RACK1-mediated Integration of Adhesion and Insulin-like Growth Factor I (IGF-I) Signaling and Cell Migration Are Defective in Cells Expressing an IGF-I Receptor Mutated at Tyrosines 1250 and 1251*

Received for publication, November 15, 2004
Published, JBC Papers in Press, December 17, 2004, DOI 10.1074/jbc.M412889200

Patrick A. Kiely, Madeline Leahy, Denise O’Gorman, and Rosemary O’Connor‡
From the Cell Biology Laboratory, Department of Biochemistry, BioSciences Institute, National University of Ireland, Cork, Ireland

The scaffolding protein receptor for activated C kinase (RACK1) has been proposed to mediate the integration of insulin-like growth factor I receptor (IGF-IR) and adhesion signaling. Here we investigated the mechanisms of this integration of signaling, by using an IGF-IR mutant (Y1250F/Y1251F) that is deficient in anti-apoptotic and transforming function. RACK1 was found to associate with the IGF-IR only in adherent cells and did not associate with the IGF-IR in nonadherent cells, lymphocytic cells, or cells expressing the Y1250F/Y1251F mutant. In R− cells transiently expressing the Y1250F/Y1251F mutant RACK1 became constitutively associated with β1 integrin and did not associate with Shc, Src, or Shp2. This was accompanied by the loss of formation of a complex containing the IGF-IR, RACK1, and β1 integrin; loss of migratory capacity; enhanced Src and FAK activity; enhanced Akt phosphorylation; and decreased p38 mitogen-activated protein kinase activity. Shc was not phosphorylated in response to IGF-I in cells expressing the Y1250F/Y1251F mutant and remained associated with protein phosphatase 2A. Similar alterations in signaling were observed in cells that were stimulated with IGF-I in nonadherent cultures. Our data suggest that disruption of RACK1 scaffolding function in cells expressing the Y1250F/Y1251F mutant results in the loss of adhesion signals that are necessary to regulate Akt activity and to promote turnover of focal adhesions and cell migration.

The IGF-IR is widely expressed and, activated by the ligands IGF-I or IGF-II, is essential for normal growth during development as well as for cell survival, differentiation, and migration in fibroblasts, muscle cells, lymphocytes, and neuronal cells. In addition, there is considerable evidence to indicate that the IGF-IR is an important contributor to the growth, survival, and migratory/invasive capacity of transformed cells and thereby to the spread of cancer (1, 2). In mouse models of tumorigenesis, enhanced IGF-IR expression promotes the acquisition of a metastatic and invasive phenotype (3). Conversely, the suppression of IGF-IR expression by antisense strategies (4) or antibodies results in decreased tumor growth and decreased metastatic capacity in tumor cell models (5–7).

Adhesion signals from the extracellular matrix are necessary to regulate the attachment and detachment of cells during cell migration and are generally considered to cooperate with growth factor signaling in normal cells as well as in tumor cell migration and invasion (8–11). The IGF-IR contributes to tumor cell migration by several mechanisms. These include interactions with the cadherin network (3), interactions with integrins and angiogenic factors (12–14), and activation of signaling responses necessary for migration and invasion (15–17).

There is also evidence that adhesion signals negatively regulate growth factor signaling. In smooth muscle cells, integrin ligation controls the availability of phosphatases that can negatively regulate IGF-I-mediated proliferation and cell movement. Integrin ligation causes the Shps-1 protein to recruit the tyrosine phosphatase Shp-2 to the cell membrane, where it dephosphorylates an active IGF-IR and attenuates signaling (18, 19). However, although there is abundant evidence for physical and functional links between IGF-IR and adhesion signaling, the mechanisms of interaction and integration of IGF-IR and adhesion signals are not well understood. One way in which these signals could be integrated is through the actions of the WD repeat scaffolding protein RACK1 (Ref. 20; reviewed in Ref. 21), which interacts with both the IGF-IR and integrins and can modulate IGF-I and integrin-mediated signaling (22, 23). Overexpression of RACK1 in transformed fibroblasts promotes cell spreading and attachment (23). Overexpression of RACK1 in MCF-7 cells promotes proliferation and MAPK activation, whereas it suppresses protection from apoptosis and Akt phosphorylation (22).

An IGF-IR mutant that has tyrosines 1250 and 1251 mutated to phenylalanine (Y1250F/Y1251F) has been shown to be deficient in cytoskeletal organization (24), suppression of apoptosis (25), promotion of anchorage-independent growth (5), and promotion of tumor metastasis in a murine tumor model (6). Recently, we demonstrated that when this mutant is expressed in FL5.12 lymphocytic cells, IGF-I-mediated phosphorylation of Shc, Ras activation, and MAPK activation are all decreased (26). These findings suggest that IGF-I-mediated activation of the Shc, Ras, and MAPK pathways have a particular role in the transforming and migratory functions of the IGF-IR. Although Shc phosphorylation is implicated in integrin signaling leading to activation of MAPK (27, 28), the requirement for Shc in IGF-IR signaling in nonhemopoietic cells is not fully understood. The potential for RACK1 scaffolding function in regulating Shc signaling from integrins has also not been investigated. All of this prompted us to investigate RACK1...
scaffolding function and Shc activity in IGF-I-mediated signaling in adherent cells expressing the Y1250F/Y1251F mutant.

We found that RACK1 does not associate with the IGF-IR in fibroblasts or MCF-7 cells when the cells were not attached to a substrate. RACK1 did not associate with the IGF-IR in FL5.12 cells or with the Y1250F/Y1251F IGF-IR mutant when expressed in adherent cells. Complexes of the IGF-IR and β1 integrins with RACK1 were not detected with this mutant, and the cells were completely deficient in migration toward IGF-I compared with R- cells expressing WT IGF-IR. IGF-I did not induce phosphorylation of Shc or its dissociation from PP2A in cells expressing the mutant IGF-IR. Src and Shp2 were associated with RACK1 in R- cells expressing WT IGF-IR, but not in cells expressing the Y1250F/Y1251F mutant. Lack of Src sequestration was accompanied by a dramatic increase in Src kinase activity. FAK and Akt were both hyperphosphorylated in cells expressing the Y1250F/Y1251F mutant, but phosphorylation of p38 was decreased. Similar IGF-I-mediated signaling responses were observed in cells that were stimulated in nonadherent cultures. Altogether the data demonstrate that the lack of RACK1-mediated integration of adhesion and IGF-IR signaling that is necessary for cell migration is deficient in cells expressing the Y1250F/Y1251F mutant.

**EXPERIMENTAL PROCEDURES**

**Materials—** Recombinant IGF-I and EGF were purchased from Pepro Tech. Inc. (Rocky Hill, NJ). The anti-IGF-IR and anti-Src were from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-phospho-Akt, anti-Akt, anti-phospho-p38, anti-phospho FAK, and anti-phospho EGFR (Type IV) polyclonal antibodies and the anti-phospho-p42/44 MAPK monokonal antibody were from Cell Signaling Technology (Beverly, MA). The anti-phosphotyrosine monoclonal antibody (4G10), anti-Grb2 and anti-Erk-2 monoclonal antibodies, and anti-IRS-1, anti-IRS-2, anti-Src and anti-PI3K antibodies were from Cell Signaling Technology, Inc. (Beverly, MA). The anti-actin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-phospho-Akt, anti phospho-p38, and anti-phospho FAK were from Cell Signaling Technology, Inc. (Beverly, MA). The anti-actin monoclonal antibody was purchased from Sigma. Okadaic acid, fibronectin, and trypsin were purchased from Sigma.

**Cell Culture, Transfection, and IGF-I Stimulation—** The MCF-7 breast carcinoma cell line, the R- cell line (mouse embryo fibroblast cell line derived from the IGF-IR knockout mouse) (29), and R+ cells (R cells that were retransfected with the IGF-IR cDNA) (29), were all maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Biowhittaker, Verviers, Belgium), supplemented with 10% (v/v) fetal bovine serum and 2 mM l-glutamine, and penicillin/streptomycin. R- cells were transiently transfected with pcDNA3 IGF-IR WT, IGF-IR Y1250F/Y1251F, or empty pcDNA3 vectors (8 μg of DNA) using Lipofectamine (Invitrogen) as described previously (22). After 18 h in culture the transfected cells were split into 6-well plates or 10-cm plates and cultured for an additional 18 h. FL5.12 cells that overexpress the wild type (FL5.12/ WT) IGF-IR have been described previously (25). The cells were maintained in Iscove’s modified defined medium supplemented with 2 mM glutamine, 10% (v/v) fetal bovine serum (all from Biowhittaker, Belgium) and 10% (v/v) conditioned medium from the interleukin 3-producing cell line WEHI-3B.

For analysis of signaling responses in adherent cells, the cells were washed extensively with PBS and starved from serum for 4 h before stimulating with IGF-I for the indicated times. For analysis of signaling responses in detached cells, confluent MCF-7 or R+ cells or HeLa cells were detached with trypsin-EDTA (Invitrogen), which was then removed by washing the cells with PBS. The cells were resuspended in serum-free media and kept in suspension for 4 h until stimulated with IGF-I for the indicated times.

**Preparation of Cellular Protein Extracts and Immunoprecipitation—** Cellular protein extracts were prepared by washing cells with PBS and then scraping into lysis buffer consisting of Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40 plus the tyrosine phosphate inhibitor Na3VO4 (1 mM), and the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), pepstatin (1 μM), and aprotonin (1.5 μg/ml). After incubation at 4 °C for 20 min, nuclear and cellular debris were removed by microcentrifugation at 20,000 × g for 15 min at 4 °C.

**Migration Assays in Boyden Chambers—** Boyden chamber apparatus were loaded with serum-free DMEM supplemented with 10 μg/ml IGF-I (final concentration), 50 ng/ml EGF or serum-free DMEM alone as a control. A 50-μl volume of cell suspension containing 50,000 cells was added to each upper well. The loaded chamber was placed in a 37 °C incubator enriched with 5% CO2. After 4 h, the chamber was removed from the incubator and disassembled. For measurement of adherent cells on the upper surface of the membrane were removed by scraping so that only cells that had migrated through the membrane remained. The membrane was then fixed with methanol, stained with 0.1% crystal violet, and air-dried. Cell counts were obtained by counting all cells, and the data are presented as the averages of counts from five fields each of triplicate wells for each test condition.

**Assay to Measure Adhesion to Fibronectin—** R- cells were transiently transfected with pcDNA3 IGF-IR WT, IGF-IR Y1250F/Y1251F, or empty pcDNA3 vectors as described above. The cells were washed and serum-starved for 4 h before removal from plates with trypsin. The wells of a 96-well plate were coated with 100 μl of fibronectin (5 μg/ml) for 2 h, and control wells were coated with bovine serum albumin (2.5% in H2O). The plates were washed extensively with PBS and blocked with 100 μl of 2.5% bovine serum albumin/well for 1 h before further washing with PBS. Cells (100 μl containing 2 × 105 cells) were added to each coated well in triplicate and allowed to attach for the indicated times. The medium was removed, the wells were washed with serum-free medium, and attached cells were fixed with Methanol. Crystal Violet was then added, and the cells were destained by extensive washing in H2O and allowed to air dry overnight. Cellular protein was solubilized in addition of X-100 through heating to 95 °C and room temperature, and absorbance for each well was read at 595 nm. The data are presented as the means and standard deviation of absorbance in triplicate wells.

**In Vitro Kinase Assays to Determine Src Activity—** R- cells transiently transfected with pcDNA3 IGF-IR WT, IGF-IR Y1250F/Y1251F, or empty pcDNA3 vectors were lysed as described above. c-Src was immunoprecipitated using anti-Src antibodies and protein G-agarose beads...
beads as described. The immune complexes were resuspended in 15 µl of kinase buffer (10 mM Tris-HCl, 10 mM MgCl₂) with 1 µl [³²P]ATP (10 μCi/µl), 0.3 µl of 100 ìCi cold ATP, with or without 5 µg of acid-denatured enolase (Sigma), and the reactions were allowed to proceed at room temperature for 3 min. The beads were washed three times with ice-cold kinase buffer, and the protein was then removed from the beads by boiling for 5 min in 20 µl of 2× SDS-PAGE sample buffer for electrophoresis. Dried gels were then assessed for incorporated radioactivity by using a phosphor imager.

RESULTS

Association of RACK1 with the IGF-IR Is Dependent on Cell Adhesion—The regulatory adapter protein RACK1 has previously been shown to associate with the IGF-IR in a ligand-independent manner in fibroblasts and MCF-7 cells (22). When R+ cells were stimulated with IGF-I for up to 60 min, the amount of RACK1 that co-precipitated with the IGF-IR remained constant regardless of the degree of tyrosine phosphorylation of the IGF-IR (Fig. 1A). Hermanto et al. (23) demonstrated that RACK1 associates with β1 integrin in response to IGF-I stimulation. We therefore asked whether adhesion regulates association of RACK1 with the IGF-IR. The IGF-IR was immunoprecipitated from either R+ cells or MCF-7 cells that were maintained in medium supplemented with 10% fetal bovine serum either while attached to tissue culture plates, or alternatively, after removal from plates and culture in suspension for 4 h. The immunoprecipitates were assessed for association of RACK1. As can be seen in Fig. 1B RACK1 was associated with the IGF-IR in R+ cells and MCF-7 cells only under adherent conditions but did not associate when the cells were maintained in suspension (Fig. 1B). This indicates that adhesion is required for association of RACK1 with the IGF-IR.

To further investigate the requirement for adhesion signals for the association of RACK1 with the IGF-IR, we tested interleukin 3-dependent murine B-lymphocytic FL5.12 cells, which grow in suspension and thus are not dependent on adhesion for viability or growth. Although FL5.12 cells express high levels of RACK1, no co-immunoprecipitation with the IGF-IR was observed (Fig. 1C). In addition, treatment of FL5.12 cells with phorbol 12-myristate 13-acetate to activate protein C kinases did not promote association of RACK1 with the IGF-IR. Together with the data from R+ cells and MCF-7 cells, this suggests that association of RACK1 with the IGF-IR requires adhesion signals, and this does not occur in lymphoid cells. The data also suggest that RACK1 associates with the IGF-IR as a consequence of adhesion.

Hermanto et al. (23) showed that both RACK1 and the IGF-IR became associated with β1 integrin in response to stimulation of cells with either IGF-I or phorbol 12-myristate 13-acetate. Here we investigated the necessity for adhesion for association of RACK1 with β1 integrin in MCF-7 cells. In adherent MCF-7 cells RACK1 was not associated with β1 integrin in the absence of IGF-I, but IGF-I-induced recruitment of RACK1 to β1 integrin. In contrast, when MCF-7 cells were detached, RACK1 was not consistently associated with β1 integrin in the absence of IGF-I. The amount of IGF-IR-associated RACK1 in detached cells was similar to that detected in cells that were stimulated with IGF-I for 10 min (Fig. 1D). Recruitment of RACK1 to β1 integrin appeared to occur in a biphasic pattern with higher levels of RACK1 detected at 20 min of IGF-I stimulation than at 10 min in both adherent and suspension cultures of MCF-7 cells (Fig. 1D). The data indicate that adhesion regulates association of RACK1 with the IGF-IR and its dissociation from β1 integrin and support the idea that RACK1 mediates adhesion-dependent regulation of IGF-IR signaling.

RACK1 Does Not Associate with the Y1250F/Y1251F Mutant IGF-IR—We previously demonstrated that overexpression of 

![Image](http://www.jbc.org/ccc/354/x354-526f1.large.png)

**FIG. 1.** RACK1 co-precipitates with the IGF-IR in adherent cells and with β1 integrin in nonadherent cells. A, R+ cells were serum-starved for 4 h and stimulated with IGF-I for the indicated times. The cell lysates were prepared, and the IGF-IR was immunoprecipitated (IP) from 500 µg of total cellular protein followed by Western blotting to measure phosphotyrosine content and associated RACK1. The membrane was stripped and reprobed with anti-IGF-IR antibody to confirm that a comparable level of IGF-IR was present in each sample. B, cell lysates were prepared from R+ and MCF-7 cells that were maintained in complete medium either attached or detached for 4 h. IGF-IR was immunoprecipitated from R+ cells or MCF-7 cells that were maintained in medium supplemented with 10% fetal bovine serum either while attached to tissue culture plates, or alternatively, after removal from plates and culture in suspension for 4 h. The immunoprecipitates were assessed for association of RACK1. As can be seen in Fig. 1B RACK1 was associated with the IGF-IR in R+ cells and MCF-7 cells only under adherent conditions but did not associate when the cells were maintained in suspension (Fig. 1B). This indicates that adhesion is required for association of RACK1 with the IGF-IR.

To further investigate the requirement for adhesion signals for the association of RACK1 with the IGF-IR, we tested interleukin 3-dependent murine B-lymphocytic FL5.12 cells, which grow in suspension and thus are not dependent on adhesion for viability or growth. Although FL5.12 cells express high levels of RACK1, no co-immunoprecipitation with the IGF-IR was observed (Fig. 1C). In addition, treatment of FL5.12 cells with phorbol 12-myristate 13-acetate to activate protein C kinases did not promote association of RACK1 with the IGF-IR. Together with the data from R+ cells and MCF-7 cells, this suggests that association of RACK1 with the IGF-IR requires adhesion signals, and this does not occur in lymphoid cells. The data also suggest that RACK1 associates with the IGF-IR as a consequence of adhesion.

Hermanto et al. (23) showed that both RACK1 and the IGF-IR became associated with β1 integrin in response to stimulation of cells with either IGF-I or phorbol 12-myristate 13-acetate. Here we investigated the necessity for adhesion for association of RACK1 with β1 integrin in MCF-7 cells. In adherent MCF-7 cells RACK1 was not associated with β1 integrin in the absence of IGF-I, but IGF-I-induced recruitment of RACK1 to β1 integrin. In contrast, when MCF-7 cells were detached, RACK1 was not consistently associated with β1 integrin in the absence of IGF-I. The amount of IGF-IR-associated RACK1 in detached cells was similar to that detected in cells that were stimulated with IGF-I for 10 min (Fig. 1D). Recruitment of RACK1 to β1 integrin appeared to occur in a biphasic pattern with higher levels of RACK1 detected at 20 min of IGF-I stimulation than at 10 min in both adherent and suspension cultures of MCF-7 cells (Fig. 1D). The data indicate that adhesion regulates association of RACK1 with the IGF-IR and its dissociation from β1 integrin and support the idea that RACK1 mediates adhesion-dependent regulation of IGF-IR signaling.

RACK1 Does Not Associate with the Y1250F/Y1251F Mutant IGF-IR—We previously demonstrated that overexpression of
Detect RACK1, IGF-IR, and cipitated from 500 tated. equal levels of each receptor were expressed and immunoprecipi- 

- IP

This pattern is very similar to that observed with nonadherent cells expressing the Y1250F/Y1251F C-terminal mutant of the IGF-IR but does interact with β1 integrin in cells expressing this mutant. A, R− cells were transiently transfected with plasmids encoding WT IGF-IR or the Y1250F/Y1251F mutant. Thirty-six hours later the cells were serum-starved and stimulated with IGF-I for the indicated times before the IGF-IR was immunoprecipitated (IP) with anti-IGF-IR antibody followed by Western blotting for RACK1. The blot was reprobed with anti-IGF-IR to show that equal levels of each receptor were expressed and immunoprecipitated. B, R− cells expressing WT IGF-IR or Y1250F/Y1251F mutant were serum-starved for 4 h and stimulated with IGF-I for the indicated times. The cell lysates were prepared, and β integrin was immunoprecipitated from 500 μg of total cellular protein followed by Western blot analysis to detect associated RACK1 and β-integrin levels in the immunoprecipitates. C, R− cells were transfected and stimulated as in B before immunoprecipitation of β1 integrin and Western blotting to detect RACK1, IGF-IR, and β1 integrin.

Therefore interested to determine whether RACK1 function was altered in cells expressing a mutant of the IGF-IR (Y1250F/Y1251F) that has previously been found to be deficient in promoting cellular transformation, survival, metastatic tumor growth, and cytoskeletal organization (5, 6, 24, 25).

We first investigated whether RACK1 associated with the Y1250F/Y1251F mutant IGF-IR by immunoprecipitating WT or Y1250F/Y1251F mutant IGF-IR that was transientsly transfected into R− cells. As can be seen in Fig. 2A, there was no RACK1 associated with the Y1250F/Y1251F mutant either in the absence or the presence of IGF-I. We also measured the ability of RACK1 to interact with β1 integrin in cells expressing the WT or Y1250F/Y1251F mutant (Fig. 2B). This demonstrated that although RACK1 was recruited to β1 integrin in response to IGF-I in a biphasic pattern, it was constitutively associated with RACK1 in cells expressing the Y1250F/Y1251F mutant but still exhibited increased recruitment at 20 min. This pattern is very similar to that observed with nonadherent cells in Fig. 1D and suggests that cells expressing the Y1250F/Y1251F mutant respond to IGF-I as if they lack an adhesion signal.

We next investigated whether a complex between the IGF-IR, RACK1, and β1 integrin may be altered in cells expressing the Y1250F/Y1251F mutant. In cells expressing WT IGF-IR, the IGF-IR was detected in β1 integrin immunoprecipitates from IGF-I-stimulated cells (Fig. 2C). However, in cells expressing the Y1250F/Y1251F mutant, there was no detectable IGF-IR in the β1 integrin complex, although RACK1 was still associated with β1 integrin (Fig. 2C). Thus, an IGF-I-mediated complex containing the IGF-IR, RACK1, and β1 integrin that forms in cells expressing WT IGF-IR cannot be detected in cells expressing the Y1250F/Y1251F mutant. This may explain why cells expressing this mutant lack adhesion signaling.

Cells Expressing the Y1250F/Y1251F Mutant Are Deficient in Migration toward IGF-I and Adhere More Rapidly than Cells Expressing WT IGF-IR—Although the Y1250F/Y1251F mutant is well characterized in terms of its lack of function in suppressing apoptosis when stably expressed in R− cells, FL5.12 cells, and tumor cell lines, its function upon transient expression in R− cells has not been analyzed. We therefore investigated the migratory capacity of cells expressing either the WT or Y1250F/Y1251F mutant in modified Boyden chamber assays compared with mock transfected vector cells. This demonstrated that although the vector-transfected cells exhibited no migration toward IGF-I, cells expressing the WT IGF-IR had greatly increased migration. However, cells expressing the Y1250F/Y1251F receptor exhibited no migration (Fig. 3A). This indicates that in R− cells the Y1250F/Y1251F mutant cannot promote IGF-I-mediated chemotaxis.

We also investigated the ability of these cells to attach to fibronectin (Fig. 3B). R− cells expressing the Y1250F/Y1251F mutant had more cells attached after 15 min than cells expressing WT IGF-IR, although there was no great difference in the number of attached cells after 20 min. This suggests that the mutant expressing cells have increased initial adhesive strength compared with WT cells. Overall, the data suggest that a signal required for motility, but not adhesion, is lacking in cells expressing the Y1250F/Y1251F mutant.

IGF-I-mediated Shc Phosphorylation and Association of Shc with RACK1 Are Deficient in R− cells Expressing the Y1250F/Y1251F Mutant IGF-IR—We previously observed that Shc phosphorylation by IGF-I was greatly impaired in cells expressing an IGF-IR mutated at tyrosines 1250 and 1251 and that this correlated with loss of MAPK signaling and cell survival (26). RACK1 does not associate with the IGF-IR in FL5.12 cells and tumor cell lines, its function upon transient expression in R− cells has not been analyzed. We therefore investigated the ability of these cells to attach to fibronectin (Fig. 3B). R− cells expressing the Y1250F/Y1251F mutant had more cells attached after 15 min than cells expressing WT IGF-IR, although there was no great difference in the number of attached cells after 20 min. This suggests that the mutant expressing cells have increased initial adhesive strength compared with WT cells. Overall, the data suggest that a signal required for motility, but not adhesion, is lacking in cells expressing the Y1250F/Y1251F mutant.

We also investigated the ability of these cells to attach to fibronectin (Fig. 3B). R− cells expressing the Y1250F/Y1251F mutant had more cells attached after 15 min than cells expressing WT IGF-IR, although there was no great difference in the number of attached cells after 20 min. This suggests that the mutant expressing cells have increased initial adhesive strength compared with WT cells. Overall, the data suggest that a signal required for motility, but not adhesion, is lacking in cells expressing the Y1250F/Y1251F mutant.

FIG. 2. RACK1 does not associate with the Y1250F/Y1251F C-terminal mutant of the IGF-IR but does interact with β1 integrin in cells expressing this mutant. A, R− cells were transiently transfected with plasmids encoding WT IGF-IR or the Y1250F/Y1251F mutant. Thirty-six hours later the cells were serum-starved and stimulated with IGF-I for the indicated times before the IGF-IR was immunoprecipitated (IP) with anti-IGF-IR antibody followed by Western blotting for RACK1. The blot was reprobed with anti-IGF-IR to show that equal levels of each receptor were expressed and immunoprecipitated. B, R− cells expressing WT IGF-IR or Y1250F/Y1251F mutant were serum-starved for 4 h and stimulated with IGF-I for the indicated times. The cell lysates were prepared, and β integrin was immunoprecipitated from 500 μg of total cellular protein followed by Western blot analysis to detect associated RACK1 and β-integrin levels in the immunoprecipitates. C, R− cells were transfected and stimulated as in B before immunoprecipitation of β1 integrin and Western blotting to detect RACK1, IGF-IR, and β1 integrin.
She phosphorylation was greatly decreased in cells expressing the Y1250F/Y1251F mutant compared with WT (Fig. 4C). RACK1 became associated with She in response to IGF-I stimulation in cells expressing the WT IGF-IR. However, association of RACK1 with She was not detected in cells expressing the Y1250F/Y1251F mutant (Fig. 4C).

To explore the mechanism of decreased She phosphorylation in cells expressing the Y1250F/Y1251F mutant, we investigated the status of PP2A association with She. It has previously been demonstrated that PP2A associates with She, and its dissociation upon stimulation with growth factors is thought to facilitate She phosphorylation (30). We found, in agreement with Ugi et al. (30), that PP2A associates with She in R– cells expressing the WT IGF-IR in the absence of IGF-I stimulation, and She rapidly becomes dissociated in response to IGF-I stimulation (Fig. 4D). However, in R– cells expressing the Y1250F/Y1251F mutant, PP2A remained associated with She both in the presence and absence of IGF-I. This lack of IGF-I-mediated phosphorylation of She and lack of dissociation of PP2A from She was also observed in R+ and MCF-7 cells that were stimulated with IGF-I when the cells were stimulated in suspension (shown for MCF-7 cells in Fig. 4E). This suggests that adhesion is necessary for IGF-I-mediated dissociation of PP2A from She and that this adhesion signal is deficient in cells expressing the Y1250F/Y1251F mutant.

If a lack of dissociation of PP2A from She prevents IGF-I-mediated phosphorylation of She, one would predict that inhibition of PP2A enzymatic activity could restore IGF-I-mediated phosphorylation of She. To investigate this we used okadaic acid to inhibit PP2A activity in R– cells transiently expressing either WT or mutant IGF-IR. As can be seen in Fig. 4E, okadaic acid restored IGF-I-mediated phosphorylation of She as well as recruitment of RACK1. This suggests that PP2A activity inhibits She phosphorylation by IGF-I and its association with RACK1.

Altogether the data indicate that cells expressing the Y1250F/Y1251F mutant have altered association of IRS-1 with RACK1 and are deficient in an adhesion signal that is necessary to stimulate dissociation of PP2A from She, phosphorylation of She, and association of RACK1 with She.

EGF Can Induce Phosphorylation of She and Association with RACK1 in R– Cells Expressing the Y1250F/Y1251F Mutant—We next sought to determine whether the lack of She phosphorylation or the lack of association of RACK1 with the Y1250F/Y1251F IGF-IR was essential for the lack of migratory capacity of cells expressing this mutant. To so this we asked whether the requirement for adhesion for IGF-I-mediated She phosphorylation was unique to IGF-I or was also a requirement for EGF, which has previously been implicated in phosphorylation of She by IGF-I (31). In attached R– cells transiently expressing the Y1250F/Y1251F mutant, EGF induced phosphorylation of She at levels comparable with those of IGF-I in cells expressing WT IGF-IR (Fig. 5A) and also induced recruitment of RACK1 to She. We also noted that EGF could induce phosphorylation of She in nonadherent cells (data not shown).

Migration toward IGF-I and EGF in Boyden chambers was then compared in R– cells expressing either the Y1250F/Y1251F mutant or the WT IGF-IR. Although R– cells transfected with empty vector exhibited no migration toward IGF-I or EGF, cells expressing the WT IGF-IR exhibited extensive migration toward IGF-I cells, and the number of cells migrating toward EGF was increased by almost 50%. Cells expressing the Y1250F/Y1251F mutant had almost no migration toward IGF-I, and migration toward EGF was decreased by ~60% compared with WT IGF-IR (Fig. 5B). These data suggest that the IGF-IR facilitates EGF-mediated cell migration and also indicates that Y1250F/Y1251F mutation abolishes this enhancement of migratory capacity toward EGF. The data also suggest that phosphorylation of She and its association with RACK1 in response to EGF is not sufficient for the migratory capacity of these cells.

RACK1 Does Not Associate with Src and Shp2, and There Is Increased Src and FAK Activity in Cells Expressing the Y1250F/Y1251F Mutant—Our data with EGF-mediated phosphorylation of She and migration suggested that She phosphorylation is not sufficient for the migratory capacity of transfected R– cells or the lack of migratory function in cells expressing the Y1250F/Y1251F mutant. We therefore investigated the status of other regulators of cell migration, in particular Src, which is a RACK1-interacting protein and which has been proposed to regulate cell migration (32–34). Association of RACK1 with Src has been shown to suppress Src activity (33), and we have previously demonstrated that IGF-I induces dissociation of Src from RACK1 (22).

We first investigated the association of Src with RACK1 in cells expressing WT/IGF-IR (Fig. 6A). As expected, Src was associated with RACK1 and became slowly dissociated after 20
min of stimulation with IGF-I. In contrast no Src was found to be associated with the Y1250F/Y1251F mutant IGF-IR. We also measured Src kinase activity toward enolase in these cells and found that Src kinase activity was greatly increased in cells expressing the Y1250F/Y1251F mutant compared with cells expressing WT IGF-IR (Fig. 6B). This indicates that a lack of sequestration of Src by RACK1 results in enhanced tyrosine kinase activity of Src.

We also measured association of the tyrosine phosphatase Shp2 with RACK1. Shp2 has previously been shown to be essential for IGF-I-mediated migration (15) and is also thought to be a regulator of Src activity (35, 36). In R− cells expressing the WT IGF-IR Shp2 was associated with RACK1, but there was greatly decreased association of Shp-2 with RACK1 in cells expressing the Y1250F/Y1251F mutant (Fig. 6C).

An increase in Src activity and altered Shp2 location in cells expressing the Y1250F/Y1251F mutant could influence the migratory capacity of these cells by their effects on components of the focal adhesion complexes. Therefore we measured the status of FAK in cells expressing the Y1250F/Y1251F mutant.
IGF-I has previously been shown to suppress FAK activity (15, 17), and this is thought to be necessary for focal adhesion turnover and cell migration. FAK phosphorylation of the Src-dependent Tyr397 site was high in cells expressing the Y1250F/Y1251F mutant (Fig. 6D), and although IGF-I caused a decreased in phosphorylation in cells expressing WT IGF-IR, there was no IGF-I-mediated decrease observed in cells expressing the mutant IGF-IR.

Altogether the data suggest that a RACK1 complex with Src and Shp2 is disrupted in cells expressing the Y1250F/Y1251F mutant IGF-IR, and this leads to enhanced Src activity as well as enhanced FAK phosphorylation. Deregulated Src and enhanced FAK may suppress the turnover of focal adhesions that is necessary for IGF-I-mediated cell migration.

Akt Activity Is Enhanced and Activation of p38 MAPK Is Reduced in Cells Expressing the Y1250F/Y1251F Mutant—The data demonstrate that cells expressing the Y1250F/Y1251F mutant are deficient in Shc phosphorylation and are deficient in RACK1-mediated regulation of Src activity. Because we previously demonstrated that overexpression of RACK1 can suppress IGF-I-mediated activation of Akt, we next investigated the status of Akt and MAPKs in cells expressing the Y1250F/Y1251F mutant. As can be seen in Fig. 7A, IGF-I-mediated activation of Akt was greatly increased in cells expressing the Y1250F/Y1251F mutant. Erk phosphorylation was comparable in cells expressing WT or mutant IGF-IR (Fig. 7A). In contrast, IGF-I-mediated phosphorylation of p38 MAPK was detected at 5 min in cells expressing WT IGF-IR but was

![Figure 5](http://www.jbc.org/)

**FIG. 5.** EGF induces phosphorylation of Shc and association of RACK1 with Shc in cells expressing the Y1250F/Y1251F mutant. A, R− cells transiently expressing either WT or Y1250F/Y1251F IGF-IR were serum-starved and stimulated with either IGF-I or EGF for the indicated times. The blot was probed for anti-Shc to measure Shc in each immunoprecipitate. B, R− cells transiently expressing WT or Y1250F/Y1251F were seeded at 5 × 10⁴ cells/chamber in modified Boyden chambers and allowed to migrate toward IGF-I or EGF for 24 h. The cells on the upper surface of the membrane were removed by scraping, and the membrane was then stained with 0.1% crystal violet and air-dried for cell counting. The data are presented as the averages of total cell counts from five fields of triplicate wells for each test condition. The inset is a Western blot showing expression levels of IGF-IR in cells transfected with empty vector (lane 1), WT (lane 2), and Y1250F/Y1251F (lane 3).

![Figure 6](http://www.jbc.org/)

**FIG. 6.** Loss of Src and Shp2 association with RACK1, and enhanced Src and FAK activity in R− cells expressing Y1250F/Y1251F mutant. A, RACK1 was immunoprecipitated (IP) from R− cells transfected with plasmids encoding WT and Y1250F/Y1251F mutant after stimulation with IGF-I. The blot was probed for associated Src and IGF-IR. The cell lysates are included to show that equal amounts of the IGF-IR are expressed. B, Src was immunoprecipitated from R− cells transfected with empty vector (Co) plasmids encoding WT and Y1250F/Y1251F mutant and subjected to an in vitro kinase assay using enolase as a substrate. The supernatant was run on a gel and exposed using a phosphorus imager to detect 32P incorporation. Immunoprecipitated Src was analyzed by Western blotting to measure Src in the immunoprecipitates. C, R− cells expressing WT IGF-IR or Y1250F/Y1251F mutant were serum-starved for 4 h and stimulated with IGF-I for the indicated times. RACK1 was immunoprecipitated from 500 μg of total cellular protein followed by Western blot analysis to detect Shp2 and RACK1. D, cell lysates were prepared from R− cells expressing WT IGF-IR or Y1250F/Y1251F mutant that were serum-starved for 4 h and stimulated with IGF-I for the indicated times. Western blot analysis was carried out using antibodies directed against phospho-FAK (Y927). The blot was stripped and reprobed for FAK and actin to determine protein loading.
To determine whether the enhanced Akt phosphorylation may be due to a lack of an adhesion mediated signal caused by loss of RACK1 association with the Y1250F/Y1251F mutant, we investigated signaling in adherent and nonadherent cultures of R+ cells and MCF-7 cells. The data for R+ cells are shown in Fig. 7C. IGF-I phosphorylation of Akt in detached R+ cells was greatly increased compared with adherent cells (Fig. 7C). Erk phosphorylation was also slightly increased when R+ cells were detached. Similar effects were seen with IGF-I-mediated Akt phosphorylation in MCF-7 cells (data not shown). This indicates that a similar effect on IGF-I-mediated Akt phosphorylation is observed in cells expressing the Y1250F/Y1251F mutant and in detached cells. This effect is opposite to the inhibition of Akt observed with RACK1 overexpression in MCF-7 cells (22). This suggests that the lack of a RACK1-mediated adhesion signal contributes to the enhanced Akt activation in cells expressing the Y1250F/Y1251F mutant.

**DISCUSSION**

A model is presented in Fig. 8 to summarize the findings of this study. In cells expressing WT IGF-IR and IGF-I signals lead to the formation of a complex containing IGF-IR, β1 integrin, Shp2, and IRS-1 associates with RACK1. Src dissociates from RACK1, and β1 integrin associates with RACK1. FAK phosphorylation decreases in response to IGF-I, and both Akt and p38 are phosphorylated. Cells migrate toward IGF-I. The right panel represents the results obtained with cells expressing Y1250F/Y1251F mutant stimulated with IGF-I. RACK1 does not associate with the IGF-IR, IRS-1, Shp2, Shc, or Src but is constitutively associated with β1 integrin. Src is not phosphorylated. Src, FAK, and Akt activity are increased, whereas p38 phosphorylation is decreased. The cells do not migrate toward IGF-I.
overexpression of the IGF-IR (23). Overexpression of RACK1 in transformed fibroblasts has previously been shown to facilitate cell spreading and contact with the extracellular matrix. In MCF-7 cells overexpression of RACK1 enhances proliferation (22) and also enhances MCF-7 cell migration.2

The data here indicate that association of the IGF-IR with RACK1 is dependent upon adhesion and that when this association occurs IGF-I can regulate the association and dissociation of other proteins from RACK1, including IRS-1, Shc, and Src. In cells expressing the Y1250F/Y1251F mutant RACK1 does not associate with the IGF-IR or with Src, Shc, and Shp2. However, association of RACK1 with β1 integrin is increased, and the cells are deficient in migratory activity. These data suggest that an essential scaffolding function of RACK1 in promoting IGF-I-mediated cell migration is to associate with the IGF-IR, Src, and Shp2. The observation that nonadherent cells have the same defects as the Y1250F/Y1251F mutant (lack of association of RACK1 with the IGF-IR, no phosphorylation of Shc, no dissociation of Shc from PP2A, increased Akt phosphorylation) suggests that the defect in the migratory capacity of cells expressing this mutant is due to a lack of integration of adhesion signals with IGF-IR signaling.

The observation that RACK1 does not associate with the IGF-IR in FL5.12 cells and that the Y1250F/Y1251F mutant in these cells is also deficient in promoting Shc phosphorylation (26) suggests that association of RACK1 with the IGF-IR and Shc phosphorylation are two distinct phenotypes of this mutant. This observation also reveals a difference in the signals that are necessary for IGF-I-mediated Shc phosphorylation in hemopoietic cells and in adherent cells. In adherent cells our findings and the findings of Ugi et al. (30) suggest that PP2A must dissociate from Shc to allow Shc phosphorylation. Our data indicate that dissociation of PP2A from Shc requires an adhesion signal because this dissociation does not occur in cells expressing the Y1250F/Y1251F mutant and does not occur in nonadherent cells. This conclusion is also supported by our unpublished observation that Shc does not associate with PP2A in FL5.12 cells. Thus, phosphorylation of Shc by IGF-I in hemoipoietic cells does not require adhesion signals, and this may explain why RACK1 apparently has no role in integration of adhesion and IGF-IR signaling in these cells.

PP2A apparently acts to regulate Akt activity in an adhesion-dependent manner, because Akt activity is increased in nonadherent cells and in cells expressing the Y1250F/Y1251F mutant. The PP2A inhibitor okadaic acid can reverse this effect and restore Shc phosphorylation. This result is in agreement with a recent publication indicating that PP2A negatively regulates an insulin signaling pathway by inhibiting Akt (41). Okadaic acid can also reverse the negative inhibitory effect of RACK1 on Akt phosphorylation in MCF-7 cells (data not shown). Overall this suggests that PP2A has a major role in adhesion-mediated regulation of IGF-IR signaling involving RACK1.

The increase in Src activity observed in cells expressing the Y1250F/Y1251F mutant where RACK1 does not associate with Src is consistent with previous reports demonstrating that association of RACK1 with Src suppresses Src kinase activity (33, 42). The increase in FAK activity in these cells and lack of IGF-I-mediated suppression of FAK activity is also consistent with increased Src kinase activity. The Tyr635 site on FAK, which is hyperphosphorylated in cells expressing the Y1250F/Y1251F mutant, has been documented as a docking site for Src family kinases. Phosphorylation of this site has been associated with enhancement of Src kinase activity as well as further phosphorylation of FAK at the Tyr861 and Tyr825 sites and subsequent recruitment of Grb2 (43, 44). It has been proposed that these phosphorylations lead to the dissociation of FAK from focal contacts (45).

There are multiple studies indicating a role for Src and FAK activity in cell migration and invasion (46), and there is elevated expression of FAK in metastatic tumor cells. Activation of FAK requires correct spatial location (with FAK Tyr397 phosphorylation occurring exclusively at the leading edge of motile cells) (47), intermolecular clustering, and association with a group of activating and regulatory molecules. Important among these are the tyrosine phosphatases including Shp2, which has previously been shown to dephosphorylate FAK and one of its interacting proteins, Paxillin (15), an event that was shown to be associated with cell adhesion and MCF-7 cell migration. In our study, the lack of Shp 2 association with RACK1 and the lack of IGF-I-mediated dephosphorylation of FAK suggests that lack of Shp-2-mediated FAK dephosphorylation may contribute to the loss of cell migration in cells expressing Y1250F/Y1251F IGF-IR. The increased contact strength with fibronectin and collagen observed in cells with Shp2 knocked out (15) and observed in cells expressing the Y1250F/Y1251F mutant also supports this conclusion.

Fibroblasts expressing the Y1250F/Y1251F mutant have previously been shown to have disrupted cytoskeletal reorganization, although they had normal Shc and Erk phosphorylation (24). In FL5.12 lymphocytes cells expressing the Y1250F/Y1251F mutant, we found that IGF-I-mediated phosphorylation of Shc on tyrosine 313 (317 in human Shc), Ras activity, and activation of the Erk, c-Jun N-terminal kinase, and p38 pathways are all deficient (26). In this study IGF-I-mediated Erk phosphorylation did not correlate with Shc phosphorylation and was also observed in Y1250F/Y1251F-expressing R− cells. This is likely due to the action of IRS proteins that are abundant in fibroblasts but not in FL5.12 cells. IRS-1 and IRS-2 were both phosphorylated by IGF-1 in R− cells expressing the Y1250F/Y1251F mutant. The observation that similar signaling responses were observed in nonadherent cells as in cells expressing the mutant together with the observation that similar effects were observed with Shc phosphorylation and RACK1-associated proteins suggests that adhesion-mediated signaling is defective in cells expressing the mutant.

In conclusion, our data indicate that RACK1 acts as a scaffolding protein to integrate adhesion and IGF-IR signals necessary for cell migration that are lost in the Y1250F/Y1251F mutant IGF-IR. Since an increase in RACK1 expression has been detected in metastatic cancer (48) and because IGF-IR activity is associated with cancer progression, it will be important to determine what other proteins RACK1 scaffolds to enhance IGF-I-mediated migration.

Acknowledgments—We are grateful to Kurt Tidmore for preparing the illustrations and to our colleagues in the Cell Biology Laboratory for helpful discussions.

REFERENCES

1. Baserga, R. (1999) Exp. Cell Res. 253, 1–6
2. LeRoith, D., and Roberts, C. T., Jr. (2000) Cancer Lett. 195, 127–137
3. Lopez, T., and Hanahan, D. (2002) Cancer Cell 1, 339–353
4. Resnick, M. (1998) Int. J. Mol. Med. 1, 883–888
5. Hongo, A., D’Ambrosio, C., Miura, M., Morrione, A., and Baserga, R. (1996) Oncogene 12, 1231–1238
6. Brodt, P., Fallavollita, L., Khatib, A. M., Samani, A. A., and Zhang, D. (2001) J. Biol. Chem. 276, 33608–33615
7. Maloney, E. K., McLaughlin, J. L., Dagdigian, N. E., Garrett, L. M., Connors, K. M., Zhou, X. M., Blattler, W. A., Chittenden, T., and Singh, R. (2003) Cancer Res. 63, 5073–5083
8. Comoglio, P. M., Boccaccio, C., and Trusolino, L. (2003) Curr. Opin. Cell Biol. 15, 565–571
9. Associa, R. K., and Schwartz, M. A. (2001) Curr. Opin. Genet. Dev. 11, 48–53
10. Regnato, M. J., Mills, K. R., Paulus, J. K., Lynch, D. K., Sgroi, D. C., Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003) Nat. Cell Biol. 5, 733–749

---

2 P. A. Kiely and R. O’Connor, unpublished observation.
Integration of IGF-I and Integrin Signaling

11. Sieg, D. J., Hauck, C. R., Bic, D., Klingbeil, C. K., Schaefer, E., Damsky, C. H., and Schlaepfer, D. D. (2000) Nat. Cell Biol. 2, 249–256
12. Doerr, M. E., and Jones, J. L. (1996) J. Biol. Chem. 271, 2443–2447
13. Dunn, S. E., Ehrlich, M., Sharp, N. J., Reiss, K., Solomon, G., Hawkins, R., Baserga, R., and Barrett, J. C. (1998) Cancer Res. 58, 3353–3361
14. Zhang, D., and Brodt, P. (2003) Oncogene 22, 974–982
15. Manes, S., Mira, E., Gomez-Mouton, C., Zhao, Z. J., Lacalle, R. A., and Martinez, A. C. (1999) Mol. Cell. Biol. 19, 3125–3135
16. Tai, Y. T., Podar, K., Catley, L., Tseng, Y. H., Akiyama, M., Shringarpure, R., Burger, R., Hideshima, T., Chauhan, D., Mitsuades, N., Richardson, P., Munshi, N. C., Kahn, C. R., Mitsiades, C., and Anderson, K. C. (2003) Cancer Res. 63, 5850–5858
17. Guvakova, M. A., and Surmacz, E. (1999) Exp. Cell Res. 251, 244–255
18. Maile, L. A., and Clemmons, D. R. (2002) Endocrinology 143, 4259–4264
19. Maile, L. A., and Clemmons, D. R. (2002) J. Biol. Chem. 277, 8955–8960
20. Ron, D., Chen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 839–843
21. McCaffhill, A., Warwicker, J., Bolger, G. B., Houssay, M. D., and Yarwood, S. J. (2002) Mol. Pharmacol. 62, 1261–1273
22. Kiely, P. A., Sant, A., and O’Connor, R. (2002) J. Biol. Chem. 277, 22581–22589
23. Hermanto, U., Zong, C. S., Li, W., and Wang, L. H. (2002) Mol. Cell. Biol. 22, 2345–2365
24. Blakeley, V. A., Koval, A. P., Stannard, B. S., Sreringsour, A., and LeRoith, D. (1998) J. Biol. Chem. 273, 18411–18422
25. O’Connor, R., Kauffmann-Zeh, A., Liu, Y., Lehar, S., Egan, G. I., Baserga, R., and Bleul, W. A. (1997) Mol. Cell. Biol. 17, 427–435
26. Lebey, M., Lyna, A., Krause, D., and O’Connor, R. (2004) J. Biol. Chem. 279, 18306–18313
27. Warly, K. K., Mainiero, F., Isakoff, S. J., Marcantonio, E. E., and Giancotti, F. G. (1996) Cell 87, 733–743
28. Barberis, L., Warly, K. K., Fiucci, G., Liu, F., Hirsch, E., Brunacccio, M., Altruda, F., Tarone, G., and Giancotti, F. G. (2000) J. Biol. Chem. 275, 36532–36540
29. Sell, C., Dumenil, G., Devaux, C., Miura, M., Coppola, D., DeAngelis, T., Rubin, R., Efstratiadis, A., and Baserga, R. (1994) Mol. Cell. Biol. 14, 3604–3612
30. Ugi, S., Imamura, T., Ricketts, W., and Olefsky, J. M. (2002) Mol. Cell. Biol. 22, 2375–2387
31. Rouladush, F. L., Pierce, K. L., Maudsley, S., Khan, K. D., and Luttrell, L. M. (2000) J. Biol. Chem. 275, 2283–2289
32. Cox, E. A., Bennin, D., Doan, A. T., O’Toole, T., and Huttenlocher, A. (2003) Mol. Cell. Biol. 14, 658–669
33. Chang, B. Y., Conroy, K. B., Machleder, E. M., and Cartwright, C. A. (1998) Mol. Cell. Biol. 18, 3245–3256
34. Chang, B. Y., Chiang, M., and Cartwright, C. A. (2001) J. Biol. Chem. 276, 20346–20356
35. Zhang, S. Q., Yang, W., Kontaridis, M. I., Bivona, T. G., Wen, G., Araki, T., Liu, J., Thompson, J. A., Schraven, B. L., Philips, M. R., and Neel, B. G. (2004) Mol. Cell. Biol. 24, 341–355
36. Ren, Y., Meng, S., Mei, L., Zhao, Z. J., Jove, R., and Wu, J. (2004) J. Biol. Chem. 279, 8497–8505
37. Buenaventura, C. S., Woodside, D., Huff, J. L., Plepper, G. E., and O’Toole, T. E. (2001) J. Cell Sci. 114, 1691–1698
38. Besson, A., Wilson, T. L., and Yong, V. W. (2002) J. Biol. Chem. 277, 22073–22084
39. Li, X., Yang, Y., Hu, Y., Dang, D., Regezi, J., Schmidt, B. L., Atakili, A., Chen, B., Ellis, D., and Rames, D. M. (2003) J. Biol. Chem. 278, 41646–41653
40. Llilental, J., and Chang, D. D. (1998) J. Biol. Chem. 273, 2379–2383
41. Ugi, S., Imamura, T., Maegawa, H., Egawa, K., Yoshizaki, T., Shi, K., Obata, T., Ebina, Y., Casshiwagi, A., and Olefsky, J. M. (2004) Mol. Cell. Biol. 24, 8778–8789
42. Miller, L. D., Lee, K. C., Mochly-Rosen, D., and Cartwright, C. A. (2004) Oncogene 23, 5682–5696
43. Schlaepfer, D. D., and Hunter, T. (1997) J. Biol. Chem. 272, 13189–13195
44. Vuori, K., Hirai, H., Aizawa, S., and Ruoslahti, E. (1996) Mol. Cell. Biol. 16, 2606–2613
45. Katz, B. Z., Romer, L., Miyamoto, S., Velberg, T., Matsumoto, K., Cukierman, E., Geiger, B., and Yamada, K. M. (2003) J. Biol. Chem. 278, 29115–29129
46. Schlaepfer, D. D., and Mitra, S. K. (2004) Curr. Opin. Genet. Dev. 14, 92–101
47. Li, L., Okura, M., and Imamoto, A. (2002) J. Biol. Chem. 278, 8497–8505
48. Berns, H., Humar, R., Hengerer, B., Kiefer, F. N., and Battegay, E. J. (2000) FASEB J. 14, 2549–2558
RACK1-mediated Integration of Adhesion and Insulin-like Growth Factor I (IGF-I) Signaling and Cell Migration Are Defective in Cells Expressing an IGF-I Receptor Mutated at Tyrosines 1250 and 1251

Patrick A. Kiely, Madeline Leahy, Denise O'Gorman and Rosemary O'Connor

J. Biol. Chem. 2005, 280:7624-7633.
doi: 10.1074/jbc.M412889200 originally published online December 17, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M412889200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 31 of which can be accessed free at http://www.jbc.org/content/280/9/7624.full.html#ref-list-1