Therapeutic Discovery

Overexpression of Mitotic Centromere–Associated Kinesin Stimulates Microtubule Detachment and Confers Resistance to Paclitaxel

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Abstract

Numerous studies have implicated mutations in tubulin or the overexpression of specific tubulin genes in resistance to microtubule-targeted drugs. Much less is known about the role of accessory proteins that modulate microtubule behavior in the genesis of drug resistance. Here, we examine mitotic centromere–associated kinesin (MCAK), a member of the kinesin family of microtubule motor proteins that has the ability to stimulate microtubule depolymerization, and show that overexpressing the protein confers resistance to paclitaxel and epothilone A, but increases sensitivity to colcemid. Cells transfected with FLAG-tagged MCAK cDNA using a tet-off–regulated expression system had a disrupted microtubule cytoskeleton and were able to survive a toxic concentration of paclitaxel in the absence, but not in the presence of tetracycline, showing that drug resistance was caused by ectopic MCAK production. Moreover, a population that was heterogeneous with respect to FLAG-MCAK expression became enriched with cells that produced the ectopic protein when it was placed under paclitaxel selection. Similar to previously isolated mutants with altered tubulin, paclitaxel resistant cells resulting from MCAK overexpression were found to have decreased microtubule polymer and a seven-fold increase in the frequency of microtubule detachment from centrosomes. These data are consistent with a model for paclitaxel resistance that is based on stability of the attachment of microtubules to their nucleating centers, and they implicate MCAK in the mechanism of microtubule detachment.

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Introduction

Previous studies have shown that tubulin mutations confer resistance to microtubule-targeted drugs by altering microtubule assembly in a way that counteracts drug action (1). For example, mutations that disrupt or inhibit microtubule assembly confer resistance to paclitaxel (structure shown in Supplementary Fig. S1) and other drugs that stabilize microtubules. In contrast, mutations that promote microtubule assembly confer resistance to colcemid and other drugs that destabilize microtubules. On the basis of these observations, it seemed reasonable to hypothesize that any treatment that disrupts microtubules should be able to confer resistance to paclitaxel. To test this hypothesis, we recently turned our attention to mitotic centromere–associated kinesin (MCAK), a protein that is known to interact with microtubules and has been reported to catalyze their depolymerization.

MCAK is a member of the kinesin superfamily of microtubule motor proteins (2). It is sometimes referred to as a kinI kinesin because its motor domain is located internally as opposed to most of the kinesin family members that have their motor domains at the N-terminus. MCAK is also sometimes called kif2c and it has been classified as a member of the Kinesin-13 subgroup of motor proteins (3). One of the remarkable properties of MCAK is that it does not carry cargo along microtubules; instead, it associates with the microtubule surface and diffuses to both the plus and minus ends to stimulate depolymerization by inducing protofilament strands to peel away from the microtubule lattice (4, 5). Although MCAK has clearly been shown to cause microtubule depolymerization (5–7) it has not previously been implicated in drug resistance. In the studies described here, cells were transfected with a FLAG-tagged MCAK cDNA under the control of a promoter that can be repressed by tetracycline and, thus, the transfected cells were tested for changes in microtubule assembly and sensitivity to microtubule-targeted drugs. The results identify MCAK as a new target involved in the generation of drug resistance and elucidate the mechanism by which it acts.
Materials and Methods

Preparation of constructs

A human MCAK cDNA (American Type Culture Collection, Image ID 3909438; GenBank No. BC014924) was cloned into the tetracycline-regulated expression vector pTOPneo (8) to produce plasmid pTOP/MCAK. A sequence encoding the FLAG epitope tag was then inserted in-frame at the 5' end of the coding sequence using the QuickChange site-directed mutagenesis kit (Invitrogen). The mutagenic primers used were TTG CTG ACT CTC CGA and its reverse complement. The underlined sequence encodes the TCG TCG CTT CAG and its reverse complement. The resulting plasmid, pTOP/FLAG-MCAK, was sequenced to make sure that it was free of any errors.

Transfection and isolation of stable transfected cell lines

Chinese hamster ovary (CHO) cells were originally obtained in 1976 from Dr. Louis Siminovitch, University of Toronto. The cells were authenticated by karyotyping and have since been reauthenticated numerous times (including 2010) by comparing the sequences of cloned tubulin genes to those deposited by others in GenBank. CHO ITA 6.6a cells were engineered to express a tetracycline-regulated transactivator (8). This transactivator binds to pTOP/FLAG-MCAK to activate transcription in the absence, but not in the presence, of tetracycline. The cells were seeded into 35-mm tissue culture dishes and transfected with pTOP/Flag-MCAK using Lipofectamine (Invitrogen) as described by the manufacturer. The transfected cells were grown overnight in alpha modification of minimum essential medium (αMEM) supplemented with 5% FBS and 1 μg/mL tetracycline. Cells were trypanosinized the next day and an aliquot was seeded onto glass coverslips in αMEM for immunofluorescence to monitor transfection efficiency. The remaining cells were replated in 100-mm dishes containing αMEM containing 1 μg/mL tetracycline and 2 mg/mL G418. After 10 days surviving cells were pooled and stored as a total G418-resistant population. From the stable G418 population about 100 individual colonies (approximately 7 days) were isolated and identified by immunofluorescence using a mouse antibody to FLAG (Sigma-Aldrich), and screened for MCAK content using Western blot analysis with a rabbit antibody to MCAK (Cytoskeleton).

Immunofluorescence

Cells were grown on glass coverslips in αMEM for 2 days, rinsed briefly in PBS, preextracted with microtubule buffer (20 mmol/L Tris-HCl, pH 6.8, 2 mmol/L EGTA, 1 mmol/L MgCl₂, 0.5% NP40, 4 μg/mL paclitaxel) for 2 minutes at 4°C, and fixed in methanol at –20°C for 20 minutes. The fixed cells were then stained with rabbit α-tubulin antibody X2 (Dr. Jeanette Bulinski, Columbia University, New York, NY), mouse Flag antibody M2 (Sigma-Aldrich), Alexa 488-conjugated goat antirabbit IgG, and Alexa 594-conjugated goat antimouse IgG (Invitrogen). All antibodies were used at 1:100 dilutions. Nuclear DNA was also stained by including 1 μg/mL DAPI (4’,6-diamidino 2-phenylindole) in the secondary antibody solution. The microtubules were visualized using an Optiphot microscope equipped with epifluorescence and a ×60 objective (Nikon Inc.). Images were captured using a Magnafire digital camera (Optronics).

Measurement of drug resistance

An equal number of cells were plated into individual wells of 24-well dishes containing 0 to 400 nmol/L paclitaxel, 0 to 10 nmol/L epothilone A, or 0 to 100 nmol/L colcemid. After incubation for 7 days at 37°C, the medium was removed, and the cells were stained with 0.25% methylene blue in water as previously described (9). The plates were rinsed with water to remove excess stain and photographed with a D50 digital camera (Nikon). Cell growth was measured by eluting the dye in 200 μL of 1% SDS and reading the optical density at 630 nm/mL with an Emax plate reader (Molecular Dynamics Inc.).

Electrophoretic techniques

Cells were grown in 24-well dishes and lysed in 1% SDS. Proteins were precipitated with 5 volumes of acetone, resuspended in SDS sample buffer (0.0625 mol/L Tris-HCl, pH 6.8, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol), fractionated on 7.5% polyacrylamide SDS minigels, and transferred to nitrocellulose membranes. Membranes were blocked with 2% milk in PBS (PBS containing 0.05% Tween-20). After washing in PBS 3 times, the membranes were incubated in 1,200 dilutions of rabbit MCAK antibody (Cytoskeleton) or mouse FLAG antibody M2. A 1:40,000 dilution of mouse actin antibody C4 (Chemicon) was also added as an internal control for sample loading. Incubation was carried out for 1 hour at room temperature. The membranes were again washed 3 times in PBST and counterstained with 1,200 dilutions of Alexa 647-conjugated goat antimouse IgG and goat antirabbit IgG (Invitrogen). Reacting protein bands were detected by capturing fluorescence emission on a STORM 860 imager (Molecular Dynamics Inc.).

Measurement of polymerized tubulin

Cells were grown in 24-well dishes in triplicate to about 70% confluence and lysed in microtubule stabilizing buffer containing 0.14 mol/L NaCl as previously described (10). The lysates were centrifuged 10 minutes at 12,000 × g to separate soluble tubulin (supernatant fraction) from polymerized tubulin (pellet fraction). Quantification was carried out by adding a constant volume of a bacterial extract containing GST-α-tubulin to each fraction to act as an internal control, precipitating the proteins in each fraction with acetone, and loading equivalent volumes on an SDS gel. The proteins were transferred onto nitrocellulose and probed with α-tubulin antibody DM1A (Sigma-Aldrich) followed by Alexa
647-conjugated goat antimouse IgG. Fluorescence emission was quantified using a STORM 860 imager. The extent of microtubule assembly was calculated as 100% times the amount of tubulin in the pellet fraction divided by the total tubulin (supernatant plus pellet).

**Live cell imaging**

Cells transfected with EGFP-MAP4 (11) to label the microtubules were grown for 2 days on glass bottom dishes (MatTek Corp.). Images were taken every 5 seconds with a DeltaVision Core system (Applied Precision, Inc.) at 37°C using a ×100 objective. Microtubule detachment was best observed by focusing at the centrosome in cells that had it positioned below the nucleus and close to the glass surface.

**Results**

**Overexpression of FLAG-MCAK disrupts the microtubule network**

CHO tTA cells were transfected with pTOP/FLAG-MCAK using a regulated expression system in which transcription of the FLAG-MCAK could be repressed with tetracycline. After overnight incubation without tetracycline to induce expression, the transfected cells were stained with an antibody to the FLAG tag and examined by immunofluorescence. Cells with weak staining for FLAG-MCAK had no visible phenotypic change, but cells with high expression were large and multinucleated, indicating that the cells had exited mitosis without dividing. To more directly assess the effects of FLAG-MCAK on microtubule assembly and cell division, we isolated stable clones resistant to G418 and screened them for their levels of expression. Three such clones are shown in Fig. 1. The production of FLAG-MCAK was well regulated in all 3 clones by the presence of tetracycline (Fig. 1A), and the relative steady state levels of protein (endogenous plus transfected, as determined with an antibody to MCAK) ranged from 2 to 8 times the level found in nontransfected wild-type cells (Fig. 1B). The clones were named according to their level of MCAK protein relative to the wild-type cells. A fourth PtxR cell line shown in Fig. 1 will be discussed later.

Immunofluorescence analysis indicated that cell lines with low FLAG-MCAK production (e.g., clone 2) had microtubule networks with normal organization and density (e.g., arrow, Fig. 2, first panel). The cells were normal in size, they had normal appearing nuclei, and many metaphase spindles were present (inset, Fig. 2, first panel) indicating that there were no serious problems in mitosis. In contrast, clones that produced 6 times the normal level of MCAK appeared to have less dense microtubule networks, the cells were larger, and they were multinucleated indicating that they had experienced problems with chromosome segregation and cell division (arrow, Fig. 2, middle panel). Consistent with this conclusion, normal

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**Figure 1.** Quantification of MCAK in cell lines stably transfected with FLAG-MCAK. A, CHO cells were transfected with FLAG-MCAK cDNA and stable cell lines were selected in G418. Western blots of 3 clones grown in the presence (+) and absence (−) of tetracycline (Tet) for 24 hours are shown. An antibody specific for the FLAG tag was used. An antibody to actin was also included as a loading control. B, similar cell lysates were compared on Western blots using an antibody that recognizes endogenous as well as transfected MCAK. The gel included nontransfected wild-type (WT) CHO cells and FLAG-MCAK transfected cells selected in paclitaxel (PtxR) as controls.

**Figure 2.** Immunofluorescence of cell lines expressing FLAG-MCAK. Stable clones expressing FLAG-MCAK were mixed with nontransfected wild-type cells for an internal control, grown for 2 days, and stained with antibodies to tubulin and FLAG tag (not shown). DNA was stained with DAPI to label nuclei. Arrows point to the cells that express FLAG-MCAK. Insets show typical mitotic cells. Scale bar, 10 μm.
metaphase spindles were seldom seen. Instead, there was an accumulation of prometaphase-like spindles with multiple spindle poles (inset, Fig. 2, middle panel). At the even higher levels of MCAK production in clone 8, the microtubules became highly disrupted, the cells were again large and multinucleated, and multipolar spindles were common (Fig. 2, third panel). It thus appears that CHO cells could tolerate low levels of MCAK overexpression without serious consequences, but high levels of overexpression had dramatic effects on microtubule assembly and cell division.

**Paclitaxel selects for cells that overexpress FLAG-MCAK**

The microtubule disruption observed with MCAK overexpression is similar to what we have observed with the overexpression of mutant forms of β1-tubulin (8), β3-tubulin (12), and β5-tubulin (13) all of which lead to paclitaxel resistance. To determine whether overproduction of MCAK is also capable of conferring resistance to this drug, we transfected CHO cells with FLAG-MCAK and selected a total, nonclonal, G418 resistant population with the expectation that it would contain a heterogeneous mixture of cells with low to high levels of expression (14). This was done to ensure that at least some cells would potentially express an optimal level of MCAK: not so low that it would be incapable of producing resistance, yet not so high that it would kill the cells independently of the drug. The G418R cells were selected and maintained in the presence of tetracycline to prevent any potential toxicity from FLAG-MCAK overexpression. They were then seeded into dishes in the presence and absence of a toxic concentration of paclitaxel under conditions that allowed FLAG-MCAK expression (i.e., no tetracycline). As shown in Fig. 3A, 300 nmol/L paclitaxel was lethal to wild-type cells, but allowed the growth of cells transfected with β1-tubulin containing an L215F mutation that we previously showed is able to confer paclitaxel resistance (8). Cells transfected with FLAG-MCAK were also able to form colonies in paclitaxel under these conditions, albeit less robustly than the mutant β-tubulin transfected cells. Importantly, the ability of both transfected cell populations to form colonies in paclitaxel was suppressed when tetracycline was present to repress transcription of the transfected cDNA.

To further validate the ability of FLAG-MCAK overexpression to confer paclitaxel resistance, we reasoned that paclitaxel should allow only the cells that produce the protein to survive selection and therefore, the abundance of FLAG-MCAK in paclitaxel selected cells should be higher than in a starting unselected G418R cell population. The results from such an experiment are shown in Fig. 3B. As predicted, cells selected in 300 nmol/L paclitaxel contained about 6 times as much FLAG-MCAK as the starting G418R cells, arguing that FLAG-MCAK overproduction is able to confer resistance to the drug. To get a semiquantitative estimate of how much overproduction was needed to confer resistance, the PtxR cells were run on a gel alongside the stable clones in

**Figure 3.** MCAK overexpression confers paclitaxel resistance. A, nontransfected wild-type (WT) CHO cells, CHO cells transfected with HA/β1-tubulin containing an L215F mutation, and a G418 selected population of CHO cells transfected with FLAG-MCAK were seeded into dishes in the presence (+) or absence (−) of tetracycline (Tet) and/or paclitaxel (Ptx). The cells were incubated for 7 days until colonies appeared and the dishes were stained with methylene blue and photographed. Cells in the first column were seeded at low density to estimate the relative number of viable cells for each cell line. Cells in the following 2 columns were under selective pressure from 300 nmol/L paclitaxel and were therefore seeded at a 50-fold higher density. B, CHO cells transfected with FLAG-MCAK were selected in G418 to obtain a stable cell population. These cells were reselected in 300 nmol/L paclitaxel (Ptx) to obtain a drug-resistant population. Both cell populations were grown with (+) and without (−) tetracycline (Tet) for 24 hours and then analyzed by Western blots with an antibody to the FLAG tag and an antibody to actin as a gel loading control.
and PtxR cells, however, had only 26% to 29% of their previously reported values for CHO cells (8, 10). Clone 8 polymerized (pellet) fraction, a value that is similar to approximately 40% of their total tubulin in the polymerized from soluble tubulin by centrifugation and then quantifying the amount of tubulin in the pellet and supernatant fractions (e.g., see Fig. 4A). The results from this assay (Fig. 4B) showed that wild-type cells had approximately 40% of their total tubulin in the polymerized (pellet) fraction, a value that is similar to previously reported values for CHO cells (8, 10). Clone 8 and PtxR cells, however, had only 26% to 29% of their tubulin polymerized, values that are similar to previously reported paclitaxel resistant cell lines (8). The addition of 300 nmol/L paclitaxel raised the polymer levels in clone 8 and PtxR cells to near normal levels, but increased the polymer levels in wild-type cells to 64%, a value that correlates with drug toxicity (16). We conclude that like other treatments that are able to confer paclitaxel resistance, high overexpression of FLAG-MCAK reduces cellular levels of microtubule polymer.

**MCAK overproduction confers resistance to microtubule stabilizing drugs but increases sensitivity to microtubule destabilizing drugs**

Previous studies have shown that paclitaxel resistant cells resulting from the expression of mutant tubulin genes or overexpression of specific tubulin isotypes are cross-resistant to agents that promote microtubule assembly but are more sensitive to drugs that inhibit assembly (1). To determine whether this same pattern holds for MCAK overexpression, clones 2, 6, and 8 were incubated with increasing drug concentrations for 7 days to allow colonies to form. The results were quantified and plotted in Fig. 5. We observed that clone 2 which has low expression of FLAG-MCAK exhibited normal sensitivity to paclitaxel (Fig. 5A) and to colcemid (Fig. 5C). Cells with high levels of FLAG-MCAK expression (clones 6 and 8), on the other hand, were 2- to 3-fold resistant to paclitaxel and epothilone A, another microtubule stabilizing drug; but they were 2- to 4-fold more sensitive to colcemid, a drug that inhibits microtubule assembly. These results are very similar, both qualitatively and quantitatively, to what we have observed with mutant tubulin overexpression (17–19), suggesting that resistance from both treatments is mediated by a common mechanism.

**MCAK overproduction stimulates microtubule detachment from centrosomes**

Recent studies with paclitaxel dependent cell lines indicated that tubulin mutations reduce microtubule assembly by increasing the frequency at which microtubules detach from centrosomes, and that paclitaxel is able to inhibit this process (20, 21). One of the hallmarks of the mutant cells is the presence of unattached microtubule fragments that arise from detachment and can easily be monitored by immunofluorescence microscopy. A close
examination of cells that overexpress FLAG-MCAK revealed a similar pattern of detached microtubule fragments (Supplementary Fig. S2). To determine whether these fragments were also generated by an elevated frequency of microtubule detachment from centrosomes, clone 8 was transfected with EGFP-MAP4 and microtubules were observed in living cells by time-lapse fluorescence microscopy. An example of microtubule detachment in this cell line is shown in Fig. 6A.

Quantification of detachment events in wild-type, clone 8, and clone 8 treated with 300 nmol/L paclitaxel is shown in Fig. 6B. The FLAG-MCAK overexpressing cells were found to have an approximately 7-fold higher detachment frequency than nontransfected wild-type cells. The increased frequency of detachment was reversed by paclitaxel which also reversed the generation of microtubule fragments in the cells.

Discussion

Previous studies have shown that alterations in α- or β-tubulin that lower the extent of microtubule assembly confer resistance to paclitaxel (8, 22). Paclitaxel resistant cells were shown to be cross-resistant to other drugs that...
promote microtubule assembly but more sensitive to drugs that act to inhibit microtubule formation (18, 19, 22). Conversely, cells selected for resistance to colcemid or vinblastine were cross-resistant to other microtubule inhibitory drugs, but more sensitive to microtubule stabilizing drugs such as paclitaxel (17). Like the paclitaxel resistant cell lines, colcemid and vinblastine resistant cells had mutations in α- or β-tubulin, but in this case the mutations increased, rather than decreased, the extent of microtubule assembly. These observations led to a model that predicted drug resistance based on microtubule polymer levels (1, 23). It was proposed that cells could only divide normally when polymer formation fell within a defined range. Tubulin mutations that decreased the amount of polymer toward the lower limit of this range conferred paclitaxel resistance whereas mutations that increased polymer toward the upper limit conferred resistance to colcemid and vinblastine. Subsequently it was shown that overexpression of the β or β5 tubulin isotypes could also confer resistance to paclitaxel (12, 13). Like the tubulin mutations found in paclitaxel selected cell lines, overexpression of β3 or β5 lowered microtubule polymer levels, made the cells paclitaxel resistant, and conferred increased sensitivity to colcemid and vinblastine. Thus, naturally occurring tubulin isotypes appear to act like mutant β1-tubulin in their ability to confer drug resistance.

The model predicted that other changes capable of reducing microtubule polymer levels should be able to confer resistance to paclitaxel. Unlike tubulin, the kinesin related protein MCAK is not required for microtubule assembly and does not coassemble into the microtubule lattice. Rather, it has been shown to associate with preformed microtubules and diffuse to either end of the tube (4). Microtubules are metastable structures that frequently transition between phases of growth and disassembly (24). During disassembly, the ends of the protofilaments that make up the wall of the tube have been seen to curve outward away from the microtubule (25) in a process that is believed to depend on whether the tubulin subunits at the microtubule ends are in a straight GTP conformation or curved GDP-bound state (26). In vitro, MCAK has been shown to catalyze disassembly at either end of the microtubule by inducing or stabilizing the curved tubulin conformation (5, 6). Studies in cultured cells confirm that overexpression of MCAK can lower microtubule polymer levels (5, 7).

Our studies are consistent with these observations and quantify how much MCAK overexpression is needed to reduce microtubule assembly and visibly disrupt the microtubule cytoskeleton. We showed that MCAK levels that cause microtubule disruption and reduce the amount of polymer confer cellular resistance to paclitaxel and epothilone A, but make the cells more sensitive to colcemid. The results thus fit the model linking decreases in microtubule polymer to paclitaxel resistance. To further link the microtubule disrupting activity of MCAK with its ability to confer paclitaxel resistance, we tested the effects of an S192A mutation. Phosphorylation of S196 in the Xenopus ortholog of MCAK by aurora B kinase has been reported to inhibit the microtubule depolymerizing activity of MCAK (27). We therefore reasoned that an S192A mutation of our human MCAK designed to prevent phosphorylation should maintain the transected MCAK in an active form and thereby increase its ability to confer paclitaxel resistance. As predicted, less overproduction of S192A MCAK was needed to allow G418R cells to survive selection in 300 nmol/L paclitaxel compared with the wild-type protein (compare Fig. 3B and Supplementary Fig. S3). These results suggest that mutation and overexpression of MCAK both can contribute to paclitaxel resistance.

Although the model linking drug resistance to microtubule polymer levels provides a reliable framework for predicting the sensitivity of cells to microtubule-targeted drugs, it does not explain how raising or lowering the polymer level beyond certain limits can affect cell division. Recent work from our laboratory argues that it is not the polymer level per se that is important. Rather, altered polymer levels are simply endpoints that reflect changes in the stability of microtubule attachment to spindle poles that are detrimental to proper spindle function (21). Although the mitotic spindle apparatus is frequently depicted as having continuous microtubules emanating from the spindle poles, electron micrographs have detected the presence of numerous microtubule fragments within mitotic spindles (28) and more recent studies have shown an abundance of fragments in meiotic spindles (29, 30). These observations have led to a model of the spindle apparatus as a tailed array of microtubule fragments held together by motor proteins (31). Our work has shown that most microtubule fragments in cultured mammalian cells originate from microtubules that detach from centrosomes and spindle poles, shorten from both the plus and minus ends, and are translocated through the cytoplasm (20, 21). Although the frequency of microtubule detachment is normally low during interphase, it increases significantly when cells enter mitosis (21). We thus suggest that microtubule detachment and fragment formation is a regulated process that is important for normal spindle assembly and function. This process can be disrupted with microtubule inhibitory drugs that increase detachment as well as with microtubule stabilizing drugs that inhibit detachment (20, 21). Similarly, tubulin mutations (20, 21) or isotype composition (32) can increase or decrease detachment and thereby counteract the effects of the microtubule inhibitors.

Our observation that MCAK overexpression causes a 7-fold increase in microtubule detachment from centrosomes represents a novel aspect of MCAK action that has not previously been described. The location of MCAK at the spindle poles (33, 34), its ability to stimulate microtubule detachment, and its ability to confer
fragments for spindle formation. We propose that in a normal cell, MCAK acts to maintain a proper ratio of attached and unattached microtubules for spindle formation (Fig. 7). Treating cells with paclitaxel decreases microtubule detachment and fragment formation, whereas treating with colcemid or vinblastine increases detachment and fragment formation. Both conditions disrupt spindle formation and the cells ultimately die. Overproducing MCAK antagonizes paclitaxel by increasing the frequency of microtubule detachment, thereby allowing normal spindle assembly and cell division. However, this activity of MCAK works in concert with vinblastine to enhance the drug effect on microtubule detachment so that the cells become more drug sensitive.

In summary, we identified increased activity of MCAK as a new mechanism of resistance to microtubule stabilizing drugs as well as enhanced sensitivity to microtubule inhibitory drugs. We further found that MCAK acts at centrosomes to increase the frequency at which microtubules detach and form fragments that may be critical for normal spindle function. Although the mechanism of microtubule detachment is unknown, our studies implicate MCAK in the process.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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