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A novel role for IQGAP1 protein in cell motility through cell retraction

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ABSTRACT

IQGAP1 has emerged as a key component in the regulation of cytoskeleton dynamics during cell migration, maintenance of adherens junctions, microbial pathogenesis and intracellular trafficking. IQGAP1 is known to localize to the protruding edge of lamellipodia in a variety of cell types and interact with regulators of actin dynamics. Here, we provide evidence suggesting a novel role of IQGAP1 in cell motility through cell edge retraction. In some of the cell lines examined, IQGAP1 was markedly separated from WAVE localization suggesting IQGAP1 may localize to retracting edges. B16F10 mouse melanoma cells exhibited the most restricted separation in which the appearance of GFP-IQGAP1 correlated with cell edge retraction velocity and the disappearance of mCherry-Arp3. These results demonstrate that in some cell types IQGAP1 may function to promote cell retraction not lamellipodium edge protrusion. In addition, we examined co-localization of IQGAP1 with adhesion site markers, myosin IIA, calmodulin and IQGAP2. In areas rich in IQGAP1 there was decreased immunofluorescence staining of vinculin, paxillin and phosphorylated-tyrosine indicating adhesion site disassembly. Interestingly, calmodulin, but not myosin IIA or IQGAP2, co-localized with IQGAP1 in areas of cell retraction. Overall these results suggest a new role of IQGAP1, distinct form IQGAP2, in cell migration through up regulation of contractility and downregulation of adhesion sites potentially through calmodulin interaction.

1. Introduction

IQGAP1 is a 190 kDa, multi-functional protein first cloned and characterized as a Ras GTPase-activating protein (RasGAP) related protein with four IQ motifs [1] responsible for binding to calmodulin and calmodulin-like proteins [2]. Through the calponin homology domain (CHD), a WW motif, IQ repeats, and the C-terminal RasGAP related protein domain, IQGAP1 interacts with numerous binding partners to mediate a multitude of cellular and biological functions [3,4]. Many of the interactions likely underlie the mechanism of IQGAP1 in cancer development and progression [5,6].

Among the diverse functions, IQGAP1 plays a role in cell–matrix interaction and actin dynamics at the cell leading edge during motility. Actin stress fibers and focal adhesions in fibroblasts induced by hyaluronan are dependent on the presence of IQGAP1 [7]. In the leading edge of vascular smooth muscle cells, PDGF stimulation recruits IQGAP1 which is necessary for focal adhesion formation and cell migration [8]. At β1 integrin activation sites, Rac1 and RhoA activities are suppressed and enhanced, respectively, through a pathway involving IQGAP1 association with RacGAP1 [9,10]. IQGAP1 has been shown to co-localize with several other proteins in actin ruffles and in the leading edge of lamellipodia. IQGAP1 co-localizes with S100P in membrane ruffles following stimulation with epidermal growth factor [11]. At the leading edge, IQGAP1 co-localizes with phosphorylated VEGF receptor [12], protein 4.1R [13], CLASP2 [14], APC, Rac1, CDC42 [15], and N-WASP and Arp3 in actin-rich structures [16,17]. In our current studies, we observed varying subcellular localization of IQGAP1 that was cell-type dependent. Surprisingly, the subcellular localization of IQGAP1 was restricted to actively retracting areas in some cell lines. Overall, our study points to a new role for IQGAP1 in cell migration, namely retraction of cell edges potentially through up regulation of contractility and downregulation of cell–matrix interactions.

2. Materials and methods

2.1. Cell culture and reagents

A375, CHO, NIH 3T3, B16F10 and B16F1 cell lines were purchased from American Type Culture Collection (Manassas, VA,

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USA) and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA) and antibiotics. Trypsin/EDTA solution (Mediatech, Manassas, VA, USA) was used for cell detachment. Fugene 6 transfection reagent was purchased from Roche Diagnostics. Mouse laminin and fibronectin from bovine plasma were purchased from Invitrogen and Sigma Aldrich (Saint Louis, MO, USA), respectively. Primary antibodies for immunofluorescence staining were purchased from the following vendors: rabbit polyclonal anti-WAVE2 and mouse monoclonal anti-IQGAP2 from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA); mouse monoclonal anti-IQGAP1 and mouse monoclonal anti-paxillin from BD Transduction Laboratories; mouse monoclonal anti-vinculin and rabbit monoclonal anti-calmodulin from Abcam (Cambridge, England); mouse monoclonal anti-phosphorylated tyrosine from EMD Millipore (Billerica, MA, USA); rabbit polyclonal anti-myosin IIA from Sigma Aldrich. Secondary anti-rabbit-Alexa 488, anti-mouse-Alexa 546 and anti-mouse Alexa-647 were from Invitrogen. pEGFP-IQGAP1 (plasmid 30112 deposited by David Sacks [18]) and Arp3-pmCherryC1 (plasmid 27682 deposited by Christien Merrifield [19]) were obtained from Addgene (Cambridge, MA, USA).

2.2. Immunofluorescence microscopy

Glass coverslips coated with 30 μg/ml mouse laminin or 30 μg/ml bovine serum fibronectin for 24 h at 4 °C were placed in 35 mm-diameter dishes containing DMEM with freshly thawed 10% FBS. CHO and NIH 3T3 cells were added to dishes with fibronectin-coated coverslips; A375, B16F10 and B16F1 cells were added to dishes with laminin-coated coverslips. Cells were incubated for 30–60 min at 37 °C with 5% CO_2. For all antibodies, except anti-IQGAP2, coverslips were fixed for 60 min at 22 °C in cytoskeleton-stabilizing buffer (80 mM PIPES, 2 mM EGTA, 3 mM $MgCl_2$, pH = 6.9) with 4% paraformaldehyde and 0.1% Triton-X 100. For anti-IQGAP2 staining, coverslips were fixed for 20 min at 22 °C in phosphate-buffered saline containing 1% glutaraldehyde and 0.1% Triton-X 100, and then incubated in 1% sodium borohydride solution for 10 min. After fixation, coverslips were washed in water, blocked with 2% bovine serum albumin for 15 min and incubated with primary antibodies for 20 min at 37 °C. Coverslips were incubated with secondary antibodies and mounted onto glass slides using Aqua Poly/Mount (Polysciences, Warrington, PA, USA). Images were acquired using a Leica DMIRE2 HC inverted epifluorescence microscope fitted with a 12-bit grayscale CCD camera.

2.3. Live-cell imaging

Delta T Dish microscope culture dishes from Bioptechs (Butler, PA, USA) were coated with 25 μg/ml laminin. Approximately 24 h post-transfection of the B16F10 cells with the GFP-IQGAP1 and mCherry-Arp3 plasmids, cells were re-seeded into the laminin-coated dishes. Cells were incubated for 30 min at 37 °C prior to initiating the imaging. During the acquisition of images, the chamber was maintained at 37 °C using the Delta T heated-lid controller system (Bioptechs) and infused with humidified, 5% CO_2-air mixture.

2.4. Video analysis

GFP-IQGAP1, mCherry-Arp3 and phase contrast image sets were acquired at 13-s intervals. For the live cell studies, a video of cell movement over 150 s was constructed from individual image sets. Positions of the cell edge, GFP-IQGAP1 intensities and mCherry-Arp3 intensities were tracked frame-by-frame using Metamorph image analysis software. GFP and mCherry intensities were corrected for photobleaching by determining the signal decay rate in whole cells. The photobleaching rate constant was 0.003/s for both GFP and mCherry in our experimental conditions.

3. Results

3.1. Cell type-dependent co-localization of IQGAP1 with WAVE

IQGAP1 has been reported to localize to protruding cell edges through association with other protein effectors in the actin protrusion machinery [12–17]. Here we examined co-immunolocalization of IQGAP1 and WAVE in a variety of cell types (Fig. 1). In all cell types WAVE localized to the extreme cell edges, consistent with the role of WAVE in polymerizing actin barbed ends [20]. WAVE therefore served as a reliable reference for examination of IQGAP1 among different cell types. Interestingly, we observed varying patterns of IQGAP1 localization within the same cell type and across cell types. In B16F1 and B16F10 mouse melanoma cells, we observed a striking difference in IQGAP1 localization compared to many previous reports [12–17]. In the mouse melanoma cell lines, there was a distinct separation of IQGAP1 from WAVE localization (Fig. 1, linescans), indicating that IQGAP1 localizes selectively behind WAVE-negative cell edges in retracting areas. In the A375 human melanoma cell line, IQGAP1 localized throughout the cell lamella and lamellipodium behind WAVE, but not in areas of cell retraction where WAVE was absent. In CHO and NIH 3T3 cells, IQGAP1 localized to areas where WAVE was both present and absent, indicating that IQGAP1 may localize to areas of edge protrusion and retraction within the same cell type. The results show that subcellular localization of IQGAP1 is cell type-dependent, and IQGAP1 localizes and may function in areas of cell retraction.

3.2. IQGAP1 and Arp3 correlation with cell edge retraction velocity

Among the variety of cell lines studied, we observed a clear intracellular separation of IQGAP1 from WAVE in B16F10 mouse melanoma cells (see Fig. 1). In order to correlate cell edge movement with IQGAP1 on a time scale of seconds, we performed live experiments with B16F10 cells co-transfected with mCherry-Arp3 and GFP-IQGAP1. Arp2/3 is part of the complex that initiates actin filament branching in actively protruding lamellipodia, and thus localizes in a pattern similar to WAVE [20]. At time = 0 s, Arp3 fluorescence intensity was at the highest point, while IQGAP1 intensity was at the lowest point indicating that the cell edge was in a state of protrusion at the beginning of the time course (Fig. 2). While the cell edge began to retract, we observed accumulation of GFP-IQGAP1 and a concomitant decrease in mCherry-Arp3 fluorescence (Fig. 2A, B). Moreover, there was a positive correlation between fluorescence intensity of GFP-IQGAP1 and velocity of cell edge retraction (Fig. 2B).

3.3. Downregulation of adhesion sites is associated with IQGAP1

IQGAP1 is known to regulate cell–cell interactions, cell–matrix adhesions [4], and Rho family GTPases activity at β1-integrin activation sites [9,10]. We determined if IQGAP1 localization was associated with a decrease or an increase in adhesion site activity. In these experiments, we performed co-immunolocalization studies of IQGAP1 with three adhesion markers in B16F10 mouse melanoma cells on laminin substrate. After 30 min on laminin, the cells typically formed broad lamellipodia with retracting edges rich in IQGAP1. We performed linescan intensity analysis of vinculin, paxillin and phosphorylated tyrosine (p-Tyr) in regions of cell retraction (Fig. 3). Linescan analysis revealed 2–3 micron separation in the peak fluorescence intensities between IQGAP1 and the