Cell Adhesion & Migration 7:5, 418–423; September/October 2013; © 2013 Landes Bioscience

Protein tyrosine phosphatase PTP1B in cell adhesion and migration

Carlos O Arregui*, Ángela González, Juan E Burdisso, and Ana E González Wusener

Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH); Universidad Nacional de San Martín; Consejo Nacional de Investigaciones Científicas y Técnicas; Buenos Aires, Argentina

Cell migration requires a highly coordinated interplay between specialized plasma membrane adhesion complexes and the cytoskeleton. Protein phosphorylation/dephosphorylation modifications regulate many aspects of the integrin-cytoskeleton interdependence, including their coupling, dynamics, and organization to support cell movement. The endoplasmic reticulum-bound protein tyrosine phosphatase PTP1B has been implicated as a regulator of cell adhesion and migration. Recent results from our laboratory shed light on potential mechanisms, such as Src/FAK signaling through Rho GTPases and integrin-cytoskeletal coupling.

Keywords: PTP1B, integrins, adhesions, migration, endoplasmic reticulum, α-actinin, Src, cytoskeleton

*Correspondence to: Carlos O Arregui; Email: carregui@iib.unsam.edu.ar

Submitted: 08/05/2013

Revised: 09/03/2013

Accepted: 09/04/2013

http://dx.doi.org/10.4161/cam.26375

Introduction

PTP1B is a non-receptor protein tyrosine phosphatase of 50 kDa, with an N-terminal catalytic domain of 240 residues, followed by a regulatory region containing SH3-binding, sumoylation, and phosphorylation motifs, and a C-terminal tail of 35 amino acids that targets the enzyme to the endoplasmic reticulum (ER).[1,15] The catalytic domain of ER-bound PTP1B faces the cytosol, having the potential for substrate dephosphorylation throughout the extensive and dynamic branching network occupied by the ER. Extension of the ER toward the cell periphery allows PTP1B to dephosphorylate substrates located at the plasma membrane, for example Src, EphA3, and proteins associated to cadherin and integrin complexes.[2-8] Alternatively, PTP1B can dephosphorylate the cytosolic domains of endocytosed receptors, which are brought into contact with the ER, as, for example, activated platelet-derived growth factor and epidermal growth factor receptors.[9] Through the proline-rich motif, PTP1B has also been shown to interact directly with components of focal adhesions, such as the SH3 domain of adaptor protein p130Cas.[10] Under specific stimulation, PTP1B is sensitive to cleavage by calpains at a site upstream from the ER-targeting sequence, producing a soluble and active 42 kDa form.[11] This truncated form of PTP1B is required for Src-dependent formation of invadopodia and breast cancer invasion.[12]

The identification of PTP1B substrates has been greatly benefited by the generation of efficient substrate trap mutants that lock the enzyme and substrate in stable "dead-end" complexes.[13] This approach combined with quantitative mass spectrometry has recently been used to identify PTP1B substrates under different stimulatory conditions.[14]

PTP1B is an important physiological modulator of insulin and leptin receptor signaling in mice and has attracted much interest as a potential drug target for diabetes and obesity.[15,16] Due to its activity on several signaling proteins that promote cell growth, PTP1B has also been implicated in oncogenesis, although its exact role is complex and apparently context dependent.[15,16] It is now recognized that PTP1B has multiple substrates, which, in turn, are involved in a wide range of fundamental cellular processes, such as cell adhesion, signaling, intracellular transport, motility, apoptosis, and proliferation.[14] In this commentary we will focus on accumulating evidence implicating PTP1B in the regulation of cell adhesion and motility.

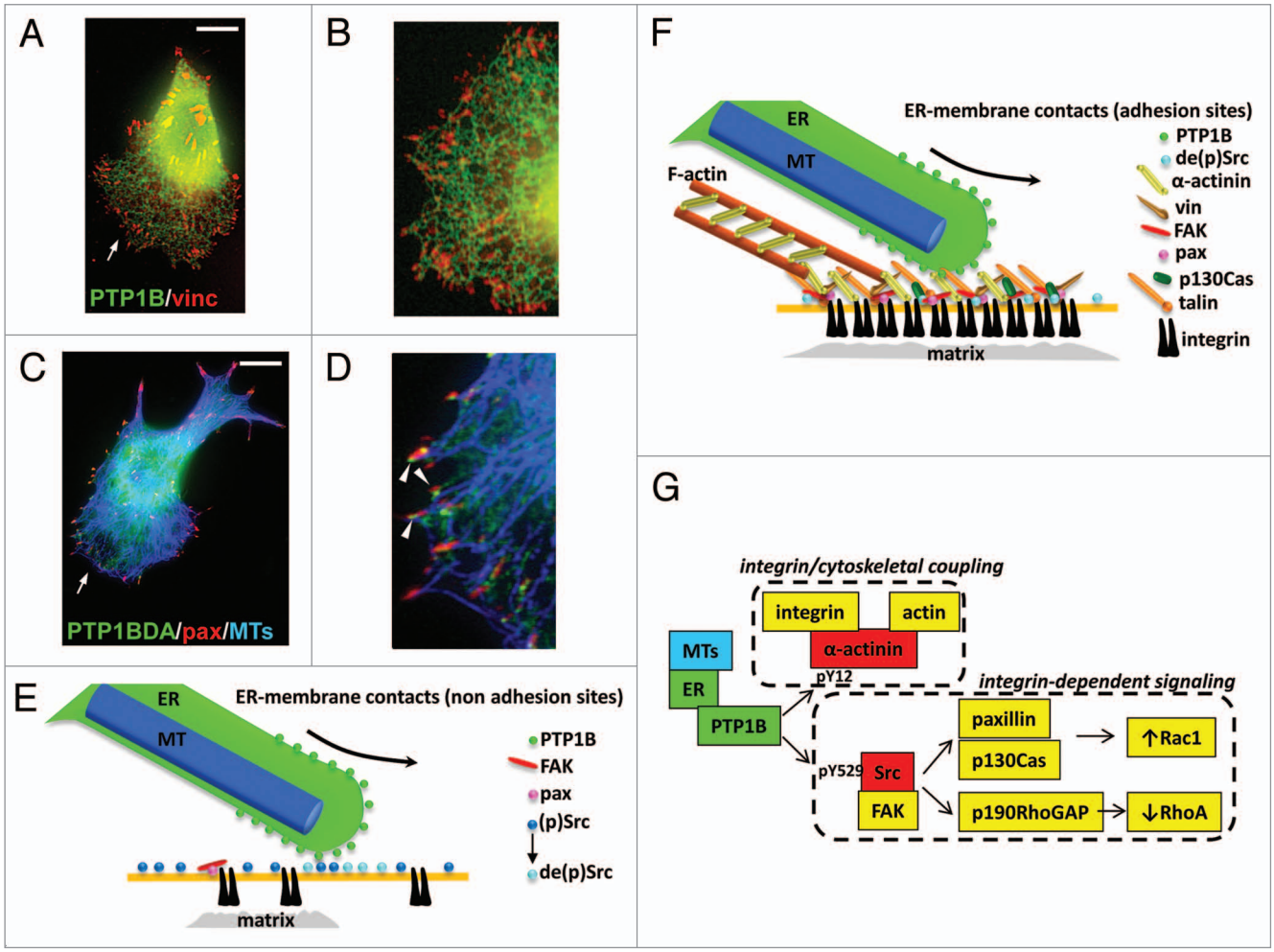

Figure 1. PTP1B localization and function on adhesion and motility in polarized, migrating cells. (**A and B**) GFP-PTP1B (green label) localizes at the ER and overlaps with vinculin adhesions (red label) at the cell periphery. (**C and D**) Microtubules (blue label) contribute to position the substrate trap GFP-PTP1BDA (green label) in paxillin adhesions (red label), where it binds to substrates and accumulates in small puncta (arrowheads in **D**). Arrows in (**A and C**) point the leading edge. (**E**) ER tubules randomly contact the plasma membrane outside adhesions in a microtubule (MT)-dependent manner. Membrane-associated, inactive Src (blue spheres, (p)Src), is targeted by ER-bound PTP1B at the pTyr-529, causing its dephosphorylation (light blue spheres, de(p)Src) and priming the kinase for activation at the plasma membrane. (**F**) MT-dependent positioning of ER-bound PTP1B over peripheral adhesions facilitates the activity of PTP1B on multiple substrates, including α-actinin, paxillin, and Src. (**G**) By targeting tyrosine 12 on α-actinin (in red) PTP1B contributes to enhance α-actinin binding to actin and to reinforce integrin/cytoskeleton linkages. By targeting Src regulatory pY529, PTP1B promotes Src activation and signaling through Rac1 and RhoA. During the protrusion phase, PTP1B promotes Rac1 activity and inhibits RhoA activity. Scale bars, 15 μm. Magnifications (**B and D**) are 400x of the original size.

Long-Range Impact of PTP1B on Cell Adhesion and Motility

As demonstrated for the regulation of cell growth and transformation, the regulatory role of PTP1B in cell adhesion and motility is complex and apparently dependent on the cell type and context. PTP1B was shown to promote cell adhesion and motility in fibroblasts,[17,18] neurons,[3,19] platelets,[4] and several tumor cell lines.[12,20-22] An inhibitory role has been described in fibroblasts,[23,24] primary aortic smooth muscle cells,[25] ovarian cancer cells,[26] and in glioblastoma multiforme tumor cell invasion in mice.[27] Remarkably, the positive role of PTP1B in cell motility was frequently associated with the stimulation of integrin-dependent signaling.[3,4,12,17,18,22] In contrast, the negative effect of PTP1B on cell motility was related to antagonizing signaling from growth factor receptor tyrosine kinases.[24-27]

In a recent quantitative study, we analyzed the intrinsic motility and migration parameters of PTP1B-null cells (KO cells) in an isotropic 2-D fibronectin substratum.[28] Directionality and average velocity of KO cells were significantly reduced when compared with KO cells reconstituted with wild-type PTP1B (WT cells). These effects could be partially explained by a higher tendency of KO cells to pause during migration. Visual inspection of cell morphology and kymograph analysis of the leading edge further revealed that migration of KO cells was characterized by fast extension/retraction of lamella produced in multiple directions, while WT cells persistently extended a broad lamella in the direction of movement.[2,28] Remarkably, neurons from hippocampus

Table 1. PTP1B substrates involved in cell adhesion and migration

Substrate and targeted Tyr (Y)	Function	Localization (A) adhesions (L) Lamellipodium	References
Caveolin-1 (Y14)	Scaffold protein	A, L	14,53–55
Cortactin (Y446)	Scaffold protein	A, L	14,56–58
Alpha-actinin (Y12)	Scaffold protein	A, L	28, 43
CrkII (Y221)	Adaptor protein	A	22,59,60
Cas-L (Y189)	Adaptor protein	A	14,50
P120 catenin (257)	Scaffold protein	L	14,61,62
Src (Y529)	Non receptor PTK	A	4,8,17,28,45
EphA3 (Y595,603,779)	Eph receptor tyrosine kinase	ND	6,14,63
Paxillin (Y118)	Adaptor protein	A	14,22

Listed proteins and candidate tyrosine residues (between brackets) identified as PTP1B substrates and as regulators of adhesion and migration. Localization was based on spatial co-localization and/or co-immunoprecipitation analysis. ND, not determined. Many of the listed proteins were isolated in a phosphoproteomic screen using PTP1B wild-type and null cells, as well as in vitro pull down with wild-type and substrate trap D181A PTP1B.[14]

and retina transfected with a dominant negative PTP1B construct, displayed axonal growth cones with similar lamellar alterations as those observed in the leading edge of KO fibroblasts. Consistently, axons also showed reduced net elongation rate and increased pause times compared with axons of control neurons.[3] Collectively, these results strongly suggest that PTP1B promotes cell migration by mechanisms that stabilize lamellar protrusions.

Microtubules Contribute to Position ER-Bound PTP1B at the Leading Edge

A wide range of cell types move by extension of morphologically, functionally, and structurally different structures called lamellipodia and filopodia at the leading edge. The lamellipodium contains a polarized and dynamic F-actin array that grows and pushes the leading edge in the direction of movement.[29] During this process, nascent adhesions assemble within the lamellipodium.[30] Microtubules are required for protrusion and dynamics of the leading edge in fibroblasts and epithelial cells, affecting F-actin assembly and organization, as well as adhesion stability.[31,32] Cortical microtubules supply and sequester regulators of RhoA and Rac1 GTPases, actin, and adhesions, modulating F-actin mechanics and intracellular tension.[31,32] Important to this discussion is the fact that microtubules interact with and pull ER tubules toward the cell periphery, partially by their association with the microtubule plus end directed kinesin-1.[33,34] Kinesin binds to the ER transmembrane protein kinectin and interfering this binding produces the collapse of the peripheral ER.[35,36]

In neurons, microtubule-dependent localization of ER-bound PTP1B at the peripheral region of axonal growth cones is critical for Src activation and filopodia stability.[3] In migrating fibroblasts, the ER network extends toward the leading edge, positioning GFP-PTP1B over new adhesions assembled at the advancing lamella (**Fig. 1A and B**). Remarkably, the substrate trap mutant PTP1B D181A, which considerably increases the steady-state population of enzyme-substrate complexes,[9,13] develops conspicuous microscopic puncta almost exclusively over adhesions, and co-localizes with microtubule ends (**Fig. 1C and D**).[2] Thus, we propose that ER association with microtubules and their elongation into the leading edge of migrating cells facilitate the activity of ER-bound PTP1B on substrates involved in cell protrusion and migration. We recently investigated the consequences of this mechanism on adhesions targeted by the enzyme. We found that adhesions assembled during lamellar protrusions and contacted with ER tubules containing wild-type GFP-PTP1B, had average lifetimes five times longer than those contacted with ER tubules bearing the catalytically inactive mutant GFP-PTP1B (C215S).[28] In addition, short-lived adhesions, which are more abundant in KO cells, displayed higher paxillin disassembly rates than those in WT cells. These results establish that by modulating adhesion lifetimes, ER-bound PTP1B promotes persistent lamellar protrusion and directional migration (**Fig. 1F**). These findings explain why the ER extension in lamellar extensions contributes to cell spreading, migration, and focal adhesion growth.[36]

PTP1B Regulation of Cell-Matrix Coupling to the Cytoskeleton

Cell-matrix interactions activate signaling pathways that regulate different aspects of cell migration, such as adhesion and cytoskeletal organization and dynamics. The focal adhesion kinase FAK is a key signaling component of cell-matrix adhesions which regulates directional cell movement and adhesion dynamics.[37] FAK is required for vinculin and talin recruitment to cell-matrix complexes, enhancing integrin-cytoskeletal coupling.[38-40] FAK may also decrease integrin-cytoskeleton linkages through α-actinin phosphorylation, which reduces its affinity for actin.[41,42] Biochemical experiments performed in COS and PTP1B-null cells suggested that PTP1B could dephosphorylate α-actinin and promote chemotaxis to fibronectin.[43] We postulated that

PTP1B dephosphorylates α-actinin in adhesions facilitating its binding to actin (**Fig. 1G**). This process could reinforce integrin-cytoskeleton linkages, contributing to extend the lifetimes of adhesions. In support to this notion, Bimolecular Fluorescence Complementation (BiFC) analysis revealed the presence of catalytic PTP1B/α-actinin complexes overlapping with paxillin adhesions at lamellar extensions.[28] In addition, we observed that small paxillin adhesions assembled during lamellar protrusion in KO cells did not incorporate α-actinin efficiently and were quickly disassembled.[28] In contrast, most adhesions assembled during lamellar protrusion in WT cells incorporated α-actinin and grew in size. These results strongly support the assumption that α-actinin is a substrate of PTP1B in adhesions, and that its dephosphorylation enhances adhesion/cytoskeleton coupling and lamellar stability (**Fig. 1G**). Our results also suggest that PTP1B function is relevant during the phase of lamellar protrusion, since PTP1B-null cells had the capacity to incorporate α-actinin in paxillin adhesions, which grew in size during lamellar retractions.[28] Two candidate protein tyrosine phosphatases that could be involved in adhesion growth and maturation during lamellar retractions are SHP2 and RPTPα, both of which were implied in matrix force transduction.[42,44] However, only SHP2 was demonstrated to modulate α-actinin dephosphorylation via the regulation of FAK activity.[42]

PTP1B Regulation of Cell-Matrix-Dependent Signaling to Rho GTPases

In an early study, we found that the expression of a dominant-negative mutant of PTP1B in fibroblasts impaired integrin-dependent signaling, including FAK and Src activation, and paxillin phosphorylation.[17] Src activation by PTP1B was further demonstrated in several different cell models.[3,4,12,18,45-47] Using a combination of time lapse, total internal reflection fluorescence microscopy, and BiFC we were able to visualize ER-bound PTP1B/Src complexes as small fluorescent puncta distributed uniformly at the plasma membrane in contact with the substrate.[8] The substrate trap mutation (D181A) in PTP1B and the tyrosine 529 at the C-tail of Src were both required for BiFC to occur, as well as the plasma membrane targeting motif of Src.[8] This study also shows dynamic projections of ER tubules toward the plasma membrane, suggesting the assembly of catalytic PTP1B/Src BiFC complexes at random point locations in the plasma membrane/substrate interface. Nevertheless, the possibility that Src could also be targeted by PTP1B at typical adhesion structures cannot be excluded (**Fig. 1E and F**). We propose that PTP1B dephosphorylates the tyrosine 529 (in mouse Src) at the C-terminal region of Src, unlocking the negative regulation imposed by the phosphorylation of this residue, and releasing the Src SH2 domain. The latter event could promote the intermolecular interaction of Src with partners localized at cell-matrix adhesions. For example, the clustering and autophosphorylation of FAK in integrin adhesions could recruit Src via an SH2-pY interaction, assembling an active Src/FAK signaling complex. This complex promotes Rac1 activation through the phosphorylation of two major adaptor proteins, paxillin and p130Cas.[48,49] Rac1 activity drives actin polymerization and adhesion formation at the extending lamellipodium. PTP1B may also reinforce this pathway by dephosphorylation of the adaptor protein CrkII, which promotes its binding to the adaptor proteins p130Cas and paxillin.[22] CrkII dephosphorylated by PTP1B preferentially binds to and protects tyrosine phosphorylated p130Cas from dephosphorylation, event which could promote cell migration by facilitating the activation of Rac1 at the membrane. It has been shown that PTP1B is able to directly dephosphorylate p130Cas and promote spreading, although the relevant tyrosine substrates were not identified.[10,47] A more recent phosphoproteomic study identified Cas-L/HEF-1/NEDD9, a member of the p130Cas family, as a potential PTP1B substrate.[14] Interestingly, the phosphorylated state of one of the target tyrosines, Tyr-189, was shown to be important for adhesion stability and cell migration.[50] The role of PTP1B in these processes involving Cas-L remains to be determined.

The Src/FAK signaling axis also promotes RhoA inactivation/activation through the temporally regulated phosphorylation and activation of p190RhoGAP and p190RhoGEF, respectively.[37] Biochemical determinations of GTPase activity in response to integrin stimulation showed that RhoA activity was downregulated and Rac1 and Cdc42 activities were upregulated at early time points, while these activities were reversed at later time points. Consistently, Rac1 and Cdc42 activities were required for protrusion and adhesion initiation, while Rho activation was required to increase actomyosin contractility and to promote adhesion maturation.[51] Our recent study showed that initial integrin-mediated downregulation of RhoA activity and induction of Rac1 activity were impaired in PTP1B KO cells.[28] Thus, microtubule-dependent positioning of PTP1B at the leading edge of migrating cells may contribute to downregulate RhoA and stimulate Rac1 activities required for adhesion stability and lamellipodium protrusion (**Fig. 1G**). This notion agrees with results suggesting that microtubules contribute to RhoA downregulation and participate in a positive feedback loop with Rac1 that promotes leading edge protrusion in slow moving cells.[32,52]

Conclusions

Recent studies from our laboratory illustrate how the seemingly spatial restriction imposed by the anchor of PTP1B to ER membranes, can eventually be compensated by the dynamics of the ER. ER tubules extend to the cell periphery in a microtubule-dependent manner, positioning PTP1B at a spatial range compatible with enzyme-substrate interactions. PTP1B regulates the activity of several substrates implied in cell adhesion and motility (see **Table 1**). Our work has identified α-actinin and Src as two PTP1B substrates playing critical roles in adhesion dynamics and signaling. By acting on α-actinin, PTP1B contributes to adhesion/cytoskeleton coupling and adhesion stability. By promoting the activation of Src, PTP1B stimulates signaling pathways that promote Rac1 activation and inhibit RhoA. Both molecular mechanisms

regulated by PTP1B may explain its long-range effects on lamellar dynamics and directional cell migration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

AG, JEB, and AEGW are recipients of fellowships from Agencia Nacional para la Promoción Científica y Tecnológica (ANPCYT) and the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). COA is research fellow from CONICET and recipient of grants (PICTs 1363 and 2129) from ANPCYT.

References

1. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 1992; 68:545-60; PMID:1739967; http://dx.doi.org/10.1016/0092-8674(92)90190-N
2. Hernández MV, Sala MG, Balsamo J, Lilien J, Arregui CO. ER-bound PTP1B is targeted to newly forming cell-matrix adhesions. J Cell Sci 2006; 119:1233-43; PMID:16522684; http://dx.doi.org/10.1242/jcs.02846
3. Fuentes F, Arregui CO. Microtubule and cell contact dependency of ER-bound PTP1B localization in growth cones. Mol Biol Cell 2009; 20:1878-89; PMID:19158394; http://dx.doi.org/10.1091/mbc.E08-07-0675
4. Arias-Salgado EG, Haj F, Dubois C, Moran B, Kasirer-Friede A, Furie BC, Furie B, Neel BG, Shattil SJ. PTP-1B is an essential positive regulator of platelet integrin signaling. J Cell Biol 2005; 170:837-45; PMID:16115959; http://dx.doi.org/10.1083/jcb.200503125
5. Anderie I, Schulz I, Schmid A. Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets. Cell Signal 2007; 19:582-92; PMID:17092689; http://dx.doi.org/10.1016/j.cellsig.2006.08.007
6. Nievergall E, Janes PW, Stegmayer C, Vail ME, Haj FG, Teng SW, Neel BG, Bastiaens PI, Lackmann M. PTP1B regulates Eph receptor function and trafficking. J Cell Biol 2010; 191:1189-203; PMID:21135139; http://dx.doi.org/10.1083/jcb.201005035
7. Haj FG, Sabet O, Kinkhabwala A, Wimmer-Kleikamp S, Roukos V, Han HM, Grabenbauer M, Bierbaum M, Antony C, Neel BG, et al. Regulation of signaling at regions of cell-cell contact by endoplasmic reticulum-bound protein-tyrosine phosphatase 1B. PLoS One 2012; 7:e36633; PMID:22655028; http://dx.doi.org/10.1371/journal.pone.0036633
8. Monteleone MC, González Wusener AE, Burdisso JE, Conde C, Cáceres A, Arregui CO. ER-bound protein tyrosine phosphatase PTP1B interacts with Src at the plasma membrane/substrate interface. PLoS One 2012; 7:e38948; PMID:22701734; http://dx.doi.org/10.1371/journal.pone.0038948
9. Haj FG, Verveer PJ, Squire A, Neel BG, Bastiaens PI. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. Science 2002; 295:1708-11; PMID:11872838; http://dx.doi.org/10.1126/science.1067566
10. Liu F, Hill DE, Chernoff J. Direct binding of the proline-rich region of protein tyrosine phosphatase 1B to the Src homology 3 domain of p130$^{(Cas)}$. J Biol Chem 1996; 271:31290-5; PMID:8940134; http://dx.doi.org/10.1074/jbc.271.49.31290
11. Frangioni JV, Oda A, Smith M, Salzman EW, Neel BG. Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets. EMBO J 1993; 12:4843-56; PMID:8223493
12. Cortesio CL, Chan KT, Perrin BJ, Burton NO, Zhang S, Zhang ZY, Huttenlocher A. Calpain 2 and PTP1B function in a novel pathway with Src to regulate invadopodia dynamics and breast cancer cell invasion. J Cell Biol 2008; 180:957-71; PMID:18332219; http://dx.doi.org/10.1083/jcb.200708048
13. Flint AJ, Tiganis T, Barford D, Tonks NK. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci U S A 1997; 94:1680-5; PMID:9050838; http://dx.doi.org/10.1073/pnas.94.5.1680
14. Mertins P, Eberl HC, Renkawitz J, Olsen JV, Tremblay ML, Mann M, Ullrich A, Daub H. Investigation of protein-tyrosine phosphatase 1B function by quantitative proteomics. Mol Cell Proteomics 2008; 7:1763-77; PMID:18515860; http://dx.doi.org/10.1074/mcp.M800196-MCP200
15. Yip SC, Saha S, Chernoff J. PTP1B: a double agent in metabolism and oncogenesis. Trends Biochem Sci 2010; 35:442-9; PMID:20381358; http://dx.doi.org/10.1016/j.tibs.2010.03.004
16. Lessard L, Stuible M, Tremblay ML. The two faces of PTP1B in cancer. Biochim Biophys Acta 2010; 1804:613-9; PMID:19782770; http://dx.doi.org/10.1016/j.bbapap.2009.09.018
17. Arregui CO, Balsamo J, Lilien J. Impaired integrin-mediated adhesion and signaling in fibroblasts expressing a dominant-negative mutant PTP1B. J Cell Biol 1998; 143:861-73; PMID:9813103; http://dx.doi.org/10.1083/jcb.143.3.861
18. Liang F, Lee SY, Liang J, Lawrence DS, Zhang ZY. The role of protein-tyrosine phosphatase 1B in integrin signaling. J Biol Chem 2005; 280:24857-63; PMID:15866871; http://dx.doi.org/10.1074/jbc.M502780200
19. Pathre P, Arregui C, Wampler T, Kue I, Leung TC, Lilien J, Balsamo J. PTP1B regulates neurite extension mediated by cell-cell and cell-matrix adhesion molecules. J Neurosci Res 2001; 63:143-50; PMID:11169624; http://dx.doi.org/10.1002/1097-4547(20010115)63:2<143::AID-JNR1006>3.0.CO;2-1
20. Blanquart C, Karouri SE, Issad T. Protein tyrosine phosphatase-1B and T-cell protein tyrosine phosphatase regulate IGF-2-induced MCF-7 cell migration. Biochem Biophys Res Commun 2010; 392:83-8; PMID:20059965; http://dx.doi.org/10.1016/j.bbrc.2009.12.176
21. Lessard L, Labbé DP, Deblois G, Bégin LR, Hardy S, Mes-Masson AM, Saad F, Trotman LC, Giguère V, Tremblay ML. PTP1B is an androgen receptor-regulated phosphatase that promotes the progression of prostate cancer. Cancer Res 2012; 72:1529-37; PMID:22282656; http://dx.doi.org/10.1158/0008-5472.CAN-11-2602
22. Takino T, Tamura M, Miyamori H, Araki M, Matsumoto K, Sato H, Yamada KM. Tyrosine phosphorylation of the CrkII adaptor protein modulates cell migration. J Cell Sci 2003; 116:3145-55; PMID:12799422; http://dx.doi.org/10.1242/jcs.00632
23. Liu F, Sells MA, Chernoff J. Protein tyrosine phosphatase 1B negatively regulates integrin signaling. Curr Biol 1998; 8:173-6; PMID:9443918; http://dx.doi.org/10.1016/S0960-9822(98)70066-1
24. Buckley DA, Cheng A, Kiely PA, Tremblay ML, O'Connor R. Regulation of insulin-like growth factor type I (IGF-I) receptor kinase activity by protein tyrosine phosphatase 1B (PTP-1B) and enhanced IGF-I-mediated suppression of apoptosis and motility in PTP-1B-deficient fibroblasts. Mol Cell Biol 2002; 22:1998-2010; PMID:11884589; http://dx.doi.org/10.1128/MCB.22.7.1998-2010.2002
25. Pu Q, Zhuang D, Thakran S, Hassid A. Mechanisms related to NO-induced motility in differentiated rat aortic smooth muscle cells. Am J Physiol Heart Circ Physiol 2011; 300:H101-8; PMID:21037226; http://dx.doi.org/10.1152/ajpheart.00342.2010
26. Fan G, Lin G, Lucito R, Tonks NK. Protein-tyrosine Phosphatase 1B Antagonized Signaling by Insulin-like Growth Factor-1 Receptor and Kinase BRK/PTK6 in Ovarian Cancer Cells. J Biol Chem 2013; 288:24923-34; PMID:23814047; http://dx.doi.org/10.1074/jbc.M113.482737
27. Lu KV, Chang JP, Parachoniak CA, Pandika MM, Aghi MK, Meyronet D, Isachenko N, Fouse SD, Phillips JJ, Cheresh DA, et al. VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer Cell 2012; 22:21-35; PMID:22789536; http://dx.doi.org/10.1016/j.ccr.2012.05.037
28. Burdisso JE, González A, Arregui CO. PTP1B promotes focal complex maturation, lamellar persistence and directional migration. J Cell Sci 2013; 126:1820-31; PMID:23444382; http://dx.doi.org/10.1242/jcs.118828
29. Gardel ML, Schneider IC, Aratyn-Schaus Y, Waterman CM. Mechanical integration of actin and adhesion dynamics in cell migration. Annu Rev Cell Dev Biol 2010; 26:315-33; PMID:19575647; http://dx.doi.org/10.1146/annurev.cellbio.011209.122036
30. Choi CK, Vicente-Manzanares M, Zareno J, Whitmore LA, Mogilner A, Horwitz AR. Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. Nat Cell Biol 2008; 10:1039-50; PMID:19160484; http://dx.doi.org/10.1038/ncb1763
31. Kaverina I, Straube A. Regulation of cell migration by dynamic microtubules. Semin Cell Dev Biol 2011; 22:968-74; PMID:22001384; http://dx.doi.org/10.1016/j.semcdb.2011.09.017
32. Stehbens S, Wittmann T. Targeting and transport: how microtubules control focal adhesion dynamics. J Cell Biol 2012; 198:481-9; PMID:22908306; http://dx.doi.org/10.1083/jcb.201206050
33. Waterman-Storer CM, Salmon ED. Endoplasmic reticulum membrane tubules are distributed by microtubules in living cells using three distinct mechanisms. Curr Biol 1998; 8:798-806; PMID:9663388; http://dx.doi.org/10.1016/S0960-9822(98)70321-5
34. Feiguin F, Ferreira A, Kosik KS, Caceres A. Kinesin-mediated organelle translocation revealed by specific cellular manipulations. J Cell Biol 1994; 127:1021-39; PMID:7962067; http://dx.doi.org/10.1083/jcb.127.4.1021
35. Santama N, Er CP, Ong LL, Yu H. Distribution and functions of kinectin isoforms. J Cell Sci 2004; 117:4537-49; PMID:15316074; http://dx.doi.org/10.1242/jcs.01326
36. Zhang X, Tee YH, Heng JK, Zhu Y, Hu X, Margadant F, Ballestrem C, Bershadsky A, Griffiths G, Yu H. Kinectin-mediated endoplasmic reticulum dynamics supports focal adhesion growth in the cellular lamella. J Cell Sci 2010; 123:3901-12; PMID:20980389; http://dx.doi.org/10.1242/jcs.069153
37. Tomar A, Schlaepfer DD. Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. Curr Opin Cell Biol 2009; 21:676-83; PMID:19525103; http://dx.doi.org/10.1016/j.ceb.2009.05.006

38. Pasapera AM, Schneider IC, Rericha E, Schlaepfer DD, Waterman CM. Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. J Cell Biol 2010; 188:877-90; PMID:20308429; http://dx.doi.org/10.1083/jcb.200906012
39. Michael KE, Dumbauld DW, Burns KL, Hanks SK, García AJ. Focal adhesion kinase modulates cell adhesion strengthening via integrin activation. Mol Biol Cell 2009; 20:2508-19; PMID:19297531; http://dx.doi.org/10.1091/mbc.E08-01-0076
40. Lawson C, Lim ST, Uryu S, Chen XL, Calderwood DA, Schlaepfer DD. FAK promotes recruitment of talin to nascent adhesions to control cell motility. J Cell Biol 2012; 196:223-32; PMID:22270917; http://dx.doi.org/10.1083/jcb.201108078
41. Izaguirre G, Aguirre L, Hu YP, Lee HY, Schlaepfer DD, Aneskievich BJ, Haimovich B. The cytoskeletal/non-muscle isoform of alpha-actinin is phosphorylated on its actin-binding domain by the focal adhesion kinase. J Biol Chem 2001; 276:28676-85; PMID:11369769; http://dx.doi.org/10.1074/jbc.M101678200
42. von Wichert G, Haimovich B, Feng GS, Sheetz MP. Force-dependent integrin-cytoskeleton linkage formation requires downregulation of focal complex dynamics by Shp2. EMBO J 2003; 22:5023-35; PMID:14517241; http://dx.doi.org/10.1093/emboj/cdg492
43. Zhang Z, Lin SY, Neel BG, Haimovich B. Phosphorylated alpha-actinin and protein-tyrosine phosphatase 1B coregulate the disassembly of the focal adhesion kinase x Src complex and promote cell migration. J Biol Chem 2006; 281:1746-54; PMID:16291744; http://dx.doi.org/10.1074/jbc.M509590200
44. Lee HH, Lee HC, Chou CC, Hur SS, Osterday K, del Álamo JC, Lasheras JC, Chien S. Shp2 plays a crucial role in cell structural orientation and force polarity in response to matrix rigidity. Proc Natl Acad Sci U S A 2013; 110:2840-5; PMID:23359696; http://dx.doi.org/10.1073/pnas.1222164110
45. Bjorge JD, Pang A, Fujita DJ. Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. J Biol Chem 2000; 275:41439-46; PMID:11007774; http://dx.doi.org/10.1074/jbc.M004852200
46. Arias-Romero LE, Saha S, Villamar-Cruz O, Yip SC, Ethier SP, Zhang ZY, Chernoff J. Activation of Src by protein tyrosine phosphatase 1B Is required for ErbB2 transformation of human breast epithelial cells. Cancer Res 2009; 69:4582-8; PMID:19435911; http://dx.doi.org/10.1158/0008-5472.CAN-08-4001
47. Cheng A, Bal GS, Kennedy BP, Tremblay ML. Attenuation of adhesion-dependent signaling and cell spreading in transformed fibroblasts lacking protein tyrosine phosphatase-1B. J Biol Chem 2001; 276:25848-55; PMID:11346638; http://dx.doi.org/10.1074/jbc.M009734200
48. Defilippi P, Di Stefano P, Cabodi S. p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol 2006; 16:257-63; PMID:16581250; http://dx.doi.org/10.1016/j.tcb.2006.03.003
49. Deakin NO, Turner CE. Paxillin comes of age. J Cell Sci 2008; 121:2435-44; PMID:18650496; http://dx.doi.org/10.1242/jcs.018044
50. Baquiran JB, Bradbury P, O'Neill GM. Tyrosine Y189 in the Substrate Domain of the Adhesion Docking Protein NEDD9 Is Conserved with p130Cas Y253 and Regulates NEDD9-Mediated Migration and Focal Adhesion Dynamics. PLoS One 2013; 8:e69304; PMID:23874939; http://dx.doi.org/10.1371/journal.pone.0069304
51. DeMali KA, Burridge K. Coupling membrane protrusion and cell adhesion. J Cell Sci 2003; 116:2389-97; PMID:12766185; http://dx.doi.org/10.1242/jcs.00605
52. Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. Trends Cell Biol 2005; 15:76-83; PMID:15695094; http://dx.doi.org/10.1016/j.tcb.2004.12.006
53. Lee H, Xie L, Luo Y, Lee SY, Lawrence DS, Wang XB, Sotgia F, Lisanti MP, Zhang ZY. Identification of phosphocaveolin-1 as a novel protein tyrosine phosphatase 1B substrate. Biochemistry 2006; 45:234-40; PMID:16388599; http://dx.doi.org/10.1021/bi051560j
54. Grande-García A, Echarri A, de Rooij J, Alderson NB, Waterman-Storer CM, Valdivielso JM, del Pozo MA. Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. J Cell Biol 2007; 177:683-94; PMID:17517963; http://dx.doi.org/10.1083/jcb.200701006
55. Nethe M, Anthony EC, Fernandez-Borja M, Dee R, Geerts D, Hensbergen PJ, Deelder AM, Schmidt G, Hordijk PL. Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. J Cell Sci 2010; 123:1948-58; PMID:20460433; http://dx.doi.org/10.1242/jcs.062919
56. Stuible M, Dubé N, Tremblay ML. PTP1B regulates cortactin tyrosine phosphorylation by targeting Tyr446. J Biol Chem 2008; 283:15740-6; PMID:18387954; http://dx.doi.org/10.1074/jbc.M710534200
57. MacGrath SM, Koleske AJ. Cortactin in cell migration and cancer at a glance. J Cell Sci 2012; 125:1621-6; PMID:22566665; http://dx.doi.org/10.1242/jcs.093781
58. Wang W, Liu Y, Liao K. Tyrosine phosphorylation of cortactin by the FAK-Src complex at focal adhesions regulates cell motility. BMC Cell Biol 2011; 12:49; PMID:22078467; http://dx.doi.org/10.1186/1471-2121-12-49
59. Chodniewicz D, Klemke RL. Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. Biochim Biophys Acta 2004; 1692:63-76; PMID:15246680; http://dx.doi.org/10.1016/j.bbamcr.2004.03.006
60. Lamorte L, Rodrigues S, Sangwan V, Turner CE, Park M. Crk associates with a multimolecular Paxillin/GIT2/beta-PIX complex and promotes Rac-dependent relocalization of Paxillin to focal contacts. Mol Biol Cell 2003; 14:2818-31; PMID:12857867; http://dx.doi.org/10.1091/mbc.E02-08-0497
61. Anastasiadis PZ. p120-ctn: A nexus for contextual signaling via Rho GTPases. Biochim Biophys Acta 2007; 1773:34-46; PMID:17028013; http://dx.doi.org/10.1016/j.bbamcr.2006.08.040
62. Boguslavsky S, Grosheva I, Landau E, Shtutman M, Cohen M, Arnold K, Feinstein E, Geiger B, Bershadsky A. p120 catenin regulates lamellipodial dynamics and cell adhesion in cooperation with cortactin. Proc Natl Acad Sci U S A 2007; 104:10882-7; PMID:17576929; http://dx.doi.org/10.1073/pnas.0702731104
63. Shi G, Yue G, Zhou R. EphA3 functions are regulated by collaborating phosphotyrosine residues. Cell Res 2010; 20:1263-75; PMID:20697431; http://dx.doi.org/10.1038/cr.2010.115