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7. CD4–chemokine receptor pseudotyped particles were produced by cotransfecting QT6 cells with three plasmids: (i) 10 μg of pNL4-3-luc-E$^-$R$^-$ (*13*), (ii) 10 μg of pT4, and (iii) 10 μg of either pCXCR4-cDNA3 or pCCR5-cDNA3. Control particles were produced by substituting pcDNA3 for plasmids encoding CD4 or a chemokine receptor. Forty-eight hours after transfection, medium was harvested, filtered (0.22 μm), aliquoted, and stored at −80°C. Pseudotyped virus was standardized by a p24 assay (Dupont).
8. Supernatants from transfected cells (*7*) were titered onto CEMx174 cells that were chronically infected by HIV-1 or SIV isolates (Fig. 1A), and luciferase activity was determined. Viral stocks were concentrated by pelleting conditioned medium for 2 hours at 50,000*g*.
9. SIV239/MT was created from SIVmac239 by substituting Tyr for Cys and introducing a premature stop codon at positions 721 and 734, respectively, in the *env* cytoplasmic domain (*14*). The Nhe I–Bgl II fragment from pCPenv was cloned into the corresponding sites of pVP-2 (*14*) to generate p239/MT-3′. To produce virus, pVP-1 and p239/MT-3′ were digested with Sph I and electroporated into CEMx174 cells; supernatants were harvested when a viral cytopathic effect was evident.
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Inhibition of Invasion of Epithelial Cells by Tiam1-Rac Signaling

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Tiam1 encodes an exchange factor for the Rho-like guanosine triphosphatase Rac. Both Tiam1 and activated RacV12 promote invasiveness of T lymphoma cells. In epithelial Madin–Darby canine kidney (MDCK) cells, Tiam1 localized to adherens junctions. Ectopic expression of Tiam1 or RacV12 inhibited hepatocyte growth factor–induced scattering by increasing E-cadherin–mediated cell-cell adhesion accompanied by actin polymerization at cell-cell contacts. In Ras-transformed MDCK cells, expression of Tiam1 or RacV12 restored E-cadherin–mediated adhesion, resulting in phenotypic reversion and loss of invasiveness. These data suggest an invasion-suppressor role for Tiam1 and Rac in epithelial cells.

Rho-like guanosine triphosphatases (GTPases) orchestrate distinct cytoskeletal changes in response to receptor stimulation (*1*). The guanine nucleotide exchange factor Tiam1 activates the Rho-like GTPase Rac, resulting in reorganization of the cortical actin cytoskeleton in fibroblasts and induction of invasiveness in T lymphoma cells (*2*, *3*). Invasion and metastasis of carcinoma cells is often associated with reduced E-cadherin–mediated cell-cell adhesion (*4*–*6*); mutations in E-cadherin, β-catenin, and α-catenin have been identified in human tumors and tumor cell lines (*7*, *8*).

We determined the distribution and localization of Tiam1 in MDCK2 cells (*9*). About 20% of Tiam1 was present in the Triton X-100–insoluble fraction (*10*) and likely represented Tiam1 associated with the cytoskeleton (Fig. 1A). Immunocytochemical analyses (*11*) of small colonies of MDCK2 cells revealed that the protein was concentrated at adherens junctions (Fig. 1B). We introduced full-length and NH_2-terminally truncated versions of Tiam1 (see Fig. 1C) into MDCK2 cells by retroviral transduction (*9*). The mutant Tiam1 proteins, which were more stable than full-length Tiam1 (*3*, *12*), were of the expected size and were expressed in equal amounts (Fig. 2A). Immunocytochemical analyses (*13*) revealed that C580Tiam1 was present in the cytoplasm (Fig. 1D). The full-length and the C1199Tiam1 proteins were concentrated at sites of cell-cell contact where they localized with cortical F-actin, as did endogenous Tiam1 (Fig. 1D). Vertical (X/Z) images showed that C1199Tiam1 was evenly distributed over the lateral side of the cells and was absent from the apical or basal side (Fig. 1D). Its localization at adherens junctions suggested a role for Tiam1 in regulation of cell-cell adhesion.

The transmembrane glycoprotein E-cadherin (where E designates epithelial), acting through calcium-dependent homotypic interactions, is the prime mediator of cell-cell adhesion in MDCK cells (*14*). Because Tiam1 localized with E-cadherin in cell-cell junctions (Fig. 1E), we studied the effect of C1199Tiam1 on cell adhesion and motility by using hepatocyte growth factor (HGF)–induced dissociation of colonies (scattering) (*13*, *15*, *16*). MDCK2 cells expressing C1199Tiam1 showed no scattering in response to HGF, whereas cells in the same microscopic field that lacked C1199Tiam1 were able to scatter (Fig. 1E). Similar but less pronounced results were obtained with MDCK2 cells that expressed activated RacV12. In contrast, cells expressing C580Tiam1 showed HGF-induced scattering similar to control cells that did not express Tiam1 (see Fig. 1E). Expression of E-cadherin and its associated proteins β- and α-catenin was not altered in the C1199Tiam1-expressing cells. To discriminate between stimulation of E-cadherin–mediated adhesion and inhibition of cell motility, we blocked E-cadherin function and added HGF to induce dissociation of colonies and stimulate cell motility. MDCK2 cells expressing C1199Tiam1 were treated (*13*) with the antibody DECMA-1 directed against the extracellular part of E-cadherin (*4*, *5*), which resulted in HGF-induced scattering and cytoplasmic localization of C1199Tiam1 (Fig. 1F). These results show that C1199Tiam1-expressing MDCK2 cells are refractory to HGF because of increased E-cadherin–mediated adhesion rather than reduced motility.

Ras-transformed MDCK cells (MDCKf3) (*5*) display a fibroblast-like phenotype, do not grow in colonies, and are highly invasive as a result of reduced E-cadherin–mediated cell-cell adhesion (*4*, *5*). We generated MDCKf3 cell lines (*9*) expressing the various Tiam1 constructs and activated RacV12 (Fig. 2A). Transient or stable expression of C1199Tiam1 or RacV12 induced reversion of the fibroblast phenotype toward the epithelial phenotype and restored cell-cell adhesion (Fig. 2B). Cytoplasmic C580Tiam1 did not revert the Ras phenotype and the effect of RacV12 was less pronounced than that of C1199Tiam1 (Fig. 2B). Reversion of the Ras phenotype by C1199Tiam1 was not

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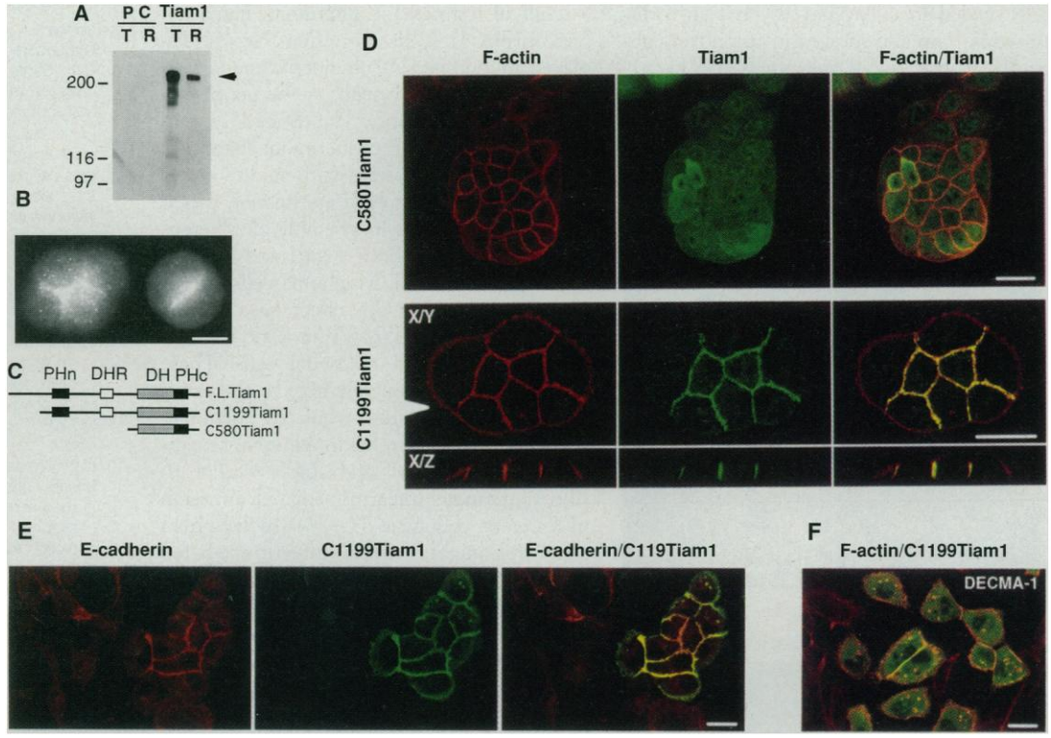

Fig. 1. Expression and effects of Tiam1 in MDCK2 cells. (**A**) Protein immunoblot showing distribution of endogenous Tiam1 after immunoprecipitation from Triton-soluble (T) and Triton-insoluble (RIPA) (R) fractions. Arrowhead indicates Tiam1; molecular size markers are in kilodaltons; PC, preclear. (**B**) Immunocytochemical staining of endogenous Tiam1. Scale bar = 10 μm. (**C**) Schematic drawing of truncated Tiam1 constructs. PHn and PHc, NH_2-terminal and COOH-terminal PH domains; DHR, Discs-Large homology region; DH, Dbl-homology domain; F.L. Tiam1, full-length Tiam1. (**D**) Confocal images showing cytoplasmic localization of C580Tiam1 and discrete localization of C1199Tiam1 at cell-cell junctions when expressed in MDCK2 cells. Tiam1 is in green, F-actin is in red, and colocalization appears in yellow. Scale bars = 25 μm. (**E**) Inhibition of HGF-induced scattering in C1199Tiam1-expressing cells; the nonexpressing cells scatter. E-cadherin is in red and C1199Tiam1 is in green. Scale bar = 25 μm. (**F**) Merged image of C1199Tiam1-expressing MDCK2 cells pretreated with DECMA-1, the antibody to E-cadherin, and HGF. F-actin is in red and C1199Tiam1 is in green. Scale bar = 25 μm.

due to changes in expression of Ras or to increased E-cadherin expression in these Ras-transformed cells. The restored cell-cell adhesion was confirmed and quantified by dissociation assays (*17*). The C1199Tiam1- and RacV12-expressing MDCKf3 cells remained as aggregates under conditions that induced almost complete dissociation of control cells (Fig. 2C).

C1199Tiam1, but not C580Tiam1, localized with cortical F-actin as well as with E-cadherin in newly formed adherens junctions in the MDCKf3 cells (Fig. 3, A to C) and prevented HGF-induced scattering. RacV12 also localized at adherens junctions, although most of the protein was cytoplasmic. This may explain why the phenotype induced by C1199Tiam1 was more pronounced than that induced by RacV12. Treatment (*13*) of the reverted MDCKf3 cells with the antibody to E-cadherin induced dissociation of C1199Tiam1-expressing cells, which regained a fibroblastoid phenotype. Subsequent addition of HGF enhanced this effect, inducing scattering of C1199Tiam1-expressing cells and cytoplasmic localization of the protein (Fig. 3D). Reversion of the fibroblast phenotype in C1199Tiam1-expressing MDCKf3 cells apparently was based on restoration of functional E-cadherin–mediated cell-cell adhesion.

Mutational analysis of the C1199Tiam1 protein revealed that a deletion in the NH_2-terminal Pleckstrin homology (PHn) domain, but not in the Discs-Large homology region, impaired the restoration of E-cadherin–mediated adhesion in MDCKf3 cells. Similar to C580Tiam1, the PHn mutant was localized in the cytoplasm, indicating that the PHn domain is essential for proper localization of C1199Tiam1 at sites of cell-cell contact. MDCK cells expressing C1199Tiam1 showed increased polymerization of cortical actin at adherens junctions (Fig. 3E). A similar but less pronounced effect was also found in RacV12-expressing cells. A role for Rac in actin polymerization at adherens junctions also was recently described in keratinocytes (*18*). Specific localization of Tiam1 at adherens junctions may lead to increased Rac-mediated, F-actin polymerization, which may facilitate firm association of the E-cadherin complex with the cortical actin cytoskeleton and thereby lead to increased cell-cell adhesion.

An inverse relationship has been demonstrated between E-cadherin–mediated cell-cell adhesion and invasion (*4*–*6*). Indeed, control and C580Tiam1-expressing MDCKf3

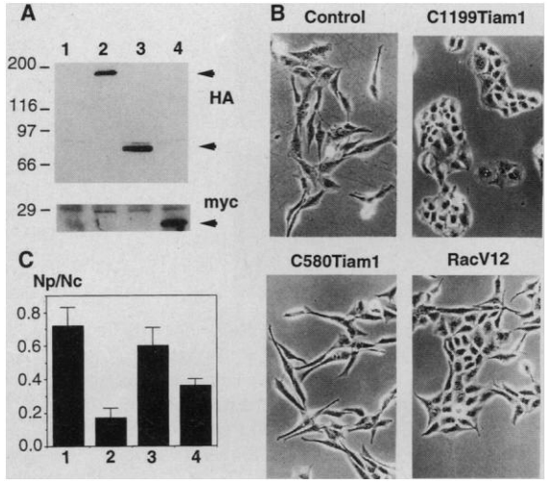

Fig. 2. Effects of RacV12 and Tiam1 mutants in Ras-transformed MDCKf3 cells. (**A**) Expression of various Tiam1 constructs and RacV12 analyzed by immunoblotting total cell lysates with antibodies to HA and Myc epitopes. Lanes: 1, control; 2, C1199Tiam1; 3, C580Tiam1; 4, RacV12. (**B**) Phase-contrast images of transduced cell lines expressing C1199Tiam1, C580Tiam1, and RacV12. Magnification, ×100. (**C**) Quantification of adhesion of the MDCKf3 cell lines determined by dissociation assays and expressed as number of particles (cell clusters) per total number of cells (Np/Nc). Bars: 1, control; 2, C1199Tiam1; 3, C580Tiam1; 4, RacV12.

cells seeded in collagen (*19*) grew in a dispersed fashion and showed invasion throughout the matrix. In contrast, the C1199Tiam1-expressing MDCKf3 cells formed compact colonies without invading into the collagen as a result of restored E-cadherin–mediated adhesion (Fig. 4). Cells expressing RacV12 displayed an intermediate phenotype and formed colonies with invasive cells at the periphery.

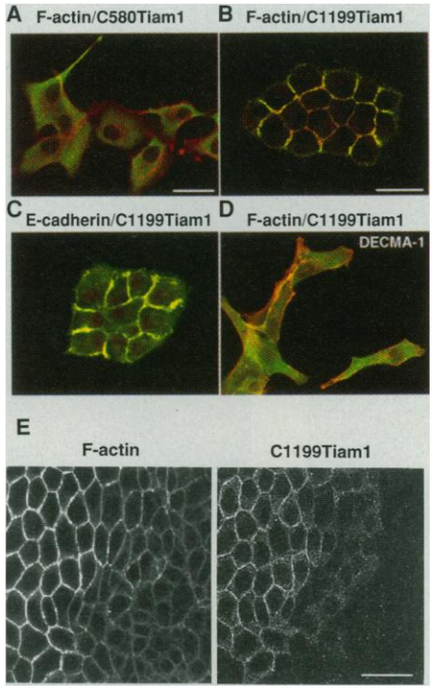

Fig. 3. Phenotypic reversion in MDCKf3 cells. Merged confocal images of cells expressing C580Tiam1 (**A**) and C1199Tiam1 (**B** to **D**). C1199Tiam1 (green) localized with F-actin (red in B) and with E-cadherin (red in C) at sites of cell-cell contact. (D) HGF-induced scattering of C1199Tiam1-expressing MDCKf3 cells treated with DECMA-1 antibody. (**E**) C1199Tiam1-transduced MDCK2 cells were stained for F-actin and C1199Tiam1. Note enhanced F-actin staining at cell-cell junctions in C1199Tiam1-expressing cells compared with nonexpressing cells. Scale bars = 25 μm.

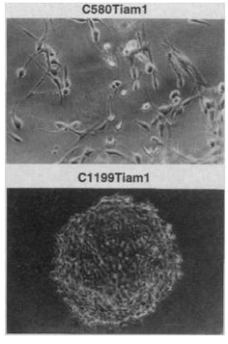

Fig. 4. Invasive behavior of MDCKf3 cells expressing various Tiam1 proteins. Phase-contrast images of cells grown in collagen matrix. C580Tiam1-expressing cells remained invasive, whereas C1199Tiam1-expressing cells formed compact colonies and were no longer invasive. Magnification, ×75.

Our results identify Tiam1 as a protein that can modulate E-cadherin–mediated adhesion and are consistent with studies in keratinocytes and *Drosophila* epithelium, which have implicated Rac in assembly of adherens junctions (*18*, *20*). Both Tiam1 and RacV12 induce invasion of T lymphoma cells (*2*, *3*). Here we show that this effect may well be cell-type specific and that Tiam1 and RacV12 suppress invasion in epithelial cells. These opposing effects ultimately may be the result of the same Tiam1-Rac–regulated phenomenon—that is, an increase in adhesive properties of cells. Tiam1 and Rac1, similar to RhoA, promote integrin-mediated adhesion in different cell types (*1*, *21*). In lymphoid cells, this might increase integrin-mediated heterotypic cell-cell and cell-matrix interactions, which could promote invasion of this cell type. In Ras-transformed epithelial cells, Tiam1-Rac signaling reverts the Ras phenotype and inhibits invasion by increasing E-cadherin–mediated homotypic cell-cell adhesion. These data suggest that the current models, which indicate Rac as a downstream effector of Ras in both tumorigenesis (*22*) and progression to metastatic disease (*2*, *3*), may not be valid for epithelial cells.

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9. MDCK2 and MDCKf3 cells (*5*) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL) with 10% fetal calf serum (FCS). Stable cell lines expressing hemagglutinin (HA)-epitope–tagged C1199Tiam1 or C580Tiam1 constructs (see Fig. 1C), or Myc-epitope–tagged RacV12 or RacN17, were generated by retroviral transduction. Construction, transfection, and production of viral vectors and amphotropic retroviruses containing the Tiam1 and Rac cDNAs are described elsewhere (*23*).
10. Cells were grown to confluency in 10-cm dishes, washed with ice-cold phosphate-buffered saline (PBS), and lysed in 0.5 ml of lysis buffer [50 mM tris-HCl (pH 7.4), 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 10 mM sodium fluoride, 0.1 mM sodium orthovanadate, 20 μM leupeptin, 20 μM aprotinin, and 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (Boehringer)]. After 10 min on ice, cell lysates (Triton-soluble fraction) were collected, and the remaining cell fraction was collected in the same buffer containing 1% sodium deoxycholate and 0.1% SDS (RIPA fraction). Tiam1 was immunoprecipitated with the antiserum to Tiam1 (anti-C16, Santa Cruz) (1 μg per immunoprecipitate) and analyzed by SDS–polyacrylamide gel electrophoresis and immunoblotting (*12*).
11. For immunocytochemical staining, cells were grown on glass coverslips for 48 hours at low density (5×10^4 cells per well). Endogenous Tiam1 was visualized with the antibody to the DH domain (1:100 in PBS) (*2*) followed by incubation with biotin-labeled mouse antiserum to rabbit immunoglobulin and fluorescein isothiocyanate (FITC)–labeled streptavidin (Zymed). The staining pattern was recorded with a charge-coupled device camera system.
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13. Cells were grown on glass coverslips as described in (*11*). Cells were stained with the monoclonal antibody to the Myc tag (9E10) to detect RacV12 or RacN17, a rabbit polyclonal antiserum to Tiam1 (anti-DH) (*2*), or the DECMA-1 antibody to E-cadherin (Sigma) (*4*, *5*). Antibodies were visualized with FITC-labeled or trimethylrhodamine isothiocyanate (TRITC)–labeled secondary antibodies (Zymed). In most cases, cells were stained simultaneously for F-actin with TRITC-labeled phalloidin (1 U/ml) (Molecular Probes). In the confocal microscopic images green and red fluorescences were recorded separately and then merged. For analysis of cell scattering, medium was replaced after 48 hours and recombinant HGF (10 ng/ml) was added as indicated. After 24 hours, cells were fixed in 3.7% paraformaldehyde and inspected by phase-contrast microscopy. For investigation of E-cadherin–dependent adhesion, C1199Tiam1-expressing cells (2×10^4 cells per well in a 12-well plate) were grown for 72 hours in the presence of control antibody or the DECMA-1 antibody (12 μg/ml). Medium and antibodies were replaced every 24 hours. HGF (10 ng/ml) was added for the last 24 hours; then the cells were fixed and processed for immunocytochemistry.
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17. Cells were grown to confluency and collected in PBS with calcium and magnesium. Cells were pipetted 30 times with a 5-ml pipette and then photographed. The extent of cell dissociation was quantified by counting the number of cell clumps (particles) (Np) and the total number of cells (Nc) (1000 to 2000 cells per different cell line) on the photographs and is represented by the ratio Np/Nc [H. Takeda *et al.*, *J. Cell Biol.* **131,** 1839 (1995)].
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19. Rat tail type IV collagen (1%, w/v) (Sigma) was dissolved in PBS (containing calcium and magnesium) and 1% acetic acid. This solution was diluted to 0.2% collagen in DMEM containing 10% FCS and adjusted to pH 7.4 with sodium hydroxide. Cells were resuspended in the collagen (4×10^3 cells per milliliter of collagen) and seeded in 12-well plates. After 7 days in culture, cells were photographed [C. P. Webb, K. Lane, A. P. Dawson, G. F. Van de Woude, R. M. Warn, *J. Cell. Sci.* **109,** 2371 (1996)].
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