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IQGAP1 translocates to the nucleus in early S-phase and contributes to cell cycle progression after DNA replication arrest

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ABSTRACT

IQGAP1 is a plasma membrane-associated protein and an important regulator of the actin cytoskeleton, contributing to cell migration, polarity and adhesion. In this study, we demonstrate the nuclear translocation of IQGAP1 using confocal microscopy and cell fractionation. Moreover, we identify a specific pool of IQGAP1 that accumulates in the nucleus during late G1-early S phase of the cell cycle. The nuclear targeting of IQGAP1 was facilitated by N- and C-terminal sequences, and its ability to slowly shuttle between nucleus and cytoplasm/membrane was partly regulated by the CRM1 export receptor. The inhibition of GSK-3β also stimulated nuclear localization of IQGAP1. The dramatic nuclear accumulation of IQGAP1 observed when cells were arrested in G1/S phase suggested a possible role in cell cycle regulation. In support of this, we used immunoprecipitation assays to show that the nuclear pool of IQGAP1 in G1/S-arrested cells associates with DNA replication complex factors RPA32 and PCNA. More important, the siRNA-mediated silencing of IQGAP1 significantly delayed cell cycle progression through S phase and G2/M in NIH 3T3 cells released from thymidine block. Our findings reveal an unexpected regulatory pathway for IQGAP1, and show that a pool of this cytoskeletal regulator translocates into the nucleus in late G1/early S phase to stimulate DNA replication and progression of the cell cycle.

1. Introduction

IQGAPs are conserved proteins that regulate cellular processes that include cytoskeletal rearrangements in cell migration and cell adhesion, proliferation and cytokinesis (Brandt and Grosse, 2007; Machesky, 1998; Noritake et al., 2005). IQGAP1 is the best characterized member of three related mammalian IQGAPs. The predominant subcellular localization of IQGAP1 is at the plasma membrane. At sites of cell:cell adhesion, IQGAP1 associates with small Rho GTPases Rac1 or Cdc42 to regulate the adherens junction complex via interaction with β-catenin (Noritake et al., 2005). At lamellipodia and membrane ruffles, IQGAP1 in concert with active Rac1/Cdc42, cross-links F-actin (Bashour et al., 1997) and stimulates actin filament nucleation by recruiting the Arp2/3-N-WASp complex (Benseñor et al., 2007; Le Clainche et al., 2007) or the formin Dia1 (Brandt et al., 2007) to promote cell migration. IQGAP1 also stimulates cell polarization during cell migration by tethering actin and microtubule networks at cortical sites via plus-end complexes comprising adenomatous polyposis coli (APC), CLIP-170 and CLASP2 (Fukata et al., 2002; Watanabe et al., 2009; Watanabe et al., 2004). The ectopic expression of IQGAP1 increases β-catenin, APC and N-cadherin at membrane ruffles and promotes their macropinocytosis (Sharma and Henderson, 2007). Photobleaching assays showed that IQGAP1 turnover at these dynamic plasma membrane sites is slow (Johnson et al., 2009b), inferring stable anchorage at the membrane. Aberrant over-expression and membrane accumulation of IQGAP1 is observed in various cancers (McDonald et al., 2007; Nabeshima et al., 2002; Takemoto et al., 2001), and thought to stimulate an invasive cancer phenotype by reducing cell adhesion and increasing migration (Johnson et al., 2009a).

There is evidence that IQGAP1 regulates certain nuclear events. IQGAP1 binds nuclear-cytoplasmic shuttling proteins such as β-catenin and ERK and contributes to the MEK/ERK cascade culminating in activation of Elk-1-mediated transcription (Bourguignon et al., 2005; Roy and Sacks, 2004). IQGAP1 can also induce β-catenin nuclear localization and signaling (Briggs et al., 2002; Wang et al., 2008). Indeed, many IQGAP1 binding partners such as actin (Hofmann, 2009), Rac1 (Michaelson et al., 2008), N-WASp (Yoo et al., 2007) and APC (Brocardo and Henderson, 2008) have described nuclear functions.

Abbreviations: APC, adenomatous polyposis coli; DMSO, dimethyl sulphoxide; GFP, green fluorescent protein; GSK-3β, glycogen synthase kinase-3β; IQGAP1, IQ-domain GTPase activating protein 1; PCNA, proliferating cell nuclear antigen; RNA Polymerase II, RNA Pol II; RPA, replication protein A.

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In recent years there were reports of nuclear localization of two IQGAPs. IQGAP2 was detected in the nuclei of rabbit gastric mucosal cells (Chew et al., 2005) and XTC fibroblast cells and embryonic cells (Yamashiro et al., 2003). In nuclei of murine oocytes and cleaving embryos, IQGAP1 was reported to envelope nucleoli (Bielak-Zmijewska et al., 2008). An N-terminal fragment (1–863 aa) of IQGAP1 expressed in L-cells showed exclusive nuclear localization (Kuroda et al., 1998) and more recently the N-terminus of Rng2, the *S. pombe* IQGAP homologue, accumulated in the nucleus when over-expressed (Takaine et al., 2009). These disparate observations suggest possible nuclear functions of IQGAPs. In this study, we first confirmed and characterized the nuclear localization of IQGAP1 in different cell types, and demonstrated that its nuclear entry is cell cycle-dependent. The potential role of nuclear IQGAP1 in cell cycle progression is described.

2. Materials and methods

2.1. Cell culture, reagents and transfection

All cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and antibiotics at 37 °C in 5% CO_2 humidified atmosphere. GSK-3β was inhibited by treating cells with LiCl (Sigma) dissolved in water, 1-azakenpaullone (Calbiochem) dissolved in DMSO, or Wnt-conditioned media (gift from Dr. Linda Bendall). Cells were arrested in S phase by treatment with thymidine (2.5 μmol/ml, Sigma), HU (2.0 μmol/ml, Calbiochem) or aphidicolin for 24 h. Leptomycin B was purchased from Sigma. All drugs were diluted to required concentrations in media immediately prior to administration. Cells were grown on glass coverslips in 6-well dishes (Nunc) and treatments were performed 24 h post-seeding. Transfection of plasmids or siRNA was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) as previously described (Sharma and Henderson, 2007).

2.2. Antibodies and plasmids

Cells were immunostained as described (Sharma et al., 2006), then visualized by fluorescence microscopy. The following antibodies and dilutions were used for immunofluorescence (IF) and western blot (WB): IQGAP1 monoclonal (WB 1:2000, BD Biosciences #610611), IQGAP1 polyclonal (IF 1:150, Santa Cruz H-109), β-catenin monoclonal (IF 1:100; BD Biosciences 610153), RPA32 rabbit polyclonal (WB 1:750; Santa Cruz H-100), PCNA polyclonal (Santa Cruz FL-261), PCNA mouse monoclonal (WB 1:1000; BD Biosciences 610664), coilin rabbit polyclonal (WB 1:800; Santa Cruz H-300), TopoII monoclonal (WB 1:800; Calbiochem NA14), β-tubulin monoclonal (WB 1:2000, Sigma) and flag monoclonal (IF 1:2000; Sigma M2). Secondary antibodies used were anti-rabbit or anti-mouse Alexa-Fluor-488 (1:500) and anti-mouse or anti-rabbit AlexaFlour-594 (1:1500) (Molecular Probes). Images were collected using an Olympus BL51 fluorescence microscope. A SPOT 32 camera and SPOT Advanced software was used for general image capture. Olympus FV1000 confocal laser scanning microscope with Fluoview Version 1.6a software or Olympus DeltaVision core deconvolution microscope with Softworx Resolve3D software were used for advanced imaging analysis. The images were compiled in Adobe Photoshop CS.

Human pEGFP-tagged IQGAP1_wt and IQGAP1_Ct (1503–1657 aa) were gifts from Dr. Kozo Kaibuchi (Watanabe et al., 2004). Flag-tagged IQGAP1_N (1–863 aa) and IQGAP1_ΔCt (1–1503 aa) were gifts from Dr. Robert Grosse (Brandt et al., 2007). RFP-Ligase was a kind gift from Cristina Cardoso (Easwaran et al., 2005).

2.3. RNA interference

Double-stranded 21-mer RNA oligonucleotides homologous to sequences in mouse IQGAP1 and β-catenin (Sharma and Henderson, 2007), or human IQGAP1 (Watanabe et al., 2004) were purchased as purified duplexes (Qiagen-Xeragon Inc). Cells were plated at medium density for siRNA transfection with 3 μg of siRNA and 6 μl of lipofectamine were used per transfection. Cells were either fixed and processed for IF 48 h post-transfection for image analysis or harvested for flow cytometry analysis.

2.4. Subcellular fractionation, immunoprecipitation and immunoblot analysis

Nuclear-cytoplasmic fractionation was performed as described (Matsuda et al., 1995 and Supplemental material). Immunoprecipitation was performed as described (Brocardo et al., 2008). Briefly, nuclear extracts were mixed with 2 μg of primary antibody or rabbit IgG (Sigma) and incubated overnight at 4 °C with mixing. Then, 30 μl of Protein A-Sepharose (CL4B, GE Healthcare Bio-Sciences) was added at 4 °C for 2 h. The immunocomplex was subjected to SDS-PAGE, followed by immunoblotting.

For Western-blot analysis, the cell fractions or total extracts were denatured in sample buffer (100 mm Tris–HCl, pH 6.8, 20% glycerol, 0.01% bromphenol blue, 10% β-mercaptoethanol, 5% SDS). 20 μg of nuclear and 60 μg of cytosolic, or 40–60 μg of total protein extracts were loaded per lane and resolved by SDS-PAGE and transferred onto nitrocellulose membrane (Millipore Corp.). The membranes were blocked using 5% skim milk powder in PBS containing 0.2% Tween 20 followed by primary antibody incubation for 2 h at room temperature using indicated antibodies. Incubation with secondary horseradish peroxidase-conjugated antibodies (1:5000; Sigma) for 1 h was followed by detection by enhanced chemiluminescence (ECL; Amersham Biosciences).Cell cycle analysis by flow cytometry

At 24 h post-transfection with indicated siRNAs, cells were detached with trypsin and resuspended in complete medium. Cells were pelleted by centrifugation at 1500 rpm for 5 min and washed in PBS and then centrifuged again at 1500 rpm for 5 min. Cells were resuspended in 400 μl of PBS and then fixed in 4.5 ml of ice-cold 85% ethanol overnight at −20 °C. Next, the cells were pelleted by centrifugation at 1500 rpm for 5 min and resuspended in 600 μl of PBS containing RNase A (1 mg/ml) and propidium iodide (2 mg/ml). Cell cycle profiles were determined using a BD Biosciences FACScalibur flow cytometer. The percentage of cells in each cell cycle phase was determined with CellQuest software. Knock-down of IQGAP1, in the asynchronous sample, was also confirmed by immunofluorescence.

3. Results

3.1. Detection of a nuclear pool of IQGAP1

IQGAP1 is primarily a plasma membrane-associated protein, however some previous studies reported detection of nuclear IQGAPs (Bielak-Zmijewska et al., 2008; Kuroda et al., 1998; Yamashiro et al., 2003). To confirm nuclear localization of IQGAP1, we performed immunofluorescence microscopy and detected a small fraction of NIH 3T3 cells displaying nucleoplasmic staining (void from nucleoli) of endogenous IQGAP1 and ectopic human IQGAP1-GFP (Fig. 1A). Biochemical fractionation of mouse 10T1/2 fibroblasts, rat NRK52e epithelial cells (Fig. 1B) and human U2OS osteosarcoma cells (Fig. S1C) confirmed a small pool of IQGAP1 in the nuclear fraction. A comparison of human tumor cell lines indicated that IQGAP1 displayed in general a poor nuclear staining, although was detectable in U2OS cells and dermal fibroblasts