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Original research

CAMSAP3-dependent microtubule dynamics regulates Golgi assembly in epithelial cells

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ABSTRACT

The Golgi assembly pattern varies among cell types. In fibroblast cells, the Golgi apparatus concentrates around the centrosome that radiates microtubules; whereas in epithelial cells, whose microtubules are mainly noncentrosomal, the Golgi apparatus accumulates around the nucleus independently of centrosome. Little is known about the mechanisms behind such cell type-specific Golgi and microtubule organization. Here, we show that the microtubule minus-end binding protein Nezha/CAMSAP3 (calmodulin-regulated spectrin-associated protein 3) plays a role in translocation of Golgi vesicles in epithelial cells. This function of CAMSAP3 is supported by CG-NAP (centrosome and Golgi localized PKN-associated protein) through their binding. Depletion of either one of these proteins similarly induces fragmentation of Golgi membranes. Furthermore, we find that stathmin-dependent microtubule dynamics is graded along the radial axis of cells with highest activity at the perinuclear region, and inhibition of this gradient disrupts perinuclear distribution of the Golgi apparatus. We propose that the assembly of the Golgi apparatus in epithelial cells is induced by a multi-step process, which includes CAMSAP3-dependent Golgi vesicle clustering and graded microtubule dynamics.

1. Introduction

Membranous organelles exhibit complex architecture, and show great morphological diversity across cell types. How such diversity is generated and how each organelle is positioned to particular subcellular sites have yet to be fully understood. The Golgi apparatus, which functions in posttranslational modification, sorting and transportation of proteins, is one of these membranous organelles; its shape and intracellular positioning vary among cell types and are altered during the cell cycle (Allan et al., 2002; Colanzi et al., 2003). In many cell types including fibroblast cells, Golgi apparatus condenses around the centrosome (Sutterlin and Colanzi, 2010), whereas in other cell types, this complex has dispersive morphologies (Burkhardt, 1998).

It is thought that Golgi assembly is dependent on microtubules because the Golgi becomes fragmented upon disruption of microtubules or inhibition of dynein motors (Burkhardt, 1998; Allan et al., 2002). One explanation of this phenomenon is that pre-Golgi vesicles exiting from the endoplasmic reticulum (ER) are transported by dynein along microtubules toward their minus ends, resulting in accumulation in peri-centrosomal regions. In the absence of microtubules or dynein, this transport system is disrupted, leaving Golgi vesicles scattered (Cole et al., 1996; Takahashi et al., 1999). Other studies have revealed an additional pathway for Golgi assembly: microtubules nucleate not only at the centrosome but also at Golgi membranes themselves; and pre-Golgi vesicles use these microtubules for clustering (Efimov et al., 2007; Miller et al., 2009; Rivero et al., 2009).

A number of factors play a decisive role in microtubule and Golgi dynamics (Schulze and Kirschner, 1986). Nezha/CAMSAP/Patronin

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family proteins, including CAMSAP (calmodulin-regulated spectrin-associated protein) 1, 2 and 3 in the case of vertebrates, bind the minus ends of noncentrosomal microtubules and stabilize them (Meng et al., 2008; Tanaka et al., 2012; Nagae et al., 2013; Jiang et al., 2014; Yau et al., 2014). In epithelial cells, whose microtubules are mainly noncentrosomal, depletion of CAMSAP3 or 2 results in significant loss of noncentrosomal microtubules, and concomitantly induces fragmentation of the Golgi apparatus (Tanaka et al., 2012; Nagae et al., 2013). Recent studies also demonstrate that CAMSAP3 mutation in mice leads to mispositioning of Golgi in intestinal epithelial cells (Toya et al., 2016), suggesting that CAMSAP3-mediated microtubule organization is important for Golgi assembly. CG-NAP (centrosome and Golgi localized PKN-associated protein), also known as AKAP450 or AKAP350, is another regulator of Golgi organization. CG-NAP localizes at the centrosome and *cis*-Golgi, and recruits various proteins to these structures (Takahashi et al., 1999; Gillingham and Munro, 2000; Sillibourne et al., 2002; Nishimura et al., 2005; Kim et al., 2007). At the Golgi apparatus, CG-NAP cooperates with the γ-tubulin ring complex (γ-TuRC) to facilitate Golgi-derived microtubule formation (Takahashi et al., 2002; Rivero et al., 2009).

The main goal of this study was to explore how CAMSAP3 regulates the assembly process of the Golgi apparatus. It was found that CAMSAP3-derived microtubules are involved in the translocation of Golgi mini-stacks, and the function of CAMSAP3 is supported by CG-NAP. Furthermore, we found that microtubule dynamics is graded along the radial axis of epithelial cells, and stathmin, a member of a family of microtubule-destabilizing factors (Sobel et al., 1989; Andersen, 2000), regulates this process. Through functional analysis of these proteins, we propose that perinuclear assembly of the Golgi apparatus is achieved by at least two steps: the CAMSAP3-CG-NAP-dependent accumulation of Golgi mini-stacks to form larger clusters, and the relocation of these clusters to perinuclear regions in a process dependent on graded microtubule dynamics. We also suggest that the organization of microtubules is a factor that determines the diversity in Golgi distribution among different cell types.

2. Results

2.1. CAMSAP3 depletion alters microtubule and Golgi assembly patterns

As reported previously (Tanaka et al., 2012), in monolayer cultures of Caco2 cells whose microtubules are essentially noncentrosomal, knockdown (KD) of CAMSAP3 caused reorganization of microtubules. In control cells, microtubules surrounded the nucleus with perinuclear localization of centrosomes, whereas in CAMSAP3-depleted cells, microtubules and centrosomes became detectable on top (Figs. 1A–C and S1A). In these cells, Golgi assembly was also altered. In control cells, Golgi membranes marked by Giantin distributed as discontinuous clusters at perinuclear regions, and a small number of Golgi apparatus concentrated at the centrosome. When CAMSAP3 was depleted, however, Golgi clusters were fragmented into mini-stacks, with some of these Golgi fragments accumulating around centrosomes as these centrosomes moved to the center of the cell (Fig. 1C and D). These observations indicate that CAMSAP3 is required for the accumulation of Golgi at perinuclear sites. We confirmed that nocodazole treatment also enhanced Golgi fragmentation, but in this case, centrosomal accumulation of Golgi did not occur (Figs. 1F and S1B), indicating that Golgi re-assembly processes are regulated by microtubules, as reported in previous studies (Lowe, 2011).

2.2. Capture of Golgi fragments by CAMSAP3 clusters

To explore the mechanism by which CAMSAP3 regulates Golgi assembly, we performed a nocodazole washout procedure using control and CAMSAP3-depleted Caco2 cells. About 1 h after removal of nocodazole in control cells, the Golgi apparatus recovered normal assembly; whereas, in the absence of CAMSAP3, the Golgi apparatus was still fragmented at this time point (Fig. 2A). In CAMSAP3-depleted cells, the average area of Golgi mini-stacks was around 0.48 μm^2, which was similar to that of Golgi mini-stacks in cells treated with a dynein inhibitor (Fig. S1B). These results are consistent with the idea that CAMSAP3-dependent regulation of microtubules is important for Golgi assembly.

In Caco2 cells cultured as flat monolayers, CAMSAP3 is detected as clusters scattered in the cytoplasm (Tanaka et al., 2012). These clusters diminished after nocodazole treatment, but they reemerged 3–5 min after nocodazole removal (Fig. S1C). Using this nocodazole washout system, we observed the spatial relationship between CAMSAP3 and Golgi mini-stacks during the process of Golgi reassembly by live imaging. We co-transfected Caco2 cells with *Camsap3-GFP* and mCherry-tagged galactosyltransferase (*GT-mCherry*), a Golgi marker, and observed their behavior after nocodazole washout. Live imaging of these cells initially detected three structures: free CAMSAP3 clusters, free Golgi mini-stacks, and Golgi mini-stacks associated with CAMSAP3 clusters (Fig. 2B). The free CAMSAP3 clusters and Golgi-CAMSAP3 complexes were still fixed at a given position, whereas the free Golgi mini-stacks moved around the cell. These free Golgi mini-stacks, however, eventually migrated toward CAMSAP3 clusters and were captured by these clusters (Fig. 2B and C; Movie S1). Although we did not visualize microtubules in live cells, in fixed samples we found that CAMSAP3-tethered noncentrosomal microtubules associated with local portions of Golgi bodies 5 min after nocodazole removal (Fig. 2D). These results suggest that Golgi fragments migrate along CAMSAP3-tethered microtubules up to their minus ends, where CAMSAP3 is associated, presumably with the aid of minus-end-directed motors such as dynein (Cole et al., 1996; Takahashi et al., 1999).

It has been shown that Golgi-derived microtubules are used for clustering of pre-Golgi vesicles (Efimov et al., 2007; Miller et al., 2009; Rivero et al., 2009). We found that Golgi-associated microtubules significantly decreased in CAMSAP3-depleted cells 3 min after nocodazole removal (Fig. 2E), suggesting the possibility that CAMSAP3 may be involved in the nucleation of microtubules from the Golgi apparatus.

2.3. CAMSAP3 binds to CG-NAP

To analyze the mechanisms by which CAMSAP3 interacts with Golgi mini-stacks, we screened for proteins that bind to CAMSAP3 by pull-down assay, and identified CG-NAP, a protein known to provide a microtubule nucleation site for the centrosome (Schmidt et al., 1999; Takahashi et al., 1999; Witczak et al., 1999), as a CAMSAP3 partner. Previous investigations have also shown that a decrease in CG-NAP expression induces vesiculation of the Golgi apparatus, similar to CAMSAP3 knockdown (Larocca et al., 2004). Immunoprecipitates of CAMSAP3 also contained CG-NAP (Fig. 3A). Co-immunostaining for CG-NAP and CAMSAP3 showed that CG-NAP closely associated with the main Golgi bodies (Fig. 3B and b′), as reported previously (Takahashi et al., 1999; Rivero et al., 2009). In addition, we noticed that there were a number of CG-NAP signals that did not associate with Golgi at peripheral regions; they overlapped instead with CAMSAP3 clusters (Fig. 3B and