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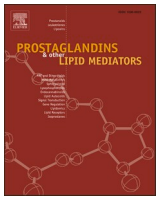

Platelets and extracellular vesicles in disease promotion via cellular cross-talk and eicosanoid biosynthesis

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ABSTRACT

New insights have been gained on the role of platelets beyond thrombosis. Platelets can accumulate in damaged and inflamed tissues, acting as a sentinel to detect and repair tissue damage. However, by releasing several soluble factors, including thromboxane A_2 (TXA_2) and 12-hydroxyeicosatetraenoic acid, and extracellular vesicles (EVs), platelets can activate vascular cells, stromal, such as fibroblasts, immune cells, and cancer cells, leading to atherosclerosis, vascular restenosis, tissue fibrosis, and tumor metastasis. Platelet-derived extracellular vesicles (PEVs) are released when platelets are activated and can transfer their cargo to other cell types, thus contributing to the development of diseases. Inhibitors of the internalization of PEVs can potentially represent novel therapeutic tools. Both platelets and PEVs contain a significant number of different types of molecules, and their omics assessment and integration with clinical data using computational approaches have the potential to detect early disease development and monitor drug treatments.

1. Introduction

Platelets have intrigued researchers since their discovery due to their remarkable characteristics. Platelets possess the ability to adhere at sites of vascular injury rapidly and to aggregate [1]. Platelets are involved in hemostasis but also the development of thrombosis. Platelets are involved in primary hemostasis and repair of the endothelium after tissue trauma and vascular damage [2]. In physiological conditions, platelets circulate close to the vascular walls. The endothelial release of soluble factors such as nitric oxide (NO) and prostacyclin (PGI_2) restrains the activation of platelets [3]. Once the platelets adhere to the extracellular matrix (ECM) components, they release some molecules [including adenosine diphosphate(ADP), serotonin, and thromboxane (TX)A_2] that amplify platelet activation. As a result, a hemostatic plug is formed that repairs the site of damage [2].

Excessive deposition of ECM is a common feature of fibrosis, which can affect different organs, such as the heart, the liver, and the lung, leading to cardiac fibrosis, liver cirrhosis, and pulmonary fibrosis [4]. Recent research has revealed the active involvement of platelets in driving fibrotic processes. Platelets release profibrotic mediators, such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and TXA_2. These mediators stimulate the activation of fibroblasts and the synthesis of collagen [5–7]. Platelets play a role in the development of tissue fibrosis by facilitating endothelial-mesenchymal transition (EndMT). EndMT is a process in which an endothelial cell undergoes a series of molecular changes that transform its phenotype into a mesenchymal cell, such as a myofibroblast or smooth muscle cell [8]. Moreover, platelet activation and enhanced TXA_2 biosynthesis in response to vascular damage and collagen exposure are early events that contribute to restenosis through vascular smooth muscle cell proliferation and migration of [9].

Platelets transmit inflammation-related signals, activating stromal cells and contributing to microenvironmental changes that drive cell plasticity. Several mechanisms can mediate these effects, such as direct interaction with target cells and the release of various mediators, including prostanoids [i.e., TXA_2 and prostaglandin (PG)E_2], angiogenic

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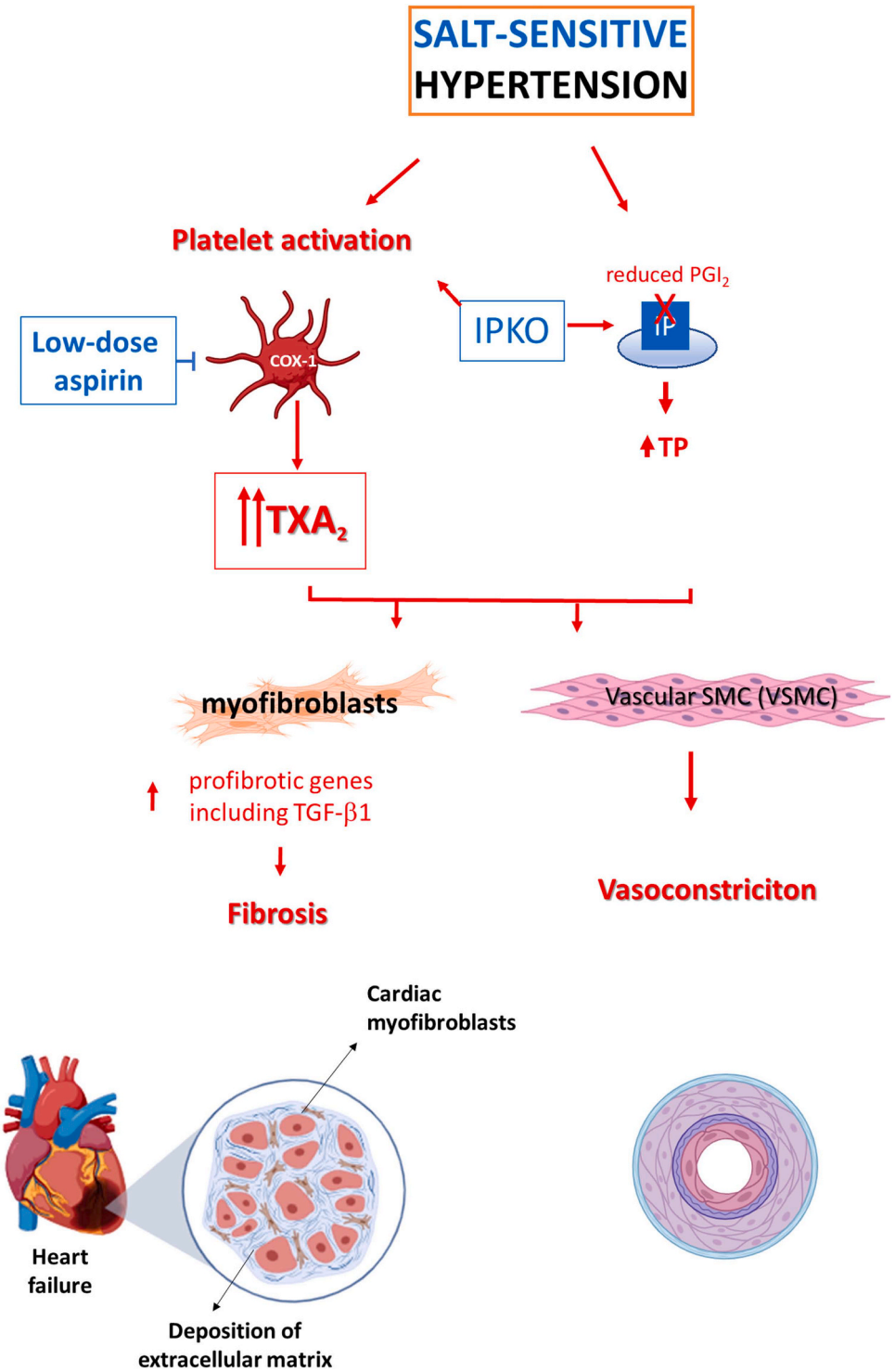

Fig. 1. Low-dose aspirin constraints enhanced blood pressure and the associated early stages of cardiac fibrosis. D'Agostino et al. (7) identified a mouse phenotype characterized by enhanced platelet TXA_2 generation, cellular TXA_2 receptor (TP) expression, and reduced prostacyclin (PGI_2) signaling, susceptible to hypertension and cardiac fibrosis. TP signaling can promote a profibrotic gene signature in myofibroblasts independent of any major hemodynamic change. Treatment with low-dose Aspirin, which inhibits platelet TXA_2 generation, mitigates hypertension and cardiac fibrosis when TXA_2 is unconstrained due to the downregulation of prostacyclin signaling. IPKO: knockout mice for prostacyclin receptor (IP); TXA_2, thromboxane A_2.

and growth factors stored in α-granules (including TGF-β and PGDF), adenosine diphosphate (ADP) stored in dense granules, and vesicles containing the genetic material, including mRNA and microRNAs [10, 11]. In this scenario, platelets contribute to chronic inflammation associated with many pathological conditions, including atherosclerosis, tissue fibrosis, and cancer.

This review discusses the evidence suggesting platelet activation's role in the early events associated with cardiac fibrosis and vascular restenosis. We also explore the potential use of both conventional and novel antiplatelet agents to prevent the development of these conditions. The role of platelets in tumor metastasis via the release of extracellular vesicles (EVs) and the transfer of their cargo to tumor cells is

also discussed [12–14]. Finally, we examine the usefulness of assessing platelets and platelet-derived extracellular vesicles (PEVs) through omics to identify human disease phenotypes.

2. Role of platelets in cardiac fibrosis and the therapeutic potential of TXA_2 inhibition

Cardiac fibrosis is a common occurrence in various cardiovascular diseases and is a major contributor to the development of heart failure (HF). Cardiac fibrosis is associated with excessive deposition of ECM proteins by cardiac fibroblasts(CFs), leading to chamber dilatation, cardiomyocyte hypertrophy, apoptosis, and the development of congestive HF. Recent studies have shown that activated myofibroblasts in fibrotic diseases can also originate from endothelial cells (EC) undergoing an EndMT process, acquiring a mesenchymal phenotype [15].

Patients with HF have been reported to experience enhanced platelet activation, leading to increased circulating platelet aggregates or surface-bound P-selectin. The activation of platelets is a complex process that various factors, including co-morbidities such as hypertension and diabetes can influence. These pathological conditions are characterized by the renin-angiotensin system (RAS) activation and the increase in catecholamines, hemodynamic changes, and alterations in vascular factors and cytokines. These factors contribute to the activation of platelets, which in turn promote HF [16].

Many pieces of evidence suggest that prostacyclin (PGI_2) protects against salt-sensitive hypertension, cardiac hypertrophy, and severe cardiac fibrosis [17], while TXA_2 promotes these conditions. PGI_2 is primarily produced by vascular cyclooxygenase (COX)-2, while TXA_2 is mainly derived from platelet COX-1. These two prostanoids have opposing effects. PGI_2 acts as a potent vasodilator and inhibitor of platelet aggregation, and TXA_2 as a pro-aggregatory and vasoconstrictor. Studies have shown that increased systemic biosynthesis of TXA_2 is detected in essential hypertension in humans and hypertensive mice with the deletion of the receptor of prostacyclin (IP) (IPKO) fed with a high-salt diet [7] (Fig. 1).

Interestingly, enhanced cellular TXA_2 receptor (TP) expression was detected in vivo in essential hypertension patients and hypertensive IPKO mice. In IPKO salt-sensitive hypertension, a profibrotic state was detected with collagen deposition in cardiac arterial vessels and epicardium [7]. However, these changes were not associated with left ventricular hypertrophy. In hypertensive IPKO mice, the expression of ECM genes, myofibroblast markers, and endothelial marker Pecam1 (platelet/endothelial cell adhesion molecule 1, known as Cd31) was enhanced in the left ventricle and indicated an increase in fibroblasts carrying an endothelial imprint (for the process of EndMT). Moreover, enhanced expression of TGF-β1 associated with TP receptors was detected. Thus, profibrotic genetic pathways are activated early, before hypertrophic remodeling, in the heart of IPKO salt-sensitive hypertensive mice. In hypertension associated with IP deficiency, an unrestrained activation of the TP receptor can promote cardiac fibrosis. TP and $PGF_{2\alpha}$ (FP) receptors contribute to blood pressure elevation in experimental models of hypertension [17].

D'Agostino et al. [7] discovered the role of platelet TXA_2 in promoting cardiac fibrosis in the IPKO mice fed with a high-salt diet, which develops salt-sensitive hypertension. The antiplatelet agent low-dose Aspirin, which profoundly affects platelet COX-1 activity while substantially sparing vascular COX-2 activity, administered to IPKO mice fed a high-salt diet reduced: (i) systolic blood pressure, (ii) systemic biosynthesis of TXA_2, (iii) epicardial fibrosis, (iv) left ventricular gene expression of ECM and myofibroblast markers, and TGF-β1, and (v) cardiac myofibroblast number and platelet extravasated (Fig. 1). The increased number of platelets extravasated can be responsible for myofibroblast activation, thus contributing to cardiac fibrosis. In vitro studies using the coculture of myofibroblasts with platelets showed that TXA_2 derived from platelets activates myofibroblasts and stimulates the expression of *TGFB1* and other profibrotic genes. Notably, the selective inhibition of platelet COX-1 by Aspirin prevented myofibroblast activation by inhibiting TXA_2 biosynthesis and TGF-β1 release. Platelets can also release intracellular TGF-β from the α-granules [7]. Moreover, or via the ability of surface constitutive expression of TGFβ-docking receptor Glycoprotein A Repetitions Predominant (GARP) to regulate latent TGF-β activation [18].

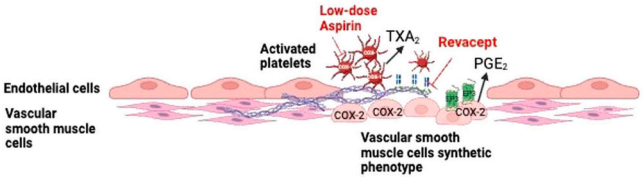

Fig. 2. Platelets play a key role in the development of restenosis. Platelets are activated when there is damage to the endothelial layer of blood vessels, which exposes the extracellular matrix (ECM) proteins, particularly collagen. Platelets then interact with vascular smooth muscle cells (VSMCs) at the site of damage, which leads to the release of platelet-derived products such as TXA_2. This interaction also induces the expression of cyclooxygenase (COX)-2, resulting in the increased production of prostaglandin E_2 (PGE2). PGE_2, in turn, activates the EP3 receptor subtype, which plays a significant role in the development of restenosis. Revacept, an antiplatelet agent in clinical development, effectively inhibits platelet adhesion and aggregation by targeting collagen at the injury site and preventing restenosis. Both low-dose Aspirin, which inhibits platelet COX-1 and TXA_2 generation, and Revacept prevent COX-2 induction in VSMCs.

These findings support the use of low-dose Aspirin for treating a subgroup of hypertensive patients with high systemic TXA_2 biosynthesis and TP expression and reduced PGI_2 signaling. This treatment can help prevent early cardiac fibrosis and the progression to HF (Fig. 1). However, these recommendations must be validated through a randomized clinical study on essential hypertensive individuals.

2.1. *The role of platelets in vascular stenosis: developing novel therapeutic approaches*

The expression of α-SMA (smooth muscle actin) is a characteristic feature of the contractile phenotype of vascular smooth muscle cells (VSMCs). At the same time, its downregulation indicates the synthetic phenotype associated with migratory capacity and inflammatory characteristics. It was discovered that when human platelets were co-cultured with coronary artery smooth muscle cells (CASMCs), they caused the downregulation of α-SMA and induced a round-shaped morphology [9]. The spindle-shaped morphology, typical of a contractile phenotype, was replaced by this synthetic phenotype of VSMCs (Fig. 2).

Enhanced biosynthesis of PGE_2 through the induction of COX-2 expression is a pivotal event in the progression of restenosis [19]. In the coculture of platelets and CASMCs, PGE_2 and TXB_2 were significantly enhanced and represented the most abundant prostanoids. The increase in TXB_2 detected in platelet-CASMC cocultures was entirely prevented by the pre-treatment of platelets with Aspirin, suggesting that enhanced TXB_2 was derived from platelets [9]. Rofecoxib, a selective COX-2 inhibitor [20], completely prevented the increased production of PGE_2 detected in platelet-CASMC cocultures, implying that COX-2 induction in CASMCs played a central role in enhanced PGE_2 biosynthesis. PGE_2 levels detected in the coculture medium were significantly reduced when platelets were pre-exposed to Aspirin before incubating with CASMCs. This effect coincided with the reduction of COX-2 expression [9]. These results suggest that a platelet factor released from activated platelets and sensitive to Aspirin was involved in COX-2 induction in CASMCs exposed to platelets. The use of the selective TP antagonist SQ 29,548 mitigated the induction of COX-2 in CASMCs in response to the interaction with platelets, supporting the role of platelet-derived TXA_2 in the enhanced generation of COX-2-dependent PGE_2 in platelets-vascular cell cocultures [9]. In the scrape wounding assay, it

was found that platelets induced the migration capacity of CASMCs, and this effect was mitigated by the incubation with platelets pre-exposed to Aspirin. Thus, Aspirin's inhibition of platelet COX-1 activity and TXA_2 biosynthesis prevented CASMC COX-2 induction and migration (Fig. 2) [9]. Moreover, the novel antiplatelet agent revacept [i.e., a fusion protein of platelet collagen receptor glycoprotein (GP)VI extracellular domain and the human immunoglobulin Fc domain], which affects both platelet adhesion to collagen and aggregation, prevented the induction of CASMC COX-2 expression by platelets in vitro [9].

These findings suggest that activated platelets are crucial in inducing neointimal formation observed in restenosis after vascular damage (Fig. 2). This happens by switching to a less differentiated state (synthetic phenotype) to acquire the proliferative, migratory, and synthetic capabilities [9]. Also, the endothelial cells that undergo EndMT could contribute to restenosis. This phenomenon involves the loss of specific properties, such as angiogenesis and anti-thrombogenicity, and favors a mesenchymal-like phenotype with increased proliferation, migration, and ECM production. This phenotype is similar to that of synthetic VSMCs [8].

Vascular injury carried out in C57BL/6 mice by bilateral femoral artery denudation was associated with the occurrence of platelet activation in vivo, assessed by measuring the urinary levels of TXM, a major urinary enzymatic metabolite of TXA_2, which is a marker of the systemic biosynthesis of TXA_2 mainly derived from activated platelets [21,22]. In the control mice, the urinary levels of TXM significantly increased versus baseline values at three days after vascular injury and returned to control levels at 28 days after injury (remodeling phase) [9]. The administration of Revacept, 2 mg/kg daily, prevented the increase in urinary TXM and caused a significant average reduction of intima-media thickness (I/M ratio) in paraffin-embedded sections of femoral arteries collected 28 days after arterial injury (remodeling phase). This effect was associated with reducing cell proliferation markers and macrophage infiltration [9].

Revacept has distinct effects, making it a promising therapeutic strategy to prevent restenosis in patients undergoing percutaneous coronary intervention (PCI) and stent implantation for coronary artery disease [23]. Importantly, it affects platelet function without causing bleeding complications [24].

Revacept was studied in the ISAR-PLASTER study (The Intracoronary Stenting and Antithrombotic Regimen: Lesion Platelet Adhesion as Selective Target of Endovenous Revacept in Patients with Chronic Coronary Syndromes Undergoing Percutaneous Coronary Intervention). The study assessed the safety and efficacy of Revacept (80 and 160 mg) [25] in 334 patients (mean age 67.4 years, 75.7 % men, and 27 % diabetic) with stable ischemic heart disease (SIHD) undergoing elective PCI co-administered with standard dual antiplatelet therapy (DAPT). The study had a primary efficacy endpoint of death and myocardial damage [i.e., an increase in high sensitivity (HS) troponin at least five times above the normal limit within 48 h of randomization]. The drug did not reduce myocardial injury in patients with SIHD undergoing elective PCI [25]. Despite providing a more robust platelet inhibition, the high dose of Revacept on top of standard DAPT was not associated with increased bleeding. This study has some limitations that need to be acknowledged. Firstly, the study only included patients with SIHD and low ischemic event risk. Secondly, being a phase II trial, ISAR-PLASTER was designed to detect large reductions of a surrogate endpoint with little prognostic value. Additionally, the post-procedural increase in HS troponin is considered a clinical surrogate of myocardial damage with a low prognostic value and has been questioned scientifically for a long time [26–28]. However, the study concludes that Revacept is a safe drug not associated with an increased risk of bleeding.

The Revacept/CS/02 phase II study assessed the safety and efficacy of plaque-specific inhibition via reduction of platelet activation using Revacept in patients with symptomatic internal carotid artery (ICA) stenosis [29]. Revacept, in combination with guideline-recommended antiplatelet therapy, showed a favorable safety profile in patients with an acute cerebrovascular ischemic event. A numerical reduction of new diffusion-weighted imaging (DWI)-lesions after the revascularization procedure was observed following treatment with 120 mg Revacept compared with placebo [29]. In addition, the combined safety and efficacy endpoint showed a 54 % risk reduction during the study period after treatment with the high dose regimen of Revacept (120 mg) compared with placebo, mainly attributable to numerically fewer bleeding complications [29]. Intensified platelet inhibition has been linked to a higher risk of bleeding and reduced safety and effectiveness. Therefore, the safety profile of Revacept makes it an option for patients who have suffered from an acute ischemic stroke caused by symptomatic carotid artery stenosis. The unique mode of action of Revacept, which inhibits platelet activation via GPVI and potentially improves hemostatic capacity [24], can pave the way for future phase III studies with underlying ruptured plaque embolization pathologies.

Other molecules that affect GPVI signaling are currently in preclinical or early clinical development [30]. One such molecule is ACT017, which is a humanized variant of Fab9O12, a monoclonal mouse antibody fragment that has a strong inhibitory effect and a high affinity for GPVI [31]. The hexa- and decay-peptideTroa6 and Troa10, which are specific GPVI antagonists derived from the C-terminal region of the GPVI-specific agonist trowaglerix, have also demonstrated potent inhibition of collagen-induced platelet aggregation and thrombus formation in animal models without prolonging the bleeding time [32].

3. Role of platelets in tumor metastasis

Growing evidence suggests that platelets play a crucial role in the metastasis of tumors through various mechanisms [12]. Specifically, activated platelets contribute to the development and spread of colorectal cancer by interacting directly and releasing various lipid mediators, proteins, and EVs [33]. Platelets help in (i) the formation of platelet aggregates surrounding tumor cells, which promotes tumor cell survival and protection from immune elimination; (ii) the increase in the adhesion of tumor cells to the endothelium, leading to tumor cell arrest and extravasation; (iii) tumor vascularization; and (iv) the induction of a malignant phenotype in epithelial cancer cells via epithelial-mesenchymal transition (EMT).

The interaction between platelets and cancer cells can result in the formation of mesenchymal-like cancer cells, which have an increased ability to move and promote platelet aggregation [34]. Guillem-Llobat et al. [34] have shown that the inhibition of platelet function by aspirin, DG-041 (an antagonist of the PGE_2 receptor subtype EP3), or ticagrelor (an antagonist of the P2Y12 receptor for ADP) prevents these changes from occurring. These treatments act by blocking direct interactions between cancer cells and platelets and the production of PGE_2 by platelets, which prevents the activation of the PGE_2 receptor subtype EP4 on colorectal cancer cells. EP4 signaling in cancer cells leads to the downregulation of E-cadherin and the induction of its transcriptional repressor Twist [34]. Interestingly, injecting mesenchymal-like HT29 colon cancer cells into the circulation of humanized immunodeficient mice activated their blood platelets, which released higher levels of TXA_2 and PGE_2 in vivo [34]. This phenomenon can contribute to developing prothrombotic properties of cancer cells undergoing EMT. Administering low-dose aspirin, which inhibits platelet activation and the biosynthesis of prostanoids, reduced formation of lung metastases [34].

Platelets can also release 12(S)-hydroxyheptadecatrienoic acid (12-HHT). It is generated from PGH_2 (the product of COX-1) by TXA_2 synthase (TXAS)-dependent and TXAS-independent pathways in vitro and in vivo [35] It has been reported that 12-HHT is an endogenous ligand for BLT2, a low-affinity leukotriene B_4 receptor [36]. Okuno & Yokomizo (2018) revealed that the 12-HHT-BLT2 axis mediates various biological functions, including the epithelial barrier, wound healing, immunosuppression, and lung protection in vivo [37]. Furthermore, 12-HHT-BLT2 signaling is reported to be involved in chemotherapy

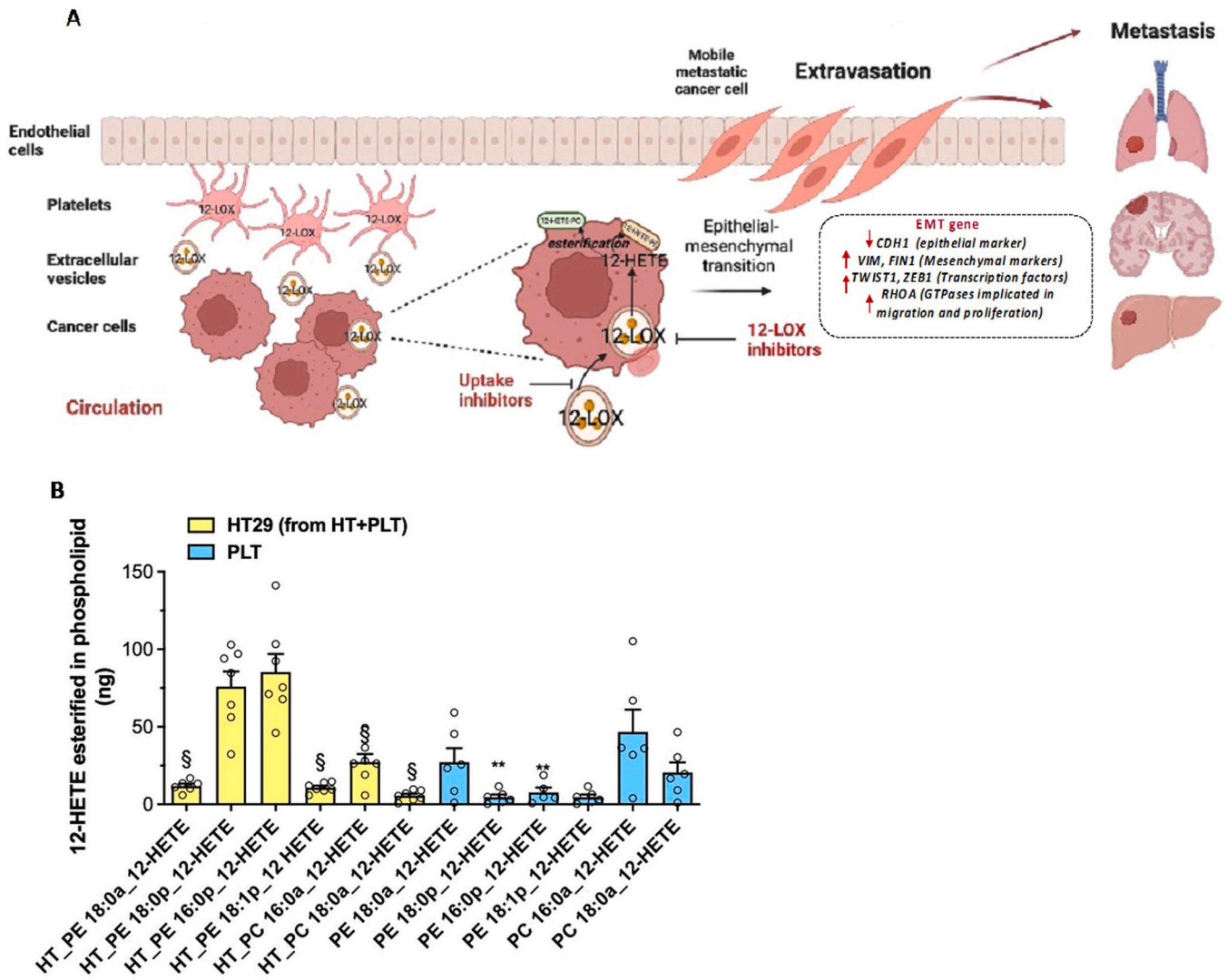

Fig. 3. Platelets can promote EMT gene expression and metastasis by generating 12-HETE via platelet type 12-LOX, which is esterified in membrane phosphatidylethanolamine (PE) and phosphatidylcholine (PC) of colon cancer cells.(A)Platelets release EVs containing 12-LOX; EVs deliver 12-LOX to cancer cells, leading to the generation of 12-HETE that can be esterified in six different species of PE and PC. (B) These 12-HETE-PLs were detected by LC-MS/MS in the cell pellet of colorectal cancer cells cocultured with platelets (in yellow) and in platelets cultured alone (in light blue) but not in colorectal cancer cells cultured alone (not shown). In cancer cells cultured with platelets, six 12-HETE containing PLs, comprising four PEs and two PCs (i.e., PE 16:0p_12-HETE, PE 18:1p_12-HETE, PE 18:0p_12-HETE, PE, and PC 18:0a_12-HETEs, and PC 16:0a_12-HETE) were detected. In cancer cells cultured with platelets, two plasmalogen PEs (PE18:0p_12-HETE and PE16:0p_12-HETE) were the major products and were higher than in platelets cultured alone. Novel strategies to prevent tumor metastasis can involve using inhibitors of 12-LOX and inhibitors of extracellular vesicle uptake [41]. Modified from Contursi et al. J Lipid Res. 2021; 62:100109.

resistance.

Platelets can transfer platelet-type 12-lipoxygenase (LOX) to human colorectal cancer cells via the release of PEVs expressing 12-LOX that are taken up by cancer cells [38]. This enzyme is responsible for converting the fatty acid arachidonic acid (AA) into 12-hydroperoxyeicosatetraenoic acid (12-HPETE), which can then be transformed into 12S-hydroxyeicosatetraenoic acid (HETE), a lipid that plays a role in tumor metastasis [39]. As a result, HT29 colon cancer cells that are unable to produce 12-HETE gain the ability to do so, and 12-HETE is incorporated into four phosphatidylethanolamines (PE) and two phosphatidylcholine (PC) phospholipids [38]. These changes led to the expression of EMT marker genes in colorectal cancer cells; 12-LOX inhibitors prevented the upregulation of EMT marker genes induced by platelets in HT29 cells [38] (Fig. 3). Novel approaches to preventing cancer and metastasis can involve the pharmacological inhibition of 12-LOX [40].

4. Role of PEVs in human diseases

Activated platelets release components from their plasma membrane and cytoplasm through medium-size extracellular vesicles (PEVs) called microparticles (MPs). The main characteristics of PEVs include the inversion of membrane phospholipids, the exposure of phosphatidylserine (PS) on the outer membrane (showing a surface 50–100 times more procoagulant than platelets), and the expression of several integrins [GPIIb/IIIa (CD41/CD61), Ib, Ia/IIa, P-selectin] and enzymes (e.g. matrix metalloproteinases) [42,43]. PEVs can transfer bioactive molecules and are involved in hemostasis, thrombosis, inflammation, tumorigenesis, angiogenesis, and immunity [38,39]. They have shown an enhanced capacity to induce EMT and EndMT genes in cancer and endothelial cells, serving as conveyors of intercellular communication [44]. Proteomics of PEVs obtained from platelets in response to thrombin from obese women vs. non-obese controls identified 214 proteins. However, only 47 proteins showed significant changes between the two groups, with 44 downregulated and three upregulated in

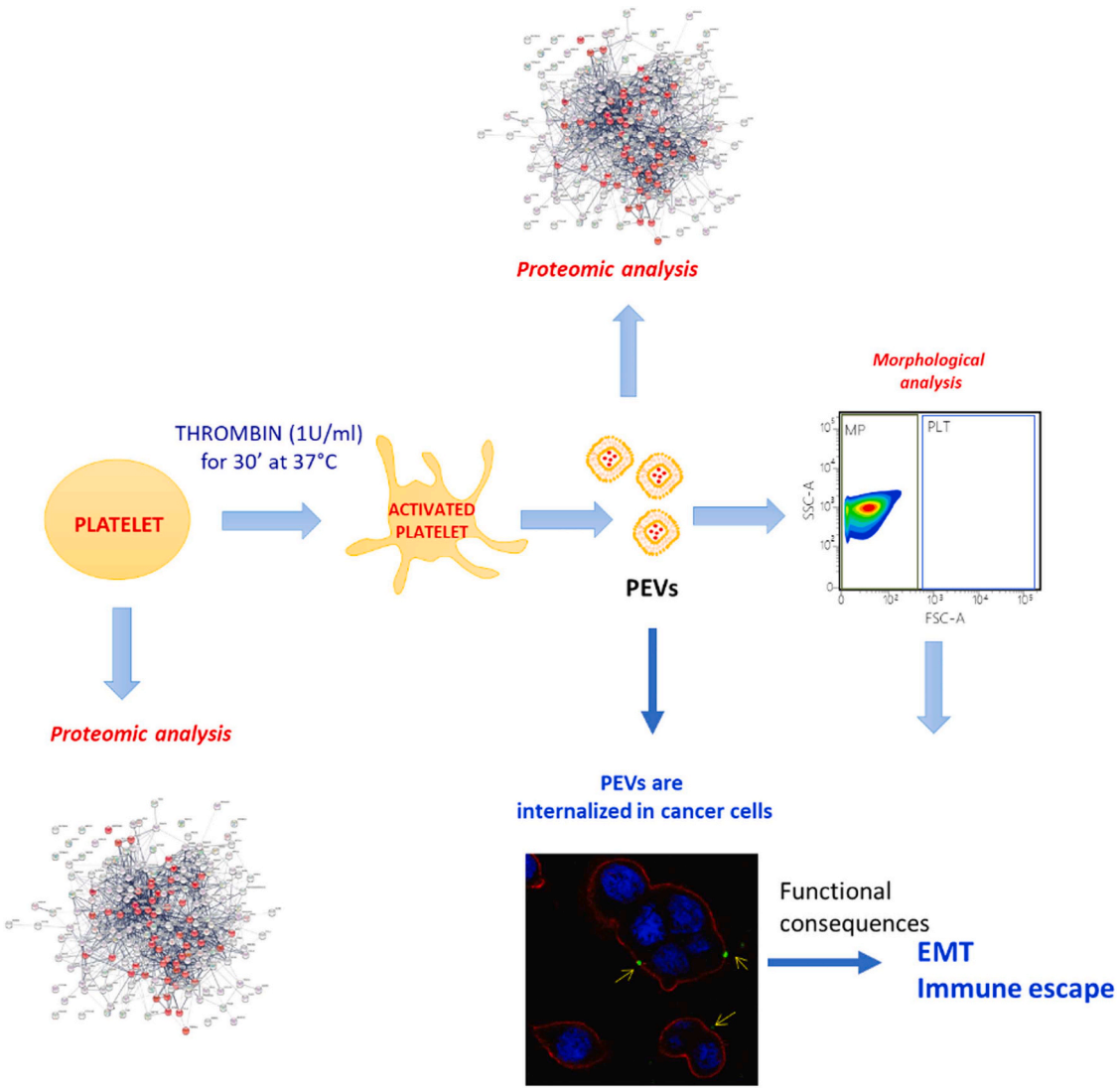

Fig. 4. Platelets and platelet-derived extracellular vesicles (PEVs) are a source of biomarkers for patient clinical condition detection and drug response. Platelets and PEVs are critical in activating cancer cells by transferring their molecule cargo. This phenomenon induces changes in cancer cells, thus promoting metastasis and immune escape.

PEVs of obese individuals compared to non-obese ones [44]. A decrease in the proteins of the α-granular membrane and those involved in mitophagy, and antioxidant defenses was observed in the PEVs of obese individuals. One protein that showed a significant increase in obese PEVs was the pro-platelet basic protein, which has pro-inflammatory and tumorigenic effects. Additionally, higher levels of the coagulation Factor V were detected in the PEVs of obese compared to non-obese individuals [44]. PEVs may contribute to the enhanced risk of thrombosis and multiple malignancies in obesity. PEVs can also influence vascular function by participating in thrombus formation, leukocyte adhesion, and interacting with the vascular endothelium. PEVs can be a source of TXA2 in the pulmonary artery and aorta and contribute to the vasoconstrictor responses to AA and methacholine [45]. Barry et al. [46] have reported that platelet activation can be amplified or modulated by concentrated delivery of AA to adjacent platelets and endothelial cells. PEVs have been shown to cause the increase in monocyte adhesion to endothelial cells in a time- and dose-dependent manner. PEV-induced adhesiveness occurs via the upregulation of monocyte CD11a and CD11b and endothelial cell intracellular adhesion molecule–1 (ICAM-1) [46]. Furthermore, PEVs increase monocyte chemotaxis [47]. PEV effects were mediated by AA of PEV acting via a protein kinase C (PKC) sensitive pathway. These data suggest that concentrated delivery of bioactive lipids in PEVs may influence thrombosis and modulate multicellular interactions relevant to the early stages of atherogenesis [47].

Recently, Contursi et al. [48] characterized the PEVs released by thrombin-stimulated platelets obtained from colorectal cancer (CRC) patients vs. healthy subjects (HS) on the capacity to induce EMT-related genes and COX-2 and the biosynthesis di TXB2 when cocultured with colorectal cancer cell lines in vitro. They showed that PEVs from CRC patients, but not HS, induced EMT marker genes, such as TWIST1 and VIM(vimentin) and *PTGS2* (COX-2), and enhanced the biosynthesis of TXB_2 [48]. The proteomics profile of PEVs released from activated platelets of CRC patients and HS revealed 208 significantly modulated proteins: 119 proteins were decreased while 89 proteins were increased in CRC compared to healthy controls [48]. The most significantly increased proteins were HLA-B class I and PSMD2. The human leukocyte antigen (HLA) system or complex is a group of related proteins encoded by the major histocompatibility complex (MHC) gene. PSMD2 is the proteasome 26 S subunit, non-ATPase 2. These data suggest the role of PEVs as critical modulators of immune response in CRC.

PEVs are known to play a significant role in various cardiovascular diseases, where they impact disease progression and complications. In atherosclerosis, PEVs participate by encapsulating and transporting

molecules such as miR-223, which can affect endothelial apoptosis and permeability and promote leukocyte accumulation [49]. Although PEVs are not found within atherosclerotic plaques, their presence in circulation suggests a potential involvement in the pathogenesis of the disease. Elevated levels of PEVs containing pro-inflammatory molecules are associated with vascular inflammation and myocardial damage in patients with coronary heart disease [50,51]. High levels of circulating PEVs have been detected in patients with an enhanced risk of venous thromboembolism (VTE) due to factor V Leiden and prothrombin G20210A mutation. This suggests a possible contribution of PEVs in the hypercoagulability of mild genetic thrombophilia [52].

5. Platelet omics

Platelets contain many proteins, lipids, and nucleic acids that can be profiled using omics technologies. By analyzing platelet omics data, researchers can identify molecular signatures associated with different disease states, such as cardiovascular disease, cancer, and autoimmune disorders [53–56]. As we move towards a post-omics era, it will become more important to find ways to bring all this information together to create a coherent picture of physiology and disease. This will require a combination of interdisciplinary fields, from omics, statistics, computational biology, and systems science. For example, network analysis and machine learning algorithms can identify key biomarkers and pathways that are dysregulated in disease. Mathematical models can simulate the behavior of complex biological systems and predict how they will respond to perturbations.

There is a wide array of high-throughput cell phenotyping tools available for clinical research that can help enhance our understanding of various diseases and their underlying mechanisms. One such tool is reverse-phase protein arrays [57], which can test hundreds of protein phosphorylation markers in platelet-rich plasma. This tool is particularly useful in monitoring the effectiveness of kinase inhibitors in cancer patients. Other tools can be combined to deeply profile platelet phenotypes. These include Western blot, ELISA, flow cytometry, microscopy, and mass spectrometry [58–61]. Combining these tools can provide a comprehensive understanding of platelet biology and changes associated with disease conditions, which can help to predict susceptibility to disease development and efficacy and toxicity of antiplatelet agents.

6. Conclusions and perspectives

Multi-omics approaches hold great promise in advancing precision medicine and enhancing our comprehension of complex diseases and the potential to transform healthcare. Nevertheless, accomplishing these goals necessitates integrating varied datasets, creating novel analytical tools, and implementing a systems-level approach to address biological issues.

The analysis of platelets and PEV phenotypes associated with disease states is a crucial source of information for understanding a patient's clinical conditions and responses to pharmacological treatments. Assessing PEV count, morphology, and proteomics content can identify early disease development without invasive procedures (Fig. 4). It is essential to recognize the value of this approach in providing critical insights into the patient's health status, but its integration into standard clinical practice requires careful validation.

Platelets and PEVs are critical in activating various vasculature, immunity, tumor microenvironment, and cancer cell types. This knowledge opens new avenues for understanding disease mechanisms and developing novel therapeutic strategies. Inhibiting platelet adhesion and interaction with other cells and using inhibitors of PEV uptake could be potential therapeutic targets. Interestingly, we found that dm-amiloride, which inhibits the Na^+/H^+ exchanger involved in macropinocytosis [62], reduced PEV uptake by colon cancer cells associated with the reduction of the levels of 12-LOX in HT29 cells cocultured with platelets [38]. Thus, macropinocytosis inhibitors [62], which prevent the internalization of PEVs have the potential to represent novel anti-cancer tools.

CRediT authorship contribution statement

Alessandra De Michele: Writing – review & editing. **Sara Di Berardino:** Writing – review & editing, Data curation. **Paola Patrignani:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Stefania Tacconelli:** Writing – review & editing, Funding acquisition, Data curation. **Annalisa Contursi:** Writing – review & editing, Writing – original draft, Funding acquisition.

Data availability

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