The Interaction of Protein-tyrosine Phosphatase α (PTPα) and RACK1 Protein Enables Insulin-like Growth Factor 1 (IGF-1)-stimulated Abl-dependent and -independent Tyrosine Phosphorylation of PTPα*

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Background: Insulin-like growth factor 1 (IGF-1) stimulates PTPα tyrosine phosphorylation to enhance cell migration.
Results: The scaffold protein RACK1 binds PTPα and the IGF-1 receptor, enabling IGF-1-stimulated phosphorylation of PTPα by Abl and other kinases.
Conclusion: RACK1 coordinates PTPα within a signaling complex to mediate PTPα phosphorylation.
Significance: RACK1 may link PTPα to diverse upstream signals that regulate PTPα tyrosine phosphorylation-dependent cell motility.

Protein tyrosine phosphatase α (PTPα) promotes integrin-stimulated cell migration in part through the role of Src-phosphorylated PTPα-Tyr(P)-789 in recruiting and localizing p130Cas to focal adhesions. The growth factor IGF-1 also stimulates PTPα-Tyr-789 phosphorylation to positively regulate cell movement. This is in contrast to integrin-induced PTPα phosphorylation, that induced by IGF-1 can occur in cells lacking Src family kinases (SFKs), indicating that an unknown kinase distinct from SFKs can target PTPα. We show that this IGF-1-stimulated tyrosine kinase is Abl. We found that PTPα binds to the scaffold protein RACK1 and that RACK1 coordinates the IGF-1 receptor, PTPα, and Abl in a complex to enable IGF-1-stimulated and Abl-dependent PTPα-Tyr-789 phosphorylation. In cells expressing SFKs, IGF-1-stimulated phosphorylation of PTPα is mediated by RACK1 but is Abl-independent. Furthermore, expressing the SFKs Src and Fyn in SFK-deficient cells switches IGF-1-induced PTPα phosphorylation to occur in an Abl-independent manner, suggesting that SFK activity dominantly regulates IGF-1/IGF-1 receptor signaling to PTPα. RACK1 is a molecular scaffold that integrates growth factor and integrin signaling, and our identification of PTPα as a RACK1 binding protein suggests that RACK1 may coordinate PTPα-Tyr-789 phosphorylation in these signaling networks to promote cell migration.

Migration is a fundamental cell process that is regulated by the cellular environment. Soluble growth factors that bind to receptor protein tyrosine kinases and extracellular matrix components that engage receptor integrins are important stimuli of cell movement. Growth factor- and extracellular matrix-induced migration signaling are often integrated at multiple levels, and both are regulated by reversible tyrosine phosphorylation.

Receptor-like protein-tyrosine phosphatase α (PTPα)5 plays important roles in integrin-stimulated cell migration, where it not only regulates, but is regulated by, tyrosine phosphorylation. Upon integrin engagement, PTPα acts catalytically to dephosphorylate and activate Src, enabling the ensuing Src-mediated activation of focal adhesion kinase (FAK) and formation of the central Src-FAK kinase complex (1–3). PTPα itself is a target of activated Src-FAK and is phosphorylated at a tyrosine site in its C-terminal tail, Tyr-789 (4). A role for PTPα-Tyr(P)-789 in signaling events that promote cell migration is evident from studies of cells expressing a mutant PTPα (Y789F) that, although catalytically active, cannot be phosphorylated at Tyr-789. In these cells, Src is activated upon integrin engagement, but defective cytoskeletal remodeling and focal adhesion formation result in impaired cell movement (4).

PTPα-Tyr(P)-789 is enriched in focal adhesions (5), indicating that its signaling is localized in these integrin-tethered multiprotein complexes. We have found that it regulates the localization of the scaffolding protein p130Cas (Cas) to the adhesion sites, thereby promoting Src-Cas interaction and Src-mediated Cas tyrosine phosphorylation, which activates migration effectors such as Rac1 and p21-activated kinase (6). The mechanism of PTPα-mediated Cas recruitment to adhesions involves the recognition of Tyr(P)-789 by the SH2 domain of the Cas-binding protein BCAR3 and formation of a PTPα-(Tyr(P)-789)-BCAR3-Cas complex (6). Therefore, in addition to its early catalytic role as an Src activator, PTPα also plays a non-catalytic role in integrin-stimulated signaling and cell migration that is dependent upon its phosphorylation.

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5 The abbreviations used are: PTP, protein tyrosine phosphatase; FAK, focal adhesion kinase; IGF, insulin-like growth factor; SFK, Src family kinase; MEF, mouse embryonic fibroblast.
The IGF-1/IGF-1R cell signaling network regulates numerous cell processes, including proliferation, survival, differentiation, and migration. It also regulates multiple malignant cell phenotypes, including invasion and metastasis (7, 8). IGF-1/IGF-1R induces the phosphorylation of PTPα at Tyr-789 (9), revealing PTPα as a new target of IGF-1R signaling. The significantly impaired migration response to IGF-1 of fibroblasts expressing mutant Y789F-PTPα suggests that PTPα phosphorylation at this site is essential for PTPα-dependent, IGF-1-regulated cell motility (9). Interestingly, IGF-1/IGF-1R-induced phosphorylation of PTPα appears most robust in SYF fibroblasts that lack the Src family kinases (SFKs) Src, Yes, and Fyn, indicating that, in contrast to integrin signaling, this is SFK-independent.

Because the SFKs are the only PTPα-Tyr-789 kinases known to date, in this study we aimed to identify the non-SFK tyrosine kinase that phosphorylates PTPα in response to IGF-1. We report that, in SYF cells, IGF-1-stimulated PTPα-Tyr-789 phosphorylation is dependent upon activity of the tyrosine kinase Abl. Furthermore, Abl-dependent PTPα phosphorylation is mediated through its interaction with RACK1, a molecular scaffold that binds and coordinates IGF-1R, Abl, and PTPα. The expression of SFKs in SYF cells switches IGF-1-stimulated PTPα-Tyr-789 phosphorylation from Abl-dependent to Abl-independent, resembling that in other SFK-expressing cell types, including several tumor cell lines. Therefore, Abl may be an alternate or “default” PTPα kinase in cells lacking active SFKs, possibly reflecting the importance of PTPα phosphorylation. The identification of RACK1 as a PTPα-interacting protein, together with the known ability of RACK1 to bind several integrin signaling proteins and regulate focal adhesion formation (10–13), suggests that it may serve as a platform to orchestrate signaling by integrins and by growth factors such as IGF-1 to PTPα to promote cell motility.

**Experimental Procedures**

**Antibodies and Reagents**—PTPα and PTPα-Tyr(P)-789 antibodies have been described previously (4). Antibodies to Crk, FAK, RACK1, and Grb2 were from BD Transduction Laboratories. Anti-IGF-1R and c-Abl antibodies were from Sigma-Aldrich. Antibodies to Akt-Ser(P)-473, Akt, p44/42 MAPK-Thr(P)-202/Tyr(P)-204, IGF-1R-Tyr(P)-1131, Abl-Tyr(P)-245, and CrkL-Tyr(P)-207 were from Cell Signaling Technology. Anti-Tyr(P) (4G10) antibody was from Upstate Biotechnology. Antibody to FAK-Tyr(P)-397 was from Invitrogen. Human recombinant IGF-1 was from Sigma-Aldrich. Imatinib and nilotinib were obtained from LC laboratories, and PF-573228 was from Selleckchem.

**Cell Culture and Treatments**—SYF cells and MEFs were cultured in DMEM, MCF-7, IMR-32, TC-32, and BV173 were cultured in RPMI medium. All culture media were supplemented with 10% FBS. Prior to 10-min treatment with IGF-1, cells were starved in medium without serum overnight. In some experiments, serum-starved cells were pretreated with rapamycin for 2 h, nilotinib or imatinib for 3 h, or PF-573228 for 1 h prior to IGF-1 stimulation.

**siRNAs, Plasmids, and Transfection**—Control non-targeting siRNA and siRNAs targeting mouse or human RACK1 (ON-TARGETplus SMARTpool, Gnb211), and mouse c-Abl (ON-TARGETplus SMARTpool, mouse AblI) were from Dharmacon. Cells were transfected with 20 nM mouse siRNA or 50–100 nM human siRNA using Lipofectamine RNAiMax reagent (Invitrogen) and grown for 48 h before further experimentation. Cells growing in a 6-cm culture dish were transfected with 2.5 μg of pXJ41neo-Src or pXJ41neo-Fyn (42) using Lipofectamine LTX (Invitrogen) and cultured for 24 h before further experimentation.

**Cell Lysis, Immunoprecipitation, and Immunoblot Analysis**—Cells were harvested in lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM Na3VO4, 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). Cell lysates were incubated on ice for 30 min and then centrifuged at 13,000 rpm for 15 min. The resulting supernatants were collected and assayed for protein concentration using Bio-Rad reagent and for IGF-1-stimulated S6 phosphorylation but has no effect on PTPα phosphorylation in response to IGF-1 stimulation, we investigated other IGF-1-mediated adhesion signaling pathways in SYF cells. The mammalian target of rapamycin inhibits treatment of rapamycin inhibits the IGF-1-stimulated S6K1-mediated tyrosine phosphorylation of the focal adhesion proteins FAK, p130Cas, and paxillin and reduces cell motility (15). We found that rapamycin treatment of SYF fibroblasts inhibits basal and IGF-1-stimulated S6 phosphorylation but has no effect on PTPα-Tyr(P)-789 phosphorylation (Fig. 1A). Another link between IGF-1 adhesion signaling is mediated by the tyrosine kinase Abl (16, 17). We tested whether the Abl inhibitors imatinib

**In Vitro Kinase Assay**—AbL immunoprecipitates were incubated with 10 μg of recombinant Crk (Millipore) and 100 μM ATP in a total volume of 40 μl of kinase buffer (20 mM HEPES (pH 7.0), 150 mM NaCl, 1% Triton X-100, 200 μM Na3VO4, 10% glycerol, 1 mM MgCl2, 2 mM MnCl2, 1 mM PMSF, 2 mM DTT, and 10 μg/ml aprotinin) for 30 min at 37 °C. The reactions were stopped with Laemmli sample buffer, and the supernatant was subjected to immunoblotting with anti-Tyr(P) (4G10) antibody to detect tyrosine-phosphorylated Crk.

**PTPα-D1 Scanning Peptide Array**—A scanning peptide array of 15-mer peptides covering the sequence of PTPα amino acids 219–468 (in the D1 catalytic domain), with a sequential two- amino acid shift (all E-O series peptides) or three-amino acid shift (all B-D and P series peptides) at the amino terminus of each peptide, was synthesized on a cellulose membrane support by automatic SPOT synthesis (14) using the AutoSpot peptide synthesizer (Intavis AG Bioanalytical Instruments). The membrane was incubated with 10 μg/ml recombinant GST-RACK1. After washing, the membrane was probed with antibody to RACK1, followed by a secondary antibody coupled to horseradish peroxidase and enhanced chemiluminescence detection.

**Results**

**Abl Is Required for IGF-1-stimulated Tyrosine Phosphorylation of PTPα in SYF Fibroblasts**—IGF-1 induces the robust phosphorylation of PTPα at Tyr-789 in SYF fibroblasts that lack the Src family kinase members Src, Yes, and Fyn, and this is independent of IGF-1-induced PI3K and MAPK signaling (9). To determine the identity of the tyrosine kinase that regulates PTPα phosphorylation in response to IGF-1 stimulation, we investigated other IGF-1-mediated adhesion signaling pathways in SYF cells. The mammalian target of rapamycin inhibitor rapamycin inhibits the IGF-1-stimulated S6K1-mediated tyrosine phosphorylation of the focal adhesion proteins FAK, p130Cas, and paxillin and reduces cell motility (15). We found that rapamycin treatment of SYF fibroblasts inhibits basal and IGF-1-stimulated S6 phosphorylation but has no effect on PTPα-Tyr(P)-789 phosphorylation (Fig. 1A). Another link between IGF-1 and adhesion signaling is mediated by the tyrosine kinase Abl (16, 17). We tested whether the Abl inhibitors imatinib
(STI571) and nilotinib blocked IGF-1-stimulated phosphorylation of PTPα. Both compounds were effective inhibitors because PTPα-Tyr-789 phosphorylation is blocked by 10 μM imatinib or 2.5 μM nilotinib (Fig. 1B), identifying Abl as a candidate PTPα kinase.

To confirm the requirement for Abl in IGF-1 signaling to PTPα, Abl expression was silenced in SYF cells using Abl-targeted siRNA. This eliminated the IGF-1-stimulated phosphorylation of PTPα while not affecting expression of the Abl-related kinase Arg (Fig. 1C), identifying Abl as a candidate PTPα kinase.

To confirm the requirement for Abl in IGF-1 signaling to PTPα, Abl expression was silenced in SYF cells using Abl-targeted siRNA. This eliminated the IGF-1-stimulated phosphorylation of PTPα while not affecting expression of the Abl-related kinase Arg (Fig. 1C). Treating the cells with control non-targeting siRNA had little, if any, effect on Abl expression and did not affect IGF-1-stimulated phosphorylation of PTPα (Fig. 1C). We further examined whether Abl was activated upon IGF-1 stimulation of SYF cells. Abl immunoprecipitates were prepared from lysates of IGF-1-treated or untreated cells and probed for phosphorylation at Tyr-245, an event that corresponds with Abl activation (18, 19). A dramatic IGF-1-induced increase in Abl-Tyr(245) was detected that, like PTPα-Tyr-789 phosphorylation, was prevented by nilotinib (Fig. 1D). Abl immunoprecipitates were also used in in vitro kinase assays where they were mixed with recombinant Crk as a substrate. Stopped reactions were centrifuged, and the supernatants were probed for Tyr(207) and Tyr(221), revealing a nilotinib-sensitive, IGF-1-stimulated tyrosine phosphorylation of Crk (Fig. 1E). CrkL-Tyr-207 and CrkII-Tyr-221 are Abl-phosphorylated sites (20), and probing cell lysates with an antibody that recognizes both phosphosites showed that IGF-1 stimulated Crk phosphorylation and that this was inhibited by nilotinib (Fig. 1F, top panel). Notably, IGF-1R, Akt, and MAPK phosphorylation/activation were unaltered in response to nilotinib treatment (Fig. 1F). Together, our results indicate that IGF-1 activates Abl and that PTPα-Tyr-789 phosphorylation is dependent on this event.

PTPα and Abl Associate in a Complex with the IGF-1R and RACK1 in SYF Fibroblasts—Because our findings suggest that IGF-1-induced phosphorylation of PTPα is dependent on Abl, we investigated whether this kinase and phosphatase interact in
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The siRNA-mediated silencing of RACK1 abolished PTPα phosphorylation, whereas control non-targeting siRNA treatment had no effect (Fig. 3A). To determine whether RACK1 is enabling IGF-1R/Ab1-mediated PTPα phosphorylation by bringing PTPα into proximity with IGF-1R and Ab1, we tested the effect of silencing RACK1 expression upon formation of the protein complex. PTPα immunoprecipitates prepared from RACK1-depleted SYF cells that were stimulated with or without IGF-1 did not contain detectable IGF-1Rβ or Ab1, in contrast to PTPα immunoprecipitates from cells treated with control nonspecific siRNA (Fig. 3B). Efficient RACK1 silencing was confirmed by probing the cell lysates for RACK1 (Fig. 3B, bottom panel).

FAK Activity Is Not Required for IGF-1-stimulated Tyrosine Phosphorylation of PTPα in SYF Fibroblasts—Integrin signaling enhances PTPα phosphorylation at Tyr-789 in a FAK-dependent manner (4). In addition, FAK, in association with RACK1, modulates cell migration in response to IGF-1 (17). Therefore, we asked whether FAK is important for PTPα-Tyr-789 phosphorylation in response to IGF-1. Our coimmunoprecipitation results demonstrate that FAK is a component of the PTPα-IGF-1R/RACK1 complex. Nilotinib treatment disrupts FAK association with the complex (Fig. 4A), consistent with the action of Ab1 in mediating the interaction of FAK with RACK1 (17). Furthermore, IGF-1 treatment induces FAK autophosphorylation at Tyr-397 in SYF cells (Fig. 4B), indicative of FAK activation. However, treatment with increasing concentrations of the FAK inhibitor PF-573228 (PF-228) (22) showed that 0.1 μM PF-228 effectively blocked IGF-1-induced FAK-Tyr-397 phosphorylation without obviously affecting PTPα-Tyr-789 phosphorylation or IGF-1R, Akt, Crk, and MAPK phosphorylation/activation (Fig. 4B). Impairment of IGF-1-induced MAPK activation was detectable at 1 μM PF-228, but reduced PTPα-Tyr-789 phosphorylation was only apparent in cells treated with 10 μM PF-228 and occurred concomitantly with reduced phosphorylation of IGF-1R, Akt, and Crk. The latter effects are therefore likely to represent decreased IGF-1R signaling arising from nonspecific inhibitory actions of PF-228 at a higher concentration (22). These results indicate that, despite the Ab1-mediated association of FAK with the RACK1 coordinated protein com-
plex, FAK activity is not required for IGF-1-stimulated PTPα tyrosine phosphorylation.

Abl Activity Is Not Required for IGF-1-stimulated Phosphorylation of PTPα in Other Cell Types—IGF-1 signaling promotes the proliferation, migration, and invasion of a wide variety of tumor cells (7, 8). PTPα has been implicated in various cancers, including colon carcinoma, oral squamous carcinoma, gastric carcinoma, and breast carcinoma (23–27). Altering PTPα expression has diverse effects on the growth, migration, invasion, and metastasis of various breast and colon cancer cell lines (26–29). To investigate whether IGF-1 effects on cancer cells may be mediated via its actions on PTPα, we determined whether IGF-1 stimulates PTPα-Tyr-789 phosphorylation in cancer cells. We found that PTPα undergoes robust tyrosine phosphorylation in all six tested cancer cell lines in response to IGF-1 stimulation (Fig. 5A). To establish whether Abl is important for this, we tested the effect of nilotinib on IGF-1-mediated PTPα-Tyr-789 phosphorylation in three of the cancer cell lines: MCF-7 (breast adenocarcinoma), IMR-32 (neuroblastoma), and TC-32 (Ewing sarcoma). Similar to SYF cells, IGF-1 induced Abl activation, as indicated by enhanced Crk phos-

FIGURE 4. IGF-1-stimulated PTPα tyrosine phosphorylation is independent of FAK activity. A, serum-starved SYF cells were treated with 2.5 μM nilotinib (Nilot) for 3 h and then treated with IGF-1 for 10 min. RACK1 immunoprecipitates (IP) were probed for protein associations as shown. B, serum-starved SYF cells were treated with the indicated concentrations of PF-228 for 1 h prior to the addition of IGF-1 for 10 min. Lysates were immunoprobbed with the indicated antibodies. Densitometric quantification of Tyr(P)-789-PTPα and total PTPα signals was carried out, and the ratios of those that represent PTPα-Tyr-789 phosphorylation per unit of PTPα are shown.

FIGURE 5. IGF-1 stimulates Abl-independent PTPα tyrosine phosphorylation in cancer cell lines. A, MCF-7, MDA-MB-231, IMR-32, SHSY-5Y, TC-32, and SMS-KCNR cells were serum-starved overnight and then treated with or without IGF-1 for 10 min. Lysates were probed for Tyr(P)-789-PTPα and PTPα. B, serum-starved MCF-7, IMR-32, and TC-32 cells were treated with 2.5 μM nilotinib (Nilot) for 3 h prior to the addition of IGF-1 for 10 min. C, BV-173 cells were treated with 2.5 μM nilotinib or 10 μM imatinib (Imat) for 3 h. DMSO, dimethyl sulfoxide. D, serum-starved MEFs were treated with 2.5 μM nilotinib and then treated with IGF-1 for 10 min. Lysates from these cells were immunoprobbed with the indicated antibodies. Densitometric quantification of Tyr(P)-789-PTPα and total PTPα signals was carried out, and the ratios of these that represent PTPα-Tyr-789 phosphorylation per unit of PTPα are shown (A, B, and D).
phosphorylation, which was sensitive to nilotinib inhibition (Fig. 5B). However, nilotinib treatment did not inhibit PTPα-Tyr-789 phosphorylation in any of the cancer cells (Fig. 5B), distinct from its effect on PTPα in SYF cells.

Human chronic myeloid leukemia arises from a chromosomal abnormality leading to the expression of the Bcr-Abl fusion protein, a constitutively active form of Abl. The Abl inhibitors imatinib and nilotinib are widely used to treat chronic myeloid leukemia. Therefore, we investigated PTPα phosphorylation in the BV173 chronic myeloid leukemia cell line, where Abl is constitutively active. Imatinib or nilotinib treatment completely inhibited Bcr-Abl activity, as evidenced by the abolition of Crk phosphorylation, but did not reduce PTPα tyrosine phosphorylation (Fig. 5C).

We next investigated the Abl dependence of IGF-1-stimulated PTPα phosphorylation in fibroblasts that represent a wild-type counterpart to the SFK-deficient SYF cells. Treating MEFs with IGF-1 induced a rapid but small increase (~1.3-fold) in PTPα tyrosine phosphorylation and enhanced Crk phosphorylation (Fig. 5D). In contrast to SYF fibroblasts, IGF-1-induced PTPα phosphorylation in MEFs was unaffected by nilotinib even though Crk phosphorylation was abolished (Fig. 5D), suggesting that the latter, but not the former event, is Abl-dependent. Similar to SYF cells, nilotinib treatment did not alter IGF-1R, Akt, and MAPK phosphorylation.

Together, the above findings indicate that IGF-1-induced PTPα tyrosine phosphorylation is regulated by mechanisms that differ between SYF fibroblasts and other cell types, with nilotinib sensitivity distinguishing the respective Abl-dependent and -independent nature of IGF-1 signaling to PTPα. Furthermore, the manifestation of this difference between the closely related SYF fibroblasts (SFK-deficient MEFs) and wild-type MEFs suggests that it may be determined by the presence or absence of the SFKs Src, Yes, and Fyn.

Reintroduction of SFKs into SYF Cells Eliminates the Abl Dependence of IGF-1-mediated PTPα Tyrosine phosphorylation—To determine whether restoring SFK expression to SYF cells would alter the requirement for Abl in IGF-1-mediated PTPα tyrosine phosphorylation, we introduced Src and Fyn into SYF fibroblasts and confirmed their expression by Western blotting (Fig. 6A, bottom two panels). In Src- or Fyn-transfected cells, IGF-1 stimulated a smaller increase (~1.5-fold) in PTPα-Tyr-789 phosphorylation compared with the more than 2-fold increase detected in mock-transfected SYF cells (Fig. 6, A and B). Crk phosphorylation was detectable in unstimulated Src- or Fyn-expressing cells, but IGF-1 did not stimulate this further (Fig. 6, A and C), suggesting that Abl is active but that its activity is IGF-1-independent. Most interestingly, the presence of Src or Fyn eliminated the nilotinib sensitivity of IGF-1-mediated PTPα tyrosine phosphorylation despite Crk phosphorylation remaining susceptible to nilotinib inhibition (Fig. 6, A and B). Therefore, the expression of Src or Fyn is sufficient to abolish the Abl dependence of IGF-1-induced PTPα-Tyr-789 phosphorylation.

Despite the altered Abl dependence of IGF-1-induced PTPα phosphorylation, wound healing assays demonstrated that IGF-1-induced migration of the parental and the Src/Fyn-expressing SYF cells was inhibited by nilotinib (data not shown). Taken together with the observation that phosphorylation of the Abl substrate and the migration-associated molecule Crk in these three types of cells is abolished by nilotinib (Fig. 6A), this indicates that Abl signaling to molecules other than PTPα, possibly including some of the Abl substrates involved in the regulation of cell shape and motil-
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ity (30, 31), is occurring in the Src/Fyn expressing cells and remains critical for migration.

**RACK1 Plays a Role in IGF-1-mediated PTPα Tyrosine Phosphorylation in MCF-7 Cells**—Because RACK1 is essential for IGF-1-stimulated and Abl-mediated PTPα-Tyr-789 phosphorylation in SYF cells, we investigated whether, as with Abl, there was a different requirement for RACK1 in IGF-1 induced PTPα phosphorylation in other cell types. To this end, we used human RACK1-targeted siRNA to deplete RACK1 expression in MCF-7 breast cancer cells. About 70% of the RACK1 was depleted by treatment with 50 nm siRNA, and this was not increased when the siRNA concentration was doubled (Fig. 7A). Nevertheless, a significant decrease in IGF-1-induced PTPα tyrosine phosphorylation was detected in RACK1-depleted cells (~1.5-fold versus 2.5-fold in control cells, respectively) (Fig. 7, A and B). This suggests that RACK1 is important for IGF-1-induced PTPα phosphorylation regardless of the presence or absence of SFKs.

**PTPα Interacts Directly with RACK1**—To investigate the interaction of PTPα and RACK1 in more detail, we first determined whether recombinant GST-PTPα fusion proteins (depicted schematically in Fig. 8A) could pull down RACK1 from SYF cell lysate. RACK1 was not pulled down by GST alone, but RACK1 associated with GST-PTPα proteins containing both or either of the homologous D1 membrane-proximal and D2 membrane-distal catalytic domains (Fig. 8B). To further refine the RACK1 binding region, we tested two forms of D1-truncated GST-PTPα in pulldown assays. PTPα-D1 lacking some carboxyl-terminal sequence (D1–442) was still able to pull down RACK1, whereas the deletion of most of the D1 catalytic domain (D1–260) completely abolished RACK1 binding (Fig. 8C), indicating that PTPα amino acids 261–442 contained a RACK1 binding site. We also found that a catalytically inactive mutant form of PTPα (D1sD2s) in which the catalytic cysteine residue in both D1 and D2 was mutated to a serine residue (C433S/C723S) was able to bind RACK1 (Fig. 8C).

To identify the RACK1 binding sequence within PTPα-D1, we constructed a scanning peptide array corresponding to the PTPα-D1 sequence. The array contains a series of 15-mer overlapping peptides with a two- or three-amino acid shift between neighboring peptides that covers the PTPα sequence from amino acids 219–468. This was probed with recombinant GST-RACK1, and peptide spots that bound to GST-RACK1 were visualized by probing with antibody to GST. Two series of peptide spots bound to GST-RACK1: C4–7/D1 and D7/E1–2 (Fig. 8D). Common amino acids within each overlapping peptide series defined the two sequences recognized by RACK1 as PTPα amino acids 261–263 (RYV) and 284–293 (SDYINAS-NASFID) (Fig. 8D), with peptides containing the former sequence exhibiting the stronger interaction. Both of these sequences are located in the minimal RACK1-interacting region of D1 defined in the GST pulldown assays. These results indicate that PTPα and RACK1 can interact directly. The ability of RACK1 to bind to the non-identical but homologous sequences in the corresponding regions of PTPα-D2, 554-RVL-556 and 577-TDYV-NASFID-586, remains to be determined.

**DISCUSSION**

Our study to elucidate the mechanism of IGF-1-stimulated PTPα-Tyr-789 phosphorylation in SYF cells has identified Abl as a novel kinase that can phosphorylate PTPα. Moreover, we report that PTPα interacts with RACK1 and is a component of a RACK1-scaffolded protein complex that coordinates IGF-1-regulated signaling to PTPα by bringing IGF-1R, Abl, and PTPα into proximity to promote Abl-catalyzed PTPα phosphorylation.
In the RACK1-coordinated signaling complex that we characterized in SYF cells, the transmembrane molecules IGF-1R and PTPα are constitutively associated with RACK1 in a manner unaltered by the presence or absence of the growth factor IGF-1 or by the activity status of Abl. On the other hand, Abl association with the complex is enhanced by IGF-1 concomitant with its IGF-1-induced activation and is abolished by nilotinib inhibition, suggesting that Abl binding to RACK1 requires the active conformation of the kinase. A third tyrosine kinase in the RACK1 complex, FAK, mimics Abl in the conditions of its association with RACK1. This is consistent with a study showing that FAK association with Tyr-52 of RACK1 is regulated by the phosphorylation of this site by Abl and, therefore, is Abl-dependent (17). Disruption of the complex prevents IGF-1-induced PTPα phosphorylation, as occurs upon the depletion of RACK1 that abrogates the physical interaction of PTPα with IGF-1Rβ and Abl and upon the depletion of Abl or nilotinib-mediated Abl inhibition that disrupts the interaction of Abl with RACK1. In addition to enabling Abl interaction with RACK1, our experiments with the kinase inhibitors nilotinib and PF-228 indicate that Abl catalytic activity, rather than that of IGF-1Rβ or FAK, is responsible for catalyzing PTPα-Tyr-789 phosphorylation. Unlike Abl, the activation of IGF-1R and its association with RACK1 as well as its signaling to downstream molecules like Akt and MAPK are not affected by nilotinib, indicating that PTPα-Tyr-789 phosphorylation is not directly catalyzed by IGF-1Rβ. Although FAK association with RACK1 is nilotinib-sensitive, FAK activity is clearly not required for PTPα phosphorylation because these can be distinguished by concentration-dependent treatment with the FAK inhibitor PF-228.
PTPα Phosphorylation Is Regulated by Interaction with RACK1

In contrast to SYF fibroblasts, we found that IGF-1-stimulated PTPα-Tyr-789 phosphorylation in other cell types, such as wild-type MEFs and various tumor cells, is Abl-independent. This was surprising because IGF-1 activates Abl in these cells, as indicated by the IGF-1-induced and nilotinib-sensitive phosphorylation of the Abl substrate Crk and as reported by others (17, 32). Interestingly, restoring SFK expression to SYF cells rendered IGF-1-induced PTPα phosphorylation Abl-independent. This switch indicates that the presence of Src or Fyn prevents Abl from phosphorylating PTPα and provides a possible explanation for the Abl independence of this reaction in normal MEFs and the tumor cell lines. In SFK-expressing SYF cells, phosphorylation of the Abl substrate Crk remained sensitive to inhibition by nilotinib, demonstrating that Src or Fyn expression does not globally inhibit Abl. However, although PTPα phosphorylation was still up-regulated by IGF-1, Abl activity was released from regulation by IGF-1, suggesting that the presence of SFKs may interfere with the presence or ability of Abl to function within the RACK1-scaffolded IGF-1R-PTPα signaling complex. Although Src and Fyn are RACK1 binding proteins (33, 34), this effect is unlikely to involve competitive displacement of Abl from RACK1 because the interaction of at least Src with Tyr-228 and/or Tyr-246 in the RACK1 WD6 repeat is distinct from the Abl binding region in the WD1–3 repeats of RACK1 (17, 35). In support of SFKs not affecting the interaction of the substrate PTPα in the complex, IGF-1-induced PTPα phosphorylation in MCF-7 cells that express SFKs was also RACK1-dependent. Therefore, the RACK1-PTPα interaction is important for PTPα-Tyr-789 phosphorylation in SFK-expressing as well as SFK-deficient cells.

RACK1 belongs to the WD repeat protein family. It can bind many proteins to regulate their localization, interaction, activation, and/or stability and functions as a hub that coordinates and integrates multiple inputs (36–38). RACK1 can regulate reversible phosphorylation in cell signaling through its interactions with protein kinases, phosphatases, and their substrates. We have identified PTPα as a new RACK1-interacting protein and a role of PTPα as a kinase substrate in a RACK1-scaffolded complex. Analysis of a peptide array spanning the sequence of the PTPα-D1 membrane-proximal catalytic domain pinpoints amino acids 261–263 (RYV) and 284–293 (SDYINASFIN) in PTPα as RACK1 binding sites. The RYV sequence lies at the top of the PTP catalytic cleft, and the central tyrosine is critical for orienting the phosphotyrosine moieties of PTP substrates for dephosphorylation (39). This raises the possibility that RACK1 binding could occlude substrate binding and prevent PTPα-D1-catalyzed protein dephosphorylation, representing a mechanism to negatively regulate PTPα activity. This would not preclude the non-catalytic role of phosphorylated PTPα in promoting cell motility. However, the precise site(s) in PTPα that mediate RACK1 binding in vivo remain to be defined, especially because GST-PTPα pulldown experiments indicate that both the D1 membrane-proximal and the homologous D2 membrane-distal catalytic domains of PTPα can interact with RACK1. Another receptor PTP, PTPμ, also undergoes a catalytic domain-mediated interaction with RACK1 (40). This and our study suggest that the highly conserved catalytic domains of classical tyrosine-specific members of the PTP family, perhaps especially those of the very closely related receptor PTPs, are capable of interaction with RACK1. These PTPs may represent important components of RACK1-coordinated protein tyrosine phosphorylation signaling.

In summary, we identified RACK1 as a novel binding partner of PTPα that couples PTPα to the IGF-1R. In the absence of SFKs, RACK1 coordinates IGF-1-stimulated Abl-dependent phosphorylation of PTPα-Tyr-789. The presence of SFKs switches the mode of IGF-1-induced PTPα phosphorylation to one that is Abl-independent and probably catalyzed by SFKs. RACK1 is gaining growing recognition as a scaffolding mediator of migration signaling, including a role in integrating signals that regulate IGF-1 and integrin-stimulated migration (37, 41). PTPα-Tyr-789 phosphorylation in response to either IGF-1 or integrin promotes cell migration (4, 9), and RACK1-regulated PTPα-Tyr-789 phosphorylation may be a shared functional component of these networks.

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