Golgi-localized KIAA0725p regulates membrane trafficking from the Golgi apparatus to the plasma membrane in mammalian cells

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Abstract

Mammals have three members of the intracellular phospholipase A₁ protein family (phosphatidic acid preferring-phospholipase A₁, p125, and KIAA0725p). In this study, we showed that KIAA0725p is localized in the Golgi, and is rapidly cycled between the Golgi and cytosol. Catalytic activity is important for targeting of KIAA0725p to Golgi membranes. RNA interference experiments suggested that KIAA0725p contributes to efficient membrane trafficking from the Golgi apparatus to the plasma membrane, but is not involved in brefeldin A-induced Golgi-to-endoplasmic reticulum retrograde transport.

1. Introduction

Phospholipase A₁ (PLA₁) comprises a group of enzymes that hydrolyze the sn-1 ester bond of phospholipids, producing 2-acyl-lysophospholipids and fatty acids. The intracellular PLA₁ (iPLA₁) family proteins were relatively recently discovered and appear to play important roles in higher organisms. In Arabidopsis thaliana and Caenorhabditis elegans, iPLA₁ mutation disrupts the shoot gravitropism [1] and the asymmetry of cell-fate specification [2], respectively.

Although there is only one iPLA₁ species in most eukaryotic organisms, mammals have three family members (PA-PLA₁, p125, and KIAA0725p). Mammalian phosphatidic acid-prefering PLA₁ (PA-PLA₁), the first identified member of the iPLA₁ family, is highly expressed in mature testis and therefore has been postulated to participate in spermiogenesis [3,4]. We identified two of these mammalian iPLA₁ family proteins, p125 and KIAA0725p [5,6]. p125 was isolated as a protein that binds to Sec23 [5,7], a coat component of COPII vesicles involved in protein transport from the endoplasmic reticulum (ER). p125 is located at ER exit sites, specialized ER subdomains where COPII vesicles are produced, and participates in their organization [8]. KIAA0725p, the most recently discovered member, was found through a database search using the amino acid sequence of p125 [6]. Differing from p125, KIAA0725p does not bind to Sec23, and its overexpression causes disorganization of the ER and Golgi [6]. In this study, we show that KIAA0725p is localized in the Golgi apparatus and is required for efficient membrane trafficking from the Golgi apparatus to the plasma membrane.

2. Materials and methods

2.1. Antibodies

Rat monoclonal antibodies (mAbs) against KIAA0725p (8F12 and 3G1) were kindly donated by Dr. H. Arai and J. Aoki (The University of Tokyo, Tokyo, Japan). The former and latter were used for immunofluorescence and immunoblotting analyses, respectively. A mouse mAb against KIAA0725p (2A9) was prepared in this laboratory, using a polypeptide (amino acids 368–486 of human KIAA0725p) as an antigen.
2.2. Fluorescence recovery after photobleaching (FRAP)

We used a Leica confocal microscope TCS SP2 equipped with a ×100 oil immersion objective (N.A. 1.4). Green fluorescent protein (GFP)-positive structures were bleached with the maximum power of a 488 nm Ar laser. Fluorescence recovery was measured with a 5% laser power at 1.68 s intervals (5 s intervals in the case of GFP-ERGIC-53). Data analysis including calculation of time constants for the recovery was performed with Leica FRAP analysis software.

Fig. 1. KIAA0725p is localized in the cis-Golgi apparatus but not in ER exit sites. (A) HeLa cells were fixed and double stained with mAb 8F12 and an antibody against β-COP or Sec31. The panels in the second and bottom rows are magnified views of a single cell in the panels in the top and third rows, respectively. Note that β-COP-positive peripheral dot-like structures, which represent its localization in the ERGIC, or Sec31-positive peripheral dot-like structures, which represent its presence in peripheral ER exit sites, were not labeled with mAb 8F12. (B) HeLa cells were treated with 10 μg/ml BFA in serum-free medium for 10 min and then processed for immunofluorescence analysis. (C) HeLa cells were treated without or with 10 μg/ml nocodazole for 3 h and then processed for immunofluorescence analysis. Because of the incompatibility of mAb 8F12 and an anti-TGN46 antibody, transiently expressed FLAG-KIAA0725p and endogenous TGN46 were stained with the respective antibodies (bottom row). (D) HeLa cells were treated with Oligonucleotide 2 as described for Fig. 3A and stained with anti-GM130.
2.3. Short interfering RNA (siRNA)-mediated protein knockdown

The RNA duplexes used for targeting KIAA0725p (Oligonucleotide 1, 5’-CAGGAUGAGUAUGGACCUUAA-3’; Oligonucleotide 2, 5’-AAGAAAGAAGAUAUUAAACUA-3’; Oligonucleotide 3, 5’-AAGGAGAAAGUAGAUAAGGAA-3’; and Oligonucleotide 4, 5’-CAGGAAAGAGGAAUCCCUUA-3’), Sec16A (5’-ACGGCCCAUCGUAAUGAAUU-3’), Rab6a and Rab6a’ (5’-AAGACAUUCUUUGAUACCCAGA-3’).
and Lamin A/C (5'-AACUGGACUUCCAGAAGAACA-3') were purchased from Japan Bioservice, Inc. Transfection of cells with RNA duplexes (200 nM final concentration) was performed using Oligofectamine (Invitrogen).

2.4. Replacement of KIAA0725p

Plasmids encoding FLAG-KIAA0725p and the FLAG-S351A mutant were constructed previously [6]. To express these proteins in KIAA0725p-siRNA-transfected cells, the siRNA targeting sequences were changed by PCR-based site-directed mutagenesis as follows: AAGAAAGAAGATATTAAACTA to TC...AAA...ATACTA for the Oligonucleotide 2 site, AAGGAGAAAGTAGATAAGGAA to AAA...GAA...GTT...AAA for the Oligonucleotide 3 site, and CAG...GAAATAGGAATTCCTTTG to CAA...GAG...ATT...GGT...ATA...CCAC...TG for the Oligonucleotide 4 site, where the mutated bases are underlined.

HeLa cells treated with siRNA for 48 h were transfected with expression plasmids and analyzed after 24 h.

2.5. ts045 vesicular stomatitis virus-encoded glycoprotein (ts045 VSVG) transport assays

The plasmid encoding ts045 VSVG fused with GFP [11] was kindly donated by Dr. J. Lippincott-Schwartz (NIH, Bethesda, MD). Transport assays were performed as described previously [9]. For determination of surface VSVG, live cells were stained with an anti-VSVG mAb (8G5) directed against the extracellular domain of VSVG. mAb 8G5 was described previously [8].

3. Results

3.1. KIAA0725p is localized in the cis-Golgi apparatus

The localization of endogenous KIAA0725p in HeLa cells was analyzed using mAbs. Anti-KIAA0725p mAb 8F12 (Fig. 1A) and 2A9 (data not shown) gave dense juxtanuclear staining with faint
cytosolic staining. Treatment of cells with a Golgi-disrupting agent, brefeldin A (BFA) [12], confirmed the Golgi localization of KIAA0725p (Fig. 1B). When compared with several Golgi markers, KIAA0725p was well colocalized with a cis-Golgi marker, GM130, but less with a medial-Golgi marker, mannosidase II, or a trans-Golgi network marker, TGN46 (Fig. 1C). The colocalization of KIAA0725p with the cis-Golgi marker was retained when Golgi was disassembled by a microtubule-depolymerizing agent, nocodazole. These data suggest that KIAA0725p is localized principally in the cis-Golgi and cytosol.

3.2. Golgi-localized KIAA0725p is dynamically cycled between the cytosolic and membrane pools

To determine whether the cytosolic and membrane pools of KIAA0725p are in dynamic equilibrium, we performed FRAP experiments. The plasmid encoding GFP-KIAA0725p was transfected into cells, and a part of the perinuclear fluorescence was photobleached. The perinuclear fluorescence of GFP-KIAA0725p was rapidly recovered (Fig. 2A and B). The time constant (8.4 s) was smaller than that of Arf1 (Fig. 2C), which undergoes fast Golgi/cytosol exchange [13]. β-1,4-Galactosyltransferase (GT)-GFP and GFP-ERGIC-53, both of which are membrane-anchored proteins localized in the Golgi and ER–Golgi intermediate compartment, respectively, showed much slower time constants.

3.3. Catalytic activity is important for targeting of KIAA0725p to Golgi membranes

To examine whether catalytic activity is related to the Golgi binding of KIAA0725p, we knocked down endogenous KIAA0725p with siRNA, and then examined the localization of the S351A mutant, which lacks phospholipase activity [6].

Fig. 5. Redistribution of GT-GFP to the ER is not inhibited by depletion of KIAA0725p. HeLa cells were transfected with an RNA duplex for Lamin A/C or KIAA0725p (A and B), or with Rab6 siRNA (B). At 24 h after transfection, the plasmid for GT-GFP was transfected into cells. After an additional 24 h incubation, the cells were treated with 10 μg/ml BFA for 30 min. (A) Localization of GT-GFP after BFA treatment. (B) Quantitation. White, gray, and black bars represent the percentages of cells with GT-GFP in the Golgi, tubular structure, and ER, respectively. Each experiment was repeated at least three times, and the data are presented as mean values with S.E.M. The asterisks indicate statistically significant difference; *P < 0.05; **P < 0.01 by Student’s t-test in comparison with Lamin A/C siRNA-transfected cells in each data set.
Although all four siRNAs (Oligonucleotides 1–4) effectively knocked down KIAA0725p, the expression of Rab6, a key factor for retrograde transport from the Golgi to the ER [10], was also reduced by Oligonucleotide 1 (Fig. 3A). We therefore used Oligonucleotides 2–4 for further experiments. In Oligonucleotide 2-transfected cells, FLAG-tagged wild-type KIAA0725p and the S351A mutant, in both of which the siRNA target sequence was mutated, were transiently expressed, and their subcellular distributions were analyzed. As shown in Fig. 3B, FLAG-tagged wild-type KIAA0725p (WT) was mainly located in the perinuclear structure in cells with a reduced expression of the endogenous protein (top left), as observed in cells transfected with control Lamin A/C siRNA (top right). Notably, the cytoplasmic staining of the expressed protein was very weak. By contrast, FLAG-tagged S351A mutant was observed not only in the perinuclear region but also in the other regions of the cytoplasm in cells depleted of endogenous KIAA0725p. Quantitative data indicate that the S351A mutant is much less efficiently targeted to the perinuclear structure (Fig. 3C).

3.4. Depletion of KIAA0725p retards the transport of VSVG from the Golgi apparatus

As depletion of KIAA0725p did not cause a significant change of the Golgi structure (Fig. 1D), we next examined if depletion of KIAA0725p interferes with VSVG transport from the ER. HeLa cells were first transfected with Oligonucleotide 2 or control Lamin A/C siRNA, and subsequently transfected with the plasmid encoding ts045 VSVG-GFP, followed by incubation at 40 °C. At 30 min after a shift to the permissive temperature (32 °C), a large portion of ts045 VSVG-GFP was detected in the perinuclear, Golgi area with some in the plasma membrane in control Lamin A/C siRNA-transfected cells (Fig. 4A, top). In KIAA0725p-depleted cells, ts045 VSVG-GFP was also observed in the perinuclear region, but the amount of ts045 VSVG-GFP reaching the plasma membrane appeared to be lower than that in control cells after 30 min incubation (bottom). This defect in transport appears to represent kinetic rather than absolute blockage, because ts045 VSVG-GFP was detected on the surface of KIAA0725p-depleted cells at 60 min after the temperature shift, as observed in control cells. To specifically label VSVG-GFP on the plasma membrane, non-permeabilized cells were incubated with mAb 8G5, followed by Texas-Red-conjugated secondary antibody (Fig. 4B). At 30 min after the temperature shift, the ratio of cells with cell surface VSVG-GFP staining to total VSVG-GFP-expressing cells was lower in KIAA0725p-depleted cells compared with in control cells (Fig. 4B, right).

To assess whether ER-to-Golgi transport is delayed in KIAA0725p-depleted cells, we examined the acquisition of endoglycosidase H (Endo H) resistance of VSVG, which is a hallmark of VSVG transport to the medial-Golgi. As shown in Fig. 4C, there was no significant delay in the acquisition of Endo H resistance of ts045 VSVG-GFP in KIAA0725p-depleted cells. These results suggest that KIAA0725p is involved in protein transport from the Golgi to the plasma membrane.

3.5. Knockdown of KIAA0725p does not retard the BFA-induced redistribution of GT-GFP to the ER

In the course of this study, Morikawa et al. [14] reported that knockdown of KIAA0725p, which they named iPLA1-γ, markedly delays the BFA-induced redistribution of GT-GFP to the ER. They proposed that KIAA0725p/iPLA1-γ is involved in a novel Golgi-to-ER transport route that is independent of COP1 or Rab6 [14]. We noticed that the siRNA they used was similar to Oligonucleotide 1, which could reduce not only the expression of KIAA0725p but also that of Rab6, which functions in BFA-induced Golgi-to-ER redistribution [15]. As shown in Fig. 5, BFA-induced redistribution of GT-GFP was inhibited in cells transfected with Oligonucleotide 1, but not Oligonucleotide 2 or 3. A little delay was observed in the case of Oligonucleotide 4. Knockdown of Rab6 in addition to KIAA0725p depletion slightly enhanced the inhibition of BFA-induced GT-GFP redistribution (Fig. 5B). These results suggest that KIAA0725p is not critical for the BFA-induced redistribution of GT-GFP and raise the possibility that the delay in the GT-GFP redistribution observed by Morikawa et al. [14] may be due to depletion of not only KIAA0725p but also Rab6 and perhaps other proteins.

4. Discussion

In the present study, we showed that KIAA0725p is localized in the cis-Golgi apparatus as well as in the cytosol, and is rapidly cycled between the Golgi and cytosolic pools. Catalytic activity is important for efficient targeting of KIAA0725p to Golgi membranes. This may imply that lysophospholipids formed by the action of KIAA0725p are important for tight association of KIAA0725p to Golgi membranes. Alternatively, the catalytic residue Ser-351 itself may be important for efficient binding to membranes. We prefer the former possibility, because our previous study [6] and present data (Fig. 3B, right) showed that the S351A mutant, like wild-type KIAA0725p, is targeted to the Golgi in cells expressing wild-type KIAA0725p.

One important finding in this study is that, in contrast to the recent report by Morikawa et al. [14], depletion of KIAA0725p does not affect the transport of VSVG-GFP from the Golgi to the plasma membrane. The difference between their and our results may be ascribed to different transport assays used. They monitored both cytoplasmic and plasma membrane fractions of VSVG-GFP, whereas we detected only the latter fraction. Some cis-Golgi-localized proteins are known to cycle between the cis- and trans-Golgi and also between the Golgi and endosomes via the plasma membrane [16,17]. KIAA0725p may contribute to the secretion of VSVG-GFP from the Golgi to the plasma membrane by regulating a pathway used by such cis-Golgi proteins. Alternatively, lysophospholipids produced by KIAA0725p at the cis-Golgi side may be required for the Golgi to be fully maturated, and their deficiency may render the trans-Golgi network partially incapable of pinching off transport carriers destined for the cell surface.

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