C chemokine receptor 2 (CXCR2).

Metastatic tumor cells must leave the primary tumor and intravasate into the blood flow and their survival therein before extravasation is a critical stage of tumor metastasis. Although the vast majority of circulating tumor cells (CTCs) are rapidly eliminated,[59] blood components such as leukocytes and platelets can be mobilized to aid the CTCs' survival and transit. Platelets are among the cells that CTCs encounter early on in the blood and are essential for their survival and transit in the blood circulation.[60] During the metastatic process, cancer cells are faced with high shear

stress and the immune system attacking blood circulation. Cancer cells activate platelets by expressing tissue factors on their surface and stimulating various mediators such as cathepsin G or thrombin. The activated platelets quickly bind to the surface of cancer cells and form a coating to protect tumor cells from shear forces and natural killer (NK) cells in the blood stream. Furthermore, platelets can reduce the recognition of cancer cells by NK cells through transferring 'normal' major histone compatibility complex (MHC) class I antigens to tumor cell membranes, which prevents NK cells from recognizing foreign cells and alleviates NK cell-mediated cytolytic activity and interferon (IFN)-γ production. Moreover, platelet can secrete TGF-β to diminish NK cell antitumor activity by downregulating NKG2D immunoreceptors. Tumor cells can exhibit "platelet receptors" such as αIIbβ3, αVβ3, or GP-Ibα" to facilitate direct tumor-endothelial and tumor-leucocyte interactions, consequently promoting tumor cell extravasation.[61, 62] For extravasation, CTCs need to adhere to the luminal side of the vascular endothelial cells and then break through the subepithelial ECM. Platelet membrane expresses multiple adhesive molecules including integrins (e.g. aIIbβ3, α6β1, αvβ3), P-selectin, GPIb-IX-V, and the immunoglobulin superfamily (e.g., GPVI, FcγRIIa, platelet/endothelial cell adhesion molecule-1 (PECAM-1)). These adhesion molecules on platelets can interact with tumor cells and leukocytes to help establish firm tumor cell arrest within the vasculature.[63, 64] Meanwhile, platelets can promote tumor cells adhesion to the vascular endothelium and binding to the exposed subendothelial matrix proteins.

Platelet integrin α6β1 could directly interact with tumor cell A Disintegrin and Metalloproteinase (ADAM-9), which induces platelet activation, and granule secretion and promotes tumor cell dissemination.[65] In addition, platelet-derived microparticles could deliver platelet-derived receptors such as CD41 to tumor cells and facilitate tumor cell adhesion to endothelium and fibrinogen.[66] GPVI on platelets is a specific collagen and fibrin receptor, mediating platelet adhesion and tumor cell arrest in the vasculature through binding tumor cells galectin-3.

Molecules stored in platelet α- and δ-granules can regulate vascular permeability. Activated platelets can release VEGF, serotonin, platelet-activating factor (PAF), thrombin, ATP/ADP, hepatocyte growth factor (HGF), and fibrinogen, which increase vascular permeability, and facilitate tumor cell transmigration.[67, 68] Tumor cell-activated platelets release ATP from dense granules, which interacts with the endothelium P2Y2 receptors, opening the transendothelial barrier and enabling tumor cells to pass through endothelial cell junctions into metastatic sites.[69] Interestingly, platelets store and release several exo-enzymes, such as MMPs, platelet hyaluronidase-2, and heparanase, which can degrade collagen-rich ECM components to help tumor cells cross the subendothelial layer.[67, 70]

2.5 Promotion of tumor growth and angiogenesis by platelets

Tumor growth needs angiogenesis formation of new blood vessels to supply adequate nutrients, oxygen, and growth factors. Tumor angiogenesis arises from a complex network of interactions between the tumor cells, tumor microenvironment as

well as cells recruited from the bone marrow. Platelets can supply a multitude of proangiogenic factors to the tumor and promote expression of proangiogenic factors by the tumor cells, thereby regulating tumor angiogenesis and vascular integrity.[71, 72] Platelet alpha (α) granules contain numerous proangiogenic factors that can directly or indirectly affect angiogenesis, such as VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), bFGF (basic fibroblast growth factor), EGF (epidermal growth factor). These factors not only promote angiogenesis and tumoral neovascularization but also induce tumor growth. Interestingly, platelets alpha (α) granules also contain anti-angiogenic factors, such as angiopoietin-1 (ANGPT1), sphingosine 1-phosphate (S1P), thrombospondin-1 (TSP1) and endostatin. Platelets can selectively release these factors to facilitate or restrain angiogenesis in a growing tumor depending on external stimuli. Stimulation of receptor PAR1 contributes to secretion of pro-angiogenic molecules, however, stimulation of receptor PAR4 leads to the release of anti-angiogenic molecules. ADP-stimulated platelets release VEGF rather than endostatin, but thromboxane A2 (TXA2)-stimulated platelets release more endostatin than VEGF. Platelets could actively take up and sequester these factors via endocytosis. MKs can selectively consign angiogenesis-related proteins and mRNAs to platelets.[73-75] Platelets participate in early and advanced stages of angiogenesis and help to stabilize newly formed vessels. Platelets adhere to differentiated endothelial cells through their surface adhesion molecules, promoting endothelial cell proliferation and inducing angiogenesis *in vivo*. Platelet-released CXCL12/stromal

cell-derived factor (SDF-1) regulates revascularization by recruiting hematopoietic progenitors.[35] Platelets not only regulate tumor angiogenesis but also regulate vascular integrity to prevent tumor hemorrhage via the anti-angiogenic factors. Platelets maintain vascular integrity by secreting granules that contain ANGPT1 and serotonin, which counteracts tumor cell-derived VEGF to stabilize tumor blood vessels.[76]

However, some studies have also shown that platelet factor-4 (CXCL4/PF-4), which is released from α-granules of activated platelets inhibits endothelial cell proliferation and migration, and thus displays anti-tumoral activity by inhibiting tumor growth and suppressing the formation of metastases.[77, 78]

3 Role of platelets in the tumor microenvironment

Various innate and adaptive immune cells, such as myeloid cells and lymphocytes within the TME are major constituents of the TME which is closely implicated in tumor progression and patient prognosis.[79-81] The composition and activity of immune cell populations in the TME dictate the state of immune phenotype of the TME.[80, 81] There is accumulating evidence that platelets play significant roles in the regulation of innate and adaptive immune responses.[82] Platelets express toll-like receptors (TLRs) on their surface, enabling recognition of pathogen- and danger-associated molecular patterns (PAMPs and DAMPs) in a manner akin to leukocytes.[82, 83] Upon activation, platelets interact with immune cells and vascular cells directly and indirectly, through released inflammatory mediators such as the intracellular adhesion

molecules (ICAM)-1, CCL2, CXCLs, IL-6, TNF-α, and TGF-β, and thereby regulate the recruitment and activity of immune cells.[82] Regulation of the TME by platelets is discussed below.

3.1 Platelet regulation of leukocyte recruitment to the TME

Feng et al. showed that depletion of platelets by a GPIbα antibody significantly lowered the infiltration of bone marrow-derived cells (BMDCs) in the B16-F10 tumor isografts whereas platelet infusion resulted in an opposite effect and deficiency of α-granule secretion completely abolished platelets' effect.[84] In a diethylnitrosamine-induced mouse model for hepatocellular carcinoma, blockage of platelet activation by clopidogrel significantly decreased the density of macrophages in the tumors.[85] Platelet-released chemokines such as CCL2, IL-8, CXCL5, CXCL7, and CXCL12 are powerful recruiters of leucocytes.[86, 87] Macrophages are positive of CXCR4 and recruited to metastatic tumors by the platelets-derived CXCL12.[87] Gil-Bernabé et al.[88] showed that platelet clot- induced by tumor cell-expressed TF enhanced macrophage recruitment in mouse B16-F10 lung metastatic tumors. In a same lung metastasis model, Labelle et al. found that platelet depletion completely inhibited granulocytic cell recruitment to the early metastatic site and the effect of platelets could be attenuated by the blockage of platelets-secreted CXCL5 and CXCL7.[89] In addition, Läubli et al.[90] showed that platelets together with polymorphonuclear leukocytes/monocytes increased monocyte infiltration in the lung metastatic tumors of colorectal cancer in mice via inducing CCL5 secretion from endothelial cells,

revealing an indirect way whereby platelets promote leukocyte recruitment to the TME. Platelets are also reported to promote monocyte recruitment in the TME through induction of CCL2 expression in tumor cells.[87]

3.2 Inhibitory regulation of the TME by platelets

Macrophages in the TME i.e. the tumor associated macrophages (TAMs) mostly acquire an immune-suppressive, M2-like phenotype, high presence of which both in the tumor tissue and blood has been linked with poor patient prognosis.[91-93] Platelets are the major source of TGF-β in the TME, which is a potent inducer of the M2-like TAMs.[94, 95] In mouse models of hepatocellular carcinoma, clopidogrel (an P2Y12 receptor inhibitor) significantly increased the expression of M1-like anti-tumor macrophage markers, such as IL-1, TNFα, inducible nitric oxide synthase (iNOS).[96] Analogous to the TAMs, tumor-associated neutrophils (TANs) mostly adopt a tumor-promoting (N2-like) phenotype in the TME, which are largely driven by TGF-β.[93, 97] Natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) are effector cells with the capacity to destroy tumor cells via multiple mechanisms including the secretion of effector cytokines, such as TNF-α and IFN-γ, and the release of cytotoxic granules.[98, 99] Protection of circulating tumor cells from NK cells-mediated immunosurveillance by platelets has been observed in the mouse models of lung metastasis involving multiple tumor cell lines including melanoma and fibrosarcoma.[100] NK cell activation is triggered by target cells with low or absent expression of MHC class I (missing-self) and/or overexpression of ligands for activating NK receptors, such as natural killer

group 2 member D (NKG2D), receptor activator of NF-κB (RANK) and glucocorticoid-induced tumor necrosis factor receptor (GITR) (induced self).[98] It is reported that platelet co-incubation with tumor cells led to transfer of platelet-derived MHC class I molecules, RANK ligands, and GITR ligands to the tumor cells, which impaired NK cell antitumor reactivity and release of IFN-γ.[61, 101, 102] Platelets coated to tumor cells were also found to reduce the levels of NKG2D ligands MHC class I polypeptide-related sequence A (MICA) and MICB in the tumor cell surface through sheddases a disintegrin and metalloproteinase (ADAM)10/17, thereby inhibiting NKG2D-mediated NK cell recognition of tumor cells.[103] In addition, it has been demonstrated that platelet-secreted TGF-β impairs IFN-γ production by and degranulation of NK cells through down-regulation of the NKG2D in NK cells.[104] Similarly, dabigatran etexilate (a thrombin inhibitor) was shown to significantly increase the infiltration of NK cells and $CD8^+$ T cells in mouse MC-38 tumors by systemically reducing active TGF-β1.[105] Upregulation of immune checkpoint proteins such as programmed death ligand 1 (PD-L1) and CTL-associated antigen 4 (CTLA-4) has been a well-documented mechanism whereby tumors suppress the CTLs-mediated immune responses.[106] Hinterleitner et al.[107] showed that co-incubation of platelets with PD-$L1^+$ NCI-H226 and NCI-H460 tumor cells resulted in an increased PD-L1 expression on the platelet surface. PD-$L1^+$ platelets obtained from non-small cell lung cancer patients were observed to suppress $CD8^+$ T cell activity, manifested as decreased release of IFN-γ and TNF-α. Moreover, TGF-β released from platelets was

found to convert $CD4^+$ T cells into Tregs that destroyed activated T cells in a granzyme B-dependent way.[94] In a mouse model of colitis-associated cancer, clopidogrel treatment inhibited the infiltration of MDSCs in the tumors.[108] Platelets isolated from mice with colitis-associated cancer were also shown to enhance splenic MDSC-mediated inhibition of T-cell proliferation. These findings collectively suggest that platelets could promote the establishment of the suppressive TME through direct or indirect interactions with immune cells.

3.3 Stimulatory regulation of the TME by platelets

Platelet interactions with immune cells in the TME are complex and seem to have both suppressive and stimulatory effects. Local treatment of platelet-rich fibrin patch (PRF-P) was found to inhibit the recruitment of Tregs to the glioma microenvironment by the secretion of soluble CD40 ligand (sCD40L).[109] Although platelets are not the only source of sCD40L in PRF-P, this finding supports the possible involvement of platelets in antitumor immunity activation. Plantureux et al.[110] showed that the interaction of platelets with colorectal cancer cell lines HT-29 and CT-26 in the absence of plasma induced the production of $CD41^+$/$Epcam^+$ (the surface markers of platelets and tumor cells) microparticles in a cadherin 6–dependent way. The $CD41^+$/$Epcam^+$ microparticles contained RANTES, macrophage migration inhibitory factor (MIF), CXCL-12, and IFN-γ that enhanced the recruitment of M1-like macrophages in the CT-26 cell tumor microenvironment, which manifested as increased expression of iNOS and Arg1. They further confirmed that the interaction of

macrophages plus platelets with tumor cells resulted in cell-cycle arrest *in vitro* and decreased tumor growth *in vivo*. This finding appears to be in contradiction with the study of Pavlović et al.[85], in which the suppression of platelet activity by clopidogrel increased the expression of M1 macrophage markers, such as IL-1, TNF-α, and iNOS, in the tumors of mice with hepatocellular carcinoma. Clopidogrel has been known to be an inhibitor of the P2Y12 receptor that mainly targets ADP-mediated TCIPA. In contrast, in the study of Plantureux et al.[110], the production of $CD41^{+}$/$Epcam^{+}$ microparticles was induced by the interaction of platelets with tumor cells through cadherin-6, independent of TCIPA. The different mechanisms of the interaction between platelets and tumor cells may be one of the possible reasons for the above contradiction, beyond this, the difference in tumor types used may be also a nonnegligible factor.

Although evidence concerning stimulatory regulation of the TME phenotype by platelet-leukocyte interaction is limited, there are hints from other diseases that platelet can act as a key amplifier in the pro-inflammatory environment. It was shown that P-selectin-mediated direct interaction between platelets with neutrophils increased neutrophil reactive oxygen species (ROS) generation efficiency.[111] Moreover, activated platelets promote the ROS production of monocytes indirectly via dissociating C-reactive protein into its proinflammatory monomeric form.[112] In a mouse model of myocardial reperfusion injury, depletion of platelet 5-hydroxytryptamine (5-HT)/serotonin significantly decreased the release of ROS and

secretory granules containing myeloperoxidase (MPO) in neutrophils, both ROS and MPO are crucial pro-inflammatory mediators.[113] It is reported that platelet 5-HT prolonged monocyte life span by preventing monocytes from spontaneous apoptosis, and enhanced their capacity to product proinflammatory cytokines including TNF-α and IL-6 after stimulation with LPS.[114] Toll-like receptor 4 (TLR4) activation on platelets during severe sepsis also could induce NETs formation and liberation.[115] Depletion of platelets diminished NET formation and lung damage in mice with transfusion-related acute lung injury.[116] Moreover, NETs also activated platelets by histones at least, thus platelet-neutrophil-mediated NETs might promote inflammation and tissue damage through the presence of positive feedback loops.[116, 117] Petersen-Uribe et al.[118] showed that platelet-derived proprotein convertase subtilisin/kexin type 9 (PCSK9) promoted monocyte differentiation into macrophages/foam cells during atherothrombosis, and then, in patient atherosclerotic tissues, they found high macrophage CD68-immunoreactivity in PCSK9-and platelet-positive areas, suggesting the possible promoting role of platelet in the atherosclerosis-favoring inflammation. In mouse models of zymosan-induced inflammation and oxazolone-induced contact dermatitis, Pierre et al.[119] showed that platelets increased the production of proinflammatory cytokines IL-6 and prostaglandin E2 (PGE2) and suppressed the expression of anti-inflammatory markers CD206 in the macrophages isolated from inflamed tissues. Pharmacological depletion of platelets decreased the upregulation of co-stimulatory molecules such as CD86 and MHC-II in the inflamed

spinal cords, supporting the influence of platelets on the extent of activation and antigen-presenting capacity of macrophages.[120, 121] In the setting of asthma and allergic diseases such as atopic dermatitis and allergic rhinitis, thymic stromal lymphopoietin (TSLP) from the epithelium might activate epithelial and dermal DCs to TSLP-DCs. Activated platelets promoted the maturation of TSLP-DCs and production of Th2-attracting chemokine CCL17 *in vitro* through RANK–RANKL and CD40–CD40L pathways, in which the Th2 cell is crucial for the initiation and maintenance of allergic inflammation.[122] Dürk et al.[123] observed consistent results using ovalbumin and house dust mite induced mouse models of experimental asthma, they showed that the lack of platelet 5-HT reduced the maturation and the Th2 priming capacity of bone marrow DCs *in vivo*. In addition to the regulation of the interaction between antigen-presenting cells (APCs) and lymphocytes, platelets influence lymphocyte differentiation, activation and cytokine production via various chemokines or by direct contact, in which the CD40-CD40L pathway is one of the key mechanisms.[124, 125] In particular, in the mouse experimental asthma models, platelet CD40L were also observed to directly inhibit the generation of Tregs both *in vitro* and *in vivo*, supplementing the mechanistic explanation for the promotion of Th2-type inflammation in asthma by platelets.[126]

In summary, the regulation of the TME phenotype by platelet appears to be two-edged. On one hand, platelets are involved in tumor immunosuppression mechanisms, directing and/or assisting various immune cells. On the other hand, platelets may be

beneficial to support immune responses in the TME. Furthermore, platelets may also amplify immune response by promoting immune cell mobilization and activation in the context of inflammation and autoimmune diseases. Thus, tumor-associated platelets have potential as a target for therapeutic modulation of the TME and augmentation of anti-tumor immune response.

4 Platelet-Leukocyte Interactions in inflammation and autoimmune diseases

As described above, the immunosuppressive TME might exhibit immune stimulation and inflammatory responses in response to the immunostimulatory effects of multiple anti-tumor therapies, which to a certain degree convert the immune-excluding cold tumors into immune-infiltrated hot tumors. Platelets are key components of the tumor immune microenvironment that play an important role in tumor immunosuppression mechanisms by directing and/or assisting the immune cells. Thus, the roles of platelets in remodeling of the suppressive tumor immune microenvironment must be considered, despite limited evidence emphasizing the immune effects of platelet-leukocyte interactions in tumor damage. Still, the involvement of platelet-leukocyte interactions in numerous pathological conditions, including acute coronary syndrome, sepsis, ulcerative colitis, asthma, rheumatoid arthritis, etc., has been well described. Increased platelet-leucocyte aggregates within the circulation and/or locally at the sites of inflammation were observed in the above inflammatory/autoimmune diseases and were associated with disease progression,

functioning as a potential biomarker of disease severity.[127-129] In recent years, with the immune regulatory role of platelets being increasingly recognized, increasing studies have focused on the consequences of platelet-leukocyte interactions that include the recruitment and activation of leukocytes which enable execution of leukocytes' pro-/anti-inflammatory functions.

4.1 Platelet-leukocyte interactions in promoting inflammation and tissue damage

During tissue inflammatory damage, activated vascular endothelial cells and platelets produce enormous amounts of adhesion molecules such as P- and E-selectin that allow them to stick to each other and function as a bridge to tether and anchor leukocytes, thus initiating leukocyte recruitment to inflammation sites.[111, 130] The importance of platelet P-selectin has been verified in multiple disease models by transferring P-selectin-deficient platelets into mice, where the impaired leukocyte recruitment accompanied by the reduction of tissue inflammatory damage was observed.[124, 131-135] In addition to P-selectin-mediated direct contact, it is reported that platelets can also upregulate endothelial selectin expression by secreting serotonin (5-HT), thereby increasing neutrophil adhesion on LPS-stimulated endothelium.[136] Using multi-channel intravital microscopy to observe inflamed microvessels of cremaster muscle in mice, Zuchtriegel et al.[133] showed that intravascularly adherent platelets not only captured neutrophils and inflammatory monocytes via CD40-CD40L-dependent interaction but promoted firm adhesion and intravascular crawling of neutrophils and

inflammatory monocytes in a P-selectin dependent manner, ultimately guiding them to the exit points of transmigration. It was found that lack of platelet CD40 led to reduced plaque area and decreased infiltration of macrophages and neutrophils in atherosclerotic mice injected with apolipoprotein E deficient platelets.[137] In mouse models of acute lung injury and permanent middle cerebral artery occlusion, neutrophils recruited to damaged vessels could also use their PSGL-1 clusters to scan for the presence of activated platelets, and then neutrophils begin to organize other receptors (e.g., CXCR2 and Mac-1) required for intravascular crawling and transmigration.[138] Indeed, platelet-derived CXCL4 has been demonstrated to enhance infiltration of neutrophils and inflamed tissue damage by upregulating plasma and tissue levels of CXCL1 and CXCL2 in mice with sepsis and acute pancreatitis, and these effects were neutralized by inhibition of CXCR2.[139, 140] Schuhmann et al.[141] also showed that inhibition of platelet GPIb limited local inflammatory response in the ischemic brain of mice with transient middle cerebral artery occlusion as manifested by lower numbers of infiltrating macrophages and T cells, which might be related to the fact that GPIb contains a binding site for Mac-1. Accordingly, in the mouse models of house dust mite-induced asthma or Leishmania major infection, platelet-derived Dkk1 was found to facilitate leukocytes infiltration including $CD4^+$ T cells, neutrophils, and eosinophils to the site of inflammatory stimulation.[142] It has been shown that platelet-derived sCD84 accumulated in the ischemic cerebral microvasculature of transient middle cerebral artery occlusion mice exerted its

proinflammatory effects by acting on $CD4^+$ T cells to promote their recruitment, resulting in aggravated cerebral ischemia/reperfusion injury.[143] Notably, CD40L-bearing T cells is reported to induce platelet activation and platelet-CCL5 release in a CD40-CD40L costimulatory mechanism-dependent manner.[144] This finding is supported by an *in vivo* study, where $CD4^+$ T cells enhanced platelet–endothelial cell interactions and neutrophil migration in postischemic hepatic microcirculation through similar costimulatory mechanisms.[145] Activated platelet and released platelet-CCL5 might, in turn, enhance $CD4^+$ T cell recruitment, thereby creating a positive feedback loop that further amplifies leukocyte recruitment to sites of immune reactivity.[144]

Beyond promoting various steps in leukocyte recruitment, platelets also modulate the immunological functions of leukocytes. P-selectin-PSGL-1 and GPIb-Mac-1 are also the key partnerships with pleiotropic actions in the regulation of leukocyte immune functions. Upon subthreshold dose of LPS stimulation, platelets promoted macrophages phenotypic and functional polarization toward proinflammatory profile depending on GPIb-Mac-1.[41] Which allowed platelets to protect mice from septic shock at early time points during sepsis development by inducing iNOS positive M1-like macrophages that enhance bacterial clearance. ROS produced and release by leukocytes can destroy invading pathogens and trigger tissue damage due to their extreme cytotoxicity.[130, 146] It has been shown that P-selectin-PSGL1 binding-mediated direct interaction between platelets with neutrophils

increases neutrophil ROS generation efficiency.[111] Moreover, activated platelets are reported to promote ROS production of monocytes indirectly via dissociating the C-reactive protein in its proinflammatory monomeric form.[112] In a mouse model of myocardial reperfusion injury, depletion of platelet 5-HT significantly decreased ROS release and secretory granules containing MPO in neutrophils. Similar to ROS, MPO is known to enhance inflammation and tissue damage.[113] It has been reported that 5-HT can prolong monocytes' life span by preventing monocytes from spontaneous apoptosis, and enhance their capacity to product proinflammatory cytokines including TNF-α and IL-6 after stimulation with LPS, indicating that platelet 5-HT may contribute to the amplification of allergic inflammation.[114] It is also reported that platelets enhanced phagocytosis of periodontitis-associated bacteria by neutrophils in a TLR2 dependent manner.[147] In addition, TLR4 activation on platelets during severe sepsis was shown to induce NETs formation and liberation.[115] NETs were described to be a netlike configuration decorated with DNA, antimicrobial molecules such as histones, defensins, and various neutrophil proteases, released by neutrophils when phagocytosis is frustrated, to enhance their capacity to capture and kill pathogens.[116, 130, 146] Since platelet-neutrophil-mediated NET formation requires the excessive activation of neutrophils, it might result in substantial endothelium and tissue damage.[115, 116] Moreover, NETs have also been reported to activate platelets at least through histones, hence platelet-neutrophil-mediated NETs might promote inflammation and tissue damage through the presence of positive feedback loops.[116,

[117] Petersen-Uribe et al.[118] showed that platelet-derived proprotein convertase subtilisin/kexin type 9 (PCSK9) promoted monocyte differentiation into macrophages/foam cells during atherothrombosis, and in patient atherosclerotic tissues they found high macrophage CD68-immunoreactivity in PCSK9-and platelet-positive areas, suggesting a possible promoting role of platelet in the atherosclerosis-favoring inflammation. In the setting of asthma and allergic diseases such as atopic dermatitis and allergic rhinitis, thymic stromal lymphopoietin (TSLP) from the epithelium might activate epithelial and dermal DCs to TSLP-DCs. Activated platelets were shown to promote the maturation of TSLP-DCs and production of Th2-attracting chemokine CCL17 *in vitro* through RANK–RANKL and CD40–CD40L pathways, in which the Th2 cells are crucial for the initiation and maintenance of allergic inflammation.[122] In addition to regulating the interactions between the antigen-presenting cells (APCs) and lymphocytes, platelets have also been reported to influence lymphocyte differentiation, activation and cytokine production via various chemokines or by direct cell contact, in which the CD40-CD40L pathway is one of the key mechanisms.[124, 125] In particular, in the mouse experimental asthma models, platelet CD40L was also observed to directly inhibit the generation of Tregs both *in vitro* and *in vivo*, supplementing the mechanistic explanation for the promotion of Th2-type inflammation in asthma by platelets.[126]

The above findings on the multitude of interactions between platelets with leukocytes make a compelling case for platelets as a vital enhancer of the innate and

adaptive immune responses in the context of inflammation and autoimmune diseases (Table 1).

4.2 Platelet-leukocyte interactions in orchestrating the resolution of inflammation

The functions of platelet-leukocyte interactions appear to be multifaceted and intimately involved in the negative feedback mechanisms of a finely tuned immune system, guarding against excessive inflammatory responses. During the early stages of mouse experimental autoimmune encephalitis, signs of platelet activation/degranulation were observed, in which soluble factors such as 5-HT, CXCL4 could exhibit proinflammatory properties by stimulating proliferation and differentiation of pathogenic Th1, Th17, and IFN-γ^+/IL-17^+ $CD4^+$ T cells. However, the capacity of activated platelets to form platelet-$CD4^+$ T cell aggregates was enhanced in the advance stages, resulting in decreased T cell activation.[148] Depletion of platelets led to an impaired $CD4^+$ Treg protective activation in an early phase after trauma in the burn injury model in mice.[149] Similar studies carried out in mouse models of sepsis showed that blockage of the P2Y12 receptor using clopidogrel alleviated TGF-β elevation in the plasma and restrained Treg population growth in the spleen during sepsis.[150] Utilizing an immune tolerance mouse model, Hotta et al.[151] showed that platelet depletion reversed the increase in the frequency of Tregs in the inflamed skin and draining lymph nodes of mice via TGF-β release and significantly enhanced the contact hypersensitivity response after tolerance. Furthermore, in a

recent study, platelets were observed to recruit neutrophils into the lungs during the onset of bacterial-induced pneumonia, whereas recruiting Tregs and transcriptionally reprogramming alveolar macrophages to an anti-inflammatory phenotype during the resolution phase. The mechanisms of this observation were postulated to involve sheddase ADAM8 cleavage of PSGL-1 on the neutrophils, which allows the preferential binding of platelets to T reg cells during the resolution phase.[152]

To summarize, platelets have emerged as a vital and active part of the TME playing crucial roles in the mechanisms of tumor-induced or -associated immunosuppression (Fig. 1). Platelets can also initiate and accelerate inflammatory processes and immune responses in the context of inflammation and autoimmune diseases. Increasing knowledge and deeper understanding in theses aspects are expected to boost the development of anti-tumor therapeutic strategies that subvert platelets' immunosuppressive properties and/or exploit their immunostimulatory properties to achieve better efficacy.

5 Platelets for antitumor drug delivery

5.1 Unmodified platelets as carriers for drug delivery

The goal of drug delivery is to enhance both therapeutic safety and efficacy, and the use of cells as delivery vehicles is a promising approach to this end. Most vehicle cells utilized thus far are immune cells e.g. the monocytes, macrophages, dendritic cells, neutrophils and platelets, which have a natural propensity to home to sites of

tissue injury and inflammation such as cancer, and are able to cross tissue barriers like the blood brain barrier. Among the candidate carrier cells for drug delivery, platelets stand out with some unique advantages. For example, platelets can take up and stably store large amounts of both endogenous and exogenous molecules including antitumor agents.[153] Healthy platelets usually maintain circulation in the blood stream for 8~10 days, affording a long half-life to the drug they carry. Platelets release contents stored in their granules upon activation by tumor, which adds to the tumor-targeting capacity of platelet-mediated delivery. Sarkar et al. showed that platelets can take up doxorubicin (DOX) and release the agent upon activation and the DOX-loaded platelets induced tumor cell toxicity with a higher efficiency than free DOX in both *in vitro* and *in vivo* models. [154] Xu et al. loaded DOX in platelets to treat mouse lymphoma models. [155] They showed that the DOX-loaded platelets (DOX@platelets) maintained integrity and biological functions with high DOX loading and encapsulation efficiency. DOX@platelets released more DOX at the tumor sites in a pH-triggered manner and increased DOX uptake by the Raji tumor cells, leading to enhanced growth inhibition and apoptosis of the Raji tumors and alleviated normal tissue damage. In our lab, we found that platelets have a small loading capacity for small molecule drugs e.g. DOX and chlorin e6 (Ce6) and platelets loaded with these drugs exhibit significant spontaneous cargo discharge, which detracts the utility of platelets as drug carriers. This problem was then overcome through employing nanoparticles as the primary drug vehicles before using platelets as the secondary

carrier of the drug-loaded nanoparticles. Our lab recently proved the concept of platelet-mediated tumor-targeted drug delivery using DOX attached to nanodiamonds (ND-DOX) as a model drug. [153] We demonstrated platelets to be drug carriers with the following features: 1) a large loading capacity and high loading efficiency, 2) good tolerance of the payload drug, 3) stable cargo retention and no cargo release without stimulation, 4) long blood circulation time, and 5) good tumor distribution and tumor-activated cargo unloading. These features eventually led to a therapeutic potency much higher than that of DOX and little systemic toxicity seen with DOX. An artificial stimulus e.g. ROS generated by photodynamic/sonodynamic therapy (PDT/SDT) or heat generated by photothermal therapy (PTT) can also cause platelet activation and cargo release. This property confers the advantage of highly controllable drug release on platelet-mediated delivery, based on which we have invented a drug delivery strategy of "platelets with photo-controlled release property".[156] To provide proof of concept for this strategy, we prepared the delivery device BNPD-Ce6@Plt by loading mouse platelets with a nano-photosensitizer (BNPD-Ce6) consisting of Ce6 loaded to boron nitride nanoparticles with a surface coating of polyglycerol and doxorubicin. Irradiation of 808 nm laser induced ROS generation in the BNPD-Ce6@Plt which exhibited rapid activation, aggregation, and quick discharge of BNPD-Ce6 into co-cultured GL261 glioma cells which in turn displayed pronounced photodynamic toxicity and cell death. *In vivo*, laser irradiation of intracranial GL261 tumors after i.v. injection of BNPD-Ce6@Plt led to extensive

distribution and accumulation of BNPD-Ce6 in the tumors which displayed massive tissue necrosis after another time of laser irradiation. A PDT regimen of intravenous BNPD-Ce6@Plt injections followed by tumor laser irradiation markedly suppressed the growth of intracranial GL261 tumors and significantly prolonged host survival. The work of Zhang et al also lends strong support for the notion of platelets as drug carriers with "photo-controlled release property". [157] Herein, the authors constructed a drug delivery device (IRDNP–PLT) that was based on platelets with encapsulated DOX nanoparticles and IR–820, a fluorescent photothermal agent. The IRDNP–PLT device featured controlled release of DOX nanoparticles by IR–820–mediated photothermal activation. Near infrared (NIR) irradiation of subcutaneous 4T1 tumors in mice after i.v. injection of IRDNP–PLT showed highly targeted and enhanced antitumor drug delivery and synergetic photo–chemotherapeutic efficacy. These findings together with ours compellingly suggest that intact platelets per se can serve as drug carriers with an active tumor–targeting property which can be greatly enhanced through external manipulation.

Like naïve platelets, platelets harboring encapsulated drugs are cleared by the reticuloendothelial system (RES) that comprises tissue macrophages and blood-borne monocytes. Recruitment and extravasation of circulating neutrophils are largely platelet-dependent, Thus, platelets might also be exploited to deliver agents to

regulate or modulate the function of the monocytes, macrophages and neutrophils which are an essential to the anti-tumor immunity. Platelets are particularly amenable to carrying agents whose actions target the nucleus as platelets are anucleate, and thus remain unscathed when carrying these agents. [158]

5.2 Platelet engineering for drug delivery

Tropism towards inflammation, adhesion properties, and dynamic changes upon activation render platelets multifunctional platforms suitable for drug delivery and immune regulation. Platelets can also be directly modified through surface engineering owing to the abundance of various binding sites on their plasma membrane.[159] Platelets can be harvested in quantities from patients with relative ease and then infused back in the same patients after *in vitro* modification. This is in line with the trend of precision medicine and personalized therapy and eliminates immunogenicity-associated adverse effects. Engineering platelets for tumor immunotherapy appears to hold great promise as this strategy can increase immune cell penetration and activation in the tumors, and thereby stimulates robust antitumor immunity. Wang et al. used a facile method to conjugate an PD-L1 antibody (aPD-L1) to the surface of mouse platelets via a maleimide linker to reduce tumor local recurrence and metastasis.[160] The aPD-L1-conjugated platelets (P-aPD-L1) were recruited to surgical wound sites due to their natural property to home to tissue damage and inflammation. Consequent activation of P-aPD-L1 at the wound sites led to the release of a large number of platelet-derived microparticles (PMPs) with aPD-

L1 presenting on the surface. This effect promoted aPD-L1 accumulation to the tumor resection site and T cell infiltration and activation at the remaining tumor residues, which eventually prevented tumor recurrence after surgery. P-aPD-L1 was also shown to interact with the CTCs to inhibit tumor metastasis. Han et al. used FDA-approved poly (lactic-co-glycolic) acid-coated indocyanine green (PLGA-ICG) as a model photothermal agent in tandem with P-aPD-L1 to treat tumor recurrence.[161] They demonstrated that thermal ablation created inflammatory sites in the tumor tissue that exhibited enhanced recruitment of aPD-L1-conjugated platelets. Activated aPD-L1-conjugated platelets then released the aPD-L1 antibodies to block the PD-L1 both on the tumor cells and the antigen-presenting cells to promote T-cell infiltration and activation, which eventually inhibited residual tumor growth and metastasis. Apart from promoting immune checkpoint inhibitor therapy, engineered platelets can also be used to combine immunotherapy with other therapies to achieve synergistic antitumor efficacy. Engineered platelets delivering therapeutic agents to the tumors can work with CAR-T cells, overcoming the immunosuppressive TME, T cell exhaustion, and the occurrence of graft-versus-host disease (GVHD).[162] After activation, platelets can serve as bioresponsive cells to release aPD-L1 antibodies intratumorally, which in turn sustain the CAR-T-cell-mediated antitumor activity and prevent T cell exhaustion.[163]

It is worth noting that binding aPD-1/aPD-L1 to platelets is relatively expensive and relatively large amounts of antibodies are required to obtain sufficient amount of

the therapeutic material. In addition, platelets that stably express PD-1 are rather difficult to obtain, necessitating the development of more advanced or refined techniques to generate engineered platelets in quantity. To overcome the technical challenges in genetic engineering of anucleate platelets, much effort has been put into their precursor cells such as MKs for producing platelets expressing certain functions. Zhang et al. recently genetically engineered MKs progenitor cells to produce platelets that express the PD-1.[164] They demonstrated that the PD-1 expressing platelets and derived microparticles could accumulate in the TME and block the PD-L1, thus inhibiting the activity of the immune-suppressive regulatory T cells (Treg) and enhancing the activity of $CD8^+$ T lymphocytes in the tumor resection site. Furthermore, they loaded low-dose cyclophosphamide (CP) into PD-1-expressing platelets which depleted the Tregs and increased reinvigorated $CD8^+$ T lymphocyte cells in the post-surgery TME. Although the viability and desired biological functions can be well preserved in genetically or chemically engineered platelets, undesirable effects on other platelet functions and long-term behavior of the engineered platelets need thorough evaluation.

5.3 Platelet membrane camouflage for drug delivery

Numerous nanoparticle (NP)-based delivery systems have been devised for tumor chemotherapy. Nanoparticle encapsulation features controlled drug release, improved antitumor drug distribution/accumulation, increased tumor tissue permeability and reduced systemic toxicity and side effects.[165, 166] Despite the great

progress, some problems remain, such as nanotoxicity, unsatisfactory targeting capacity, unfavorable immune responses, and short circulating time in the blood stream. To address these issues, various cell membranes are introduced onto the nanoparticle surface to form biomimetic cell membrane-camouflaged drug delivery devices. These devices have cell surface proteins retaining their biological functions but are bereft of disadvantages associated with live cells, such as activation, cell toxicity and low drug loading capacity. Among the many candidate cell membranes, platelet membrane is particularly attractive.[167, 168] Platelet membrane integrated with various nanomaterials display distinct properties of platelets, e.g. long blood circulation time, cell adhesion, interaction with immune cells, and targeting of tumor cells as well as additional features e.g. biocompatibility, biodegradability, and translocation cross biological barriers. Coating nanoparticles with platelet membrane also reduces undesired bio-distribution , unexpected activation-related drug toxicity, and decreased efficacy.[155, 156] The combination of platelet membrane with chemotherapy, photothermal or photodynamic therapy may generate synergistic antitumor effects. Pei et al. developed platelet membrane-coated poly lactic-co-glycolic acid nanoparticles (PM-NPs) loaded with IR780, a photothermal agent with tumor targeting and superior optical properties and, the chemotherapeutic agent DOX. [169] The PM-NPs featured good stability, prolonged blood circulation time, NIR-enhanced tumor accumulation and active targeting of cancer cells, which resulted in much enhanced anti-tumor PTT efficiency. Platelet membrane-camouflaged devices

can also target circulating tumor cells to suppress tumor metastasis. CTCs can induce platelet activation, fibrin deposition and local thrombosis to protect from immune attack. Inspired thereby, Li et al. coated silica (Si) particles with membrane-derived vesicles (PMDVs) from activated platelets and conjugated tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to the platelet membrane-covered Si particles.[170] The resultant PMDV-Si particles exhibited reduced phagocytic clearance thanks to the presence of membrane proteins and glycans from platelet membranes such as CD47. The PMDV-coated Si particles could adhere to fibrin in flow and the TRAIL-conjugated PMDV-coated Si particles could also induce tumor cells apoptosis via the tumoricidal activity of immune cells. Eventually, the TRAIL-conjugated PMDV-Si particles killed CTCs in the lung vasculature and reduced lung metastasis. Combination of a platelet membrane-camouflaged drug delivery device with immunotherapy has also been shown to enhance antitumor immunity in solid tumors. Resiquimod (R848), a TLR agonist, was coated with platelet membrane (PNP-R848) to achieve tumor-localized delivery and elicit antitumor immune responses.[171] The biomimetic platelet-derived membrane of PNP-R848 displayed enhance affinity to tumor cells and improved tumor retention. PNP-R848 promoted the activation of antigen-presenting cells (APCs) in the draining lymph node (DLN), resulting in enhanced T cell infiltration in the tumor, and ultimately leading to tumor eradication, metastasis suppression, and generation of memory T cells that thwarted subsequent tumor re-challenge. These findings suggest the potential of platelet membrane-

camouflaged delivery devices for tumor immunotherapy. Although the multifaceted biointerfacing supported by the platelet membrane cloaking technology provides a new approach to developing functional nanoparticles for targeted tumor therapy, biomembrane-derived biomimetic nanomedicines must address the issue of reproducibility of membrane protein quantities and their large-scale production. Moreover, the integrity of the platelet membranes and the integrity and functions of the NP core in the blood are not easily characterized. Despite a general acceptance of the safety of platelet membrane-disguised NPs, there is currently little information regarding their long-term biocompatibility and toxicity profiles.

5.4 Platelet-derived microparticles for antitumor drug delivery

Platelets activated by tumor cells can generate platelet-derived microparticles (PMPs) that are released into the blood. They are formed by cell membrane budding and vary between 0.1 and 1 μm in size. Platelet-derived microparticles, also known as microvesicles, participate in cell-to-cell communication. Microparticles deliver payloads of lipids, proteins, miRNAs, mRNAs, and non-coding RNAs into tumor cells, and thereby modulate gene expression and functions of the latter.[172] Recent studies have shown promising results in employing microparticles as novel therapeutic vehicles for drug delivery. Microparticles are poorly immunogenic, and capable of shielding ‘therapeutic cargoes’ from fast degradation *in vivo* and crossing biological barriers such as the blood-brain barrier. Kailashiya et al. engineered human platelets to generate microparticles loaded with DOX and other agents by a top-down

approach, which targeted leukemia cells and showed good biocompatibility.[173] They showed that PMPs could carry multiple drug payloads, had a long shelf life, and could be harvested in large quantities in a short period of time. Importantly, PMPs exhibited remarkably higher toxicity towards cancer cells than free drugs and less escape into extravascular spaces. PMP-mediated delivery resulted in significantly higher drug contents in cancer cells of leukemia patients than free drugs.

Despite the promise of nanovesicle drug delivery, there are still several challenges that need to be addressed before they can be used clinically. For all cell types isolated from blood, they must undergo a rigorous sterilization process to avoid the risk of infection, and if the blood of non-autologous origin is used, blood screening is required after blood type matching to ensure maximum compatibility.

6 Conclusions and Outlook

In summary, platelets have emerged as promising and versatile candidates for delivery of tumor-targeted therapy owing to their unique, advantageous biological properties including stable and high capacity to drug loading, amenability to modification and engineering, long blood circulation time, tumor homing, ability to cross biological barriers, and tumor- or stimulus-induced activation and aggregation (Table 2). Furthermore, platelets have close interactions with tumor cells and tumor-associated immune cells both in the blood circulation and at local tissue level, exerting fundamental influence on the immune system in the context of tumor. Hence,

platelets have come forth both as an intriguing therapeutic target and a powerful, maneuverable tool for the treatment of tumors. (Fig. 2).

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China [82272718] and the Incubation Fund of Wuhan University Basic-Clinical Co-Construction Project [JCZN2022001].

Conflict of interest

The authors declare that they have no conflicts of interest.

Reference

1. K. R. Machlus and J. E. Italiano, Jr.: The incredible journey: From megakaryocyte development to platelet formation, *J Cell Biol*, 2013, 201: 785–796.

2. R. Grozovsky, S. Giannini, H. Falet and K. M. Hoffmeister: Regulating billions of blood platelets: glycans and beyond, *Blood*, 2015, 126: 1877–1884.

3. J. N. Thon and J. E. Italiano, Jr.: Does size matter in platelet production?, *Blood*, 2012, 120: 1552–1561.

4. G. Perrella, J. Huang, I. Provenzale, F. Swieringa, F. Heubel–Moenen, R. W. Farndale, M. Roest, P. E. J. van der Meijden, M. Thomas, R. A. S. Ariëns, M. Jandrot–Perrus, S. P. Watson and J. W. M. Heemskerk: Nonredundant Roles of Platelet Glycoprotein VI and Integrin αIIbβ3 in Fibrin–Mediated Microthrombus Formation, *Arteriosclerosis, thrombosis, and vascular biology*, 2021, 41: e97–e111.

5. M. Crescente, F. G. Pluthero, L. Li, R. W. Lo, T. G. Walsh, M. P. Schenk, L. M. Holbrook, S. Louriero, M. S. Ali, S. Vaiyapuri, H. Falet, I. M. Jones, A. W. Poole, W. H. Kahr and J. M. Gibbins: Intracellular Trafficking, Localization, and Mobilization of Platelet–Borne Thiol Isomerases, *Arterioscler Thromb Vasc Biol*, 2016, 36: 1164–1173.

6. P. Blair and R. Flaumenhaft: Platelet alpha–granules: basic biology and clinical correlates, *Blood Rev*, 2009, 23: 177–189.

7. A. L. Ambrosio and S. M. Di Pietro: Storage pool diseases illuminate platelet dense granule biogenesis, *Platelets*, 2017, 28: 138–146.

8. J. Polasek: Platelet lysosomal acid phosphatase enzyme activity as a marker of platelet procoagulant activity, *Blood Transfus*, 2009, 7: 155–156.

9. M. Holinstat: Normal platelet function, *Cancer Metastasis Reviews*, 2017, 36: 195–198.

10. J. Yeung, W. Li and M. Holinstat: Platelet Signaling and Disease: Targeted Therapy for Thrombosis and Other Related Diseases, *Pharmacol Rev*, 2018, 70: 526–548.

11. V. Rumjantseva and K. M. Hoffmeister: Novel and unexpected clearance mechanisms for cold platelets, *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*, 2010, 42: 63–70.

12. M. E. Quach, W. Chen and R. Li: Mechanisms of platelet clearance and translation to improve platelet storage, *Blood*, 2018, 131: 1512–1521.

13. N. Maugeri, P. Rovere-Querini, V. Evangelista, C. Covino, A. Capobianco, M. T. Bertilaccio, A. Piccoli, L. Totani, D. Cianflone, A. Maseri and A. A. Manfredi: Neutrophils phagocytose activated platelets in vivo: a phosphatidylserine, P-selectin, and β2 integrin-dependent cell clearance program, *Blood*, 2009, 113: 5254–5265.

14. M. Koupenova, L. Clancy, H. A. Corkrey and J. E. Freedman: Circulating Platelets as Mediators of Immunity, Inflammation, and Thrombosis, *Circ Res*, 2018, 122: 337–351.

15. L. Repsold and A. M. Joubert: Platelet Function, Role in Thrombosis, Inflammation, and Consequences in Chronic Myeloproliferative Disorders, *Cells*, 2021, 10.

16. S. E. Bailey, O. C. Ukoumunne, E. A. Shephard and W. Hamilton: Clinical relevance of thrombocytosis in primary care: a prospective cohort study of cancer incidence using English electronic medical records and cancer registry data, *Br J Gen Pract*, 2017, 67: e405–e413.

17. V. Josa, M. Krzystanek, A. C. Eklund, F. Salamon, A. Zarand, Z. Szallasi and Z. Baranyai: Relationship of postoperative thrombocytosis and survival of patients with colorectal cancer, *Int J Surg*, 2015, 18: 1–6.

18. L. T. Mounce, W. Hamilton and S. E. Bailey: Cancer incidence following a high-normal platelet count: cohort study using electronic healthcare records from English primary care, *Br J Gen Pract*, 2020, 70: e622–e628.

19. R. J. Lin, V. Afshar-Kharghan and A. I. Schafer: Paraneoplastic thrombocytosis: the secrets of tumor self-promotion, *Blood*, 2014, 124: 184–187.

20. V. Josa, V. Brodszky, A. Zarand, T. Mezei, Z. Szilasi, K. Merkel, A. Feher, Z. Szallasi and Z. Baranyai: The relationship between IL-6 and thrombocytosis accompanying gastrointestinal tumours, *Prz Gastroenterol*, 2020, 15: 215–219.

21. Y. Hisada, J. E. Geddings, C. Ay and N. Mackman: Venous thrombosis and cancer: from mouse models to clinical trials, *J Thromb Haemost*, 2015, 13: 1372–1382.

22. R. Sungaran, B. Markovic and B. H. Chong: Localization and regulation of

thrombopoietin mRNA expression in human kidney, liver, bone marrow, and spleen using in situ hybridization, *Blood*, 1997, 89: 101–107.

23. A. Kaser, G. Brandacher, W. Steurer, S. Kaser, F. A. Offner, H. Zoller, I. Theurl, W. Widder, C. Molnar, O. Ludwiczek, M. B. Atkins, J. W. Mier and H. Tilg: Interleukin–6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis, *Blood*, 2001, 98: 2720–2725.

24. S. Lazar and L. E. Goldfinger: Platelets and extracellular vesicles and their cross talk with cancer, *Blood*, 2021, 137: 3192–3200.

25. Z. Chen, X. Wei, S. Dong, F. Han, R. He and W. Zhou: Challenges and Opportunities Associated With Platelets in Pancreatic Cancer, *Front Oncol*, 2022, 12: 850485.

26. S. Mai and I. Inkielewicz–Stepniak: Pancreatic Cancer and Platelets Crosstalk: A Potential Biomarker and Target, *Front Cell Dev Biol*, 2021, 9: 749689.

27. L. Sun, Q. Li, Y. Guo, Q. Yang, J. Yin, Q. Ran, L. Liu, Z. Zhao, Y. Wang, Y. Li, Y. Chen, X. Weng, W. Cai and X. Zhu: Extract of Caulis Spatholobi, a novel platelet inhibitor,efficiently suppresses metastasis of colorectal cancer by targeting tumor cell–induced platelet aggregation, *Biomed Pharmacother*, 2020, 123: 109718.

28. L. Lian, W. Li, Z. Y. Li, Y. X. Mao, Y. T. Zhang, Y. M. Zhao, K. Chen, W. M. Duan and M. Tao: Inhibition of MCF–7 breast cancer cell–induced platelet aggregation using a combination of antiplatelet drugs, *Oncol Lett*, 2013, 5: 675–680.

29. L. Plantureux, D. Mege, L. Crescence, F. Dignat–George, C. Dubois and L. Panicot–Dubois: Impacts of Cancer on Platelet Production, Activation and Education and Mechanisms of Cancer–Associated Thrombosis, *Cancers (Basel)*, 2018, 10.

30. Y. Hisada and N. Mackman: Cancer cell–derived tissue factor–positive extracellular vesicles: biomarkers of thrombosis and survival, *Curr Opin Hematol*, 2019, 26: 349–356.

31. F. Schaffner and W. Ruf: Tissue factor and PAR2 signaling in the tumor microenvironment, *Arterioscler Thromb Vasc Biol*, 2009, 29: 1999–2004.

32. J. Yeung, W. Li and M. Holinstat: Platelet Signaling and Disease: Targeted Therapy for Thrombosis and Other Related Diseases, *Pharmacological Reviews*, 2018, 70: 526–548.

33. M. Schlesinger: Role of platelets and platelet receptors in cancer metastasis, *J Hematol Oncol*, 2018, 11: 125.

34. Y. Hisada and N. Mackman: Tissue Factor and Cancer: Regulation, Tumor Growth, and Metastasis, *Semin Thromb Hemost*, 2019, 45: 385–395.

35. N. Maishi and K. Hida: Tumor endothelial cells accelerate tumor metastasis, *Cancer Sci*, 2017, 108: 1921–1926.

36. M. Dovizio, P. Ballerini, R. Fullone, S. Tacconelli, A. Contursi and P. Patrignani: Multifaceted Functions of Platelets in Cancer: From Tumorigenesis to Liquid Biopsy Tool and Drug Delivery System, *Int J Mol Sci*, 2020, 21.

37. R. J. Nilsson, L. Balaj, E. Hulleman, S. van Rijn, D. M. Pegtel, M.

Walraven, A. Widmark, W. R. Gerritsen, H. M. Verheul, W. P. Vandertop, D. P. Noske, J. Skog and T. Wurdinger: Blood platelets contain tumor-derived RNA biomarkers, *Blood*, 2011, 118: 3680-3683.

38. M. Banerjee and S. W. Whiteheart: The ins and outs of endocytic trafficking in platelet functions, *Current opinion in hematology*, 2017, 24: 467-474.

39. G. Carrasco, M. A. Cruz, V. Gallardo, P. Miguel, M. Lagos and C. González: Plasma and platelet concentration and platelet uptake of serotonin in normal and pre-eclamptic pregnancies, *Life sciences*, 1998, 62: 1323-1332.

40. G. L. Klement, T. T. Yip, F. Cassiola, L. Kikuchi, D. Cervi, V. Podust, J. E. Italiano, E. Wheatley, A. Abou-Slaybi, E. Bender, N. Almog, M. W. Kieran and J. Folkman: Platelets actively sequester angiogenesis regulators, *Blood*, 2009, 113: 2835-2842.

41. A. Carestia, H. A. Mena, C. M. Olexen, J. M. Ortiz Wilczyñski, S. Negrotto, A. E. Errasti, R. M. Gómez, C. N. Jenne, E. A. Carrera Silva and M. Schattner: Platelets Promote Macrophage Polarization toward Pro-inflammatory Phenotype and Increase Survival of Septic Mice, *Cell reports*, 2019, 28: 896-908.e895.

42. S. In 't Veld and T. Wurdinger: Tumor-educated platelets, *Blood*, 2019, 133: 2359-2364.

43. N. Sol and T. Wurdinger: Platelet RNA signatures for the detection of cancer, *Cancer Metastasis Rev*, 2017, 36: 263-272.

44. S. D'Ambrosi, R. J. Nilsson and T. Wurdinger: Platelets and tumor-associated RNA transfer, *Blood*, 2021, 137: 3181-3191.

45. M. G. Best, N. Sol, I. Kooi, J. Tannous, B. A. Westerman, F. Rustenburg, P. Schellen, H. Verschueren, E. Post, J. Koster, B. Ylstra, N. Ameziane, J. Dorsman, E. F. Smit, H. M. Verheul, D. P. Noske, J. C. Reijneveld, R. J. A. Nilsson, B. A. Tannous, P. Wesseling and T. Wurdinger: RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics, *Cancer Cell*, 2015, 28: 666-676.

46. K. M. Heinhuis, S. In 't Veld, G. Dwarshuis, D. van den Broek, N. Sol, M. G. Best, F. V. Coevorden, R. L. Haas, J. H. Beijnen, W. J. van Houdt, T. Wurdinger and N. Steeghs: RNA-Sequencing of Tumor-Educated Platelets, a Novel Biomarker for Blood-Based Sarcoma Diagnostics, *Cancers (Basel)*, 2020, 12.

47. M. G. Best, A. Vancura and T. Wurdinger: Platelet RNA as a circulating biomarker trove for cancer diagnostics, *J Thromb Haemost*, 2017, 15: 1295-1306.

48. L. J. Gay and B. Felding-Habermann: Platelets alter tumor cell attributes to propel metastasis: programming in transit, *Cancer Cell*, 2011, 20: 553-554.

49. N. Li: Platelets in cancer metastasis: To help the "villain" to do evil, *Int J Cancer*, 2016, 138: 2078-2087.

50. R. Saito, K. Shoda, S. Maruyama, A. Yamamoto, K. Takiguchi, S. Furuya, N. Hosomura, H. Akaike, Y. Kawaguchi, H. Amemiya, H. Kawaida, M. Sudo, S. Inoue, H. Kono, K. Suzuki-Inoue and D. Ichikawa: Platelets enhance malignant behaviours of gastric cancer cells via direct contacts, *Br J Cancer*, 2021, 124: 570-573.

51. M. W. Klymkowsky and P. Savagner: Epithelial—mesenchymal transition: a cancer researcher's conceptual friend and foe, *Am J Pathol*, 2009, 174: 1588—1593.

52. E. J. Tan, A. K. Olsson and A. Moustakas: Reprogramming during epithelial to mesenchymal transition under the control of TGFbeta, *Cell Adh Migr*, 2015, 9: 233—246.

53. M. Labelle, S. Begum and R. O. Hynes: Direct signaling between platelets and cancer cells induces an epithelial—mesenchymal—like transition and promotes metastasis, *Cancer Cell*, 2011, 20: 576—590.

54. B. O. Hwang, S. Y. Park, E. S. Cho, X. Zhang, S. K. Lee, H. J. Ahn, K. S. Chun, W. Y. Chung and N. Y. Song: Platelet CLEC2—Podoplanin Axis as a Promising Target for Oral Cancer Treatment, *Front Immunol*, 2021, 12: 807600.

55. T. Hauck, S. Kadam, K. Heinz, M. Garcia Peraza, R. Schmid, A. E. Kremer, K. Wolf, A. Bauer, R. E. Horch, A. Arkudas and A. Kengelbach—Weigand: Influence of the autotaxin—lysophosphatidic acid axis on cellular function and cytokine expression in different breast cancer cell lines, *Sci Rep*, 2022, 12: 5565.

56. K. E. Johnson, J. R. Ceglowski, H. G. Roweth, J. A. Forward, M. D. Tippy, S. El—Husayni, R. Kulenthirarajan, M. W. Malloy, K. R. Machlus, W. Y. Chen, J. E. Italiano, Jr. and E. M. Battinelli: Aspirin inhibits platelets from reprogramming breast tumor cells and promoting metastasis, *Blood Adv*, 2019, 3: 198—211.

57. W. Wang, H. Y. Chu, Z. M. Zhong, X. Qi, R. Cheng, R. J. Qin, J. Liang, X. F. Zhu, M. S. Zeng and C. Z. Sun: Platelet—secreted CCL3 and its receptor CCR5 promote invasive and migratory abilities of anaplastic thyroid carcinoma cells via MMP—1, *Cell Signal*, 2019, 63: 109363.

58. M. Labelle, S. Begum and R. O. Hynes: Platelets guide the formation of early metastatic niches, *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111: E3053—3061.

59. D. Lin, L. Shen, M. Luo, K. Zhang, J. Li, Q. Yang, F. Zhu, D. Zhou, S. Zheng, Y. Chen and J. Zhou: Circulating tumor cells: biology and clinical significance, *Signal Transduct Target Ther*, 2021, 6: 404.

60. X. L. Lou, J. Sun, S. Q. Gong, X. F. Yu, R. Gong and H. Deng: Interaction between circulating cancer cells and platelets: clinical implication, *Chin J Cancer Res*, 2015, 27: 450—460.

61. T. Placke, M. Örgel, M. Schaller, G. Jung, H.—G. Rammensee, H.—G. Kopp and H. R. Salih: Platelet—derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells, *Cancer Research*, 2012, 72: 440—448.

62. N. Li: Platelets in cancer metastasis: To help the "villain" to do evil, *International Journal of Cancer*, 2016, 138: 2078—2087.

63. L. J. Gay and B. Felding—Habermann: Contribution of platelets to tumour metastasis, *Nat Rev Cancer*, 2011, 11: 123—134.

64. B. M. Szczerba, F. Castro—Giner, M. Vetter, I. Krol, S. Gkountela, J. Landin, M. C. Scheidmann, C. Donato, R. Scherrer, J. Singer, C. Beisel, C.

Kurzeder, V. Heinzelmann—Schwarz, C. Rochlitz, W. P. Weber, N. Beerenwinkel and N. Aceto: Neutrophils escort circulating tumour cells to enable cell cycle progression, *Nature*, 2019, 566: 553—557.

65. E. Mammadova—Bach, P. Zigrino, C. Brucker, C. Bourdon, M. Freund, A. De Arcangelis, S. I. Abrams, G. Orend, C. Gachet and P. H. Mangin: Platelet integrin alpha6beta1 controls lung metastasis through direct binding to cancer cell—derived ADAM9, *JCI Insight*, 2016, 1: e88245.

66. A. Janowska—Wieczorek, M. Wysoczynski, J. Kijowski, L. Marquez—Curtis, B. Machalinski, J. Ratajczak and M. Z. Ratajczak: Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer, *International Journal of Cancer*, 2005, 113: 752—760.

67. S. Albeiroti, K. Ayasoufi, D. R. Hill, B. Shen and C. A. de la Motte: Platelet hyaluronidase—2: an enzyme that translocates to the surface upon activation to function in extracellular matrix degradation, *Blood*, 2015, 125: 1460—1469.

68. N. Cloutier, A. Paré, R. W. Farndale, H. R. Schumacher, P. A. Nigrovic, S. Lacroix and E. Boilard: Platelets can enhance vascular permeability, *Blood*, 2012, 120: 1334—1343.

69. D. Schumacher, B. Strilic, K. K. Sivaraj, N. Wettschureck and S. Offermanns: Platelet—derived nucleotides promote tumor—cell transendothelial migration and metastasis via P2Y2 receptor, *Cancer Cell*, 2013, 24: 130—137.

70. V. Masola, G. Zaza, G. Gambaro, M. Franchi and M. Onisto: Role of heparanase in tumor progression: Molecular aspects and therapeutic options, *Semin Cancer Biol*, 2020, 62: 86—98.

71. M. Z. Wojtukiewicz, E. Sierko, D. Hempel, S. C. Tucker and K. V. Honn: Platelets and cancer angiogenesis nexus, *Cancer Metastasis Rev*, 2017, 36: 249—262.

72. S. Sabrkhany, A. W. Griffioen and M. G. Oude Egbrink: The role of blood platelets in tumor angiogenesis, *Biochim Biophys Acta*, 2011, 1815: 189—196.

73. J. E. Italiano, Jr., J. L. Richardson, S. Patel—Hett, E. Battinelli, A. Zaslavsky, S. Short, S. Ryeom, J. Folkman and G. L. Klement: Angiogenesis is regulated by a novel mechanism: pro— and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released, *Blood*, 2008, 111: 1227—1233.

74. M. Chatterjee, Z. Huang, W. Zhang, L. Jiang, K. Hultenby, L. Zhu, H. Hu, G. P. Nilsson and N. Li: Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli, *Blood*, 2011, 117: 3907—3911.

75. E. M. Battinelli, J. N. Thon, R. Okazaki, C. G. Peters, P. Vijey, A. R. Wilkie, L. J. Noetzli, R. Flaumenhaft and J. E. Italiano: Megakaryocytes package contents into separate alpha—granules that are differentially distributed in platelets, *Blood Adv*, 2019, 3: 3092—3098.

76. B. Ho—Tin—Noe, T. Goerge, S. M. Cifuni, D. Duerschmied and D. D. Wagner: Platelet granule secretion continuously prevents intratumor hemorrhage, *Cancer*

Res, 2008, 68: 6851–6858.

77. Z. Wang and H. Huang: Platelet factor–4 (CXCL4/PF–4): an angiostatic chemokine for cancer therapy, *Cancer letters*, 2013, 331: 147–153.

78. J. Vandercappellen, S. Liekens, A. Bronckaers, S. Noppen, I. Ronsse, C. Dillen, M. Belleri, S. Mitola, P. Proost, M. Presta, S. Struyf and J. Van Damme: The COOH–terminal peptide of platelet factor–4 variant (CXCL4L1/PF–4var47–70) strongly inhibits angiogenesis and suppresses B16 melanoma growth in vivo, *Molecular cancer research : MCR*, 2010, 8: 322–334.

79. J. Tian, Y. Cai, Y. Li, Z. Lu, J. Huang, Y. Deng, N. Yang, X. Wang, P. Ying, S. Zhang, Y. Zhu, H. Zhang, R. Zhong, J. Chang and X. Miao: CancerImmunityQTL: a database to systematically evaluate the impact of genetic variants on immune infiltration in human cancer, *Nucleic Acids Res*, 2021, 49: D1065–D1073.

80. J. M. Pitt, A. Marabelle, A. Eggermont, J. C. Soria, G. Kroemer and L. Zitvogel: Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy, *Ann Oncol*, 2016, 27: 1482–1492.

81. M. Binnewies, E. W. Roberts, K. Kersten, V. Chan, D. F. Fearon, M. Merad, L. M. Coussens, D. I. Gabrilovich, S. Ostrand–Rosenberg, C. C. Hedrick, R. H. Vonderheide, M. J. Pittet, R. K. Jain, W. Zou, T. K. Howcroft, E. C. Woodhouse, R. A. Weinberg and M. F. Krummel: Understanding the tumor immune microenvironment (TIME) for effective therapy, *Nat Med*, 2018, 24: 541–550.

82. C. N. Morrell, D. N. Pariser, Z. T. Hilt and D. Vega Ocasio: The Platelet Napoleon Complex–Small Cells, but Big Immune Regulatory Functions, *Annu Rev Immunol*, 2019, 37: 125–144.

83. R. Aslam, E. R. Speck, M. Kim, A. R. Crow, K. W. A. Bang, F. P. Nestel, H. Ni, A. H. Lazarus, J. Freedman and J. W. Semple: Platelet Toll–like receptor expression modulates lipopolysaccharide–induced thrombocytopenia and tumor necrosis factor–alpha production in vivo, *Blood*, 2006, 107: 637–641.

84. W. Feng, M. Madajka, B. A. Kerr, G. H. Mahabeleshwar, S. W. Whiteheart and T. V. Byzova: A novel role for platelet secretion in angiogenesis: mediating bone marrow–derived cell mobilization and homing, *Blood*, 2011, 117: 3893–3902.

85. N. Pavlović, M. Kopsida, P. Gerwins and F. Heindryckx: Activated platelets contribute to the progression of hepatocellular carcinoma by altering the tumor environment, *Life Sciences*, 2021, 277: 119612.

86. A. L. Palacios–Acedo, D. Mège, L. Crescence, F. Dignat–George, C. Dubois and L. Panicot–Dubois: Platelets, Thrombo–Inflammation, and Cancer: Collaborating With the Enemy, *Frontiers In Immunology*, 2019, 10: 1805.

87. A. K. Olsson and J. Cedervall: The pro–inflammatory role of platelets in cancer, *Platelets*, 2018, 29: 569–573.

88. A. M. Gil–Bernabé, S. Ferjancic, M. Tlalka, L. Zhao, P. D. Allen, J. H. Im, K. Watson, S. A. Hill, A. Amirkhosravi, J. L. Francis, J. W. Pollard, W. Ruf and R. J. Muschel: Recruitment of monocytes/macrophages by tissue factor–mediated coagulation is essential for metastatic cell survival and premetastatic niche

establishment in mice, *Blood*, 2012, 119: 3164–3175.

89. M. Labelle, S. Begum and R. O. Hynes: Platelets guide the formation of early metastatic niches, *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111: E3053–E3061.

90. H. Läubli, K.–S. Spanaus and L. Borsig: Selectin–mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes, *Blood*, 2009, 114: 4583–4591.

91. V. Dymicka–Piekarska, O. M. Koper–Lenkiewicz, J. Zińczuk, E. Kratz and J. Kamińska: Inflammatory cell–associated tumors. Not only macrophages (TAMs), fibroblasts (TAFs) and neutrophils (TANs) can infiltrate the tumor microenvironment. The unique role of tumor associated platelets (TAPs), *Cancer Immunol Immunother*, 2021, 70: 1497–1510.

92. Q.–w. Zhang, L. Liu, C.–y. Gong, H.–s. Shi, Y.–h. Zeng, X.–z. Wang, Y.–w. Zhao and Y.–q. Wei: Prognostic significance of tumor–associated macrophages in solid tumor: a meta–analysis of the literature, *Plos One*, 2012, 7: e50946.

93. J. Kim and J.–S. Bae: Tumor–Associated Macrophages and Neutrophils in Tumor Microenvironment, *Mediators Inflamm*, 2016, 2016: 6058147.

94. S. Rachidi, A. Metelli, B. Riesenberg, B. X. Wu, M. H. Nelson, C. Wallace, C. M. Paulos, M. P. Rubinstein, E. Garrett–Mayer, M. Hennig, D. W. Bearden, Y. Yang, B. Liu and Z. Li: Platelets subvert T cell immunity against cancer via GARP–TGFβ axis, *Sci Immunol*, 2017, 2.

95. S. Malekghasemi, J. Majidi, A. Baghbanzadeh, J. Abdolalizadeh, B. Baradaran and L. Aghebati–Maleki: Tumor–Associated Macrophages: Protumoral Macrophages in Inflammatory Tumor Microenvironment, *Adv Pharm Bull*, 2020, 10: 556–565.

96. N. Pavlović, M. Kopsida, P. Gerwins and F. Heindryckx: Activated platelets contribute to the progression of hepatocellular carcinoma by altering the tumor environment, *Life sciences*, 2021, 277: 119612.

97. M. T. Masucci, M. Minopoli and M. V. Carriero: Tumor Associated Neutrophils. Their Role in Tumorigenesis, Metastasis, Prognosis and Therapy, *Front Oncol*, 2019, 9: 1146.

98. S. Maurer and L. Ferrari de Andrade: NK Cell Interaction With Platelets and Myeloid Cells in the Tumor Milieu, *Frontiers In Immunology*, 2020, 11: 608849.

99. D. Stoiber and A. Assinger: Platelet–Leukocyte Interplay in Cancer Development and Progression, *Cells*, 2020, 9.

100. L. Schmied, P. Höglund and S. Meinke: Platelet–Mediated Protection of Cancer Cells From Immune Surveillance – Possible Implications for Cancer Immunotherapy, *Frontiers In Immunology*, 2021, 12: 640578.

101. K. L. Clar, C. Hinterleitner, P. Schneider, H. R. Salih and S. Maurer: Inhibition of NK Reactivity Against Solid Tumors by Platelet–Derived RANKL, *Cancers (Basel)*, 2019, 11.

102. Y. Zhou, J. S. Heitmann, K. L. Clar, K. N. Kropp, M. Hinterleitner, T.

Engler, A. Koch, A. D. Hartkopf, L. Zender, H. R. Salih, S. Maurer and C. Hinterleitner: Platelet—expressed immune checkpoint regulator GITRL in breast cancer, *Cancer Immunol Immunother*, 2021, 70: 2483—2496.

103. S. Maurer, K. N. Kropp, G. Klein, A. Steinle, S. P. Haen, J. S. Walz, C. Hinterleitner, M. Märklin, H.—G. Kopp and H. R. Salih: Platelet—mediated shedding of NKG2D ligands impairs NK cell immune—surveillance of tumor cells, *Oncoimmunology*, 2018, 7: e1364827.

104. H.—G. Kopp, T. Placke and H. R. Salih: Platelet—derived transforming growth factor—beta down—regulates NKG2D thereby inhibiting natural killer cell antitumor reactivity, *Cancer Research*, 2009, 69: 7775—7783.

105. A. Metelli, B. X. Wu, B. Riesenberg, S. Guglietta, J. D. Huck, C. Mills, A. Li, S. Rachidi, C. Krieg, M. P. Rubinstein, D. T. Gewirth, S. Sun, M. B. Lilly, A. H. Wahlquist, D. P. Carbone, Y. Yang, B. Liu and Z. Li: Thrombin contributes to cancer immune evasion via proteolysis of platelet—bound GARP to activate LTGF—β, *Sci Transl Med*, 2020, 12.

106. F. Del Vecchio, V. Martinez—Rodriguez, M. Schukking, A. Cocks, E. Broseghini and M. Fabbri: Professional killers: The role of extracellular vesicles in the reciprocal interactions between natural killer, CD8+ cytotoxic T—cells and tumour cells, *J Extracell Vesicles*, 2021, 10: e12075.

107. C. Hinterleitner, J. Strähle, E. Malenke, M. Hinterleitner, M. Henning, M. Seehawer, T. Bilich, J. Heitmann, M. Lutz, S. Mattern, S. Scheuermann, M. Horger, S. Maurer, J. Walz, F. Fend, R. Handgretinger, C. Seitz, B. Weigelin, S. Singer, H. Salih, O. Borst, H.—G. Kopp and L. Zender: Platelet PD—L1 reflects collective intratumoral PD—L1 expression and predicts immunotherapy response in non—small cell lung cancer, *Nature Communications*, 2021, 12: 7005.

108. L. Servais, O. Wéra, J. Dibato Epoh, C. Delierneux, N. Bouznad, S. Rahmouni, G. Mazzucchelli, D. Baiwir, P. Delvenne, P. Lancellotti and C. Oury: Platelets contribute to the initiation of colitis—associated cancer by promoting immunosuppression, *J Thromb Haemost*, 2018, 16: 762—777.

109. W. K. Panek, K. C. Pituch, J. Miska, J. W. Kim, A. Rashidi, D. Kanojia, A. Lopez—Rosas, Y. Han, D. Yu, C. L. Chang, J. R. Kane, P. Zhang, A. Cordero and M. S. Lesniak: Local Application of Autologous Platelet—Rich Fibrin Patch (PRF—P) Suppresses Regulatory T Cell Recruitment in a Murine Glioma Model, *Mol Neurobiol*, 2019, 56: 5032—5040.

110. L. Plantureux, D. Mege, L. Crescence, E. Carminita, S. Robert, S. Cointe, N. Brouilly, W. Ezzedine, F. Dignat—George, C. Dubois and L. Panicot—Dubois: The Interaction of Platelets with Colorectal Cancer Cells Inhibits Tumor Growth but Promotes Metastasis, *CANCER RESEARCH*, 2020, 80: 291—303.

111. J. Rossaint, A. Margraf and A. Zarbock: Role of Platelets in Leukocyte Recruitment and Resolution of Inflammation, *Frontiers In Immunology*, 2018, 9: 2712.

112. S. U. Eisenhardt, J. Habersberger, A. Murphy, Y.—C. Chen, K. J. Woollard, N. Bassler, H. Qian, C. von Zur Muhlen, C. E. Hagemeyer, I. Ahrens, J. Chin—Dusting, A. Bobik and K. Peter: Dissociation of pentameric to monomeric C—

reactive protein on activated platelets localizes inflammation to atherosclerotic plaques, *Circulation Research*, 2009, 105: 128–137.

113. M. Mauler, N. Herr, C. Schoenichen, T. Witsch, T. Marchini, C. Härdtner, C. Koentges, K. Kienle, V. Ollivier, M. Schell, L. Dorner, C. Wippel, D. Stallmann, C. Normann, H. Bugger, P. Walther, D. Wolf, I. Ahrens, T. Lämmermann, B. Ho-Tin-Noé, K. Ley, C. Bode, I. Hilgendorf and D. Duerschmied: Platelet Serotonin Aggravates Myocardial Ischemia/Reperfusion Injury via Neutrophil Degranulation, *Circulation*, 2019, 139: 918–931.

114. F. Soga, N. Katoh, T. Inoue and S. Kishimoto: Serotonin activates human monocytes and prevents apoptosis, *J Invest Dermatol*, 2007, 127: 1947–1955.

115. S. R. Clark, A. C. Ma, S. A. Tavener, B. McDonald, Z. Goodarzi, M. M. Kelly, K. D. Patel, S. Chakrabarti, E. McAvoy, G. D. Sinclair, E. M. Keys, E. Allen-Vercoe, R. Devinney, C. J. Doig, F. H. Y. Green and P. Kubes: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood, *Nat Med*, 2007, 13: 463–469.

116. S.-J. Kim and C. N. Jenne: Role of platelets in neutrophil extracellular trap (NET) production and tissue injury, *Semin Immunol*, 2016, 28: 546–554.

117. P. Patel, J. V. Michael, U. P. Naik and S. E. McKenzie: Platelet FcγRIIA in immunity and thrombosis: Adaptive immunothrombosis, *J Thromb Haemost*, 2021, 19: 1149–1160.

118. Á. Petersen-Uribe, M. Kremser, A.-K. Rohlfing, T. Castor, K. Kolb, V. Dicenta, F. Emschermann, B. Li, O. Borst, D. Rath, K. A. L. Müller and M. P. Gawaz: Platelet-Derived PCSK9 Is Associated with LDL Metabolism and Modulates Atherothrombotic Mechanisms in Coronary Artery Disease, *Int J Mol Sci*, 2021, 22.

119. S. Pierre, B. Linke, J. Suo, N. Tarighi, D. Del Turco, D. Thomas, N. Ferreiros, D. Stegner, S. Frölich, M. Sisignano, S. Meyer Dos Santos, N. deBruin, R. M. Nüsing, T. Deller, B. Nieswandt, G. Geisslinger and K. Scholich: GPVI and Thromboxane Receptor on Platelets Promote Proinflammatory Macrophage Phenotypes during Cutaneous Inflammation, *J Invest Dermatol*, 2017, 137: 686–695.

120. S. C. Starossom, T. Veremeyko, M. Dukhinova, A. W. Y. Yung and E. D. Ponomarev: Glatiramer acetate (copaxone) modulates platelet activation and inhibits thrombin-induced calcium influx: possible role of copaxone in targeting platelets during autoimmune neuroinflammation, *Plos One*, 2014, 9: e96256.

121. H. F. Langer, E. Y. Choi, H. Zhou, R. Schleicher, K.-J. Chung, Z. Tang, K. Göbel, K. Bdeir, A. Chatzigeorgiou, C. Wong, S. Bhatia, M. J. Kruhlak, J. W. Rose, J. B. Burns, K. E. Hill, H. Qu, Y. Zhang, E. Lehrmann, K. G. Becker, Y. Wang, D. I. Simon, B. Nieswandt, J. D. Lambris, X. Li, S. G. Meuth, P. Kubes and T. Chavakis: Platelets contribute to the pathogenesis of experimental autoimmune encephalomyelitis, *Circulation Research*, 2012, 110: 1202–1210.

122. T. Nakanishi, M. Inaba, N. Inagaki-Katashiba, A. Tanaka, P. T. X. Vien, K. Kibata, T. Ito and S. Nomura: Platelet-derived RANK ligand enhances CCL17 secretion from dendritic cells mediated by thymic stromal lymphopoietin, *Platelets*, 2015, 26: 425–431.

123. T. Dürk, D. Duerschmied, T. Müller, M. Grimm, S. Reuter, R. P. Vieira, K. Ayata, S. Cicko, S. Sorichter, D. J. Walther, J. C. Virchow, C. Taube and M. Idzko: Production of serotonin by tryptophan hydroxylase 1 and release via platelets contribute to allergic airway inflammation, *Am J Respir Crit Care Med*, 2013, 187: 476–485.

124. M. Tomczyńska and J. Saluk-Bijak: The mutual cooperation of blood platelets and lymphocytes in the development of autoimmune thyroid diseases, *Acta Biochim Pol*, 2018, 65: 17–24.

125. J. M. Sowa, S. A. Crist, T. L. Ratliff and B. D. Elzey: Platelet influence on T- and B-cell responses, *Archivum Immunologiae Et Therapiae Experimentalis*, 2009, 57: 235–241.

126. J. Tian, T. Zhu, J. Liu, Z. Guo and X. Cao: Platelets promote allergic asthma through the expression of CD154, *Cell Mol Immunol*, 2015, 12: 700–707.

127. A. Tabuchi and W. M. Kuebler: Endothelium–platelet interactions in inflammatory lung disease, *VASCULAR PHARMACOLOGY*, 2008, 49: 141–150.

128. E. Oikonomou, M. Leopoulou, P. Theofilis, A. S. Antonopoulos, G. Siasos, G. Latsios, V. C. Mystakidi, C. Antoniades and D. Tousoulis: A link between inflammation and thrombosis in atherosclerotic cardiovascular diseases: Clinical and therapeutic implications, *Atherosclerosis*, 2020, 309: 16–26.

129. M. Finsterbusch, W. C. Schrottmaier, J. B. Kral-Pointner, M. Salzmann and A. Assinger: Measuring and interpreting platelet–leukocyte aggregates, *Platelets*, 2018, 29: 677–685.

130. P. X. Liew and P. Kubes: The Neutrophil's Role During Health and Disease, *Physiological Reviews*, 2019, 99: 1223–1248.

131. A. Zarbock, K. Singbartl and K. Ley: Complete reversal of acid–induced acute lung injury by blocking of platelet–neutrophil aggregation, *The Journal of Clinical Investigation*, 2006, 116: 3211–3219.

132. T. Hara, K. Shimizu, F. Ogawa, K. Yanaba, Y. Iwata, E. Muroi, M. Takenaka, K. Komura, M. Hasegawa, M. Fujimoto and S. Sato: Platelets control leukocyte recruitment in a murine model of cutaneous arthus reaction, *The American Journal of Pathology*, 2010, 176: 259–269.

133. G. Zuchtriegel, B. Uhl, D. Puhr-Westerheide, M. Pörnbacher, K. Lauber, F. Krombach and C. A. Reichel: Platelets Guide Leukocytes to Their Sites of Extravasation, *PLoS Biol*, 2016, 14: e1002459.

134. A. Gros, V. Ollivier and B. Ho-Tin-Noé: Platelets in inflammation: regulation of leukocyte activities and vascular repair, *Frontiers In Immunology*, 2014, 5: 678.

135. J. A. Giles, A. D. Greenhalgh, A. Denes, B. Nieswandt, G. Coutts, B. W. McColl and S. M. Allan: Neutrophil infiltration to the brain is platelet-dependent, and is reversed by blockade of platelet GPIbα, *Immunology*, 2018, 154: 322–328.

136. D. Duerschmied, G. L. Suidan, M. Demers, N. Herr, C. Carbo, A. Brill, S. M. Cifuni, M. Mauler, S. Cicko, M. Bader, M. Idzko, C. Bode and D. D. Wagner: Platelet serotonin promotes the recruitment of neutrophils to sites of acute

inflammation in mice, *Blood*, 2013, 121: 1008–1015.

137. N. Gerdes, T. Seijkens, D. Lievens, M. J. E. Kuijpers, H. Winkels, D. Projahn, H. Hartwig, L. Beckers, R. T. A. Megens, L. Boon, R. J. Noelle, O. Soehnlein, J. W. M. Heemskerk, C. Weber and E. Lutgens: Platelet CD40 Exacerbates Atherosclerosis by Transcellular Activation of Endothelial Cells and Leukocytes, *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2016, 36: 482–490.

138. V. Sreeramkumar, J. M. Adrover, I. Ballesteros, M. I. Cuartero, J. Rossaint, I. Bilbao, M. Nácher, C. Pitaval, I. Radovanovic, Y. Fukui, R. P. McEver, M.-D. Filippi, I. Lizasoain, J. Ruiz-Cabello, A. Zarbock, M. A. Moro and A. Hidalgo: Neutrophils scan for activated platelets to initiate inflammation, *Science*, 2014, 346: 1234–1238.

139. R. Hwaiz, M. Rahman, E. Zhang and H. Thorlacius: Platelet secretion of CXCL4 is Rac1-dependent and regulates neutrophil infiltration and tissue damage in septic lung damage, *British Journal of Pharmacology*, 2015, 172: 5347–5359.

140. E. Wetterholm, J. Linders, M. Merza, S. Regner and H. Thorlacius: Platelet-derived CXCL4 regulates neutrophil infiltration and tissue damage in severe acute pancreatitis, *Transl Res*, 2016, 176: 105–118.

141. M. K. Schuhmann, J. Guthmann, G. Stoll, B. Nieswandt, P. Kraft and C. Kleinschnitz: Blocking of platelet glycoprotein receptor Ib reduces "thrombo-inflammation" in mice with acute ischemic stroke, *J Neuroinflammation*, 2017, 14: 18.

142. W.-J. Chae, A. K. Ehrlich, P. Y. Chan, A. M. Teixeira, O. Henegariu, L. Hao, J. H. Shin, J.-H. Park, W. H. Tang, S.-T. Kim, S. E. Maher, K. Goldsmith-Pestana, P. Shan, J. Hwa, P. J. Lee, D. S. Krause, C. V. Rothlin, D. McMahon-Pratt and A. L. M. Bothwell: The Wnt Antagonist Dickkopf-1 Promotes Pathological Type 2 Cell-Mediated Inflammation, *Immunity*, 2016, 44: 246–258.

143. M. K. Schuhmann, G. Stoll, M. Bieber, T. Vögtle, S. Hofmann, V. Klaus, P. Kraft, M. Seyhan, A. M. Kollikowski, L. Papp, P. U. Heuschmann, M. Pham, B. Nieswandt and D. Stegner: CD84 Links T Cell and Platelet Activity in Cerebral Thrombo-Inflammation in Acute Stroke, *Circulation Research*, 2020, 127: 1023–1035.

144. S. Danese, C. de la Motte, B. M. R. Reyes, M. Sans, A. D. Levine and C. Fiocchi: Cutting edge: T cells trigger CD40-dependent platelet activation and granular RANTES release: a novel pathway for immune response amplification, *Journal of Immunology (Baltimore, Md. : 1950)*, 2004, 172: 2011–2015.

145. A. Khandoga, M. Hanschen, J. S. Kessler and F. Krombach: CD4+ T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets, *Hepatology*, 2006, 43: 306–315.

146. G. A. Ramirez, A. A. Manfredi and N. Maugeri: Misunderstandings Between Platelets and Neutrophils Build in Chronic Inflammation, *Frontiers In Immunology*, 2019, 10: 2491.

147. A. Assinger, M. Laky, G. Schabbauer, A. M. Hirschl, E. Buchberger, B. R. Binder and I. Volf: Efficient phagocytosis of periodontopathogens by neutrophils requires plasma factors, platelets and TLR2, *J Thromb Haemost*, 2011, 9: 799–809.

148. S. C. Starossom, T. Veremeyko, A. W. Y. Yung, M. Dukhinova, C. Au,

A. Y. Lau, H. L. Weiner and E. D. Ponomarev: Platelets Play Differential Role During the Initiation and Progression of Autoimmune Neuroinflammation, *Circulation Research*, 2015, 117: 779–792.

149. C. B. Bergmann, F. Hefele, M. Unger, S. Huber–Wagner, P. Biberthaler, M. van Griensven and M. Hanschen: Platelets modulate the immune response following trauma by interaction with CD4+ T regulatory cells in a mouse model, *Immunol Res*, 2016, 64: 508–517.

150. S. Albayati, H. Vemulapalli, A. Y. Tsygankov and E. Liverani: P2Y antagonism results in altered interactions between platelets and regulatory T cells during sepsis, *Journal of Leukocyte Biology*, 2021, 110: 141–153.

151. E. Hotta, R. Tamagawa–Mineoka and N. Katoh: Platelets are important for the development of immune tolerance: Possible involvement of TGF– β in the mechanism, *Exp Dermatol*, 2019, 28: 801–808.

152. J. Rossaint, K. Thomas, S. Mersmann, J. Skupski, A. Margraf, T. Tekath, C. C. Jouvene, J. Dalli, A. Hidalgo, S. G. Meuth, O. Soehnlein and A. Zarbock: Platelets orchestrate the resolution of pulmonary inflammation in mice by T reg cell repositioning and macrophage education, *J Exp Med*, 2021, 218.

153. Q.–R. Li, H.–Z. Xu, R.–C. Xiao, Y. Liu, J.–M. Tang, J. Li, T.–T. Yu, B. Liu, L.–G. Li, M.–F. Wang, N. Han, Y.–H. Xu, C. Wang, N. Komatsu, L. Zhao, X.–C. Peng, T.–F. Li and X. Chen: Platelets are highly efficient and efficacious carriers for tumor–targeted nano–drug delivery, *Drug Deliv*, 2022, 29: 937–949.

154. S. Sarkar, M. A. Alam, J. Shaw and A. K. Dasgupta: Drug delivery using platelet cancer cell interaction, *Pharm Res*, 2013, 30: 2785–2794.

155. P. Xu, H. Zuo, B. Chen, R. Wang, A. Ahmed, Y. Hu and J. Ouyang: Doxorubicin–loaded platelets as a smart drug delivery system: An improved therapy for lymphoma, *Sci Rep*, 2017, 7: 42632.

156. H.–Z. Xu, T.–F. Li, Y. Ma, K. Li, Q. Zhang, Y.–H. Xu, Y.–C. Zhang, L. Zhao and X. Chen: Targeted photodynamic therapy of glioblastoma mediated by platelets with photo–controlled release property, *Biomaterials*, 2022, 290: 121833.

157. Y. Zhang, Y. Sun, X. Dong, Q.–S. Wang, D. Zhu, L. Mei, H. Yan and F. Lv: A Platelet Intelligent Vehicle with Navigation for Cancer Photothermal–Chemotherapy, *ACS NANO*, 2022, 16: 6359–6371.

158. L. Wang, X. Wang, E. Guo, X. Mao and S. Miao: Emerging roles of platelets in cancer biology and their potential as therapeutic targets, *Front Oncol*, 2022, 12: 939089.

159. G. Marriott: Engineering platelets for tumour–targeting, *Aging (Albany NY)*, 2016, 8: 1572–1573.

160. C. Wang, W. Sun, Y. Ye, Q. Hu, H. N. Bomba and Z. Gu: In situ activation of platelets with checkpoint inhibitors for post–surgical cancer immunotherapy, *Nature Biomedical Engineering*, 2017, 1: 0011.

161. X. Han, H. Li, D. Zhou, Z. Chen and Z. Gu: Local and Targeted Delivery of Immune Checkpoint Blockade Therapeutics, *Acc Chem Res*, 2020, 53: 2521–2533.

162. Y. X. Wang, Z. T. Li, F. Y. Mo, Z. Gu and Q. Y. Hu: Engineered platelets: Advocates for tumor immunotherapy, *NANO TODAY*, 2021, 40.

163. Q. Hu, H. Li, E. Archibong, Q. Chen, H. Ruan, S. Ahn, E. Dukhovlinova, Y. Kang, D. Wen, G. Dotti and Z. Gu: Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets, *Nature Biomedical Engineering*, 2021, 5: 1038-1047.

164. X. Zhang, J. Wang, Z. Chen, Q. Hu, C. Wang, J. Yan, G. Dotti, P. Huang and Z. Gu: Engineering PD-1-Presenting Platelets for Cancer Immunotherapy, *Nano Lett*, 2018, 18: 5716-5725.

165. E. Pérez-Herrero and A. Fernández-Medarde: Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy, *Eur J Pharm Biopharm*, 2015, 93: 52-79.

166. Y. Huang, C.-Q. Fan, H. Dong, S.-M. Wang, X.-C. Yang and S.-M. Yang: Current applications and future prospects of nanomaterials in tumor therapy, *Int J Nanomedicine*, 2017, 12: 1815-1825.

167. S. T. Yurkin and Z. Wang: Cell membrane-derived nanoparticles: emerging clinical opportunities for targeted drug delivery, *Nanomedicine (Lond)*, 2017, 12: 2007-2019.

168. Z. Li, S. Hu and K. Cheng: Platelets and their biomimetics for regenerative medicine and cancer therapies, *J Mater Chem B*, 2018, 6: 7354-7365.

169. W. Pei, B. Huang, S. Chen, L. Wang, Y. Xu and C. Niu: Platelet-Mimicking Drug Delivery Nanoparticles for Enhanced Chemo-Photothermal Therapy of Breast Cancer, *Int J Nanomedicine*, 2020, 15: 10151-10167.

170. J. Li, Y. Ai, L. Wang, P. Bu, C. C. Sharkey, Q. Wu, B. Wun, S. Roy, X. Shen and M. R. King: Targeted drug delivery to circulating tumor cells via platelet membrane-functionalized particles, *Biomaterials*, 2016, 76: 52-65.

171. B. Bahmani, H. Gong, B. T. Luk, K. J. Haushalter, E. DeTeresa, M. Previti, J. R. Zhou, W. W. Gao, J. D. Bui, L. F. Zhang, R. N. H. Fang and J. Zhang: Intratumoral immunotherapy using platelet-cloaked nanoparticles enhances antitumor immunity in solid tumors, *NATURE COMMUNICATIONS*, 2021, 12.

172. S. Lazar and L. E. Goldfinger: Platelet Microparticles and miRNA Transfer in Cancer Progression: Many Targets, Modes of Action, and Effects Across Cancer Stages, *Front Cardiovasc Med*, 2018, 5: 13.

173. J. Kailashiya, V. Gupta and D. Dash: Engineered human platelet-derived microparticles as natural vectors for targeted drug delivery, *Oncotarget*, 2019, 10: 5835-5846.

https://doi.org/10.55415/deep-2023-0030.v1

Legends

Fig.1. Model of the possible involvement of platelet-tumor cell and -immune cell interactions in tumor development and TME.

Platelet-tumor cells interactions contribute to tumor proliferation, angiogenesis, and metastasis. Platelet-leukocyte interactions also play crucial roles in the mechanisms of tumor-associated immunosuppression. While platelet-leukocyte interactions can initiate and accelerate inflammatory processes and immune responses in the context of inflammation and autoimmune diseases.

Fig.2. Schematic illustration of different platelet-based drug delivery systems.

(1) Platelets deliver antitumor drugs.

(2) Platelet membrane-camouflaged antitumor drug delivery systems.

(3) Platelet engineering for drug delivery.

(5) Platelet-derived microparticles for antitumor drug delivery.