Stably transfected Chinese hamster ovary cell lines expressing increasing levels of 34a, a class IV neuronal-specific β-tubulin, were compared for effects on microtubule organization, assembly, and sensitivity to antimitotic drugs. It was found that 34a reduced microtubule assembly in proportion to its abundance and thereby caused supersensitivity to microtubule disruptive drugs such as colcemid, vinblastine, and nocodazole. However, the response to paclitaxel was more complex. Low expression of 34a caused supersensitivity to paclitaxel, whereas higher expression resulted in the loss of supersensitivity. The results suggest that 34a may possess an enhanced ability to bind paclitaxel that increases sensitivity to the drug and acts substoichiometrically. At high levels of 34a expression, however, microtubule disruptive effects counteract the assembly promoting pressure exerted by paclitaxel binding, and drug supersensitivity is lost. 34a-Tubulin differs from the more ubiquitous 34b isotype at relatively few amino acid residues, yet 34b expression has little effect on microtubule assembly or drug response. To determine which amino acids mediate the effects of 34a expression, 34a and 34b were altered by site-directed mutagenesis and expressed in Chinese hamster ovary cells. The introduction of N332S or N335S mutations into 34b-tubulin was sufficient to confer microtubule disruption and increased colcemid sensitivity. On the other hand, mutation of Ala115 to serine in 34a-tubulin almost completely reversed heightened sensitivity to paclitaxel, but introduction of an S115A mutation into 34b had no effect, suggesting that a complex interaction of multiple amino acids are necessary to produce this phenotype.

Vertebrate tubulin is encoded by at least six α- and seven β-tubulin genes that produce a highly conserved family of proteins (1). A region of especially high variability among the β-tubulins in any given species, however, occurs in the C-terminal 15 amino acids, and these variable sequences are conserved across species. This observation has led to the classification of β-tubulin into discreet classes or isotypes (2). Some of these isotypes are expressed in virtually all tissues of an organism, whereas expression of others is tissue-restricted (3). The presence of multiple tubulin genes along with evidence of tissue-specific expression has long fueled speculation that specific tubulin isotypes subserve specific functions (4), but there has been little evidence to support this notion.

Early transfection studies in mammalian cells established that microtubules are composed from all of the available tubulin proteins but provided little evidence of unique roles for any of the examined isotypes (5, 6). In vitro studies, however, gave hints that some isotypes of β-tubulin may possess unique properties that could potentially alter the behavior of the microtubules into which they incorporate. For example, differences in rates of assembly, dynamic behavior, and drug binding for some of the isotypes have been reported (3). More recently, several laboratories have observed overexpression of specific β-tubulin isotypes in cell lines selected for resistance to paclitaxel and have suggested that this overexpression was responsible for the drug resistance phenotype (7).

To explore how tubulin composition might affect microtubule assembly and drug response in living cells, we undertook a direct approach in which we transfected Chinese hamster ovary (CHO) cells with cDNAs encoding various β-tubulin isotypes and placed the transcription of the cDNAs under the control of a tetracycline-regulated promoter to limit potential toxicity from their overexpression. In previous work, we reported that overexpression of 31-, 32-, and 34b-tubulin had no effect on microtubule assembly or response of the cells to antimitotic drugs (8). We later reported that overexpression of β3-tubulin, a brain-specific isotype, did cause microtubule disruption and weak paclitaxel resistance when expressed at high levels (9) and that overexpression of β5-tubulin, a minor ubiquitous isotype, produced major disruption of microtubules and paclitaxel resistance even at moderate levels of expression (10). In contradiction to previous studies linking its overexpression to paclitaxel resistance (11, 12), we now report that low expression of 34a-tubulin, an isotype normally restricted to brain (13), produces increased paclitaxel sensitivity. Higher expression, however, inhibits microtubule assembly, causes increased sensitivity to microtubule disruptive drugs, and counteracts the paclitaxel-supersensitive phenotype.

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids—CB1 (14) is a hamster class 1 β-tubulin (GenBank™ accession number U08342); Mβ4 (GenBank™ accession number NM_009451) and Mβ3 (Gen-
Bank™ accession number NM_146116) are mouse class IVa and IVb β-tubulins (15). All three cDNAs were cloned into tetracycline-regulated expression vector pTOPneo (16) and modified to encode a 9-amino acid hemagglutinin antigen (HA) epitope (YPYDVPDYA) at their C termini. These tubulins are hereafter called HAβ1, HAβ2a, and HAβ4b. HAβ2a mutations (N58K, A115S, F159Y, S332N, and S335N), and HAβ4b mutations (K58N, S115A, Y159F, N332S, and N335S) were created using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The plasmids were sequenced to ensure that the expected mutations were present and that no additional changes in the tubulin coding sequences were introduced during the process.

**Transfection and Selection of Stable Cell Lines**—CHO/tTA 6.6a cells, which express the tetracycline-regulated transactivator (16), were maintained in α modification of minimum essential medium (αMEM; Sigma-Aldrich), supplemented with 5% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 50 units/ml penicillin, and 50 µg/ml streptomycin (Invitrogen). Transfections were performed using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. After transfection, the cells were trypsinized and seeded into 60-mm dishes containing αMEM with 1 µg/ml tetracycline (Sigma-Aldrich) and 2 mg/ml G418 (Invitrogen) for the selection of stable cell lines. After 7 days, G418-resistant colonies were isolated and screened by immunofluorescence and Western blots for tetracycline-regulated expression of exogenous tubulin. Stable cell lines were maintained in αMEM containing G418 and tetracycline to repress the expression of the cDNAs until the time of analysis.

**Electrophoretic Techniques**—The cells were grown in 24-well dishes for 24 h with or without tetracycline and lysed in 1% SDS. The proteins were precipitated with 5 volume of acetone, resuspended in SDS loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol), resolved in 8% SDS-PAGE gels, and transferred to nitrocellulose (Pall Life Sciences, Ann Arbor, MI). The membranes were stained with a mixture of TUB 2.1 (1:2,000 dilution; Sigma-Aldrich) that recognizes both endogenous and transfected tubulin, and actin antibody C4 (1:30,000; Chemicon International Inc., Temecula, CA) as a loading control. Chemiluminescence was carried out by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG (Bethyl Laboratories, Montgomery, TX), followed by SuperSignal chemiluminescence detection (Pierce) and exposure to x-ray film.

**Immunofluorescence Microscopy**—The cells were grown on sterile glass coverslips for 48 h to ∼50% confluence. They were briefly washed with PBS and gently lysed in a microtubule-stabilizing buffer (MTB; 20 mM Tris-HCl, pH 6.8, 1 mM MgCl₂, 2 mM EGTA, 0.5% Triton X-100) containing 4 µg/ml paclitaxel (all from Sigma-Aldrich) for 90 s at 4 °C and fixed in methanol at −20 °C for at least 30 min. The fixed cells were rehydrated in PBS for 15 min and incubated in affinity purified rabbit HA antibody (Bethyl Laboratories; 1:100 dilution) for 1 h at 37 °C followed by Alexa 488-conjugated goat anti-rabbit IgG (Invitrogen, 1:50 dilution) and 1 µg/ml 4’,6’-diamino-2-phenylindole. After washing in PBS, the coverslips were inverted onto 5 µl of Gel/Mount (BioMeda Corp., Foster City, CA) and viewed with an Optiphot microscope equipped with a Plan Apochromat 60×, 1.4 numerical aperture oil objective (Nikon Inc., Melville, NY). The images were acquired using a MagnaFire digital camera (Optronics, Goleta, CA).

**Measurement of Drug Sensitivity**—A colony formation assay was used to determine the sensitivities of stable cell lines to various drugs. Approximately 200 cells were seeded into replicate wells of 24-well dishes containing increasing concentrations of drug in αMEM with or without tetracycline and incubated for 7 days until visible colonies appeared. The cells were then stained with 0.5% methylene blue in water for 2 h. The excess dye was washed away, and the dishes were dried. The dye from each well was extracted with 200 µl of 1% SDS, and optical density was measured at 630 nm using an Emax plate reader (Molecular Dynamics Inc., Sunnyvale, CA).

**Measurement of Steady State Microtubule Assembly**—Stable cell lines were plated in triplicate wells of a 24-well dish and incubated in αMEM with or without tetracycline for 24 h. The cells were gently washed with PBS and scraped into 200 µl of MTB containing 0.14 M NaCl and 4 µg/ml paclitaxel. This buffer prevents the depolymerization of microtubules but does not promote further assembly (17). The lysates were transferred to 1.5-ml microcentrifuge tubes, briefly vortexed, and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant fractions containing free tubulin dimers were transferred to a new tube, and the pellet fractions containing microtubules were resuspended in 50 µl of water. To dissolve any residual cellular material remaining in the dish, 100 µl of 1% SDS was added to each well and transferred to the corresponding pellet fraction. An equal volume of bacterial lysate containing glutathione S-transferase fused to α-tubulin (GST-α) was added to each fraction as an external loading control, and the proteins were precipitated with 5 volumes of acetone. The precipitated proteins were dissolved in 50 µl of SDS loading buffer. The proteins were resolved on 8% SDS-PAGE gels, transferred to nitrocellulose, and blotted with a mouse monoclonal antibody to either α-tubulin (DM1A; Sigma-Aldrich) or β-tubulin (TUB 2.1; Sigma-Aldrich), followed by an Alexa 647-conjugated goat anti-mouse IgG (Invitrogen). The fluorescence emission from Alexa 647 was captured using a STORM 860 imager (Molecular Dynamics Inc.) and quantified with NIH Image analysis software. The percentage of total tubulin polymerized into microtubules was calculated by dividing tubulin in the pellet fraction by the total tubulin in pellet and supernatant fractions after first normalizing each value to the amount of GST-α in the corresponding fraction.

**Statistics**—Individual experiments were repeated three to five times, and the significance of any differences between samples was analyzed using Student’s t test.

**RESULTS**

**Isolation of Stable Cell Lines**—CHO microtubules are composed of αβ heterodimers containing three β-tubulin isotypes (β1, β4b, and β5) in a ratio of 75:25:5 (18, 19). To assess the effects of incorporating β4a-tubulin, stably transfected CHO cell lines expressing an HA-tagged β4a cDNA under the control of a tetracycline-regulated promoter were isolated, and four individual cell lines with different expression levels were cho-
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![Figure 1](image1)

**FIGURE 1. Expression of HAβ-tubulin in stably transfected cell lines.** The cells were incubated in the presence (+) or absence (−) of tetracycline for 24 h, lysed in SDS, and examined by Western blot analysis with Tub2.1, an antibody that recognizes both ectopic (HAβ) and endogenous β-tubulin (β). An antibody to actin was also included to act as a loading control. Cell lines transfected with HAβ1 (A), HAβ4b (B), and HAβ4a (C) are shown. The clones are numbered in order of increasing expression. %, the percent of total tubulin produced by the transfected cDNA.

![Figure 2](image2)

**FIGURE 2. Microtubule organization in cells transfected with HAβ-tubulin.** The cells were cultured without tetracycline for 2 days to induce expression of ectopic β-tubulin, extracted with microtubule-stabilizing buffer to remove background soluble tubulin, and fixed in methanol. The cells were then labeled with an antibody to the HA tag followed by Alexa 488-conjugated goat anti-mouse IgG. A, HAβ1 clone 1; B, HAβ4b clone 1; C, HAβ4a clone 1 (clones 2 and 3 produced similar results); D, HAβ4a clone 4; E, nucleus of HAβ4a clone 1; F, nucleus of HAβ4a clone 4. Mitotic spindles for each of the cell lines are shown in the insets. The arrows point to nuclear fragments produced by nuclear membrane formation around scattered chromosomes.

...
remained that it might assemble with a lower efficiency compared with the endogenous tubulin. To test this possibility, we induced expression for 24 h, lysed the cells with a microtubule-stabilizing buffer, centrifuged to separate nonassembled tubulin (supernatant) from the polymer (pellet), and compared the relative content of endogenous and ectopic tubulin in the two fractions using Western blots with an antibody that recognizes all the β-tubulins. As shown in Fig. 3, the ratio of HAβ4a to endogenous β-tubulin is similar in the supernatant and pellet fractions for all of the clones, arguing that the ectopic tubulin assembles into microtubules with the same efficiency as the endogenous tubulin. Thus, at this level of analysis, HAβ4a appears to be used interchangeably with the endogenous tubulin. To examine this possibility, the extent of cellular microtubule assembly was analyzed by lysing cells with a microtubule-stabilizing buffer and centrifuging the lysate to separate polymerized from nonpolymerized tubulin. Western blot analysis of the two fractions using an antibody to α-tubulin is shown in Fig. 4. In agreement with previous studies (9, 10, 16, 17, 23, 24), ~38% of the cellular tubulin was found in the microtubule fraction for both HAβ1- and HAβ4b-transfected cells. HAβ4a, however, caused a small but significant reduction in microtubule assembly in an expression-dependent manner, i.e. the percentage of total tubulin in the microtubule fraction decreased progressively from 38% to 26% as the proportion of HAβ4a in the cell increased up to 89%.

CHO Cells Expressing HAβ4a Have Altered Sensitivity to Microtubule Drugs—In extensive previous studies into the mechanisms of drug resistance among CHO cells with mutations in tubulin, we have noted that paclitaxel resistance and increased sensitivity to colcemid are almost always found in cells with reduced tubulin assembly (see Ref. 25 for review). Given the decrease in microtubule assembly that we observed in HAβ4a-expressing cells, we therefore expected them to exhibit paclitaxel resistance, a result that would have confirmed the correlation between paclitaxel resistance and elevated β4a expression reported by others (11, 12). Instead we found that cells with low levels of HAβ4a expression (e.g. clone 1) were more sensitive to paclitaxel than nontransfected cells (Fig. 5A and Table 1). Moreover, this increased sensitivity was lost as the level of HAβ4a expression increased (e.g. clones 2 and 3). It is unlikely that the increased sensitivity to paclitaxel exhibited by clone 1 is due to some other random change in the cell line because multiple clones with low expression possessed the phenotype, and the phenotype was lost when the same cells were cultured in the presence of tetracycline to inhibit HAβ4a expression (data not shown). Also, the phenotype was specific to paclitaxel because it did not extend to another microtubule-stabilizing drug, epothilone A (Fig. 5B), even though the two drugs share the same binding site and mechanism of action (26, 27).

On the other hand, the response to microtubule-depolymerizing agents was exactly as predicted for cell lines with decreased tubulin assembly, i.e. the cells were ~2-fold more sensitive to colcemid and vinblastine (Figs. 5, C and D), even though the two drugs bind to different sites (28, 29). Similar results were obtained with two additional microtubule-stabilizing drugs, nocodazole and CI-980, which bind to the colce-
mid site (data not shown). In contrast with the results obtained with HAβ4a, cells overexpressing HAβ1 or HAβ4b had normal sensitivity to the microtubule-depolymerizing drugs colcemid and vinblastine, and they had normal sensitivity to the microtubule-stabilizing drug paclitaxel. HAβ4b-expressing cells did, however, exhibit a slight increase in sensitivity to epothilone A (Table 1).

We conclude that HAβ4a expression has a small disruptive effect on microtubules that causes increased sensitivity to drugs that inhibit microtubule assembly. The response to paclitaxel, however, is more complex. Low expression causes a shift in the dose-response curve to the left, i.e. toward higher sensitivity to paclitaxel. We propose that this response is caused by an increased binding affinity of HAβ4a for paclitaxel, a mechanism that is known to act in a substoichiometric manner (30).

As the level of HAβ4a expression increases, however, the microtubule disruptive effect shifts the dose-response curve to the right, i.e. toward paclitaxel resistance, thereby counteracting the supersensitivity caused by enhanced paclitaxel binding.

Site-directed Mutagenesis of β4a and β4b—In contrast to β4a, overexpression of β1 and β4b did not alter microtubule assembly or affect sensitivity to paclitaxel or colcemid. To identify the amino acid residues in β4a that might be important in mediating its unique properties, we reasoned that amino acids in β4a, which differed from their counterparts in both β4b and β1, would be the most likely candidates. The amino acid sequences of all three isotypes are compared in Fig. 6. There are multiple differences in sequence between β1 and β4b near the C terminus, whereas β4a and β4b differ by a single amino acid; thus, we discarded this region as one of prime importance.

Within the remaining sequence, we identified five amino acids (highlighted residues in Fig. 6 that span three sequences) that could potentially account for the differences in behavior between β4a and the other two isotypes. Each of these amino acids in HAβ4a was converted, one at a time, to its β4b counterpart to determine whether the amino acid was essential for conferring supersensitivity to paclitaxel or colcemid. Similarly, each amino acid in HAβ4b was converted to its β4a counterpart to determine whether the change was sufficient to confer supersensitivity to those drugs. Because supersensitivity to paclitaxel was only seen at low levels of expression, we used transfected cell lines with low expression levels to examine this phenotype. For colcemid supersensitivity, however, we used cell lines with higher expression because this phenotype required microtubule disruption that was expression-dependent. The mutations that were analyzed and their locations in the tubulin structure are summarized in Table 2.

**TABLE 1**

IC50 (nM) of nontransfected and HAβ4a- and HAβ4b-expressing CHO cells to microtubule drugs

| Cell line          | IC50  | Paclitaxel | Epothilone A | Colcemid | Vinblastine |
|--------------------|-------|------------|--------------|----------|-------------|
| CHO wild type      | 0     | 26.3 ± 2.2 (1) | 1.8 ± 0.5 (1) | 19.8 ± 2.9 (1) | 11.1 ± 0.6 (1) |
| HAβ4a-1            | 18    | 13.0 ± 2.4 (2)b | 1.8 ± 0.5 (1) | 17.3 ± 0.7 (1.1) | 8.0 ± 0.5 (1.4)b |
| HAβ4a-2            | 47    | 23.1 ± 5.2 (1.1) | 2.3 ± 0.1 (1.3) | 11.3 ± 1.2 (1.8)b | 7.0 ± 0.9 (1.6)b |
| HAβ4b-3            | 71    | 25.3 ± 2.1 (1) | 2.6 ± 0.5 (1.5)b | 9.8 ± 1.7 (2.0)b | 5.2 ± 0.2 (3.1)b |
| HAβ4b-1            | 34    | 24.8 ± 2.7 (1.1) | 1.2 ± 0.2 (1.5)b | 19.0 ± 4.1 (1) | 10.7 ± 0.7 (1) |
| HAβ4b-2            | 64    | 29.2 ± 5.4 (1.1) | 1.2 ± 0.2 (1.5)b | 17.8 ± 1.0 (1.1) | 10.5 ± 0.1 (1) |

* The percentage of total cellular tubulin contributed by the transfected cDNA.

* p < 0.05 compared with nontransfected CHO wild-type cells. n = 3–5.
Following transfection and selection of stable cell lines, immunofluorescence was used to ensure that at least 95% of the cells in the population had similar expression of the transfected mutant cDNA, and Western blot analysis was used to determine the proportion of exogenous and endogenous tubulin for each of the mutant cell lines. IC\textsubscript{50} values for paclitaxel and colcemid were determined, and they are summarized in Table 3. In the case of supersensitivity to paclitaxel, all five amino acid substitutions in HA\textsubscript{134}/H9252\textsubscript{4a} produced at least some reversal of the phenotype, implying that a complex interaction among the amino acids is involved, but the amino acid change with the greatest effect in reversing the phenotype was A115S. The fact that no single amino acid substitution was found to be uniquely required for paclitaxel supersensitivity was reinforced by the observation that none of the amino acid substitutions introduced into HA\textsubscript{111}/H9252\textsubscript{4b} was sufficient to recreate the phenotype, although N332S did appear to partially increase sensitivity to the drug.

The results for colcemid supersensitivity were more straightforward. The A115S mutation in HA\textsubscript{206}/H9252\textsubscript{4a}, which completely reversed paclitaxel supersensitivity, had essentially no effect on reversing supersensitivity to colcemid. All other substitutions had very small effects in reversing the phenotype, with only the S335N substitution rising to statistical significance. Thus, no single amino acid change in HA\textsubscript{179}/H9252\textsubscript{4a} completely abrogated the ability of that isotype to confer colcemid supersensitivity. However, three amino acid substitutions introduced into HA\textsubscript{398}/H9252\textsubscript{4b} (S115A, N332S, and N335S) significantly increased the ability of that isotype to confer supersensitivity to colcemid. Interestingly, the degree of colcemid supersensitivity conferred by each of the latter two substitutions was greater than that observed for the wild-type HA\textsubscript{404}/H9252\textsubscript{4a} isotype, suggesting that even though some of the substitutions are capable of causing supersensitivity to colcemid by themselves, their combined effect is somewhat antagonistic in producing the final phenotype.

**DISCUSSION**

In previous studies we found that most cells selected for resistance to paclitaxel are cross-resistant to other microtubule-stabilizing drugs such as epothilone A, are more sensitive to microtubule-disrupting drugs, and have reduced microtubule assembly. Conversely, the cells selected for resistance to colcemid or vinblastine are cross-resistant to other microtubule-disrupting drugs, are more sensitive to microtubule-stabilizing drugs, and have increased microtubule assembly. Thus, the tubulin mutations causing resistance appear to act by altering microtubule assembly in a direction that opposes the action of the selecting drug (reviewed in Ref. 25). Many of these changes in microtubule assembly are small enough that they do not alter the ability of the mutant cells to proliferate, but some mutations cause larger perturbations in microtubule assembly that interfere with cell division. Examination of a large number of mutants has established that normal proliferation of CHO cells occurs when \(22\% - 58\%\) of the total cellular tubulin is assembled into microtubules (31). Outside of this range, spindle function is compromised, chromosomes mis-segregate, and cells fail to complete cytokinesis (22). The effect of these mutations on microtubule assembly and drug resistance is dose-dependent, i.e. the magnitude of the change in microtubule assembly and the degree of drug resistance both increase as the amount of mutant tubulin increases in transfected cells (24).
In related studies we made a number of amino acid substitutions of Leu$^{215}$ by site-directed mutagenesis of β1-tubulin cDNA and tested for drug sensitivity in transfected cells. Most of the mutations produced phenotypes similar to the paclitaxel resistance mutations already described, i.e. they decreased microtubule assembly, conferred resistance to paclitaxel and epothilone A, and made the cells more sensitive to colcemid. However, one mutation, L215I, behaved differently. This mutation did not affect microtubule assembly, sensitivity to epothilone A, and made the cells more sensitive to colcemid. Moreover, superimposed on this, however, confers increased sensitivity to paclitaxel. Therefore, it is unlikely that toxicity is due to an inherent inability of β4a to assemble into microtubules because in vitro studies have indicated that purified β4 tubulin subunits actually assemble faster than heterogeneous brain tubulin (32).

The decrease in microtubule assembly caused by β4a expression is relatively mild and causes no problems in growth until β4a accumulates to very high levels (89% of total tubulin). At this level of overexpression, the extent of microtubule assembly drops from 38% (in wild-type cells) to 26% (in HAβ4a clone 4) of total cellular tubulin, a level that is only slightly higher than the 22% at which growth problems are encountered in cells with mutant forms of β1-tubulin (see Ref. 31). The reasons for this small difference are unclear, but the toxic end points for β4a and mutant β1 transfections are similar: defective spindle function that results in mis-segregated chromosomes and a failure of cytokinesis. How the decrease in microtubule assembly produces defects in spindle function is unclear, but it is unlikely to involve alterations in tubulin synthesis. Previous studies have demonstrated that overexpression β-tubulin in CHO cells has only minor effects on steady state α- and β-tubulin levels because any overproduced β-tubulin that cannot find an α-tubulin partner for heterodimer formation is degraded (16). It is also unlikely that toxicity is due to an inherent inability of β4a to assemble into microtubules because in vitro studies have indicated that purified β4 tubulin subunits actually assemble faster than heterogeneous brain tubulin (32). On the other hand, it has been reported that inhibition of microtubule dynamics can inhibit mitotic spindle function (33). It is therefore possible that particular ratios of tubulin isoforms may cause changes in microtubule structure or alter the binding of microtubule-interacting proteins and thereby affect microtubule dynamics or other behavior essential for spindle function.

**TABLE 3**

### HAβ4a and HAβ4b mutations and their effects on drug sensitivity

Controls are shown, and the corresponding mutations are listed below each isotype. The numbers in parentheses represent fold resistance (positive numbers) or sensitivity (negative numbers) relative to the nonmutated control. In all cases, the IC$_{50}$ values determined in the presence of tetracycline to inhibit transcription of the transfected cDNA were not significantly different from nontransfected controls.

| Cell line | Paclitaxel | Colcemid |
|-----------|------------|----------|
| CHO wild type | 26.3 ± 2.2 | 19.8 ± 2.9 |
| HAβ4a | 18.3 ± 2.4 (1) | 9.8 ± 1.7 (1) |
| N58K | 16.9 ± 2.1 (1.3)$^a$ | 11.3 ± 1.4 (1.2) |
| A115S | 23.7 ± 6.3 (1.8)$^a$ | 8.2 ± 0.6 (1.2) |
| F159Y | 19.3 ± 1.7 (1.5)$^a$ | 11.7 ± 0.4 (1.2) |
| S332N | 18.6 ± 2.7 (1.4)$^a$ | 11.9 ± 2.6 (1.2) |
| S335N | 19.6 ± 3.1 (1.5)$^a$ | 16.5 ± 2.1 (1.7)$^a$ |
| HAβ4b | 24.8 ± 2.7 (1) | 17.8 ± 1.0 (1) |
| K58N | 22.6 ± 0.8 (1.1) | 17.3 ± 1.5 (1.1) |
| S115A | 23.3 ± 3.5 (1.1) | 11.0 ± 0.7 (1.7)$^a$ |
| Y159F | 22.6 ± 1.6 (1.1) | 15.4 ± 1.2 (1.2) |
| N332S | 18.2 ± 2.1 (1.4)$^a$ | 4.7 ± 0.3 (3.9)$^a$ |
| N335S | 25.7 ± 5.9 (1) | 5.9 ± 2.3 (5.1)$^a$ |

$^a$ The percentage of total cellular tubulin contributed by the transfected cDNA.

$^b$ Cells with low expression of HAβ4a and HAβ4b were used to assay paclitaxel sensitivity.

$^c$ Cells with high expression were used to assay colcemid sensitivity.

$^d$p < 0.05 compared with nonmutated controls. n = 3–6.

Most of the mutations produced phenotypes similar to the paclitaxel resistance mutations already described, i.e. they decreased microtubule assembly, conferred resistance to paclitaxel and epothilone A, and made the cells more sensitive to colcemid. However, one mutation, L215I, behaved differently. This mutation did not affect microtubule assembly, sensitivity to epothilone A, and made the cells more sensitive to colcemid. Moreover, superimposed on this, however, confers increased sensitivity to paclitaxel. Therefore, it is unlikely that toxicity is due to an inherent inability of β4a to assemble into microtubules because in vitro studies have indicated that purified β4 tubulin subunits actually assemble faster than heterogeneous brain tubulin (32). On the other hand, it has been reported that inhibition of microtubule dynamics can inhibit mitotic spindle function (33). It is therefore possible that particular ratios of tubulin isoforms may cause changes in microtubule structure or alter the binding of microtubule-interacting proteins and thereby affect microtubule dynamics or other behavior essential for spindle function.

HAβ4a and HAβ4b are very similar in amino acid sequence, yet only HAβ4a produces a phenotype when overexpressed. We therefore attempted to identify the amino acid residues that are responsible for differences in their behavior and focused on five residues that are distinct in β4a from their counterparts in β1 and β4b (Fig. 7). For both paclitaxel supersensitivity (because of altered drug binding) and colcemid supersensitivity...
Tubulin Isotypes and Drug Sensitivity

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Tubulin Isotypes and Drug Sensitivity

Finally, the results of this study reinforce the idea that some β-tubulin isotypes act like “mutant” tubulins that affect microtubule assembly and sensitivity to antimitotic drugs. Based on an alignment of vertebrate β-tubulin sequences, Sullivan (1) previously proposed two groups of tubulin: one highly conserved group (outside of the C-terminal 15 amino acids) included β1, β2, and β4; the other more divergent group included β3, β5, and β6. Results from our laboratory showing that changes in expression of members from the second, but not the first, group produce distinctive changes in microtubule assembly and drug resistance support Sullivan’s observation, the lone exception being β4α, which is very similar to β4β but produces significant effects in transfected CHO cells. Thus, differences in cellular isotype composition can impart subtle to rather dramatic effects on the properties of microtubules, leading to potential functional consequences. Exactly how expression of specific tubulin isotypes subserves microtubule function in any particular cell such as a neuron remains a mystery, but it could potentially involve the need to control microtubule dynamics differently than might be needed for a dividing cell, or it might involve a need for the interaction of brain-specific proteins with the microtubule network. In addition to their potential role in mediating some aspects of cellular behavior, the existence of distinct tubulin isotypes in specific cells opens up an opportunity to exploit microtubule isotype composition for therapeutic intervention.

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