RACK1 inhibits the serum- and anchorage-independent growth of v-Src transformed cells

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Abstract Cancer cells are capable of serum- and anchorage-independent growth, and focus formation on monolayers of normal cells. Previously, we showed that RACK1 inhibits c-Src kinase activity and NIH3T3 cell growth. Here, we show that RACK1 partially inhibits v-Src kinase activity, and the serum- and anchorage-independent growth of v-Src transformed cells, but has no effect on focus formation. RACK1-overexpressing v-Src cells show disassembly of podosomes, which are actin-rich structures that are distinctive to fully transformed cells. Together, our results demonstrate that RACK1 overexpression in v-Src cells partially reverses the transformed phenotype of the cells. Our results identify an endogenous inhibitor of the oncogenic Src tyrosine kinase and of cell transformation.

Keywords: Src; Tyrosine kinase; RACK1; Cell transformation; Cell adhesion

1. Introduction

When regulated, the Src tyrosine kinase participates in diverse signaling pathways that control cell proliferation, differentiation, adhesion and survival [1]. Deregulated Src is oncogenic [2–5]. Thus, it is important to search for cellular mechanisms that regulate Src. In doing so, we will learn about mechanisms by which normal cells regulate their growth.

RACK1 (a homolog of the β subunit of G proteins) was originally identified as an intracellular receptor for protein kinase C (PKC) [6]. We identified RACK1 as a Src substrate and an inhibitor of c-Src kinase activity and NIH 3T3 cell growth [7–9]. However, it is not yet known what influence RACK1 has on v-Src kinase activity or cell transformation. To answer these questions, we stably expressed HA-RACK1 in v-Src-transformed NIH 3T3 cells and analyzed the cells for v-Src activity and for anchorage- or serum-independent growth and focus formation on monolayers of normal cells, all capabilities that fully transformed v-Src cells have and normal cells lack. We observed that RACK1 expression partially inhibited the anchorage- and serum-independent growth of v-Src transformed cells, and in doing so, partially reversed their transformed phenotype.

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2. Materials and methods

2.1. Cell culture

NIH 3T3 cells and NIH(pmvsrc/foc/EP)A1 [3] (gift from David Shalloway, Cornell University, Ithaca, NY) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Herndon, VA) supplemented with 10% calf serum (Sigma, St. Louis, MO), unless otherwise stated.

2.2. Plasmids and transfection assays

Construction of pcDNA3-RACK1 and pcDNA3-HA-RACK1 (by insertion of the influenza virus hemagglutinin (HA) tag into the *Bgl*II site of pcDNA3-RACK1) has been previously described [7]. NIH(pmvsrc/foc/EP)A1 cells were transfected with 1 μg pcDNA3-HA-RACK1 or pcDNA3 using Lipofectamine (Gibco-BRL) according to the manufacturer's protocol and as previously described [7]. Colonies were selected for neomycin resistance with the addition of G418 (400 μg/ml) and subclones were examined for stable expression of HA-RACK1 by immunoblotting with anti-RACK1 [2,7].

2.3. Antibodies

Src monoclonal antibody (MAb) 327 was produced from mouse hybridoma cells [10]. MAbs that recognize RACK1, phosphotyrosine (PY20), paxillin and focal adhesion kinase (FAK) were purchased from Transduction Laboratories (Lexington, KY). Other commercially available antibodies included Sam68 (Santa Cruz Biotechnology, Santa Cruz, CA), FAK Y397 and Y576 (BioSource International, Camarillo, CA), rhodamine–phalloidin (Molecular Probe, Eugene, OR) and cortactin (p80/85; Upstate Biotechnology, Lake Placid, NY).

2.4. Immunoblot analysis and in vitro protein kinase assays

Lysate proteins or immunoprecipitates were resolved by SDS–PAGE and analyzed by immunoblotting with the specified mouse MAb or rabbit polyclonal antibody, as previously described [2,7–9]. Methods to evaluate the phosphorylating activity of Src by kinase assays in vitro have also been described [2,7–9].

2.5. Cell growth assays

Growth rates of NIH(pmvsrc/foc/EP)A1 cells that were stably expressing HA-RACK1 or vector alone were analyzed as described [2,7]. Briefly, 5×10^4 cells were suspended in DMEM supplemented with 0.2% FBS and seeded onto 35-mm diameter plates. Triplicate plates of cells were counted daily with a hemocytometer from days 2 to 5. Cell number was plotted versus time.

2.6. Agar colony assays

Agar colony assays were performed as described [2]. Briefly, NIH(pmvsrc/foc/EP)A1 cells that were stably expressing HA-RACK1 or vector alone were suspended at varying densities in 1 ml of DMEM supplemented with 10% FBS and 0.33% Bacto-Agar (Difco Laboratories, Detroit, MI). The cell suspension was added to chilled, 35-mm diameter plates containing a 2 ml base of DMEM supplemented with 10% FBS and 0.5% agar. After the agar had solidified, plates were returned to a 37 °C incubator. Fourteen days later, colonies were counted on triplicate plates at each dilution.

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2.7. Transformation assays

Transformation assays were performed as described [2]. Briefly, a mixture of 10^4 NIH 3T3 cells and various numbers (50, 100 or 500) of NIH(pmvsrc/foc/EP)A1 cells that were stably expressing HA-RACK1 or vector alone were suspended in DMEM supplemented with 5% serum and seeded onto 35-mm diameter plates. The media were changed every fourth day and the cells were rinsed, fixed and stained with crystal violet 21 days after plating. Foci were counted on triplicate plates at each dilution.

2.8. Fluorescence microscopy

v-Src transformed NIH 3T3 cells that were stably expressing HA-RACK1 or vector alone were grown overnight in DMEM supplemented with 10% FBS in Lab-Tek 8 well permanox chamber slides (Nalgene Nunc International, Rochester, NY). Cells were fixed in 4% paraformaldehyde in PBS for 30 min, quenched twice in 0.1 M glycine for 10 min and permeabilized in 0.4% saponin, 1% BSA and 5% goat serum for 15 min [11]. For actin staining, cells were incubated with rhodamine–phalloidin (33 nM; Molecular Probe, Eugene, OR) for 30 min in the dark. For cortactin staining, cells were incubated with anti-cortactin (20 ng/μl) for 1 h, washed and incubated with FITC goat anti-mouse IgG (1:100) for 30 min in the dark. Slides were mounted with vectashield mounting medium (Vector Laboratories, Burlingame, CA).

3. Results

3.1. RACK1 expression partially inhibits the tyrosine kinase activity of v-Src

Previously, we found that RACK1 expression in NIH 3T3 cells inhibits c-Src kinase activity and cell growth [7–9]. To determine whether RACK1 expression influences v-Src activity and cell transformation, we transfected v-Src-transformed NIH 3T3 cells with pcDNA3-HA-RACK1 and screened isolated clones for stable expression of HA-RACK1 by subjecting cell lysates to immunoblot analysis with anti-RACK1. We selected two clones that were expressing the highest levels of HA-RACK1 for further investigation (Fig. 1A, lanes 2 and 3, upper band). We observed that HA-RACK1 was approximately one kDa larger than endogenous RACK1, as would be predicted for a RACK1 protein containing nine additional amino acids from a HA tag.

To assess the effect of RACK1 overexpression on v-Src in vitro kinase activity, we incubated Src or IgG immunoprecipitates of HA-RACK1- or vector-transfected v-Src clones with [γ-^{32}P]ATP, $MnCl_2$ and the exogenous substrate enolase, and performed in vitro protein kinase assays (Fig. 1B). We observed that Src activity, as measured by autophosphorylation or enolase phosphorylation, was lower in the two HA-RACK1-expressing clones (lanes 2 and 3) than in the vector-transfected clone (lane 1). Immunoblot analysis with anti-Src showed that Src immunoprecipitates contained equivalent amounts of Src protein (data not shown). As expected, proteins were not phosphorylated in control IgG immunoprecipitates (lanes 4–6). Thus, RACK1 overexpression partially inhibited the in vitro tyrosine kinase activity of v-Src.

To assess the effect of RACK1 expression on Src in vivo kinase activity, we examined v-Src or NIH 3T3 clones that were overexpressing HA-RACK for tyrosine phosphorylation of Sam68, a mitotic-specific Src substrate [12,13] involved in cell cycle regulation and RNA splicing (reviewed in [14]) (Fig. 1C). To do so, lysate proteins were first precipitated with an MAb that is specific for phosphotyrosine (PY20) and subjected to immunoblot analysis with anti-Sam68 (left upper panel). We observed that tyrosine phosphorylation of Sam68

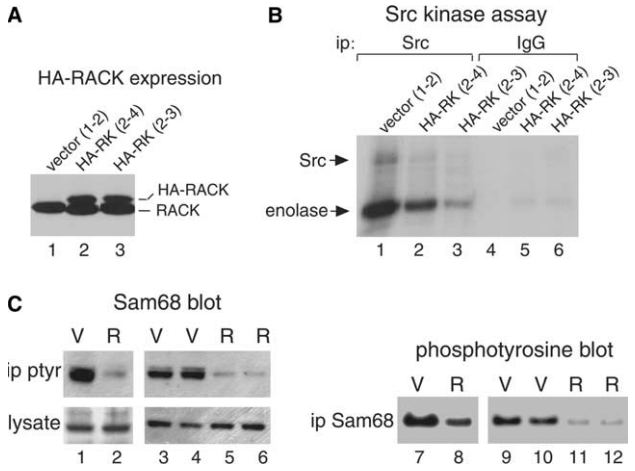

Fig. 1. Effect of HA-RACK1 expression of v-Src protein-kinase activity. (A) v-Src transformed NIH 3T3 cells were transfected with pcDNA3-HA-RACK1 or pcDNA3 and selected for neomycin resistance as described in Section 2. Colonies were isolated and lysates containing 20 μg of total cellular protein were assayed for stable expression of HA-RACK1 by immunoblot analysis with anti-RACK1. Lane 1: pcDNA3 transfected clone; Lanes 2 and 3: pcDNA3-HA-RACK1-transfected clones. (B) Proteins were immunoprecipitated (ip) with excess Src MAb 327 (lanes 1–3) or mouse IgG (lanes 4–6) from lysates of HA-RACK1-expressing clones (lanes 2, 3, 5 and 6) or a vector-transfected clone (lanes 1 and 4) that contained 100 μg of total cellular protein. Immunoprecipitates were incubated with [γ-^{32}P]ATP, $MnCl_2$ and enolase for 10 min at 30 °C in an in vitro protein kinase assay. (C) v-Src transformed NIH 3T3 cells (lanes 1, 2, 7 and 8) or parental NIH 3T3 cells (lanes 3–6 and 9–12) that were stably expressing HA-RACK1 (R) or vector alone (V) were lysed. Proteins from lysate containing 200 μg of total cellular protein were precipitated with anti-phosphotyrosine MAb PY20 and subjected to immunoblot analysis with anti-Sam68 (left upper panel), or precipitated with anti-Sam68 and subjected to immunoblot analysis with MAb PY20 (right panel). Left lower panel: proteins from lysate containing 10 μg of total cellular protein were subjected to immunoblot analysis with anti-Sam68. Data are representatives of three independent experiments.

was lower in v-Src (lane 2) or NIH3T3 (lanes 5 and 6) clones that were overexpressing RACK1 than in the corresponding vector-transfected clones (lane 1 or 3 and 4, respectively). Immunoblot analysis of cell lysate proteins with anti-Sam68 (lower panel) showed that similar amounts of Sam68 were expressed in the HA-RACK1 and vector-transfected clones (compare lanes 1 and 2 or 3–6). In a complementary approach, we immunoprecipitated proteins with anti-Sam68 and immunoblotted with anti-phosphotyrosine and again observed decreased tyrosine phosphorylation of Sam68 in the RACK1-overexpressing cells (Fig. 1C, lanes 8, 11 and 12). Therefore, RACK1 overexpression partially inhibited the in vivo tyrosine kinase activity of Src, as measured by tyrosine phosphorylation of a mitotic-specific Src substrate.

3.2. RACK1 expression partially inhibits serum- and anchorage-independent growth of v-Src transformed NIH3T3 cells

One feature of cancer cells is their ability to grow in low serum, presumably because of constitutive activation of growth factor-dependent signaling pathways [1]. Thus, to assess the effect of RACK1 expression on serum-independent growth of v-Src-transformed cells, we seeded equivalent numbers of HA-RACK1- or vector-transfected v-Src cells in

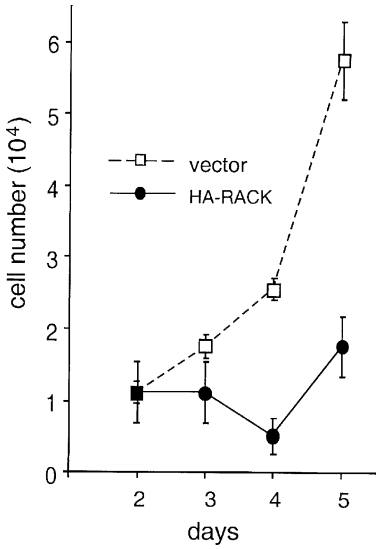

Fig. 2. Effect of HA-RACK1 expression on serum-independent growth of v-Src-transformed cells. NIH(pmvsrc/foc/EP)A1 clones that were stably expressing HA-RACK1 (2–4) or vector alone (1–2) were assayed for cell growth rates in low serum. 5×10^4 cells were suspended in DMEM supplemented with 0.2% FBS and seeded onto a 35-mm diameter plate. Triplicate plates of HA-RACK1-expressing cells (closed circles) or vector-transfected cells (open squares) were counted daily from days 2 to 5. Data represent mean values ± S.E. from triplicate plates at each time point. Data are representatives of two independent experiments.

0.2% FBS and analyzed cell growth rates over 5 days (Fig. 2). We observed that the HA-RACK1-expressing v-Src cells grew more slowly than the vector-transfected cells. After 5 days of growth, there were approximately threefold fewer HA-RACK1-expressing v-Src cells than vector-transfected cells. Thus, RACK1 expression partially inhibited the serum-independent growth of v-Src transformed cells.

The ability of cancer cells to grow without adhering to extracellular matrix proteins (anchorage-independent growth) correlates closely with their ability to form malignant tumors [15]. Anchorage-independent growth presumably allows the cells to invade and metastasize, characteristics that distinguish malignant from benign tumors. The ability of cancer cells to form colonies in soft agar is a measure of their ability for anchorage-independent growth. Thus, to assess the effect of RACK1 expression on anchorage-independent growth of v-Src-transformed cells, we serially diluted HA-RACK1- or vector-transfected v-Src cells, seeded the cells in DMEM supplemented with 10% FBS and 0.33% agar, and counted colonies 14 days later (Table 1). At all cell dilutions, we observed fivefold fewer colonies of HA-RACK1-expressing v-Src cells than vector-transfected v-Src cells after 14 days of growth in soft agar. Thus, RACK1 expression partially inhibited the anchorage-independent growth of v-Src transformed cells. The RACK1-overexpressing v-Src cells formed slightly smaller colonies in soft agar than did the vector-transfected cells.

Another feature of v-Src transformed cells is their ability to form foci on monolayers of normal cells, presumably because the v-Src transformed cells are no longer contact inhibited [16]. Thus, to determine whether RACK1 expression inhibits the focus-forming ability of v-Src-transformed cells, we seeded a mixture of 10^4 NIH 3T3 cells and various numbers of HA-RACK1- or vector-transfected v-Src cells in DMEM supplemented with 10% FBS, and counted foci 21 days later. At all cell dilutions, we observed a similar number of foci formed by the HA-RACK1-expressing as the vector-transfected v-Src clones (data not shown). Thus, RACK1 expression had no apparent effect on the ability of v-Src transformed cells to form foci on monolayers of normal cells.

Table 1
Effect of HA-RACK1 expression on anchorage-independent growth of v-Src transformed NIH 3T3 cells

No. cells seeded/plate	No. colonies/plate	
	Vector (1–2)	HA-RACK(2–4)
50	1 ± 1	0
500	36 ± 6	7 ± 2
1000	74 ± 6	14 ± 4
2000	175 ± 10	32 ± 4
5000	514 ± 51	117 ± 14

NIH(pmvsrc/foc/EP)A1 cells that were stably expressing HA-RACK1 (2–4) or vector alone (1–2) were suspended at various densities in 1 ml of DMEM supplemented with 10% FBS and 0.33% agar. The cell suspension was added to a chilled, 35-mm diameter plate containing a 2 ml base of DMEM supplemented with 10% FCS and 0.5% agar. After the agar had solidified, triplicate plates at each dilution were returned to a 37 °C incubator. Colonies were counted 14 days later. Data represent mean values ± S.E. from triplicate plates at each cell dilution.

3.3. *RACK1 expression modulates tyrosine phosphorylation of focal adhesion proteins*

The reduced ability of RACK1-overexpressing cells to grow in soft agar suggested that the cells require anchorage to extracellular matrix. Adhesion to extracellular matrix is mediated by integrins [1], and β integrins interact with RACK1 [17]. Upon binding to extracellular matrix proteins, integrin receptors are activated and induce tyrosine phosphorylation of FAK and paxillin; proteins whose tyrosine phosphorylation is important for integrin-mediated cell adhesion [1,18,19]. Thus, to explore potential mechanisms whereby RACK1 overexpression inhibits the ability of v-Src cells to grow in anchorage-independent conditions, we examined the HA-RACK1-expressing v-Src cells or NIH 3T3 cells for tyrosine phosphorylation of paxillin (Fig. 3A) and FAK (Fig. 3B). To do so, we first immunoprecipitated proteins with anti-phosphotyrosine and immunoblotted with anti-FAK or paxillin (left upper panels). We observed that tyrosine phosphorylation of both paxillin and FAK was higher in the RACK1-overexpressing v-Src (lane 2) and NIH 3T3 (lanes 5 and 6) cells than in the vector-transfected cells (lane 1 or 3 and 4, respectively). Immunoblot analysis of cell lysates with anti-paxillin or anti-FAK (lower panels) demonstrated that equivalent amounts of paxillin or FAK were expressed in the HA-RACK1 and vector-transfected clones (compare lanes 1 and 2 or 3–6). In a complementary approach, we immunoprecipitated proteins with anti-paxillin or anti-FAK and immunoblotted with anti-phosphotyrosine (Figs. 3A and B, respectively; lanes 7–12), and again observed increased tyrosine phosphorylation of paxillin and FAK in the RACK1-overexpressing cells. Thus, RACK1 overexpression increased the tyrosine phosphorylation of paxillin and FAK; proteins whose tyrosine phosphorylation is important for integrin-mediated cell adhesion [18–22].

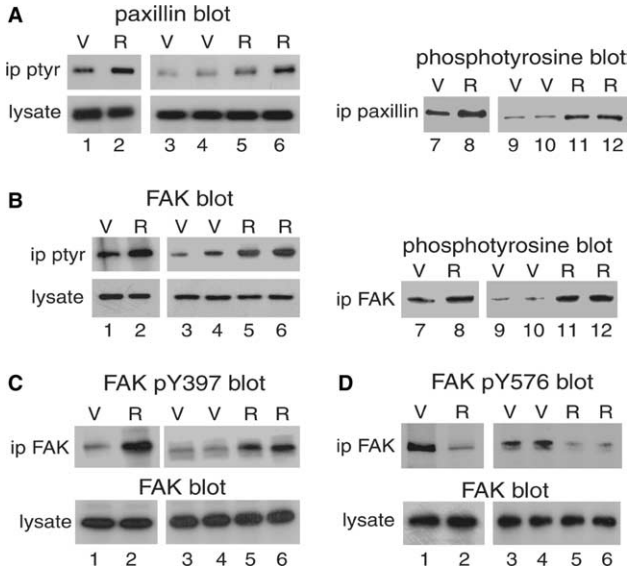

Fig. 3. Effect of HA-RACK1 expression on the tyrosine phosphorylation of paxillin and FAK. (A–D) v-Src transformed NIH 3T3 cells (lanes 1, 2, 7 and 8) or parental NIH 3T3 cells (lanes 3–6 and 9–12) that were stably expressing HA-RACK1 (R) or vector alone (V) were lysed. (A and B) Proteins from lysate containing 200 μg of total cellular protein were precipitated with anti-phosphotyrosine and subjected to immunoblot analysis with anti-paxillin or FAK (left upper panels), or precipitated with anti-paxillin or FAK and subjected to immunoblot analysis with anti-phosphotyrosine (right panels). Left lower panels: proteins from lysate containing 10 μg of total cellular protein were subjected to immunoblot analysis with anti-paxillin or FAK. (C and D) Upper panels: proteins precipitated with anti-FAK from lysate containing 200 μg of total cellular protein were subjected to immunoblot analysis with FAK antibody that specifically recognizes phosphorylated Y397 or Y576. Lower panels: proteins from lysate containing 10 μg of total cellular protein were subjected to immunoblot analysis with anti-FAK. Data are representatives of three independent experiments.

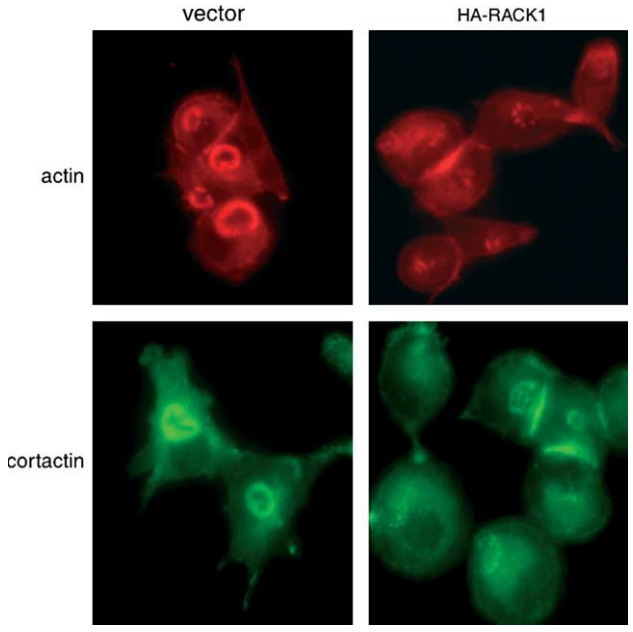

Fig. 4. Effect of HA-RACK1 expression on the actin cytoskeleton. v-Src transformed NIH 3T3 cells that were stably expressing HA-RACK1 (right panels) or vector (left panels) were fixed in 4% paraformaldehyde in PBS for 30 min, quenched twice in 0.1 M glycine for 10 min and permeabilized in 0.4% saponin, 1% BSA and 5% goat serum for 15 min. Cells were stained with rhodamine–phalloidin (upper panels) or antibody to cortactin (lower panels) for 30 min in the dark, as described in Section 2. Data are representatives of three independent experiments.

Integrin activation induces FAK autophosphorylation at Y397 [19] and this is critical to subsequent downstream signaling events involved in cell adhesion. One downstream event involves Src, which binds with high affinity via its SH2 domain to pY397 of FAK, and then phosphorylates other sites on FAK. A major Src phosphorylation site on FAK is Y576, which lies in the activation loop of the catalytic domain [23–25]. Thus, to assess RACK1's influence on phosphorylation of specific tyrosines in FAK, we subjected FAK immunoprecipitates to immunoblot analysis with antibodies that recognize phosphorylated Y397 or Y576. We observed that tyrosine phosphorylation of FAK at the autophosphorylation site was increased (Fig. 3C, upper panel), whereas tyrosine phosphorylation of FAK at a major Src phosphorylation site was decreased (Fig. 3D, upper panel) in the RACK1-overexpressing cells. This observation was consistent with previous work showing that FAK autophosphorylation at Y397 is independent of Src kinase activity [26].

3.4. *RACK1 expression alters cytoskeletal features of v-Src transformed cells*

A rapid consequence of activation of v-Src is the replacement of organized actin stress fibers and focal adhesions (into which the actin fibers are tethered) with actin-rich ring structures called podosomes [27,28]. While podosomes contain similar proteins to those found in focal adhesions, their architecture is distinct in that podosomes do not have actin stress fibers tethered to them, but rather have a peculiar structure with an actin core [29] surrounded by a membrane domain enriched in integrins and adaptor proteins such as vinculin and paxillin. To assess the influence of RACK1 expression on the actin cytoskeleton in v-Src transformed cells, we stained RACK1-overexpressing and vector-transfected v-Src cells with phalloidin (Fig. 4). We observed numerous dense, actin-rich, ring structures in v-Src transformed cells that had the appearance of podosomes (upper left panel). Additional staining for cortactin (lower left panel), an F-actin bundling protein that distinctly localizes to podosomes [28,30], confirmed that the structures were podosomes. We observed a striking disassembly and disappearance of podosomes in the RACK1-overexpressing cells (right panels). However, we did not detect an increase in actin stress fibers or focal-adhesion structures (vinculin or paxillin) in v-Src cells that were stably or transiently overexpressing RACK1 (data not shown). Thus, RACK1 overexpression in v-Src cells partially disassembled actin cytoskeleton features that are distinctive to fully transformed cells (podosomes), but did not reassemble cytoskeleton features that are characteristic of normal cells.

4. Discussion

This study shows that RACK1 expression partially inhibits v-Src kinase activity (Fig. 1) and the serum- and anchorage-independent growth of v-Src transformed cells (Fig. 2 and

Table 1, respectively), and in doing so partially reverses the transformed phenotype of the cells. These findings extend our previous observations that RACK1 expression inhibits c-Src kinase activity and the growth of NIH 3T3 cells [7]. They provide new information about the influence of RACK1 on cell transformation by v-Src.

The reduced ability of RACK1-overexpressing v-Src cells to grow in low serum (Fig. 2) suggests that RACK1 may function in growth factor-dependent signaling pathways. Previously, we showed that treatment of cells with platelet-derived growth factor (PDGF) induces the association of Src and RACK1 and the tyrosine phosphorylation of RACK1 by Src [8]. Thus, RACK1 appears to function, in part, by inhibiting the PDGF receptor-Src signaling pathway. RACK1 also functions in the insulin-like growth factor I receptor (IGF-IR) dependent signaling pathway [31]. In both pathways, RACK1 partially inhibits cell growth by prolonging the G_0/G_1 phase of the cell cycle [7,31].

The finding that RACK1-overexpressing v-Src cells formed slightly smaller colonies than did the vector-transfected cells is probably a reflection of the slightly slower growth rates of the RACK1-overexpressing cells observed in 10% serum (data not shown). However, this would not account for the fivefold decrease in ability of the RACK1-overexpressing v-Src cells to form colonies in soft agar (Table 1). Thus, RACK1 overexpression significantly reduces the ability of v-Src transformed cells to grow in anchorage-independent conditions.

v-Src transformed cells form foci on monolayers of normal cells, presumably because the cells are no longer constrained by density-dependent inhibition of cell division (contact inhibition) [16]. Our finding that RACK1 expression in v-Src transformed cells did not affect focus formation suggests that overexpression of RACK1 is not sufficient to override the mechanism by which v-Src drives cell division forward despite increasing cell density, or that the mechanism is independent of v-Src's interaction with RACK1.

Emerging evidence suggests a role for RACK1 in integrin-dependent cell adhesion [31–33]. RACK1 overexpression in untransformed cells is known to increase the number of actin stress fibers and focal adhesions, enhance cell spreading and inhibit cell migration. RACK1 appears to mediate cell migration via its interaction with PKC [32] and integrin-dependent cell adhesion via its interaction with Src [33]. Consistent with these observations, we find that overexpression of RACK1 in v-Src transformed cells strikingly reduces the ability of the cells to grow in suspension (Table 1). However, overexpressing RACK1 in Src-transformed cells was not sufficient to increase formation of actin stress fibers or focal adhesion structures (vinculin or paxillin), as occurs when RACK1 is overexpressed in untransformed cells. Thus, RACK1's inhibitory influence on v-Src activity is not powerful enough to completely reverse the transformed phenotype of the cells and restore a normal phenotype.

RACK1 overexpression in NIH 3T3 cells that express elevated levels of IGF-IR also induces tyrosine phosphorylation of paxillin and FAK [31]. The mechanism is thought to involve binding of RACK1 to β integrins and activated PKC [34]. Consequently, FAK autophosphorylates, recruits paxillin to the complex, and directs the tyrosine phosphorylation of paxillin [17,18, reviewed in]. Expression of various Src molecules in fibroblasts lacking other Src family kinases revealed that FAK can autophosphorylate at Y397 independently of Src [26]. However, Src kinase activity is required for phosphorylation of additional sites on FAK and for other integrin-directed functions, including cell spreading. Our observations that RACK1 overexpression in v-Src cells results in increased tyrosine phosphorylation of paxillin and FAK (at the autophosphorylation site), and decreased tyrosine phosphorylation of FAK at a major Src phosphorylation site (Fig. 3), are consistent with these findings.

RACK1's influence on normal cellular responses is broad-reaching, affecting biologic functions as diverse as those that regulate cell growth [7], adhesion [31–33], migration [32,33], protrusion [33], death [35,36] and cell-cell interactions [37]. Some of the RACK1 effects (e.g., cell protrusion) appear to be due to RACK1's influence on Src kinase activity, others (e.g., cell migration) do not. Together, the findings signify the powerful and pervasive influence that RACK1 has on a wide array of signals that regulated distinct cellular functions.

In summary, we have shown that RACK1 overexpression partially inhibits anchorage- and serum-independent growth of v-Src-transformed cells and alters cytoskeletal features of the transformed cells. We believe that RACK1 is an important Src substrate that signals downstream of growth factor receptor tyrosine kinases and is involved in the regulation of Src activity and cell growth. The significance of these findings is that endogenous inhibitors of oncogenic tyrosine kinases are potentially tumor suppressors; they represent exciting new targets for cancer therapy.

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