Platelet Signaling Pathways and New Inhibitors

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The major physiological role of platelets is in the formation of hemostatic plugs at sites of penetrating vascular injury that serve to limit blood loss. The aberrant intravascular activation of platelets can, if unchecked, lead to thrombotic events that cause myocardial infarction and stroke. Many antiplatelet agents are used clinically to limit platelet activation in patients at risk of arterial thrombotic events. However, their use can be associated with a significant risk of bleeding. An improved understanding of platelet signaling mechanisms should identify safer targets for antiplatelet therapy.

Our understanding of the breadth and complexity of signaling pathways that marshal platelet activation has expanded rapidly over the past decade.[1–4] Recent work published in *Arteriosclerosis, Thrombosis, and Vascular Biology* (*ATVB*) and other journals has provided further insight into the regulation of platelet signaling events and identified new targets against which to develop novel antiplatelet agents.

Purinergic Receptors

One of the cornerstones of current antiplatelet therapy targets ADP-mediated platelet activation and aggregation via the P2Y12 receptor. However, a major challenge of the thienopyridine-based P2Y12 inhibitors, such as clopidogrel and prasugrel, is the occurrence of high on-treatment platelet reactivity, defined as a higher than expected platelet response to agonist.[5] Armstrong et al[6] have recently described an important contribution of platelet turnover to high on-treatment platelet reactivity. In vitro and ex vivo studies demonstrated that the relatively short half-life of clopidogrel and prasugrel was associated with poor inhibition of aggregation of newly formed reticulated platelets upon ADP stimulation.[6] By comparison nonthienopyridine-based P2Y12 inhibitors, such as ticagrelor, maintained a good level of platelet inhibition even when untreated platelets were introduced.[6] However, further studies are required to establish if there is any clinical benefit of nonthienopyridine-based P2Y12 inhibitors in the context of high on-treatment platelet reactivity.

In addition to expressing P2Y12, platelets also express P2Y1, that may regulate initial Ca^{2+} mobilization and shape change. Currently available therapies, however, only target P2Y12 leaving P2Y1-mediated platelet activation intact. The diadenosine tetraphosphate derivative, GLS-409, has recently been developed as an inhibitor of both P2Y1 and P2Y12 (Figure; Table).[14] GLS-409 potently inhibited platelet aggregation and protected against recurrent coronary thrombosis in a canine model.[7] Encouragingly, bleeding was only marginally, and nonsignificantly, prolonged after administration of GLS-409. It remains to be determined if dual P2Y1/P2Y12 antagonists will be used in the clinic in place of the classical unimodal P2Y12 inhibitors.[15]

P2Y12-dependent platelet activation also plays a role in the pathological response to sepsis. Wild-type mice treated with clopidogrel and P2Y12-deficient mice had reduced platelet aggregation, platelet–leukocyte aggregate formation, and lung injury in a sepsis-induced inflammation model.[16] Interestingly, no protection was observed in P2Y1-deficient mice, which indicates that platelet activation in response to septic challenge is primarily driven by the activation of P2Y12.[16] These findings are consistent with a study showing reduced neutrophil recruitment and lung injury in ticagrelor-treated mice subject to abdominal sepsis. Together, these studies suggest that platelets contribute to sepsis-induced lung injury by enhancing the recruitment of neutrophils.[17]

Protease-Activated Receptors

Thrombin is an extremely potent agonist activating human platelets by proteolytic cleavage of PAR (protease-activated receptor)1 and PAR4, which are high- and low-affinity receptors, respectively (Figure).[18] Interestingly, PAR1 activation leads to rapid and transient signaling, whereas PAR4 activation leads to prolonged signaling that is required for stable thrombus formation.[19] Two PAR1-specific antagonists, vorapaxar and atopaxar, have been developed.[20,21] Recent studies analyzed the effect of triple antiplatelet therapy on cardiovascular death by adding vorapaxar to standard antiplatelet therapy (aspirin and clopidogrel). Patients receiving triple therapy had reduced cardiovascular death but also had an increase in intracranial hemorrhage.[20,22] Nevertheless, vorapaxar has been approved for use in patients with a history of cardiovascular disease with no history of a stroke, but it needs to be used with another platelet inhibitor.

Although effective in inhibiting PAR1 activation, a limitation of vorapaxar is its long half-life and slow off-rate. This limitation has driven the search for alternative PAR1 antagonists with improved pharmacokinetic profiles. One such agent is the cell-permeable peptide–based inhibitor PZ-128, which binds to the intracellular C terminus of PAR1 and blocks downstream G-protein signaling (Figure;

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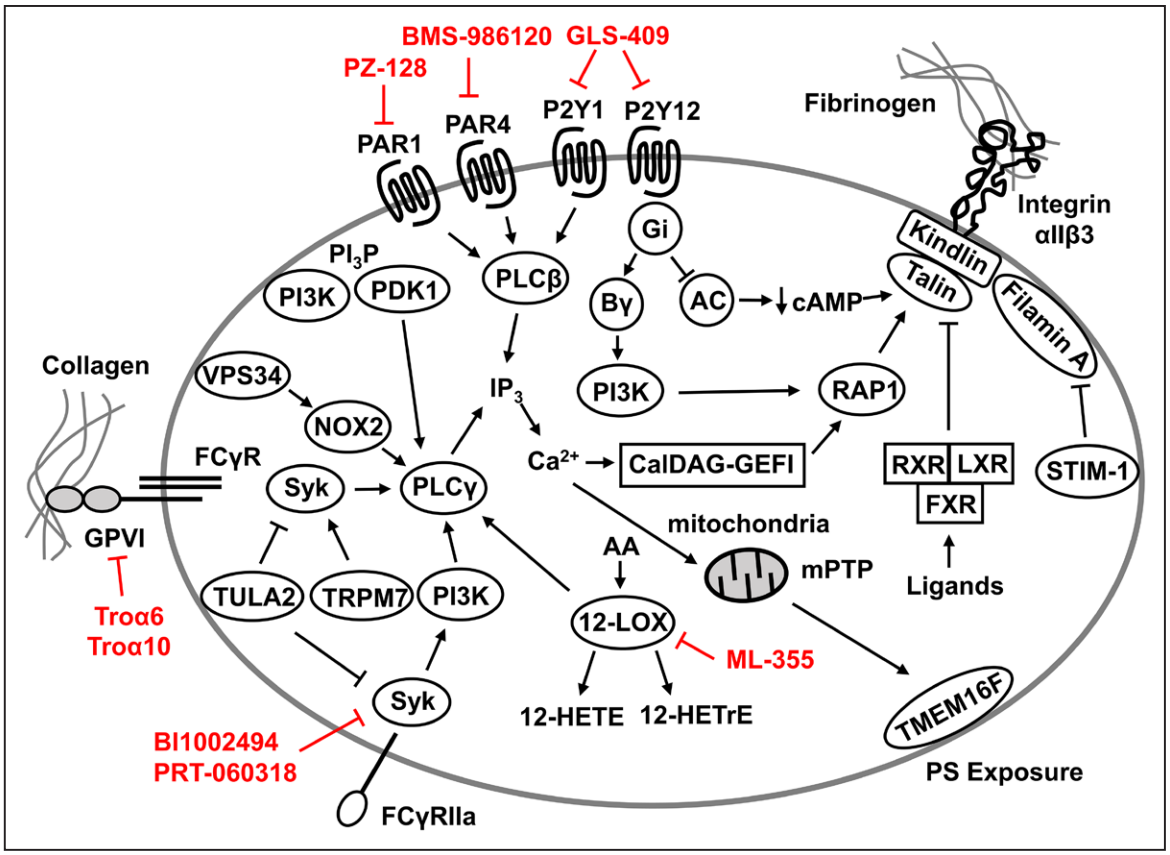

Figure. Platelet signaling pathways. Overview of some of the intracellular platelet signaling pathways required for platelet activation. Novel antiplatelet agents in various stages of preclinical and clinical development are annotated. 12-HETE indicates 12(S)-hydroxy-5,8,10,14-eicosatetraenoic; 12-HETrE, 12S-hydroxy-8Z,10E,14Z-eicosatrienoic acid; 12-LOX, 12-lipoxygenase; AC, adenylyl cyclase; FCγR, Fcγ receptor; FXR, farnesoid X receptor; GPVI, glycoprotein VI; IP3, inositol 1,4,5-triphosphate; LXR, liver X receptor; mPTP, mitochondrial membrane transition pore; NOX2, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2; PAR, protease-activated receptors; PDK1, phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; PI3P, phosphatidylinositol 3-phosphate; PLC, phospholipase C; RAP1, Ras-related protein 1; RXR, retinoid X receptor; STIM-1, stromal interaction molecule 1; Syk, spleen tyrosine kinase; TMEM16F, transmembrane protein 16F; Tro, trowaglerix; TRPM7, transient receptor potential melastatin-like 7; TULA2, T-cell ubiquitin ligand-2; and VPS34, vacuolar protein sorting 34.

Table).[23] In preclinical studies, PZ-128 inhibited platelet aggregation and thrombus formation.[23] In a recent phase I study published in *ATVB,* intravenous administration of PZ-128 resulted in inhibition of platelet activation.[8] These studies indicate that PZ-128 is a rapid, specific, and reversible inhibitor that can be used for short-term inhibition of platelet activation.

To date, the majority of studies have focused on PAR1. Recently, there has been a shift to understanding the contribution of PAR4 to platelet activation. For instance, a recent study identified 7 PAR4 variants in a cohort of 236 cardiac patients. One of these PAR4 variants, Tyr157Cys, was predicted to lead to significant structural changes and partial loss of function.[24] Indeed, platelets isolated from patients with the Tyr157Cys PAR4 variant demonstrated impaired aggregation in response to a PAR4-specific agonist and thrombin. Interestingly, the Tyr157Cys PAR4 variant localized primarily to the perinuclear space likely limiting PAR4 availability at the plasma membrane.[24] In addition, a racially dimorphic gain-of-function PAR4 variant Thr120 has been described.[25,26] This PAR4 variant is associated with increased PAR4 reactivity and calcium flux in platelets.[26] These clinical studies highlight the potential for genetic diversity in PAR4 in the human population.

PAR4 is also being considered as an alternative target for the development of antiplatelet agents. Indeed, a first-in-class small molecule PAR4 inhibitor, BMS-986120, has been developed (Table).[27] In a nonhuman primate model, BMS-986120 potently inhibited arterial thrombosis with only a minor prolongation of bleeding time.[27] In a recent phase I clinical trial published in *ATVB*, BMS-986120 was well tolerated and inhibited both platelet aggregation and thrombosis ex vivo.[9] It will be interesting to see if these PAR1 and PAR4 inhibitors can overcome some of the limitations of current therapies and join our arsenal of antiplatelet agents.

Collagen–GPVI–Fcγ Receptor

The platelet-specific collagen receptor GP (glycoprotein) VI, in complex with the FcγR (Fcγ receptor), plays a critical role in both platelet adhesion and activation (Figure).[28] GPVI inhibitors have been developed as possible antiplatelet agents, including an inhibitory anti-GPVI antibodies and a soluble GPVI-Fc fusion protein called Revacept.[29] In addition,

Table. Novel Antiplatelet Agents Under Development

Target	Drug	Ref
P2Y1/P2Y12	GLS-409	7
PAR1	PZ-128	8
PAR4	BMS-986120	9
GPVI	Troα6, Troα10	10
Syk	BI1002494, PRT-060318	11,12
12-LOX	ML-355	13

12-LOX indicates 12-lipoxygenase; GP, glycoprotein; PAR, protease-activated receptor; Syk, spleen tyrosine kinase; and Tro, trowaglerix.

many C-type lectin-like proteins have been identified in snake venoms that modulate GPVI activity. This includes the GPVI-specific agonist Tro (trowaglerix), which was purified from *Tropidolaemus wagleri* venom and potently induces platelet aggregation.[30] In contrast to full-length Tro protein, however, hexa and decapeptides (Troα6 and Troα10) derived from the C-terminal region of Tro function as potent inhibitors of collagen-induced platelet aggregation (Figure; Table).[10] Interestingly, docking studies indicate that the decapeptide binds to a cleft between the D1 and D2 domains but does not disrupt GPVI–collagen binding.[31] Both the hexa and decapeptide also strongly inhibited thrombus formation in mesenteric and carotid ferric chloride models without prolonging bleeding after tail transection.[10]

GPVI inhibitors are also effective in protecting against cardiac ischemia–reperfusion injury. For instance, both anti-GPVI monoclonal antibody infusion, which induces shedding of GPVI from platelets, and Revacept administration reduced infarct size in a murine cardiac ischemia–reperfusion model.[32,33] These studies indicate that GPVI-dependent activation of platelets contributes to cardiac injury and that anti-GPVI therapies may be useful adjuvants in patients with myocardial infarction undergoing revascularization.

Collagen-mediated activation of GPVI can also be negatively regulated by endogenous mechanisms. LAIR1 (leukocyte-associated immunoglobulin-like receptor-1) is expressed in megakaryocytes and negatively regulates GPVI signaling by binding to collagen (Figure).[34,35] Smith et al[36] generated megakaryocyte-specific LAIR1 knockouts to study the effect of LAIR1 on megakaryocyte and platelet function. LAIR1 deficiency did not affect megakaryocyte development. Consistent with serving as a negative regulator of GPVI, collagen-related peptide stimulation of GPVI on platelets from megakaryocyte-specific LAIR1 knockouts caused an enhancement in aggregation that was accompanied by increased phosphorylation of FcγR, Src, and Syk (spleen tyrosine kinase).[36] It should be noted that LAIR1 expression has not been detected in platelets.[37] It is proposed that LAIR1 impairs the capacity to activate GPVI in megakaryocytes and that this may, in some way, be transmitted to and persist in formed platelets, although the mechanism behind this remains to be elucidated.[36]

GPVI-mediated platelet activation can also be interrupted at a point further downstream in the intracellular signaling pathway. TULA2 (T-cell ubiquitin ligand-2) is a histidine phosphatase that binds and dephosphorylates Syk (Figure).[38] Studies with TULA2 knockout mice showed that TULA2 limits Syk phosphorylation in collagen-related peptide-stimulated platelets leading to a restriction of GPVI-mediated activation.[39] In addition, another study found that dephosphorylation of Syk by TULA2 may also serve to limit activation downstream of FcγRIIA.[40] Mice with reduced TULA2 and expressing human FcγRIIA had enhanced Syk and PLCγ2 (phospholipase Cγ2) phosphorylation after stimulation with an FcγRIIA-specific agonist when compared with wild-type controls.[40] Finally, in a model of anti-GPIX antibody–mediated heparin-induced thrombocytopenia, the absence of TULA2 expression markedly worsened thrombocytopenia and shortened bleeding times consistent with increased activation of TULA2-deficient platelets.[40]

Activation of Syk downstream of the tyrosine kinase–coupled receptors GPVI, C-type lectin-like receptor 2, and FcγRIIA is critical for platelet activation. Despite early studies suggesting a central role of Syk in regulating platelet activation, deletion of Syk was not associated with increased bleeding.[41] Recent work by van Eeuwijk et al[11] revealed a relatively mild hemostatic defect in platelet-specific Syk knockout mice. However, these mice were strongly protected in a model of arterial thrombosis.[11] Similar findings were observed when a selective Syk inhibitor, BI1002494, was administered to wild-type mice (Table).[11] Syk inhibitors have also been investigated as a possible therapy for heparin-induced thrombocytopenia. For instance, administration of PRT-060318 protected against spontaneous formation of thrombi in the pulmonary vasculature and preserved platelet counts in a humanized FcγRIIA and platelet factor 4 mouse model (Table).[12]

Calcium- and DAG-Regulated Guanine Nucleotide Exchange Factor-1

The calcium- and DAG-regulated guanine nucleotide exchange factor-1 (CalDAG-GEFI) is a critical activator of the small GTPases of the Rap1 (Ras-related protein 1) subfamily.[42] GTP loading of Rap1 by CalDAG-GEFI induces platelet adhesion, in part, through activation of integrin αIIbβ3.[43,44] Rap1 activation is also required for many other platelet processes, including granule secretion, thromboxane A2 generation, spreading, and clot retraction.[42] Platelets from CalDAG-GEFI knockout mice revealed a blunted aggregatory response after stimulation with a range of physiological agonists.[45] These results indicated that CalDAG-GEFI–mediated activation of Rap1 provides a common signaling pathway for platelet activation downstream of numerous receptors, including the PARs, P2Y12, and GPVI (Figure).[45] In addition, CalDAG-GEFI knockout mice had reduced thrombosis in a variety of experimental models.[45–47] However, loss of CalDAG-GEFI also resulted in a pronounced bleeding phenotype after tail transection indicating an important function in primary hemostasis. In recent work published in *ATVB*, Piatt et al[48] generated CalDAG-GEFI transgenic mice that express ≈10% of wild-type levels of CalDAG-GEFI. Platelets from these mice had an impaired response to PAR4-, GPVI-, and P2Y12/P2Y1-specific agonists, but it was less severe than that observed in platelets from CalDAG-GEFI knockout mice.[48] Importantly, CalDAG-GEFI low mice had a similar level of

protection against thrombosis compared with CalDAG-GEFI knockouts but had a much milder bleeding diathesis.[48]

Platelets have also been shown to play a role in the development of atherosclerotic plaques in mouse models.[49–51] These studies highlight the potential of antiplatelet therapies to limit atherogenesis.[52] Recent studies have shown that P2Y12-deficient, Apolipoprotein E double-deficient mice had smaller lesions than controls.[53] Similarly, LDL (low-density lipoprotein) receptor–deficient mice reconstituted with CalDAG-GEFI–deficient bone marrow had significantly smaller lesions than controls.[54] These studies indicate that platelet activation contributes to atherogenesis in mice and an additional benefit of antiplatelet therapy in humans maybe a reduction in lesion progression.

Nuclear Receptor Subfamily 1 Members

A recent study found that 2 members of the nuclear receptor subfamily 1 of transcription factors, FXR (farnesoid X receptor) and LXR (liver X receptor), and the associated RXR (retinoid X receptor), are expressed in human platelets (Figure).[55–57] RXR forms heterodimeric complexes with both FXR and LXR.[57] Recent studies revealed a surprising nongenomic function for these transcription factors in the regulation of platelet activation. Stimulation of human platelets with FXR and LXR ligands strongly inhibited both platelet aggregation and granule release.[55,56] The capacity of FXR ligands to induce accumulation of cGMP likely accounts for the observed impairment in platelet activation.[56] Interestingly, treatment of platelets with FXR and LXR ligands impaired integrin αIIbβ3 activation and outside-in signaling.[57] Moreover, infusion of either FXR or LXR ligands into mice inhibited platelet accumulation at sites of vascular injury.[55,56] Stimulation of platelets with RXR ligands also results in inhibition of platelet aggregation, granule secretion, and integrin αIIbβ3 outside-in signaling.[57] Further work is required to determine if FXR, LXR, and RXR ligands could serve as viable antiplatelet agents.

Phosphoinositides and Platelet Kinases

Activation of platelet receptors initiates downstream signaling events that includes generation of the small molecule signaling intermediate phosphatidylinositol 3-phosphate through the action of phosphatidylinositol 3-kinases (Figure). PDK1 (phosphoinositide-dependent protein kinase 1) is activated through binding of phosphatidylinositol 3-phosphate enabling phosphorylation of downstream signaling targets that include members of the Akt family of kinases.[58] Platelet-specific PDK1 deficiency is associated with reduced platelet activation because of impaired integrin αIIbβ3-mediated outside-in signaling.[59] A small molecule PDK1 inhibitor has also been found to inhibit activation of human platelets.[60] Another study showed that PDK1-deficient platelets had reduced aggregation and granule release after stimulation with the GPVI-specific agonist collagen-related peptide.[61] PDK1 was also found to be essential for the collagen-induced increase in intracellular [Ca^{2+}] in part through a Rac1-PLCγ2–dependent pathway.[61] As in an earlier report,[59] platelet-specific PDK1 knockout mice exhibit reduced arterial thrombosis and were also protected from ischemic stroke.[61]

Additional platelet kinases have been found to regulate the metabolism of phosphoinositides in platelets, including the TRPM7 (transient receptor potential melastatin-like 7) channel (Figure). TRMP7 is a constitutively active divalent cation-selective channel that regulates intracellular [Ca^{2+}] and [Mg^{2+}] but also functions as a serine/threonine kinase.[62] Studies with TRPM7-deficient megakaryocytes and megakaryocytes expressing kinase-dead TRPM7 revealed an important role of the cation channel, but not kinase, function in platelet biogenesis.[63] TRPM7 deficiency in megakaryocytes was associated with impaired proplatelet formation and abnormal megakaryocyte microtubule assembly that led to macrothrombocytopenia.[63] In a subsequent study, Gotru et al[64] explored the kinase function of TRPM7 in platelets. Although platelet biogenesis was normal in mice expressing a kinase-dead TRPM7, loss of kinase activity markedly reduced arterial thrombosis.[64] Interrogation of signaling downstream of GPVI and C-type lectin-like receptor-2–mediated receptor activation in platelets containing kinase-dead TRPM7 revealed a common deficit in Syk, linker for activation of T cells and PLCγ2 phosphorylation. Consistent with an impaired activation of PLCγ2, platelets containing a kinase-dead TRPM7 produced significantly less inositol 1,4,5-trisphosphate. Additionally, TRPM7 activity is dependent on the PLCγ substrate phosphatidylinositol 4,5-bisphosphate.[65]

Platelet Oxidases

LOXs (lipoxygenases) are a family of enzymes that catalyze the oxygenation of polyunsaturated fatty acids that leads to the generation of a variety of active signaling molecules. 12-LOX, named for the ability of this family member to oxidize arachidonic acid at carbon 12, is expressed in both megakaryocytes and platelets.[66] Oxidation of arachidonic acid by 12-LOX results in the formation of 12(S)-hydroperoxyicosa-5,8,10,14-tetraenoic acid that is reduced to 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid by glutathione peroxidase (Figure). Oxidation of dihomo-γ-linolenic acid by 12-LOX also generates 12(S)-hydroxy-8Z,10E,14Z-eicosatrienoic acid (Figure). Administration of dihomo-γ-linolenic acid or 12(S)-hydroxy-8Z,10E,14Z-eicosatrienoic acid to mice resulted in a 12-LOX–dependent inhibition of platelet activation and thrombosis.[67,68] There is a growing body of evidence supporting the involvement of 12-LOX in platelet activation with inhibition or gene deletion effectively abrogating platelet aggregation in response to stimulation through PAR1, PAR4, GPVI, and FcγRIIa.[13,69,70] A recent study showed that a novel and selective 12-LOX small molecule inhibitor, ML-355 (Table), reduced platelet activation and arterial thrombosis in mice with a minimal effect on hemostasis.[13]

NOX2 (NADPH [nicotinamide adenine dinucleotide phosphate] oxidase 2) is a membrane-bound enzyme that catalyzes the generation of superoxide from NADPH. Studies of patients with Chronic Granulomatous Disease, which is caused by a genetic deficiency for NOX2, indicate that NOX2 is the primary producer of superoxide in stimulated platelets.[71] Reactive oxygen species (ROS) generated from superoxide can markedly increase platelet reactivity.[72] ROS contribute significantly to thrombosis with deletion of the antioxidant enzyme glutathione peroxidase-3 resulting in increased

platelet activation and thrombosis.[73] Consistent with these findings, NOX2-deficient platelets had a selective defect in response to collagen-related peptide stimulation accompanied by a reduced capacity for ROS production and impaired phosphorylation of PLCγ2 and Syk.[74] In addition, NOX2-deficient mice had reduced thrombosis in the laser injury model but had no impairment of hemostasis.[74] However, a conflicting study has reported that NOX1, and not NOX2, is the primary generator of platelet ROS in response to GPVI activation.[75]

A recent study suggested that platelet NOX activity is regulated by the class III phosphatidylinositol 3-kinase VPS34 (vacuolar protein sorting 34).[76] VPS34-dependent generation of phosphatidylinositol 3-phosphate has previously been found to regulate NOX-dependent ROS generation in leukocytes by binding to the gp40phox subunit.[77,78] Studies with platelets from platelet-specific VPS34 knockout mice demonstrated that VPS34 is also essential for NOX-dependent ROS generation.[76] Importantly, VPS34-deficient platelets had a reduced capacity to form the active NOX complex at the plasma membrane leading to impaired ROS generation.[76] This impairment led to reduced platelet aggregation and reduced thrombus formation.[76] However, an independent study found that VPS34 deficiency in megakaryocytes disrupts platelet biogenesis and resulted in lower platelet counts and abnormal granule formation. These changes may also contribute to the reduced thrombosis.[79]

Filamin A

The actin-binding protein filamin A functions as an important regulator of platelet activation and shape change. Filamin A mediates platelet activation through interaction with a variety of binding partners, including GPIbα, GPVI, and Syk.[80] Studies have suggested that filamin A-GPIbα binding facilitates activation of integrin αIIbβ3.[81] Structural studies, however, indicate that filamin A may also directly interact with and regulate the activation of integrin αIIbβ3 (Figure).[82] Interestingly, platelets isolated from a patient with a filamin A gain-of-function mutation that potentiates its interaction with integrin αIIbβ3 had increased ADP-induced platelet aggregation, dense granule release, and integrin αIIbβ3 activation.[83] Despite enhanced recruitment of talin to integrin αIIbβ3 in ADP-stimulated platelets from the patient, Rap1 activation was unchanged supporting a GPIbα-independent mechanism.[83] The retinue of confirmed filamin A binding partners increased recently with the identification of stromal interaction molecule 1, which is a critical regulator of store-operated calcium entry.[84] In contrast to other filamin A interactions, binding of stromal interaction molecule 1 likely inhibits platelet activation by limiting store-operated calcium entry.[84]

Phosphatidylserine Exposure

Strong agonist-mediated activation of platelets leads to exposure of phosphatidylserine on the outer leaflet of the plasma membrane in a subpopulation of platelets. Exposure of phosphatidylserine confers procoagulant activity on this subpopulation by increasing the assembly of coagulation protease/cofactor complexes, such as prothrombinase complex, which enhances thrombin generation. Agonist-mediated phosphatidylserine exposure requires intracellular and mitochondrial accumulation of calcium, which facilitates cyclophilin D–mediated formation of the mitochondrial transition pore and the disruption of the inner mitochondrial membrane.[85,86] Activation of apoptotic pathways, involving Bcl-2-associated X protein/Bcl-2 homologous antagonist killer mediated activation of caspase-9, can also lead to platelet phosphatidylserine exposure.[87] It was previously unclear if phosphatidylserine exposure driven by apoptosis caused inner mitochondrial membrane disruption. Recent work published in *ATVB* demonstrated that both agonist and apoptosis-initiated phosphatidylserine exposure involve disruption of the inner, but not the outer mitochondrial membrane.[88] Furthermore, the authors showed that activation of caspase-9 is required for inner mitochondrial membrane disruption and phosphatidylserine exposure after initiation of apoptosis.[88]

Exposure of phosphatidylserine also requires activation of phospholipid scramblases that transfer phosphatidylserine from the inner leaflet of the membrane to the outer plasma membrane surface. Scott syndrome is a bleeding disorder that is caused by defective phosphatidylserine exposure on platelets. Scott syndrome patients have a truncated version of the critical calcium-sensitive phospholipid scramblase TMEM16F (transmembrane protein 16F) present in the plasma membrane of platelets and other cells (Figure).[89,90] Platelets from TMEM16F knockout mice showed impaired exposure of phosphatidylserine after stimulation with calcium ionophore or a combination of collagen and thrombin.[90,91] This evidence strongly supports the involvement of TMEM16F in phosphatidylserine exposure on platelets. Consistent with the importance of TMEM16F-mediated phosphatidylserine exposure TMEM16F gene-specific knockout mice were also protected against arterial thrombosis.[90] The role of TMEM16F on platelets has been further explored using platelet-specific TMEM16F knockout mice.[92] The thromboprotection observed in platelet-specific TMEM16F largely phenocopies that seen in global TMEM16F knockouts.[92] Although TMEM16F is also expressed in the endothelium, these findings indicate that TMEM16F-mediated phosphatidylserine exposure on the platelet surface is sufficient to support the formation of procoagulant platelets. An additional study showed that phosphatidylserine exposure on platelets stimulated with convulxin/thrombin was dependent on TMEM16F, whereas phosphatidylserine exposure on platelets stimulated with collagen/thrombin was dependent on 2 pathways one of which involved mitochondrial depolarization mediated by TMEM16F.[93]

Summary

Recent studies have revealed a series of novel mechanisms that either positively or negatively regulate signaling events downstream of receptor-mediated platelet activation. In several cases, it seems that disruption of these pathways can selectively inhibit thrombosis while leaving essential hemostatic processes largely intact. These pathways may be of considerable interest as potential targets for development of a new generation of antiplatelet agents. Other work has focused on the development of novel antiplatelet agents that inhibit established targets such as P2Y12, PAR1, and GPVI. These

agents may overcome some of the limitations of established therapies. It remains to be determined if these novel agents will find clinical use.

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Disclosures

None.

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