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Multiple factors other than p53 influence colon cancer sensitivity to paclitaxel

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Abstract *Purpose*: To determine factors which influence the sensitivity of human colorectal carcinoma cell lines to paclitaxel. *Methods*: The paclitaxel sensitivity of ten human colorectal carcinoma cell lines, and a panel of RKO colon carcinoma cell lines, isogenic except for p53 status, were studied. The inhibitory concentrations causing a 50% decrease in growth (IC_{50}) were assayed after 3, 24, and 96 h after paclitaxel exposure. The doubling time (DT) and cell cycle parameters of cells were also measured. The expression of the multidrug resistance glycoprotein-1 (MDR-1), bcl-2 and bax was quantitatively assessed by immunoblotting. *Results*: Mean IC_{50} values at 24 and 96 h drug exposure were about 1.5 logs lower than the IC_{50} values at 3 h, regardless of the p53 status. No difference was found between the IC_{50} values of wild-type and mutant p53 cells, or among the RKO panel of cells. Correlation analysis showed that: (1) resistance was associated with longer DTs, but this was generally abated by a 96-h exposure; (2) with a 3-h exposure, the combination of MDR, bcl-2 and bax parameters with DT (DT + MDR + bcl-2–bax) best correlated with IC_{50} values ($r = 0.77$); (3) with a 96-h exposure, in spite of the generally decreased IC_{50} values, a combination of MDR-1, bcl-2 and bax parameters (MDR + bcl-2–bax) best correlated with the IC_{50} values ($r = 0.71$). *Conclusions*: These results suggest that the exposure duration, DT, and expression of MDR-1, bcl-2 and bax each contribute to paclitaxel sensitivity of human colorectal carcinoma cells. In assessing paclitaxel drug resistance, multiple factors should always be considered. There may be a therapeutic window for taxanes in colon cancer by optimizing pharmacokinetics and modulating MDR-1 and bcl-2 resistance factors.

Key words Paclitaxel · p53 · MDR-1 · bcl-2 · bax

Introduction

There are a limited number of drugs which have clinical efficacy for patients with advanced colorectal cancer [2, 30]. Therefore, a study was initiated to study various parameters which might increase or decrease the sensitivity of human colon cancer cells to the microtubule spindle toxin, paclitaxel. A better understanding of paclitaxel resistance factors in colorectal cancer cells may facilitate the use of taxanes in this type of cancer.

We were initially interested in whether p53 alterations altered sensitivity to paclitaxel. Alteration in the p53 tumor suppressor gene in human colorectal carcinoma occurs in 70–80% of cases [1, 34, 45]. Paclitaxel overstabilizes the tubulin polymer and is most active in cycling cells at the G_2M phase of the cell cycle [17, 27]. Wild-type p53 (WT-p53) controls cell cycle progression through the G_1/S checkpoint via induction of p21/WAF expression [21, 25]. Loss of p53 function may lead to increased paclitaxel sensitivity by several mechanisms, such as through an increase in the percentage of cells entering G_2M [7, 24, 28, 33], or through the loss of potential DNA repair activity [39, 49]. Conversely, loss of p53 function may decrease paclitaxel sensitivity due to the impairment of p53-dependent apoptotic mechanisms, such as the induction of bax relative to bcl-2 expression [15, 31, 32]. In addition, since others have

This work is dedicated to the memory of the sister of the senior author, Vivian Lee, whose faith in Christ overcame the burden of her cancer, and whose life instilled hope and joy in others.

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observed that prolonged exposure to paclitaxel increases the sensitivity of breast [26] and non-small-cell lung cancer cells [12], we also investigated how exposure duration, cell cycle and other molecular parameters might influence chemosensitivity of human colorectal cancer cells to paclitaxel.

Material and methods

Tumor cell lines and confirmation of p53 status

Nine human colon carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, Md.). The RKO colon cancer cell line, RKO-RC10.1 in which p53 is degraded through constitutive expression of HPV-16E6, along with a control clone, RKO-RCneo were gifts from K. Cho [23]. All culture reagents were obtained from GIBCO/BRL (Gaithersburg, Md.). All cells from ATCC were cultured in RPMI-1640 supplemented with 10% fetal calf serum, penicillin 100 U/ml and streptomycin 100 mg/ml, and glutamine 2 m*M* (complete medium). RKO cells were cultured in McCoy's 5A, instead of RPMI-1640, with the addition of 0.5 mg/ml G418, for the RKO-RCneo and RKO-RC10.1 sublines. P53 status was confirmed by single-strand conformation polymorphism (SSCP) analysis. Total genomic DNA was extracted from cell lines using an established protocol [38]. Exons four through nine of the p53 gene were amplified with human p53 primers (Clontech, Palo Alto, Calif.). A volume of 25 µl polymerase chain reactions (PCR) mix contained 1 U Taq polymerase (Roche, Branchburg, N.J.), 1× standard buffer (Roche), 200 µ*M* (final concentration) deoxyribonucleotide mix (Roche), 2 µCi ^{32}P-deoxycytidine triphosphate (Amersham, Arlington Heights, Ill.), 0.4 µ*M* (final concentration) of each primer and 200 ng of template DNA. The reactions were thermocycled at 94 °C for 5 min, 40 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min, followed by 72 °C for 10 min. PCR reaction (3 µl) was loaded on MDE gels (AT Biochem, Malvern, Pa.), run at 15 °C with the Stratatherm Cold System (Stratagene, La Jolla, Calif.). Radiograph exposures were examined for band shifts distinct from control DNA.

In vitro drug sensitivity and doubling time assays

Actively growing cells were trypsinized, resuspended in complete medium, and 90 µl containing 1×10^3 cells (for SW620, SW948, COLO 205, HT29 and the RKO panel) or 2×10^3 cells (for SW48, SW1116, SW1417 or Lovo) was distributed to quadruplicate wells of multiple columns of three 96-well plates (Corning, Corning, N.Y.). The cells were incubated for 48 h to allow adherence. Paclitaxel (Taxol) and Cremophor (negative control) supplied by Albert Favioletti (Drug Management and Authorization Section, NCI, Bethesda, Md.) were diluted on the day of administration in RPMI-1640 and added in 10-µl volumes to achieve final concentrations ranging from 0.0001 to 10 µ*M*. After 3 or 24 h of drug exposure, the wells of two of the plates were gently rinsed twice with 100 µl 1 × PBS (Biofluids, Rockville Md.), and refed with 100 µl complete medium. After 96 h from the time drug was first added, cell mass was assessed with the MTS assay (Promega, Madison, Wis.). Absorption values were corrected for the background in the wells with medium only, and normalized to the absorption of wells with cells without drug. The mean and standard deviation of the quadruplicate values was plotted as a dose response curve. The concentrations causing a 50% inhibition in cell growth (IC_{50}) were calculated based on a nonlinear regression fit with the Prism 2.0 software (GraphPad Software, San Diego, Calif.). Doubling times (DT) were determined by growing 1000 or 2000 cells per well (as above) in replicate dishes, and the cell mass was assayed by the MTS assay on day zero and daily for six consecutive days. DT was also calculated based on a nonlinear regression fit with Prism 2.0. The final IC_{50} and DT values for each cell line were derived from the mean of values from two independent experiments.

Cell cycle analysis

Actively growing cells were trypsinized and washed twice in cold sample buffer (PBS with glucose, 1 g/l), and fixed in ice-cold 70% ethanol. Cells were stained with 100 µg/ml propidium iodide (Sigma) with RNAse A 100 U/ml (Sigma) for 30 min at room temperature. DNA flow cytometry was performed with a FACSCAN (Becton-Dickinson, Mountain View, Calif.), and data were analyzed with Multicycle software (Phoenix/Flow Systems, San Diego, Calif.). The final values representing the percentage of cells in the G_2M phase (%G_2M) are the means from two independent cell cycle studies.

Immunoblotting

Immunoblotting was performed as previously described [48] with the following modifications. Actively growing cells were harvested and 50 µg of each sample was electrophoresed on a polyacrylamide gel and transferred to nitrocellulose membrane (Hybond-C Extra, Amersham, Arlington Heights Ill.). The membranes were incubated with the anti-MDR-1 monoclonal antibody, C-219 (Signet Laboratories, Dedham, Mass.) at 2 µg/ml, anti-bcl-2 monoclonal antibody, Ab-1 (Oncogene Science, Cambridge, Mass.) at 2 µg/ml, and the polyclonal anti-bax antibody, 13666E (Pharmingen, San Diego, Calif.) at a dilution of 1:1000. Equal loading of protein was confirmed by stripping and reprobing blots with the antiactin monoclonal antibody, Ab-1 (Oncogene Science), diluted at 1:500. The blots were probed with appropriate horseradish peroxidase (HRP)-linked secondary antibodies (Amersham, Arlington, Ill., or Jackson ImmunoResearch Laboratory, West Grove, Pa.), and developed with ECL (Amersham) or Super Signal Substrate (Pierce, Rockford, Ill.) reagents. Exposed films were scanned with a ScanJet IIcx scanner (Hewlett-Packard, Minneapolis, Minn.) and densitometric analysis was performed with SigmaGel software (Jandel Scientific, San Rafael, Calif.). Densitometric values for the protein bands of interest were all normalized to the relative actin band intensity. This value was divided by the signal from a selected positive control cell extract (whose normalized signal was assigned a value of 10). The final expression values for each cell line were derived from the mean of values from two independent immunoblots.

Statistics

The means of the IC_{50} values for the ten cell lines at 3, 24 and 96 h were compared using the paired *t*-test performed with Excel software (Microsoft, Seattle Wash.). The means of the IC_{50} values (as well the other parameters) of the three WT- versus the seven M-p53 cell lines were compared using Student's two-tailed *t*-test with correction for heteroscedasticity as performed on Excel. The IC_{50} values were correlated with the other continuous variables using the Pearson correlation (*r*-value), calculated and graphed using Prism 2.0 or Cricket Graph (Cricket Software, Malvern, Pa.) software.

Results

The human carcinoma cell lines used and their p53 status [22, 23, 37] are shown in Table 1. SSCP was performed for all the cells except for the RKO panel, and in each case SSCP confirmed the presence of p53 mutations as previously reported. The collective data on all the parameters are presented in Table 2. With *every*

Table 1 Colon carcinoma cell lines and their p53 status

Cell lines	p53 status	Exon mutated	Confirmed by SSCP	Author (year)
Lovo	Wild-type	–	Yes	Kas (1995)
LS174T	Wild-type	–	Yes	Rodrigues (1990)
RKO	Wild-type	–	No	Kessis (1993)
Colo 205	Mutant	4	Yes	Kas (1995)
SW620	Mutant	8, 9	Yes	Rodrigues (1990)
SW1417	Mutant	7	Yes	Kas (1995)
SW48	Mutant	6	Yes	Kas (1995)
SW948	Mutant	4	Yes	Kas (1995)
HT29	Mutant	8	Yes	Rodrigues (1990)
SW1116	Mutant	5	Yes	Kas (1995)
RKO-RCneo	Wild-type	–	No	Kessis (1993)
RKO-RC10.1	Not expressed	Degraded by E6	No	Kessis (1993)

Table 2 IC_{50} values and other parameters of paclitaxel (*DT* doubling time, *ND* not done)

Cell line	P53 status	IC_{50} (n*M*)			DT (h)	G_2/M (%)[a]	MDR[b]	bcl-2[b]	bax[b]
		3 h	24 h	96 h					
Lovo	Wild-type	1116.0	80.0	43.0	33.6	16.8	2.3	2.8	0.0
LS174T	Wild-type	1957.0	82.0	71.0	29.1	4.6	4.1	3.8	0.0
RKO	Wild-type	358.0	6.0	5.2	36.8	15.8	0.0	2.4	5.1
Colo 205	Mutant	1083.0	17.0	4.2	41.1	12.0	0.0	0.0	3.8
SW620	Mutant	297.0	6.3	8.2	32.0	9.0	0.42	0.11	3.6
SW1417	Mutant	1500.0	106.0	7.4	72.5	9.2	0.02	0.0	4.6
SW48	Mutant	660.0	681.0	99.0	34.9	7.0	0.0	7.1	6.7
SW948	Mutant	741.0	70.0	46.0	48.5	7.8	12.4	0.0	3.3
HT29	Mutant	184.0	10.3	7.3	40.0	1.2	0.0	0.0	4.2
SW1116	Mutant	3338.0	1217.0	82.0	78.0	10.6	9.4	0.0	1.2
RKO-RCneo	Wild-type	216.0	7.5	11.1	48.2	13.4	0.0	ND	6.3
RKO-RC10.1	Absent	251.0	10.0	7.3	49.3	22.4	0.0	ND	2.2

[a] Percentage in G_2/M phases of cell cycle
[b] Based on duplicate normalized immunoblot expression relative to respective positive control

cell line a significant decrease in IC_{50} was observed as the duration of paclitaxel exposure was increased from 3 to 96 h. Representative dose-inhibition curves for the LS174T and HT29 cell lines, which have WT- and M-p53, respectively, are shown in Fig. 1. The effect of exposure duration on mean IC_{50} values of all ten distinct cell lines is presented in Fig. 2. Overall, about a 1.5 log decrease in IC_{50} occurred when exposure duration was increased from three to 96 h. In contrast, p53 status had no influence on sensitivity to paclitaxel. This was true whether the mean log IC_{50} values of three WT-p53 were compared with those of the seven M-p53 cell lines (Fig. 3A), or the IC_{50} values of the WT-p53 RKO and RKO-RCneo clones were compared with those of the RKO-RC10.1 cells (Fig. 3B), in which p53 function is abrogated through HPV-E6 gene expression [23]. Moreover, there was no influence of p53 status whether the IC_{50} values at 3, 24 or 96 h were compared (Fig. 3).

Since paclitaxel sensitivity may be linked with cell cycle or growth kinetics, we sought to determine whether the IC_{50} values of all ten unrelated cell lines correlated with either the percentage of cells in the G_2/M phase (%G_2/M) or DT. There was no significant association between IC_{50} and %G_2/M values of all ten distinct cell lines (Table 3). Moreover, in the RKO panel, although there was the expected increase in %G_2/M in the p53-incompetent RKO-RC10.1 clone (22.4%) compared with the two clones with WT-p53 function (10.6% and 13.4%, respectively), the IC_{50} values were comparable (Table 2). This suggests that the sensitivity to paclitaxel does not appear to be limited to the G_2M phase of the cell cycle.

In contrast, IC_{50} values directly correlated with DTs after 3 h ($r = 0.65$) and after 24 h paclitaxel exposure ($r = 0.56$), but not after 96 h ($r = 0.07$; Table 3, Fig. 4). Among individual parameters, with 3 or 24 h exposure, DT correlated best with the IC_{50} (Table 3). Thus, slower growing cells were more resistant to a 3-h exposure. However, with a 96-h drug exposure (an exposure longer than the longest DT of 78 h), all cell lines became five-fold or more sensitive to paclitaxel, regardless of the expression of MDR-1, bcl-2 or bax (Table 2). Together, these results suggest that in unsynchronized colorectal carcinoma cells, a longer drug exposure can significantly overcome intrinsic resistance to paclitaxel.

The individual influences of MDR-1, bcl-2 and bax protein expression levels, as evaluated by immunoblotting (Fig. 5), on paclitaxel sensitivity were also assessed for all ten unrelated cell lines. MDR-1 expression was moderately correlated with IC_{50}, irrespective of duration of exposure to paclitaxel. Bcl-2 was directly correlated with IC_{50} but only with the 96-h exposure, while bax was

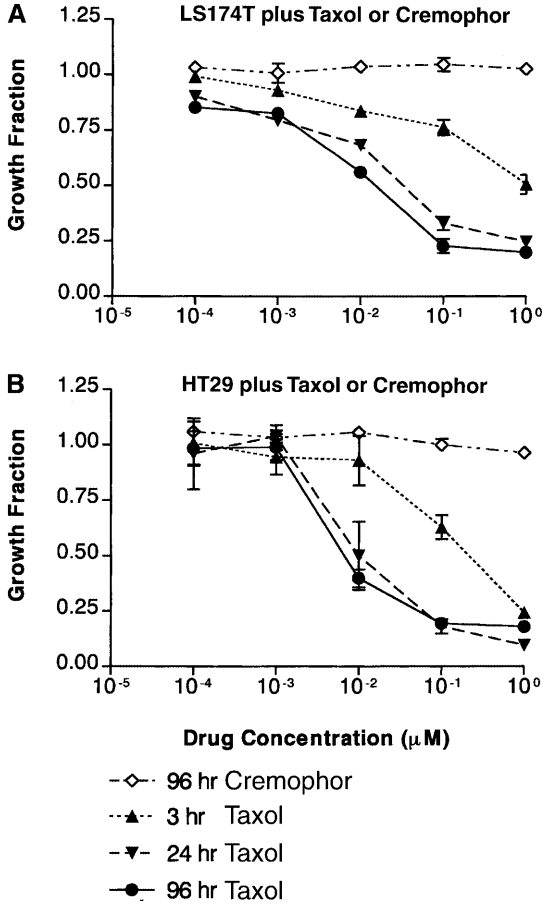

Fig. 1A,B Dose response curves in colon carcinoma cells showing increased sensitivity with longer paclitaxel (Taxol) exposure. **A** LS174T cells which have WT p53. **B** HT29 cells which have mutant p53 (◇ 96-h Cremophor, ▲ 3-h paclitaxel, ▼ 24-h paclitaxel, ● 96-h paclitaxel)

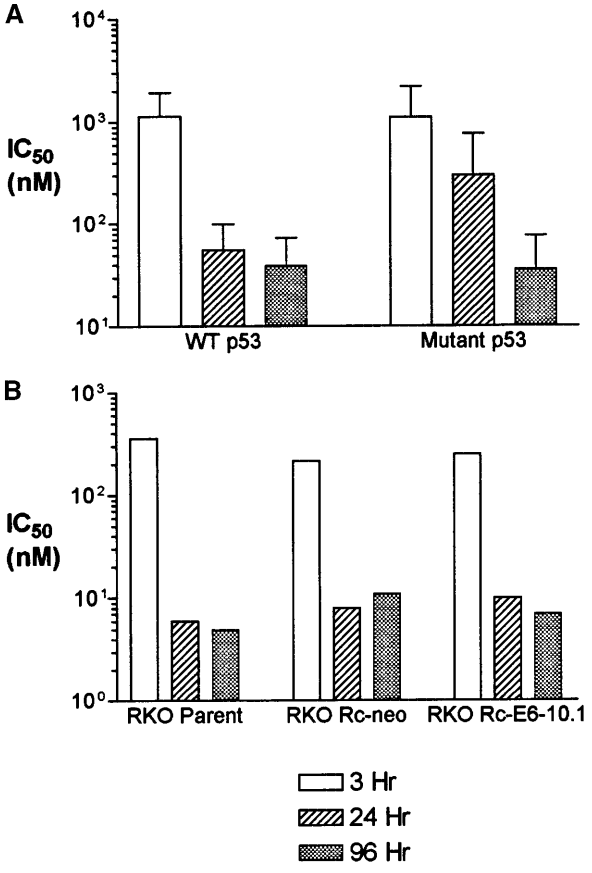

Fig. 3A,B P53 status has no influence on sensitivity of colon carcinoma cells to 3, 24 or 96 h of paclitaxel exposure. **A** The mean ± SD (*bars*) IC_{50} values of the three cell lines with WT p53 compared with those of the seven with mutant p53. The two-tailed *t*-test *P*-values between the WT and M IC_{50} values at 3, 24, and 96 h are 0.96, 0.22, and 0.89, respectively. **B** Comparison of the IC_{50} values of the RKO panel of cells

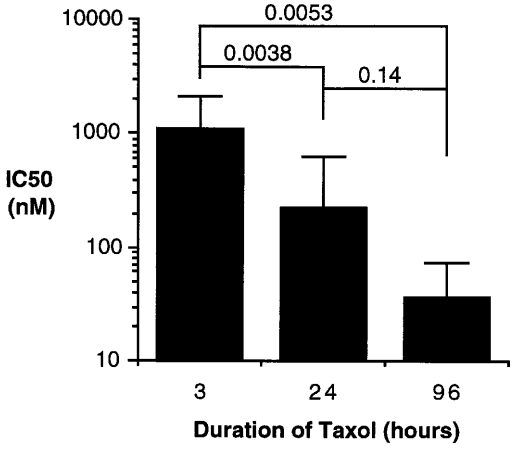

Fig. 2 Longer paclitaxel (Taxol) exposure associated with significantly decreased IC_{50}. The mean ± SD IC_{50} values of the ten unrelated cell lines (excluding RKO-RCneo and -RC10.1) after 3, 24 or 96 h paclitaxel exposure are shown. The *P*-values of the two-tailed Student's *t*-test comparing the three groups are shown above the bars

inversely correlated with IC_{50} but only with the 3-h exposure (Table 3). Next, combinations of parameters were examined for correlation with the IC_{50}. Since others have suggested that the combination of or ratio between bcl-2 and bax may more accurately reflect resistance to apoptosis, we observed that the difference between bcl-2 and bax expression (bcl-2–bax) appeared to better correlate with IC_{50} at 96 h than either factor alone. With the 96-h exposure, the combination of MDR + bcl-2–bax showed the best direct correlation with IC_{50} ($r = 0.71$), with an inferior correlation at shorter exposures (Table 3). In contrast, the addition of DT to MDR + bcl-2–bax (DT + MDR + bcl-2–bax) showed the best correlation with IC_{50} after the 3-h exposure ($r = 0.77$), and less so with longer exposures. This was expected, since our results showed that DT noticeably correlated with IC_{50} after 3 h ($r = 0.65$), but not with IC_{50} after 96 h of exposure ($r = 0.07$). The correlation of MDR + bcl-2–bax or DT + MDR + bcl-2–bax with the IC_{50} is graphically presented in Fig. 6. This analysis suggests that longer exposures to paclitaxel may overcome the resistance due to slow cell

Table 3 Correlation between IC_{50} and individual or combination parameters (*r*-values)

Exposure duration (h)	Doubling time	G_2/M (%)	MDR-1 expression	bcl-2 expression	bax expression	bcl-2–bax	MDR + bcl-2–bax	DT + MDR + bcl-2–bax
3	0.65	0.08	0.49	−0.10	−0.58	0.34	0.57	0.77
24	0.56	0.00	0.39	0.19	−0.06	0.20	0.41	0.64
96	0.07	−0.16	0.45	0.64	−0.23	0.68	0.71	0.28

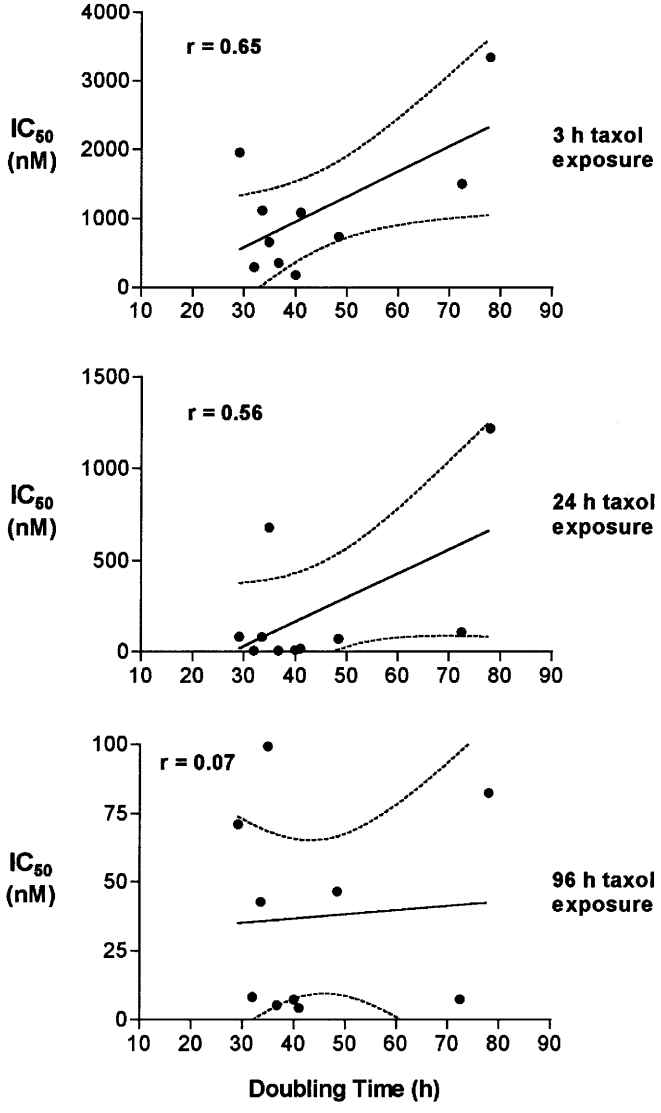

Fig. 4 Sensitivity to paclitaxel (Taxol) of the ten independent colon cancer cell lines is correlated with doubling time after 3 and 24 h, but not after 96 h of exposure

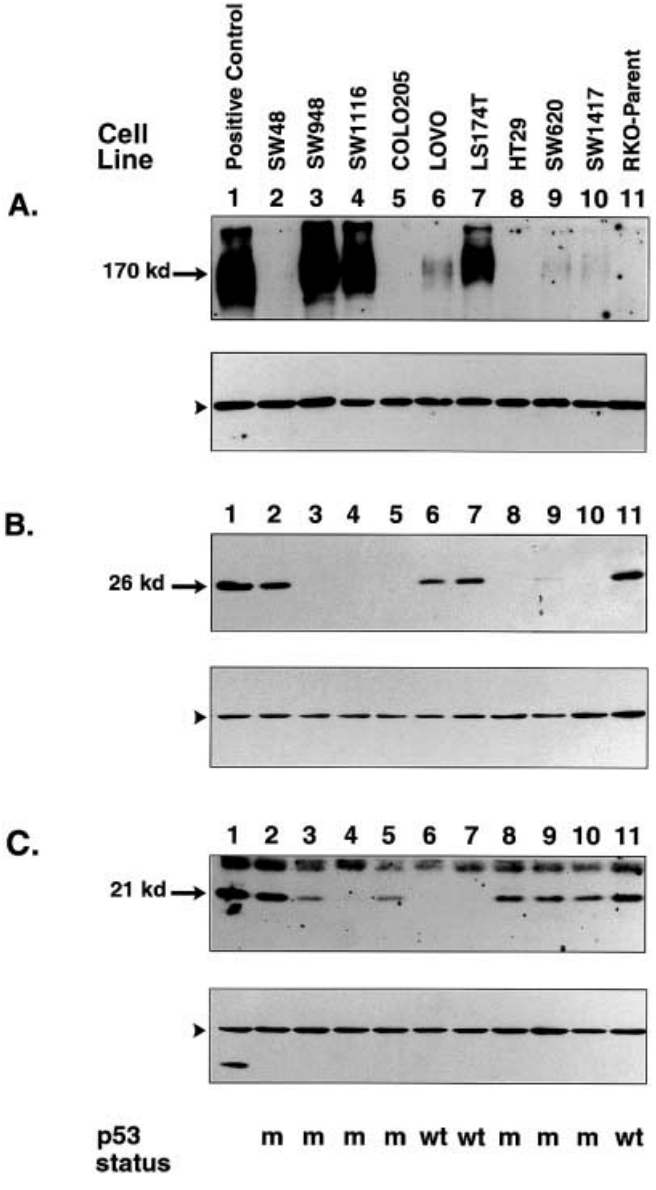

Fig. 5A–C Immunoblots of ten colon cancer cell lines (**A** MDR-1, **B** Bcl-2, **C** Bax). *Arrows* indicate expected products. The lower respective panels show the results of stripping and reprobing of the same respective blots for actin (*arrow heads*), indicating equivalent loading. Values from Table 2 are derived from the mean data of two independent immunoblots of two separate sets of cell extracts, as described in Methods

kinetics. However, even with the 96-h exposure, resistance was still generally increased with high MDR-1 or bcl-2 expression (relative to bax). With short 3-h paclitaxel exposures, the DT + MDR + bcl-2–bax parameter best correlated with resistance.

The status of p53 did not significantly influence DT, $\%G_2$/M, MDR-1, bcl-2, or bax expression. This is based upon paired *t*-tests for these parameters between the two groups (three WT-p53 versus seven M-p53 cell lines; data not shown), with the recognition that statistical power was low due to the relatively small number of cell lines. Although there was a trend for M-p53 cell lines to have longer DT (which seems paradoxical), the DTs of

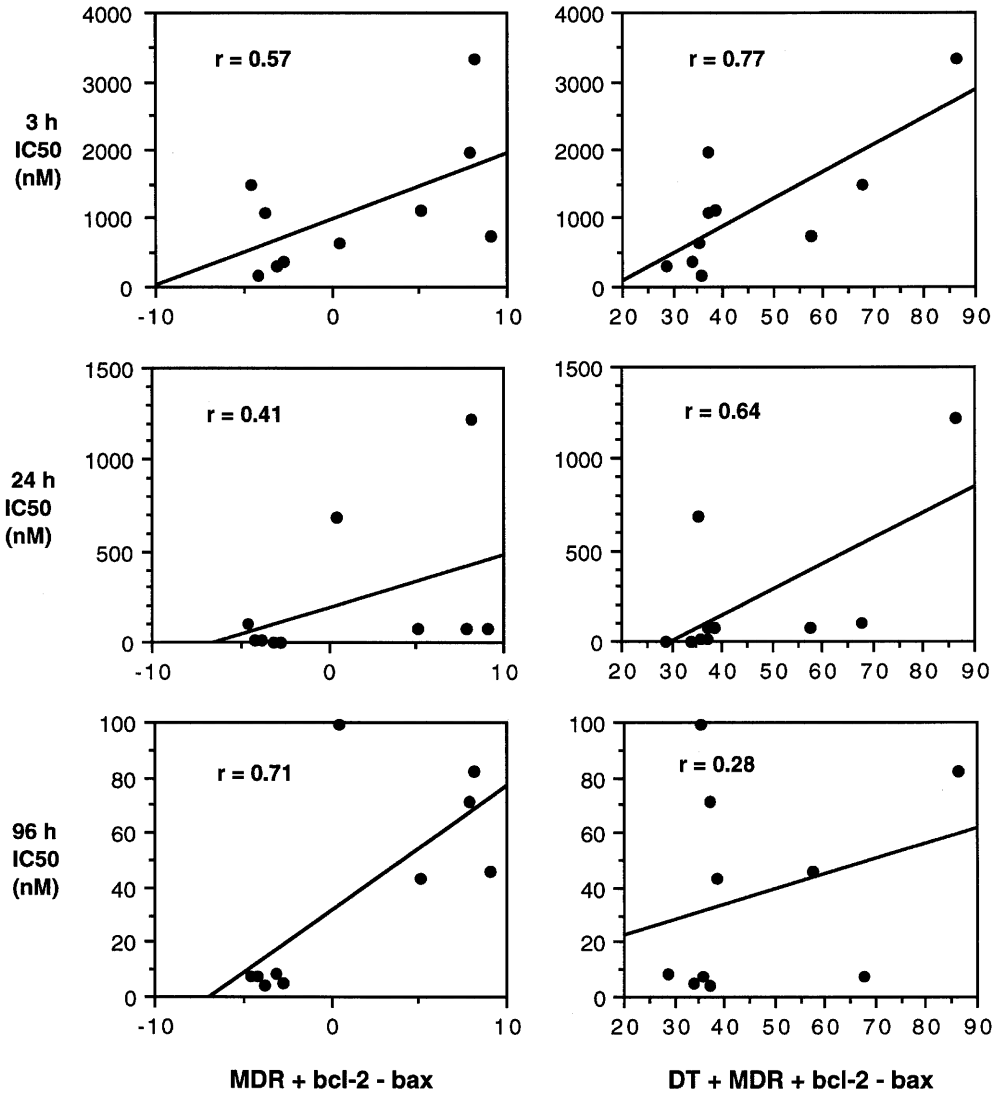

Fig. 6 IC_{50} values are correlated with DT, MDR, bcl-2 and bax expression of the ten colon cancer cell lines. *Left column* Correlations between IC_{50} values and the parameter combining relative MDR, bcl-2, and bax expression (MDR + bcl-2–bax); *right column* correlations between IC_{50}s and the combined parameter including DT (DT + MDR + bcl-2–bax)

the isogenic pair of cell lines (RKO-RCneo and RKO-RC10.1) were virtually the same (Table 2). This finding suggests that in nonisogenic malignant colorectal carcinoma cell lines, mechanisms other than p53 status influence these parameters.

Discussion

Prolonged exposure (96 h) to paclitaxel significantly increased sensitivity of human colorectal carcinoma cells (reducing the mean IC_{50} by about 1.5 log), regardless of p53 status (Fig. 3), consistent with in vitro studies of human breast and lung cancer cells [12, 26, 50]. All colon cancer cell lines showed a decrease in IC_{50} of fivefold or more regardless of expression of other resistance factors (Table 2), and overall there was a decrease in mean IC_{50} to 37 n*M* after a 96-h exposure (Fig. 2). A preliminary study in 28 lung cancer cell lines showed that after a 120-h paclitaxel exposure there was about a 3-log drop in IC_{50} to a mean of 27 n*M* [12]. Despite this similar in vitro pattern, it is unclear why paclitaxel lacks clinical activity in patients with colorectal cancer, even with 96-h paclitaxel infusions [44]. It is possible that, in the three-dimensional microenviroment of metastatic colon cancer, the malignant cells are more resistant to paclitaxel-induced apoptosis, a phenomenon observed for ovarian cancer cells [11]. However, the results of this study suggest that other resistance factors also contribute to resistance, even in the context of long paclitaxel exposures.

Our results suggest that DT, MDR-1, bcl-2 and bax expression may each contribute to the cellular resistance of colon cancer to paclitaxel, and that through the combination of DT, MDR, bcl-2 and bax parameters, much of the intrinsic differences in in vitro paclitaxel sensitivity between carcinoma cell lines may be explainable. MDR-1 expression has been established as an important mechanism of resistance to paclitaxel [17, 29]. Compatible with this, the correlation between IC_{50} and MDR-1 expression was more consistent than either bcl-2 or bax for all of the three exposure durations (Table 3).

Bcl-2 and bax appear to influence sensitivity of cancer cells to paclitaxel [18, 19, 40, 41]. Notably, bax may enhance paclitaxel-induced apoptosis independent of the p53 pathway [40]. In an attempt to assess the relative contributions of multiple parameters, the composite parameter MDR + bcl-2–bax was conceived. This parameter included the difference between bcl-2 and bax, since they appear to influence chemosensitivity in an inverse manner. MDR + bcl-2–bax provided direct correlation to the IC_{50} generally superior to any single parameter (Table 3). The correlation between MDR + bcl-2–bax and IC_{50} was best with long (96-h) paclitaxel exposure ($r = 0.71$), *despite* the general decrease in resistance with that duration. This is consistent with the finding that, at the 96-h exposure, DT was no longer influential (Table 3, Fig. 6). As expected, the combination including DT (DT + MDR + bcl-2–bax) showed the best correlation with IC_{50} at the 3-h exposure ($r = 0.77$; Table 3, Fig. 6).

The status of p53 does not significantly alter the in vitro sensitivity of human colorectal cancer cells to paclitaxel, confirming the recent findings of Fan et al. [10], who showed that in a panel of HCT-116 colon cancer cell lines isogenic except for WT or abrogated p53 function there was no difference in growth inhibition in response to paclitaxel. Of seven studies (including the study reported here) of human ovarian, lung, breast or colon carcinoma cell lines in which p53 function is altered by stable gene transfection, six have shown no effect of p53 status on sensitivity to paclitaxel [5, 10, 14, 47]. Only in a human ovarian teratocarcinoma cell line was it shown that disruption of p53 function (by HPV-E6 expression) conferred an increased resistance [47]. The observed resistance with p53 disruption in this study may have been due to the context of this rather rare type carcinoma. In addition to the RKO-derived colon cancer cell line panel, a panel of ten unrelated colon cell lines was also surveyed in this study. This confirmed that p53 status did not influence sensitivity to paclitaxel. The only other survey of a group of unrelated carcinoma cell lines of one histologic type (nine ovarian carcinoma cell lines) has also shown that there is no influence of p53 status on paclitaxel sensitivity [5]. NCI has systematically tested the growth inhibitory potency of 123 anticancer agents against 60 human cancer cell lines which have been characterized for their p53 mutation status. No significant differences in median sensitivity to paclitaxel between the 19 cell lines with WT-p53 versus the 38 with M-p53 gene sequence were found [35]. In summary, it appears that p53 does not influence the in vitro sensitivity of human cancer cells to paclitaxel.

In contrast, the loss of p53 function in immortalized but nontransformed cells appears to confer sensitivity to paclitaxel. For example, Wahl et al. have reported that human foreskin fibroblasts depleted of functional p53 by the human papilloma virus type 16 E6 expression, or mouse embryo fibroblasts derived from p53 null transgenic mice, have seven- and tenfold lower 72-h exposure IC_{50} values, respectively, than control cells with intact p53 function [46]. Hawkins et al. have reported similar findings in both types of cell lines [16]. Two other studies have shown no difference in sensitivity to paclitaxel between lymphoblastoid cell lines with either WT or abrogated p53 function [6, 10]. The divergence of these results may be related to different origins of these cell lines. Thus for non-malignant cells, loss of p53 function may increase sensitivity to paclitaxel, but with additional transforming events, the influence diminishes.

There are several implications from these results. First, that molecular studies that try to evaluate mechanisms of clinical paclitaxel resistance should include multiple relevant parameters, as in some clinical studies in which only MDR-1 expression has been evaluated no association with resistance has been found [20, 42, 43]. Second, longer taxane exposures may overcome some of the resistance related to cell kinetics or MDR-1 overexpression [50], especially since the DT of tumors in vivo is measured in terms of weeks to months. Third, since the same resistance factors appear to influence sensitivity of colon cancer cells as in other tumor types, paclitaxel should be combined with other agents that can inhibit MDR-1-mediated drug efflux [3] or reduce the antiapoptotic influence of bcl-2 (e.g. through antisense oligonucleotides [4, 36]). Efforts to combine taxanes with MDR-1-reversal agents may be fruitful, since MDR-1 expression is detected in the majority of colorectal carcinomas [13]. Fourth, newer taxane derivatives which have superior efficacy in MDR-1-expressing tumor cells should be considered in the treatment of colorectal carcinoma [8, 9]. Thus, further work may identify combinations containing taxanes which may be effective against colorectal carcinoma tumors.

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