Differential Effect of Taxol in Rat Primary and Metastatic Prostate Tumors: Site-Dependent Pharmacodynamics

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Purpose. This study compared the sensitivity of rat prostate MAT-LyLu primary and lymph node metastatic tumors to taxol.

Methods. Tumors were established by subcutaneous implantation of tumor cells in a hind leg (primary site) of male Copenhagen rats. Lymph node metastases were used for serial transplantation. Eleven pairs of primary and metastatic tumors between the sixth and twentieth generations were harvested and maintained as 3-dimensional histocultures. The effects of taxol (24 hr treatment at 1 nM to 10 μM) were measured by the appearance of apoptotic cells, and by the inhibition of DNA precursor (thymidine) incorporation. To determine the basis of differential sensitivity of primary and metastatic tumors to the DNA inhibition, we examined the expression of multidrug resistance p-glycoprotein (Pgp) and the accumulation of ^{3}H-taxol after 24 hr exposure and the retention after a 48 hr washout period.

Results. The fraction of apoptotic cells increased linearly with the logarithm of taxol concentration to a maximal value of 25%; the concentration-response curves for primary and metastatic tumors were superimposable. Taxol produced a sigmoidal, concentration-dependent inhibition of thymidine incorporation; the maximal inhibition of ~40% was reached at 0.1 and 1 μM for primary and metastatic tumors, respectively. Within the primary or metastatic subgroups, the IC_{30} (drug concentration that produced a 30% inhibition of DNA synthesis) among consecutive generations varied by < 5 fold, but the primary tumor consistently showed a lower IC_{30} than the daughter or the parent metastatic tumor (mean, 20-fold; median, 15-fold; range, 6- to 56-fold). The finding that the lower drug sensitivity in metastatic tumors was not exhibited in its daughter primary tumor but was regained in its daughter metastatic tumors suggests that the chemoresistant phenotype is maintained only in lymph nodes and not in the primary site. There were no differences in the Pgp status (neither tumor expressed Pgp), accumulation and retention of taxol in primary and metastatic tumors.

Conclusions. Taxol induced apoptosis and inhibited DNA synthesis in the rat MAT-LyLu primary and lymph node metastatic tumors. The apoptotic effect was not different among the two tumors, whereas the primary tumor was more sensitive to the inhibition of DNA synthesis. The differential sensitivity of the two tumors to the DNA effect is not associated with a difference in Pgp expression, drug accumulation nor drug retention, and appears to be associated with changes that are linked to lymph node metastasis.

KEY WORDS: taxol; pharmacodynamics; rat MAT-LyLu prostate tumors; primary and metastatic tumors; apoptosis; p-glycoprotein.

INTRODUCTION

Taxol is an important anticancer agent with significant activity in human cancers including ovarian, breast, non-small cell lung, and head and neck cancers (1). The encouraging results of taxol in multiple human solid tumors have prompted phase I/II trials of taxol as a single agent in advanced prostate cancer patients. The initial trials of taxol at doses of 135–170 mg/m^2 showed only minor activity with a <5% response against advanced prostate cancer (2). Taxol has not been studied in early stage prostate disease which is treated by hormone ablation and seldom by chemotherapy. Advanced disease is usually accompanied by metastatic spread. It is not known if taxol has differential effects against primary and metastatic prostate tumors.

Taxol has multiple pharmacologic effects. It promotes the polymerization and stabilizes microtubules, induces abnormal mitotic spindle asters during cell division, causes blockade at the G_2/M interphase, inhibits DNA synthesis, inhibits cell motility, and induces apoptosis, or programmed cell death (3–8). The mechanism by which taxol induces apoptosis is unclear.

The multidrug resistance (MDR1) p-glycoprotein (Pgp) has been implicated in tumor resistance to chemotherapeutic agents. The structural features of taxol, i.e. natural product, high molecular weight, and hydrophobicity, favor it as a substrate for Pgp (9). Over-expression of Pgp has been associated with taxol resistance (10,11). Decreased intracellular taxol accumulation was observed in several Pgp-positive and taxol-resistant tumor cell lines (10–12). Transgenic mice expressing the transferred MDR1 gene in their bone marrow cells were about 10-fold more resistant to taxol-induced leukopenia compared to mice with normal bone marrow (13). Furthermore, the taxol resistance can be reversed by MDR1-reversal drugs, supporting the role of MDR1 in taxol resistance (14,15). Additional mechanisms of taxol resistance include overproduction of tubulin, acetylation of α-tubulin, mutation of the α-tubulin gene, and alteration of β-tubulin subunits (9), formation of polyploid cells (16), and reduction of cellular glutathione (17).

The present study was to compare the taxol-induced inhibition of proliferation and apoptosis in primary and metastatic tumors, and to determine if differential expression of Pgp protein, intratumoral accumulation and retention of taxol accounted for the differential pharmacodynamics of taxol in primary and metastatic tumors. The tumor model was the rat prostate MAT-LyLu tumor. This tumor when implanted subcutaneously metastasizes to lungs and regional lymph nodes (18). The comparative chemosensitivity studies were done using 3-dimensional solid tumor histocultures. The major advantages of the histoculture system are the maintenance of a 3-dimensional tissue structure and organization, co-existence of tumor and stromal cells, cell-cell interaction, and intra-tumoral heterogeneity (19). The maintenance of tissue architecture is critical because the interaction between the tumor and normal cells may be important for prostatic epithelial growth and response to androgen stimulation (20,21).

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ABBREVIATIONS: LI, labeling index; MDR1, multidrug resistance gene; Pgp, multidrug resistance p-glycoprotein; E_{max}, maximal inhibition of thymidine LI; IC_{30}, drug concentration needed to produce a 30% inhibition of thymidine LI; PBS, phosphate buffered saline.

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MATERIALS AND METHODS

Chemicals and Supplies

Taxol was a gift from Bristol Myers Squibb Co. (Wallingford, CT). 3″-^{3}H-taxol (specific activity 19.3 Ci/mmol) was supplied by the National Cancer Institute (Bethesda, MD). Sterile pigskin collagen (Spongostan standard) was purchased from Health Designs Industries (Rochester, NY), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), gentamycin from Solo Pak Laboratories (Franklin Park, IL), RPMI medium from GIBCO Laboratories (Grand Island, NY), methyl-^{3}H-thymidine (specific activity, 61 Ci/mmole) from Moravek Biochemicals (Brea, CA), NTB-2 nuclear track emulsion from Eastman Chemicals (Rochester, NY), and the mouse antihuman Pgp monoclonal antibody (JSB-1) from BioGenex (San Ramon, CA). All chemicals and reagents were used as received. Male Copenhagen rats were obtained from Harlen Biomedicals (Dawley, OH). The research for experimental animals were adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Tumor Implantation and Procurement

The rat MAT-LyLu tumor cells was a gift from Dr. J. Isaacs of the Johns Hopkins University (Baltimore, MD). This tumor can be maintained as a cell line or as a solid tumor in vivo in rats. As a cell line, cells were maintained in RPMI medium supplemented with 9% heat-inactivated fetal bovine serum, 200 mM glutamine, 100 μg/ml gentamycin and 95 μg/ml cefotaxime. Cells were harvested from subconfluent cultures and resuspended in serum-free medium after trypsinization. Cells with greater than 90% viability, as determined by trypan blue exclusion, were used for transplantation. The initial tumor establishment was achieved by subcutaneously implanting 10^6 cells (0.1 ml) with a 18 gauge needle to a hindlimb of a male Copenhagen rat. The primary tumor was removed when it was between 0.5 to 1 g in size, disected into 1 mg fragments, and implanted subcutaneously into recipient animals with a 15 gauge needle. Transplants of this initial primary tumor resulted in metastases to lungs and inguinal lymph nodes in 100% and 50% of animals, respectively. We found that re-implantation of lymph node metastatic tumors subcutaneously to the primary site increased the incidence of nodal metastasis to 80%, occurring between 14 to 16 days. Subsequent studies used lymph node metastases for serial transplantation.

Tumor Histocultures

Histocultures of solid tumor fragments used tumor transplants of the sixth to the twentieth generation. We used 11 pairs of primary and lymph node metastatic tumors from the same hosts. The average animal body weight was 250 ± 30 g.

Following surgical removal, tumor specimens were placed immediately in RPMI medium and prepared for culturing within 30 min postsurgery. The non-necrotic portions of the tumor were cut into 1mm^3 fragments and cultured on 1 cm^2 presoaked collagen gels at 37°C, as previously described (22). The culture medium was identical to that used for maintaining the cell line. All tumors were used for experiments after 2 days in culture.

Pharmacologic Effects of Taxol

Taxol stock solution was prepared in ethanol. Sufficient volume of stock solution was added to the culture medium so that the final ethanol concentration was <0.1%.

Two effects of taxol were determined, i.e., inhibition of DNA synthesis and apoptosis. Inhibition of DNA synthesis was measured by the inhibition of thymidine incorporation in tumor cells. Tumor histocultures were exposed to various concentrations of taxol ranging from 1 nM to 10 μM for 24 hr. These concentrations are equivalent to about 1% to 1,000% of the clinically achievable concentrations (23). After drug treatment, the medium was exchanged and the tumors were washed three times with 4 ml of drug-free medium, and incubated with 4 ml of [^{3}H]thymidine (1 μCi/ml) for 60 hr. This was the optimal time that yielded maximal thymidine labeling before the culture medium turned acidic. Thereafter, histocultures were fixed in 10% neutralized formalin and embedded in paraffin. The embedded tissues were cut into 5 μm sections using a microtome, deparaffinized and analyzed for thymidine labeling using autoradiography. Controls were processed similarly, with the exception of drug treatment. The thymidine-labeled cells in the most active area were counted using a Zeiss Axiovert 35 microscope (Zeiss, Thornwood, NY) equipped with a 7 × 7 mm disc micrometer in the right eyepiece (Thomas Scientific, Swedesboro, NJ). Thymidine labeling index (LI) was defined as the number of labeled nuclei divided by the total number of nuclei within the grid at 400 × magnification. Apoptosis was evaluated using light microscopy by morphological changes, i.e., chromatin condensation, disappearance of nucleoli, formation of membrane blebs, apoptotic bodies and cell shrinkage (24). A separate study in our laboratory showed that this method gave identical results to the terminal deoxynucleotide transferase-mediated DNA-end labeling method (25). A typical experiment used a total of 12 to 20 tumor pieces for each drug concentration. A minimum of 100 cells per tumor piece, of ≥1,200 cells were counted per concentration.

Pharmacodynamic Data Analysis

The plot of thymidine LI, expressed as the percent of control, versus the logarithm of taxol concentration was analyzed using equation 1 and nonlinear least-squares regression (NLIN, SAS, Cary, NC). Values for IC_{30} (the drug concentrations needed to produce 30% inhibition) were determined.

$$E = (E_0 - Re) \cdot (1 - \frac{C^n}{K^n + C^n}) + Re \qquad (1)$$

where E is the LI of drug-treated tissues, C is the drug concentration, E_0 is the LI of untreated controls, K is the drug concentration at one-half E_0, n is a curve shape parameter, and Re is the residual fraction. Equation 1 is a modification of the more commonly used equation that describes a sigmoidal concentration-effect relationship for a spectrum of effect from 0% to 100%. Inclusion of the Re term is necessary to describe the less-than-complete effect, i.e., the average maximum inhibition of DNA synthesis by taxol was about 40% (see Results).

Accumulation and Retention of Taxol in Tumors

Tumor histocultures were incubated with culture medium containing 50 μCi/ml ^{3}H-taxol in 10 nM or 1 μM of unlabeled taxol (the final concentration of ^{3}H-taxol was 3 nM). Three pairs of primary and metastatic tumors were studied. Twenty to thirty fragments per tumor were used for each concentration. After being exposed to taxol for 24 hr, tumor histocultures were removed. One-half (about 12 histocultures) were used to determine drug accumulation and one-half for retention. For the accumulation study, the collected histocultures were processed immediately. For the retention study, the histocultures were washed 3 times with drug-free medium and incubated in drug-free medium for an additional 48 hr. A separate experiment showed that washing the fragments in drug-free medium removed <2% of total drug taken up by tumors (Kang, H.J.K. and Au, J.L.-S., manuscript in preparation). To determine drug concentration, the histocultures were weighed, and solubilized in 1 ml of 0.5 M solubilizer (Packard Instrument, Meriden, CT) at 55°C in an oven overnight. The total radioactivity were determined using a liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

Immunohistochemistry

The expression of Pgp was detected by immunohistochemical staining as described elsewhere (25). Briefly, tissue sections were de-paraffinized and rehydrated sequentially in xylene, ethanol and phosphate buffered saline (PBS), and boiled in a 0.1 M citrate buffer, pH 6.0, in a microwave oven for antigen retrieval. The slides were then cooled and washed in PBS, incubated in Dako blocking solution for 10 min to eliminate non-specific binding, and then with the JSB-1 antibody (1:100 dilution in 5 mg/ml bovine serum albumin) for 2 hr. Incubation was carried out in a humidified chamber at room temperature. After washing with PBS, the slides were incubated with biotin-conjugated secondary antibody and peroxidase-conjugated streptavidin solutions, again washed with PBS, and then incubated with the chromogen diaminobenzidine for 5 to 8 min and counterstained with hematoxylin. Rat renal proxima tubules were used as positive control.

Statistical Analysis

Differences in mean IC_{30} and E_{max} values between groups were analyzed using the paired Student's t test. Linear regression analysis was used to determine if there was a relationship between IC_{30} values and tumor generation numbers. Software for statistical analysis was by SPSS Inc. (Chicago, IL).

RESULTS

Tumor Histocultures and Pgp Expression

A pilot study to determine the culturing conditions for histocultures indicated that tumor histocultures were viable for at least one week, as indicated by the presence of more than 100 viable tumor cells per histoculture. For both primary and lymph node metastatic tumors, visible size enlargement of the histocultures was evident after 4 days of culturing. The 60 hr cumulative thymidine LI in untreated controls was 66.3 ± 7.0 and 64.3 ± 6.6 for primary and nodal metastatic tumors, respectively (n = 11 each, $p > 0.5$).

Rat renal proximal tubules, known to express Pgp, were stained positive by the JSB1 antibody (Figure 1A), indicating that the antibody detected Pgp in rat tissues. However, all eleven pairs of primary and nodal metastatic tumors were Pgp-negative before (Figures 1B & 1C) and after taxol treatment (Figures 1D & 1E), indicating that the rat MAT-LyLu tumors did not express Pgp.

Pharmacodynamics of Taxol-Induced Apoptosis

Figure 2 shows the detection of apoptotic cells by morphological changes. For both primary and metastatic tumors, the maximum fraction of apoptotic cells in untreated controls was about 4%. Induction of apoptosis by taxol was concentration-dependent. No increase was evident at 1 nM, whereas at higher concentrations, the apoptotic fraction increases linearly with the logarithm of taxol concentrations (Figure 3). There were no differences in the apoptotic indices for primary and metastatic tumors at all taxol concentrations (Figure 3). The maximal apoptotic index at the highest concentration of 10 μM was 23.8 ± 4.5% in primary tumors and 23.5 ± 3.0% in metastatic tumors. The coefficients of variation in the apoptotic fractions were between 10 to 28% at all taxol concentrations.

Pharmacodynamics of Inhibition of Thymidine Labeling

Figure 4 shows the concentration-response relationship for taxol-induced inhibition of thymidine labeling. Table 1 summarizes the pharmacodynamic data. Taxol produced a sigmoidal, concentration-dependent inhibition of thymidine labeling. Compared to metastatic tumors, primary tumors showed a significantly higher maximal inhibition (E_{max}) and required a significantly lower taxol concentration to produce E_{max} (0.1 μM vs 1 μM). After reaching E_{max}, a 10 to 100-fold increase in drug concentration to 10 μM did not significantly enhance the inhibition.

Within the subgroups of primary or metastatic tumors from different generations, the IC_{30} showed a relatively small variation of between 4 to 5 fold. For example, the lowest and highest IC_{30} were 5.5 and 23.5 nM for primary tumors, and 96.3 and 418.1 nM for metastatic tumors (Table 1). The IC_{30} values were randomly distributed with respect to the generation number, indicating no relationship between taxol sensitivity and the number of tumor passage ($r^2 = 0.09$ and $p = 0.92$ for primary tumors; $r^2 = 0.16$ and $p = 0.88$ for metastatic tumors). The replica experiment performed using the same generation of tumors derived from two hosts (tumors 10 and 11) showed a similar variation. These data indicate relatively small inter-tumor variations. In contrast, IC_{30} for all primary tumors were consistently lower than their parent or paired metastatic tumors ($p = 0.0001$ for both cases). The median and mean ratio of IC_{30} for primary tumors and paired or parent metastatic tumors were 15- and 20-fold, respectively. This indicates a lower taxol sensitivity in the metastatic tumors.

Accumulation and Retention of Taxol in Tumors

Table 2 summarizes the accumulation and retention of taxol. The concentration of taxol in tumor histocultures, after 24 hr exposure to 10 nM or 1 μM taxol, were between 20 to

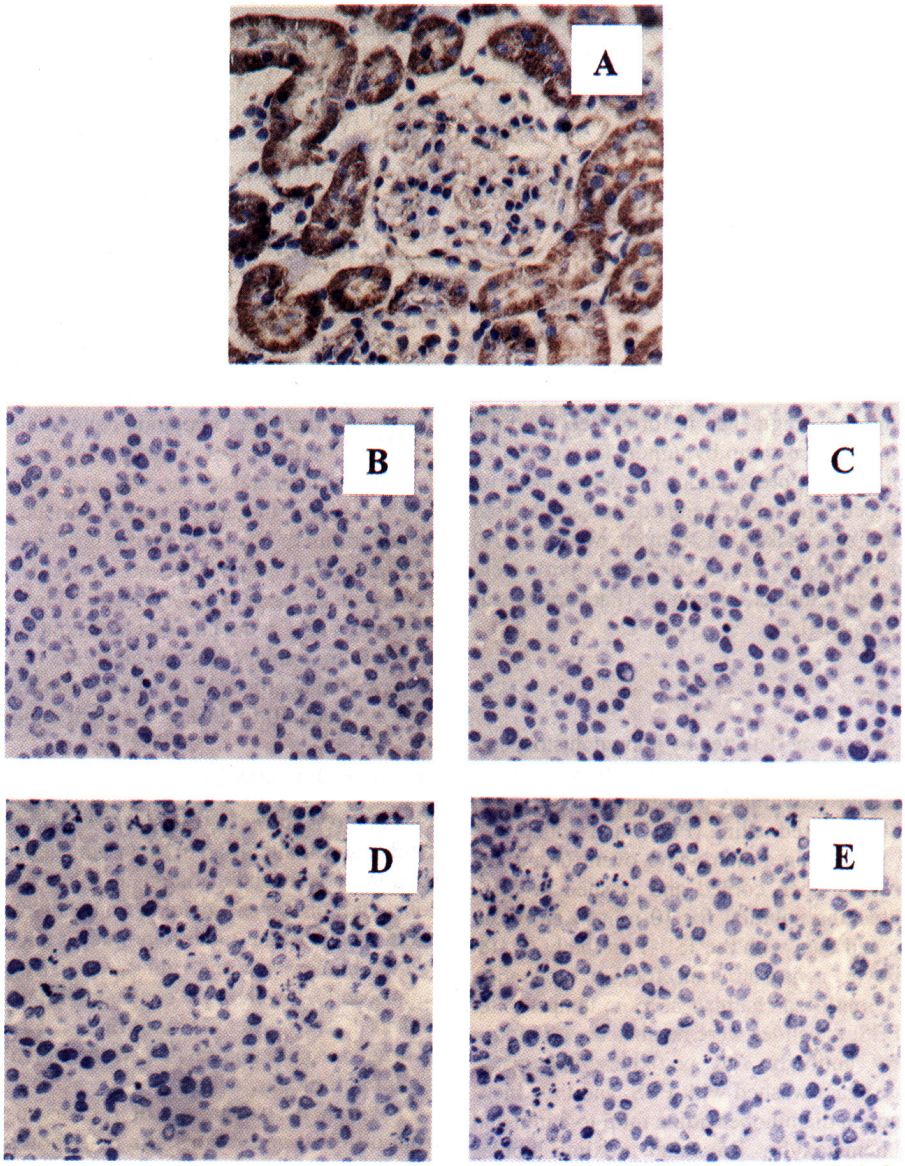

Fig. 1. Pgp expression in rat renal proximal tubules (A) and lack of Pgp expression in rat MAT-LyLu primary and metastatic tumors untreated controls (B & C) and treated with 10 μM taxol (D & E). Pgp was detected by immunohistochemical staining (brown color) with the JSB-1 antibody.

30 times the initial extracellular concentration in the medium. About 30% of the drug was retained after a 48 hr washout period. No significant differences in accumulation or retention were found between primary and metastatic tumors.

DISCUSSION

The present study demonstrated that taxol inhibited DNA synthesis and induced apoptosis in rat MAT-LyLu primary and nodal metastatic tumor histocultures. Inhibition of DNA synthesis is an antiproliferation and therefore cytostatic effect, whereas apoptosis is a cytocidal event. Neither effect was complete at 10 μM, a concentration that is 10 times the maximal plasma concentration achieved at clinically relevant doses in patients (23). Primary and metastatic tumors responded equally to the apoptotic effect of taxol, and there were little inter-tumor variations in the maximal apoptotic fraction. In contrast, primary and metastatic tumors responded differently to the DNA effect with the primary tumor being 19 fold more sensitive (Table 1). The inter-tumor variation in the IC_{30} of DNA synthesis was <5-fold, which is relatively small compared to the >160-fold variation observed for head and neck, bladder, and prostate tumors from human patients (24,28). The lower variability in chemosensitivity in rat tumors as compared to human tumors may be because the animal tumor is a transplanted tumor propagated in inbred rats, whereas human tumors are from patients of different genetic background and environmental exposure.

Our finding of an incomplete inhibition of DNA synthesis in rat MAT-LyLu tumors is in agreement with findings in human cancer cell lines by other investigators (26,27) and our findings in other human solid tumors (24,28). The incomplete inhibition indicates that a fraction of tumor cells, in the presence of taxol, is capable to enter the S phase. This observation is unique to

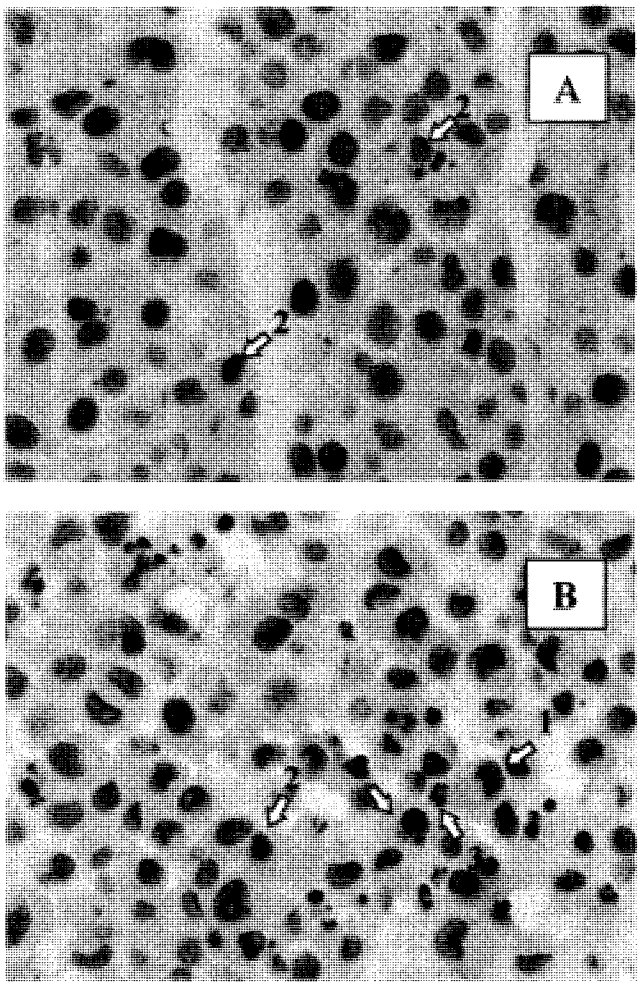

Fig. 2. Taxol-induced apoptotic cells. Apoptotic cells were evaluated by morphological changes: condensation and migration of nuclear chromatin (arrow 1), progression of condensed chromatin with loss of nuclear membrane (arrow 2), formation of apoptotic bodies and cell shrinkage (arrow 3). The apoptotic fraction was 3.6% (3/83 cells) in untreated control (A) and 21.7% (20/92 cells) in treated with 10 μM taxol (B) of a primary tumor (tumor number 8).

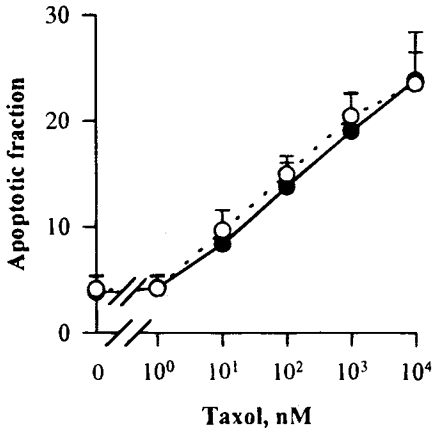

Fig. 3. Concentration-response of taxol-induced apoptosis. The fraction of apoptotic cells increased linearly with respect to the initial taxol concentration in culture medium. Solid symbols: primary tumors, open symbol: metastatic tumors. Mean + one SEM., n = 11 each.

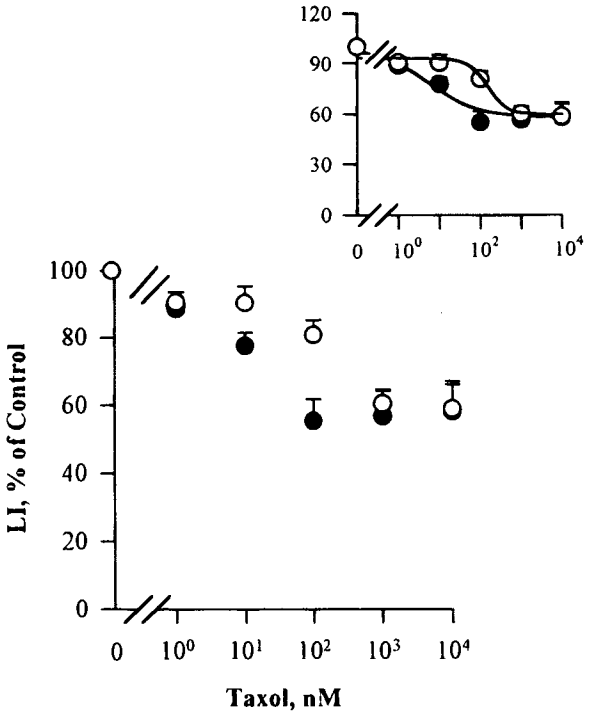

Fig. 4. Concentration-response of taxol-induced inhibition of thymidine labeling. The inhibition of thymidine labeling as a function of initial taxol concentration in culture medium. Solid symbols: primary tumors, open symbols: metastatic tumors. Mean + one SEM., n = 11 each. Inset: data from one pair of primary (solid symbols) and lymph node metastatic (open symbols) tumors. The lines are computer-fitted lines using equation 1.

taxol, since most anticancer agents produce complete inhibition. For example, we reported complete inhibition of DNA synthesis, accompanied by necrosis, in human bladder and head and neck tumor histocultures by mitomycin C, doxorubicin, cisplatin, and 5-fluorouracil (29–31). The therapeutic implications of an incomplete cytostatic effect are not known.

We examined the basis of the differential sensitivity among primary and metastatic tumors. The lack of Pgp expression before and after taxol treatment as well as the similar amount of intra-tumoral ^{3}H-taxol in primary and metastatic tumors (Table 2) indicates that the differential chemosensitivity was not due to differential expression of Pgp and differential drug accumulation and retention. It is noted that we measured total radioactivity. Although extensive intracellular metabolism of taxol is unlikely because taxol is mainly metabolized by liver microsomal p450 isozymes (32), a different taxol activation or deactivation in the two tumors cannot be ruled out.

Other possible causes of the differential sensitivity among primary and metastatic tumors to the DNA effect of taxol are host-related inter-tumor heterogeneity, differences in proliferation rates, and tissue-specific environment-related changes. The inter-tumoral variations in the IC_{30} within each of the subgroups of primary and metastatic tumors (<5-fold) was significantly lower than the 19-fold differences in sensitivity, indicating that host-related inter-tumor heterogeneity is not likely to be the major cause. The identical thymidine LI in primary and metastatic tumors rules out differences in proliferative status as the cause of differential taxol sensitivity. The

Table 1. Differential Sensitivity of Primary and Nodal Metastatic Tumors to Taxol-induced DNA Precursor Incorporation. Lymph Node Metastatic Rat MAT-Lylu Tumors Were Harvested and Implanted in Hind Legs (primary site) of Recipient Rats. This Sequence Was Repeated to Create a Series of Generations. Primary and Lymph Node Metastases from the Sixth to Twentieth Generations Were Used. Tumors Were Removed from Hosts, Cultured as Solid Tumor Histocultures, and Treated with Taxol. The IC_{30} and E_{max} Were Calculated Using Equation 1. Tumors 10 and 11 Were from Two Hosts of the Same Generation. NA, Not Applicable

		Primary		Metastatic		Ratio of IC_{30} for metastatic:primary	
Experiment	Generation	IC_{30} μM	E_{max} %	IC_{30} μM	E_{max} %	paired[a]	parent: daughter[b]
1	6	7.4	38.1	204.4	36.5	27.6	NA
2	7	9.0	65.9	96.3	35.7	10.7	22.7
3	8	15.3	50.6	294.0	41.0	19.2	6.3
4	10	18.2	40.7	274.0	42.1	15.1	16.2
5	11	19.4	42.7	143.1	34.2	7.4	14.1
6	13	11.1	42.2	125.5	35.1	11.3	12.9
7	14	23.5	37.5	154.2	25.4	6.6	5.3
8	17	14.4	37.1	418.1	31.2	29.0	10.7
9	18	16.3	42.0	228.6	36.8	14.0	25.7
10	20	13.2	40.3	287.1	30.8	21.8	17.3
11	20	5.1	46.7	272.0	27.1	53.3	56.3
Mean	NA	13.9[c]	44.0[d]	227.0[c]	34.2[d]	19.6	18.8
S.D.	NA	5.5	8.3	94.4	5.2	13.5	14.7
Median	NA	14.4	42.0	228.6	35.1	15.1	16.2

[a] Ratio of IC_{30} for metastatic and primary tumors from the same host (i.e., same generation).
[b] Ratio of IC_{30} for parent metastatic tumor (i.e., the previous generation) and daughter primary tumor. In the cases where the immediately preceding generation was not available, the ratio was calculated using the next preceding generation, e.g., the ratio for tumor number 8 (17th generation) was calculated using the IC_{30} of the fourteenth generation metastatic tumor and the IC_{30} of the seventeenth generation primary tumor.
[c] IC_{30} of primary tumors is smaller than IC_{30} of metastatic tumors ($p < 0.0001$).
[d] E_{max} of primary tumors is greater than E_{max} of metastatic tumors ($p < 0.0001$).

remaining likely causes are tissue-specific changes associated with lymph node metastasis such as environment and/or preferential growth of pre-existing resistant clones in lymph nodes. This possibility is supported by literature data and our present findings, as follows. Other investigators used animal models to show that implantation of tumor cells from the same source in different organs or allowing the implanted cells to metastasize to other organs result in differential chemosensitivity among tumors from different organs (either primary implant site or metastatic sites) (33–37). These data suggest a link between the tissue-specific environment and chemosensitivity (33–37). The experimental design of these earlier studies, i.e., evaluation of drug effect on the in vivo growth of tumors in different organs derived from transplanting cells of the same generation was limited to establishing the influence of organ environment (33–37). The experimental design of the present study, in addition to establishing the differential taxol sensitivity of primary and lymph node metastatic tumors, also addressed the question whether the lymph node-specific chemoresistance was unstable and/or reversible. Data in Table 1 show that the lower sensitivity of parent lymph node metastases was not exhibited in its daughter primary tumors but was regained in its daughter metastatic tumors. This novel finding indicates that the mechanism by which microenvironment affects tumor chemosensitivity is the maintenance of the chemoresistant phenotype in the metastatic lymph node site and not in the primary site.

Instability of drug-induced chemoresistance has been shown for alkylating agents in murine tumors; the chemoresistance observed in mice disappeared when tumors were harvested and grown in monolayer cultures (35,38). The rat MAT-LyLu primary and lymph node metastatic tumor histocultures,

Table 2. Uptake and Retention of Taxol in Tumor Histocultures. Tumors Were Removed from Hosts, Cultured as Solid Tumor Histocultures, and Incubated with ^{3}H-taxol. The Amounts of Radioactivity in Tumors After a 24 hr Uptake Period and After a 48 hr Washout Period Were Determined. Mean ± S.D. of Three Experiments (tumor numbers 9, 10, and 11 listed in Table 1). Each Experiment Used 20–30 Tumor Histocultures for each Concentration

	Tissue concentration, μM					
	Accumulation at 24 hr			Retention after 48 hr washout		
Initial extracellular concentration μM	Primary	Metastatic	p	Primary	Metastatic	p
0.01	0.19 ± 0.02	0.23 ± 0.1	0.59	0.06 ± 0.02	0.07 ± 0.03	0.64
1	21.6 ± 3.3	28.2 ± 3.0	0.17	7.64 ± 1.31	8.01 ± 0.95	0.76

because of their reproducible differential sensitivity to taxol, represents a unique, renewable model for in vitro investigation of tissue- and/or metastasis-specific chemosensitivity. Studies are ongoing in our laboratory to elucidate the tissue-specific cellular and molecular changes associated with differential taxol sensitivity.

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