βIII-Tubulin Induces Paclitaxel Resistance in Association with Reduced Effects on Microtubule Dynamic Instability*

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The development of resistance to paclitaxel in tumors is one of the most significant obstacles to successful therapy. Overexpression of the βIII-tubulin isotype has been associated with paclitaxel resistance in a number of cancer cell lines and in tumors, but the mechanism of resistance has remained unclear. Paclitaxel inhibits cancer cell proliferation by binding to the β-subunit of tubulin in microtubules and suppressing microtubule dynamic instability, leading to mitotic arrest and cell death. We hypothesized that βIII-tubulin overexpression induces resistance to paclitaxel either by constitutively enhancing microtubule dynamic instability in resistant cells or by rendering the microtubules less sensitive to the suppression of dynamics by paclitaxel. Using Chinese hamster ovary cells that inducibly overexpress either βI- or βIII-tubulin, we analyzed microtubule dynamic instability during interphase by microinjection of rhodamine-labeled tubulin and time-lapse fluorescence microscopy. In the absence of paclitaxel, there were no differences in any aspect of dynamic instability between the two β-tubulin-overexpressing cell types. However, in the presence of 150 nM paclitaxel, dynamic instability was suppressed to a significantly lesser extent (suppressed only 12%) in cells overexpressing βIII-tubulin than in cells overexpressing similar levels of βII-tubulin (suppressed 47%). The results suggest that overexpression of βIII-tubulin induces paclitaxel resistance by reducing the ability of paclitaxel to suppress microtubule dynamics. The results also suggest that endogenous regulators of microtubule dynamics may differentially interact with individual tubulin isotypes, supporting the idea that differential expression of tubulin isotypes has functional consequences in cells.

Microtubules are dynamic polymers composed of αβ-tubulin heterodimers that, both in vitro and in living cells, can continuously grow and shorten through tubulin dimer addition and loss at the microtubule ends. Dynamic microtubules are required for many processes in cells including cell migration, cell signaling, and mitosis (1). Mitosis is particularly sensitive to changes in microtubule dynamics, and mitotic progression depends upon the maintenance of microtubule dynamics and microtubule polymer levels within a narrow range (2–6). Paclitaxel is an extremely effective microtubule-targeted antican-

er drug used to treat a wide range of tumor types (7). The binding of paclitaxel to tubulin in microtubules arrests cells in mitosis, leading to cell death (8). Acquired resistance to paclitaxel is one of the most significant reasons for its failure in chemotherapy (3). Determining the molecular mechanisms of paclitaxel resistance is of great clinical value both in the design of chemotherapeutic treatment strategies and in the development of drugs to avoid or overcome resistance.

The antimitotic and antiproliferative effects of paclitaxel are attributed to its ability to suppress microtubule dynamics and to induce microtubule polymerization and bundling (9–11), driving dynamics and polymer levels outside of an acceptable range. Because the lowest concentrations of paclitaxel that effectively inhibit cell proliferation and block mitosis suppress microtubule dynamics (6, 12, 13) without significantly increasing microtubule polymer levels, suppression of microtubule dynamics appears to be its most potent mechanism of mitotic arrest (14).

Paclitaxel binds to the β-subunit of tubulin, of which at least seven isoforms exist at the protein level in humans: βI, βII, βIII, βIVa, βIVb, βV, and βVI. The β-isotypes differ primarily within the C-terminal 15–20 amino acids, a region of the protein that lies on the exterior of the microtubule and is the putative binding site for several microtubule-associated proteins (MAPs)1 (15–17). Expression of some tubulin isoforms is restricted to specific tissues, whereas other isoforms are constitutively expressed, resulting in a unique pattern of expression for each tissue. In non-neuronal cells, βI is often the predominant tubulin isotype, whereas βIII-tubulin is generally expressed at very low levels. Tumor cells often express a different complement of β-tubulin isoforms than their normal counterparts (18). The functional significance of variations in tubulin isotype expression in both normal and tumor cells is not known.

Overexpression of βIII-tubulin has been associated with paclitaxel resistance in cell lines and in tumors (19). Kavallaris et al. (20) showed that paclitaxel-resistant A549 cells overexpress βIII-tubulin compared with their sensitive counterparts and that partial sensitivity to paclitaxel was regained by down-regulation of βIII-tubulin in these cells. Hari et al. (21) recently showed that overexpression of βIII-tubulin conferred 1.5–2-fold resistance to paclitaxel in CHO cells; however, the mechanism of resistance remains unclear.

We hypothesized that βIII-tubulin induces paclitaxel resistance by generating inherently more dynamic microtubules or by rendering the microtubules less responsive to the suppressive effects of paclitaxel on microtubule dynamics, thus allowing cells to maintain sufficiently rapid microtubule dynamics in the presence of paclitaxel to complete mitosis. To test this hypothesis, we

1 The abbreviations used are: MAP, microtubule-associated protein, CHO, Chinese hamster ovary cells; HA, hemagglutinin.

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analyzed microtubule dynamic instability in CHO cells induced to overexpress βIII-tubulin or β-tubulin, in the absence and presence of paclitaxel. We found that increased levels of βIII-tubulin did not affect microtubule dynamic instability in the absence of paclitaxel. However, in the presence of paclitaxel, microtubules in βIII-tubulin-overexpressing cells were significantly more dynamic than in either β-tubulin-overexpressing cells or uninduced controls. Our results support the hypothesis that increased levels of βIII-tubulin directly induce paclitaxel resistance by rendering microtubules less sensitive to the effects of paclitaxel. In addition, our results suggest that tubulin isotype composition can regulate dynamic instability through the differential effects of endogenous regulators on microtubules of different isotype compositions.

MATERIALS AND METHODS

All materials were purchased from Sigma unless otherwise noted.

Cell Culture, Microinjection, and Analysis of Microtubule Dynamics—CHO cells were stably transfected with C-terminal HA-tagged β- or βIII-tubulin under the control of a tetracycline-regulatable promoter (21) such that in the presence of tetracycline, expression of the transfected β-tubulin was repressed (“off”) and expression of the protein was induced when tetracycline was removed from the medium. In normal CHO cells, β-tubulin is the predominant β-isotype comprising ~70% of the total β-tubulin pool. CHO cells additionally express lower levels of βVb-tubulin (25%) and βV-tubulin (5%) (22, 23). βIII-tubulin is normally not expressed. After induction of HA-β- or HA-βIII-tubulin expression, the percentages of each β-tubulin isotype were 80, 14, 5, and 1% for the HA-β-tubulin, endogenous β, βVb, and βV, respectively (21).

Cells were maintained in a modification of minimal essential medium supplemented with 5% fetal bovine serum (HyClone, Logan, UT), non-essential amino acids, 0.1% penicillin/streptomycin, 2 mg/ml G418 (BioWhittaker, Walkersville, MD), and 2 µg/ml tetracycline. Cells were seeded at a density of 8 x 10^4 cells/ml in a 6-well plate onto poly-t-lysine-treated, gridded glass CELLCulture coverslips (Eppendorf, Westbury, NY) with/without tetracycline. The cells were washed in phosphate-buffered saline after 8 h to remove any residual tetracycline and incubated for an additional 16 h, followed by a 24-h incubation in medium containing a reduced concentration of fetal bovine serum (2%) to promote cell flattening.

Rhodamine tubulin was prepared by carboxyryhodamine labeling (Molecular Probes, Eugene, OR) of microtubules assembled from phosphocellulose-purified bovine brain tubulin (24). To visualize microtubule dynamic instability, cells were microinjected with untagged tubulin (2.5 mg/ml) as described previously (2). Paclitaxel was dissolved in dimethyl sulfoxide and stored at −80 °C until use. For experiments involving paclitaxel, cells were incubated with 150 nm paclitaxel for an additional 5 h prior to imaging and transferred to recording medium lacking paclitaxel during imaging (15 min-2 h).

Time-lapse Microscopy, Image Acquisition, and Analysis of Dynamic Instability—Microscopy and analysis of dynamic instability have been described elsewhere (2, 12). Briefly, cells were seeded in a Rose chamber in recording medium containing 30 µl/ml Oxyrase (Oxyrase Inc., Mansfield, OH). 31–46 images were captured at 4-s intervals. The growth and shortening dynamics of individual microtubules were tracked using the track points function of Metamorph, converted to life history plots, and analyzed using real time measurement software (25). Dynamicity and time- and length-based catastrophe and rescue frequencies were calculated as described previously (12).

Mitotic Index—Cells were plated at a concentration of 8 x 10^4 cells/ml into 6-well plates. After 48 h, cells were incubated in the absence or presence of paclitaxel at a range of concentrations (0.03–10 µM) for 6 h. Fixation, staining, and determination of mitotic indices were performed on all cells, both floating and attached, as described in Ref. 12. Results are the mean and S.D. of five experiments, in each of which 500 cells were counted per concentration.

Immunocytochemistry—Cells were pre-extracted for 30 s in TBS (20 mM Tris, 150 mM sodium chloride) containing 0.5% Triton X and fixed in 10% formalin (37 °C) or methanol (0 °C). Non-specific antibody staining was blocked with TBS containing 2% bovine serum albumin and 0.1% Triton X. Cells were incubated with mouse anti-HA antibody (1:100; AbCAM, Cambridge, UK) or mouse anti-HA antibody and YL1/2 rat anti-tubulin antibody, followed by fluorescein isothiocyanate goat anti-mouse secondary antibody (Sigma) and Rhodamine Red-X goat anti-rat secondary antibody (Jackson Immunolaboratories, Westgrove, PA). Nuclei were stained with 4', 6 diamidino-2-phenylindole.

RESULTS

We used paclitaxel-resistant CHO cells overexpressing HA-tagged βIII-tubulin and paclitaxel-sensitive HA-β-tubulin-overexpressing cells to determine whether βIII-tubulin expression differentially affects microtubule dynamics in the presence or absence of paclitaxel. In normal CHO cells, β-tubulin is the predominant β-tubulin isotype. βIII-tubulin is normally not expressed (21). Because we analyzed microtubule dynamics in individual cells, it was important to confirm that all the cells expressed similar levels of HA-β- or HA-βIII-tubulin after inducing expression under the conditions used to analyze dynamic instability. Cells were incubated in the presence or absence of tetracycline for 48 h and processed for immunofluorescence microscopy (see “Materials and Methods”). In the absence of tetracycline (Fig. 1, A, C, E, G), all of the cells expressed HA-β- (A, E) or HA-βIII- (C, G) tubulin at approximately equivalent levels, whereas in the presence of tetracycline, expression of the transfected β-tubulin was repressed and levels were negligible (B, D, F, H). In Fig. 1, E–H, cells were incubated in the presence or absence of tetracycline (to repress or induce HA-β-tubulin expression, respectively), and cells were labeled with an antibody to HA (green) and tubulin (red) and the images were overlaid. In induced cells, HA-βIII- (E) or HA-βIII-tubulin (G) coassembled with endogenous tubulin (red) producing yellow microtubules. Uninduced cells (F, H) expressed extremely low levels of HA-β-tubulin, indicated by red microtubules.

To determine the effects of overexpressed βIII-tubulin on microtubule dynamic instability, cells overexpressing βIII- or β-tubulin were microinjected with rhodamine-labeled tubulin. The dynamic instability of individual microtubules in the peripheral regions of the two cell types was observed and recorded by time-lapse fluorescence microscopy and quantified (see “Materials and Methods”). As expected from previous studies of microtubule dynamic instability, in β-overexpressing cells many of the individual microtubules underwent periods of slow growth and more rapid shortening within the 3-min observation period. For example, as shown in Table I, in β-overexpressing cells, microtubules grew at a mean rate of 14.4 ± 6.8 µm/min and shortened approximately twice as fast, at a mean rate of 33.2 ± 12.8 µm/min. The parameters of microtubule dynamics in βIII-overexpressing cells were virtually identical to those of β-overexpressing cells (Table I, compare data columns 1 and 2). Thus, by itself, overexpression of βIII-tubulin in CHO cells does not significantly affect the dynamic instability of interphase microtubules.

To determine the paclitaxel concentration to use in microtubule dynamics experiments, we first determined the mitotic index after incubating cells for 5 h with a range of concentrations of paclitaxel (see “Materials and Methods”). As shown in Fig. 2, in the absence of paclitaxel 3.2% of the cells were in mitosis. The mitotic index increased to 4.8% at 0.1 µM paclitaxel, and arrest was maximal by 1 µM paclitaxel. Therefore, a concentration of 0.03 µM paclitaxel was selected as the lowest concentration consistent with minimal mitotic index (6%). We analyzed microtubule dynamic instability in cells incubated with 150 nm paclitaxel, at which the mitotic index was ~6%. At this concentration, the microtubules remained somewhat dynamic, and all of the effects of paclitaxel were clearly detectable.

Paclitaxel had relatively minor effects on dynamic instability in βIII-overexpressing cells, whereas the drug significantly suppressed many parameters of microtubule dynamics in β-overexpressing cells (Table I, compare data columns 1 and 3). The major effects of paclitaxel in β-overexpressing cells are summarized in data column 1 of Table II; the growth rate was suppressed by 31%, the growth length by 45%, the shortening...
overexpressing cells under non-inducing conditions in the presence of paclitaxel. These cells expressed very low levels of βIII-tubulin (Fig. 1, D, H). Paclitaxel (150 nM) suppressed microtubule dynamics to levels in βIII-uninduced cells nearly identical to those in βI cells. (Compare columns 2 and 5 in Table I and compare percentages in data columns 1 and 3 in Table II.)

**Discussion**

We found that βIII-tubulin overexpression induces paclitaxel resistance by decreasing the efficacy of paclitaxel binding to βIII-tubulin, resulting in a weaker suppressive effect on microtubule dynamics. The results are also the first direct demonstration that drugs can differentially interact with different tubulin isotypes in cells, suggesting that microtubule dynamic instability might be regulated by the differential interaction of endogenous regulators with the individual isotypes as well.

**Overexpression of βIII-Tubulin Does Not Cause an Inherent Increase in Dynamic Instability as Compared with Overexpression of βI-Tubulin**—One mechanism by which βIII-tubulin has been proposed to mediate resistance to paclitaxel is to constitutively increase microtubule dynamics, such that in the presence of paclitaxel microtubules would remain sufficiently dynamic to complete mitosis (2). In living interphase CHO cells in the absence of paclitaxel, overexpression of βIII-tubulin did not significantly alter any parameters of microtubule dynamic instability. Thus, in these cells a high level of βIII-tubulin overexpression does not cause an inherent increase in dynamic instability, indicating that this is not the mechanism of βIII-tubulin-induced resistance. Hari et al. (21) showed previously that in the βIII-tubulin-overexpressing CHO cells polymer mass was decreased by ~30%. Taken together, these results suggest that a reduction in polymer mass does not necessitate a change in cellular microtubule dynamics. This result also suggests that in our previous study of paclitaxel-resistant and -dependent A549 cells (2), the increased microtubule dynamics resulted not from the increased levels of βIII-tubulin but rather from other changes associated with resistance (a mutation in the putative stathmin and MAP4 binding site in conjunction with increased levels of unphosphorylated (active) stathmin and phosphorylated (inactive) MAP4 (26)).

The lack of a difference in microtubule dynamics parameters in the absence of paclitaxel observed in the present study in cells overexpressing either βI- or βIII-tubulin is perhaps surprising. In two previous in vitro studies, the microtubules assemled from αβIII-tubulin were significantly more dynamic than microtubules assembled from αβIV-tubulin. For example, comparison of their dynamicities indicated that αβIII-microtubules were 2.2-fold more dynamic than αβIV-microtubules in one study (27) and 1.7-fold more dynamic in a second study (28). The sequence of βIV resembles that of βI; thus one might expect that microtubules in cells overexpressing βIII-tubulin might be more dynamic than those in cells overexpressing βI-tubulin. In cells, tubulin undergoes post-translational modifications and interacts with a large number of microtubule regulatory proteins (ranging from proteins that induce catastrophe to those that stabilize microtubule dynamics) (11); thus the dynamics of individual isotypes may be significantly altered in cells. Paclitaxel Has a Weaker Effect on Dynamic Instability in βIII-Tubulin-overexpressing Cells—Microtubules in cells overexpressing βIII-tubulin were significantly less susceptible to the suppressive effects of paclitaxel than in control cells. In controls (βI-overexpressing cells or uninduced βIII-tubulin-transfected cells), paclitaxel significantly reduced the mean growth and shortening rates and lengths and dynamicity. In βIII-overexpressing cells, only the mean shortening rate and length were reduced, and these parameters were affected to a...
Values are means ± S.D. Tests of significance cannot be performed on percentages of time spent in each phase but were performed on all other parameters of dynamics.

| Parameter                  | βIII-Tubulin | βIII-Tubulin | βIII-Tubulin | βIII-Tubulin |
|----------------------------|--------------|--------------|--------------|--------------|
|                            | 150 nm paclitaxel | 150 nm paclitaxel | 150 nm paclitaxel |
| Growth rate (μm/min)       | 14.4 ± 6.8   | 14.2 ± 6.4   | 9.9 ± 3.9b,c | 13.4 ± 7.5   | 10.4 ± 5.0c   |
| Growth duration (min)      | 0.25 ± 0.16  | 0.25 ± 0.17  | 0.22 ± 0.17  | 0.24 ± 0.17  | 0.22 ± 0.16  |
| Shortening rate (μm/min)   | 3.6 ± 3.0    | 3.7 ± 3.3    | 2.0 ± 1.9b,c | 3.0 ± 2.3    | 2.0 ± 1.3c   |
| Shortening duration (min)  | 0.18 ± 0.11  | 0.18 ± 0.10  | 0.20 ± 0.19  | 0.18 ± 0.13  | 0.19 ± 0.19  |
| Shortening length (μm)     | 5.8 ± 3.4    | 5.6 ± 3.8    | 2.6 ± 1.7b,c | 3.8 ± 2.6b   | 2.4 ± 1.8c   |
| Pause duration (min)       | 0.35 ± 0.34  | 0.29 ± 0.23  | 0.35 ± 0.35  | 0.30 ± 0.29  | 0.31 ± 0.31  |
| Catastrophe/mina           | 0.74 ± 0.75  | 0.89 ± 0.60  | 0.68 ± 1.1   | 1.2 ± 1.0    | 1.5 ± 1.5    |
| Rescue/mina                | 5.9 ± 4.1    | 6.4 ± 3.3    | 5.6 ± 2.7    | 6.8 ± 4.7    | 5.7 ± 3.1    |
| Catastrophe/μmada          | 0.23 ± 0.31  | 0.24 ± 0.21  | 0.23 ± 0.33  | 0.28 ± 0.34  | 0.68 ± 0.56  |
| Rescue/μmd                | 0.21 ± 0.17  | 0.25 ± 0.16  | 0.42 ± 0.29  | 0.41 ± 0.44  | 0.48 ± 0.32  |
| % Growing                | 33.4         | 35.4         | 28.0         | 35.6         | 23.3         |
| % Shortening             | 11.4         | 14.8         | 12.2         | 16.0         | 19.9         |
| % Paused                 | 53.2         | 50.7         | 58.3         | 49.3         | 56.8         |
| Dynamicity (μm/min)       | 9.1 ± 4.8    | 10.1 ± 5.8   | 4.8 ± 2.9b,c | 8.0 ± 5.5    | 5.0 ± 2.6b,c |
| No. of microtubules and cells | 59/14     | 75/16        | 57/14        | 55/12        | 41/9         |

* CHO cells transfected with, but not expressing, HA-βIII-tubulin.  
* Means are significantly different from β cells without paclitaxel at ≥99% confidence level by Student's t-test.  
* Means are significantly different from βIII cells with paclitaxel at ≥99% confidence level by Student's t-test.  
* A catastrophe is a transition from growth or pause to shortening; a rescue is a transition from shortening to growth or pause.

**FIG. 2. Paclitaxel concentration dependence for mitotic arrest of uninduced CHO cells.** Results are the mean ± S.D. of five experiments. Cells were incubated with a range of concentrations of paclitaxel (0.3–10 μM) for 5 h, and the percentage of mitotic cells was determined by counting 4',6-diamidino-2-phenylindole-stained cells.

**significantly lesser extent than in controls (Tables I and II and Fig. 3).**

**Dynamics** is calculated as a value representing the overall dynamic instability of a population of microtubules. In previous studies, dynamics was reduced by 25–64% in conjunction with drug-induced inhibition of cell proliferation or mitotic arrest in A498, CaOV3, A549, and MCF7 cancer cell lines (6, 12, 13). Consistent with these results, in βIII-overexpressing CHO cells, dynamicity was reduced by 47%. However, in βIII-overexpressing paclitaxel-resistant cells, the dynamicity was reduced by only a statistically insignificant 12%. The lack of an effect of paclitaxel on dynamic instability in βIII-overexpressing cells is also consistent with the in vitro study of Derry et al. (28), who found that microtubules composed exclusively of αβIII-tubulin were 7-fold less sensitive to suppression by paclitaxel than microtubules composed of αβII- or unfractionated tubulin. Thus, both in vitro and in living cells, increased levels of βIII-tubulin reduce the ability of paclitaxel to suppress microtubule dynamics. The possibility that the C-terminal HA tag on the transfected β-tubulin artifically produced the observed results is highly unlikely. First, previous studies have shown that the presence of the HA tag does not affect microtubule assembly or endogenous MAP4 binding nor does it change the growth or drug resistance properties of the cells when compared with its transfected untagged β-tubulin counterpart (21, 29). Second, the HA tag was present on both overexpressed isotypes. Finally, transfection of βIII-tubulin with or without the HA tag induced the same degree of paclitaxel resistance, indicating that the βIII-tubulin alone is responsible for resistance (21). Suppression of microtubule dynamics correlates with inhibition of proliferation and sensitivity to paclitaxel (2, 6); thus, the lack of suppression of microtubule dynamics is the most likely explanation for the resistance phenotype.

**What Is the Mechanism of βIII-Tubulin-mediated Paclitaxel Resistance?**—Our results indicate that the weaker effect of paclitaxel on microtubules in βIII-tubulin-overexpressing cells is due to reduced drug binding or reduced drug efficacy (i.e. reduced ability of the drug to induce the conformational change required to suppress microtubule dynamics). Other microtubule-targeted drugs, including estramustine and colchicine, interact more weakly with βIII-tubulin than with several other isotypes in vitro (34, 35). In addition, colchicine binding studies and cysteine cross-linking studies suggest that βIII-tubulin has a more rigid conformation than the other β-isotypes examined (18, 36). Thus, microtubules composed largely of βIII-tubulin might resist the paclitaxel-induced conformational changes that suppress microtubule dynamic instability. Tubulin dimers are thought to undergo a conformational change upon addition to the microtubule end (15). The rigidity of βIII-tubulin might also cause it to resist this conformational change, which may account for the reduced microtubule polymer in βIII-overexpressing cells. It is also conceivable that paclitaxel binding is prevented by a MAP with higher affinity for βIII-tubulin than the other isotypes. However, this possibility is less likely be-
cause the in vitro studies indicating altered interactions between paclitaxel and the β-tubulin isotypes were performed in the absence of MAPs (28), suggesting a weaker or less efficacious interaction between paclitaxel and βIII-tubulin.

One important question is how the levels of βIII-tubulin overexpression in the paclitaxel-resistant CHO cells used here (80% of total tubulin) (21) relate to levels of isotype overexpression in the paclitaxel-resistant CHO cells used here. In tumor samples, these studies are further complicated by the presence of normal cells as well as many other cell types (39).

Development of more sophisticated methods to measure tubulin isotype expression will be necessary to understand what level of overexpression of βIII-tubulin significantly contributes to paclitaxel resistance. In addition, understanding how βIII-tubulin expression is regulated will be important in preventing or overcoming this type of drug resistance.

In summary, we have shown directly in living human tumor cells that increasing the expression of βIII-tubulin can induce paclitaxel resistance by rendering microtubules relatively insensitive to the effects of paclitaxel. Clinical determination and regulation of βIII-tubulin expression levels in tumors may lead to significantly improved prediction of tumor response to paclitaxel and ultimately to overcoming one source of clinical resistance to paclitaxel.

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REFERENCES

1. Valiron, O., Caudron, N., and Job, D. (2001) Cell Mol. Life Sci. 58, 2069–2084
2. Gonçalves, A., Braguer, D., Kamath, K., Martello, L., Briaud, C., Horwitz, S., Wilson, L., and Jordan, M. A. (2003) Proc. Natl. Acad. Sci. U. S. A. 98, 11737–11741
3. Cabral, F. (2001) Drug Resist. Update 4, 3–8
4. Barlow, S. B., Gonzalez-Garay, M. L., and Cabral, F. (2002) J. Cell Sci. 115, 3469–3478
5. Minotti, A. M., Barlow, S. B., and Cabral, F. (1991) J. Biol. Chem. 266, 3987–3994
6. Yvon, A.-M., Wadsworth, P., and Jordan, M. A. (1999) Mol. Biol. Cell 10, 947–949
7. Jordan, M. A. (2002) Curr. Med. Chem.-Anti-Cancer Agents 2, 1–17
8. Jordan, M. A., Wendell, K. L., Gardiner, S., Derry, W. B., Copp, H., and Wilson, L. (1996) Cancer Res. 56, 816–825
9. Schif, P. B., and Horwitz, S. B. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1561–1565
10. Derry, W. B., Wilson, L., and Jordan, M. A. (1995) Biochemistry 34, 2203–2211
11. Jordan, M. A., and Wilson, L. (2004) Nat. Rev. Cancer 4, 253–265
12. Kamath, K., and Jordan, M. A. (2003) Cancer Res. 63, 6026–6031
13. Honore, S., Kamath, K., Braguer, D., Horwitz, S. B., Wilson, L., Briaud, C., and Jordan, M. A. (2004) Cancer Res. 64, 4857–4861
14. Jordan, M. A., Tsoe, R. J., Thrower, D., and Wilson, L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9552–9556
15. Downing, K., and Nogales, E. (1998) Curr. Opin. Cell Biol. 10, 16–22
16. Cross, D., Dominguez, J., Macciocci, R. B., and Avila, J. (1991) Biochemistry 30, 4362–4366
17. Macciocci, R. B., Vera, J. C., Dominguez, J., and Avila, J. (1989) Arch. Biochem. Biophys. 275, 568–579
18. Luduena, R. P. (1998) Int. Rev. Cytology 178, 207–275
19. Kavallaris, M., Kuo, D. Y.-S., Burkhardt, C. A., Reel, D. L., Norris, M. D., Haber, M., and Horwitz, S. B. (1997) J. Clin. Investig. 100, 1–12
20. Kavallaris, M., Burkhardt, C. A., and Horwitz, S. B. (1999) Br. J. Cancer 80, 1020–1025
21. Hari, M., Yang, H., Zeng, C., Canizales, M., and Cabral, P. (2003) Cell Motil. Cytoskeleton 56, 45–56
22. Sawoda, T., and Cabral, P. (1989) J. Biol. Chem. 264, 3031–3020
23. Ahmad, S., Singh, B., and Gupta, R. S. (1991) Biochim. Biophys. Acta 1090, 252–254
24. Hyman, A., Drochael, D., Kellogg, D., Sailer, S., Sawin, K., Steffen, P., Wordeeman, L., and Mitchison, T. (1991) Methods Enzymol. 196, 478–485
25. Walker, R. A., O’Brien, E. T., Pryer, N. K., Soohoo, M. F., Voter, W. A., Erickson, H., and Salmon, E. D. (1988) J. Cell Biol. 107, 1437–1448
26. Martello, L. A., Verdier-Pinard, P., He, L., Torres, K., Orr, G. A., and Horwitz, S. B. (2003) Cancer Res. 63, 1207–1213
27. Panda, D., Miller, H. P., Barnerjea, A., Luduena, R. F., and Wilson, L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11358–11362
28. Derry, W. B., Wilson, L., Khan, I. A., Luduena, R. F., and Jordan, M. A. (1997) Biochemistry 36, 3554–3562
29. Gonzalez-Garay, M. L., and Cabral, F. (1995) Cell Motil. Cytoskeleton 31, 259–272

TABLE II

| Parameter               | β-Tubulin | βIII-Tubulin | βIII-Tubulin (uninduced) |
|-------------------------|-----------|--------------|--------------------------|
| Growth rate % change from β control | -31       | -7           | -28                      |
| Growth length            | -45       | -17          | -45                      |
| Shortening rate % change from β control | -54       | -32          | -56                      |
| Shortening length        | -55       | -34          | -59                      |
| Dynamics % change from β control | -47       | -12          | -45                      |

FIG. 3. Histograms indicating the differential effects of 150 nM paclitaxel on growth rate (a), shortening rate (b), growth length (c), shortening length (d), and dynamics (e) in cells induced to express βI- or βIII-tubulin. Black bars, no paclitaxel; gray bars, 150 nM paclitaxel. * Values significantly differ from β controls (no paclitaxel) at >99% confidence interval.
30. Luduena, R. F. (1993) *Mol. Biol. Cell* **4**, 445–457
31. Cassimeris, L. (1999) *Curr. Opin. Cell Biol.* **11**, 134–141
32. Kar, S., Fan, J., Smith, M. J., Goedert, M., and Amos, L. A. (2003) *EMBO J.* **22**, 70–77
33. Bunker, J. M., Wilson, L., Jordan, M. A., and Feinstein, S. C. (2004) *Mol. Biol. Cell* **15**, 2720–2729
34. Banerjee, A., and Luduena, R. F. (1994) *J. Biol. Chem.* **269**, 2041–2047
35. Laing, N., Dahllof, B., Hartley-Asp, B., Ranganathan, S., and Tew, K. D. (1997) *Biochemistry* **36**, 871–878
36. Sharma, J., and Luduena, R. (1994) *J. Protein Chem.* **13**, 165–176
37. Gygi, S. P., Rochon, Y., Franza, B. R., and Aebersold, R. (1999) *Mol. Cell. Biol.* **19**, 1720–1730
38. Chen, G., Gharib, T., Huang, C., Taylor, J., Misek, D., Kardia, S., Giordano, T., Iannettoni, M., Orringer, M., Hanash, S., and Beer, D. (2002) *Mol. Cell Proteomics* **1**, 304–313
39. Dozier, J., Hiser, L., Davis, J., Thomas, N., Tucci, M., Benghuzzi, H., Franklin, A., Correia, J., and Lobert, S. (2003) *Breast Cancer Res.* **5**, R157–69
40. Verdier-Pinard, P., Wang, F., Martello, L., Burd, B., Orr, G. A., and Horwitz, S. B. (2003) *Biochemistry* **42**, 5349–5357