



Platelet Shape Changes and Cytoskeleton Dynamics as Novel Therapeutic Targets for Anti-Thrombotic Drugs

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Abstract

Platelets play an essential role in hemostasis through aggregation and adhesion to vascular injury sites but their unnecessary activation can often lead to thrombotic diseases. Upon exposure to physical or biochemical stimuli, remarkable platelet shape changes precede aggregation or adhesion. Platelet shape changes facilitate the formation and adhesion of platelet aggregates, but are readily reversible in contrast to the irrevocable characteristics of aggregation and adhesion. In this dynamic phenomenon, complex molecular signaling pathways and a host of diverse cytoskeleton proteins are involved. Platelet shape change is easily primed by diverse pro-thrombotic xenobiotics and stimuli, and its inhibition can modulate thrombosis, which can ultimately contribute to the development or prevention of thrombotic diseases. In this review, we discussed the current knowledge on the mechanisms of platelet shape change and also pathological implications and therapeutic opportunities for regulating the related cytoskeleton dynamics.

Key Words: Platelet shape changes, Cytoskeleton dynamics, Thrombosis, Aggregation, Adhesion

INTRODUCTION

Platelets play a key role in hemostasis and thrombosis through aggregation, adhesion and procoagulant activation. However, their unnecessary activation is often linked to life-threatening thrombotic diseases including venous and arterial thrombosis, embolism and stroke (Coller, 2013). Numerous drugs modulating platelet activation have been developed and are hugely successful in the market for the prevention of thrombotic diseases and thrombotic complications in diverse cardiovascular diseases, exemplified by aspirin, Plavix (Bristol-Myers Squibb, NY, USA; clopidogrel), Efient (Lilly, Indianapolis, IN, USA; prasugrel) and Pletal (Otsuka, Tokyo, Japan; cilostazol) (Collins and Hollidge, 2003; Rao *et al.*, 2006). In contrast to aggregation or adhesion, functions that are well-illustrated in the thrombus formation, role of the morphological changes of platelet, i.e., platelet shape change, is relatively less established. Platelets dynamically undergo remarkable morphological alterations when exposed to physical or biochemical stimuli such as high shear or endogenous thrombot-

ic agonists (Hartwig, 2013). Platelet shape changes frequently occur even when the cells are exposed to minimal degrees of perturbation that cannot result in aggregation or adhesion, such as sub-threshold concentrations of platelet agonists or shear stress. In this review, we discuss the current knowledge concerning the mechanisms of platelet shape changes, its toxicological and pathological implications and also the therapeutic opportunities for regulating the associated cytoskeleton dynamics.

PROCESS OF THE SHAPE CHANGES AND CYTOSKELETON DYNAMICS IN PLATELETS

Resting platelets are small discoid shapes around 1.5-3 μm in size. The cytoskeleton of platelets mainly consists of actin, tubulin, spectrin and filamin. Spectrin, which has a two-dimensional web-like structure, laminates the cytoplasmic side of the plasma membrane, both ends of which are connected to actin filaments. Actin exists in polymer where filamin and α -actinin

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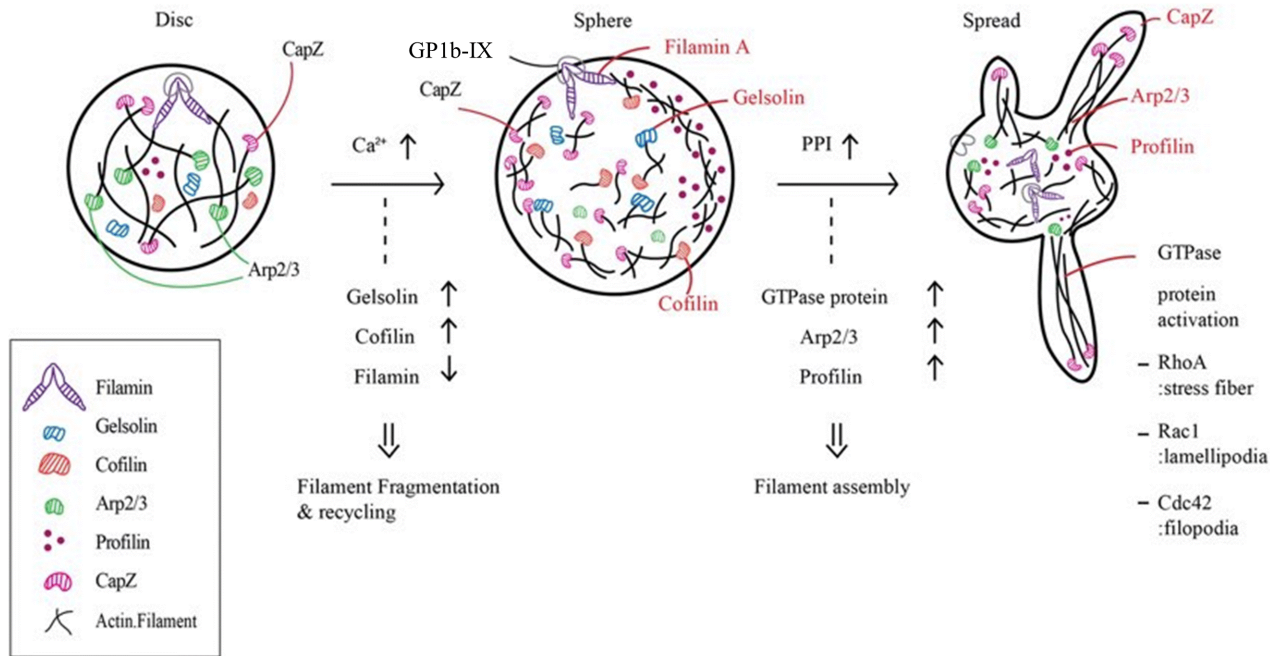


Fig. 1. Alteration of cytoskeletal proteins during platelet shape change. PPI: polyphosphoinositides.

act as cross-linkers with approximately 1:10 ratios (Smyth *et al.*, 2010). In resting platelets, microtubule coils, polymers of $\alpha\beta$ -tubulin, are located beneath the plasma membrane, maintaining the discoid shape (Smyth *et al.*, 2010). Upon exposure to various biological stimuli and agonists, shape change occurs via dismantling and reorganization of the cytoskeletons. There are generally two steps in agonist-induced platelet shape change. First, platelets become spherical as actin filaments are fragmented, which is followed by the dismantling and release of spectrin networks composed of filamin A, GP1b/IX and spectrin. Second, the membranes of the cells protrude due to actin filament assembly, which manifests as lamellipodia and filopodia (Sandmann and Koster, 2016). Filopodia protrudes from the core actin fibers while lamellipodia grows from the short actin filament templates (Hartwig, 1992).

Each step in platelet shape change involves the participation of a variety of actin filament-related proteins that are highly concentrated in platelets (Fig. 1). In resting human platelets, the actin filaments from the core to the membrane skeleton are tightly bound to the plasma membrane by GP1b/IX-filamin A complexes (Kovacsovics and Hartwig, 1996). When an agonist binds to its receptor, the activation of phospholipase C γ induces inositol-1,4,5-triphosphate (IP3) generation and increases intracellular calcium up to 5-10 μ M (Berridge *et al.*, 2000; Zheng *et al.*, 2015). Gelsolin is an actin binding protein that severs and caps the barbed-end actin filaments to prevent actin monomer exchange upon intracellular calcium increase in the initial step. Cofilin also binds to actin and contributes to the disassembly of actin filaments and the subsequent release of actin monomers. The actin cross-linking complex, GP1b/IX-filamin, translocates from the plasma membrane to the cytoskeleton during this step. Therefore, severance of actin filaments unlocks the membrane skeleton and induces cell deformation with marginal short actin filaments. This leads to the

swelling of the membrane, probably in collaboration with the open canalicular system (OCS), which is considered a reservoir of membrane substances like GP1b/IX and some portion of integrin $\alpha_{IIb}\beta_3$ (Smyth *et al.*, 2010) for platelet shape change owing to its invaginated membranous structure connected to the surface. Polyphosphoinositides (PPI) such as PI-3,4-P $_2$, PI-4,5-P $_2$, and PI-3,4,5-P $_3$ are known to deactivate the capping proteins in actin filaments and expose barbed-end nucleation sites. Also, the Arp2/3 complex stimulates the assembly of actin filaments. There are many actin sequestering proteins such as profilin and thymosin- β 4 in platelets that carry an actin monomer to the barbed end and protrude filopodia. Profilin binds to both formin and actin monomers to increase the addition of actin monomers to the barbed end of the filament.

Meanwhile, intracellular organelles and granules as well as fragmented microtubules are concentrated around the cell center. Finally, the capZ protein recaps the barbed filament ends to complete the assembly and these actin cytoskeletons can be used for cellular contraction, resulting in the final activated platelet shape: spiny spheres with long, thin filopodia extending several micrometers from the platelets.

PLATELET SHAPE CHANGES PRECEDING AGGREGATION

Platelet shape change is regarded as a prerequisite for platelet aggregation. Shape changes of platelets facilitate their adhesion to the site of vascular injury and cohesion with other platelets or erythrocytes, ultimately contributing to the formation of stable aggregates (Jackson, 2007). The degree of platelet aggregation can be easily measured by light transmission aggregometry (LTA) since platelet aggregates consume and precipitate floating platelets, thereby, reducing

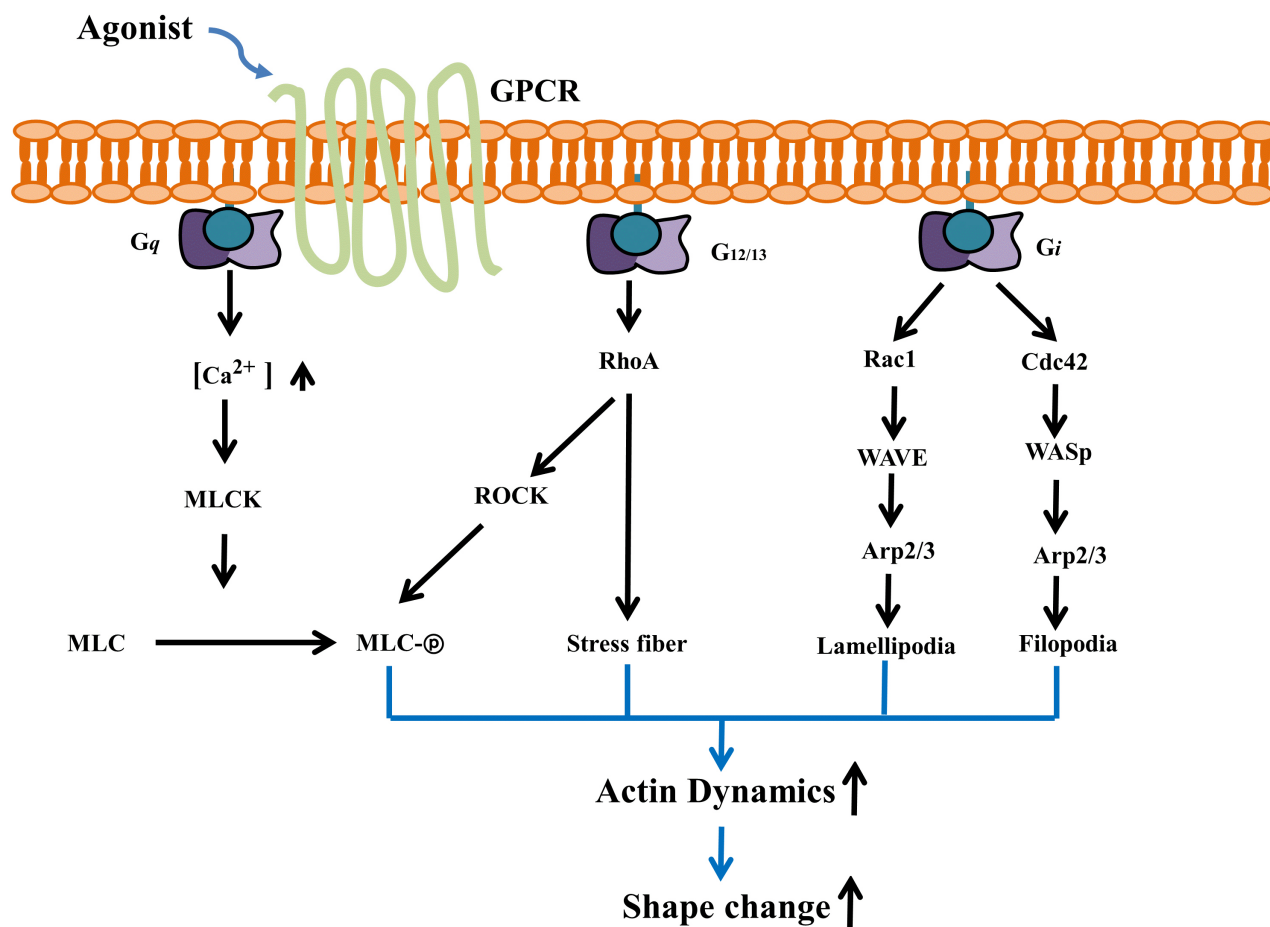


Fig. 2. Signal transduction pathway underlying platelet shape change. WASp: Wiskott-Aldrich syndrome proteins, WAVE: WASP-family verprolin-homologous, MLCK: MLC kinase, ROCK: Rho associated coiled-coil-forming kinase.

the turbidity of platelet suspension. In LTA, upon the addition of agonists or exposure to stimuli, light transmission through platelet suspension decreases initially and then increases thereafter. This initial phenomenon observed in LTA, i.e., the decrease of light transmission, is thought to be attributable to platelet shape changes (Maurer-Spurej and Devine, 2001), while some ascribe it to micro-aggregation (Born *et al.*, 1978). It is unclear why platelets change their shapes prior to aggregation, but one explanation is that platelet shape change reduces electrostatic repulsion between two negatively surface-charged platelets.

Many studies have been conducted to understand the mechanisms and interaction of shape change and aggregation. G_{α_q} /PLC β and PLC γ pathways are important for platelet aggregations activated by thrombin, ADP and collagen, mediated through G-protein coupled receptors, PAR1, PAR4, P2Y1 and GPVI receptor, respectively. The activation of the G_{α_q} /PLC β pathway results in platelet shape changes through activating IP $_3$ -calcium signaling (Offermanns *et al.*, 1997). In $G_{\alpha_q}^{-/-}$ mice, however, while thrombin and collagen induce typical platelet shape changes, aggregation is not triggered, suggesting that the morphological change does not initiate platelet aggregation and the pathways for the two phenomena do

not fully coincide. There are some examples of platelet aggregation without preceding shape changes. Again, in the $G_{\alpha_q}^{-/-}$ mice model, platelets did not undergo platelet shape change or aggregation by ADP at the effective dose (10 μ M). Interestingly, high concentrations (~100 μ M) of ADP prompted platelet aggregation, presumably due to the G_{i2} -mediated adenylyl cyclase inhibition (Ohlmann *et al.*, 2000). This suggests that the shape changes are accomplished by the specific interaction between G proteins, G_{α_q} and G_{i2} , which is distinct from that for the platelet aggregation.

PLATELET SHAPE CHANGES OBSERVED DURING ADHESION

Another distinctive pattern of platelet shape change, spreading to the adhesion molecules, is different from that observed prior to platelet aggregation. While rolling ball-shaped platelets or hemisphere-shaped platelets with filopodia observed before aggregation are reversible, platelets that exhibit a spreading shape, which is flat with a large adhesive surface (platelet diameter >2.5 μ m), are terminal. In this case, the cells make more lamellipodia than filopodia, which consists of ac-

tin networks cross-linked orthogonally by filamin A (Hartwig *et al.*, 1999). Platelet spreading can further recruit other platelets or neutrophils to increase procoagulant activity. Kuwahara *et al.* (2002) tried to explain the molecular mechanism occurring during these adhesion steps by testing the effects of the PI3K inhibitor wortmannin, $\alpha_{IIb}\beta_3$ blocker c7E3 and an intracellular calcium chelator, dimethyl BAPTA. They reported that while GP1b-vWF (von Willebrand factor) interaction is essential to initiate the platelet shape change with filopodia formation, both $\alpha_{IIb}\beta_3$ activation and intracellular calcium increase are necessary for platelet spreading.

SIGNAL TRANSDUCTION FOR PLATELET SHAPE CHANGES

Rho GTPases, Formins, WASp and WAVE

In the platelet, a family of small (~21 kDa) signaling G proteins, Rho GTPases, RhoA, Cdc42 and Rac1 play key roles in the dynamics of the actin cytoskeleton, platelet aggregation, secretion, and spreading as well as in thrombus formation. It has been reported that Rho GTPase-related actin remodeling, filopodia and lamellipodia formation are mediated through the activation of formins and the Arp2/3 complex (Fig. 2) (Heasman and Ridley, 2008). Cdc42 can induce Arp2/3-mediated filopodia formation through the activation of WASp (Wiskott-Aldrich syndrome proteins) and neuronal N-WASp (Rohatgi *et al.*, 1999). Similarly, Rac1-enhanced lamellipodia formation is related to Arp2/3 activation by the WAVE (WASP-family verprolin-homologous) complex (Miki *et al.*, 1998). Not only WASp and WAVE, but formin proteins (formins) have also emerged as vital effectors of Rho GTPases (Kuhn and Geyer, 2014). Among the 15 formins in mammalian cells, mDia and Daam are shown to be activated upon interaction with Rho GTPases, which causes the central domain for actin polymerization to become exposed and elongated into actin filaments. The Arp2/3 complex provides a platform for new actin polymerization through activating the barbed-end where formin serves as an elongation factor.

Myosin light chain phosphorylation, Calcium, small GTPase proteins

Phosphorylation of the myosin light chain and moesin, an actomyosin filament/plasma membrane cross-linker molecule, is essential for the initiation of shape change in platelets (Daniel *et al.*, 1984; Klages *et al.*, 1999; Retzer and Essler, 2000). Phosphorylation of adhesion site proteins such as myosin facilitates their binding to actin filaments, ultimately generating contractile force on the actin framework. Previously, it has been reported that both calcium dependent and independent mechanisms result in myosin light chain phosphorylation (Paul *et al.*, 1999). Agonists induce intracellular calcium influx through the $G_{\alpha q}/PLC\gamma-IP_3$ pathway and increased calcium can cooperate with calmodulin to activate MLC kinase (MLCK) (Fig. 2). $PLC\gamma$ activation is mediated by the binding of Syk kinase to the ITAM (immunoreceptor tyrosine-based activation motif) of the GPVI and $Fc\gamma RIIa$ receptors and the association of adaptor proteins, LAT and SLP-76. Shape changes also occur in the absence of calcium peaks. Calcium-chelated cells were observed to elongate and form abnormal filopodia (Hartwig *et al.*, 1995). Even without calcium peaks, calcium-independent RhoA can activate Rho associated coiled-coil-forming kinase

(ROCK), which induces shape changes directly by phosphorylating MLC or indirectly through inhibiting myosin phosphatase (PP1M).

ACTIVATORS OF PLATELET SHAPE CHANGES

Physiological stimuli

Prior to aggregation, many agonists such as ADP, platelet-activating factor (PAF), 5-hydroxytryptamine (5-HT, serotonin), thrombin and collagen induce remarkable morphological changes (Maurer-Spurej and Devine, 2001). Previously, Sanderson *et al.* (1996) determined the time and dose dependency forward/side-scatter profile of platelets following exposure to platelet activating agonists using a flow cytometer. Depending on the agonist exposed, the onset of platelet forward/side scatter changes was different. For instance, ADP, PAF and 5-HT initiated platelet shape changes rapidly and strongly, whereas other agonists like A23187 and collagen induced strong but slower responses. Interestingly, although each agonist could induce both platelet shape change and aggregation, it is not always mediated via a single receptor activation. For instance, a selective P2Y purinoceptor antagonist ARL66096 was effective against aggregation but had no effect on ADP-induced platelet shape changes, indicating that two different receptors and distinct signaling pathways could be involved in agonist-stimulated platelet activation. Epinephrine is well known for its potentiating effects on platelet shape change, aggregation and granule secretion induced by ADP or thrombin (Haaland and Holmsen, 2011). Epinephrine alone can also induce shape changes and platelet aggregation but with a much slower time-frame and to a lesser degree compared with that by ADP. It has been shown that the epinephrine-induced shape change results in pseudopods without changing the main cell-body size (Milton and Frojmovic, 1984).

In addition to platelet agonists, shape changes can occur without apparent aggregation by various stimuli. Angiotensin II, an important hormone for hypertension and vascular remodeling (Crowley *et al.*, 2006), causes a significant increase in median platelet volume (MPV) in platelet rich plasma *in vitro* and potentiates platelet shape changes induced by ADP and serotonin, indicating that angiotensin II and the related signal transduction pathway may be linked with shape changes (Jagroop and Mikhailidis, 2000). This was further corroborated by the attenuation of angiotensin II-induced MPV increase and U46619 (a thromboxane A2 analogue)-induced platelet shape changes by losartan (a selective angiotensin II antagonist) at its therapeutic concentrations for antihypertensive effects. Immunological activation is also associated with platelet shape changes. Oponized zymosan A, an immunological stimulus, can induce platelet shape changes that accompany reactive oxygen species (ROS) generation and mitochondrial membrane hyperpolarization (Matarrese *et al.*, 2009). Immunoglobulin G (IgG) antiplatelet antibody can also induce morphological changes, as can be observed under microscopy, as a disk-to-sphere transformation associated with the formation of bulbous, short surface projections or pseudopodia (Colman *et al.*, 1983).

Pathologically high shear stress, as observed in various disease states such as atherosclerosis, cancer or stenotic area, can induce platelet shape changes (Nesbitt *et al.*, 2009). Shear rate can increase significantly up to 10,000 s^{-1} in car-

diovascular diseases. The normal shear rate is around 200 s^{-1} . Platelets respond to blood vessel injury and start rolling through the interaction between the GP1b/IX complex and the von Willebrand factor (vWF). The shear stress induces the rolling platelets which tightly adhere to the vessel wall by interactions between integrin $\alpha_{IIb}\beta_3$ and surface matrices.

Exogenous stimuli

Many environmental toxicants and xenobiotics can induce platelet activation and cardiovascular diseases (Savouret *et al.*, 2003; Abrescia and Golino, 2005; Kim *et al.*, 2014). Methylmercury, a cardiovascular toxicant that can induce platelet aggregation and prime granular release by activating the prostaglandin synthesis pathway, also primes platelet shape changes (Macfarlane, 1981; Jennrich, 2013). Xenobiotics that can affect actin dynamics also induce morphological changes in platelets. Cytochalasin D, which interferes with actin polymerization, can induce platelet shape changes (Fox and Phillips, 1981). The microtubule disassembling drug, vincristine, induces the transformation of discoid platelets to rounded cells. Nanoparticles have gained a lot of attention due to their effects on platelets. Platelets tend to be more susceptible to nanoparticles compared to other cells with larger sizes. A variety of nanoparticles can induce cytoskeletal alterations and the aggregation of platelets. Carbon nanoparticles (Radomski *et al.*, 2005), silver nanoparticles (Jun *et al.*, 2011) and more recently, polystyrene latex nanoparticles (PLNPs) (Smyth *et al.*, 2015) have been reported to induce platelet shape changes along with aggregation.

In cold environments, most eukaryotic cells either assume discoid shapes or do not change but platelets undergo deformation (Winokur and Hartwig, 1995). Chilling platelets (4 degree) *in vitro* results in an increase in volume and the formation of loose marginal microtubules as well as pseudopods. The shape changes are observed even at 0 degree or in ice water but are reversible. They can also be mediated by myosin phosphorylation, cytosolic calcium increase and actin filament elongation (Van Poucke *et al.*, 2014).

PATHOLOGICAL IMPLICATION OF PLATELET SHAPE CHANGES AND CYTOSKELETON DYNAMICS

Platelets with morphological changes become hyper-reactive to agonists (Maurer-Spurej and Devine, 2001) and xenobiotics that can prime platelet shape changes can potentiate the platelet aggregation responses and ultimately thrombosis. Hyper-reactivity or prothrombotic traits in morphologically changed platelets can be explained by granule secretion. Platelet shape change can trigger granule secretion through actin-myosin contraction. After platelet shape change is completed, granules and organelles are centralized in the cells (Furman *et al.*, 1993). Although it is not clearly defined whether granule secretion occurs in platelets through OCS or plasma membrane fusion, the movement of granules following platelet shape change is considered a hallmark of granule secretion. A couple of Rab proteins including Rab27 (Tolmachova *et al.*, 2007), SNAREs (Ren *et al.*, 2007) and Munc (Schraw *et al.*, 2003) proteins are known to induce the process in platelets. In the mice cremaster muscle arteriole thrombosis model, platelets are integrated and thrombi are formed upon laser-induced injury. Interestingly, platelets are disaggregated and

easily washed off by blood flow with mild injury, whereas tight thrombi can form in accordance with P-selectin expression after severe injury (Stalker TJ *et al.*, 2014). This suggests that stable thrombus formation is related to the irreversible platelet shape change and subsequent α -granule secretion.

Platelet shape change and concomitant actin filament turnover can release activating molecules that are bound to the cytoskeleton as inactive forms. Integrin $\alpha_{IIb}\beta_3$ is retained by the actin cytoskeleton in resting platelets but is released to the cell surface during platelet activation. It provides a receptor site for fibrinogen as well as vWF and promotes further adhesion of platelets. Therefore, it could increase pathologic thrombosis if platelet $\alpha_{IIb}\beta_3$ is expressed through thrombogenic agent-driven shape change. Down-regulation of $\alpha_{IIb}\beta_3$ as witnessed in some inherent diseases like Glanzmann's thrombasthenia (Nurden, 2006) or by antiplatelet agents or anticancer drugs can lead to bleeding, namely compromised thrombosis.

In order to understand the mechanism underlying the turnover of these functional receptors, cytoskeleton related regulation has to be discussed. OCS might play a key role in platelet shape change and spreading, as the OCS occupies 50% of the total plasma membrane (White and Clawson, 1980). Patients with the Budd-Chiari syndrome, a condition caused by the formation of a blood clot within the hepatic veins, display dilated OCS in their platelets (Dayal *et al.*, 1995). In line with this, it has been reported that integrin $\alpha_{IIb}\beta_3$ is highly expressed in the membrane of the OCS (Escobar *et al.*, 1989). Recently, Mountford *et al.* (2015) showed that PI3KC2 α knockdown significantly increases shear stress-induced platelet adhesion to fibrinogen and the collagen surface. Interestingly, the PI3KC2 α disrupted platelets display enlarged OCS, suggesting that the pathologic platelet shape change may enhance thromboembolism (Mountford *et al.*, 2015).

THERAPEUTIC OPPORTUNITY FOR REGULATING CYTOSKELETON DYNAMICS AND PLATELET SHAPE CHANGES

The polymerization of actin filaments and tubulin has been explored as therapeutic targets for a variety of diseases including cancer, cardiovascular diseases and thrombosis over the years. Since the morphological changes of platelets are closely associated with the pathophysiologic states, modulation of the platelet cytoskeleton can be a good therapeutic approach to improve disease states. Semaphorins were originally identified to induce growth cone collapse in developing neurites. Many studies have revealed the involvement of semaphorins in angiogenesis, immune cell regulation, and organogenesis. Among 25 Semaphorin family members, Semaphorin 3A (Sema3A) was found to be produced and secreted by endothelial cells. To investigate its role in blood, Kashiwagi *et al.* (2005) applied Sema3A to platelets in various conditions and demonstrated the inhibitory effects of Sema3A in platelet aggregation, $\alpha_{IIb}\beta_3$ expression, granular secretion, adhesion, and spreading through its receptors on the cells. In platelets, sema3A inhibited agonist-induced Rac1 activation and phosphorylation of actin-depolymerizing protein, cofilin, and indeed, it reduced F-actin contents. Interestingly, they could not detect any effect on the major platelet inhibitory pathways including cAMP and cGMP contents or alterations in the intracellular Ca^{2+} concentration. Therefore, sema3A mediated

platelet inhibition appears to be related to the modulation of actin polymerization and morphological changes. The authors proposed that sema3A supports the maintenance of circulating platelets in a quiescent state and that the sema3A signaling pathway could be a novel target for antiplatelet therapy.

Similarly, 3 semaphorins and 8 plexins, receptors of semaphorin, have been detected in platelets (Wannemacher *et al.*, 2011). Their roles in platelet function and in cell to cell interaction remain to be elucidated, although studies on sema4D^{-/-} mice have demonstrated that sema4D plays a positive role in thrombus formation *in vivo* and platelet aggregation *in vitro* (Zhu *et al.*, 2007). Importantly, sema4D^{-/-} mice did not show gross bleeding and therefore, the authors suggested that sema4D could be a promising drug target to limit excessive platelet activation without disrupting normal platelet function.

Recently, Estevez *et al.* (2013) investigated the role of LIM kinase 1 (LIMK1) in platelet activation and thrombosis. LIMK1 is one of the effectors of Rac1, which facilitates actin polymerization, mediated through the phosphorylation and deactivation of cofilin. In the FeCl₃-induced arterial thrombosis model as well as the tail bleeding time analysis model, the LIMK1-null mice revealed increased thrombotic occlusion time, but no difference in bleeding time compared with the wild-type. GP1bIX signaling-induced actin polymerization was indeed reduced in platelets from KO mice and more importantly, cytosolic phospholipase A₂ (cPLA₂)-dependent thromboxane A₂ (TXA₂) generation was inhibited.

It should be clarified further whether the rearrangement of modified actin directly affects platelet function. Many attempts have also been made to answer the question by using inhibitors of actin polymerization such as cytochalasins (Lefebvre *et al.*, 1993; Torti *et al.*, 1996; Natarajan *et al.*, 2000). The results are contradicting, however, depending on the concentration of drugs used. Cytochalasin treatment inhibited α_{IIb}β₃ activation at high concentrations, but enhanced platelet activation at low concentrations (Bennett *et al.*, 1999). Nevertheless, it has been reported that talin is essential for integrin activation in platelets by linking the integrin cytoplasmic tail to actin filaments, suggesting that the dynamics of actin filaments by agonists can be important for platelet activation (Tadokoro *et al.*, 2003).

The generation of platelet microparticles (PMP) could be another important result of platelet cytoskeleton rearrangement and shape change. PMPs are found in both normal and disease states. PMPs are also proposed to play active roles in many diseases including rheumatoid arthritis (Knijff-Dutmer *et al.*, 2002), breast cancer (Janowska-Wieczorek *et al.*, 2006), and cardiovascular diseases (Nomura *et al.*, 2003). A recent study on the protein compositions of PMPs using a proteomics approach has revealed that the characteristics of PMPs are determined by the activating agonist, suggesting that PMPs are generated in specific pathophysiological states (Milioli *et al.*, 2015).

Investigating platelet cytoskeleton regulation also represents an opportunity for the development of novel therapeutic targets. Gelsolin has been recently suggested as one of the targets (Liu *et al.*, 2013), because platelet gelsolin level is positively correlated with cardiovascular diseases (CVD). The Xiongshao capsule, a Chinese herbal medicine used to improve blood circulation, reduced platelet gelsolin and platelet activation markers, similar to the antiplatelet drug aspirin (Liu *et al.*, 2013).

Previous study has shown that filamin A (FlnA) null platelets are rapidly removed from the circulation (Nakamura *et al.*, 2011; Thon and Italiano, 2012). FlnA-null megakaryocytes (MKs) did not show many differences in the expression of vWF receptor components such as GP1bα, ADAM17, metalloproteinase-9 (MMP-9) compared with wild-type MKs. However, the number of MKs was increased by 2.5-5 fold in the KO. The authors suggested that this is because of the fast removal of large and fragile platelets containing GP1bα degradation products, increased ADAM17 and MMP-9 (Jurak Begonja *et al.*, 2011). Together with another report (Kanaji *et al.*, 2012), the researchers have clarified that FlnA and GP1bα play crucial roles in determining platelet size during thrombopoiesis.

CONCLUSIONS

Platelet shape changes have many important implications in pathophysiology and toxicology, therefore, understanding the underlying mechanism and the interaction with xenobiotics and thrombotic activators will illustrate the pathophysiology of thrombotic diseases and provide important insights into molecular drug targets. In the same vein, modulation of the targets related to platelet shape changes would provide a novel therapeutic opportunity that can satisfy unmet medical needs in thrombotic diseases and prevent toxicity from prothrombotic xenobiotics.

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