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IQGAP1 interacts with Aurora-A and enhances its stability and its role in cancer

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ABSTRACT

IQGAP1, a ubiquitously expressed scaffold protein, has been identified in a wide range of organisms. It participates in multiple aspects of cellular events by binding to and regulating numerous interacting proteins. In our present study, we identified a new IQGAP1 binding protein named Aurora-A which is an oncogenic protein and overexpressed in various types of human tumors. *In vitro* analysis with GST-Aurora-A fusion proteins showed a physical interaction between Aurora-A and IQGAP1. Moreover, the binding also occurred in HeLa cells as endogenous Aurora-A co-immunoprecipitated with IQGAP1 from the cell lysates. Overexpression of IQGAP1 resulted in an elevation of both expression and activity of Aurora-A kinase. Endogenous IQGAP1 knockdown by siRNA promoted Aurora-A degradation whereas IQGAP1 overexpression enhanced the stability of Aurora-A. Additionally, we documented that the IQGAP1-induced cell proliferation was suppressed by knocking down Aurora-A expression. Taken together, our results showed an unidentified relationship between Aurora-A and IQGAP1, and provided a new insight into the molecular mechanism by which IQGAP1 played a regulatory role in cancer.

1. Introduction

IQGAP1 is an important member of the IQGAP (IQ-domain GTPase-activating protein) protein family [1–3]. It is the best characterized member, which contains many functional binding domains for F-actin, calmodulin, S100B, Rac/Cdc42, β-catenin, E-cadherin, CLIP-170, adenomatous polyposis coli (APC), ERK1/2 and MEK1/2. By interacting with these proteins, IQGAP1 plays a crucial role in multiple fundamental cellular activities, such as cytoskeletal regulation, cadherin-mediated cell to cell adhesion, cell polarization and actin reorganization [4–7]. IQGAP1 also enhances cell proliferation and differentiation through modulating signal transduction pathways, such as the MAPK (mitogen-activated protein kinase) cascade and Wnt pathway [8–10].

Aurora-A which belongs to the Aurora kinase family, was first discovered in the screening for Drosophila mutations affecting the poles of the mitotic spindle function [11,12]. Human Aurora-A is located on the chromosome 20q13 and found overexpressed in several human cancers. Many research groups have proved that the overexpression of Aurora-A induces several cancer-associated phenotypes, including enhanced cell proliferation and colony formation, and inhibition of apoptosis [13–15]. In mammalian cells, degradation of Aurora-A depends mainly on the Anaphase Promoting Complex/Cyclosome (APC/C) with its auxiliary subunit CDH1 [16]. As a multi-subunit ubiquitin ligase, APC/C is composed of at least 13 subunits, including APC2, a structural component, and CDC27, which regulates activation of APC/C by association with CDH1 and CDC20. APC/C is activated by its association with CDH1 through recognizing either a D or a KEN box, whereas APC/C-CDC20 is activated by direct binding to the D box of the substrate protein [17,18].

In this study, we report for the first time that IQGAP1 interacts with Aurora-A, and reveal how the level of Aurora-A is regulated by IQGAP1 in cancer cells.

2. Materials and methods

2.1. Cell cultures and treatment

MCF-7, HEK293 and HeLa were obtained from the Cell Resource Center of Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China). All the cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 °C in 5% CO_2. For transfection, Lipofectamine-2000 (Invitrogen, Carlsbad, CA, USA) and Attractene Transfection Reagent (Qiagen, Valencia, CA, USA) were used. For analyzing protein stability, cells were treated with 100 μg/ml CHX (cycloheximide) (Sigma, St. Louis, MO, USA) or 10 μM MG132 (Z-Leu-Leu-Leu-al)

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(Sigma, St. Louis, MO, USA). In order to synchronize cells at G2/M phase, cells were treated with 400 ng/ml nocodazole (Sigma, St. Louis, MO, USA) for 16 h.

2.2. Plasmid construction

To obtain myc-tagged human IQGAP1 (amino acids 1–1657), PCR was performed on human cDNA using primers flanking nucleotides 1 and 4971. The forward primer 5′-CCGCTCGAGATGT-CCGCCGCAGACGAGGTTG-3′ and the reverse primer 5′-TCCGGGC-CCCTTCCCGTAGAACTTTTTGTTGAGA-3′ were designed to generate a 4971-bp DNA product containing an XhoI site at the 5′ end and ApaI site at the 3′ end. Digestion with XhoI and ApaI generated a fragment that was subcloned into the XhoI-ApaI site of pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) yielding a plasmid containing the cDNA for the 1657-amino acid residues of IQGAP1 fused in-frame at its N terminus to a myc epitope tag. A similar cloning strategy was employed to construct IQGAP1-C (amino acids 864–1657), IQ-GAP1-N (amino acids 1–863), IQGAP1-ΔCT (amino acids 1–1502) and IQGAP1-CT (amino acids 1503–1657) using primers flanking nucleotides 2289 and 4974, 1 and 2588, 1 and 4506, and 4506 and 4974, of IQGAP1 respectively. The forward primers 5′-CCGCTCGAGATGGAATTCCGATCCAGGATGAAT-3′, 5′-CCGCTCGA-GAT GTCCGCCGCAGACGAGGTTG-3′, 5′-CCGCTCGAGATGTCCGCCG-CAGACGA GGTTG-3′ and 5′-CCGCTCGAGATGCTA-GTGAAACTGCA ACAGACAT-3′ and the reverse primers 5′-TCCGGGCCCCTTCCCGTA-GAACTTTTT GTTGAGA-3′, 5′-TCCGGGCCCATCCTCAGCATTGATGAG AGTCTTG-3′, 5′-TCCGGGCCCTT CGGCCTTTCTCCTCTGTCG-3′ and 5′-TCCGGGCCCTTCGGCCTTTCTCCTCT GTCG-3′ were used to gener-ate the IQGAP1-(864–1657), IQGAP1-(1–86 3), IQGAP1-(1–1502), and IQGAP1-(1503–1657), respectively. Sequences were confirmed by DNA sequencing. All deletion mutants and wild type migrated to the expected positions on SDS–PAGE.

2.3. RNA interference

siRNA were purchased from Santa Cruz Technology. For MCF-7, 0.8×10^6 cells were transfected with control siRNA (sc-37007), IQ-GAP1 siRNA (sc-35700), or ARK-1 siRNA (sc-29731) using Lipofect-amine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. All siRNA products generally consist of pools of three to five target-specific 19–25 nt siRNAs designed to knockdown gene expression.

2.4. Reverse transcription and Real-time quantitative PCR

Total RNA was purified and reverse transcription was carried out as described previously [22]. Real-time PCR was performed with ABI PRISM 7000 (Applied Biosystems, Foster City, CA, USA) and SYBR Premix Ex Taq II Kit (TaKaRa, Dalian, China). The specific primer pairs used were as follows: IQGAP1, 5′-CACTGGCT AAGACGGAAGTGTC-3′ (forward), 5′-TCCTGGCTGGAACCGGAT-3′ (reverse); Aurora-A, 5′-ACTCAGCAATTTCCTTGTCAGA-3′ (forward), 5′-GATTATTTTC AGGTGCCGATG-3′ (reverse) and GAPDH, 5′-ATGA-CATCAAGAAGGTGGTG-3′ (forward), 5′-CATACCAGGAAA TGA-GCTTG-3′ (reverse).

2.5. Co-immunoprecipitation

Cellular extracts were precleared with protein A/G Sepharose beads (Santa Cruz, CA, USA) and incubated with indicated antibod-ies for 4 h at 4 °C. Then, protein A/G Sepharose beads were added and samples were incubated overnight at 4 °C. The beads were washed five times with lysis buffer followed by western blot assay.

2.6. GST pull-down assay

GST-pull down assay was performed essentially as previously described [19]. Briefly, MCF-7 cells were lysed and equal amounts of protein lysate were incubated with GST-Aurora-A on glutathi-one-Sepharose beads for 6 h at 4 °C. GST alone was used as a control.

2.7. BrdU incorporation and flow cytometry

To label MCF-7 cells *in vitro,* 10 μl BrdU (1 mM) were added di-rectly to each milliliter of cell culture media. After 4 h, cells were harvested and treated by using the BrdU Flow Kits (BD Biosciences Pharmingen, San Diego, CA, USA) according to user's manual. The stained cell samples were then used for flow cytometry analysis.

2.8. Antibodies

Antibody against IQGAP1 was purchased from BD Biotechnol-ogy (610612). Antibodies for Aurora-A (sc-25425), GST (sc-80004), APC2 (sc-20984), CDC27 (sc-5618), CDC20 (sc-8358), Ubiq-uitin (sc-9133), and β-actin (sc-8432) were obtained from Santa Cruz Technology. Antibodies for CDH1 (c-7855) were purchased from Sigma. Antibodies for Phospho-Aurora A (#3079) were pur-chased from Cell Signaling Technology.

3. Results

3.1. IQGAP1 interacts with Aurora-A

To investigate the role of IQGAP1 in the tumorigenesis, we first examined the interaction between Aurora-A, a key mitotic regula-tor and strong oncogenic protein, and IQGAP1. Using the immuno-precipitation approach, we found that IQGAP1 was able to bind specifically to Aurora-A. Neither of the proteins was detected in samples precipitated with non-specific IgG (Fig. 1A and B). GST-pull down experiment also proved that IQGAP1 could bind to GST-Aurora-A fusion protein (Fig. 1C and D). The binding was specific, as no IQGAP1 was present in the samples incubated with GST alone.

3.2. IQGAP1 increases Aurora-A protein expression

To determine whether the abnormal expression of IQGAP1 has an effect on Aurora-A, we measured the protein levels of Aurora-A. Two complementary strategies were adopted. First, we tran-siently overexpressed IQGAP1 in MCF-7 cells, pcDNA3.1 (vector) as a control. It was shown in Fig. 2A that Aurora-A was up-regu-lated by IQGAP1 overexpression, whereas it was dramatically down-regulated when IQGAP1 was knocked down (shown in Fig. 2B). Additionally, the level of Aurora-A mRNA was examined via semi-quantitative RT-PCR and Real-time quantitative PCR, and there was no evidence of a decrease in Aurora-A mRNA level following IQGAP1 RNAi (Supplementary Fig. 1). This result suggests that IQGAP1 may regulate Aurora-A expression through a post-transcriptional mechanism.

3.3. Overexpression of IQGAP1 delays the degradation of Aurora-A

We employed CHX (cycloheximide), a protein synthesis inhibi-tor, to treat HeLa cells transfected with myc-tagged IQGAP1 or pcDNA3.1. The protein levels of Aurora-A were detected at differ-ent time points (0, 2, 4, 6 and 8 h) by western blot. It was shown in Fig. 3A that when transfected with myc-tagged IQGAP1, Aurora-A became more stable and had a longer half-life. Several