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Characterization of a Taxol-resistant Human Small-cell Lung Cancer Cell Line

Sei Ohta,[1,2] Kazuto Nishio,[1] Naohiro Kubota,[1] Tohru Ohmori,[1,2] Yasunori Funayama,[1] Tatsuo Ohira,[1] Hiroaki Nakajima,[2] Mitsuru Adachi[2] and Nagahiro Saijo[1,3]

[1]*Pharmacology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104 and* [2]*First Department of Internal Medicine, Showa University, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142*

Taxol is a novel anticancer agent with activity against a broad range of tumors. It has a unique ability to stabilize polymerized tubulin into microtubule bundles within the cell. We have established a taxol-resistant human small-cell lung cancer cell line (H69/Txl) by exposing H69 cells to stepwise increases in taxol concentration. The resistance of H69/Txl cells to taxol was 4.7-fold that of the original H69 cells: the IC_{50} values for H69 and H69/Txl were 113.7±56.54 n*M* and 538.7±214.7 n*M* by the tetrazolium dye assay, respectively. Removal of the drug from the medium resulted in a 38% decrease in the growth rate of H69/Txl as compared with that in the presence of 30 n*M* taxol, suggesting that the growth of H69/Txl was partially dependent on taxol. H69/Txl showed higher sensitivity to vinca alkaloids such as vindesine, vincristine and vinblastine than the parental H69. There was no significant difference in intracellular [^{3}H]taxol content between H69 and H69/Txl cells. No MDR-1 mRNA was detected in H69/Txl by the reverse transcription polymerase chain reaction. There was no significant difference of total and polymerized tubulin content between H69 and H69/Txl cells. Altered mobility of one of the α-tubulin isoforms in H69/Txl was revealed by using isoelectric focusing and Western blotting with anti-α-tubulin antibody. In H69, two α-tubulin isoforms were observed, whereas three were evident in H69/Txl, two of them comigrating with the isoforms of H69 and the other being more acidic. We observed the increased acetylation of α-tubulin in H69/Txl cells as compared with that in H69 cells. The acetylation of α-tubulin may be responsible for the taxol resistance and/or taxol-dependent growth of H69/Txl.

Key words: Taxol — Drug resistance — Tubulin — Polymerization — Acetylation

Taxol is a novel anticancer agent with activity against a broad range of human tumors, particularly drug-refractory ovarian[1] and breast[2] carcinomas, and malignant melanoma.[3] Taxol's main target is the equilibrium between microtubules and their basic subunits, tubulin dimers. The drug has a characteristic ability to bind directly to β-tubulin *in vitro*.[4] The ability of taxol to stabilize polymerized tubulin into microtubule bundles within the cell by preventing depolymerization is a unique property, not shared by other clinically employed antimicrotubule agents (vincristine, vinblastine and vindesine) that induce microtubule disassembly. Taxol is a potent inhibitor of replication, blocking cells in the late G_2 or mitotic phase of the cell cycle.[5]

One of the main reasons for chemotherapy failure is believed to be the emergence of cellular drug resistance. It is therefore very important to identify the mechanisms of drug resistance in order to improve the clinical efficacy of chemotherapy. Resistance to taxol involves a multidrug resistance phenotype caused by the MDR-1 gene product, P-glycoprotein.[6] However, mechanisms other than P-glycoprotein-mediated drug efflux may also be important in clinical resistance of human lung cancer.[7]

[3] To whom requests for reprints should be addressed.

We attempted to establish a taxol-resistant human small-cell lung cancer cell line with a mechanism different to that of typical MDR-1-mediated multidrug resistance, and were successful in producing a taxol-resistant cell line without elevated expression of the MDR-1 gene or increased drug efflux. We believe that this taxol-resistant cell line is unique and may be useful for elucidating the mechanism of taxol resistance and for selection of drugs active against human small-cell lung cancer.

MATERIALS AND METHODS

Chemicals Taxol was obtained from Bristol-Myers Squibb (Tokyo). Vindesine sulfate was purchased from Shionogi Pharmaceutical Co. (Osaka). RPMI 1640 medium and phosphate-buffered saline (PBS) were purchased from Nissui (Tokyo). [^{3}H]Taxol was purchased from Moravek (Brea, CA). ^{35}S-Labeled anti-mouse IgG antibody, [α-^{32}P]deoxycytidine triphosphate ([α-^{32}P]-dCTP), and [^{14}C]acetyl-coenzyme A were purchased from Amersham Japan. For the slab gel for isoelectric focusing (IEF), an Ampholine[R] PAG plate, pH 4.0–6.5, for IEF and Protein G Sepharose 4 Fast Flow (Pharmacia LKB, Uppsala, Sweden) were used. Mouse monoclonal anti-α- or anti-β-tubulin antibody, vincri-

stine sulfate and vinblastine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were also purchased from Sigma Chemical Co. unless otherwise stated.

Establishment of a taxol-resistant human small-cell lung cancer cell line A taxol-resistant cell line was established by stepwise exposure of H69 cells to increasing concentrations of the drug. The H69 human small-cell lung cancer cell line was originally established at the National Cancer Institute (Bethesda, USA), and stock cultures were obtained from Dr. Y. Shimosato (National Cancer Center Research Institute, Tokyo). The cells were propagated in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml) (RPMI-FBS) at 37°C in a balanced air humidified incubator with an atmosphere of 5% CO_2. The resistant cell line was developed by continuous exposure to taxol starting with a concentration of 8 nM, which was increased stepwise to 20 nM. We isolated an H69 subline resistant to taxol-induced growth inhibition (H69/Txl) by using a limiting dilution method twice. The established taxol-resistant cell line grew continuously in the medium containing 20 nM taxol.

Growth inhibition assay To determine the growth-inhibitory effect of drugs, we used the tetrazolium dye assay of Mosmann.[8] A well containing only RPMI-FBS and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used as a control. Each experiment was performed in 6 wells for each concentration, and carried out 3 times. The IC_{50} was defined as the concentration required for 50% reduction of the optical density in each test, and was calculated as (mean absorbance in 6 wells containing drug − mean absorbance in 6 control wells)/(mean absorbance in 6 drug-free wells − mean absorbance in 6 control wells) × 100. The relative resistance was defined as IC_{50} of the resistant subline/IC_{50} of the parental cell line.

Accumulation assay For determination of [^{3}H]taxol accumulation, exponentially growing cells (2×10^6 cells/ml) were incubated with 10, 100 or 1,000 nM [^{3}H]taxol at 37°C for 3 h. The cells were washed twice with ice-cold PBS, and cell pellets obtained after centrifugation were dissolved in 1 ml of 90% formic acid. Then 4 ml of Clear-sol^R 1 solution was added (Nacalai Tesque, Kyoto) to the tubes and radioactivity was measured with a liquid scintillation counter (LS6000TA, Beckman, Irvine, CA).

RNA extraction and reverse transcriptase reaction The MDR-1 mRNA was detected by the reverse transcription polymerase chain reaction (RT-PCR) method reported by Noonan *et al.*[9] Total cellular RNA was extracted from the H69 and H69/Txl cells by the acid guanidine thiocyanate-phenol-chloroform extraction method,[10] and 1-μg samples were annealed with random hexadeoxynucleotide primer (Takara Shuzo, Tokyo). The mixtures (14 μl) were then transcribed with M-MLV reverse transcriptase (Bethesda Research Laboratories) in tubes containing the ribonuclease inhibitor, RNasein, as described by Kawasaki *et al.*[11]

PCR Etoposide-resistant H69/VP cells were used as a positive control for typical MDR-1-expressing cells.[12] Oligonucleotide primers were synthesized by the phosphoramidite method with a 391 DNA synthesizer and purified on OPC columns (Applied Biosystems, Foster City, CA). The names and nucleotide sequences of the primers used for amplification of cDNA are listed in Table I. Five microliters of reverse transcriptase mixture was subjected directly to PCR to amplify the region of MDR-1, β_2-microglobulin (β_2-MG) and β-actin cDNA in the mixture (50 μl), as described previously.[9] For quantitation, 0.5 μCi (1 Ci=37 GBq) of [α-^{32}P]dCTP was added to each reaction mixture. PCR products were diluted with 20 volumes of loading solution containing 20 mM ethylenediaminetetraacetic acid (EDTA), 0.05% bromophenol blue and xylene cyanol, 0.1% sodium dodecyl

Table I. Oligonucleotides Used for Amplification of cDNA

mRNA species	Sequence	Size of PCR product (bp)
MDR-1		
(upstream)	5′CCCATCATTGCAATAGCAGG 3′	167
(downstream)	5′GTTCAAACTTCTGCTCCTGA 3′	
β_2-Microglobulin		
(upstream)	5′ACCCCCACTGAAAAAGATGA 3′	120
(downstream)	5′ATCTTCAAACCTCCATGATG 3′	
β-Actin		
(upstream)	5′TACATGGCTGGGGTGTTGAA 3′	218
(downstream)	5′AAGAGAGGCATCCTCACCCT 3′	

Primer sequences for MDR-1 and β_2-microglobulin are according to Noonan *et al.* (1990),[9] that of β-actin is according to Kinoshita *et al.* (1992).[28]

sulfate (SDS) and 5% glycerol and applied (1 μl/lane) to 6% polyacrylamide gel. Electrophoresis was performed at 15 W for 45 min. The gel was dried on filter paper and the amount of radioactivity in each sample was determined using a BAS 2000 image analyzer (Fuji Film, Tokyo).

Preparation of whole-cell lysates for determination of total tubulin content Total tubulin was isolated by a modification of the method reported by Thrower *et al.*[13] and Minotti *et al.*[14] Exponentially growing cells were washed twice with PBS, collected and adjusted to 2×10^6 cells/ml. One milliliter of each cell suspension was centrifuged at 200*g* for 5 min, and resuspended in 0.3 ml of depolymerization buffer (0.1 *M* 2-[N-morpholino]ethanesulfonic acid, 1 m*M* $MgSO_4$, 10 m*M* $CaCl_2$, and 5 m*M* guanine triphosphate, pH 6.9). The cells were lysed by sonication on ice with a Branson Sonifier 450 (Branson Ultrasonics, Danbury, CT) at 15 W output for two intervals of 15 s each. Lysate fractions were incubated in depolymerization buffer for 1 h on ice to depolymerize the microtubules, then the samples were centrifuged at 50,000*g* for 15 min at 4°C using a TL-100 centrifuge (Beckman) with a fixed-angle TL-45 rotor (Beckman). The supernatant was transferred to a new centrifuge tube. After addition of 30 μl of protein G Sephrose 4 Fast Flow to samples and overnight mixing at 4°C, the tube was centrifuged at 250*g* for 5 min. The supernatant was transferred to a new centrifuge tube, anti-α- or anti-β-antibody was added and the tube was mixed for 1 h at 4°C. To each sample was then added 30 μl of protein G Sepharose, followed by mixing for 1 h at 4°C and washing 3 times with TNE buffer (10 m*M* Tris-HCl, pH 7.6, 1% (w/v) Nonidet P-40 (Iwai Chem. Co., Tokyo), 0.15 *M* NaCl, and 1 m*M* EDTA.

Preparation of whole-cell lysates for determination of polymerized tubulin content Polymerized tubulin was isolated by a modification of the method of Thrower *et al.*[13] and Minotti *et al.*[14] Briefly, exponentially growing cells were washed twice with warmed PBS, collected and adjusted to 2×10^6 cells/ml. One milliliter of each cell suspension was centrifuged at 200*g* for 5 min. The pellet was resuspended in 1 ml of stabilization buffer (20 m*M* Tris-HCl, pH 6.8, 0.14 *M* NaCl, 0.5% Nonidet P-40, 1 m*M* $MgCl_2$, 2 m*M* ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 4 μg/ml taxol) and incubated at 37°C for 30 min. Each sample was centrifuged at 50,000*g* for 15 min at 37°C. The supernatant was aspirated and the pellet was resuspended in 0.3 ml of depolymerization buffer. The lysate was treated after this step in a manner similar to that for the total tubulin isolates, with 1 h incubation on ice, centrifugation and immunoprecipitation.

Western blot analysis of total and polymerized tubulin The contents of total and polymerized tubulin were analyzed on 10% SDS-polyacrylamide gel. After electrophoresis, the protein on the gel was transferred electrophoretically to a nitrocellulose membrane as reported by Towbin *et al.*[15] The membrane was incubated with blocking buffer (4% skim milk with PBS) for 1 h and was then allowed to react with anti-α- or anti-β-tubulin antibody for 12 h at 4°C. After incubation, the membrane was washed four times with PBS containing 0.1% Tween-20 and then incubated with ^{35}S-labeled anti-mouse IgG antibody (Amersham, Japan) at room temperature for 1 h. After washing of the membrane four times with PBS containing 0.1% Tween-20, it was exposed to an imaging plate (BAS-III, Fuji Film). The plate was finally analyzed by a BAS 2000 Bio-image analyzer (Fuji Film).

IEF and Western blotting The methods of IEF and Western blotting were modifications of those reported by Matthaei *et al.*[16] Samples for IEF were dissociated in sample buffer (7.5 *M* urea, 4% Nonidet P-40, 3.2% Ampholine^R (Pharmacia-LKB) (pH 4.0–6.0), 0.8% Ampholine^R (pH 3.5–10), and 10% 2-mercaptoethanol). Samples were loaded and focused at 2000 V for 2.5 h and the protein on the gel was transferred electrophoretically to a nitrocellulose membrane. The membrane was incubated with PBS containing 4% skim milk for 1 h and then allowed to react with anti-α-tubulin antibody for 12 h at 4°C. After incubation, the membrane was washed four times with PBS containing 0.1% Tween-20 and then incubated with biotinylated anti-mouse IgG antibody at room temperature for 1 h. Color development was done by the avidin-biotin-peroxidase complex method with 0.5 mg/ml 3,3'-diaminobenzidine as a substrate.

Assay for *in vitro* acetylation of α-tubulin Assay for *in vitro* acetylation of α-tubulin followed Greer's report.[17] Reactions were started by the addition of [^{14}C]acetyl-coenzyme A, and were carried out at 37°C. The contents of acetylated α-tubulin were analyzed on 10% SDS-polyacrylamide gel. After electrophoresis, the protein on the gel was fixed with 10% glacial acetic acid and 30% methanol, and was impregnated with EN^{3}HANCE (Du Pont de Nemours & Co., Boston, MA). Following impregnation, the gel was washed with water, dried and exposed to Hyperfilm MP (Amersham) at −70°C for 3 month for the autoradiography. For normalization, the same samples were compared by Western blotting with anti-α-tubulin antibody.

RESULTS

General characteristics of H69 and H69/Txl Fig. 1a shows the cell proliferation curves of H69/Txl exposed or not exposed to 30 n*M* taxol, when they were seeded in RPMI 1640 at an initial cell concentration of 2×10^5 cells/ml and cultured without a change of medium for 11 days. Removal of the drug from the medium resulted in

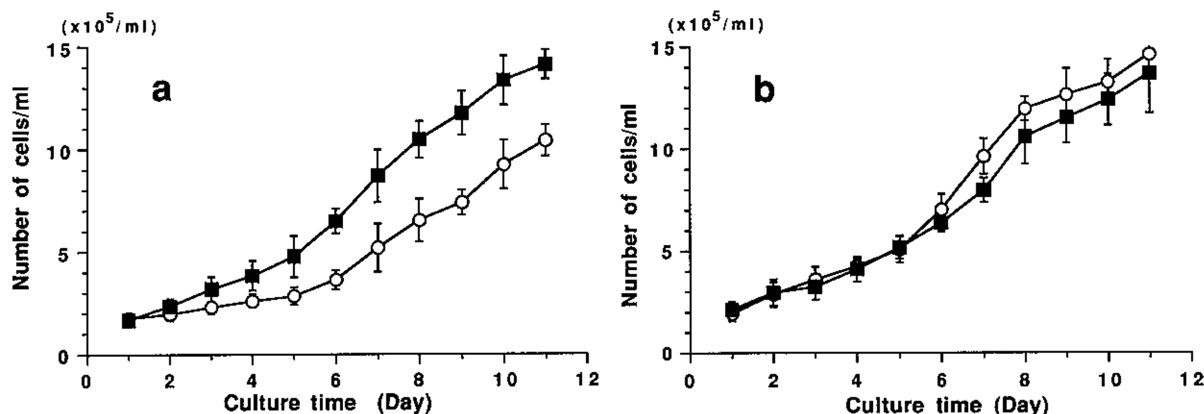

Fig. 1. Cell proliferation curves of H69/Txl (a) and H69 parental cells (b) when seeded in RPMI 1640 with (■) or without (○) 30 n*M* taxol at an initial cell concentration of 2×10^5 cells/ml and cultured without change of medium for 11 days. Removal of the drug from the medium of H69/Txl cells resulted in a 38% decrease in growth rate compared with the cells exposed to 30 n*M* taxol. The growth of H69/Txl was partially dependent on taxol. However, the growth of the parental H69 cells was not increased by taxol.

Table II. Characteristics of H69 and H69/Txl

	H69	H69/Txl
Cell size (μm)[a]	8.11±0.24[b]	6.95±0.24
Doubling time (h)	52.5	85.7 (taxol-free) 62.5 (taxol 30 n*M*)
Protein content (μg/1×10⁶ cells)	270	150

a) Diameter was measured by using a Coulter Channelyzer 256.
b) Each value is the mean±SD of 3 independent experiments.

Table III. Sensitivities to Various Agents in the MTT Assay

Drug	IC_{50}[a] (n*M*) H69	IC_{50}[a] (n*M*) H69/Txl	Relative resistance[b]
Taxol	113.7±56.54[c]	538.7±214.7	4.73[d]
VDS	1.757±0.430	0.900±0.350	0.51[d]
VCR	2.080±0.470	1.047±0.350	0.50[d]
VBL	0.074±0.011	0.041±0.021	0.55[d]

a) Drug concentration inhibiting cell growth by 50%.
b) Relative resistance value equals the IC_{50} value of the resistant cell line divided by that of the parent cell line.
c) Each value is the mean±SD of 3 independent experiments.
d) $P<0.01$ (unpaired Student's *t* test).

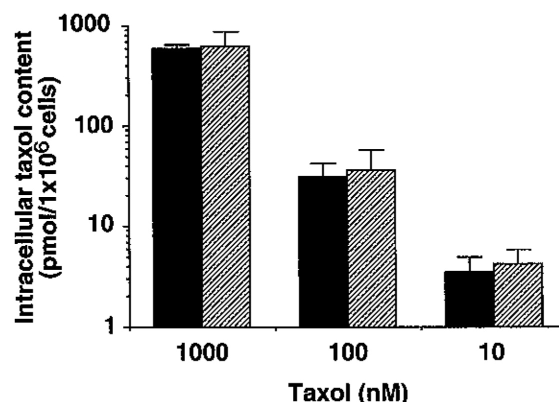

Fig. 2. Intracellular concentrations of [³H]taxol after addition of 10, 100, or 1,000 n*M* [³H]taxol to the culture medium for 3 h. The amount of intracellular radioactivity of [³H]taxol increased in both H69 (solid column) and H69/Txl cells (hatched column) with increasing concentration of [³H]taxol in the medium. There was no significant difference in the intracellular [³H]taxol content in H69 and H69/Txl cells at any of the concentrations tested.

a 38% decrease in the growth rate of H69/Txl as compared with cells in the presence of 30 n*M* taxol, suggesting that the growth of H69/Txl was partially dependent on taxol. Fig. 1b shows the cell proliferation curves of H69 parental cells exposed or not exposed to 30 n*M* taxol. The growth of H69 parental cells was decreased by exposure to 30 n*M* taxol, although there was no significant difference between exposure and non-exposure to 30 n*M* taxol. Table II lists the general characteristics of the parental H69 and taxol-resistant H69/Txl cells. The doubling time for H69/Txl cells (62.5 h) exposed to 30 n*M* taxol was slightly longer than that of H69 cells (52.5 h) and shorter than that of H69/Txl cells not exposed to taxol (85.7 h). Cell diameter was measured using a Coulter Channelyzer 256 (Nikkaki, Tokyo), and the diameter of H69/Txl cells was found to be smaller than

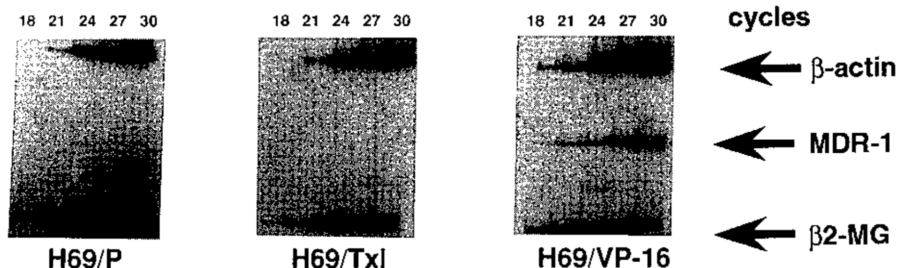

Fig. 3. Examination of MDR-1 mRNA expression in H69/Txl by RT-PCR analysis. An autoradiogram is shown for the PCR product analyzed by the sequential cycling method. H69/VP cells were used as a positive control for typical MDR-1 cells. Amplified β-actin and β_2-MG mRNA were used as internal controls. An increase in β-actin and β_2-MG mRNA with increasing cycle number was observed in H69, and H69/VP, and H69/Txl cells. In H69/VP cells, increased expression of MDR-1 mRNA was detected. On the other hand, MDR-1 mRNA was not detected in H69 parental and H69/Txl cells.

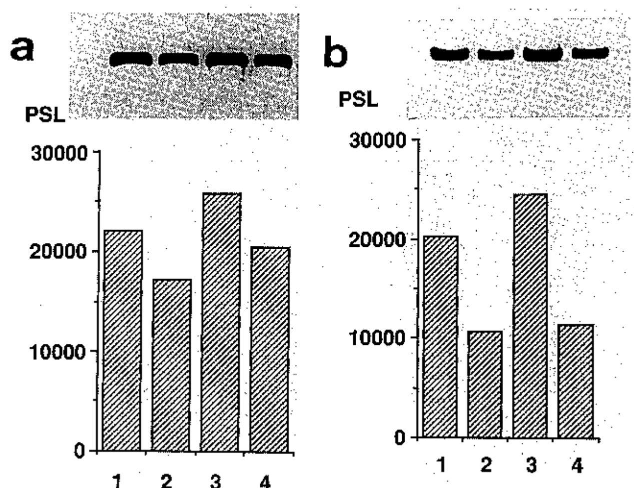

Fig. 4. Lack of significant difference in the total α- (a) and β-tubulin (b) contents between H69 and H69/Txl cells. Polymerized tubulin ratios of α- and β-tubulin were 77.9% and 52.6%, respectively, in parental H69 cells. In H69/Txl, the polymerized tubulin ratios were 79.0% and 46.4%, respectively. There was no significant difference in polymerized tubulin ratio between the parental H69 and H69/Txl cells. 1, total tubulin of parental cells. 2, polymerized tubulin of parental cells. 3, total tubulin of taxol-resistant cells. 4, polymerized tubulin of taxol-resistant cells.

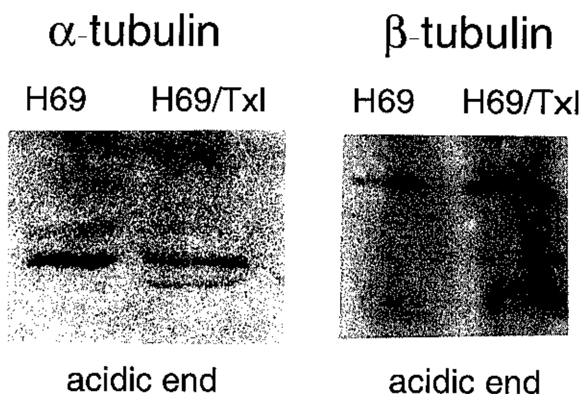

Fig. 5. Estimation of tubulin in H69 parental and H69/Txl cells using isoelectric focusing and Western blotting with anti-α- and β-tubulin antibodies. Altered mobilities of α-tubulin in H69/Txl were observed. In H69, two α-tubulin isoforms were evident, compared with three in H69/Txl. Two of the latter comigrated with the isoforms of H69, and the other was more acidic.

that of H69 cells. The protein content of H69/Txl (150 μg/1×10^6 cells) was lower than that of H69 (270 μg/1×10^6 cells).

Growth-inhibitory effect of anti-microtubule agents The growth-inhibitory effects of taxol and anti-microtubule agents in H69 and H69/Txl cells were evaluated by MTT assay, and the IC_{50}'s values are shown in Table III. The relative taxol resistance of H69/Txl compared with H69 was 4.73-fold. Higher sensitivity to vinca alkaloids such as vindesine ($\times$0.51), vincristine ($\times$0.50), and vinblastine ($\times$0.55), which can induce microtubule disassembly, was observed as compared with the parental H69 cells (Table III). Thus, the resistance pattern of H69/Txl cells seemed to be different from that of the typical multidrug resistance phenotype.

Accumulation study To determine whether decreased drug accumulation was a cause of taxol resistance, we examined the intracellular accumulation of [^{3}H]taxol in H69 parental and H69/Txl cells. Fig. 2 shows the intracellular concentrations of [^{3}H]taxol after addition of 10, 100 or 1,000 nM [^{3}H]taxol to the culture medium for 3 h. The amounts of intracellular radioactivity in both H69 and H69/Txl cells increased with the increase in concentration of [^{3}H]taxol in the medium. There was no

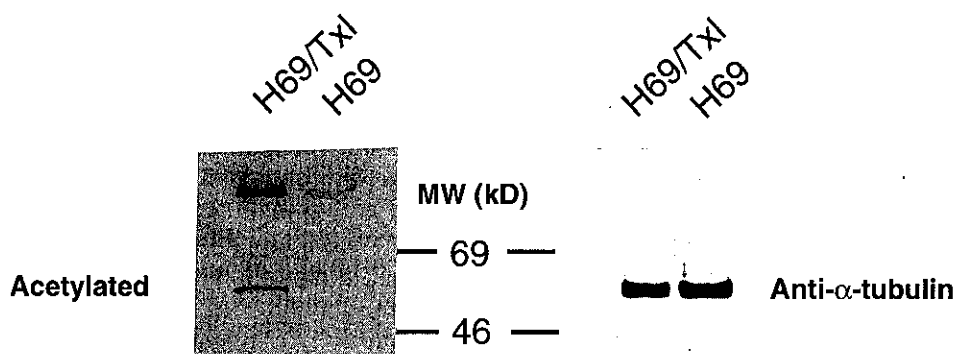

Fig. 6. Estimation of acetylated tubulin in H69 parental and H69/Txl cells using [^{14}C]acetyl-coenzyme A. Western blotting with anti-α-tubulin antibody was performed for normalization. We observed acetylation of α-tubulin in H69/Txl, but not in H69 parental cells.

significant difference in the intracellular [^{3}H]taxol contents of H69 and H69/Txl cells at any of the concentrations tested. This result suggests that an alteration of intracellular accumulation of [^{3}H]taxol is not responsible for the resistance of H69/Txl cells to taxol.

RT-PCR To confirm that H69/Txl did not have the P-glycoprotein-mediated multidrug resistance phenotype, we examined the MDR-1 mRNA expression in H69/Txl by RT-PCR analysis. Etoposide-resistant H69/VP cells were used as a positive control for typical MDR-1-expressing cells. The cDNA was synthesized from the total cellular RNA isolated from the H69, H69/VP, and H69/Txl cell lines. The amplified cDNA fragments of MDR-1, β_2-MG, and β-actin were separated by electrophoresis. Fig. 3 shows an autoradiogram of the PCR product analyzed by the sequential cycling method. The amplified β-actin and β_2-MG mRNAs were used as internal controls. An increase of β-actin and β_2-MG mRNA with increasing cycle number was observed in the H69, H69/VP, and H69/Txl cell lines. In the H69/VP cell line, increased expression of MDR-1 mRNA was detected. On the other hand, the MDR-1 mRNA was not detected in the parental H69 and H69/Txl cell lines. Thus, it appears that the H69/Txl cell line does not show a typical multidrug resistance phenotype.

Tubulin content We examined other mechanisms which might be responsible for the resistance of H69/Txl cells to taxol (Fig. 4). As H69/Txl showed higher sensitivity to drugs such as vindesine, vincristine and vinblastine, which are known to interact with microtubules, we considered that the changes in total and polymerized tubulin might be related to the taxol resistance of the H69/Txl cell line. There was no significant difference in the total α- and β-tubulin contents between H69 and H69/Txl cells. The polymerized tubulin ratio is defined as the ratio of polymerized tubulin content to total tubulin content. The polymerized tubulin ratios of α-tubulin and β-tubulin were 77.9% and 52.6%, respectively, in the parental H69 cells. In H69/Txl, the polymerized α- and β-tubulin ratios were 79.0% and 46.4%, respectively. There was no significant difference in polymerized tubulin ratio between the parental H69 and H69/Txl cells.

Isoelectric focusing To determine whether the alteration of tubulin itself was a cause of resistance, we estimated the tubulin in H69 parental and H69/Txl cells using isoelectric focusing and Western blotting with anti-α- and -β-tubulin antibodies (Fig. 5). We observed altered mobilities of α-tubulin in H69/Txl. In H69, two α-tubulin isoforms were observed, whereas three were observed in H69/Txl. Two of them comigrated with the isoforms of H69, and the other was more acidic. On the other hand, no difference in β-tubulin was detectable in H69 and H69/Txl.

Acetylation of α-tubulin To clarify the cause of this altered mobility of α-tubulin in H69/Txl, we estimated the acetylation of α-tubulin in H69 parental and H69/Txl cells using [^{14}C]acetyl-coenzyme A (Fig. 6). We observed the acetylation of α-tubulin in H69/Txl, but not in H69 parental cells. These observations suggest that the acetylation of α-tubulin may be responsible for the taxol resistance and/or taxol-dependent growth of H69/Txl.

DISCUSSION

Resistance to taxol and vinca alkaloids has been reported to involve amplification of the gene for a 170-kDa membrane glycoprotein (P-glycoprotein), whose apparent function is to pump various hydrophobic agents (taxol, vinca alkaloids, doxorubicin, and etoposide) out of the cell in an ATP-dependent manner.[6, 18, 19] Not only do P-glycoprotein-positive cells show resistance to taxol, but also resistant cells selected using taxol contain P-

glycoprotein. P-glycoprotein-positive cells exhibit cross-resistance to hydrophobic drugs that diffuse easily across the plasma membrane of the cell, but intracellular accumulation is limited by P-glycoprotein. In the taxol-resistant small-cell lung cancer cell line H69/Txl, which we established, hypersensitivity to tubulin-depolymerizing agents such as vindesine, vincristine and vinblastine was apparent (Table III). The resistance pattern of H69/Txl seemed to be different from that of the typical multidrug resistance phenotype. The drug accumulation was equal in parental H69 and H69/Txl cells. Furthermore, the expression of MDR-1 mRNA was negative by RT-PCR analysis in H69/Txl cells. These data clearly demonstrated that H69/Txl did not have a typical multidrug resistance phenotype involving decreased cellular drug accumulation.

The site of action of taxol and vinca alkaloids is generally considered to be cellular microtubules. We therefore considered that altered expression of tubulin may be important for taxol and vinca alkaloid resistance. Mutant clones of β-tubulin have been isolated from Chinese hamster ovary (CHO) cells.[20] Colcemid resistance is associated with reduction in the binding affinity of tubulin for colcemid.[21] A characteristic property of taxol is its ability to bind directly to β-tubulin *in vitro*, as in the case of colchicine.[4, 22] On the other hand, we observed altered mobility of α-tubulin in H69/Txl. The tubulin dimer is constructed from one α-tubulin and one β-tubulin unit, and thirteen tubulin dimers comprise one microtubule. In H69/Txl, therefore, some changes in α-tubulin may be responsible for taxol resistance and/or taxol-dependent growth. This altered mobility of α-tubulin in H69/Txl suggests a change at either the gene or post-transduction level. Several post-translational modifications are known to occur; phosphorylation of β-tubulin,[23] tyrosination of α-tubulin,[24] and acetylation of α-tubulin[25] have been documented. We observed acetylation of α-tubulin in H69/Txl, but not in H69 parental cells. These observations suggest that the acetylation of α-tubulin may be responsible for the taxol resistance and/or taxol-dependent growth of H69/Txl. Further investigations will be necessary to clarify this. Minotti *et al.*[14] have reported that taxol-resistant CHO mutants have a lower fraction of total tubulin in assembled form, whereas colcemid-resistant mutants have a higher fraction of polymerized tubulin. However, we did not observe any difference in the polymerized tubulin ratio between H69 and H69/Txl.

Several observations have suggested that the integrity of cytoplasmic microtubules influences the initiation of proliferative events after treatment with mitogen, and that microtubule integrity itself may play a regulatory role in the initiation of proliferation.[26, 27] The growth of H69/Txl was partially dependent on taxol, a feature found with other taxol-resistant cell lines. Therefore, in H69/Txl, some changes in α-tubulin may be responsible for taxol-dependent growth.

Basic information about this taxol-resistant cell line could provide a rationale for the development of strategies for the identification and prevention of drug resistance, leading to more effective therapy.

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