Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 368 (2008) 157–161
BBRC
www.elsevier.com/locate/ybbrc

Regulation of adaptor protein GIT1 in platelets, leading to the interaction between GIT1 and integrin $\alpha_{IIb}\beta_3$

Hitomi Sato, Katsue Suzuki-Inoue *, Osamu Inoue, Yukio Ozaki

Department of Clinical and Laboratory Medicine, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan

Received 15 January 2008
Available online 22 January 2008

Abstract

GIT1 is an adaptor protein, which links signaling proteins to focal adhesion, thereby regulating cytoskeletal reorganization. Platelets undergo dynamic cytoskeletal reorganization during platelet activation, for which a large number of adaptor proteins are required. However, there has been no report of GIT1 in platelets. We found that GIT1 was abundantly expressed in platelets and underwent tyrosine phosphorylation downstream of integrin $\alpha_{IIb}\beta_3$, which was inhibited by the Src kinase inhibitor PP2. Furthermore, GIT1 constitutively associated with βPIX, a guanine nucleotide exchange factor (GEF) for Rac. The GIT1/βPIX complex associated with $\alpha_{IIb}\beta_3$, concomitantly with GIT1 tyrosine phosphorylation. Moreover, both GIT1 and $\alpha_{IIb}\beta_3$ rapidly translocated to the cytoskeletal fraction during platelet aggregation, which was not observed in the absence of aggregation. These results suggest that tyrosine phosphorylation of GIT1 by Src kinases may regulate cytoskeletal reorganization downstream of $\alpha_{IIb}\beta_3$ by bringing the Rac GEF βPIX to the vicinity of the integrin.

Keywords: GIT1; Integrin $\alpha_{IIb}\beta_3$; PIX; Blood platelets

Platelets play a critical role in physiological hemostasis and pathological thrombus formation. Platelet activation is an integrated process involving subendothelial matrix proteins, including collagen and laminin, and soluble agonists such as thrombin, ADP, and thromboxane A_2 [1]. These agonists bind to their receptors in platelets and generate inside-out activation signals, which result in activation of integrin $\alpha_{IIb}\beta_3$. Activation of $\alpha_{IIb}\beta_3$ by 'inside-out' signals leads to binding of fibrinogen and platelet aggregation. In turn, clustering of $\alpha_{IIb}\beta_3$ as a consequence of engagement of $\alpha_{IIb}\beta_3$ by fibrinogen mediates 'outside-in' signals that stimulate tyrosine phosphorylation of β_3 integrin tail [2] and activation of a Src-based signaling cascade [3] that involves Syk [4,5], SLP-76 [6], and PLCγ2 [7,8]. These outside-in signals have been shown to be essential for platelet spreading (lamellipodia formation) on fibrinogen [3,7,8].

Adaptor proteins play important roles in transducing both inside-out and outside-in signals in platelets. Adaptor proteins lack intrinsic activity, yet possess a range of modular domains that facilitate protein–protein and protein–lipid interactions. A large number of adaptor proteins have been described in platelets, including LAT, SLP-76, Grb2, Gab2 [9], SLAP-130, Cas [10], and recently identified Dok1/2 [11]. Some of these adopter proteins undergo tyrosine phosphorylation, which results in creation of new-binding sites for signaling molecules.

An adaptor protein, GIT1 (G protein-coupled receptor kinase interacting protein 1) is a multidomain protein that has an N-terminal ARF GTPase-activating protein (ARF-GAP) domain, three ankyrin (ANK) repeats, a Spa2-homology domain (SHD), a coiled-coil domain and a paxillin-binding site (PBS) [12]. GIT1 is a GTPase-activating protein for the ADP-ribosylation factor family of small GTP-binding proteins [13], and also regulates cytoskeletal

* Corresponding author. Fax: +81 55 273 6713.
E-mail address: katsuei@yamanashi.ac.jp (K. Suzuki-Inoue).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.01.064

reorganization by serving as an adaptor to link signaling proteins to distinct cellular locations. GIT1 tightly forms complex with βPIX (p21-activated kinase interacting exchange factor), which is a guanine nucleotide exchange factor for Rac, a small GTP-binding protein [14]. By binding to molecules having distinct cellular localizations, such as paxillin/Hic-5, liprin-α, or shank, the GIT/PIX complex can be transiently localized to many parts of the cell, including focal adhesions, membrane ruffles, intracellular vesicles, regulating cell motility, lamellipodia formation, and vesicular transport [15–18]. GIT1 has been reported to undergo tyrosine phosphorylation during cell spreading on extracellular matrix by the tyrosine kinases Src and FAK [19–21]. However, a role of GIT1 tyrosine phosphorylation for cellular responses has not been elucidated to date.

Although platelets undergo dynamic cytoskeletal reorganization upon activation and a number of adaptor proteins are required for platelet activation, the role of GIT1 in platelet activation has not been elucidated to date. In this study, we demonstrate that GIT1 is constitutively associates with the Rac GEF βPIX, and the complex associates with integrin $\alpha_{IIb}\beta_3$, concomitantly with GIT1 tyrosine phosphorylation. We propose that GIT1 may recruit the Rac GEF βPIX in the vicinity of $\alpha_{IIb}\beta_3$, regulating lamellipodia formation downstream of integrin $\alpha_{IIb}\beta_3$.

Materials and methods

Antibodies and reagents. Rhodocytin was kindly donated by Prof. Takashi Morita (Meiji Pharmaceutical University, Tokyo, Japan). Goat anti-GIT1 antibody, rabbit anti-βPIX antibody, mouse anti-integrin β_3 antibody were from Santa Cruz (CA, USA). Mouse anti-phosphotyrosine antibody (4G10) was obtained from Millipore (MA, USA). Mouse anti-GIT1 antibody was from Becton–Dickinson. (NJ, USA). Gly-Arg-Gly-Asp-Ser (GRGDS) peptide was purchased from Peptide Institute (Osaka, Japan). Thrombin receptor agonist peptide: TRAP (Ser-Phe-Leu-Leu-Arg-Asn: SFLLRN peptide) was from Bachem Biochemica (Heidelberg, Germany). Convulxin was obtained from Pentapharm (Basel, Switzerland). Fibrinogen was purchased from Sigma–Aldrich (St. Louis, USA). PP2 and Syk inhibitor were from Calbiochem (Bad Soden, Germany).

Platelet preparation. Venous blood from healthy drug-free volunteers was taken into 10% sodium citrate. This study was approved by the Ethical Committees in University of Yamanashi and informed consent was provided according to the Declaration of Helsinki. Washed human platelets were obtained by centrifugation as previously described [22]. Washed platelets were resuspended in modified Tyrode buffer [22] at a cell density of 1×10^9/ml or 5×10^8/ml.

Immunoprecipitation and Western blotting. Washed human platelets (1×10^9/ml) were incubated with or without 1 mM GRGDS peptide, 10 μM PP2 (a Src kinase inhibitor), 10 μM Syk inhibitor, 10 μM indomethacin, or 100 nM wortmannin at 37 °C. Then, they were stimulated by 100 μM TRAP, 250 ng/ml convulxin, or 50 nM rhodocytin for indicated durations of time. Reactions were terminated by addition of 2× ice-cold lysis buffer containing 2% nonidet P-40 [22]. Platelet lysates were precleared and detergent-insoluble debris was cleared as described [22]. GIT1, βPIX, or integrin β_3 was immunoprecipitated by addition of anti-GIT1, βPIX, or integrin β_3 antibody together with protein G. Precipitated proteins were separated by 8% sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE), electrotransferred, and Western blotted by the indicated antibodies.

Platelet adhesion assay. Tissue culture plates (10 cm) were coated with 200 μg/ml fibrinogen or phosphate-buffered saline (PBS) at 4 °C overnight. After washing twice with PBS, the plates were blocked with 1% bovine serum albumin in PBS for 2 h at room temperature and washed twice with modified Tyrodes buffer before incubation with 500 μl washed platelets (5×10^8/ml) at 37 °C for 1 h. Five hundred microliters of 2× ice-cold lysis buffer was added to the plates without removing unbound platelets. Immunoprecipitation and Western blotting were performed as described above.

Platelet subcellular fractions. Human washed platelets (1×10^9/ml) were stimulated by 100 μM TRAP in the presence or absence of 1 mM GRGDS. Reactions were terminated by addition of 2× ice-cold lysis buffer containing Triton X-100 instead of NP-40. The cytoskeleton fraction (CS) was isolated by centrifugation of the lysate at 15,000*g* for 10 min. The membrane skeleton fraction (MS) was separated from the resultant supernatant by centrifugation at 100,000*g* for 3 h with a Himac CS 100 FX (Hitachi Koki Co., Tokyo, Japan). The pellets (CS and MS) were solubilized with 1× SDS sample buffer. For analysis of cytosol fraction (CY), the final supernatant was solubilized with 4× SDS sample buffer. The proteins were separated by 8% SDS–PAGE. GIT1 and integrin β_3 in each fraction was detected by Western blotting.

Results and discussion

GIT1 is tyrosine-phosphorylated downstream of $\alpha_{IIb}\beta_3$ in platelets

Since there has been no previous report addressing the presence of GIT1 in human platelets, we initially investigated whether GIT1 is present in platelets. We demonstrated the presence of GIT1 in both murine and human platelets as well as in rat testis as previously reported [23] (Fig. 1A). It has been reported that GIT1 is tyrosine-phosphorylated in growing NIH 3T3 fibroblasts, and the phosphorylation is increased following cell spreading on fibronectin [19]. We next examined whether GIT1 undergoes tyrosine phosphorylation upon platelet activation, by way of immunoprecipitation and Western blotting for phosphotyrosine. Both thrombin receptor agonist peptide (TRAP) and convulxin, a specific agonist for the collagen receptor glycoprotein (GP) VI, induced tyrosine phosphorylation of GIT1 at 30 s–2 min, which was maintained until 5 min after stimulation (Fig. 1B). Since not only inside-out signals from GPVI or thrombin receptor, but also subsequent outside-in signals from integrin $\alpha_{IIb}\beta_3$ activate tyrosine kinases, we next investigated whether GIT1 tyrosine phosphorylation occurs downstream of GPVI, thrombin receptor, and/or the integrin. Pretreatment of platelets with GRGDS before stimulation blocks fibrinogen binding to $\alpha_{IIb}\beta_3$, thereby inhibiting outside-in signals downstream of the integrin, but not inside-out signals from the agonist receptors. GRGDS completely inhibited tyrosine phosphorylation of GIT1 induced by TRAP, convulxin, or the snake toxin rhodocytin that generates tyrosine kinase-dependent signals through recently identified c-type lectin-like receptor 2 (CLEC-2) [24] (Fig. 1C). These findings suggest that GIT1 tyrosine phosphorylation is located in the process of outside-in signals downstream of $\alpha_{IIb}\beta_3$, but not in the process of inside-out signals downstream of thrombin receptor, GPVI, or CLEC-2. This is a charac-

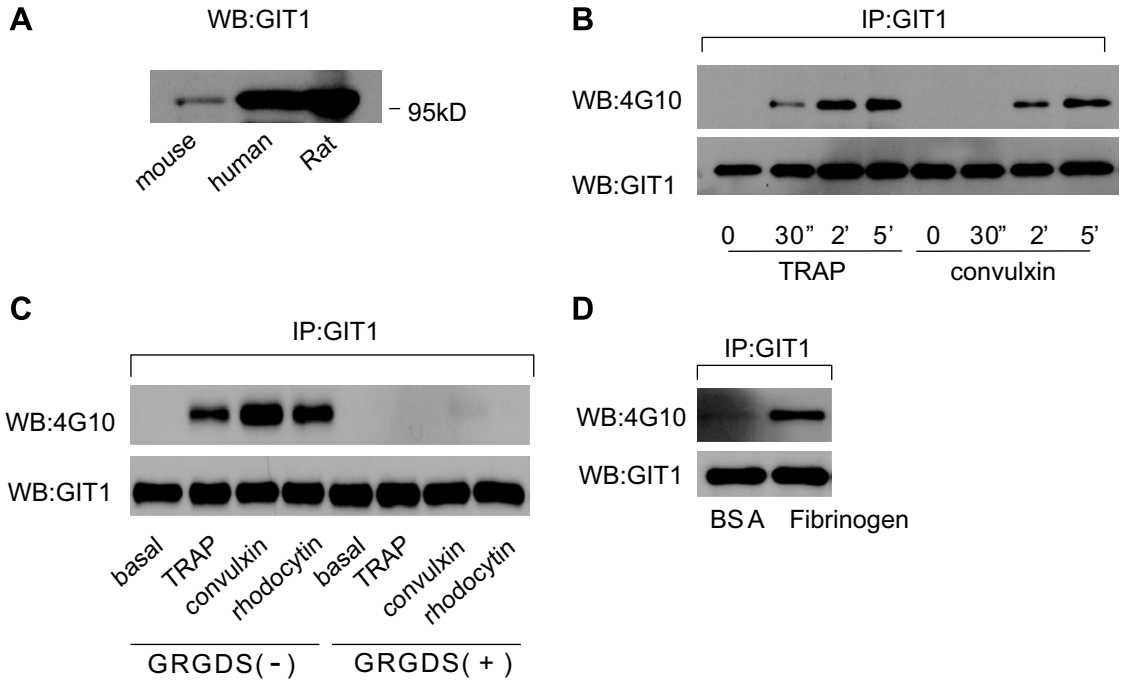

Fig. 1. GIT1 is tyrosine-phosphorylated depending on platelet aggregation. (A) Whole cell lysates of human platelets, murine platelets, and rat testis were separated by 8% SDS–PAGE, electrotransferred, and blotted with anti-GIT1 antibody. (B) Washed human platelets stimulated with 100 μM TRAP or 250 ng/ml of convulxin. At indicated time points, the reactions were terminated by addition of 2× lysis buffer. GIT1 was immunoprecipitated and immunoblotted with anti-phosphotyrosine antibody (4G10) or anti-GIT1 antibody. (C) Washed human platelets were preincubated with or without GRGDS (1 mM), and then stimulated with TRAP (100 μM), convulxin (250 ng/ml), or rhodocytin (50 nM) for 2 min. GIT1 was immunoprecipitated and immunoblotted with anti-phosphotyrosine antibody (4G10) or anti-GIT1 antibody. (D) Five hundred microliters of washed human platelets (5×10^8/ml) were seeded on fibrinogen-coated plates for 1 h at 37 °C. The same amount of 2× ice-cold lysis buffer was added to the plates without removing unbound platelets. GIT1 was immunoprecipitated and immunoblotted with anti-phosphotyrosine antibody (4G10) and anti-GIT1 antibody. The data were representative of at least two experiments.

teristic unique to GIT1, as most of the signaling molecules including Syk, SLP-76, and PLCγ2, undergo tyrosine phosphorylation downstream of both the integrin and GPVI, with the exception of LAT, which is not phosphorylated downstream of the integrin [1].

To further confirm tyrosine phosphorylation of GIT1 downstream of $\alpha_{IIb}\beta_3$, we utilized immobilized fibrinogen that generates outside-in activation through the integrin without inside-out activation mediated by agonist receptors. Immobilized fibrinogen stimulated tyrosine phosphorylation of GIT1, further confirming that GIT1 tyrosine phosphorylation is mediated by $\alpha_{IIb}\beta_3$ (Fig. 1D). Since we could induce GIT1 tyrosine phosphorylation more steadily by soluble agonists than by immobilized fibrinogen, we used TRAP to simulate platelets in the experiments, hereafter.

GIT1 tyrosine phosphorylation is dependent on Src kinases

The contributions of Src kinases and Syk to GIT1 tyrosine phosphorylation were evaluated, as both kinases are crucial in generating activation signals downstream of $\alpha_{IIb}\beta_3$. The Src kinase inhibitor PP2, but not Syk inhibitor, almost completely inhibited an increase in GIT1 tyrosine phosphorylation (Fig. 2). Combination of PP2 and Syk inhibitor did not result in further inhibition of the phosphorylation compared with PP2 alone (Fig. 2). These finding suggest that GIT1 tyrosine phosphorylation is almost

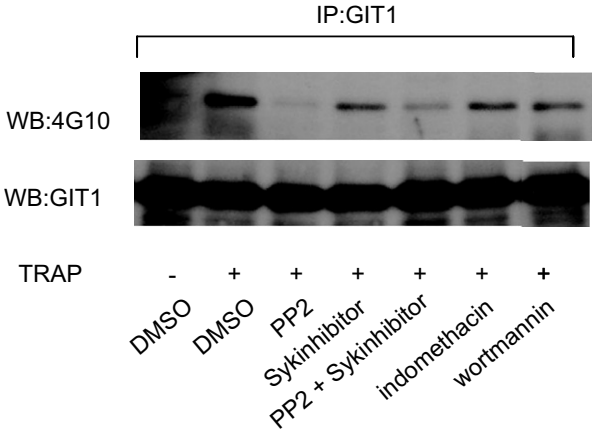

Fig. 2. Phosphorylation of GIT1 is dependent on Src kinases. Washed human platelets were preincubated with DMSO, 10 μM PP2, 10 μM Syk inhibitor, PP2 plus Syk inhibitor, 10 μg/ml indomethacin, or 100 nM wortmannin. Then, they were stimulated with 100 μM TRAP for 3 min before the reactions were terminated by addition of 2× lysis buffer. GIT1 was immunoprecipitated and immunoblotted with anti-phosphotyrosine antibody (4G10) or anti-GIT1 antibody. The data were representative of at least two experiments.

dependent on Src kinases. We further investigated whether the secondary feedback agonist, thromboxane A_2 and the second messenger pathway, PI3-kinase regulate tyrosine phosphorylation of GIT1. Thromboxane A_2 and PI3-kinase play a role in potentiation of activation signals mediated through both inside-out and outside-in pathway. Fig. 2 showed that the cyclooxygenase inhibitor indomethacin and the broad-spectrum inhibitor of PI3-kinase wortmannin had only marginal inhibitory effect on GIT1 tyrosine phosphorylation. Importantly, these inhibitors did not inhibit platelet aggregation induced by TRAP (data not shown). These findings, taken together, suggest that GIT1 is tyrosine-phosphorylated downstream of integrin $\alpha_{IIb}\beta_3$ by Src kinases.

GIT1 forms complex with βPIX and associated with integrin $\alpha_{IIb}\beta_3$ concomitantly with GIT1 tyrosine phosphorylation

Recent study indicated that GIT1 forms oligomers with the Rac GEF βPIX and the oligomers are recruited to focal complexes regulating cytoskeletal dynamics [12]. We examined whether GIT1 associates with βPIX in platelets by immunoprecipitation and Western blotting. GIT1 constitutively associated with βPIX, irrespective of platelet stimulation with TRAP or $\alpha_{IIb}\beta_3$ blockade by GRGDS (Fig. 3A). Further, GIT1/βPIX complex associated with $\alpha_{IIb}\beta_3$ during platelet aggregation, which was inhibited by GRGDS (Fig. 3A). The interaction between GIT1/βPIX complex and $\alpha_{IIb}\beta_3$ appears to be dependent on outside-in signaling downstream of $\alpha_{IIb}\beta_3$ and may be mediated by phosphorylation of GIT1 and/or β_3. However, another signaling molecule may be required for association between GIT1/βPIX complex and $\alpha_{IIb}\beta_3$, since both GIT1/βPIX and β_3 lack a phosphotyrosine-binding motif.

We next examined the subcellular distribution of GIT1 and integrin $\alpha_{IIb}\beta_3$ in relevance to cytoskeletal reorganization. The platelet cytoskeleton contains two actin filament-based components. One is the cytoplasmic actin filaments (cytoskeleton fraction) that fill the cytoplasm and mediate contractile events. The other is the membrane skeleton (membrane skeleton), which coats the plasma membrane and regulates the membrane contours and stability. Upon platelet aggregation, it has been reported that $\alpha_{IIb}\beta_3$ associated with cytoplasmic actin [25]. As was reported previously, integrin β_3 existed in the cytosol and membrane cytoskeletal fraction and then, translocated to the cytoskeleton fraction during platelet aggregation, which was completely inhibited under non-aggregating conditions (Fig. 3B). GIT1 was detected mainly in membrane cytoskeleton fractions in the resting state. Concomitantly with

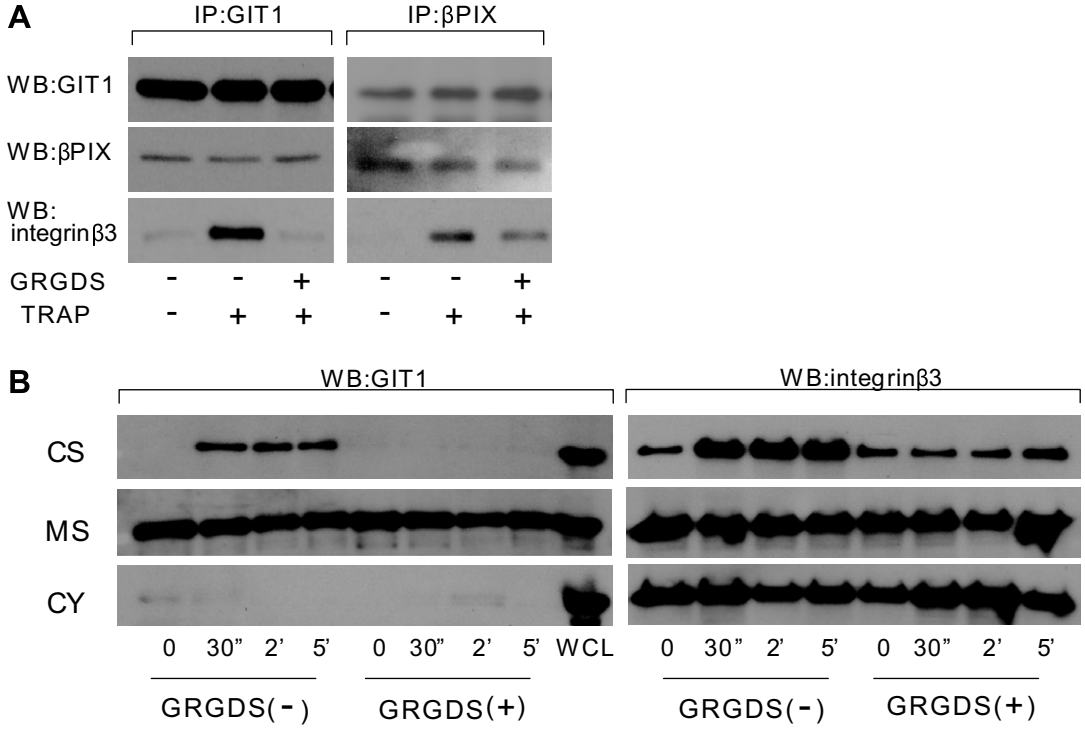

Fig. 3. GIT1/βPIX complex associated with $\alpha_{IIb}\beta_3$ and translocated to the cytoskeleton fraction depending on platelet aggregation. (A) Washed human platelets were preincubated with or without GRGDS (1 mM), and then stimulated with TRAP (100 μM) for 2 min. Reactions were terminated by the addition of an equal volume of 2× lysis buffer. GIT1, βPIX, and integrin β_3 were immunoprecipitated, separated by SDS–PAGE, and blotted with the indicated antibodies. (B) Washed human platelets were preincubated with or without GRGDS (1 mM), and then stimulated with TRAP (100 μM) for various time periods as indicated. Reactions were terminated by addition of 2× ice-cold lysis buffer containing Triton X-100. Cytoskeleton fraction (CS), membrane skeleton fraction (MS), and cytosol fraction (CY) were separated as described in Materials and methods. The proteins were separated by SDS–PAGE. GIT1 and integrin β_3 in each fraction was detected by Western blotting. WCL indicates whole cell lysates. The data were representative of at least three experiments.

integrin β_3, GIT1 rapidly translocated to the cytoskeleton fraction after platelet aggregation, which was completely inhibited by GRGDS (Fig. 3B). These findings are in agreement with the finding that GIT1 associated with $\alpha_{IIb}\beta_3$ depending on platelet aggregation, when GIT1 undergoes tyrosine phosphorylation. Translocation of GIT1 to cytoskeleton fraction also suggests a role of GIT1 for cytoskeletal reorganization during platelet aggregation.

These data, taken together, suggest that GIT1 constitutively forms complex with βPIX and the complex is associated with $\alpha_{IIb}\beta_3$, depending on platelet aggregation and GIT1 tyrosine phosphorylation by Src kinases. We speculate that GIT1 tyrosine phosphorylation leads to recruitment of the Rac GEF βPIX to $\alpha_{IIb}\beta_3$, thereby promoting lamellipodia formation through Rac activation downstream of the integrin. Alternatively, it may be possible that the complex inhibits lamellipodia formation due to inhibition of Rac activity by GIT1 ARF-GAP activity [26]. Further investigation is required for addressing this issue.

Acknowledgments

We are very grateful to Ms. Chiaki Komatsu, Mr. Hisaichiro Nakazawa, and Ms. Kayoko Ishimaru for their excellent technical assistance.

References

[1] B. Nieswandt, S.P. Watson, Platelet–collagen interaction: is GPVI the central receptor? Blood 102 (2003) 449–461.
[2] D.A. Law, L. Nannizzi-Alaimo, D.R. Phillips, Outside-in integrin signal transduction. alpha IIb beta 3-(GP IIb IIIa) tyrosine phosphorylation induced by platelet aggregation, J. Biol. Chem. 271 (1996) 10811–10815.
[3] A. Obergfell, K. Eto, A. Mocsai, C. Buensuceso, S.L. Moores, J.S. Brugge, C.A. Lowell, S.J. Shattil, Coordinate interactions of Csk, Src, and Syk kinases with [alpha]IIb[beta]3 initiate integrin signaling to the cytoskeleton, J. Cell. Biol. 157 (2002) 265–275.
[4] E.A. Clark, S.J. Shattil, M.H. Ginsberg, J. Bolen, J.S. Brugge, Regulation of the protein tyrosine kinase pp72syk by platelet agonists and the integrin alpha IIb beta 3, J. Biol. Chem. 269 (1994) 28859–28864.
[5] J. Gao, K.E. Zoller, M.H. Ginsberg, J.S. Brugge, S.J. Shattil, Regulation of the pp72syk protein tyrosine kinase by platelet integrin alpha IIb beta 3, EMBO J. 16 (1997) 6414–6425.
[6] A. Obergfell, B.A. Judd, M.A. del Pozo, M.A. Schwartz, G.A. Koretzky, S.J. Shattil, The molecular adapter SLP-76 relays signals from platelet integrin alpha IIb beta 3 to the actin cytoskeleton, J. Biol. Chem. 276 (2001) 5916–5923.
[7] P. Wonerow, A.C. Pearce, D.J. Vaux, S.P. Watson, A critical role for phospholipase C gamma 2 in alpha II beta 3-mediated platelet spreading, J. Biol. Chem. 278 (2003) 37520–37529.
[8] I. Goncalves, S.C. Hughan, S.M. Schoenwaelder, C.L. Yap, Y. Yuan, S.P. Jackson, Integrin alpha IIb beta 3-dependent calcium signals regulate platelet–fibrinogen interactions under flow: involvement of PLC gamma2, J. Biol. Chem. 278 (2003) 34812–34822.
[9] Q. Yi, K. Suzuki-Inoue, N. Asazuma, O. Inoue, S.P. Watson, Y. Ozaki, Docking protein Gab2 positively regulates glycoprotein VI-mediated platelet activation, Biochem. Biophys. Res. Commun. 337 (2005) 446–451.
[10] T. Ohmori, Y. Yatomi, K. Inoue, K. Satoh, Y. Ozaki, Tyrosine dephosphorylation, but not phosphorylation, of p130Cas is dependent on integrin alpha IIb beta 3-mediated aggregation in platelets: implication of p130Cas involvement in pathways unrelated to cytoskeletal reorganization, Biochemistry 39 (2000) 5797–5807.
[11] S.C. Hughan, S.P. Watson, Differential regulation of adapter proteins Dok2 and Dok1 in platelets, leading to an association of Dok2 with integrin alpha IIb beta 3, J. Thromb. Haemost. 5 (2007) 387–394.
[12] R.J. Hoefen, B.C. Berk, The multifunctional GIT family of proteins, J. Cell Sci. 119 (2006) 1469–1475.
[13] R.T. Premont, A. Claing, N. Vitale, J.L. Freeman, J.A. Pitcher, W.A. Patton, J. Moss, M. Vaughan, R.J. Lefkowitz, beta2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein, Proc. Natl. Acad. Sci. USA 95 (1998) 14082–14087.
[14] E. Manser, T.H. Loo, C.G. Koh, Z.S. Zhao, X.Q. Chen, L. Tan, I. Tan, T. Leung, L. Lim, PAK kinases are directly coupled to the PIX family of nucleotide exchange factors, Mol. Cell 1 (1998) 183–192.
[15] C.E. Turner, M.C. Brown, J.A. Perrotta, M.C. Riedy, S.N. Nikolopoulos, A.R. McDonald, S. Bagrodia, S. Thomas, P.S. Leventhal, Paxillin LD4 motif binds PAK and PIX through a novel 95-kDa ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling, J. Cell Biol. 145 (1999) 851–863.
[16] J. Ko, S. Kim, J.G. Valtschanoff, H. Shin, J.R. Lee, M. Sheng, R.T. Premont, R.J. Weinberg, E. Kim, Interaction between liprin-α and GIT1 is required for AMPA receptor targeting, J. Neurosci. 23 (2003) 1667–1677.
[17] S. Kim, J. Ko, H. Shin, J.R. Lee, C. Lim, J.H. Han, W.D. Altrock, C.C. Garner, E.D. Gundelfinger, R.T. Premont, B.K. Kaang, E. Kim, The GIT family of proteins forms multimers and associates with the presynaptic cytomatrix protein piccolo, J. Biol. Chem. 278 (2003) 6291–6300.
[18] S. Audebert, C. Navarro, C. Nourry, S. Chasserot-Golaz, P. Lécine, Y. Bellaiche, J.L. Dupont, R.T. Premont, C. Sempéré, J.M. Strub, A.V. Dorsselaer, N. Vitale, J.P. Borg, Mammalian scribble forms a tight complex with the βPIX exchange factor, Curr. Biol. 14 (2004) 987–995.
[19] S. Bagrodia, D. Bailey, Z. Lenard, M. Hart, J.L. Guan, R.T. Premont, S.J. Taylor, R.A. Cerione, A tyrosine-phosphorylated protein that binds to an important regulatory region on the cool family of p21-activated kinase-binding proteins, J. Biol. Chem. 274 (1999) 22393–22400.
[20] H. Kawachi, A. Fujikawa, N. Maeda, M. Noda, Identification of GIT1/Cat-1 as a substrate molecule of protein tyrosine phosphatase zeta/beta by the yeast substrate-trapping system, Proc. Natl. Acad. Sci. USA 98 (2001) 6593–6598.
[21] G. Yin, J. Haendeler, C. Yan, B.C. Berk, GIT1 Functions as a scaffold for MEK1-extracellular signal-regulated kinase 1 and 2 activation by angiotensin II and epidermal growth factor, Mol. Cell. Biol. 24 (2004) 875–885.
[22] K. Suzuki-Inoue, O. Inoue, J. Frampton, S.P. Watson, Murine GPVI stimulates weak integrin activation in PLC gamma 2 −/− platelets: involvement of PLC gamma 1 and PI3-kinase, Blood 102 (2003) 1367–1373.
[23] R.T. Premont, A. Claing, N. Vitale, S.J. Perry, R.J. Lefkowitz, The GIT family of ADP-ribosylation factor GTPase-activating proteins, J. Biol. Chem. 275 (2000) 22373–22380.
[24] K. Suzuki-Inoue, G.L. Fuller, Á. García, J.A. Eble, S. Pöhlmann, O. Inoue, T.K. Gartner, S.C. Hughan, A.C. Pearce, G.D. Laing, R.D. Theakston, E. Schweighoffer, N. Zitzmann, T. Morita, V.L. Tybulewicz, Y. Ozaki, S.P. Watson, A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2, Blood 107 (2006) 542–549.
[25] J.E. Fox, The platelet cytoskeleton, Thromb. Haemost. 70 (1993) 884–893.
[26] N. Nishiya, W.B. Kiosses, J. Han, M.H. Ginsberg, An alpha4 integrin–paxillin-Arf-GAP complex restricts Rac activation to the leading edge of migrating cells, Nat. Cell Biol. 7 (2005) 343–352.