

no sequence homology with human TPO and there have been no reports of the production of clinically significant antibodies against romiplostim.

A short-term (24 weeks), double-blind randomized controlled trial in adult cITP patients demonstrated 88 % (36/41) and 79 % (33/42) overall platelet response rates in non-splenectomised and splenectomised patients given romiplostim, respectively [67]. A 52-week study in 234 non-splenectomised adult cITP patients, in which randomly assigned romiplostim or standard of care (SOC), showed a higher rate of a platelet response and lower incidence of treatment failure in the romiplostim group [68]. It has been reported that cITP patients experience poor quality-of-life (QOL) [69], and this study showed that the romiplostim group had significantly greater improvement of QOL compared with the SOC group at 52 weeks [68]. Thromboembolic events were reported in 4 % (6/154) in the romiplostim group and 3 % (2/75) in the SOC group, with no statistically significant differences, and no hematologic malignancy was reported in either group. An open-label extension study of romiplostim for up to 3.5 years in 44 Japanese patients also demonstrated very high response rate (96 %) [70]. The median dose of romiplostim was similar compared with the studies of other ethnic origins (3.8 µg/kg in the Japanese study, 4.0 µg/kg in the other). The only thromboembolic event was transient ischemic attack in a patient who had a history of paroxysmal atrial fibrillation [70].

Rituximab

Rituximab is a chimeric monoclonal antibody that specifically depletes B cells from the blood, lymph nodes, and bone marrow by targeting CD20, which is expressed on the surface of premature and mature B cells [71]. Rituximab has been widely used for the treatment of B cell malignancies, such as indolent and aggressive non-Hodgkin lymphoma, and chronic lymphocytic leukemia, and its effectiveness have been well-established [72, 73]. In addition, rituximab has been used off-label for autoimmune diseases, especially for ITP patients in Europe and the United States [74]. Recent reviews have suggested that the usual rituximab treatment (375 mg/m² weekly × 4) is effective in around 40–60 % cITP patients, and that the response in more than 50 % of responders lasts at least 1 year, although the 5-year response rate was as low as 20 % in adults [75, 76]. Some reports have shown that smaller doses of rituximab (100 mg weekly × 4) have almost the same effects as the usual dose [77, 78]. One report showed that retreatment with standard dose rituximab was successful in ~75 % of patients who had responded to their initial course of rituximab [79]. Evidence-based ASH 2011 guidelines suggest that rituximab may be considered for patients at risk of bleeding who have failed one line of

therapy such as corticosteroids, intravenous immunoglobulin or splenectomy [80]. Overall, the safety profile of the rituximab therapy is good. However, the possible occurrence of life-threatening infectious complications, such as hepatitis B virus reactivation and progressive multifocal leukoencephalopathy, should be noted, especially in countries, such as Japan, with high incidence of HBV carriage.

The mechanism of action of rituximab in ITP may not be as straightforward as it first appears [74]. In most studies published to date, there were two patterns of response; the majority of responders responded to rituximab within 4 weeks, whereas, the rest responded more slowly, several weeks or even months after rituximab treatment. The response in early responders to rituximab is too rapid to be explained by the depletion of autoantibodies. Instead, it has been proposed that opsonized B cells with rituximab block macrophage Fc-receptor function, reducing platelets destruction in the spleen [81, 82]. Very recently, Audia et al. [83] reported that rituximab-induced total B cell depletion may not be correlated with its therapeutic effects. In this context, Stasi et al. [26, 33] demonstrated that the therapeutic efficacy of rituximab may be due to normalization of the abnormal autoreactive T cell responses and Treg function in ITP patients. A combination of these mechanisms likely plays a role in the efficacy of rituximab in ITP.

Conclusion

It is now clear that ITP is caused by both increased platelet clearance and decreased platelet production. However, the underlying abnormalities involved in the development of ITP appear to be complex and mostly remain to be determined. Recent progress of ITP treatment enables us to use three distinct approaches to increase platelet mass: (1) by suppression of platelet clearance, e.g. splenectomy, (2) by suppression or modification of abnormal immune responses, e.g. corticosteroids or rituximab, and (3) by stimulation of platelet production, e.g. TPO-RAs. Further clinical studies are needed to establish the best strategies using these approaches for the treatment of ITP patients, together with the development of new treatments aimed at curing ITP through elucidation of its pathophysiology.

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The Integrin-Linked Kinase-PINCH-Parvin Complex Supports Integrin α IIb β 3 Activation

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Abstract

Integrin-linked kinase (ILK) is an important signaling regulator that assembles into the heteroternary complex with adaptor proteins PINCH and parvin (termed the IPP complex). We recently reported that ILK is important for integrin activation in a Chinese hamster ovary (CHO) cell system. We previously established parental CHO cells expressing a constitutively active chimeric integrin (α IIb β 3) and mutant CHO cells expressing inactive α IIb β 3 due to ILK deficiency. In this study, we further investigated the underlying mechanisms for ILK-dependent integrin activation. ILK-deficient mutant cells had trace levels of PINCH and α -parvin, and transfection of ILK cDNA into the mutant cells increased not only ILK but also PINCH and α -parvin, resulting in the restoration of α IIb β 3 activation. In the parental cells expressing active α IIb β 3, ILK, PINCH, and α -parvin were co-immunoprecipitated, indicating the formation of the IPP complex. Moreover, short interfering RNA (siRNA) experiments targeting PINCH-1 or both α - and β -parvin mRNA in the parent cells impaired the α IIb β 3 activation as well as the expression of the other components of the IPP complex. In addition, ILK mutants possessing defects in either PINCH or parvin binding failed to restore α IIb β 3 activation in the mutant cells. Kindlin-2 siRNA in the parental cells impaired α IIb β 3 activation without disturbing the expression of ILK. For CHO cells stably expressing wild-type α IIb β 3 that is an inactive form, overexpression of a talin head domain (THD) induced α IIb β 3 activation and the THD-induced α IIb β 3 activation was impaired by ILK siRNA through a significant reduction in the expression of the IPP complex. In contrast, overexpression of all IPP components in the α IIb β 3-expressing CHO cells further augmented THD-induced α IIb β 3 activation, whereas they did not induce α IIb β 3 activation without THD. These data suggest that the IPP complex rather than ILK plays an important role and supports integrin activation probably through stabilization of the active conformation.

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Introduction

Cell adhesions are critical for hemostasis processes composed of interactions between vessel walls, platelets and coagulation-related proteins. During these processes, cells react with several elements such as extracellular matrix (ECM) proteins and cell surface receptors. As one of the main elements, an integrin family is known to play a key role in cell-ECM interactions. Integrins, transmembrane glycoprotein adhesion receptors, are composed of α and β subunits and are linked non-covalently. Both subunits include long extracellular domains, transmembrane domains, and short cytoplasmic domains. There are at least two conformational states of integrin presenting low affinity (inactive) or high affinity (active) against its ligands and this heterodimeric receptor acts as a

bidirectional signaling transducer. The binding of the cytoplasmic proteins such as talin and kindlins to the integrin β cytoplasmic domain upregulates the ligand-binding affinity of integrin (inside-out signaling). In contrast, ligand binding to integrins and the subsequent clustering of ligand-bound integrins result in intracellular molecular rearrangements such as focal adhesion formation and cell spreading (outside-in signaling) [1].

α IIb β 3, a major integrin expressed on platelets, is critical for platelet aggregation mediated by bindings of fibrinogen and von Willebrand factor. Since inside-out signaling pathways of α IIb β 3 induce striking conformational changes between inactive and active states, the activation processes of α IIb β 3 have been extensively investigated [2]. Talin, a cytoskeletal protein consisting of an N-terminal head and a C-terminal rod,

has been well characterized as an integrin activator [3,4]. The talin head domain (THD) contains four subdomains: F0, F1, F2, and F3. The F3 domain itself can bind to the $\beta 3$ cytoplasmic domain and exert $\alpha \text{IIb}\beta 3$ activation [5]. Other subdomains also have important roles in the activation [6-8]. The kindlin family members (kindlin-1, -2, and -3), which are focal adhesion proteins, have recently been proven to be critical for integrin activation [9,10]. Kindlin-1 and -2 are widely expressed and kindlin-3 expression is restricted mainly to hematopoietic cells [11]. Several studies suggest that the binding of talin and kindlins to the integrin $\beta 3$ cytoplasmic domain is pivotal for the final step in the inside-out activation of $\alpha \text{IIb}\beta 3$. Moreover, since kindlins synergistically augment talin-dependent $\alpha \text{IIb}\beta 3$ activation, they act as a co-activator of talin [12,13]. However, regulatory molecules other than talin and kindlins necessary to $\alpha \text{IIb}\beta 3$ activation remain to be fully clarified.

Since platelets are inadequate for gene manipulation, the CHO cell system has been used to study essential regulators of integrin $\alpha \text{IIb}\beta 3$ function. For example, $\alpha \text{IIb}\beta 3$ -expressing CHO cells contributed to the elucidation of the functional importance of kindlin-1 and -2 as co-activators and of THD as a direct activator of integrin [10,12]. It was also shown that the Rap1-GTP-interacting adaptor molecule promotes talin-dependent integrin activation in the CHO cell system [14]. A chimeric integrin, $\alpha \text{IIb}\alpha 6\beta 3\beta 1$ or $\alpha \text{IIb}\alpha 5\beta 3$, expressed on CHO cells having the extracellular and transmembrane domains of $\alpha \text{IIb}\beta 3$ connected to the cytoplasmic domains of $\alpha 6\beta 1$ or $\alpha 5\beta 3$ has been constitutively active on CHO cells but susceptible to integrin regulatory proteins [15]. Several integrin regulatory proteins including H-ras, PEA-15, CD98, and talin were characterized in this cell system [15-18]. Thus, the CHO cell system has been utilized to analyze the mechanisms by which integrin function is regulated.

Integrin-linked kinase (ILK) was originally identified as a serine/threonine kinase associated with integrin $\beta 1$ and $\beta 3$ cytoplasmic domains. It consists of three domains: an N-terminal ankyrin repeat domain, a putative pleckstrin homology domain, and a C-terminal kinase domain [19]. Many studies have shown that ILK is widely expressed and involved in interactions between integrins, cytoskeletal proteins, and signaling molecules. A deficiency or aberrant function of ILK resulted in the impairment of adhesion, spreading, migration, proliferation, and survival of the cells [20]. ILK seems to have two functions: that of a scaffold protein and that of a protein kinase, whereas the kinase activity is controversial [21,22]. At focal adhesion sites, ILK forms a heterotrimeric complex composed of the adaptor proteins PINCH and parvin [23-28]. PINCH consists of two members, PINCH-1 and -2, each of which consists of five LIM domains. PINCH-1 and -2 are ubiquitously expressed in mammalian tissues and show overlapping expression in many tissues. Parvin comprises three members, α -, β -, and γ -parvin, and contains N-terminal nuclear localization sequences and two calponin homology domains. In mammalian tissues, α - and β -parvin are ubiquitously expressed but γ -parvin is expressed mainly in hematopoietic tissues. These adaptor proteins are known to directly bind to several cytoplasmic proteins including Nck2 for PINCH and filamentous actin for parvin [25,29]. The ankyrin

repeat domain of ILK binds to PINCH and the kinase domain binds to parvin. ILK interacts directly or indirectly with several other cytoskeletal and signaling proteins and exerts diverse roles in different tissues [30].

In our previous study, we identified ILK as a molecule important for integrin activation, using an expression cloning system as follows. First, we established CHO cells expressing constitutively active integrin $\alpha \text{IIb}\alpha 6\beta 3$ whose αIIb cytoplasmic domain we replaced by that of integrin $\alpha 6\beta$ (parental cells). Next we obtained mutant cells with inactive integrin using genome-wide mutagenesis, and finally isolated an ILK cDNA as a factor that complements the function of inactive $\alpha \text{IIb}\alpha 6\beta 3$ in mutant cells by expression cloning [31]. Although the role of ILK at focal adhesion sites has been well studied, there are only a few reports on the involvement of ILK in integrin activation [32,33]. In the present study, we further investigated the mechanisms by which ILK regulates integrin activation in the CHO cell system.

Materials and Methods

Plasmids

Human wild-type (WT) αIIb and $\beta 3$ subcloned into expression plasmid pcDNA3 (Invitrogen, San Diego, CA) were provided by Drs P. Newman and G. White (Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI). pRKHA, including full-length mouse talin-1 was a gift from Dr K. Yamada (NIH, Bethesda, MD). The N-terminal head region (residues 1-433) of talin-1 was constructed by polymerase chain reaction (PCR) and subcloned into green fluorescence protein (GFP) containing vector pEGFP-N1 to make a fusion protein of THD with GFP (THD-GFP) (Clontech, Mountain View, CA). Mouse α -parvin cDNA and mouse PINCH-1 cDNA (Thermo Scientific Open Biosystems, Lafayette, CO) were amplified by PCR and then were subcloned into expression plasmids, pcDNA3.1 (Invitrogen) for α -parvin and pBApo-CMV Pur DNA (Takara Bio, Shiga, Japan) for PINCH-1. pcDNA3- $\alpha \text{IIb}\alpha 6\beta$ was created using PCR-based mutagenesis as previously described [31]. Nucleotide and amino acid numbers begin with the start codon (ATG) and the first Met residue, respectively. The full length of rat ILK cDNA was amplified by PCR then subcloned into pcDNA3 and GFP-encoding plasmid pAcGFP1-Hyg-C1 to make a fusion protein of ILK with GFP (GFPILK-WT) (Clontech). Three point mutations (H99D/F109A/W110A) in the ankyrin repeat domain of ILK were introduced into pAcGFP1-Hyg-C1 to make a fusion protein of the ILK mutant with GFP (GFPILK-H99D/F109A/W110A). Two point mutations (M402A/K403A) in the ILK kinase domain were introduced in pAcGFP1-Hyg-C1 to make a fusion protein of the ILK mutant with GFP (GFPILK-M402/K403A). The ILK mutant (H99D/F109A/W110A) was designed to disrupt the PINCH binding based on the crystal structure of a complex of the ankyrin repeat domain of ILK with the LIM1 domain of PINCH, PDB 3F6Q [34]. The ILK mutant (M402A/K403A) was designed to disrupt the parvin binding as previously reported [35]. Expression plasmid pCMV-SPORT6, containing full-length mouse kindlin-2 (Thermo Scientific Open Biosystems) was obtained. All PCR-generated DNA inserts were verified by

sequencing using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Cell Cultures

CHO-K1 cells from ATCC were cultured in DMEM supplemented with 10% fetal bovine serum and 1% non-essential amino acids (Sigma-Aldrich, St. Louis, MO). CHO cells stably expressing constitutively active α IIb β 3 (parental cells) were previously established [31]. CHO-K1 cells were cotransfected with pcDNA3- α IIb β 3 and pcDNA3- β 3 using Lipofectamine 2000 (Invitrogen) and selected with 1 mg/ml G418 (Nacalai Tesque, Kyoto, Japan). G418-resistant cells expressing α IIb β 3 were cloned to isolate parental cells by a limiting dilution method. ILK-deficient mutant cells, which result in its inactive form from active α IIb β 3 (mutant cells), were previously established by the introduction of random mutations into the parental cells using a chemical mutagen, ethyl methane sulfonate (EMS) [31]. For α IIb β 3-expressing CHO cells, pBApo-CMV Pur DNA- α IIb and pcDNA3- β 3 were cotransfected into CHO-K1 cells by Lipofectamine 2000. After selection with 12 μ g/ml puromycin (Clontech) and 1 mg/ml G418, clones expressing α IIb β 3 were established by the limiting dilution method.

Flow cytometry

Flow cytometry analyses were performed as previously described [31]. Cells suspended in Tyrode's buffer containing 1.5 mM CaCl₂, 1 mM MgCl₂, and 1% bovine serum albumin were incubated with the primary antibody of 5 μ g/ml of a mouse monoclonal antibody (mAb) specific for α IIb β 3, HIP8 (BD Biosciences) for 30 minutes at 4°C. After washing, the cells were incubated with the secondary Ab of ~1 μ g/ml phycoerythrin (PE)-conjugated rat anti-mouse IgG (BD Biosciences) for 30 minutes at 4°C, washed once, stained with 1 μ g/ml 7-aminoactinomycin D (7AAD) (Sigma-Aldrich) to discriminate dead cells, and then analyzed on a flow cytometer (FACSCalibur; BD Biosciences). As a negative control, cells were incubated with the secondary Ab alone. For the binding of a ligand-mimetic, activation-specific anti- α IIb β 3 mAb, PAC-1 (BD Biosciences), cells were incubated with 10 μ g/ml PAC-1 for 30 minutes at room temperature in the absence or presence of 10 μ M of a peptidomimetic antagonist of α IIb β 3, Ro44-9883 (a gift from Astellas Pharma, Tokyo, Japan), washed once, and then incubated with 10 μ g/ml PE-conjugated anti-mouse IgM (eBioscience, San Diego, CA) for 30 minutes at 4°C. After washing, cells were stained with 7AAD and then analyzed. As a positive control for PAC-1 binding, cells were incubated with 15 mM dithiothreitol (DTT) for 10 minutes at 37°C to activate integrin α IIb β 3 and incubated with PAC-1 as mentioned above. Integrin activation was quantified as an activation index calculated using the following formula: $100 \times (F - F_0) / (F_{max} - F_0)$, where F is the median fluorescence intensity (MFI) of PAC-1 binding, F_0 is the PAC-1 binding in the presence of Ro44-9883, and F_{max} is the maximal PAC-1 binding in the cells treated with DTT. For fibrinogen binding, cells were incubated with 150 μ g/ml Alexa-Fluor 647-conjugated fibrinogen (Molecular Probes, Eugene, OR) under similar conditions to the above assay. In some experiments using

α IIb β 3-expressing CHO cells, the activation indexes were normalized by α IIb β 3 expression, as shown by the following formula: $100 \times (F - F_0) / (F_1 - F_2)$, where F and F_0 are the same as mentioned above, F_1 is the HIP8 binding, and F_2 is the binding of the secondary Ab alone.

Immunoblotting

Immunoblotting was performed using procedures previously described [31]. In brief, cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were incubated with either one of the following primary Abs: 0.125 μ g/ml mouse mAb specific for ILK, 3/ILK (BD Biosciences), 0.25 μ g/ml mouse mAb specific for PINCH, 49/PINCH (BD Biosciences), rabbit polyclonal Ab specific for α -parvin (IgG fraction; 1:3,000) (Sigma-Aldrich), 1 μ g/ml mouse mAb specific for β -parvin, 11A5 (Millipore, Temecula, CA), mouse mAb specific for talin, 8D4 (ascites fluid; 1:2,000) (Sigma-Aldrich), rabbit polyclonal Ab specific for kindlin-2 (IgG fraction; 1:1,000) (ProteinTech Group, Chicago, IL), 0.5 μ g/ml rabbit polyclonal Ab specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), FL-335 (Santa Cruz Biotechnology), horseradish peroxidase (HRP)-conjugated rabbit polyclonal Ab specific for β -actin (IgG fraction; 1:2000) (MBL, Woburn, MA), or 1 μ g/ml mouse mAb specific for GFP, B-2 (Santa Cruz Biotechnology) for 90 minutes at room temperature. After washing, bound Abs except for the HRP-conjugated Abs were incubated with peroxidase-conjugated secondary Abs (Kirkegaard & Perry Labs, Gaithersburg, MD). Detection was performed using a chemiluminescence kit (Immobilon Western; Millipore, Bedford, MA). Chemiluminescence was visualized by an image analyzer, LAS-3000PLUS (Fuji Photo Film, Kanagawa, Japan).

Immunoprecipitation

Parental cells were solubilized at concentrations of 2×10^7 cells/ml in a lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, and 1% Triton X-100) containing proteinase inhibitors. After centrifugation at 15,000 \times g for 12 min, the supernatant (200 μ l) was subjected to immunoprecipitation using protein A/G agarose (Santa Cruz Biotechnology) and the following Abs: 1 μ g mouse mAb specific for ILK, 3/ILK, 1 μ g mouse mAb specific for PINCH, 49/PINCH, 1 μ g mouse IgG₁ isotype control, MOPC 21 (Sigma-Aldrich), 1 μ g mouse IgG_{2a} isotype control, UPC-10 (Sigma-Aldrich), 1 μ g rabbit polyclonal Ab specific for α -parvin, and 1 μ g pooled rabbit IgG (Invitrogen). The immunoprecipitants were analyzed by immunoblotting as described above. As a positive control, cell lysates (15 μ l) were loaded onto a lane.

Short interfering RNA (siRNA) and transfection

Total RNA from parental cells was extracted with Trizol reagent (Invitrogen). PINCH-1, α - and β -parvin, and kindlin-2 mRNA were amplified by a one-step RT-PCR kit (Qiagen, Valencia, CA) using primers specific to both mouse and rat homologues according to the manufacturer's instructions. RT-PCR products were directly sequenced using specific primers.

siRNAs against RNA targets were designed and synthesized by Invitrogen (Stealth RNAi). The siRNA target sequences of hamster mRNA are as follows: PINCH-1 (p) 157 sense 5'-CGGGUUAUUAAGCCAUGAACACA-3'; PINCH-1 (p) 755 sense 5'-CCTGCAATACCAAATTAACACTCAA-3'; α -parvin (pa) 503 sense 5'-CCAGGAGCATCAAGTGGAAATGTAGA-3'; α -parvin (pa) 761 sense 5'-CAGACAAGCTCAACGTGGTAAAGAA-3'; β -parvin (pb) 900 sense 5'-UCCACAACUUCUACCUGACACCUGA-3'; β -parvin (pb) 1011 sense 5'-AAGAUGUGUAAACUUGGACCUCAA-3'; kindlin-2 (k) 770 sense 5'-GAUCGCUAAUGGAACAAGAUGUGUGAA-3'; kindlin-2 (k) 770 scrambled control sense 5'-GAUAUCGUAAAGAACUAGUGCGGAA-3'; kindlin-2 (k) 1733 sense 5'-AAGGCGCAAGAGAGAAGAACUUUAU-3'; kindlin-2 (k) 1733 scrambled control sense 5'-AAGCGGGAAAGAAAGAAGUCGCUAU-3'. The sequences of ILK siRNA (Ilk1255) and its scrambled control were previously described [31]. Stealth RNAi-negative control duplexes (Invitrogen) were used as controls in knockdown experiments targeting PINCH-1 and parvins. Cells cultured in six-well plates were transfected with 12.5–50 nM siRNA using Lipofectamine RNAiMAX (Invitrogen) or cotransfected with 30 nM siRNA and the indicated amounts of plasmid (0.5 μ g pEGFP-N1 encoding THD-fused GFP or 0.015 μ g pEGFP-N1 plus 0.485 μ g pcDNA3 as a negative control) using Lipofectamine 2000. Transfected cells were usually analyzed at 72 hours after transfection. For transfections with plasmid DNA alone, Lipofectamine 2000 was employed.

Statistical analysis

The statistical significance of observed data was determined using one-way analysis of variance followed by Bonferroni's post hoc test using PRISM 5 software (GraphPad Software; La Jolla, CA, USA). P values of 0.05 or less were considered statistically significant.

Results

Evaluation of PINCH, α -parvin, talin, and kindlin-2 in ILK-deficient α IIb β 3-inactive mutant CHO cells and α IIb β 3-active parental CHO cells

We previously obtained ILK-deficient mutant cells by treating parental cells expressing constitutively active α IIb β 3 with the chemical mutagen EMS [31]. In the mutant cells, ILK mRNAs contained two nonsense mutations, R317X and W383X, in a compound heterozygous state, resulting in a complete lack of ILK expression. It has been shown that ILK forms a ternary complex with PINCH and parvins to make an IPP complex [25]. To assess the role of ILK-binding proteins in ILK-deficient mutant cells with the inactive state of α IIb β 3, we examined the protein expression of ILK-binding adaptor proteins, PINCH and α -parvin. In addition to a lack of ILK expression, mutant cells showed severe reductions in the protein expression of PINCH and α -parvin as compared to parental cells. In contrast, talin and kindlin-2, which play critical roles in integrin activation, were present at normal levels of protein expression (Figure 1A). Transfection of a plasmid

encoding ILK cDNA into mutant cells showed the increased expression of ILK and concomitant increases in PINCH and α -parvin expression levels but did not affect talin and kindlin-2 expression levels. Moreover, flow cytometry using an activation-specific anti- α IIb β 3 mAb, PAC-1, showed that ILK-plasmid transfection increased PAC-1 binding compared to empty-plasmid transfection (Figure 1B). These data suggest that ILK, PINCH, and α -parvin are necessary to restore the active state of α IIb β 3 in mutant cells.

Detection and assessment of ILK, PINCH, and parvin (IPP) complex in α IIb β 3-active parental cells

Since ILK, PINCH, and parvins form the IPP complex, we assessed IPP complex formation in α IIb β 3-active parental cells, which show constitutively active α IIb β 3. Immunoprecipitation experiments revealed that ILK is coprecipitated with PINCH and α -parvin, indicating the presence of the IPP complex in those cells (Figure 2). To evaluate the importance of these proteins comprising the complex on the active state of α IIb β 3, we performed RNA interference (RNAi) experiments targeting PINCH or parvins, and we analyzed the active state of α IIb β 3 by flow cytometry using PAC-1. For PINCH siRNA, we targeted PINCH-1, one of the two PINCH isoforms, because we failed to find sequences of PINCH-2 mRNA in CHO cells. Each of the two PINCH-1 siRNAs (p157 and p755) decreased PINCH expression and concomitantly decreased ILK and α -parvin expression compared to nontargeting negative control siRNA in parental cells (Figure 3A), leading to a decreased integrin activation index, which was determined by flow cytometry analysis of PAC-1 binding (Figure 3B). For parvin siRNA, α - and β -parvins were knocked down since both parvins are thought to bind to ILK. A mixture of two α -parvin siRNAs (pa503 and pa761) or two β -parvin siRNAs (pb900 and pb1011) reduced α -parvin or β -parvin expression, respectively; however, ILK and PINCH expression levels were less significantly affected (Figure 3C). When a mixture of α -parvin and β -parvin siRNAs was transfected into parental cells, the expression levels of α -parvin and β -parvin were decreased and concomitant decreases in ILK and PINCH expression were observed. Flow cytometry analysis evaluating the activation state exhibited that the transfection of both α - and β -parvin siRNAs, but not that of either α - or β -parvin siRNA significantly decreased PAC-1 binding (Figure 3D). These data suggest that the IPP complex formation of ILK, PINCH, and parvins is necessary for α IIb β 3 activation in a CHO cell system.

Knockdown of kindlin-2 in α IIb β 3-active parental cells

In our previous work, talin siRNA decreased PAC-1 binding to α IIb β 3-active parental cells [31]. To confirm that kindlin-2 plays an important role in integrin activation in the CHO cell system, we performed the kindlin-2 siRNA experiment in parental cells (Figure 3E, F). Each of two different siRNAs (k770 and k1733) reduced kindlin-2 expression and decreased PAC-1 binding in association without significantly affecting ILK or talin expression. In addition, when an ILK cDNA was cotransfected with kindlin-2 siRNA into ILK-deficient mutant

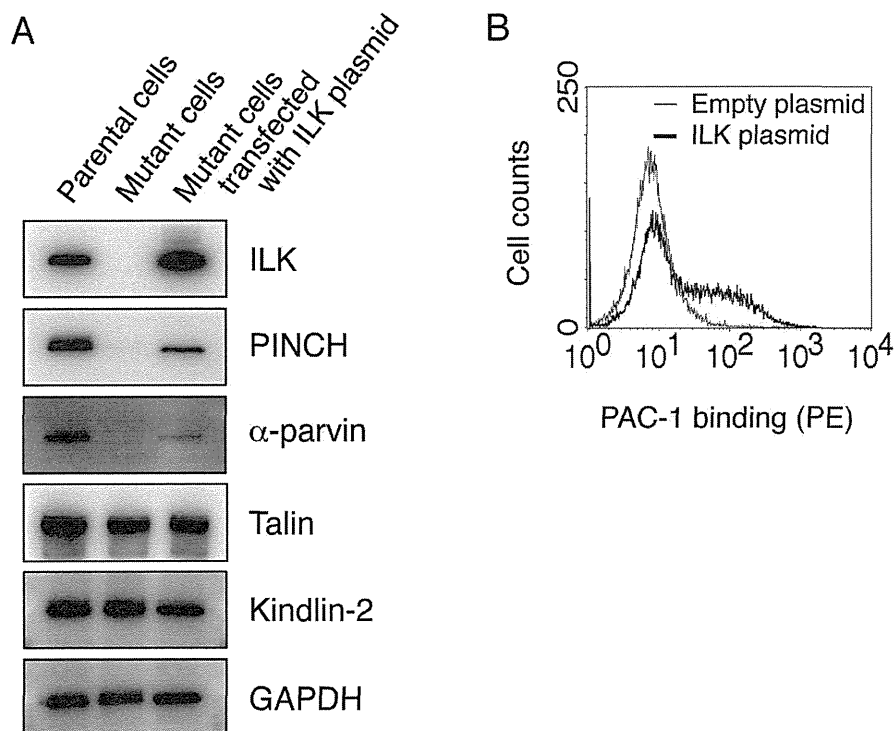


Fig. 1

Figure 1. Characterization of ILK-deficient mutant cells expressing inactive α IIb β 3. (A) Immunoblotting for ILK, PINCH, α -parvin, talin, and kindlin-2. Cell lysates obtained from parental cells with constitutively active α IIb β 3, ILK-deficient mutant cells with inactive α IIb β 3, and mutant cells transiently transfected with rat ILK cDNA were electrophoresed on SDS-PAGE gels and immunoblotted with indicated Abs. GAPDH shows an internal loading control. (B) Flow cytometry analysis showing PAC-1 (an activation-specific mAb for α IIb β 3) binding to mutant cells transiently transfected with either ILK plasmid or empty plasmid. Bound PAC-1 was detected with a PE-conjugated secondary mAb.

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cells, PAC-1 binding was decreased as compared to those of the cotransfection of both the ILK cDNA and scrambled kindlin-2 siRNA (data not shown). These data indicate that kindlin-2 is required for α IIb β 3 activation in parental cells.

Role in binding of ILK to PINCH and parvin for integrin activation

To examine the significance of ILK-PINCH binding for integrin activation, we generated a GFP-fused ILK mutant (GFPILK-H99D/F109A/W110A) in which mutations were introduced into the binding sites for the LIM1 domain of PINCH in the ankyrin repeat domain of ILK. This ILK mutant is designed to disrupt ILK-PINCH binding but not ILK- α -parvin binding. When GFPILK-WT cDNA was transfected into mutant cells, PAC-1 binding was increased (Figure 4A). Transfection of the GFPILK-H99D/F109A/W110A cDNA into mutant cells failed to recover PAC-1 binding and did not induce an obvious upregulation of PINCH expression, whereas the ILK mutant

protein was well expressed and α -parvin was similarly increased compared to the case with GFPILK-WT cDNA transfection, indicating the ILK- α -parvin complex (Figure 4B). These data suggest that ILK-PINCH binding is required for stable PINCH expression even in the presence of ILK, as well as for α IIb β 3 activation in the CHO cell system. When cell lysates of the mutant cells transfected with the GFPILK-H99D/F109A/W110A cDNA was subjected to immunoprecipitation with anti- α -parvin Ab, the ILK mutant was coprecipitated (data not shown). In addition, we generated a GFP-fused ILK mutant (GFPILK-M402A/K403A) that disrupts the parvin binding and that impairs the localization of ILK to focal adhesions as shown in the previous report [35]. Transfection of GFPILK-M402A/K403A cDNA into mutant cells showed strongly impaired PAC-1 binding and did not induce an overt upregulation of α -parvin expression (Figure 4C, D). These data suggest that ILK- α -parvin binding is necessary for stable parvin expression, as well as for α IIb β 3 activation in the CHO cell system.

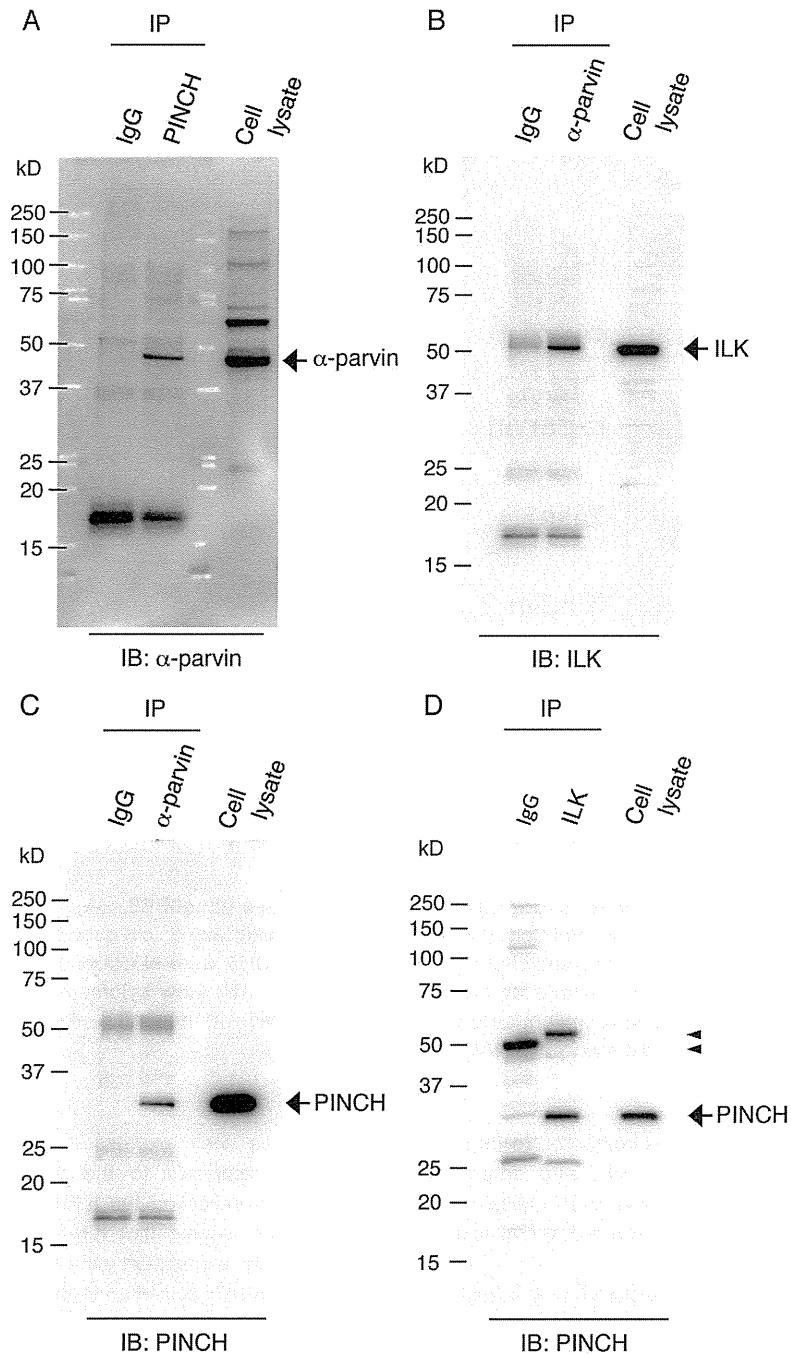


Fig. 2

Figure 2. Detection of IPP complex proteins in $\alpha 11\beta 6\beta 3$ -active parental cells. Cell lysates obtained from $\alpha 11\beta 6\beta 3$ -active parental cells were immunoprecipitated with Abs against PINCH (A), α -parvin (B, C), and ILK (D). The co-precipitates were detected by Abs for α -parvin (A), ILK (B), and PINCH (C, D). IgG means immunoprecipitation (IP) using non-immune control IgG. IB stands for immunoblotting. Arrows indicate the predicted sizes of the indicated proteins. Arrowheads (D) indicate the antibody heavy chains used in the IP. Different mobilities between those of the two IgG antibodies are probably caused by differences in the amino acid compositions of them.

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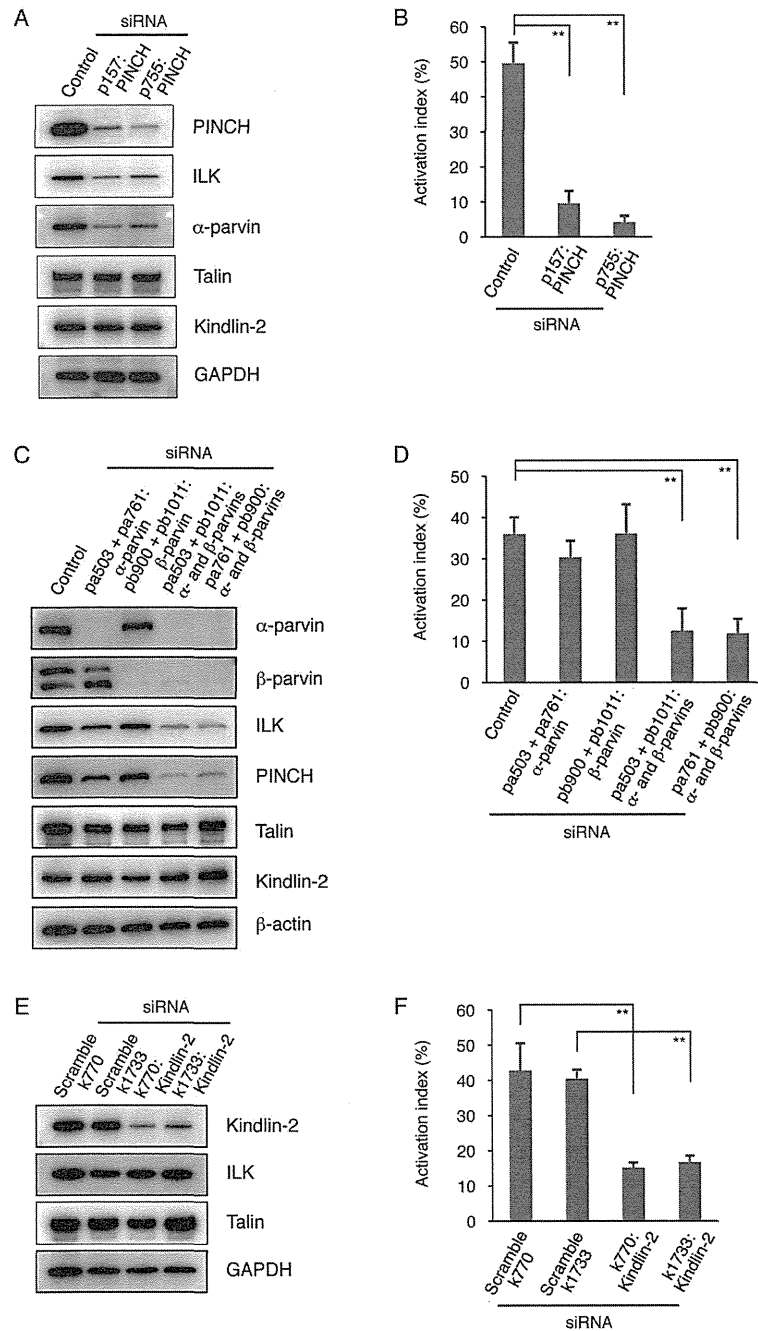


Fig. 3

Figure 3. Knockdown effects of PINCH, parvins, and kindlin-2 in $\alpha 5 \beta 1 \beta 3$ -active parental cells. $\alpha 5 \beta 1 \beta 3$ -active parental cells were transiently transfected with PINCH siRNAs (p157 and p755) (A), α -parvin siRNAs (pa503 and pa761) (C), β -parvin siRNAs (pb900 and pb1011) (C), kindlin-2 siRNAs (k770 and k1733) (E), negative control siRNAs, and scrambled siRNAs. Cell lysates were electrophoresed on SDS-PAGE gels, and the separated proteins were immunoblotted with the indicated Abs. GAPDH and β -actin are shown as internal loading controls. The activation indexes of transfected cells (B, D, F) were calculated using the formula shown in Materials and Methods. A value of 100% implies the maximum PAC-1 binding to the cells treated with dithiothreitol (DTT). Data represent means \pm standard deviation (SD) of three (B, F) or four (D) independent experiments. ** indicates $P < 0.01$.

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Moreover, when fibrinogen, a natural ligand for α IIb β 3, was used instead of PAC-1 in these experiments, similar results were obtained (Figure 4E).

Analysis of ILK in inactive α IIb β 3-expressing CHO cells

α IIb β 3 is present in an inactive state on CHO cells, and overexpression of the THD into those cells can induce α IIb β 3 activation [36]. The THD directly binds the integrin β 3 cytoplasmic domain and causes integrin activation. To examine ILK's contribution to THD-mediated α IIb β 3 activation, we performed knockdown experiments targeting ILK under transient THD expression (Figure 5). For the cotransfection of THD-GFP cDNA with scrambled ILK siRNA (scramble Ilk1255), the THD-GFP highly expressing cells exhibited a significant increase in the PAC-1 binding as compared to the case with GFP cDNA cotransfection (Figure 5A, B). In contrast, the cotransfection of THD-GFP cDNA with ILK siRNA (Ilk1255) showed decreased PAC-1 binding in the cells with high expression of THD-GFP (Figure 5A, B). The protein expression levels of ILK, PINCH, and α -parvin were suppressed by the cotransfection of ILK siRNA and THD-GFP cDNA, whereas the expression level of THD-GFP was not changed in the presence of ILK siRNA (Figure 5C). These results suggest that THD is sufficient to restore partially the integrin activation upon elimination of ILK by its siRNA (Ilk1255) and that ILK may assist THD in regulating the integrin activation state by assembling the IPP complex. In addition, these findings obtained from α IIb β 3-expressing CHO cells support the importance of the IPP complex observed in the α IIb β 3-expressing CHO cells. □

Overexpression of the IPP complex in inactive α IIb β 3-expressing CHO cells

To examine the IPP complex's role in THD-mediated α IIb β 3 activation, we performed overexpression experiments of ILK, PINCH-1, and α -parvin in inactive α IIb β 3-expressing CHO cells. As expected, THD-GFP overexpression into α IIb β 3-expressing CHO cells induced PAC-1 binding in the cells with high expression of THD-GFP, as compared to the case with GFP cDNA transfection (Figure 6A, B). Interestingly, although quadruple overexpression of GFP and IPP did not significantly increase PAC-1 binding, quadruple overexpression of THD-GFP and IPP caused higher PAC-1 binding compared to the case with THD-GFP overexpression, suggesting a supportive effect of IPP on THD-mediated α IIb β 3 activation (Figure 6A, B). Kindlin-2 binds to the integrin β 3 cytoplasmic domain and functions as a co-activator of talin [12,37]. As expected, double overexpression of THD-GFP and kindlin-2 cooperatively increased PAC-1 binding (Figure 6A, B), suggesting that both THD and kindlin-2 are required for the full activation of α IIb β 3. Regarding protein expression, THD-GFP was adequately expressed in each transfection, and the expression levels of ILK, PINCH, α -parvin, and kindlin-2 were higher than their endogenous expression levels in the cells with indicated transfection (Figure 6C). Thus, these data suggest that the IPP complex supports the THD for integrin α IIb β 3 activation.

Discussion

ILK is a multidomain scaffold protein that interacts with several cytoplasmic proteins [38,39]. In integrin adhesion sites, ILK exists in a ternary complex composed of the two other proteins PINCH and parvin. The ternary complex formation can stabilize each component and exert its function. In fact, ILK-deficient mutant CHO cells exhibited profoundly reduced PINCH and α -parvin expression levels, leading to inactive α IIb β 3 (Figure 1). The introduction of ILK expression into ILK-deficient mutant cells increased the expression levels of PINCH and α -parvin, accompanied by α IIb β 3 activation (Figure 1). The involvement of the IPP complex formation in integrin activation was confirmed in the knockdown experiments of PINCH and parvins in α IIb β 3-active parental cells (Figure 3). The ILK mutants with defects in either PINCH or parvin binding did not activate α IIb β 3 in ILK-deficient mutant cells (Figure 4). Since it has been reported that the parvin-binding defective ILK mutant (M402A/K403A) fails to localize to focal adhesions [35], these two ILK mutants are probably not recruited to the α IIb β 3 sites in a process of integrin activation. Thus, our data support a previous report that the proper complex formation of ILK, PINCH, and parvin is necessary for ILK recruitment to the integrin adhesion sites [35,40].

Recent studies of integrin regulatory proteins have shown that both talin and kindlins directly bind to different regions in the integrin β cytoplasmic domain and cooperate in a final step of integrin activation [9,12]. In our experiments, kindlin-2 knockdown in α IIb β 3-active parental cells reduced the activation state of α IIb β 3, and talin knockdown exhibited similar results in our previous work [31]. The knockdown of PINCH and of parvins in the IPP complex decreased the activation state of α IIb β 3 in parental cells to a similar extent as did the knockdown of either kindlin-2 or talin. In inactive α IIb β 3-expressing CHO cells, overexpression of THD-GFP induced α IIb β 3 activation, and ILK knockdown reduced THD-GFP-mediated α IIb β 3 activation (Figure 5). Moreover, overexpression of the IPP complex further augmented the activation state of α IIb β 3 induced by the THD in inactive α IIb β 3-expressing CHO cells (Figure 6). These data suggest that the IPP complex participates in the cooperation of talin and kindlin-2 during the activation processes of not only α IIb β 3 but also α IIb β 3. The precise binding sites of ILK in the integrin β cytoplasmic domain remain to be determined, although their interaction has been reported [19]. There seem to be two possible direct and indirect manners of ILK binding to the integrin cytoplasmic domain. It was recently reported that the binding of PAT-4 (ILK) to UNC-112 (kindlin) in *C. elegans* is necessary for UNC-112 recruitment to adhesion sites [41]. While kindlin alone appears to bind to integrin in mammalian cells, the IPP complex would contribute to effective binding of kindlin to the β integrin cytoplasmic domain to fully induce conformational changes of integrin.

Adaptor proteins, PINCH-1 and -2, share high amino acid sequence identity [27]. Those are ubiquitously expressed in different tissues and show overlapping expression in many tissues. Both isoforms bind equally well to ILK, but its binding is

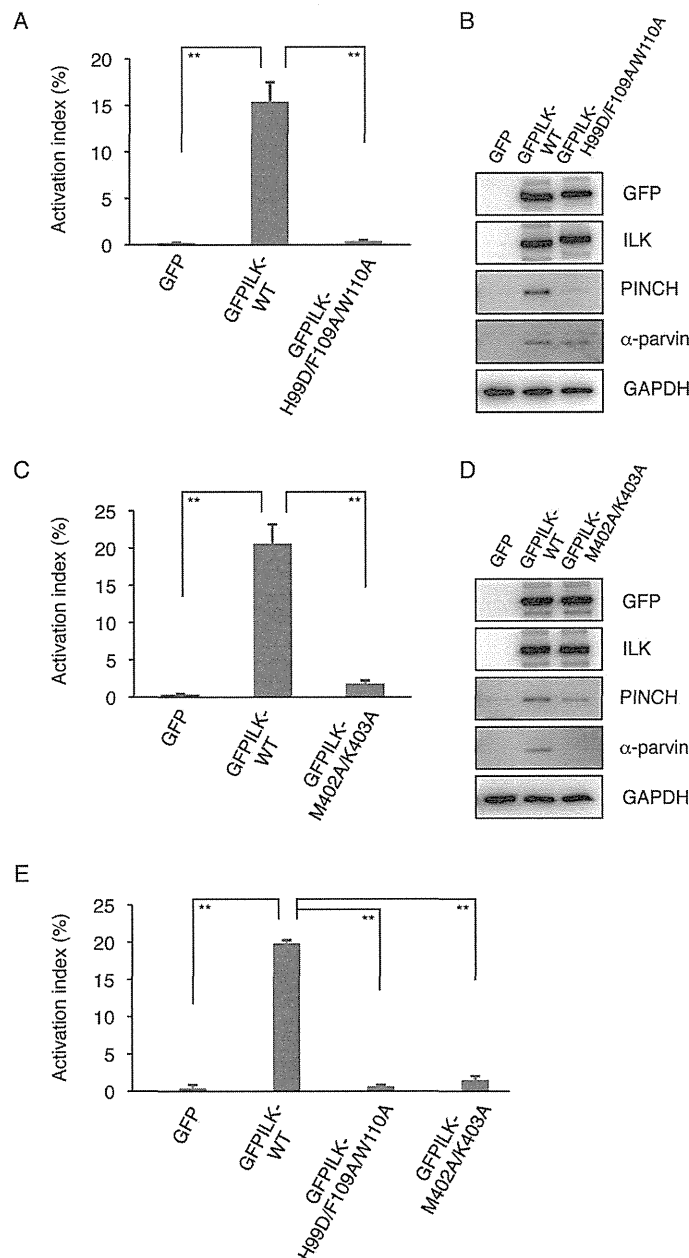


Fig.4

Figure 4. Effects of ILK mutants with defects in either PINCH or parvin binding. The activation indexes of transfected cells (A, C, E). ILK-deficient mutant cells were transiently transfected with GFP cDNA, GFP-fused wild-type ILK (GFPILK-WT) cDNA, GFP-fused ILK mutant with defective PINCH binding (GFPILK-H99D/F109A/W110A) cDNA (A, E), or GFP-fused ILK mutant with defective parvin binding (GFPILK-M402A/K403A) cDNA (C, E). After transfection, the binding of either PAC-1 (A, C) or fibrinogen (E) to the cells was analyzed by flow cytometry. The activation index was determined by the formula shown in Materials and Methods. A value of 100% represents the maximal binding of PAC-1 or fibrinogen to the cells treated with dithiothreitol. Data represent means \pm SD of three independent experiments. ** indicates $P < 0.01$. Immunoblotting showing protein expression of GFP (B, D), GFP-fused wild-type ILK (GFPILK-WT) (B, D), GFP-fused ILK mutant with defective PINCH binding (GFPILK-H99D/F109A/W110A) (B), and GFP-fused ILK mutant with defective parvin binding (GFPILK-M402A/K403A) (D) in ILK-deficient mutant cells. Cell lysates were electrophoresed and immunoblotted with indicated Abs.

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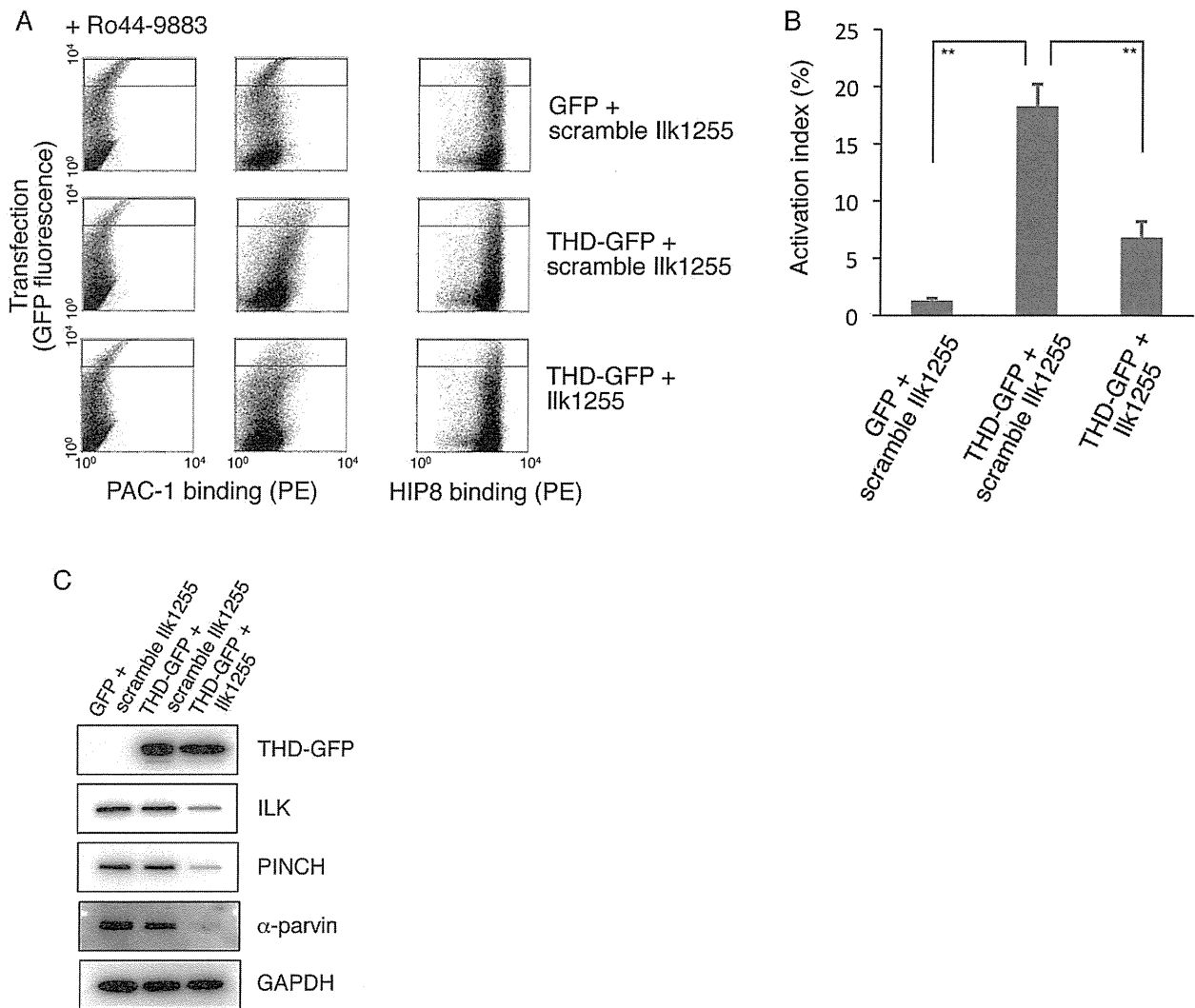


Fig. 5

Figure 5. Knockdown effects of ILK on THD-mediated α IIb β 3 activation. (A) Dot plot detecting PAC-1 binding to cotransfected cells. Inactive α IIb β 3-expressing CHO cells were transiently cotransfected with GFP cDNA plus scrambled ILK siRNA (scramble Ilk1255), with THD-GFP cDNA plus scrambled ILK siRNA (scramble Ilk1255), or with THD-GFP cDNA plus ILK siRNA (Ilk1255). Highly transfected cells (cells in gated regions) were analyzed for PAC-1 binding or HIP8 (an α IIb β 3-specific mAb) binding. (B) The activation indexes of transfected cells. The activation index was determined by the formula shown in Materials and Methods. A value of 100% implies the median fluorescence intensity of HIP8 binding to the cells in gated regions. Data represent means \pm SD of three independent experiments. ** indicates $P < 0.01$. (C) Immunoblotting to evaluate expression levels of IPP and THD-GFP. Cell lysates were electrophoresed and immunoblotted with indicated Abs.

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mutually exclusive [34,42]. PINCH-1 is expressed in hematopoietic systems, and strong expression of it has been observed in megakaryocytes during fetal liver hematopoiesis [27]. PINCH-2 also joins in the IPP complex and contributes to the stabilization of individual proteins. We examined only

PINCH-1 expression in CHO cells since we were unable to find PINCH-2 mRNA in parental CHO cells. Our knockdown experiment using PINCH-1-specific siRNA revealed the reduction of both ILK and α -parvin expression levels. In addition, published amino acid sequences of hamster PINCH-2

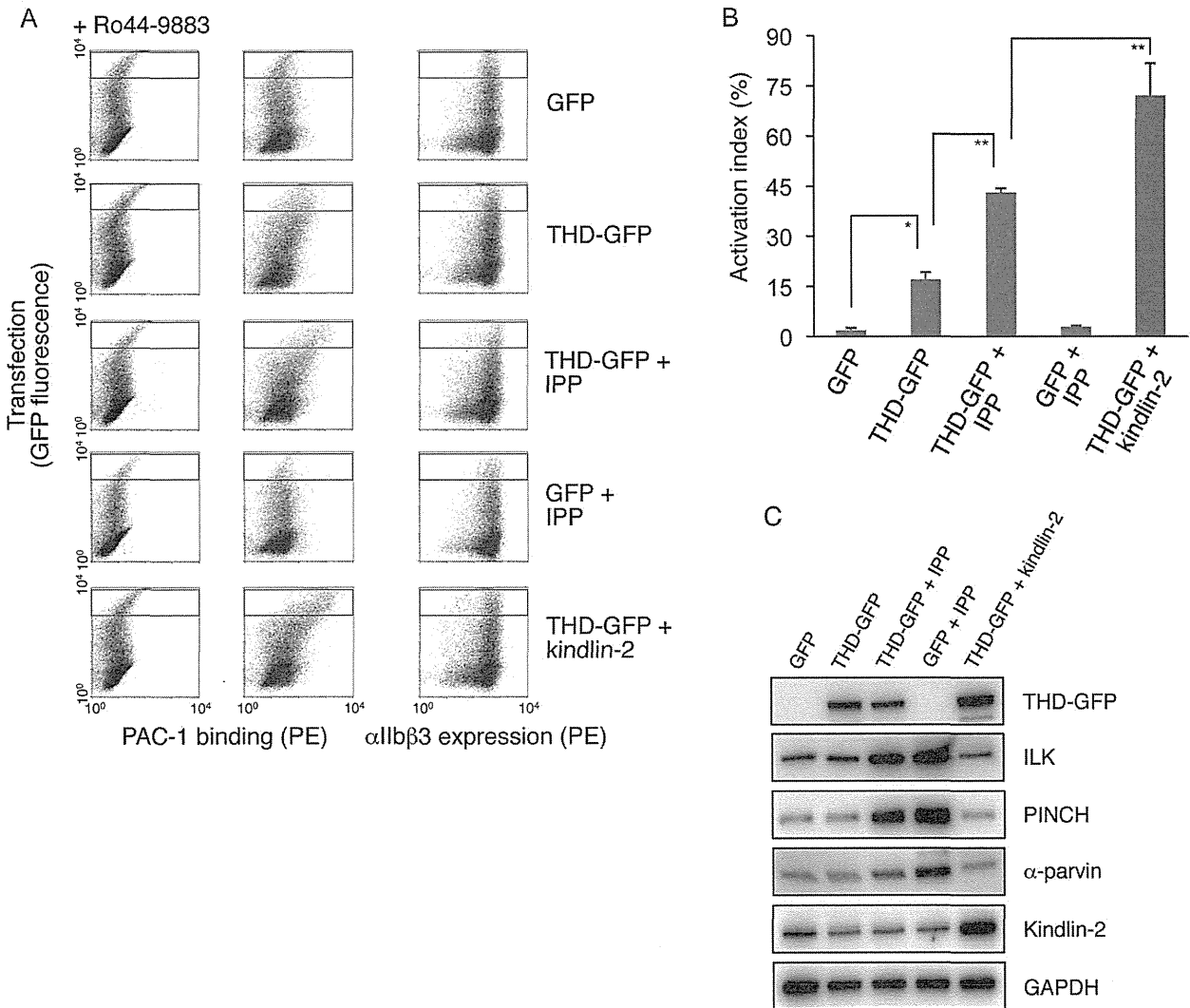


Fig. 6

Figure 6. Effects of IPP overexpression on THD-mediated integrin activation in inactive α IIb β 3-expressing CHO cells. (A) Dot plot detecting PAC-1 binding to transfected cells. Inactive α IIb β 3-expressing CHO cells were transiently transfected with GFP cDNA, with THD-GFP cDNA, with THD-GFP cDNA plus IPP (ILK, PINCH, and α -parvin) cDNAs, with GFP cDNA plus IPP cDNAs, or with THD-GFP cDNA plus kindlin-2 cDNA. Highly transfected cells in the gated regions were analyzed for PAC-1 binding or HIP8 (an α IIb β 3-specific mAb) binding. (B) The activation indexes of transfected cells. The index was determined by the formula shown in Materials and Methods. A value of 100% implies the median fluorescence intensity of HIP8 binding to the cells in gated regions. Data represent means \pm SD of three independent experiments. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively. (C) Immunoblotting to evaluate expression levels of IPP, THD-GFP, and kindlin-2. Cell lysates obtained from transfected cells were electrophoresed and immunoblotted with indicated Abs.

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(GenBank accession number EGW10997) showed an amino acid length composed of 144 residues, shorter than that of mouse PINCH-2 (accession number NP659111) composed of

341 residues. This suggested that proper PINCH-2 may not be expressed in CHO cells. Unlike PINCH, α - and β -parvins were expressed in CHO cells and the knockdown of both parvins but

not either α - or β -parvin decreased ILK and PINCH expression to a similar extent as the parvins. Thus, the parvins are complemented with each other in the formation of the IPP complex, and either one seems to support integrin activation by maintaining the IPP complex.

Platelets are likely to have α - and β -parvins, and both parvins contribute to the formation of the IPP complex [43,44]. The functional importance of the IPP complex for platelet integrin regulation has not been fully elucidated. There are only a few reports in which the IPP complex stably exists to a similar extent between resting and stimulated platelets [43,44]. It has been shown in human platelets that ILK is activated and binds to the β subunit of α IIb β 3 and the integrin collagen receptor α 2 β 1 after stimulation with thrombin, phorbol 12-myristate 13-acetate, and collagen [45,46]. These processes seem to aggregation-dependently occur in α IIb β 3 or aggregation-independently arise in α 2 β 1. In a recent study using an ILK-conditional knockout mouse, ILK-deficient platelets exhibited reduced abilities of aggregation, fibrinogen binding, and α -granule secretion [33]. The ILK-deficient platelets also showed decreased expression levels of PINCH and α -parvin, suggesting that the IPP complex is involved in the regulation of integrin affinity. In platelets, the IPP complex may be translocated from the cytoplasm to the integrin β cytoplasmic domain in response to agonist stimulation and may participate in the control of integrin affinity.

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V 血小板の異常

血小板減少症

特発性血小板減少性紫斑病

急性特発性血小板減少性紫斑病

Acute ITP

Key words : 小児, ウイルス感染, 予防接種, 6カ月, 急性発症

富山佳昭

1. 疾患・定義

特発性血小板減少性紫斑病 (idiopathic thrombocytopenic purpura: ITP) は, 他の基礎疾患や薬剤などの原因が明らかではないにもかかわらず, 血小板の破壊が亢進し減少する後天性の自己免疫疾患である^{1,2)}. ITPに関する国際作業部会により primary immune thrombocytopenia (primary ITP) との名称が新たに提唱されている³⁾.

ITPはその発症様式と経過より, 便宜上, 急性型と慢性型に分類され6カ月以内に自然寛解する病型は急性型, それ以後も血小板減少が持続する病型は慢性型と分類される(表1). しか

しながら, 発症時に急性型か慢性型かを区別することは極めて困難であり, 実際には発症後6カ月経過した時点において寛解したものを急性型, そうでないものを慢性型として分類することになる. 小児ITPでは急性ITPが約75-80%を占め, ウイルス感染や予防接種を先行事象として有する場合が多い. 急性ITPでは数週間で血小板が増加することが多いが, 特に小児においては6カ月以上かけて自然軽快する症例も経験されるため, 6カ月という期間が急性と慢性を分ける分岐点であるとはいいがたく, あくまで便宜上の期間といえる^{1,4)}.

前述のITP国際作業部会では, ITPの持続期間を予測するマーカーがないため, 新規発症

表1 特発性血小板減少性紫斑病の分類

| | 急性ITP | 慢性ITP |
|------|------------------------|--------------------------------|
| 好発年齢 | 2-5歳 | 20-40歳, 60-80歳 小児では10歳以上 |
| 性差 | 男1:女1 | 若年発症例では男1:女3 高齢者では性差なし |
| 好発時期 | 冬~春 | とくになし |
| 発症様式 | 急性の発症 発症時期が明確なことが多い | 発症時期が不明なことが多い 検診などで見つかることあり |
| 先行事象 | ウイルス感染 予防接種 | なし |
| 出血症状 | 強い | 症状を欠く場合もあり |
| 経過 | 6カ月以内に寛解 | 慢性に経過し6カ月以上 |
| 頻度 | 小児ITPの75-80% | 小児ITPの20-25% |

のITPで発症3カ月以内はすべてnewly diagnosed ITPと表記, また新たなカテゴリーとして血小板減少が3-12カ月持続する場合は, persistent ITPと表記することを提唱している。Persistent ITPでは自然寛解する可能性がまだ残っていると考えられており, 脾摘などのより強力な治療法はpersistent ITPに関しては延期してもよいとの意見である。また, ITP国際作業部会案ではchronic ITPは12カ月以上持続する場合となる³⁾。しかしながら, この案はまだまだ世界で認知, 承認されたとはいいがたい。重要なのは, ITPにおいて6カ月以内(あるいは1年以内)で自然寛解する症例が存在することを常に念頭に置くべきであり, この点において‘急性ITP’との表現は重要であるといえる。成人ITPにおいても, その頻度は不明であるが急性ITPは決してまれではない。

2. 病 因

小児における急性ITPの約半数は, 風疹, 水痘, ムンプス, 麻疹, 伝染性単核球症, その他の上気道感染など, ウイルス感染症が先行し急激に発症する。また, 一部(小児の新規診断ITPの約1%)は, これら特定感染症に対する予防接種, 特に生ワクチン接種に引き続いて起こる。血小板膜糖タンパクに対する抗体(特に抗GP IIb-IIIa抗体)は慢性型のみならず急性型においても検出されるが, 予後との関係は明らかではない⁴⁾。

3. 病 態

ITPの主症状は, 皮下出血, 歯肉出血, 鼻出血, 性器出血など皮膚粘膜出血である。血尿, 消化管出血, 吐血, 網膜出血を認めることもある。慢性ITPは, 発症時期が不明なことが多く検診などで偶然血小板減少を指摘されることが多いが, 急性ITPは慢性型に比べ急激に血小板数が減少することが多く, 発症時期が明確であることが多い。冬から春にかけて好発時期である。小児ITPでは, 新規発症例の約2/3において血小板数1万/ μ L未満と著減しており, その約半数に鼻出血や粘膜出血がみられるが, 頭蓋

表2 鑑別すべき先天性血小板減少症

| |
|----------------------------|
| 先天性無巨核球性血小板減少症 |
| 橈骨欠損に伴う血小板減少症(TAR) |
| MYH9異常症 |
| May-Hegglin異常 |
| Sebastian症候群 |
| Fechtner症候群 |
| Epstein症候群 |
| Bernard-Soulier症候群 |
| Wiskott-Aldrich症候群 |
| GP II b-III a異常に起因する血小板減少症 |

内出血, 消化管出血などの重篤な出血は全体の約1%と少ない⁴⁾。

4. 診断と鑑別診断

ITPの診断に関しては, いまだに他の疾患の除外診断が主体となる。詳しい病歴の聴取や身体所見に加え, 特に小児ITPの診断に関しては家族歴の聴取が重要であり, 家族歴を有する場合はMay-Hegglin異常やBernard-Soulier症候群, GP IIb-IIIa異常に起因する血小板減少症などの先天性血小板減少症を念頭に置く必要がある(表2)(本誌における関連稿参照)。

5. 治療と予後

小児急性ITPの場合, 治療しなくても予後が良好であることが多いため, 初期治療のタイミングに関しては一定の見解をみない。実際のところ治療に関しては, 頭蓋内出血の懸念や患児が極度の日常生活の制限を順守できるかが治療開始の判断材料となっている。小児急性ITPでは, 頭蓋内出血の頻度は0.2-1%と報告されており, 血小板1万/ μ L未満にてその危険性が増す。また, 頭蓋内出血は, ITP発症後4週間以内(特に1週間以内)に起こることが示されており, 更に頭部の打撲や血小板機能を抑制する薬剤の投与などが増悪因子となる^{1,4)}。

我が国の‘小児新規診断ITPに対する治療ガイドライン’では血小板1万/ μ L未満なら治療を行うとしている⁵⁾。しかしながら, 小児のICIS group(Intercontinental Childhood ITP Study Group)では血小板数に関係なく出血症状がな

ければ治療の必要はなく、注意深く観察している。出血を伴う場合の治療目標は、可及的速やかに血小板を増加させ出血症状の制御と予防を行うことである。免疫グロブリン大量療法(1g/kg)や高用量副腎皮質ステロイドを用い

る。

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