IQGAP1 Promotes Cell Motility and Invasion*

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Jennifer M. Mataraza‡, Michael W. Briggs‡, Zhigang Li‡, Alan Entwistle§, Anne J. Ridley§§, and David B. Sacks¶¶

From the ‡Department of Pathology, Brigham and Women's Hospital and Harvard Medical School Boston, Massachusetts 02115, §Ludwig Institute for Cancer Research, Royal Free and University College School of Medicine, 91 Riding House Street, London W1F 7BS, United Kingdom, and the ¶¶Department of Biochemistry and Molecular Biology, University College London, London WC1E 6BT, United Kingdom

The dynamic processes of cell migration and invasion are largely coordinated by Rho family GTPases. The scaffolding protein IQGAP1 binds to Cdc42, increasing the amount of active Cdc42 both in vitro and in cells. Here we show that overexpression of IQGAP1 in mamalian cells enhances cell migration in a Cdc42- and Rac1-dependent manner. Importantly, cell motility was significantly decreased both by knock down of endogenous IQGAP1 using small interfering RNA and by transfection of a dominant negative IQGAP1 construct, IQGAP1ΔGRD. Cell invasion was similarly altered by manipulating intracellular IQGAP1 concentrations. Moreover, invasion mediated by constitutive active Cdc42 was attenuated by IQGAP1ΔGRD. Thus, IQGAP1 has a fundamental role in cell motility and invasion.

Cell migration is a multistep process that is essential for normal development, angiogenesis, wound repair, and metastasis (1). Specifically, it involves protrusion of the plasma membrane at the leading edge, configuration of new sites of adhesion to the extracellular matrix at the front, the release of old adhesions in the back of the cell, and finally, contraction of actomyosin-based cytoskeletal filaments in the cell body (2). Coordination of the actin cytoskeleton, adhesion molecules, and microtubules is required for cell movement; this function is largely orchestrated by the Rho family of GTPases. For example, Cdc42 and Rac, which regulate the production of filopodia and lamellipodia, respectively (3, 4), are important for mediating new protrusions and adhesions at the cell periphery during migration.

Cdc42 and Rac1 participate in cell function by interacting with a diverse array of proteins (5). One of these, IQGAP1, regulates cytoskeletal function by integrating multiple targets, including Cdc42 and Rac1 (6, 7), actin (8, 9), calmodulin (7, 10), and CLIP-170 (11). We previously documented that IQGAP1 inhibits the intrinsic GTPase activity of Cdc42 (10), and CLIP-170 have been described previously (12). In addition, a dominant negative IQGAP1 construct, IQGAP1ΔGRD, substantially reduced active Cdc42, preventing the formation of filopodia (12). Moreover, in vitro analysis revealed that IQGAP1 induced superficial ectodermal lesions in Xenopus embryos, indicating that IQGAP1 is likely to affect cytoskeletal architecture and cell adhesion (13).

Here we therefore hypothesized that IQGAP1 may play a role in mediating cell motility and invasion. Here we demonstrate that IQGAP1 overexpression significantly increased cell migration in several different cell types. IQGAP1 also increased cell speed on both glass and plastic substrata. Dominant negative Cdc42 and Rac1, but not RhoA, inhibited the IQGAP1-mediated increase in motility. Moreover, cell migration was significantly slowed by both IQGAP1ΔGRD and knock down of IQGAP1 by both transient and stable expression of small interfering RNA (siRNA) for IQGAP1. Stable overexpression of IQGAP1 also led to a significant increase in cell invasive capacity. These data imply that IQGAP1 is an important component of cell motility and invasion.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Cells were cultured and transfected as described (14). MCF-7 cells which stably overexpress either pcDNA3 (MCFV) or pcDNA3-Myc-IQGAP1 (MCFI) have been described previously; MCFI cells have 3-fold more IQGAP1 than MCFV cells (12).

Plasmid Constructs—Wild type human IQGAP1 in pcDNA3 vector was used (6, 10). The construction of IQGAP1ΔGRD (residues 1122–1324 deleted) and dual promoter plasmids co-expressing green fluorescent protein (GFP) and IQGAP1 or IQGAP1ΔGRD have been described previously (12, 13, 15). GST fusion proteins were expressed in Escherichia coli and isolated with glutathione-Sepharose as described (16). Myc-tagged forms of N17Cdc42, N17RhoA, and N17Rac1 (3) were kindly provided by Alan Hall (University College London).

siRNA—siRNAs were engineered by oligonucleotide hybridization as 19-mer duplexes with 3-nucleotide spacer loops and were targeted to the following regions of IQGAP1 mRNA (with +1 representing the first nucleotide of the start codon, gttcaggggaattg): siRNA 2, 145–163; siRNA 3, 315–333; siRNA 5, 2061–2079; siRNA 6, 2551–2569; siRNA 8, 4959–4977; siRNA 9, 6705–6723. Each oligonucleotide pair was designed to produce restriction site overhangs upon annealing (BStI at the 5′-end and XhoI at the 3′-end) for cloning into vector mU6pro (16) (kindly provided by David Turner (University of Michigan)) digested with the same enzymes. mU6pro and mU6siRNA 2, 3, 5, 6, 8, or 9 were transfected into MCF-7 cells, and lysates were prepared 48 h later for analysis by Western blotting (12).

Stable expression of siRNA for IQGAP1 was performed with the pSUPER(SUPpression of Endogenous RNA) retroviral vector construct (OligoEngine) (17). Oligonucleotide IQ8 was synthesized with a 5′ BamHI and 3′ HindIII site to directionally clone into the BgII and HindIII sites of pSUPER. The H1 promoter and targeting inserts were excised from pSUPER-IQ8 and cloned into the self-inactivating murine stem cell virus pMSCV to generate pRETSUPER-IQ8. Replication-defective pseudotyped amphotropic retrovirus was produced by cotransfection of the Ras-GTPase-activating protein-related domain; GST, glutathione S-transferase; GFP, green fluorescent protein; siRNA, small interfering RNA; DMEM, Dulbecco’s modified Eagle’s medium.
fecting HEK-293H cells with pMKat, pCMV-YSV-G, and pRETRO-SPEH-IQGAP1. MCF-7 cells were infected with cancer supernatants containing active retrovirus for 6 h by standard centrifugation-mediated infection (18). After recovery for 24 h, stable integrants were selected with 1 μg/ml puromycin. One stable cell line, termed MCF-silIQ8, was chosen for these studies.

**Wound Healing Assay and Time Lapse Microscopy**—HEK-293H cells were transiently transfected with a dual promoter plasmid that expresses both GFP and either wild type IQGAP1, IQGAP1ΔGRD, or pcdNA3 vector (12, 15). Cells were plated onto Lab-Tek II chambered coverglasses (Nalge Nunc International) in DMEM containing 10% fetal bovine serum. 24 h after plating, cells were scraped with a 28-gauge needle and rinsed twice with DMEM containing 10% fetal bovine serum to remove dislodged cells, and images were acquired immediately using a Zeiss LSM 510 upright confocal microscope. Cells were imaged again 8 or 14 h after wounding.

For time lapse microscopy, MCF/I and MCF/V cells were plated into 35-mm plastic Petri dishes with (Mat Tek Corp.) or without (Nalge Nunc International) a central poly-o-lysine-coated glass coverslip insert and grown to confluence in DMEM containing 10% fetal bovine serum. Cell monolayers were wounded with a 200-μl disposable plastic pipette tip (Sarstedt), a layer of embryo-tested mineral oil (Sigma) was spread across the medium to prevent evaporation, and the dishes were then placed in a chamber affixed to the stage of an Axiovert 135 inverted microscope (Carl Zeiss) and maintained at 37 °C with an atmosphere containing 5% CO2. Sequences of time lapse images were collected through a 20× objective and projected onto a KPM1E CCD camera (Hitachi Denshi Ltd.) using a 10%/90% pellicle beam splitter (Melles Griot). The acquisition of image data and synchronization of the illumination were controlled by Tempus Meteor software (Kinetic Imaging). Images were collected every 4 min for 14 h.

**Active Cdc42 and Active Rac1 Assays**—Measurement of active Cdc42 was performed essentially as previously described (12, 19). Briefly, HEK-293H cells were washed twice in PBS and lysed in 500 μl of buffer A (20 mM Hapes, pH 7.4, 150 mM NaCl, 1% (v/v) Nonidet P-40, 20 mM NaF, 1 mM MgCl2, and protease inhibitors) containing 20 μg/ml GTP. Cell lysates were quick frozen, thawed, sonicated at 4 °C, and subjected to centrifugation at 15,000 × g for 10 min at 4 °C. Equal amounts of lysates were prewarmed for 1 h at 4 °C with 40 μl of glutathione-Sepharose. An equal aliquot of each sample (50 μg of total protein) was examined directly as whole cell lysate, and equal amounts of protein lysate were incubated with 40 μg of GST-WASP-GBD (for active Cdc42) or GST-PAK-CRIB (Cdc42-Rac-interactive binding) (for active Rac1) for 2 h at 4 °C. Following collection of complexes with glutathione-Sepharose, the beads were washed six times with buffer A. SDS-PAGE was performed and proteins were transferred to polyvinylidene difluoride membranes. Blots were probed with anti-Cdc42 or anti-Rac1 antibodies (BD Biosciences), followed by horseradish peroxidase-conjugated sheep-anti-mouse antibodies, and ECL was used for detection.

**Cell Division Analysis**—The coordinates of MCF/F and MCF/I cells were recorded in Excel data files (Microsoft Corp.) semiautomatically from each frame of time lapse data sequentially using Motion Analysis software version 1.1 (Kinetic Imaging) employing a minimum difference over a region-of-interest algorithm searching with eight-way connectivity. The speeds were calculated using a series of Mathematica notebooks (20), and the significance of the results was established through analysis of variance and the method of Sheffe’s multiple comparisons. The number of mitotic figures in each time lapse sequence was counted manually during the tracking process.

**Migration and Invasion Assays**—Migration assays were performed as previously described (21). Briefly, Transwells with 8-μm pores were placed into 24-well tissue culture plates (NUNC), and the underside of the membranes was coated with human collagen I at 37 °C (Collaborative Biomedical Products). Collagen was removed after 18 h and replaced with DMEM. Cells were trypsinized, washed once in DMEM, and counted with a hemacytometer. Cells were resuspended at a concentration of 200,000/ml in DMEM containing 5 mg/ml bovine serum albumin (BSA). 600 μl of this suspension was added to the top chamber of the Transwell, whereas 600 μl of DMEM was added to the lower chamber. Cells were trypsinized, washed, counted, and resuspended at a concentration of 200,000/ml in DMEM. 500 μl of this suspension was added to the top chamber of the Transwell. After 24 h at 37 °C, assays were terminated, and cells were quantified as described above for migration analysis. (at least 400 cells were counted in each well).

**Microinjection**—Subconfluent Swiss 3T3 cells were grown on ethanol-washed coverslips and microinjected with GST or GST-IQGAP1 (>90% pure; data not shown) using an Eppendorf microinjector with a Zeiss Axiovert microscope essentially as described (4). Cells maintained at 37 °C under 5% CO2 were imaged at 60-s intervals by time lapse video microscopy.

**Immunocytochemistry and Confocal Microscopy**—Cells were grown to confluence on Lab Tek II chambered glass slides and processed for immunocytochemistry as described (12). Images were generated by confocal laser scanning with a Zeiss LSM 510 upright confocal microscope using separate channels for red and green image collection. Quantification of green and red channel pixel intensity (collection at 488 and 543 nm (BF505-550 and LF560)) was performed using the Zeiss LSM 510 Meta Excitation system.

**RESULTS**

**IQGAP1 Localizes at the Leading Edge of Migrating Cells**—Previous studies from our laboratory indicate that IQGAP1 is a regulator of the actin cytoskeleton (12). Specifically, overexpression of IQGAP1 increases levels of active Cdc42, leading to increased production of filopodia. To investigate a possible role for IQGAP1 in cell motility, GST-IQGAP1 was microinjected into Swiss 3T3 cells. Time lapse imaging revealed that IQGAP1 induced filopodium formation predominantly in one area of the cell, followed by directional extension of lamellipodia (Fig. 1A). This phenotype was not observed with a 10-fold molar excess of GST alone. These results are very similar to the formation of filopodia and subsequent lamellipodia seen in cells microinjected with Cdc42 (4).

To investigate whether the localization of IQGAP1 is consistent with a role in migration, we examined the location of endogenous IQGAP1 in migrating Swiss 3T3 cells. In sessile cells, IQGAP1 is normally distributed throughout the cytoplasm, with accumulation at cell-cell junctions and in the Golgi (14, 22). After wounding a confluent monolayer of cells, immunofluorescence showed that IQGAP1 was localized at the leading edge of migrating cells (Fig. 1, B and C). To confirm that IQGAP1 is enriched at the leading edge, we transfected MCF-7 cells with GFP and stained for endogenous IQGAP1. Because GFP is distributed throughout the cytoplasm and nucleus, comparison of GFP and IQGAP1 distribution at the front of the cell will reveal whether IQGAP1 accumulates at the leading edge. We quantified pixel intensities of both green (GFP) and red (IQGAP1) channels (collection at 488 and 543 nm, respectively), starting at the front edge and moving toward the back of a single cell along the wound edge. The pixel intensity of GFP was distributed relatively evenly over the distance measured. In contrast, IQGAP1 was increased at the front of the cell (Fig. 1D). These data clearly demonstrate that endogenous IQGAP1 localization is enhanced at the leading edge of migrating cells.

**IQGAP1 Increases Cell Migration**—The data in Fig. 1 suggested that IQGAP1 could play a role in cell motility. We employed several strategies to examine this hypothesis. Confluent monolayers of MCF-7 cells stably transfected with pcDNA3 vector (MCF/V) or pcDNA3-IQGAP1 (termed MCF/I, these cells express IQGAP1 at 3 times the levels expressed in MCF/V cells) (12) were wounded, and cells along the wound edge were imaged by time lapse microscopy over a 12-h time period (Fig. 2A). Both MCF/I and MCF/V cells migrated into the wound primarily by cell spreading, not cell division (Fig. 2A). In addition, some MCF/I cells at the wound edge spread out into the wound space and nearly detached from their neighbors (Fig. 2A, arrowhead). This behavior was not observed in MCF/V cells. The migration speed of MCF/I cells was signifi-
cantly faster than MCF/V cells on both glass and plastic (Table I), and this led to more rapid healing of wounded monolayers. MCF/V cells reduced the width of the wound (200 μm) by 37.7 ± 7.2% (mean ± S.E., n = 3, p < 0.01) after 14 h (Fig. 2, B and C) (note that MCF-7 cells are not very motile) (23). In contrast, MCF/I cells completely filled the wound in the same
FIG. 2. IQGAP1 enhances migration of MCF-7 cells. Wound healing of MCF-7 cells stably expressing either vector (MCF/V) or IQGAP1 (MCF/I) was examined. A, MCF/I cells (upper panel) and MCF/V cells (lower panel) plated onto poly-lysine-coated glass coverslips were wounded, and images were collected every 4 min for a period of 13–15 h starting 10–15 min after scraping (0 h). The arrows indicate examples of cells that clearly displayed an increase in spread area during the process of wound repair. The arrowheads indicate an example of a cell at the wound edge spread out into the wound space and nearly detached from neighboring cells. B, cells, plated onto slides, were scraped with a 26-gauge needle. Images were captured immediately after rinsing at 0 h, and at 14 h. Data are representative of at least three independent experimental determinations. Scale bar, 40 μm. C, percentage of wound closure was quantified by measuring the average width of the wounds from three separate experiments; data are expressed relative to the wound closure of MCF/I cells. D, migration of MCF/V and MCF/I cells through Transwell pores was quantified by counting fields of migratory cells under a light microscope. Data, expressed relative to migration of MCF/V cells, represent the means ± S.E. (n = 16). *, significantly different from MCF/V (p < 0.005).
time interval. This difference was not due merely to increased proliferation of MCF/I cells, since the number of cells entering mitosis was not increased (Table I). Moreover, the growth rate of MCF/I cells was indistinguishable from that of MCF/V cells (data not shown).

Closure of the wound by MCF/I cells in 14 h is consistent with the data in Table I. The distance moved by MCF/V cells in Fig. 2B, however, is less than that predicted from the measured mean speed (Table I). This apparent disparity may reflect different experimental conditions and parameters measured. Mean cell speed (Table I) measures displacement of nuclei, which includes movement in all directions including sideways movement that does not contribute to wound closure, whereas wound healing (Fig. 2, B and C) measures distance covered by the wound edge. In addition, mean cell speed does not take into account the length of forward protrusions, which contributes to the speed of wound closure. Because the increase in cell motility is predominantly via increased cell spreading (Fig. 2A), analysis of cell speed (which tracks nuclei) and cell migration (which involves cell spreading) may not give identical results.

The effect of IQGAP1 on cell motility was confirmed by evaluating migration through Transwell pores. MCF/I cells exhibited a 2.82 ± 0.17-fold (mean ± S.E., n = 16, p < 0.005) greater motility than MCF/V cells (Fig. 2D). Similarly, transient overexpression of IQGAP1 accelerated motility by 1.60 ±

| Substratum | Mean speed (μm/h) | No. of cells tracked | No. of cells entering mitosis |
|------------|-------------------|----------------------|-----------------------------|
| MCF/I Plastic | 6.33 ± 0.15* | 48 | 11 |
| MCF/V Plastic | 5.01 ± 0.09* | 46 | 9 |
| MCF/I Glass | 6.86 ± 0.14** | 50 | 4 |
| MCF/V Glass | 5.78 ± 0.12** | 50 | 14 |

Table I

Mean cell speed of MCF/I and MCF/V cells

Confluent monolayers of MCF/I and MCF/V cells were wounded and imaged by time lapse microscopy for 14 h. Each mean value represents the cumulated results from two independent experiments ± SD. * and **, these pairs of values are significantly different, p < 0.01.

![Image](https://via.placeholder.com/150)

Fig. 3. IQGAP1ΔGRD decreases cell motility. A, wound healing of HEK-293H cells transiently transfected with a plasmid that expresses both GFP and either vector (V), wild type IQGAP1 (IQGAP1), or IQGAP1ΔGRD. Assays were performed as described in the legend to Fig. 2. Data are representative of at least three independent experimental determinations. Scale bar, 40 μm. B, wound closure was quantified as described in the legend to Fig. 2 and is expressed relative to vector-transfected cells. C, migration through Transwells of HEK-293H and MDA-MB-231 cells transiently transfected with vector (V), IQGAP1 (WT), or IQGAP1ΔGRD (ΔGRD) was examined. Data are expressed relative to the migration of vector-transfected cells and represent the means ± S.E. (n = 4). *, significantly different from vector-transfected cells (p < 0.005). **, significantly different from vector-transfected cells (p < 0.01).
0.07- and 1.67-fold in HEK-293H cells and highly motile MDA-MB-231 cells, respectively (Fig. 3C).

**IQGAP1**Δ**GRD Reduces Cell Motility**—To gain insight into the mechanism by which IQGAP1 enhances cell motility, we utilized IQGAP1ΔGRD, which reduces levels of endogenous active Cdc42 (12). HEK-293H cells were transiently transfected with dual promoter vectors that co-express GFP (to easily identify transfected cells) and either IQGAP1 or IQGAP1ΔGRD. A pcDNA3 vector expressing GFP was used as a control. Cells transfected with vector or wild type IQGAP1 closed the wound by 8 h (Fig. 3A). By contrast, transfection of IQGAP1ΔGRD slowed motility by 85.1 ± 5.3% (mean ± S.E., n = 3, p < 0.001) (Fig. 3, A and B). Nontransfected cells in the upper right quadrant of the IQGAP1ΔGRD panel (Fig. 3A, compare 0 and 8 h) appeared to move the same distance as vector-transfected cells. However, when nontransfected cells are adjacent to cells transfected with IQGAP1ΔGRD, their ability to migrate seemed to be impaired. The molecular mechanism responsible for this effect, which we have observed repeatedly, is unknown.

Analysis of the effect of IQGAP1ΔGRD on cell motility was also examined with the Transwell assay. Transient expression of IQGAP1ΔGRD in HEK-293H cells significantly slowed migration (Fig. 3C). To confirm this observation in another cell line, migration assays were performed using MDA-MB-231 cells. Transient transfection of IQGAP1ΔGRD dramatically reduced cell motility in this highly motile breast epithelial cell line (Fig. 3C).

Cdc42 and Rac1 Are Necessary for the IQGAP1-mediated Increase in Cell Migration—Wild type IQGAP1 and IQGAP1ΔGRD increase and decrease active Cdc42, respectively (12) (Fig. 4A). Similarly, overexpression of IQGAP1 increased levels of active Rac1 in HEK-293H cells. Analogous to its effect on Cdc42, IQGAP1ΔGRD reduced the amount of active Rac1 (Fig. 4A). The possible participation of Rho GTPases with dual promoter vectors that co-express GFP (to easily identify transfected cells) and either IQGAP1 or IQGAP1ΔGRD. A pcDNA3 vector expressing GFP was used as a control. Cells transfected with vector or wild type IQGAP1 closed the wound by 8 h (Fig. 3A). By contrast, transfection of IQGAP1ΔGRD slowed motility by 85.1 ± 5.3% (mean ± S.E., n = 3, p < 0.001) (Fig. 3, A and B). Nontransfected cells in the upper right quadrant of the IQGAP1ΔGRD panel (Fig. 3A, compare 0 and 8 h) appeared to move the same distance as vector-transfected cells. However, when nontransfected cells are adjacent to cells transfected with IQGAP1ΔGRD, their ability to migrate seemed to be impaired. The molecular mechanism responsible for this effect, which we have observed repeatedly, is unknown.

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**FIG. 4. IQGAP1 increases cell migration in a Cdc42- and Rac-dependent manner.** A. HEK-293H cells were transiently transfected with 10 µg of either wild type IQGAP1 (WT), vector (V), or IQGAP1ΔGRD (ΔGRD). Equal amounts of protein were subjected to SDS-PAGE, and blots were probed with anti-Myc (which detects only transfected IQGAP1) (Myc), anti-Rac1 (Total Rac1), and anti-Cdc42 antibodies (Total Cdc42), followed by horseradish peroxidase-conjugated goat anti-mouse antibodies, and developed with ECL. Equal amounts of protein were also incubated with GST-PAR-CRIB (Active Rac1) or GST-WASP-GBD (Active Cdc42) as described under “Experimental Procedures.” Complexes were collected with glutathione-Sepharose and resolved by SDS-PAGE, and Western blots were probed with anti-Rac1 or anti-Cdc42 antibodies. B. MCF/V or MCF/I cells were transfected with equal amounts of vector (V), N17Cdc42, N17Rac1, or N19RhoA. Migration through Transwell pores was assessed as described under “Experimental Procedures.” Data represent the means ± S.E. (n = 4). *, significantly different from MCF/V cells transiently transfected with vector (p < 0.005); **, significantly different from MCF/I cells transiently transfected with vector (p < 0.005).

**FIG. 5. Reduction of IQGAP1 by siRNA attenuates cell migration.** A. MCF-7 cells were transiently transfected with mU6pro (V) or mU6siRNA 2, 3, 5, 6, 8, or 9 as described under “Experimental Procedures.” Equal amounts of protein lysates were analyzed by Western blotting with anti-IQGAP1 and anti-actin antibodies. B. MCF-7 cells were transfected with vector (V), IQGAP1 (WT), or mU6siRNA 2, 3, 5, 6, 8, or 9. Migration data, expressed relative to vector-transfected cells, represent the means ± S.E. (n = 6 for vector, wild type, and mU6siRNA 8, and n = 2 for mU6siRNA 2, 3, 5, 6, and 9). *, significantly different from vector-transfected cells (p < 0.001).
in IQGAP1-stimulated motility was examined. In cells overexpressing IQGAP1, N17Cdc42 and N17Rac1 completely eliminated the IQGAP1-mediated enhancement of cell motility (Fig. 4B). N17Cdc42 and N17Rac1 reduced migration of MCF/V cells only slightly (Fig. 4B). The latter finding is in agreement with a previous study, which showed that N17Cdc42-expressing Rat1 fibroblasts do not exhibit reduced motility (24). RhoA does not bind to IQGAP1 (6), and N19RhoA did not significantly attenuate IQGAP1-induced motility (Fig. 4B). Expression levels of N17Cdc42, N17Rac1, and N19RhoA, monitored by lysing an aliquot of cells prior to plating for migration assays and Western blotting with anti-Myc antibodies, were found to be similar (data not shown). The finding that N19RhoA did not affect migration was somewhat surprising, since several groups have shown that RhoA is required for cell migration (20, 25). However, N19RhoA does not abrogate the Vav-3-mediated increase in motility of NIH-3T3 cells (26), nor does it affect the migration speed of endothelial cells (27) and thus the relative contribution of RhoA to cell migration differs among various cell types. We also observed that an IQGAP1 mutant unable to bind Cdc42 or Rac1 failed to alter motility of HEK-293H cells (data not shown). Collectively, these data suggest that IQGAP1 increases cell motility in a Cdc42- and Rac1-dependent manner.

**Reduction in IQGAP1 Expression Inhibits Cell Motility**—siRNA has recently been shown to be an efficient method to specifically knock down individual proteins in mammalian cells (16, 28). Several siRNA oligonucleotides were designed, targeting various regions of IQGAP1. Transient expression of a 19-mer stem-loop oligonucleotide complementary to bp 4959–4977 of IQGAP1 cDNA, termed siRNA 8, reduced IQGAP1 protein levels by over 50% (Fig. 5A). Importantly, reduction of IQGAP1 protein by siRNA 8 significantly retarded the ability of MCF-7 cells to migrate (Fig. 5B). In order to verify the specificity of the siRNA, another oligonucleotide was utilized, termed siRNA 9, which is directed against bp 6705–6723 of IQGAP1. Transfection of siRNA 9 significantly reduced both endogenous IQGAP1 (Fig. 5A) and cell migration through
Transwell pores (Fig. 5B). By contrast, siRNAs 2, 3, 5, and 6, which do not reduce IQGAP1 protein expression, had no effect on cell motility (Fig. 5, A and B).

We employed a retroviral system to stably integrate siRNA 8 into the genome of MCF-7 cells. IQGAP1 protein expression in these cells (termed MCF-siIQ8 cells) was reduced by 80% (Fig. 6A). Compared with native MCF-7 cells, cell migration of MCF-siIQ8 cells was decreased by 71% (Fig. 6B). Note that the magnitude of reduction of cell migration correlates with the extent of the decrease in IQGAP1 protein levels. These data strongly suggest that IQGAP1 is required for cell motility. The possible contribution of IQGAP2 to cell motility was not addressed in this study, since the protein is expressed primarily in liver (29).

Cdc42 is an important component of cell motility. Stable transfection with constitutively active Cdc42 increases migration of T47D cells across collagen (30) (Fig. 6C). Because IQGAP1 appears to function both upstream and downstream of Cdc42 (10, 12, 13), we investigated the function of IQGAP1 in Cdc42-induced cell migration. Knock down of IQGAP1 by

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**Fig. 7. IQGAP1 increases invasion.** A, invasion of MCF/V and MCF/I cells, transfected with mU6pro (V) or mU6siRNA 8 (siRNA) represents the means ± S.E. (n = 4 for vector, n = 2 for siRNA). *, significantly different from MCF/V cells transfected with vector (p < 0.005). B, invasion of MCF-7 and MCF-siIQ8 cells (mean ± S.E., n = 3). *, significantly different from uninfected MCF-7 cells (p < 0.0001). C, invasion of T47D and T47D/V12Cdc42 cells transfected with vector (V) or IQGAP1∆GRD (ΔGRD) (n = 2).
IQGAP1 markedly reduced migration of these T47D/V12Cdc42 cells (Fig. 6C), implying that IQGAP1 is necessary for the increased epithelial cell motility produced by Cdc42.

**IQGAP1 Enhances Cell Invasion**—The enhanced motility induced by IQGAP1 suggested that it could contribute to cell invasion. Analysis revealed that MCF/1 cells were significantly more invasive than MCF/1 cells (Fig. 7A). By contrast, reduction of endogenous IQGAP1 by siRNA attenuated cell invasion (Fig. 7, A and B). Cdc42 contributes to cell invasion; stable transfection of V12Cdc42 in the modestly invasive T47D cell line enhances invasive capacity by 9-fold (21) (Fig. 7C). Because IQGAP1ΔGRD reduced the amount of active Cdc42 and eliminated the morphological changes produced by constitutively active Cdc42 in T47D/V12Cdc42 cells (12), we examined the role of IQGAP1 in Cdc42-induced invasion. Transfecting IQGAP1ΔGRD into T47D/V12Cdc42 cells reduced their invasiveness by 54% (Fig. 7C), suggesting that the interaction of IQGAP1 with Cdc42 contributes to cell invasion.

**DISCUSSION**

This study shows that increasing and decreasing intracellular IQGAP1 concentrations leads to a concomitant augmentation and reduction, respectively, of cell migration. In addition, cells overexpressing IQGAP1 move faster and some cells at the leading edge of the wound tend to move far forward of neighboring cells without detaching.

A complex interaction among many proteins and signaling pathways contributes to cell migration, and Rho GTPases have a prominent role in this process (1). Several lines of evidence suggest that the interaction of IQGAP1 with Cdc42/Rac1 contributes to its effect on cell migration. A dominant negative IQGAP1, which decreases levels of active Cdc42 (12) and active Rac1, significantly decreased cell motility. IQGAP1-stimulated migration was inhibited by dominant negative Rac1 and Cdc42. In addition, dominant negative IQGAP1 attenuated the enhanced invasion produced by constitutively active Cdc42 in T47D/V12Cdc42 cells. Earlier work from our laboratory demonstrated that IQGAP1 is required for active Cdc42 to localize at the plasma membrane (12), and in the current study we show that IQGAP1 localizes at the leading edge. Therefore, we propose that IQGAP1 may influence cell motility by increasing levels of active Cdc42 (and Rac1) at the leading edge of migrating cells.

IQGAP1 was shown to disrupt the E-cadherin-β-catenin complex at cell-cell junctions, thereby reducing cell-cell adhesion (14, 31). We cannot exclude the possibility that attenuation of E-cadherin function contributes to the enhanced migration speed induced by IQGAP1 in MCF-7 cells. However, IQGAP1 increased migration in MDA-MB-231 cells, which lack E-cadherin (14), indicating that in these cells IQGAP1 promotes cell motility independently of E-cadherin.

Collectively, our data provide a possible molecular mechanism underlying the observation by Richard Hynes’ group that IQGAP1 gene expression is enhanced in highly metastatic melanoma cells (32). Our findings indicate that IQGAP1 is an integral component of cell motility and participates in Cdc42-induced cell migration and invasion.

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