Nucleophosmin/B23 Inhibits Eg5-mediated Microtubule Depolymerization by Inactivating Its ATPase Activity*

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Nucleophosmin/B23, an abundant nucleolar protein, plays multiple roles in cell growth and proliferation, and yet, little has been studied about its function in regulating dynamics of microtubules. Here, we report that B23 directly interacts with Eg5, a member of the kinesin family, in the cytosol. The DNA/RNA binding domain of B23 and the motor domain of Eg5 were found to be involved in their interaction. Both in vivo and in vitro evidences showed that B23 acts as an upstream regulator of Eg5 in promoting microtubule polymerization. Moreover, we further demonstrated that B23 regulates microtubule dynamics by directly inhibiting Eg5 ATPase activity.

Microtubule (MT)³ dynamics exist in two major states, either growing or shrinking, and switch stochastically between these two states. The transition from growth to shrinkage is called a catastrophe, and in contrast, transition from shrinkage to growth is called a rescue (1). Both growing and shrinking processes are regulated by various cellular factors that stabilize and/or destabilize the MT lattice. Eg5 (also known as kinesin-5, KIF11), a motor protein that belongs to the kinesin-like protein family, was recently reported to mediate length-dependent control of MT assembly (2, 3). It is proposed that kinesins disassemble longer kinetochore microtubule by generating a concentration gradient along a MT and hydrolyze ATP to move toward the plus (+) end, where it causes MT depolymerization (4, 5). It is now a well established fact that kinesin-related proteins can destabilize microtubules, and the kinesins that destabilize the ends of microtubules are known as “catastrophe kinesins” (6). However, how catastrophe kinesins are regulated remains to be further investigated. Nucleophosmin (also known as B23) is a multifunctional phosphoprotein that shuttles rapidly between the nucleus and the cytoplasm (7, 8). B23 provokes cell proliferation and transformation, and B23 is frequently mutated in many different tumor types such as acute myeloid leukemia (AML) (9–11). Despite the fact that its functions inside of the nucleus have been well studied, the functional role of B23 in the cytoplasm remains elusive. Previous studies have shown that B23 regulates centrosome duplication during mitosis (12–14), which hints that the protein B23 also may play roles in the global control of the MT lattice.

In this study, we identified that B23/nucleophosmin interacts directly with Eg5, and through this interaction, B23 inhibited ATPase activity of the Eg5 motor domain and thus down-regulated the Eg5-mediated ATP-dependent microtubule catastrophe. The physiological effects of Eg5 on microtubules are more significant upon the elimination of B23 protein levels, indicating that B23 normally plays a protective role in MT assembly.

EXPERIMENTAL PROCEDURES

Cell Culture, Drug Treatments, Transfection, and siRNA—Cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum. Cells were transfected by Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The human Eg5 synthetic siRNA (Santa Cruz Biotechnology) was used according to the manufacturer’s instructions. Stable B23 knockdown (KD) cell line was prepared using pSIREN-RetroQ short hairpin RNA system according to the manufacturer’s protocol (Clontech).

Plasmid Clones and Protein Purification—Total RNA was purified from HeLa cells using Trizol reagent (Invitrogen). Statmin, B23, and the B23 deletions were amplified from HeLa RNA using the PrimeScript™ One Step RT-PCR kit (Takara Bio). Eg5 and its dominant-negative mutation T112N were amplified from GFP-Eg5 and GFP-Eg5 T112N (gifts from Peter W. Baas) (2). The primers were listed in supplemental Table 1. GST-statmin, GST-B23, GST-Eg5-(1–437), GST-Eg5-(1–437) T112N, and GST-B23 deletions were constructed by inserting their open reading frames into the EcoRI/XhoI sites of pGEX-5X-3 plasmid. His₆-Eg5-(1–111) was constructed by inserting its PCR fragment into the EcoRI/XhoI sites of the pET22b(+) plasmid. These proteins were purified as described previously (15). Porcine brain tubulin was purified by two warm/cold polymerization cycles followed by phosphocellulose chromatography (GE Healthcare HiTrap SP HP), as described (16, 17).

Antibodies—The following antibodies were used in this study: anti-GFP monoclonal antibody, anti-FLAG M2 monoclonal antibody, and anti-B23 monoclonal antibody (Sigma);
anti-human Eg5 antibody (BD Pharmingen); anti-PARP antibody (Upstate); and anti-His antibody, anti-GAPDH, and anti-human β-tubulin antibody (Cell Signaling).

Subcellular Fractionation, Immunoprecipitation (IP), GST Pulldown, and Western Blot Analysis—Subcellular fractionation was described as previously (18). GST pulldown, Western blot analysis, and IP were performed as described elsewhere (15).

Immunofluorescence Microscopy—Cells were cultured in 24-well chamber slides. After washing once with PEM buffer (100 mM Pipes-KOH, 2 mM EGTA, 1 mM MgCl₂, and protease inhibitors, pH 6.8), cells were permeabilized in PEM containing 0.5% Triton X-100 for 5 min at room temperature and fixed in 3.7% paraformaldehyde. After blocking with 5% bovine serum albumin in phosphate-buffered saline for 1 h, cells were incubated with primary antibody and donkey anti-mouse immunoglobulin G conjugated with rhodamine antibody (Jackson ImmunoResearch Laboratories), respectively. Hoechst 33342 (Sigma) was used for nuclear staining. Stained cells were mounted with VECTASHIELD mounting medium (Vector Laboratories, Burlingame, CA) and analyzed under an Olympus IX71 inverted microscope coupled to an Olympus DP70 high resolution color camera.

Yeast Two-hybrid Assay—The B23 open reading frame was cloned into the pGBK-T7 plasmid and was used as bait in a yeast two-hybrid screen of a HeLa cDNA library (Clontech) as described previously (15). After elimination of false positive clones by a β-galactosidase colony lift filter assay, plasmids were isolated from positive colonies, transformed into DH5α, and were sequenced thereafter.

In Vivo Polymerized Tubulin Assay—The in vivo assay of polymerized tubulin was performed as described previously (19). Briefly, cells were washed twice by PEM buffer and then incubated with PEM supplemented with 0.05% Triton X-100 at 37 °C for 30 min. The supernatant (designated as S) containing solubilized tubulin was clarified by centrifugation (15,000 × g for 30 min) to separate from the pellet (designated as P) containing sedimented polymerized tubulin. The pellet was washed once in PEM buffer before being denatured in Laemmli buffer.

In Vitro Tubulin Polymerization Assay—In vitro tubulin polymerization assay was used as described previously (20). Briefly, porcine tubulin was mixed with proper proteins in tubulin assembly buffer (80 mM Pipes, pH 6.8, 0.5 mM EGTA, 2 mM MgCl₂, 1 mM GTP, 1 mM ATP, and 10% glycerol) and incubated at 37 °C for 1 h. The polymerized tubulin was clarified by centrifugation (15,000 × g for 30 min). After a wash with tubulin assembly buffer, the pellet was denatured in Laemmli buffer.

Steady-state ATPase Assay—The steady-state ATPase assay was used as described previously (20). In brief, a temperature-controlled spectrophotometer (Beckman Coulter™ DU® 640) was set at 37 °C to monitor A₃₄₈ absorbance change after cuvettes were added with Eg5 and B23 in the indicated combinations: polymerized porcine tubulin, Tris-MgCl₂-dithiothreitol, Mg·ATP, PEP (Sigma), NADH, and PK/LDH (Sigma). Data points were analyzed with a Michaelis-Menten equation using the Kaleidagraph program, and the kₜₐₙ values (s⁻¹) were plotted.

Live Cell Imaging—Live cell imaging was performed as described previously (21). Briefly, GFP-β-tubulin was transiently transfected into B23-WT or B23-KD HeLa cells, transfected cells were replated on gridded coverglass bottom dishes (MatTek, Cat. P35G-1.5–7-C-grid). Then, images were automatically acquired at multiple locations on the coverslip using a Nikon Te2000E inverted microscope fitted with a 60×Nikon Plan Fluor objective, a linearly encoded stage (Proscan, Prior) and a Hamamatsu Orca-ER CCD camera. Fluorescence illumination was implemented by a mercury arc lamp with two neutral density filters (for a total 32-fold reduction in intensity). The microscope was controlled using Simple PCI (Compix) software and housed in a custom-designed 37 °C chamber with a secondary internal chamber that delivered humidified 5% CO₂. Fluorescence and differential interference contrast images were obtained every 5 min for a period of 2–3 h.

RESULTS

B23 Interacts with Eg5—To find novel B23 binding partners, we made use of a yeast two-hybrid system to screen a pretransformed human HeLa cDNA library using B23 as bait. Eg5 (residues 1–111) was detected as one of its novel binding partners (data not shown). The interaction of endogenous B23 with endogenous Eg5 was further verified by co-IP experiments. B23 was able to be co-precipitated with antibody against Eg5 but not with control IgG (Fig. 1a, upper panel, lane 2 versus lane 3). Reciprocally, Eg5 was able to be co-precipitated by an anti-B23 antibody but not by control IgG (Fig. 1b, upper panel, lane 2 versus lane 3). The B23-Eg5 interaction is likely to be direct, as shown by a pulldown assay with purified bacterially expressed GST-B23 and His₆-Eg5-(1–111) (Fig. 1c, upper panel, lane 1 versus lane 2). We also conducted a co-IP experiment with nuclear and cytosolic fractions, and as shown in Fig. 1d, B23 was found to interact with Eg5 mainly in the cytosolic fraction (lane 3 versus lane 4).

The DNA/RNA Binding Domain (DRBD) of B23 and the Motor Domain of Eg5 Are Involved in Their Interaction—B23 is a multifunctional protein and consists of three distinct functional domains (7), including the oligomerization molecular chaperone domain, histone binding domain, and DRBD. To delineate the region of B23 that interacts with Eg5, a panel of B23 mutants, each fused with GST, was tested (Fig. 2a). All of the B23 mutants that contained the C-terminal DRBD were able to bind Eg5 (Fig. 2b, lanes 3, 4, and 6), whereas a DRBD deletion mutant failed to bind to Eg5, (Fig. 2b, lane 5), suggesting that the DRBD of B23 mediates its binding to Eg5.

Reciprocally, domains of Eg5 that responsible for binding to B23 were also determined. Eg5, the Eg5 motor domain, and the Eg5 tail domain were individually expressed in HeLa cells and followed by co-IP using an anti-B23 antibody. GFP-Eg5 and the GFP-Eg5 motor were able to be co-precipitated with endogenous B23 (Fig. 2d, lanes 6 and 7), whereas the Eg5 motor domain deletion mutant (Eg5 tail domain) failed to be co-precipitated by B23 (Fig. 2d, lane 8), indicating that the motor domain of Eg5 is involved in its interaction with B23.

In Contrast to Eg5, B23 Promotes MT Polymerization—Recent reports suggest that Eg5 might possess the property of a
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catastrophe kinesin, and the ATPase activity of its motor domain contributes to its ability to destabilize MTs (2, 3). To verify the proposition that human Eg5 is indeed a catastrophe kinesin, we made use of an assay to separate dynamic MTs (designated as S for soluble) from stable (designated as P for pelletable) MTs. When GFP-Eg5 was ectopically expressed, MTs undergo catastrophes (Fig. 3a, lanes 3 and 4 versus lanes 5 and 6). However, overexpression of an Eg5 dominant-negative mutant (ATPase rigor mutant with a T112N point mutation) caused no effect on ratio of dynamic/stable MTs in HeLa cells (Fig. 3a, lanes 3 and 4 versus lanes 7 and 8). This evidence confirms the role of Eg5 in MT catastrophe.

B23 is an abundant nucleolar protein that shuttles rapidly between the nucleus and cytoplasm (22). Given the fact that B23 directly binds to the motor domain of Eg5, which was solely responsible for its MT depolymerizing activity (2), and B23 could also regulate centrosome cycle (12), we sought to investigate whether B23 can also regulate MT dynamics. First, we examined whether B23 regulates tubulin protein level. As shown in Fig. 3b, tubulin levels in both HeLa and 293T cells were not affected by short hairpin RNA-mediated B23 gene silencing, suggesting that B23 does not regulate tubulin protein expression. Next, we performed in vivo polymerized tubulin assay to compare the change in the ratio of dynamic/stable MTs between B23 wild type (B23-WT) and B23 knockdown (B23-KD) cells. Results showed that knockdown of B23 induced significant MT catastrophe compared with wild-type B23 (Fig. 3c; lanes 1 and 2 versus lanes 3 and 4). To exclude the possibility that MT catastrophe by B23 knockdown was due to an off-target effect, an siRNA-resistant plasmid FLAG-B23 was reintroduced back into HeLa B23-KD cells and the normal ratio of dynamic/stable MTs was restored (Fig. 3c; lanes 5 and 6 versus lanes 7 and 8). Furthermore, reintroducing B23-ΔDRBD, an interaction-defective mutant, failed to rescue the MT catastrophe (Fig. 3c; lanes 5 and 6 versus lanes 9 and 10), suggesting that the interaction between B23 and Eg5 is necessary for inhibiting MT catastrophe. Immunofluorescence experiments further support our conclusion. Stable MTs in B23-KD and B23-WT HeLa cells were immunostained with anti-tubulin antibody after the highly dynamic MTs were prereleased by Triton treatment. As shown in Fig. 3d, the MT network was less stable in B23-KD cells compared with that in B23-WT cells. Live cell imaging was also performed to study the role of B23 in MT regulation. As shown in Fig. 3e, B23-KD cells displayed less polymerized MTs compared with B23-WT cells. Together, these data indicate that B23 promotes MT polymerization and inhibits MT catastrophe.

B23 Regulates MT Dynamics by Directly Inhibiting Eg5 ATPase Activity—As demonstrated above, whereas Eg5 inhibits MT polymerization, B23 promotes its polymerization. Given the fact that B23 physically interacts with the Eg5 motor domain (specifically amino acids 1–111), we naturally hypothesized that the function of B23 in promoting MT assembly

FIGURE 1. B23 interacts with Eg5. a, HeLa cell lysates were incubated separately with anti-B23 antibody and a control antibody (IgG). Immunoprecipitated proteins and 5% input were analyzed by Western blot (WB) using antibodies against Eg5 and B23, respectively. b, HeLa cell lysates were incubated separately with anti-Eg5 antibody and a control antibody (IgG). The lysates (5%) and immunoprecipitates were analyzed by WB using antibodies as indicated. c, the His6-tagged Eg5 fragment (amino acids 1–111) was incubated separately with GST or GST-B23 immobilized on glutathione-Sepharose beads. The bead-bound proteins were analyzed by WB using an anti-His antibody (upper panel) and by Coomassie Blue staining (lower panel). d, lysates from HeLa cells were separated into cytosolic and nuclear fractions. Each fraction was immunoprecipitated with an anti-B23 antibody. Immunoprecipitates (IP) and 5% input were subjected to WB using antibodies for B23 and Eg5. PARP and GAPDH in the lysates were analyzed for fractional marker. C, cytoplasmic fraction; N, nuclear fraction; Lc, light chain; Hc, heavy chain; PARP, poly(ADP-ribose) polymerase.
could be through alleviating the MT destabilizing effect of Eg5, specifically inactivating its ATPase activity. To test this hypothesis, HeLa cells with or without a reduced B23 level by knockdown were further treated with or without Eg5 siRNA. Stable and dynamic MTs were fractionated for each treated cell, and the results showed that knockdown of Eg5 decreases dynamic MTs (Fig. 4a, lanes 1 and 2 versus lanes 3 and 4); knockdown of B23, in contrast, increases dynamic MTs (Fig. 4a, lanes 1 and 2 versus lanes 5 and 6). However, transfecting Eg5 siRNA into B23-KD cells could rescue MT catastrophe caused by B23 knockdown (Fig. 4a, lanes 5 and 6 versus lanes 7 and 8). These data indicate that the MT assembly promoting function for B23 is dependent on Eg5. This conclusion was further reinforced by the immunofluorescence experiment. As shown in Fig. 4c, g versus j, transfecting Eg5 siRNA into B23-KD cells was shown to rescue MT disruption.

To test whether B23 inhibits Eg5-mediated MT depolymerization through direct protein-protein interactions, an in vitro experiment was conducted. Tubulin was purified from porcine brain using a method described previously (16). GST, GST-Eg5-(1–437), GST-B23, and GST-stathmin fusion proteins were expressed and purified from *Escherichia coli*. Stathmin, a well known MT-depolymerizing factor (23), was used as a positive control. Polymerized tubulin and its associated proteins were visualized on gel (Fig. 4b, lanes 6–12). Although similar to GST-stathmin, the GST-Eg5 motor domain also caused extensive disruption of the microtubules (Fig. 4b, lane 7 versus lane 10). GST-B23 alone, however, showed little, if any, effects on
In contrast to Eg5, B23 promotes microtubule (MT) polymerization. a, HeLa cells individually expressing GFP, GFP-Eg5, GFP-Eg5 (T112N) ATPase rigor mutant or mock were permeabilized in PEM buffer containing 0.05% Triton X-100. Soluble fractions containing dynamic MTs (designated as s for soluble) were collected and the pellets containing stable MTs (designated as p for pellet) were washed twice with detergent-free PEM buffer, and all fractions were subjected to SDS-PAGE and probed with anti-GFP, anti-Eg5, and anti-β-tubulin antibodies. GAPDH was used as a soluble MT fractional marker. All of the biochemical pelleting experiments were repeated three times; the histogram depicts the average ratio of polymerized MTs to total MTs. Roman numerals I, II, III, and IV denote the respective ratios of polymerized MTs to total MTs from various transfections as indicated (**, p < 0.01; error bars are ± S.E.). A representative Western blot was shown below the histogram. b, lysates from HeLa and 293T cells with or without B23-KD were collected and subjected to WB. Levels of β-tubulin were compared by WB analysis. Successful knockdown of B23 was verified using antibody against B23. Equal sample loading was assessed by GAPDH. c, MTs in cell lysates from HeLa cells with and without B23-KD or from B23-KD cells reintroduced with siRNA-resistant (siRNA was designed to targeted for the 3' UTR of B23 mRNA, therefore the coding region fragment of B23 cloned into plasmid will not be affected) FLAG-B23 or FLAG-B23-ΔDRBD plasmids were fractionated into dynamic and stable fractions. The distributions of MTs were detected by WB using an anti-β-tubulin antibody. Densitometry was performed using ImageJ to ensure an accurate assessment. Knockdown efficiency was shown in the right panel. Each fractionation was verified for the presence or absence of GAPDH, a marker for dynamic MT fraction. Expression of reintroduced FLAG-B23 was confirmed by anti-FLAG antibody. All of the biochemical pelleting experiments were repeated three times, the histogram depicts the average ratio of polymerized MTs to total MTs. Roman numerals I, II, III, IV, and V denote the respective ratios of polymerized MTs to total MTs from various transfections as indicated (*, p < 0.05); NS, no significance; error bars are ± S.E. A representative Western blot was shown below the histogram. d, unpolymerized tubulins of B23-WT or B23-KD cells were prereleased using 0.5% Triton X-100 at room temperature, and cells were then fixed and subjected to immunostaining using an antibody against β-tubulin (red). The nuclei were visualized by Hoechst staining. The merged images were shown in the right panels. e, B23-WT and B23-KD HeLa cells expressing GFP-β-tubulin were subjected to time-lapse imaging for 2 h to analyze the MT (green) polymerizing state.
**FIGURE 4.** B23 regulates MT dynamics by directly inhibiting Eg5 ATPase activity. 

*a*, HeLa cells both with and without B23-KD were further transfected with or without siRNA against Eg5. *si-ctrl*, non-specific control siRNA. Lysates from each treated cells were subjected to dynamic/stable MT fractionation using the method described in Fig. 3a. The distributions of MTs were detected by WB using an β-tubulin antibody. Densitometry was performed using ImageJ. B23 and Eg5 knockdown efficiencies were examined by WB (right). Each fractionation was verified for the presence or absence of GAPDH, a marker for dynamic MT fraction (bottom panels). All of the biochemical pelleting experiments were repeated three times. The histogram depicts the average ratio of polymerized MTs to total MTs. Roman numerals I, II, III, and IV denote the respective ratios as indicated (*, p < 0.05). NS, no significance; error bars are ± S.E. A representative Western blot was shown below.

*b*, GST alone and GST fusion proteins including GST-B23, GST-stathmin, and GST-Eg5 (1–437) were expressed and purified from *E. coli*. Tubulins were purified from porcine brain. Mixtures with indicated combinations of GST, GST-B23, GST-stathmin and GST-Eg5 (1–437) were added into reaction buffer containing tubulin (1 mg/ml) and ATP (1 mM) for incubation at 37 °C for 1 h; pellets containing polymerized tubulin and polymerized binding proteins were collected by centrifugation at 15,000 × g for 30 min. Complexes were resolved on SDS-PAGE and visualized using Coomassie Blue staining; the in vitro tubulin polymerization assay was repeated three times; and the histogram depicts the relative density of polymerized tubulin (**, p < 0.01; *** p < 0.001; error bars are ± S.E.). Input proteins were indicated on the left side of the gel (lanes 1–5). c, cells used in a were further treated under the same conditions described in Fig. 3d. Fixed cells were immunostained with antibody against β-tubulin (red). The nuclei were visualized by Hoechst staining. *si-ctrl*, non-specific control siRNA.

d, mixture from indicated combinations of GST-B23 fusion proteins and GST-Eg5 fusion proteins were added into ATPase assay buffer containing ATP (1 mM), NADH (0.2 mM), PEP (3 mM), and PK/LDH (13/16 units/ml). ATPase activity was assayed at 37 °C using a PK/LDH linked system. A340 was monitored using a spectrometer (Beckman Coulter TM DU 640), and kcat (s−1) of each sample was analyzed (bottom panel). The steady-state ATPase assay was repeated three times (***, p < 0.001; error bars are ± S.E.). Protein compositions were resolved on SDS-PAGE using Coomassie Blue staining (bottom panel). Molecular mass markers are indicated on the left side of the gel.
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MT dynamics (Fig. 4b, lane 6 versus lane 8). When the GST-Eg5 motor domain was added together with GST-B23, the ability of Eg5 to depolymerize MTs was significantly inhibited (Fig. 4b, lane 11 versus lane 12). Thus, results from in vitro assay are consistent with our in vivo findings.

Kinesins were known to use the energy of ATP hydrolysis to move in discrete steps along microtubules (24), and then made the use of a coupled enzyme ATPase assay described previously (20) to probe possible ATPase regulatory functions of B23 on Eg5 in vitro. Results in Fig. 4d indicated that while GST alone and GST-B23 and its deletions showed no ATPase activity (Fig. 4d, lanes 1–4), GST-Eg5 motor was shown to vigorously hydrolyze ATP (Fig. 4d, lane 5). As expected, its ATPase rigor mutant (T112N point mutation) or GST-Eg5 C-terminal domain (amino acids 675–1057), failed to show obvious ATPase activity (Fig. 4d, lanes 6 and 7), indicating that only the wild-type Eg5 motor domain is able to hydrolyze ATP. Next, purified GST, GST-B23, GST-B23-ΔDRBD, and GST-B23-DRBD fusion proteins were added into the ATPase assay mixture. GST-B23 and GST-B23-DRBD showed effective reduction of ATPase activity of the Eg5 motor domain (Fig. 4d, lanes 9 and 11), whereas GST alone and GST-B23-ΔDRBD showed no obvious inhibitory effects on reduction of Eg5 ATPase activity (Fig. 4d, lanes 8 and 10). Taken together, the above results demonstrate that B23 inhibits Eg5 motor ATPase activity via its DRBD domain (raw data in supplemental Fig. S1). A proposed model illustrating how B23 regulates Eg5 in MT dynamics is shown in Fig. 5.

DISCUSSION

Here, we identified the kinesin Eg5 as a novel B23-interacting protein. To date, the functional role of B23 has been attributed primarily to the nucleus (25). The few functions known outside of the nucleus include the control of centrosome duplication (26) and the regulation of K-Ras nanocluster formation (27). In this study, we presented evidence demonstrating that the cyto-nuclear shuttling protein B23 is able to maintain the proper MT organization in the cytosolic compartment of a HeLa cell. This observation has also been confirmed in other cell lines as well (supplemental Fig. S3).

We demonstrated that the association of B23 with Eg5 was involved in modulating the MT-destabilizing activity of the latter. Cells lacking B23 clearly exhibited a disrupted MT network with a lower level of polymerized tubulin. This MT disruption was able to be reversed upon the reintroduction of B23 (Fig. 3c). Together, these results indicated that B23 was involved in maintaining proper MT organization. Additionally, it had been reported that cells lacking B23 showed aberrant formation of the mitotic bipolar spindle (26), which supports our finding that B23 has an important role in MT dynamics regulation.

We have demonstrated above that B23 acts as an upstream regulator of Eg5 in promoting MT polymerization. It is interesting to note that in B23-KD cells, no matter whether the cells were mock-treated or arrested (at early G2/M phase by nocodazole or late G2/M phase by taxol), microtubules were found to be less polymerized compared with B23-WT cells (supplemental Fig. S2); thus, the effect of B23 on MT dynamics appears to be cell cycle-independent, although this conclusion needs to be further verified experimentally.

Kinesins have been shown to be a major microtubule dynamics regulator. Most kinesins were thought to promote microtubule disassembly ever since the first catastrophe kinesin XKCM1 was discovered in frog eggs by Walczak et al. (28). However, Daire et al. (29) have recently identified a novel kinesin-1 that promotes microtubule assembly. Our study showed that B23 binds to the motor domain of the kinesin Eg5, which was known to contribute solely to its length-dependent control of kinetochore microtubule and works as a brake of MT array (2, 3). This suggests that B23 may antagonize Eg5-mediated MT destabilizing function via inhibiting its enzymatic activity. Although it is still unclear how B23 interferes Eg5 ATPase activity aside from its direct binding to Eg5 motor motif, our results showed that the DRBD domain of B23 is essential for Eg5 ATPase inhibition (Fig. 4), suggesting that some RNA, DNA, or functional noncoding RNA, which normally bind to the DRBD domain of B23, may be involved in maintaining MT morphology. The exact underlying mechanisms still await further investigation. Because Eg5 is an important regulator of kinetochore microtubule length determination, it is of interest to further examine whether B23 is also involved in controlling kinetochore microtubule length in mitosis.

As a part of the MT apparatus, the centrosome is often found to be defective in primary AML patients (30). Because B23 is involved in the process of centrosome duplication and mitosis (12), the B23 is expected to significantly affect genomic stability. The C-terminal region of B23 is necessary for its nucleolar localization (31), and aberrant cytosolic B23 (npy cytoplasmic), which contains an altered C terminus due to frameshift mutation, frequently occurred in AML patients (10). We have demonstrated that the C terminus, specifically the DRBD of B23, is solely responsible for the regulation of Eg5 ATPase activity (Fig. 4), which implicates that Eg5 ATPase activity in AML patients may be deregulated and functional aberrancy of Eg5 in AML patients may be of interest for future research. Furthermore, as Eg5 is an important antineoplastic drug target (32), and WT B23 could inhibit AML development (11, 33); whether the inhibitors of Eg5 such as mimics of DRBD of B23 could be used in treating AML patients is of clinical interest.
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