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REVIEW ARTICLE

Platelet GPIb-IX-V-dependent signaling

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Summary. Although the signaling pathways related to GPIb-IX-V have not been fully elucidated, an accumulating body of evidence suggests that phospholipase C (PLC)γ2 activation, subsequent Ca^{++} release and oscillations constitute an essential signal transduction pathway related to GPIb-IX-V. Src family kinases are required for PLCγ2 activation, while FcRγ-chain/FcγRIIA may be dispensable for PLCγ2 activation. Although PI-3K serves to potentiate various signaling events culminating in $\alpha_{IIb}\beta_3$ activation, PI-3K activity may be dispensable for Src-PLCγ2 activation in GPIb-IX-V-mediated signaling. Glycosphingolipid-enriched microdomains (GEMs) appear to provide platforms for the signal transduction pathway related to GIb-IX-V, as the interaction between GPIb-IX-V and Src or PLCγ2 tyrosine phosphorylation occurs exclusively in GEMs.

Keywords: Ca^{++} mobilization, GPIb-IX-V, Platelets, PLCγ2, tyrosine kinases.

Introduction

Platelets play an important role in the physiological process of hemostasis and are also closely involved in pathologic thrombus formation. At sites of vessel injury, platelets first adhere to various components of the subendothelial matrices (SEM) through the interaction between adhesive receptors on platelet membranes and SEM elements. The collagen receptors on platelet membranes, integrin $\alpha_2\beta_1$ and glycoprotein VI (GPVI), interact with collagen, one of the major compoments of SEM, exposed at sites of endothelial cell damage. Collagen also interacts with plasma von Willebrand factor (VWF), which then gains the capacity to bind the glycoprotein GPIb-IX-V complex on platelet membranes [1]. It is also likely that other components in SEM, such as laminin and vitronectin, contribute to platelet adhesion under certain conditions. Adhered platelets are activated by intracellular signaling pathways elicited by receptor-ligand interactions, and resultant activation of integrin $\alpha_{IIb}\beta_3$ on platelet membranes lead to platelet aggregation by its interaction with VWF [2,3] or with fibrinogen [4]. Although it has been long recognized that the interaction between collagen receptors and collagen can elicit intracellular activation signals that finally culminate in integrin $\alpha_{IIb}\beta_3$ activation [5], the role of GPIb-IX-V for intracellular signaling and integrin $\alpha_{IIb}\beta_3$ activation has remained controversial for a relatively long time. This is because of the fact that platelets fixed with paraformaldehyde can form platelet aggregates when mixed with VWF and VWF-modulating agents such as ristocetin or botrocetin [6], and that the rapid on/off rate between GPIb-IX-V and VWF may not allow enough time for initiation of efficient activation signaling [4,7]. Thus, it was assumed that the GPIb-IX-V/VWF interaction only provides physical force which fixes platelets to SEM, whereby allowing enough time for the interaction of collagen in SEM and the collagen receptors to elicit intracellular activation signals. A series of recent reports have corrected this concept, and now it is evident that GPIb-IX-V mediates intracellular signaling which leads to full activation and aggregate formation of platelets with integrin $\alpha_{IIb}\beta_3$ activation.

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Secondary mediators and calcium mobilization

The intracellular signals or secondary mediators reported first include thromboxane A_2 (TXA_2), protein kinase C (PKC), and Ca^{++} mobilization [8]. Cyclooxygenase inhibitors which block conversion of arachidonic acid into TXA_2 impairs a wide variety of platelet reactions elicited by the GPIb-IX-V/VWF interaction, and the production of TXB_2, a stable metabolite of TXA_2, upon GPIb-IX-V stimulation has been documented in a few reports [9]. The activation of p38MAPK and $cPLA_2$ which lead to TXA_2 production has been also documented upon GPIb-IX-V/VWF interaction [10]. However, a number of events including shear-dependent platelet aggregation and tyrosine phosphorylation of several proteins occur irrespective of TXA_2 production [11–13]. These findings suggest that GPIb-IX-V-mediated platelet activation involves TXA_2-dependent and TXA_2-independent signaling pathways, and collectively it may be concluded that it does not constitute the essential signal transduction pathway related to GPIb. It is most likely that the TXA_2-dependent pathway only serves to amplify platelet responses in a manner similar to those observed with other agonists of platelet activation. The involvement of PKC in

GPIb-IX-V-mediated platelet activation has been suggested by a few reports [8,14]. However, PKC inhibitors only have partial effects on platelet activation assessed with various markers [15]. VWF-induced tyrosine phosphorylation of Pyk2 which has high homology with focal adhesion kinase (FAK) was totally independent of PKC [16]. Thus, it appears that PKC like TXA_2 lies downstream of a certain essential signal transduction pathway related to the GPIb-IX-V complex, and that it serves as an amplifier of GPIb-induced platelet activation.

Ca^{++} mobilization has been a controversial issue with GPIb-IX-V-related platelet activation. Earliest evidence was reported in 1991, which observed an increase in intracellular Ca^{++} in platelets treated with VWF and ristocetin [8]. In subsequent years, a number of studies supported intracellular Ca^{++} mobilization does occur upon GPIb-IX-V/VWF interaction. A considerable number of studies have suggested that intracellular Ca^{++} mobilization mediated by GPIb-IX-V is attributed to Ca^{++} influx rather than to Ca^{++} release from intracellular Ca^{++} stores [2,11,17]. Some reported on Ca^{++} release from intracellular Ca^{++} stores, which suggest phospholipase C (PLC) activation [18]. The others failed to detect any Ca^{++} mobilization [19]. Attempts to directly measure inositol trisphosphate, a PLC product, produced contradictory results [9, 20, 21].

Whether or not these discrepancies could be ascribed to the different techniques or agents used in these studies, it could be concluded that GPIb-IX-V/VWF interaction would induce only a weak level of Ca^{++} mobilization, and if it indeed did activate PLC with inositol trisphosphate production and Ca^{++} release from intracellular Ca^{++} stores, it should be at a level far less than those of G protein-coupled receptors or collagen. Ca^{++} release from intracellular Ca^{++} induced by the GPIb-IX-V/VWF interaction was finally established by microscopic analysis of single platelet Ca^{++} oscillation profiles which clearly shows that at least a portion of Ca^{++} mobilization mediated by GPIb-IX-V is attributed to intracellular Ca^{++} release [21,22]. This premise has been further substantiated by a recent paper which demonstrated Ca^{++} release induced by dimeric VWF A1 domain in platelets from human GPIb-transgenic mice [23].

Ca^{++} release from intracellular Ca^{++} storage sites is mediated by inositol trisphosphate, a product of PLC. While agonists such as ADP and TXA_2 which bind to seven-transmembrane receptors activate members of the PLCβ subfamily, there has been an accumulating body of evidence to suggest that PLCγ2 instead of PLCβ is activated in GPIb-IX-V-related signaling. Shape change on VWF-coated surfaces occurs normally with $G\alpha_q$-deficient mice, excluding a role of PLCβ in this process [21]. In a wide variety of cell types, PLCγ2 activity is regulated by its association with tyrosine kinases and subsequent tyrosine phosphorylation of PLCγ2 [24]. The GPIb-IX-V/VWF interaction in platelets indeed induces a considerable level of PLCγ2 tyrosine phosphorylation [13,19–21]. Furthermore, Ca^{++} mobilization mediated by GPIb-IX-V is significantly reduced in mice lacking PLCγ2 [21]. However, a residual level of Ca^{++} mobilization in PLCγ2 (−/−) knockout mice suggests that other isoforms of PLC may also be involved. In this context, PLCγ1 has been reported to play a role in GPVI-activated PLCγ2 (−/−) knockout platelets [25]. It is also of interest that inositol trisphosphate production and Ca^{++} mobilization in GPIb-mediated platelets activation is only minimal, in great contrast to considerable levels of PLCγ2 tyrosine phosphorylation, which presumably represents its activity. In this respect, it has been recently reported that tyrosine phosphorylation sites of PLCγ2 induced by GPVI is distinct from that of PLCγ2 induced by GPIb [26]; virtually equal levels of PLCγ2 tyrosine phosphorylation between GPIb-mediated or GPVI-mediated platelet activation reported in a few papers may be attributed to the use of anti-phosphotyrosine antibodies that cannot differentiate specific phosphotyrosine residues.

However limited it may be, PLCγ activation, subsequent Ca^{++} release and oscillation constitute an essential signal transduction pathway related to GPIb-IX-V, as platelet responses to VWF including filopodia formation are almost completely abrogated in PLCγ2 −/− platelets or chelation of intracellular Ca^{++} by BAPTA [21].

Src family tyrosine kinases and signaling molecules related to tyrosine phosphorylation

The studies on Ca^{++} mobilization and PLCγ2 aforementioned strongly suggest that signaling events related to tyrosine phosphorylation are involved in GPIb-IX-V-mediated platelet activation. Initial reports suggesting for the role of signaling molecules related to tyrosine phosphorylation date back to 1994 and 1995 when cytoskeletal association of Src and the appearance of multiple tyrosine-phosphorylated proteins were observed in GPIb-IX-V-mediated platelet activation [11,27,28]. Later, Syk, another tyrosine kinase, and shc, an adaptor protein, were reported to be tyrosine phosphorylated, and a tyrosine kinase activity, although not identified, associated with the GPIb-IX-V complex upon VWF stimulation [12]. In 1999, it was reported that a snake venom, alboaggregin A, which presumably interacted with the GPIb-IX-V complex induced tyrosine phosphorylation of FcRγ-chain, Syk activation, PLCγ2 tyrosine phosphorylation, and complex formation between GPIb and two Src family tyrosine kinases, Lyn and Fyn [29]. This report was a great surprise to the investigators involved in the GPIb-IX-V-mediated signaling pathways, as the proposed model of signal transduction was exactly the same as that of the collagen receptor, GPVI. GPVI-mediated platelet activation involves the sequential activation of signaling molecules, Src family kinases, Lyn and Fyn, FcRγ-chain, Syk, and PLCγ2 [15,30]. However, it was later found that alboaggregin A also interacts with GPVI, and the signal transduction pathway characteristic of the GPIb-IX-V remained to be determined [31].

In 2001, using the combination of VWF and a VWF modulator, botrocetin, which is accepted to react with GPIb-IX-V but not with GPVI, it was found that GPIb-IX-V-mediated platelet activation induces tyrosine phosphorylation

of FcRγ-chain, Syk, LAT and PLCγ2 [20]. Src kinase inhibition markedly suppressed these events, and Src kinases, Src and Lyn, formed a complex with FcRγ-chain and Syk upon GPIb-IX-V/VWF interaction, suggesting an important role of Src kinases in these processes [32]. It was also reported that FcγRIIA, another ITAM-containing molecule, undergoes tyrosine phosphorylation upon platelet activation induced by the addition of VWF and ristocetin, followed by Syk and PLCγ2 activation [33]. A selective Src kinase inhibitor PP1 severely abrogated these events. One of the most proximal signaling molecules downstream of GPIb-IX-V is suggested to be Src [32]. p85 subunit of PI-3K constitutively associates with GPIb-IX-V, and this binding is not affected by PI-3K inhibitors [34]. Upon platelet activation with VWF/GPIb-IX-V interaction, Src with its SH3 domain binds GPIb-associated p85, the regulatory subunit of PI-3K [32]. The role of Src kinases and its downstream signaling molecule, PLCγ2 was also confirmed with a number of platelet responses, including spreading and Ca^{++} mobilization on VWF-coated surfaces [13,19,21,35]. While there has been an accumulating body of evidence in addition to the studies described above to suggest that tyrosine kinases, Syk, and Src family kinases, and PLCγ2 are involved in GPIb-IX-V-mediated platelet activation, there remained some room for criticism that VWF modulators such as botrocetin or ristocetin might interact with certain membrane molecules or that VWF through its C1 domain might interact with $\alpha_{IIb}\beta_3$ and the outside-in signals elicited by $\alpha_{IIb}\beta_3$ might confound the analysis of GPIb-IX-V-mediated signaling. A most recent paper utilizing dimeric A1 domains of VWF and human GPIbα transgenic mice has revealed that GPI-IX-V itself can indeed signal to activate $\alpha_{IIb}\beta_3$ through sequential actions of Src kinases and Ca^{++} oscillation, a marker of PLC activation [23]. Although most studies to date support Src kinase-dependent signaling in platelet activation induced by VWF/GPIb-IX-V interaction, Src kinase-independent platelet activation has been reported with platelet spreading on surfaces coated with echicetin, a GPIb-interacting snake venom [36]. Whether echicetin binding to GPIb elicits intracellular activation signals distinct from that of VWF/GPIb-IX-V interaction with a rapid on-off rate awaits to be elucidated.

On the whole, it can be concluded that there are striking similarities in signal transduction pathways between GPIb-IX-V and GPVI except for several points; Src and Lyn appear to be recruited to GPIb-IX-V upon platelet activation, while Lyn and Fyn constitutively associate with GPVI. GPVI activation induces a robust level of inositol phosphate production and PLCγ2 activity, while with GPIb-IX-V activation PLCγ2 activation is only modest and the tyrosine phosphorylation sites pf PLCγ2 is distinct from that of GPVI stimulation [26].

FcRγ-chain and FcγRIIA

The GPIb-IX-V-mediated activation of platelets leads to tyrosine phosphorylation of two ITAM-containing molecules, FcRγ-chain and FcγRIIA [13,20]. FcRγ-chain forms a complex with Syk, and GPIb-IX-V and FcRγ-chain are co-precipitated with Brij 35 lysates of platelets, suggesting a functional link between GPIb-IX-V. Some of the activation signals are attenuated in FcRγ-chain knockout mice [20,23]. A physical proximity of < 10 nm between GPIb-IX-V and FcγRIIA was also shown by fluorescent energy transfer, and indeed they may associate on the platelet membrane, based on the results of a two-hybrid system [37,38]. On the contrary, normal shape change and Ca^{++} mobilization was observed in platelets treated with anti-FcγRIIA antibodies or in FcRγ-chain deficient platelets [21]. A most recent paper observed only slight reduction in Ca^{++} oscillation and $\alpha_{IIb}\beta_3$ activation in FcRγ-chain-deficient mice, while confirming tyrosine phosphorylation of FcRγ-chain upon GPIb-IX-V stimulation [23]. These studies taken together suggest that FcRγ-chain and FcγRIIA is not required for GPIb-IX-V-mediated signal transduction, while it may have a limited potentiating effect on downstream signals.

PI-3K

As the regulatory subunit of PI-3K, p85, associates with GPIb-IX-V, and appears to mediate Src binding to GPIb [32], the role of PI-3K in GPIb-IX-V signaling should be thoroughly explored. PI-3K is activated by VWF in the presence of ristocetin, or in platelets adhering to VWF-coated surfaces [39]. PI-3K activity is also increased by high shear stress, as assessed by PIP(3) production [40]. PI-3K inhibition leads to a decrease level of platelet spreading and aggregate formation under flow conditions [39,41]. However, there are several cellular events unaffected by PI-3K inhibitors, wortmannin or LY294002. Irrespective of shear stress, filopodia formation and Ca^{++} spikes are insensitive to PI-3K inhibition [39,42]. Under static conditions, wortmannin did not inhibit tyrosine phosphorylation of Src and PLCγ2 tyrosine phosphorylation [32] or Ca^{++} oscillations [23]. Thus, at least under static conditions, the GPIb-IX-V-mediated signal transduction pathway sequentially involving Src, PLCγ2 activation and Ca^{++} oscillations is unaffected by the PI-3K activity, and platelet aggregation and spreading on VWF-coated surfaces supported by $\alpha_{IIb}\beta_3$ is dependent upon PI-3K. Under high shear stress, the roles of PI-3K are somewhat at variance, probably because of the various degrees of involvement of $\alpha_{IIb}\beta_3$ outside-in signaling in experimental settings. By activating Src and Syk, $\alpha_{IIb}\beta_3$ leads to PLCγ2 activation, and the resulting Ca^{++} mobilization along with secondary mediators such as ADP and TXA_2 potentiate PI-3K activity, which can then regulate PLCγ activity [42,43]. That the full activation of $\alpha_{IIb}\beta_3$ requires PI-3K activity also makes the story complicated. However, because of experimental difficulties, the effects of PI-3K inhibitors have not been evaluated along with $\alpha_{IIb}\beta_3$ blockade in most flow-condition studies. While facing difficulties in elucidating the exact role of PI-3K in GPIb-IX-V signaling, it may be safely concluded that there are PI-3K-independent and PI-3K-dependent processes, and that most probably PI-3K activity is dispensable for Src-PLCγ2 activation in GPIb-IX-V-mediated signaling, while it serves to potentiate various signaling events culminating in $\alpha_{IIb}\beta_3$ activation.

14-3-3ζ

The 14-3-3ζ belongs to a family of proteins involved in regulation of a diverse number of intracellular signaling proteins through its interaction with serine-phosphorylated signaling molecules [44]. A wide variety of proteins including Raf-1 kinase, Bad and PI-3K associate with 14-3-3ζ [45]. GPIb-IX-V has several specific binding sites for 14-3-3ζ, and phosphorylation of these sites ensures constitutive association between 14-3-3ζ and GPIb-IX-V [46]. It has been reported that GPIb-IX-V-mediated activation of $\alpha_{IIb}\beta_3$ requires 14-3-3ζ binding to the cytoplasmic domain of GPIbα [47]. The heterotrimeric complex of GPIb-IX-V, 14-3-3ζ and p85 subunit of PI-3K is present in resting platelets [34]. As GPIb-IX-V has no apparent binding sites for PI-3K, it is most likely that p85 PI-3K binds to GPIb-IX-V via 14-3-3ζ. Based on the findings that Src associates with p85 PI-3K bound to GPIb-IX-V upon VWF/GPIb-IX-V interaction [34], and that the downstream signaling pathways of Src and PLCγ2 activate $\alpha_{IIb}\beta_3$, the requirement for 14-3-3ζin GPIb-IX-V-mediated $\alpha_{IIb}\beta_3$ activation, as shown in a previous paper, may be explained as its adaptor role for binding sequentially p85 PI-3K and then Src to GPIb-IX-V.

In addition to the role as an adaptor protein to recruit PI-3K and Src to GPIb-IX-V, another functional role has been recently suggested for 14-3-3ζ. Shear stress induces dissociation of 14-3-3ζ from GPIb-IX-V, concomitant with dephosphorylation of 14-3-3ζ-binding sites of GPIb-IX-V [46]. Released 14-3-3ζ somehow activates Rac and Cdc42 which regulate cytoskeletal reorganization in integrin-dependent cell activation [48]. It is conceivable that the dephosphorylation process of GPIb-IX-V and release of 14-3-3ζ appears not to be required in the initial stage of platelet adhesion to a VWF matrix, but may be required for the phase of platelet spreading supported by $\alpha_{IIb}\beta_3$ activation. Thus, the role of 14-3-3ζ in GPIb-IX-V-mediated platelet activation needs to be considered in a dynamic mode, with its role changing at different stages of platelet activation.

Glycosphingolipid-enriched microdomains (GEMs; also known as rafts)

Lipids and proteins on cell membranes are unequally distributed and form distinct microdomains which have specific lipid and protein components. Glycolipid-enriched microdomains (GEMs) which are rich in glycosphingolipids, saturated phospholipids and cholesterol, have been identified in many cell types. Molecules present in GEMs have diffusion velocities much lower than those present in non-GEM areas of cell membranes, and thus GEMs appear to provide organized milleu on cell membranes which otherwise are chaotic [49]. GEMs appear to act as platforms for signal transduction and ligand localization, selectively recruiting a certain set of signaling molecules while excluding others [50].

There is an increasing body of evidence to suggest that GEMs also have functional roles in platelets. Phosphatidylinositol 3,4,5-triphosphate is produced in platelet GEMs [51]. GEMs accumulate at the extended tips of the formed filopodia, and this concentration process of GEMs is accompanied by the simultaneous enrichment of Src and the tetraspanin CD63 [52]. GPVI co-localizes with FcRγ-chain in GEMs, and destruction GEMs in platelets leads to a lower response to GPVI agonists [53]. With regard to GPIb-IX-V, it appears that a minor portion of GPIb-IX-V molecules (8%) on platelet membranes reside in GEMs in the resting state, and this portion increases three- to sixfold with platelet activation by VWF/GPIb-IX-V interaction [54]. Although fractionation of platelet membranes on sucrose gradient suggests that GPIb-IX-V and FcγRIIA are present both in GEMs and non-GEM fractions, GPIb-IX-V colocalize with FcγRIIA exclusively in GEMs.

We have previously reported that VWF/GPIb-IX-V interaction involves a set of signaling molecules, Src, FcRγ-chain, Syk, PLCγ2 and PI-3K [20,32]. We thus sought to see whether these signaling molecules play their roles in GEMs. In agreement with the report of Shrimpton *et al*. [54], we found that portion of GPIb-IX-V (10–13%) localizes in GEMs in resting platelets, and that this portion increases in a time-dependent manner after platelet activation induced by VWF/botrocetin. The association between GPIb-IX-V and PI-3K occurs constitutively regardless of their localization. However, Src association with GPIb-IX-V induced by VWF/GPIb-IX-V interaction is restricted to GEMs, and this recruitment of Src is confined to the activated form of Src with its 416 tyrosine residue phosphorylated. While FcRγ-chain is present both in GEMs and non-GEMs, the association between GPIb-IX-V and FcRγ-chain occurs only in GEMs, similar to that of FcγRIIA. Only the FcRγ-chain molecules present in GEMs undergo tyrosine phosphorylation upon VWF/GPIb-IX-V interaction, and this event is marked inhibited by the treatment with methyl-β-cyclodextrin to disrupt GEMs. Syk and PLCγ2 are present both in GEMs and non-GEMs, and complex formation between GPIb-IX-V and Syk or that between GPIb/IX-V and PLCγ2 is not detected. However, only Syk and PLCγ2 in GEMs are tyrosine-phosphorylated by VWF/GPIb-IX-V interaction, and their tyrosine phosphorylation is marked suppressed by methyl-β-cyclodextrin treatment of platelets. Thus, the association between GPIb-IX-V and active Src, tyrosine phosphorylation of GPIb-IX-V-associated FcRγ-chain, Syk and PLCγ2 tyrosine phosphorylation all occur in GEMs and are dependent upon the intact structure of GEMs (manuscript in preparation). These findings suggest that GEMs indeed provide platforms for the signal transduction pathway related to GIb-IX-V (Fig. 1).

GPIb-IX-V and the cytoskeleton

The platelet cytoskeleton constitutes the contractile machinery that regulates platelet shape change and spreading. Furthermore, it forms an intracellular network which connects and orchestrates a number of signaling molecules. In this context, it has been demonstrated that GPIb-VWF interaction, particularly under high shear stress, stimulates the assembly of

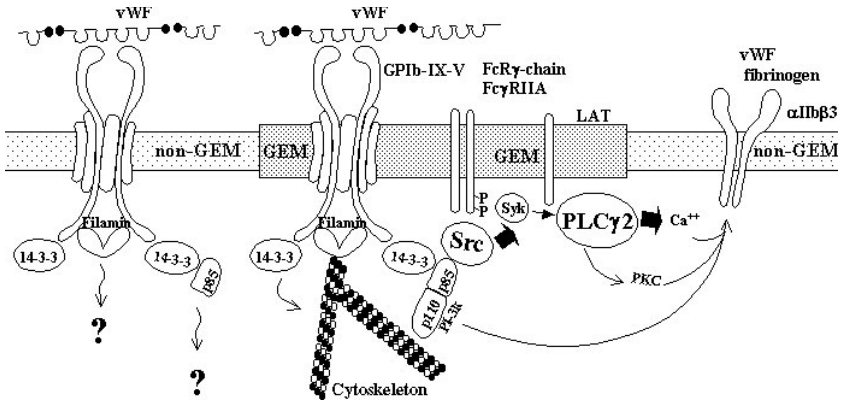

Fig. 1. Hypothetical signal transduction pathway mediated by GPIb-IX-V, leading to $\alpha_{IIb}\beta3$ activation. GPIb-IX-V constitutively associates with p85 subunit of PI-3K via 14-3-3ζ. The interaction between GPIb-IX-V and VWF induces the binding between P85 PI-3K and Src, which then elicits downstream signals leading to PLCγ2 activation. These processes appear to take place predominantly in GEMs.

filamentous actin to GPIb-IX-V [55,56]. Shear stress-induced GPIb-IX-V stimulation leads to the assembly of α-actinin, PI-3K and its products [40]. VWF/GPIb-IX-V interaction induces the formation of SHIP-2, filamin, actin and GPIb-IX-V complexes [57]. The cytoplasmic domain of GPIbα has been shown to be important for cytoskeletal reorganization induced by VWF-GPIb interaction, and studies using cells transfected with truncated GPIbα lacking filamin A binding sites suggest that the association of GPIb-IX-V with actin filaments via filamin A is critical for maintaining platelet adhesion at high shear rates [58]. A more recent report, employing intracellular delivery of peptide sequences into platelets, has demonstrated that filamin A binding to GPIbα is required for cytoskeletal reorganization, platelet aggregation, and tyrosine phosphorylation of some signaling molecules [59]. Thus, although interaction of GPIb-IX-V with the cytoskeleton has been proposed to strengthen the receptor anchorage to VWF under high shear stress, and this premise should be duly valued, the association of the actin network (filamin A, α-actinin, actin filaments etc.) with GPIb-IX-V appears to provide more than just physical support to GPIb-IX-V. The cytoskeleton associated with GPIb-IX-V may act as platforms for interactions among signaling molecules. It is also likely that the cytoskeletons play a role in lateral clustering of GPIb-IX-V which has been shown to lead to $\alpha_{IIb}\beta_3$ activation [60]. With reference to GEMs, it has been suggested that signaling molecules recruited to GEMs are enriched with the actin network, probably required for their translocation or anchorage in GEMs [61]. As a portion of GPIb-IX-V is recruited to GEMs upon platelet activation, and this process appears to be related to its function, it is also likely that the cytoskeleton associated with GPIb-IX-V plays a role in directing GPIb-IX-V to GEMs.

cGMP-dependent protein kinase

It is generally accepted that an elevated level of cGMP and cGMP-dependent protein kinase (PKG) inhibits platelet activation [62]. Recently, it has been suggested that the cGMP-PKG pathway plays an important stimulatory role in GPIb-IX-V-mediated platelet activation. Expression of recombinant PKG in a cell model enhanced VWF-induced activation of $\alpha_{IIb}\beta3$. PKG-knockout mice showed impaired platelet responses to VWF [63]. A more recent paper by the same group suggests that the stimulatory role for cGMP-PKG in platelet activation is not restricted to GPIb-IX-V-mediated platelet activation, but appears to promote platelet secretion response mediated by a number of G-protein-coupled receptors [64]. However, there is also a report that contradicts these findings in that VWF does not increase cGMP levels in platelets and cGMP-elevating agents inhibit platelet activation induced by VWF/GPIb-IX-V interaction [35]. Although this issue is potentially important, with a limited number of contradictory publications, it is too early to draw a conclusion on this issue.

Concluding remark

Although the signaling pathways related to GPIb-IX-V have not been fully elucidated, an accumulating body of evidence suggests that Src family kinase- and PLCγ2-related signaling plays an important role in GPIb-IX-V-mediated platelet activation.

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