FEBS Letters 589 (2015) 1771–1777

FEBS Letters

journal homepage: www.FEBSLetters.org

Cep70 regulates microtubule stability by interacting with HDAC6

Xingjuan Shi [a,*], Yanjun Yao [a], Yujue Wang [a], Yu Zhang [a], Qinghai Huang [a], Jun Zhou [b], Min Liu [b], Dengwen Li [b,*]

[a] Key Laboratory of Developmental Genes and Human Disease, Institute of Life Sciences, Southeast University, Nanjing 210096, China
[b] Department of Genetics and Cell Biology, College of Life Sciences, Nankai University, Tianjin, China

ARTICLE INFO

Article history:
Received 13 April 2015
Revised 25 May 2015
Accepted 4 June 2015
Available online 22 June 2015

Edited by Judit Ovadi

Keywords:
Cep70
Tubulin
Microtubule stability
Tubulin acetylation
Histone deacetylase 6

ABSTRACT

Microtubules, highly dynamic components of the cytoskeleton, are involved in mitosis, cell migration and intracellular trafficking. Our previous work has shown that the centrosomal protein Cep70 regulates microtubule organization and mitotic spindle orientation in mammalian cells. However, it remains elusive whether Cep70 is implicated in microtubule stability. Here we demonstrate that Cep70 enhances microtubule resistance to cold or nocodazole treatment. Our data further show that Cep70 promotes microtubule stability by regulating tubulin acetylation, and plays an important role in stabilizing microtubules. Mechanistic studies reveal that Cep70 interacts and colocalizes with histone deacetylase 6 (HDAC6) in the cytoplasm. These findings suggest that Cep70 promotes microtubule stability by interaction with HDAC6 and regulation of tubulin acetylation.

1. Introduction

The centrosome, acts as major microtubule-organizing center in animal cells, is essential for many cellular functions such as mitosis, cell migration and polarization [1,2]. In many mammalian cells, microtubules are organized radially with minus ends anchored to the centrosome whereas plus end oriented toward the periphery of the cell. Cellular homeostasis relies on a dynamic microtubule network, which can be perturbed by alterations in microtubule stability and dynamics [3–5]. Microtubules display dynamic instability at plus ends and relative stability at minus ends [6]. Dysregulation of microtubule stability is causative for developmental defects, neurodegenerative diseases and cancer [7–9]. Many centrosomal proteins have been proved to regulate microtubule stability [10,11]. For example, centrosomal CAP350 protein stabilizes microtubules associated with the Golgi complex [12]. Besides, centrosomal protein CDK5RAP2 interacts directly with microtubule plus-end tracking protein EB1, and promotes microtubule stability [13].

Cep70, a centrosomal protein of 70kDa, was initially described in a proteomic study of human centrosome [14]. Cep70 has been showed to contribute to ciliogenesis in zebrafish embryos [15]. CRC70, the Chlamydomonas homolog of Cep70, functions as a scaffold for the assembly of the centriole precursor [16]. In our previous study, Cep70 has been shown to participate in microtubule assembly and mitotic spindle orientation [17,18]. In addition, Cep70 contributes to cell polarization and migration by regulating microtubule rearrangement [19]. However, the role of Cep70 plays in microtubule stability remains unknown. In this study, we provide the first evidence that Cep70 promotes microtubule stability by enhancing tubulin acetylation and interacting with histone deacetylase 6 (HDAC6).

2. Materials and methods

2.1. Chemicals and antibodies

Nocodazole, 4′,6-diamidino-2-phenylindole (DAPI), and antibodies against α-tubulin, acetylated α-tubulin (T7451), glutathione S-transferase (GST), Hemagglutinin (HA) and maltose-binding protein (MBP) were purchased from Sigma–Aldrich. Antibodies against HDAC6 were from Abcam, antibodies against GFP were from Roche, and the antibodies against acetylated-lysine were from Cell Signaling Technology. The anti-Cep70 antibody was generated as described previously. Horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, and fluorescein- and

Author contributions: XS, DL, ML and JZ conceived and designed the experiments; YY, YW, YZ, and QH performed the experiments; XS analyzed the data and wrote the paper.

* Corresponding authors.
E-mail addresses: xingjuanshi@seu.edu.cn (X. Shi), dwli@nankai.edu.cn (D. Li).

http://dx.doi.org/10.1016/j.febslet.2015.06.017
0014-5793/

rhodamine-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories.

2.2. Cell culture and transfection

HeLa cells and 293T cells were obtained from the American Type Culture Collection, and cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2. Plasmids were transfected into cells with Entranster-D reagent (Engreen Biosystem). siRNA oligonucleotides targeting human Cep70 (siCep70#1: GAGGAUGAAUCACUAAGUA, siCep70#2: CCUCAUACCGUCUUGGAUA) were synthesized (RiboBio) and transfected into cells with Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) according to manufacturers' protocols.

2.3. Plasmids and recombinant proteins

Mammalian expression plasmids for GFP-Cep70 (1–597 full-length and various truncated form) and HA-HDAC6 were generated by using the pEGFPC1 and pCMV-HA vectors, respectively. Bacterial expression plasmids for MBP-Cep70 and GST-HDAC6 were constructed using the pMALp2T and pGEX-6P3 vectors, respectively. MBP and MBP-Cep70 fusion proteins were purified with the amylose resin following the manufacturer's protocol (New England Biolabs). GST and GST-HDAC6 fusion proteins were purified with Glutathione Sepharose 4B (GE Healthcare). MAP-free tubulin and MAP-rich tubulin were purchased from Cytoskeleton.

2.4. Immunoblotting

Cells were lysed in a buffer containing 1% Triton X-100, 150 mM NaCl, and 50 mM Tris (pH7.5). Proteins were resolved by SDS–PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked in Tris-buffered saline containing 0.2% Tween 20 and 5% fat-free dry milk and incubated with primary antibodies and then horseradish peroxidase-conjugated secondary antibodies. Specific proteins were visualized with enhanced chemiluminescence detection reagent according to the manufacturer's instructions (Pierce).

2.5. Immunofluorescence microscopy

Cells grown on coverslips were fixed with methanol at −20 °C or fixed with 4% paraformaldehyde for 30 min at room

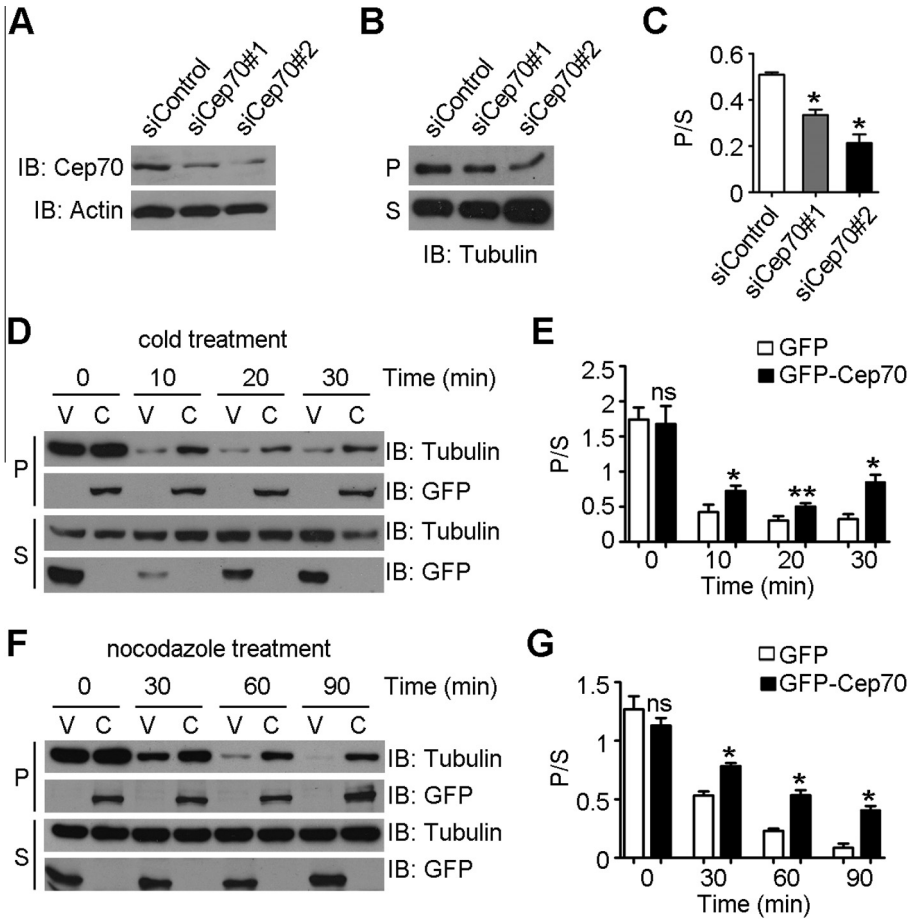

Fig. 1. Cep70 enhances microtubule resistance to cold or nocodazole treatment. (A) HeLa cells were transfected with control or Cep70 siRNAs. The levels of Cep70 and actin were then examined by immunoblotting with antibodies against Cep70 and actin. (B) HeLa cells were transfected with control or Cep70 siRNAs, and treated with nocodazole for 90 min. Tubulin partitioning between the polymer (P) and soluble dimer (S) forms was examined by immunoblotting with the anti-α-tubulin antibody. (C) Experiments were performed as in (B), and the ratio of tubulin polymer to soluble dimer was analyzed. (D) HeLa cells were transfected with GFP (V) or GFP-Cep70 (C) and placed on ice for 0, 10, 20 or 30 min. Tubulin partitioning between the polymer and soluble dimer forms was examined by immunoblotting with the anti-α-tubulin antibody, and the expression of GFP and GFP-Cep70 was examined by immunoblotting with the anti-GFP antibody. (E) Experiments were performed as in (D), and the ratio of tubulin polymer to soluble dimer was analyzed. (F) HeLa cells were transfected with GFP or GFP-Cep70 and treated with nocodazole for 0, 30, 60 or 90 min. Tubulin partitioning between the polymer and soluble dimer forms was examined by immunoblotting with the anti-α-tubulin antibody, and the expression of GFP and GFP-Cep70 was examined by immunoblotting with the anti-GFP antibody. (G) Experiments were performed as in (F), and the ratio of tubulin polymer to soluble dimer was analyzed. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, not significant ($P \geqslant 0.05$).