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Modulation of drug resistance by α-tubulin in paclitaxel-resistant human lung cancer cell lines

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Abstract

Beta(β)-tubulin isotype variation has recently been implicated in the modulation of resistance to paclitaxel in human lung cancer cells and in primary human ovarian tumour samples. Whether α-tubulin is involved in drug resistance has not been reported. We have generated a paclitaxel-resistant cell line (H460/T800) from the sensitive human lung carcinoma parental cell line NCI-H460. The resistant cells are more than 1000-fold resistant to taxol and overexpress P-glycoprotein. Interestingly, H460/T800 cells also overexpress α- and β-tubulin as detected by Western blot analysis. From Northern blot analysis, the mechanism of tubulin overexpression appears to be post-transcriptional. To understand whether α-tubulin plays a role in drug resistance, we transfected antisense human kα1 cDNA construct into the H460/T800 paclitaxel-resistant cells. The antisense clones displayed a reduced α-tubulin expression, and the cells were 45–51% more sensitive to paclitaxel and other known antimitotic drugs, compared with vector transfected controls. Complementary experiments of transfecting the sense kα1 cDNA into H460 cells conferred a 1.8- to 3.3-fold increase in the IC_{50} of several antimitotic agents. Our study suggests that α-tubulin is one of the factors that contributes to drug resistance.

Keywords: Multidrug resistance; α-Tubulin; P-glycoprotein; Antimitotics

1. Introduction

Paclitaxel is an antimitotic drug that promotes the formation of stable microtubules in the absence of guanosine triphosphate and prevents depolymerisation. It has been used for the treatment of ovarian, breast, head and neck and lung cancers, as well as metastatic melanomas [1–3]. However, its efficacy in the clinic has been hampered by the acquisition of drug resistance by cancer cells. Multidrug resistance (MDR) is a phenomenon in which cancer cells become resistant to a wide range of chemotherapeutic agents with diverse structures and biological mechanisms. Although MDR is a multifactorial phenomenon, little is known about its overall mechanism with the exception of the efflux pump P-glycoprotein (Pgp), a probable key component in MDR [4–7].

Microtubules, which primarily consist of alpha(α)- and beta(β)-tubulin heterodimers, play several important cellular functions. Microtubules are involved in mitosis, intracellular vesicle transport, intracellular organisation and determination of cell shape and motility. Recent reports suggest that β-tubulin isotypes are involved in drug resistance [8–12]. However, although β-tubulin has been intensively studied, very few studies have examined the role of α-tubulin isotypes with respect to drug resistance. Photoaffinity labelled paclitaxel showed that paclitaxel binds to both α- and β-tubulin thus confirming that both α and β-tubulins contribute to the paclitaxel binding site [13]. However, recent electron crystallography analysis showed that paclitaxel binds to a single site on β-tubulin rather than α-tubulin [14]. To understand further whether α-tubulin has any role in drug resistance, we developed a paclitaxel-

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resistant H460 cell line (H460/T800) for studying the role of α- and β-tubulin in drug resistance.

2. Materials and methods

2.1. Cell culture

Lung cancer cell line NCI-H460 (hereafter referred to as H460) was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The H460 cell line was grown and maintained in RPMI medium plus 10% fetal bovine serum (FBS). Paclitaxel-resistant cell lines were derived from the H460 parental cell line. H460 cells were initially selected using the concentration of paclitaxel that results in 50% inhibition [IC_{50}] and thereafter paclitaxel concentration was doubled every 2–3 weeks until 800 nM paclitaxel resistance was obtained. Paclitaxel and all other chemicals were obtained from Sigma (St Louis, MO, USA). Cell cultures were maintained in a 37°C incubator with 5% CO_2.

2.2. Determination of IC_{50} and growth curves

Cytotoxicity assays were performed in 96-well microtitre plates using the colorimetric method as previously described [15]. To measure cell proliferation, the sulphorhodamine binding assay (SRB) was performed in the presence of paclitaxel, vinblastine, colchicine, doxorubicin or nocodazole (10^{-4}–10^{-11} M) in 96-well plates containing cells at 60–70% confluency. Cells were cultured in the presence of drug for 48 h, after which time they were fixed with 10% trichloroacetic acid, stained with 0.4% SRB in 10% acetic acid, washed and dried. The remaining dye was solubilised by 10 mM Tris buffer, and the plates were read at an optical density (O.D.) of 540 nm. For growth curves, cells were plated onto 60-mm tissue culture dishes at 1×10^5 cells/plate, and were counted every 24 h for 3–5 days.

2.3. Kα1 tubulin cDNA transfection

A full-length Kα1 tubulin cDNA was purchased from the ATCC (ATCC#769444), Rockville, MD, USA. The insert (~1.6 kb, *Bam*HI/*Xho*I fragment) was subcloned into a pcDNA 3.1 (−) expression vector (Invitrogen, Carlsbad, CA, USA). This is an antisense cDNA construct. The *Bam*HI/*Xho*I fragment was subcloned into a pcDNA 3.1 (+) for the sense cDNA construct. After subcloning into the respective vectors, a large-scale preparation was performed using Qiagen plasmid purification columns (Qiagen, Chatsworth, CA, USA). Transfection into either H460 or H460/T800 cells was carried out using the Lipofectamine Plus kit (GIBCO-BRL, Grand Island, NY, USA). After transfection, cells were selected in the presence of 1 mg/ml G418, colonies were isolated and expanded.

2.4. Protein extraction and Western blot analysis

Protein extracts were prepared from exponentially growing cells as described [16,17]. Briefly, cells were collected and the cell pellets were resuspended in lysis buffer [(20 mM Tris–HCl pH 7.4, 2 mM EGTA, 2 mM EDTA, 6 mM β-mercaptoethanol, 1% NP-40, 0.1% sodium dodecyl sulphate (SDS) and 10 mM NaF, plus the protease inhibitors aprotinin (10 μg/ml), leupeptin (10 μg/ml) and phenylmethyl sulphonyl fluoride (PMSF) (1 mM)]. This suspension was sonicated three times with a Sonifier Cell Disruptor (Branson Ultrsonics Co., Danbury, CT, USA). Cells were spun briefly (13 000*g* for 1 min) and supernatants were collected for determination of protein concentration by the Bio-Rad assay. Membrane preparations from paclitaxel-resistant cells were prepared as previously described [18]. For Western blotting, 20 μg of protein from the total cell lysates was fractionated by SDS polyacrylamide gel electrophoresis (PAGE). The proteins on these gels were then transferred, using transfer buffer (25 mM Tris, 190 mM glycine, 10% methanol), to immobilon-P membranes (Millipore, Bedford, MA, USA). Membranes were blocked with blocking buffer (50 mM Tris, 200 mM NaCl, 0.2% Tween 20, 3% non-fat dry milk), and the membranes were then incubated with the indicated antibodies. Human anti-α tubulin (1:1000, Sigma), anti-β tubulin (1:1000, BioGenex, San Ramon, CA, USA), anti-β II, -β III and -β IV (1:500, BioGenex) and anti-mdr (1:100, CalBiochem) antibodies were used. After treatment with blocking buffer without 3% non-fat dry milk (washing buffer), a dilute solution (1:2000–1:5000) of horseradish peroxidase linked anti-rabbit donkey serum (Amersham, Arlington Heights, IL, USA) was added. Membranes were then washed with washing buffer and immune detection was performed using the ECL Western blotting detection system (Amersham). All immunoblotting experiments were carried out twice.

2.5. Northern blot analysis

Cells from exponentially dividing cultures were collected with a rubber policeman, washed three times with ice-cold phosphate buffered saline (PBS) and lysed in TRIZOL Reagent (GIBCO BRL, Gaithersburg, MD, USA). Total mRNA isolation was performed according to the manufacturer's instructions. Poly-A RNAs were isolated according to manufacturer's instructions using the Poly A Tract mRNA system (Promega, Madison, WI, USA). The poly-A RNA samples (2 μg) were fractionated by electrophoresis in 1% agar–6% formaldehyde gels and blotted onto Hybond-N+ membranes

(Amersham). The membranes were then pre-incubated in Church buffer at 50°C and hybridised with ^{32}P-labelled oligo probes to human α-tubulin coding sequence (cattggtgatctctgctacagaaagctgttcatggtaggc, 843–880 base pair) [19] and human GAPDH (Clontech, cat#5840-1). The oligos were 5′-end labelled with [^{32}P]-ATP using 5′-end labelling kit (Promega). The membranes were washed with 1×SSC containing 0.2% SDS for 20 min at room temperature and then washed for 20 min at 50°C, using the same washing solution. The membranes were exposed to a Phosphor Imager screen and images were developed using a Phosphor Imager (Molecular Dynamics). All Northern blotting experiments were carried out twice.

2.6. Flow cytometric analysis

H460 cells cultured in the presence or absence of paclitaxel were trypsinised, collected and washed twice with PBS. Cell pellets were fixed in 90% methanol and stored at −20°C. On the day of the assay, the fixed cells were collected by centrifugation, and the pellets were resuspended in 0.8 ml, containing 0.2 mg/ml of propidium iodide, 0.6% NP-40 and 1 mg/ml RNase. The suspension was incubated in the dark at room temperature for 30 min. The cell suspension was then filtered through a 35 μm Spectra mesh filter and analysed on a fluorescent activated cell sorter (FACS) Calibur™ flow cytometer (Becton Dickinson, San Jose, CA, USA) for DNA content. The per cent of cells in different phases of the cell cycle was determined using the ModFit 5.2 program (Becton Dickinson).

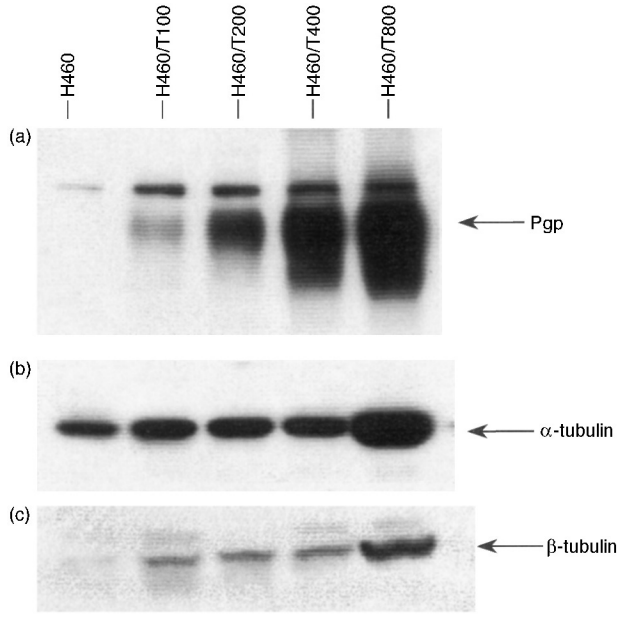


Fig. 1. Expression of (a) Pgp; (b) α-tubulin; and (c) β-tubulin in the paclitaxel-resistant NCI-H460 cell lines. Membrane fractions and cell extracts from each of the indicated cell lines was prepared and resolved on an 8% SDS-PAGE gel. The protein gel was transferred to PVDF membrane and probed with anti-Pgp, anti-α or anti-β-tubulin antibody. H460, parental cells; H460/T100, T200, T400 and T800 cells grown in the indicated concentrations of paclitaxel (nM).

3. Results

3.1. H460/T800 paclitaxel-resistant cells are more than a 1000-fold resistant to paclitaxel than the parental H460 cells

To understand the mechanism of paclitaxel resistance, we developed a series of paclitaxel-resistant cell lines from H460 lung cancer cells. The parental cell line was shown to express a very low level of Pgp mRNA [18] and an undetectable level of protein for Pgp (Fig. 1). Parental cells were initially selected with 10 nM paclitaxel, and thereafter the concentration was gradually increased until it reached 800 nM paclitaxel. The H460/T800 cell line was maintained at 800 nM paclitaxel. During each selection step (T100, T200, T400, T800; T stands for paclitaxel and each number represents nM concentration of paclitaxel maintained in the medium), cells were analysed by the SRB proliferation assay in the presence of a number of antimitotic compounds. As shown in Table 1(a), cells became more resistant to paclitaxel as its concentration in the culture medium was systematically increased. Paclitaxel-resistant cells displayed a cross-resistance to other MDR compounds such as colchicine, vinblastine and doxorubicin. However, cells did not become resistant to nocodazole, a drug which is an inhibitor of mitosis. Doubling times of paclitaxel-resistant cell lines were not altered (Table 1b). The H460/T800 cell line, which is the cell line most resistant to paclitaxel (>1000-fold, Table 1a), was used for most of the work presented below.

3.2. H460/T800 cells are resistant to paclitaxel-induced G2/M cell cycle arrest

To determine if there was any alteration in the cell cycle distribution of paclitaxel-resistant T800 cells, both H460 and H460/T800 cells were treated with or without the indicated concentrations (0.01 μM–1 μM) of paclitaxel for 24 h. As shown in Table 2, both control H460 and H460/T800 cells displayed similar cell cycle profiles in the absence of paclitaxel as measured by flow cytometry. When H460 cells were treated with increasing concentrations of paclitaxel, there was a proportional increase in the per cent of cells in G2/M in a dose-dependent manner. After treatment with 0.1 μM paclitaxel, nearly all the cells (94.2%) arrested at G2/M. When H460 cells were treated with either 0.5 or 1.0 μM paclitaxel, most of the cells accumulated at G2/M. In contrast, when H460/T800 cells were treated with the same concentrations of paclitaxel, there was only a

Table 1
Determination of cell proliferation [IC_{50}] (a) and doubling times (b) for H460 paclitaxel-resistant variants[a]
(a) Determination of IC_{50} by the SRB assay from H460-paclitaxel-resistant cell lines

	Mean [IC_{50}] (nM)±SEM				
	H460	T100	T200	T400	T800
TAX	5.5±1.2	355.0±63.6	764.5±89.8	1370±42.4	5985±190.9
CLC	31.4±9.4	43.5±17.6	189.5±6.4	608±56.6	1065±35.4
VBL	2.8±0.3	48.3±22.5	177.5±60.1	254±41.7	832±97.7
DOX	14.8±2.4	49.5±4.9	289.5±27.6	343±9.9	1250±14.1
NOC	49.5±11.2	14.4±4.9	47.8±25.2	14.9±5.7	75.7±0.9
(b) Determination of doubling times					
	Mean time (h)±SEM				
	H460	T100	T200	T400	T800
Untreated	18.2±1.6	19.4±0.3	21.0±0.8	21.0±0.8	19.5±0.3

TAX, paclitaxel; CLC, colchicine; VBL, vinblastine; DOX, doxorubicin; NOC, nocodazole.
[a] SEM (standard error of the mean) were derived from three independent experiments.

slight increase in the accumulation of cells at G2/M. At the highest concentration of paclitaxel (1 μM) treatment, only 42.8% of the cells accumulated in G2/M compared with 22.1% in the untreated cells. To demonstrate further that H460/T800 cells were resistant to paclitaxel, cells were stained with an antitubulin antibody and analysed by immunohistochemistry. Following paclitaxel-treatment, the microtubule distribution in the H460/T800 cells was not altered, however, in the control H460 cells, paclitaxel-treatment resulted in the bundling and condensation of tubulin (data not shown).

3.3. H460/T800 cells overexpress P-glycoprotein and α-tubulin

To elucidate the mechanism of paclitaxel resistance in the H460/T800 cell line, we examined P-glycoprotein expression by Western blot analysis. Membrane fractions from each of the indicated cells were analysed by Western blot using an antibody directed against Pgp. As shown in Fig. 1(a) as the cells became more resistant to paclitaxel, there was a proportional increase in the expression of Pgp. The most paclitaxel-resistant cell line H460/T800 expressed the highest level of Pgp. The expression of Pgp was undetectable in the parental H460 cell line. Thus, Pgp is one of the factors responsible for paclitaxel resistance in H460/T800 cells.

Previous reports show that β-tubulin is also involved in drug resistance [8–12,20]. This prompted us to investigate the expression of α- and β-tubulin in paclitaxel-resistant cell lines by Western blot analysis. As shown in Fig. 1, α- and β-tubulin are also overexpressed in our paclitaxel-resistant variants. The H460/T800 cell line expressed the highest levels of α- and β-tubulin. When we examined the expression of β-tubulin isotypes, we found that β II and β III, but not β-IV isotypes were overexpressed in the H460/T800 cells (data not shown). When α-tubulin mRNA expression was analysed by Northern analysis, no increase in the α-tubulin mRNA level was found (Fig. 2). This suggests that α-tubulin overexpression is regulated at either the post-transcriptional or translational level.

Table 2
Flow cytometric analyses of H460 and H460/T800 cells following different concentrations of paclitaxel treatment

(a) H460	Paclitaxel (μM)	% G0/G1	%S	% G2/M
	0	64.8	22.9	12.3
	0.01	23.5	15.1	61.5
	0.1	2.0	3.8	94.2
	0.5	1.3	4.8	93.9
	1	2.8	0.6	96.6
(b) H460/T800	Paclitaxel (μM)	% G0/G1	%S	% G2/M
	0	54.8	23.1	22.1
	0.01	54.7	21.9	23.4
	0.1	58.1	22.7	19.2
	0.5	41.4	21.3	37.3
	1	21.6	35.6	42.8

3.4. Antisense (AS) kα1 cDNA transfection sensitises H460/T800 cells to the cytotoxic effects of antimitotic agents

To further understand the mechanism of α-tubulin overexpression in H460/T800 cells, we used a full-length antisense kα1 cDNA construct to downregulate the expression of α-tubulin. H460/T800 cells express kα1, bα1 and Hα44 as detected by reverse transcriptase–polymerase chain reaction (RT–PCR) (data not shown). RT–PCR studies did not reveal that one form of α-tubulin

isotype is overexpressed over another in H460/T800 cells (data not shown). Antibodies that are specific to each of the α-tubulin isotypes would be extremely important in determining the expression of individual α-tubulin isotypes. Currently, these antibodies are not commercially available, and this limits our study. We chose kα1 tubulin isotype for our antisense study since it is ubiquitously expressed in various tissues [19]. In contrast, bα1 and Hα44 isotypes are specific for brain and skeletal muscle, respectively [8,19,21,22]. Furthermore, coding regions are conserved among all α-tubulin isotypes. For example, there is only one amino acid difference between kα1 and bα1 in the entire coding region. In terms of nucleotide sequence, there is a greater than 95% homology [19] among the α-tubulin isotypes.

Following transfection of kα1 antisense cDNA construct into H460/T800 cells, we obtained several G418-resistant antisense (AS) clones (AS5, AS8 and AS9) which displayed a reduced expression of α-tubulin compared with vector controls (Vt1 and Vt4) (Fig. 3a). The level of β-tubulin expression was slightly reduced in the antisense clones compared with the vector controls (Fig. 3b).

We performed SRB assay on the AS clones to see whether the reduced expression of α-tubulin has any modulating cytotoxic effects on H460 cells. As shown in Table 3, the [IC_{50}]s for three AS clones (5, 8 and 9) were reduced by 45–51% for paclitaxel, colchicine and vinblastine compared with the vector controls. The mean IC_{50} values for two vectors and three AS clones exposed to paclitaxel were 6835 and 3375 nM, respectively. Similarly, the mean [IC_{50}] values of two vectors exposed to colchicine and vinblastine were 1043 and 812 nM, respectively. In contrast, the mean [IC_{50}] values of three AS clones exposed to colchicine and vinblastine were 575 and 433 nM, respectively. However, the cellular response to nocodazole was least affected by the reduced expression of α-tubulin. Since the expression of Pgp or multidrug resistance-associated protein (MRP) was not affected in AS clones compared with vector controls (data not shown), our data show that in addition to Pgp overexpression that the overexpression of α-tubulin also contributes to drug resistance.

3.5. Overexpression of α-tubulin in parental H460 cells confers resistance to antimitotic agents

To examine further the role of kα1 in drug resistance, we transfected a kα1 sense cDNA construct into parental H460 cells. As shown in Fig. 1, this cell line did not express a detectable level of Pgp. Although we obtained a number of G418-resistant clones following transfection, only a single kα1 overexpressor clone was obtained. This kα1 overexpressor (kα1 5) and a vector control (V2) were used in the SRB assay. As shown in

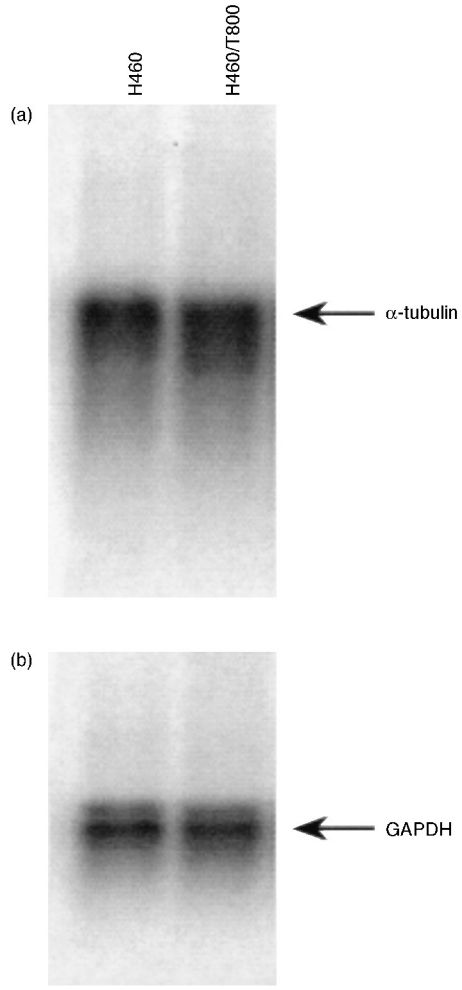


Fig. 2. Expression of α-tubulin mRNA by Northern blot analysis. Poly-A RNAs from either H460 or H460/T800 cells were isolated and electrophoresed on agarose gels, transferred to Nylon membrane and probed with either an α-tubulin oligo probe (a) or GAPDH oligo probe (b).

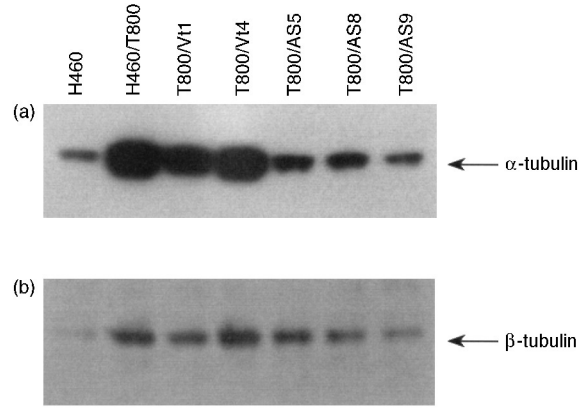


Fig. 3. Expression of α- and β-tubulin in antisense clones by Western blot analysis. Cell extracts from each of the cell line were prepared, loaded (20 μg), electrophoresed on 8% SDS-PAGE and transferred to PVDF membranes. Ponceau-S staining of the transferred PVDF membranes showed equal loading in each lane (data not shown). The blots were probed with either α- or β-tubulin antibody.

Table 3
Determination of cell proliferation [IC_{50}] of α-tubulin AS clones[a]

	Mean [IC_{50}] (nM)±SEM						
	V1	V4	Mean	AS5	AS8	AS9	Mean
TAX	5415±7	8255±262	6835	2500±14	3530±269	4095±32	3375
CLC	1080±14	1005±7	1043	634±33	466±86	625±23	575
VBL	748±54	876±111	812	414±67	322±62	564±49	433
NOC	177±9	144±46	161	110±12	108±6	100±11	106

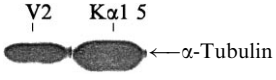
SEM, standard error of the mean; TAX, paclitaxel; CLC, colchicine; VBL, vinblastine; NOC, nocodazole.
[a] V1 and V4 represent two individual vector clones. AS5, AS8 and AS9 indicate three individual antisense clones. SEM were derived from three independent experiments.

Table 4a, kα1 overexpressor displayed a 1.8–3.3-fold increase in [IC_{50}] against paclitaxel, colchicine and vinblastine when compared with the vector control (V2). In contrast, the kα1 overexpressor showed a 1.2-fold increase in IC_{50} against nocodazole when compared with the vector control. The kα1 clone showed an approximately 3-fold overexpression of kα1 tubulin compared with the V2 vector control (Table 4b). No morphological differences were observed between the vector clone and kα1 overexpressing clone (data not shown). This complementary study further confirms that α-tubulin plays a role in drug resistance.

4. Discussion

Mechanisms responsible for MDR have been extensively studied for many years since MDR is a major obstacle to the successful treatment of cancer. A number of factors have been shown to contribute to the MDR phenomenon. For example, a drug transporter, Pgp has been shown to confer MDR in tissue cultures [5,6]. Recently, several reports have shown that β-tubulin is involved in drug resistance [8–12,20]. For example, the paclitaxel-resistant human prostate carcinoma, DU-145, showed an increase in the expression of the βIII tubulin isotype [12,20]. Furthermore, the estramustine-resistant human prostate carcinoma DU-145 displayed overexpression of βIII and βIVa tubulin isotypes [11] and the paclitaxel-resistant J774.2 cell line also overexpressed class II β-tubulin isotype [8]. In addition, three classes of β-tubulin isotypes (I, III and IVa), were shown to be overexpressed in paclitaxel-resistant ovarian tumours [9]. However, the involvement of α-tubulin in drug resistance had not been reported.

Table 4
Determination of [IC_{50}] of a kα1-tubulin sense clone (a) and its expression by Western blot analysis (b)[a]

	Mean [IC_{50}] (nM)±SEM	
	V2	Kα1 5
(a)		
TAX	2.9±0.2	7.3±0.1
CLC	39.7±7.0	72.6±0.9
VBL	1.5±0.2	5.0±1.0
NOC	62.1±9.1	72.4±0.1
(b)		

TAX, paclitaxel; CLC, colchicine; VBL, vinblastine; NOC, nocodazole.
[a] V2 and Kα1 5 represent a vector and kα1 sense clone, respectively. SEM (standard error of the mean) was derived from three independent experiments.

Our present study shows that reduction of α-tubulin by a kα1 antisense cDNA construct resulted in H460/T800 cells becoming more sensitive to antimitotic drugs, such as paclitaxel, colchicine and vinblastine (Table 3). Based on the [IC_{50}] data (Table 3), three AS clones showed approximately 45–51% increased sensitivity toward these drugs. This is within the same range of efficacy as a very recent report where antisense oligonucleotides to class III β-tubulin resulted in a 39% increase in sensitivity to paclitaxel [10]. Furthermore, when we transfected kα1 sense cDNA construct into the H460 cell line, a kα1 overexpressor was 1.8–3.3-fold more resistant to paclitaxel, colchicine and vinblastine compared with a vector control. As shown in Fig. 1a and b, H460/T800 cells overexpress Pgp in addition to α-tubulin. However, Pgp protein was not expressed in parental H460 cells (Fig. 1a). As shown in the results, two independent but complementary sets of experiments demonstrated that α-tubulin plays a role in drug resistance. Thus, our study suggests that α-tubulin is one of the factors that can contribute to drug resistance.

The α- and β-tubulin proteins are encoded by multigene families and exist as several isotypes in cells [23,24]. α-Tubulin exists in six isotypes, and the role for each isotype in cells is not well known. Among all six isotypes, kα1 tubulin is the most widely expressed in tissues [19]. At the present time, we do not know which α-tubulin isotype is overexpressed in H460/T800 cells. Our RT–PCR data suggest that the H460/T800 cells expressed kα1, bα1 and Hα44 isotypes (data not shown). However, the RT–PCR study did not show that one isotype was preferentially expressed over the others.

Based on our study, α-tubulin expression was not regulated at the RNA level but rather at the post-transcriptional level. One possible mechanism for an increase in the α-tubulin protein level is the increase in the α-tubulin mRNA half-life. Alternatively, an increase in the stability of the α-tubulin protein may also contribute to an increase in the α-tubulin protein level. We are in the process of looking at individual α-tubulin isotypes using transfection studies. Since studies have shown mutations in *β-tubulin* may be responsible for drug resistance or tubulin polymerisation [25,26], it is also possible that mutations in *α-tubulin* might be responsible for drug resistance. Indeed, it was shown that *α-tubulin* is altered in paclitaxel-resistant Chinese hamster ovary cells [27]. Furthermore, it was recently shown that a mutation of the *α-tubulin* gene is responsible for herbicide resistance [28].

In summary, we developed a paclitaxel-resistant cell line which overexpresses α-tubulin in addition to Pgp. Reduction of α-tubulin expression by antisense kα1 cDNA construct led to cells becoming more sensitive to antimitotic drugs. Conversely, overexpression of a sense kα1 cDNA construct resulted in an increased resistance to antimitotic compounds. To our knowledge, this is the first report to demonstrate that α-tubulin is involved in drug resistance. The role of each of the α-tubulin isotypes is currently being investigated.

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