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Loss of *atm* sensitises *p53*-deficient cells to topoisomerase poisons and antimetabolites

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Background: Ataxia-telangiectasia is a pleiotropic autosomal recessive disorder caused by mutations in the *ATM* gene. In addition to a profound cancer predisposition, another hallmark of ataxia-telangiectasia is radiosensitivity. Recently, *p53*-null mouse fibroblasts have been reported to be radiosensitised by the concurrent loss of *ATM*.

Materials and methods: We compared the sensitivity of $atm^{+/+}/p53^{-/-}$ and $atm^{-/-}/p53^{-/-}$ mouse embryonic fibroblasts to different classes of chemotherapeutic agents using the MTT assay, Trypan Blue exclusion and fluorescence-activated cell sorting for cell cycle and apoptosis analyses.

Results: Loss of ATM function in p53-deficient cells resulted in a 2- to 4-fold increase in sensitivity to the topoisomerase I poisons camptothecin and topotecan, to the topoisomerase II poisons doxorubicin, epirubicin and etoposide, and to the antimetabolites 5-fluorouracil and gemcitabine, but not to the platinum compounds cisplatin, carboplatin and oxaliplatin, the taxanes docetaxel and paclitaxel, or to busulfan. Loss of ATM function did not result in increased apoptosis, but resulted in increased Trypan Blue staining in response to epirubicin, suggesting that processes other than apoptosis may mediate cytotoxicity. ATM deficiency did not alter the extent of G_1/S or G_2/M cell cycle phase accumulation produced by epirubicin, suggesting that enhanced sensitivity was not due to failure of checkpoint activation.

Conclusions: We provide further evidence that ATM is involved in regulating cellular defences against some cytotoxic agents in the absence of p53. Tumour-targeted functional inhibition of ATM may be a valuable strategy for increasing the efficacy of anticancer agents in the treatment of *p53*-mutant cancers.

Key words: antimetabolites, ATM, cancer, drug sensitivity, p53, topoisomerase poisons

Introduction

The *p53* tumour suppressor gene is the most frequently mutated gene in human cancers [1]. p53 is involved in regulating cell cycle checkpoints, apoptosis and DNA repair in response to DNA damage, and thus plays a pivotal role in maintaining the integrity of the genome [2]. Lack of functional p53 is associated with abrogation of cell cycle checkpoint control and of apoptosis induction. This contributes to resistance to radiotherapy and to certain chemotherapeutic agents in some tissues [3]. The failure of *p53*-mutant tumours to respond to therapeutic regimens is of increasing concern. Strategies that cause cells to override the DNA damage checkpoints, to block protective pathways or to trigger apoptosis via p53-independent pathways are predicted to sensitise cells to killing by genotoxic agents.

ATM is mutated in patients with ataxia-telangiectasia, a pleiotropic autosomal recessive disorder characterised by progressive neurodegeneration, premature senescence, immunodeficiency, predisposition to cancer, chromosomal instability and hypersensitivity to γ-irradiation [4, 5]. ATM is a PI-3-like protein kinase operating upstream of p53 [6] and it has been shown to bind to free DNA ends produced by DNA double-strand breaks that occur during normal replication and recombination, or in response to exogenous genotoxic stress [7]. It is known to interact with a variety of other targets in addition to p53, including c-Abl, BRCAl, CHK2, MDM2 and DNA-PK [8, 9]. ATM plays a key role in sensing DNA damage and in propagating signals that modulate protective cellular responses to genotoxic agents. It serves a surveillance function that helps maintain genomic integrity by promoting cell cycle arrest and damage repair, and possibly by recruiting repair proteins to the site of damage to prevent double-strand break repair from entering an error-prone pathway [10]. Ataxia-telangiectasia cells display defective p53 induction, abrogation of G_1/S and G_2/M cell cycle checkpoints, and hypersensitivity to γ-irradiation [5], suggesting that ATM and p53 interact in a common pathway in response to this type of DNA damage. However, some *p53*-null mouse tissues have been shown to be rendered radiosensitive by the concurrent loss of the *ATM* gene [11], suggesting the existence of an ATM effector pathway that is activated in response to ionising radiation but which does not depend on p53.

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Because a very large fraction of human cancers is functionally p53 deficient, and hence may be resistant to chemotherapeutic agents, a means of sensitising p53-deficient tumours assumes great importance. Therefore, we were interested in determining whether p53-deficient cells could be sensitised to a panel of clinically important chemotherapeutic agents by the additional loss of ATM function. We report here that loss of ATM function in *p53*-null mouse embryonic fibroblasts results in hypersensitivity to a panel of chemotherapeutic agents, indicating that ATM plays a role in protective responses to DNA damage independently of p53. The increased drug sensitivity of ATM-deficient cells was not accompanied by increased apoptosis or by further alteration of cell cycle checkpoint activation. Our data support the concept that tumour-targeted functional inhibition of ATM may be a valuable approach to improving the effectiveness of chemotherapeutic agents in the treatment of *p53*-mutant cancers.

Materials and methods

Cell lines and culture conditions

The $atm^{+/+}/p53^{-/-}$ and $atm^{-/-}/p53^{-/-}$ mouse embryonic fibroblasts were generously provided by Dr P. Leder. Mice were generated by crossing $p53^{-/-}$ mice in a FVB background to $atm^{-/-}$ mice [12]. The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 4 mM L-glutamine (Life Technologies, Basel, Switzerland), 15% heat-inactivated fetal calf serum (Oxoid, Basel, Switzerland) and penicillin/streptomycin (100 U/ml, 100 µg/ml; Life Technologies) at 37°C in a humidified atmosphere containing 5% carbon dioxide. The human breast adenocarcinoma cell line MCF-7 was obtained from the American Type Culture Collection (ATCC HTB 22; Manassas, VA, USA) and maintained in Opti-MEM (Life Technologies supplemented with 10% heat-inactivated fetal calf serum. All cell lines tested negative for contamination with *Mycoplasma* spp.

Drugs

Camptothecin and busulfan were purchased from Sigma (Buchs, Switzerland). The following drugs were generous gifts: epirubicin and doxorubicin (Pharmacia & Upjohn, Dubendorf, Switzerland), topotecan (SmithKline Beecham, Thorishaus, Switzerland), docetaxel (Aventis, Zurich, Switzerland), oxaliplatin (Sanofi-Synthelabo, Meyrin, Switzerland), etoposide, paclitaxel, cisplatin and carboplatin (Bristol-Myers Squibb, Baar, Switzerland), 5-fluorouracil (Roche, Reinach, Switzerland) and gemcitabine (Eli Lilly, Vernier, Switzerland).

Immunoblot analysis

To provoke p53 induction, exponentially growing cells were exposed to 25 nM epirubicin for 24 h and then collected as described. Cells were washed in cold phosphate-buffered saline (PBS) and lysed on ice in 150 mM NaCl containing 5 mM EDTA, 1% Triton X-100, 10 mM Tris–HCl (pH 7.4), 5 mM dithiothreitol (DTT), 100 µg/ml phenylmethylsulfonyl fluoride and 1 µg/ml aprotinin, followed by centrifugation at 14 000 *g* for 20 min at 4°C. The protein amount was determined using the Bio-Rad protein assay dye (Bio-Rad, Glattbrugg, Switzerland). After centrifugation, 150 µg (for ATM) or 50 µg (for p53) of protein was boiled in an equal volume of 100 mM Tris–HCl (pH 6.8) containing 20% glycerol, 200 mM DTT, 4% sodium dodecylsulphate (SDS) and 0.2% bromophenol blue. The proteins were separated using SDS–PAGE on a 5% gel for ATM and on a 10% gel for p53 analysis, followed by blotting onto a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). ATM protein was detected using a mouse monoclonal antibody directed against amino acids 2577–3056 (ATM-2CI; GeneTex, Inc., San Antonio, TX, USA), whereas p53 protein was detected using the mouse monoclonal antibody pAb 240 (Santa Cruz Biotechnology Inc., Basel, Switzerland). After washing the blots, horseradish peroxidase-conjugated antimouse antibody (BD Biosciences, Allschwil, Switzerland) was added and the complexes were visualised by enhanced chemiluminescence (Amersham Pharmacia Biotech).

Growth inhibition assay

Epirubicin, doxorubicin, topotecan, cisplatin, carboplatin, oxaliplatin, 5-fluorouracil and gemcitabine were diluted in 0.9% NaCl immediately before use. Stock solutions of etoposide, camptothecin, paclitaxel and busulfan were prepared in dimethyl sulphoxide (DMSO), whereas docetaxel was dissolved in methanol. The final concentration of DMSO or methanol in the cultures was <0.1% at all drug concentrations and in controls. Previous experiments (data not shown) have established that neither 0.1% DMSO nor 0.1% methanol affects the viability or growth of these cell lines. Growth inhibition in response to drug treatment was determined by the MTT assay [13]. Cells growing in the log phase were harvested by brief trypsinisation and washed once with medium containing 15% fetal calf serum. MTT assays were performed by seeding 500 ($atm^{-/-}/p53^{-/-}$) or 1000 ($atm^{+/+}/p53^{-/-}$) cells into 96-well plates 24 h before incubation without or with the drug for 5 days. A volume of 20 µl MTT in PBS was added to a final concentration of 0.5 mg/ml, followed by incubation at 37°C for 4 h, aspiration of the medium and addition of DMSO 200 µl. Optical density was measured by the Emax microplate reader E9336 (Molecular Devices, Clearwater, MN, USA) at 540 nm, setting the value of the cell lines in medium to 1.0 (control) and the value of the no cells blank to zero. Differences in drug sensitivity of the respective cell lines were determined from at least four independent experiments and are reported as the concentration required to suppress growth by 50% (IC_{50}).

TUNEL apoptosis assay

Cells were grown to 70% confluence in 60 mm dishes in triplicate cultures and then treated with 25 nM epirubicin. Adherent and floating cells were collected at the time points indicated and washed in PBS. Cells were then fixed in 4% paraformaldehyde on ice for 30 min, followed by dropwise addition of ice-cold 70% ethanol. Samples were stored at 4°C until further use. Samples were washed twice with PBS and resuspended in the TUNEL reaction mixture and incubated at 37°C for 2 h, according to the manufacturer's protocol (Roche Molecular Biochemicals, Basel, Switzerland). Cells were analysed by flow cytometry (EPICS ELITE; Beckmann-Coulter, Hialeah, FL, USA).

Trypan Blue exclusion assay

Cell viability after drug treatment was determined by means of the Trypan Blue exclusion assay. Cells were grown to 60% confluence and incubated without (controls) or with 25 nM epirubicin for 24, 48 or 72 h. At the indicated time points, floating and adherent cells were collected. Cells were then incubated with Trypan Blue solution (0.1% final concentration) for 1 min, and the number of Trypan Blue-positive and -negative cells was determined using a haematocytometer.

Cell cycle analysis

Cells were grown to 50% confluence in 60 mm dishes. Cells were then incubated with or without (controls) epirubicin 10 nM. This dose inhibited growth of ATM-deficient cells by at least 70% in the MTT assay. Before collection by trypsinisation at the times indicated, cells were incubated with BrdU 10 µM (Serva, Heidelberg, Germany) at 37°C for 8 h. Samples were washed in ice-cold PBS, resuspended in PBS 100 µl, fixed by dropwise addition of ice-cold 70% ethanol and stored at 4°C until use. Then, ethanol was removed by centrifugation (3000 *g*) and cells were resuspended and incubated in 2 N HCl/0.5% Triton X-100 for 30 min at room temperature, followed by centrifugation (3000 *g*) and resuspension in 0.1 M $Na_2B_4O_7$ to neutralise the acid. After

collection of the pellet by centrifugation, cells were resuspended and incubated in PBS 500 µl /0.5% Tween-20/1% bovine serum albumin (BSA) and further incubated with FITC-conjugated anti-BrdU antibody (5 µg/ml; BD Biosciences) at room temperature for 30 min. Nuclei were then collected by centrifugation (3000 *g*) and incubated in 1 ml of propidium iodide staining solution (50 µg/ml propidium iodide and 100 U/ml RNase A in PBS) at room temperature for 1 h, followed by washing once in PBS containing 0.5% BSA. All light-sensitive steps were carried out in twilight. Samples were analysed for BrdU incorporation and DNA content by flow cytometry (EPICS ELITE; Beckmann-Coulter), and the percentage of cells in each phase was determined using the MultiCycle for Windows Software (Phoenix Flow Systems, San Diego, CA, USA).

Statistical analysis

Mean ± SD are indicated for all data sets. The two-sided paired *t*-test was performed to compare the effects of loss of ATM function on drug sensitivity. $P < 0.05$ was considered to be statistically significant.

Results

Loss of ATM and increased sensitivity to topoisomerase I and II poisons and to antimetabolites

Loss of the *ATM* gene has previously been reported to render *p53*-null mouse tissues more sensitive to γ-irradiation [11]. Using a pair of mouse knockout cell lines, we determined the effect of loss of ATM function in a *p53*-null background on sensitivity to a panel of anticancer agents. $atm^{+/+}/p53^{-/-}$ cells are replete with respect to ATM but lack expression of p53, whereas $atm^{-/-}/p53^{-/-}$ cells express neither ATM nor p53 protein (Figure 1).

Figure 2 shows the extent of growth inhibition for both cell lines as a function of drug concentration in response to continuous exposure to the topoisomerase II poisons epirubicin and etoposide, to the topoisomerase I poisons camptothecin and topotecan, and to the antimetabolites 5-fluorouracil and gemcitabine. The IC_{50} values for all the drugs tested are summarised in Table 1. The $atm^{-/-}/p53^{-/-}$ cell line was 3.2-fold ($P = 0.0004$, $n = 5$) more sensitive to epirubicin and 3.1-fold ($P = 0.003$, $n = 5$) more sensitive to etoposide than the $atm^{+/+}/p53^{-/-}$ cell line. Likewise, a 3-fold ($P = 0.0001$, $n = 5$) greater sensitivity was also found for doxorubicin. In addition, $atm^{-/-}/p53^{-/-}$ cells displayed a 3.6-fold increased sensitivity to camptothecin ($P = 0.0001$, $n = 4$) and a 2.7-fold increased sensitivity to topotecan ($P = 0.01$, $n = 4$) as compared with the ATM-proficient cells. They were also 2-fold hypersensitive to 5-fluorouracil ($P = 0.0001$, $n = 5$) and 2.1-fold hypersensitive to gemcitabine ($P = 0.003$, $n = 5$) as compared with $atm^{+/+}/p53^{-/-}$ cells. Thus, the additional loss of the *ATM* gene in a *p53*-deficient genetic background resulted in a 2- to 4-fold increased sensitivity to the topoisomerase I and II poisons, and to some types of antimetabolites.

Loss of ATM and unaltered sensitivity to platinum compounds, taxanes or the alkylating agent busulfan

Figure 3 shows the extent of growth inhibition for $atm^{+/+}/p53^{-/-}$ and $atm^{-/-}/p53^{-/-}$ cells in response to a continuous exposure to the platinum compounds cisplatin, carboplatin and oxaliplatin, to the alkylating agent busulfan, and to the microtubule poisons

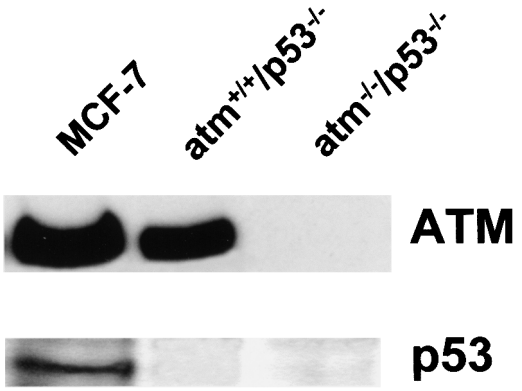

Figure 1. Immunoblot analysis of the expression of ATM and/or p53 protein in $atm^{+/+}/p53^{-/-}$ and $atm^{-/-}/p53^{-/-}$ cells. The human breast cancer cell line MCF-7 was used as a positive control. $atm^{+/+}/p53^{-/-}$ cells lack the expression of p53, whereas $atm^{-/-}/p53^{-/-}$ cells express neither ATM nor p53.

docetaxel and paclitaxel. The IC_{50} values for these drugs are reported in Table 1. There was no difference in sensitivity between the $atm^{+/+}/p53^{-/-}$ and $atm^{-/-}/p53^{-/-}$ cells with respect to treatment with cisplatin ($P = 0.38$, $n = 5$), carboplatin ($P = 0.36$, $n = 5$), oxaliplatin ($P = 0.15$, $n = 5$), docetaxel ($P = 0.81$, $n = 4$), paclitaxel ($P = 0.51$, $n = 4$) or busulfan ($P = 0.14$, $n = 5$). Thus, loss of the ATM function in *p53*-null cells does not alter the sensitivity to platinum compounds, to taxanes, or to the alkylating agent busulfan.

ATM deficiency and increased apoptosis

The TUNEL apoptosis assay was performed to determine whether the increased sensitivity to epirubicin due to loss of ATM function was accompanied by increased apoptosis. Treatment with 25 nM epirubicin, which inhibited growth of ATM-deficient cells by more than 85%, produced 3- to 4-fold more apoptotic $atm^{+/+}/p53^{-/-}$ cells than $atm^{-/-}/p53^{-/-}$cells (Figure 4). The respective values were 9 ± 6% versus 2 ± 1% at 34 h after epirubicin treatment, and 13 ± 3% versus 4 ± 1% at 57 h after treatment. These results indicate that the hypersensitivity to the cytotoxic effect of epirubicin in ATM-deficient cells is not associated with increased apoptosis.

Loss of ATM and overall cell viability in response to the topoisomerase II poison epirubicin

The Trypan Blue exclusion assay was performed to clarify the apparently discrepant results observed with epirubicin in the different assay systems. The data indicate that loss of ATM function was associated with a 2- to 3-fold increase in the number of Trypan Blue-positive cells following exposure to 25 nM epirubicin (Figure 5). The changes in the proportion of Trypan Blue excluding cells with the time after drug treatment were also monitored. The decrease in viability was faster in the ATM-deficient cells than in the ATM-proficient cells (55 ± 8% versus 20 ± 8% at 48 h, 71 ± 11% versus 42 ± 12% at 72 h). This parallels the data obtained in the MTT assay. Thus, both the growth rate inhibition assay and the Trypan Blue assay indicate that loss of

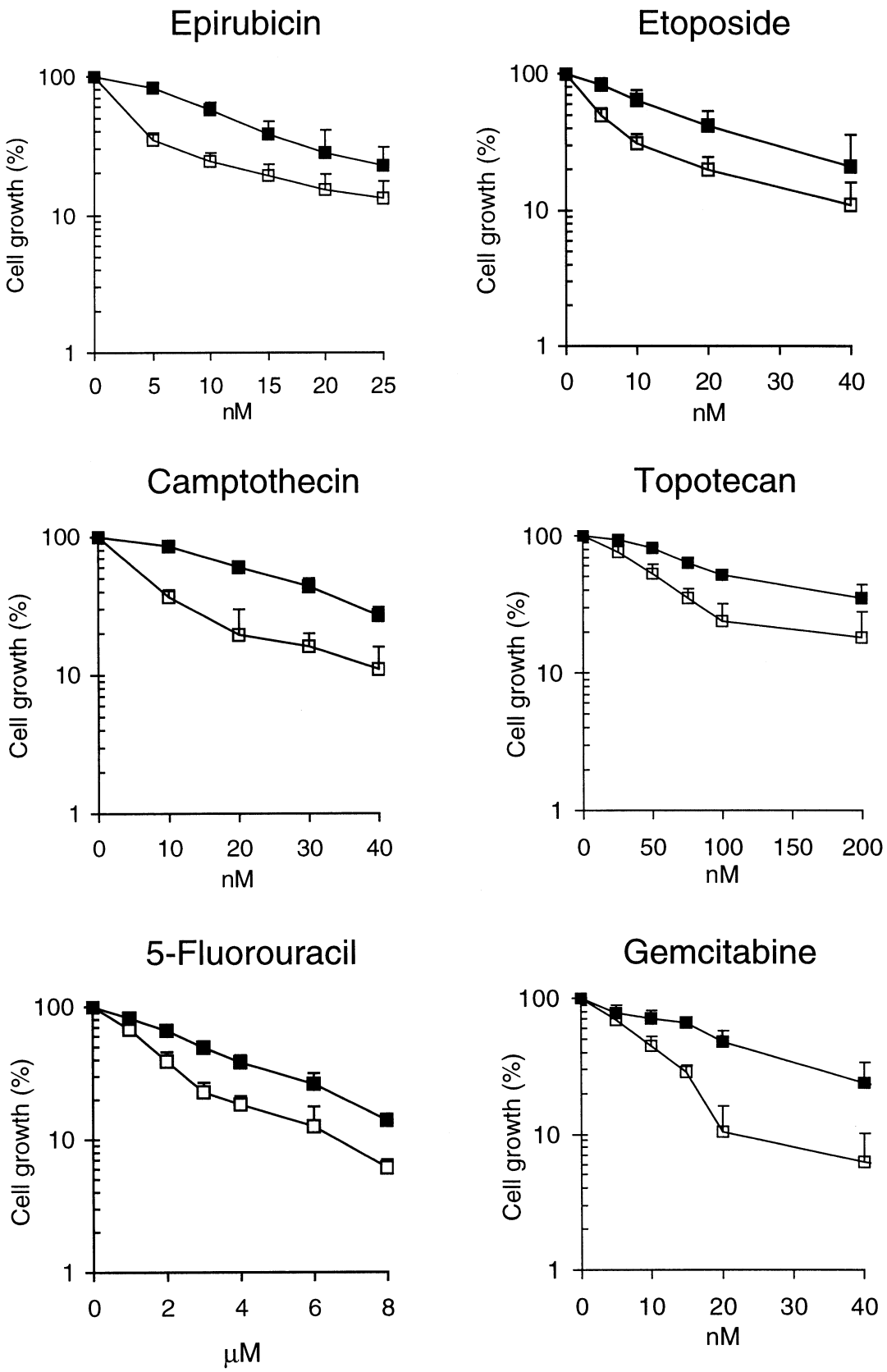

Figure 2. Sensitivity to a continuous exposure to epirubicin, etoposide, camptothecin, topotecan, 5-fluorouracil and gemcitabine in $atm^{+/+}/p53^{-/-}$ (filled squares) and $atm^{-/-}/p53^{-/-}$ (open squares) cells as determined by the MTT assay. Each point represents the mean of at least four independent experiments. Bars = SD.

Table 1. IC_{50} for ATM-proficient or -deficient *p53*-null cells determined by MTT assay[a]

Drug	$atm^{+/+}/p53^{-/-}$	$atm^{-/-}/p53^{-/-}$	S[b]	*P*[c]
Epirubicin (nM)	12.0 ± 2.0	3.7 ± 0.6	3.2	0.0004
Doxorubicin (nM)	34.4 ± 5.3	11.6 ± 4.8	3.0	0.0001
Etoposide (nM)	16.2 ± 5.3	5.2 ± 1.5	3.1	0.003
Camptothecin (nM)	26.4 ± 3.2	7.4 ± 1.1	3.6	0.0001
Topotecan (nM)	91.8 ± 24.6	34.6 ± 8.8	2.7	0.01
5-Fluorouracil (μM)	3.1 ± 0.4	1.6 ± 0.2	2.0	0.0001
Gemcitabine (nM)	19.1 ± 5.1	8.9 ± 1.6	2.1	0.003
Cisplatin (μM)	0.37 ± 0.14	0.44 ± 0.14	0.9	0.38
Carboplatin (μM)	5.4 ± 1.5	6.1 ± 1.3	0.9	0.36
Oxaliplatin (μM)	2.9 ± 0.3	2.4 ± 0.6	1.2	0.15
Busulfan (mM)	0.20 ± 0.07	0.14 ± 0.03	1.4	0.14
Docetaxel (nM)	5.4 ± 1.5	5.0 ± 2.4	1.1	0.81
Paclitaxel (nM)	35.6 ± 5.5	33.0 ± 6.3	1.1	0.51

[a]Data are expressed as the mean ± SD of at least four independent experiments.
[b]S represents the sensitisation factor.
[c]*P* values for statistical significance were determined by the two-sided paired *t*-test.

ATM function reduces the threshold for cell kill by a process that does not depend on apoptosis.

Loss of ATM and cell cycle phase distribution after treatment with the topoisomerase II poison epirubicin

To determine whether the differential sensitivity of ATM-proficient and -deficient cells to epirubicin was due to alteration in the ability to trigger G_1/S phase arrest, the fraction of cells in S phase was quantified as a function of drug exposure. Table 2 shows that 10 nM epirubicin reduced the fraction of ATM-proficient and -deficient cells in S phase after 24 h to about the same extent.

An alternative hypothesis is that the differential sensitivity to epirubicin is due to altered ability to activate the G_2/M checkpoint. Cells were exposed to 10 nM epirubicin, a concentration that inhibited the growth of ATM-deficient cells in the MTT assay by at least 70%, and the fraction of cells in G_2/M was determined at various time points. The data presented in Table 3 demonstrate that epirubicin produced a transient 1.3-fold accumulation of ATM-deficient cells in G_2/M at 24 h, largely at the expense of cells in G_1. Thus, ATM-deficient *p53*-null cells seemed to retain, at least partially, the ability to arrest in G_2/M in response to epirubicin. A comparable analysis of the response of ATM-proficient cells to epirubicin could not be interpreted due to the rapid formation of hyperploid cells.

Taken together, the cell cycle data suggest that ATM does not play a central role in the ability of epirubicin to trigger either the G_1/S or G_2/M checkpoint, and that the differential sensitivity of ATM-proficient and -deficient *p53*-null cells cannot be accounted for by loss of either of these cell cycle control elements.

Discussion

We report here that *p53*-null cells are sensitised by the loss of the *ATM* gene to topoisomerase I and II poisons and several types of antimetabolites, but not to the major platinum compounds, the major taxanes and one alkylating agent. The data permit several conclusions to be drawn. First, they extend the previous observation that p53-deficient mouse tissues are rendered more sensitive to γ-irradiation by the concurrent loss of *ATM* [11] by identifying classes of anticancer agents to which these cells are also hypersensitive. Secondly, they support the existence of an ATM-dependent, p53-independent DNA damage-induced effector pathway and the role of ATM as a sensor for specific types of DNA damage [5, 14]. Thirdly, the effector pathway activated by ATM in the absence of p53 must involve some as yet unravelled cellular defence mechanism(s), because the increased chemosensitivity of ATM-deficient cells was not accompanied by increased apoptosis or by altered activation of either the G_1/S or G_2/M checkpoints.

ATM may function as a sensor of DNA double-strand breaks arising from γ-irradiation [11] and, as reported here, from some types of anticancer agents including topoisomerase poisons or antimetabolites. However, it does not sense lesions produced by microtubule poisons or platinum compounds. This is perhaps not surprising for docetaxel and paclitaxel, since they are not known to interfere with DNA. Perhaps, as for UV radiation-introduced DNA damage [14], ATR, another member of the PIK family [9, 15], is responsible for sensing damage produced by platinum drugs. ATR and ATM are partially redundant, and they act in parallel but overlapping DNA damage signalling pathways, but respond primarily to different kinds of lesions [7, 15].

ATM regulates cell cycle checkpoint activation upon DNA damage causing cells to arrest in G_1/S and/or G_2/M, ensuring that cells delay entry into mitosis and putatively permitting time for damage repair prior to the onset of mitosis [16]. Although stress may cause overriding of the G_1/S and the G_2/M arrests in *p53*-mutant cells [2], damage-mediated activation of ATM may stop cells at the G_2/M checkpoint in these cells [16]. However, loss of ATM does not substantially affect either G_1/S or G_2/M checkpoint activation in p53-deficient cells after γ-irradiation [17]. Our data show that the increased sensitivity of these cells to epirubicin cannot be explained by the lack of ATM-mediated cell cycle checkpoint control. Indeed, ATM, unlike ATR, does not seem to modulate the length and magnitude of the G_2 arrest induction [18, 19].

The primary mechanism by which ATM exerts its protective effect may be through modulating damage repair and decreasing the threshold for cell kill [10]. ATM immunoprecipitates with DNA damage repair proteins including MLH1, MSH2, MSH6 and BRCA1, and may facilitate recruitment of repair proteins to the site of the lesion [20]. When such damage remains unrepaired as the cell enters mitosis, the increased rate of chromosome breaks and aberrant chromosome segregation results in increased cell killing [11]. Our results indicate that the increased chemosensitivity of the ATM-deficient cells may be a result of processes other than apoptosis.

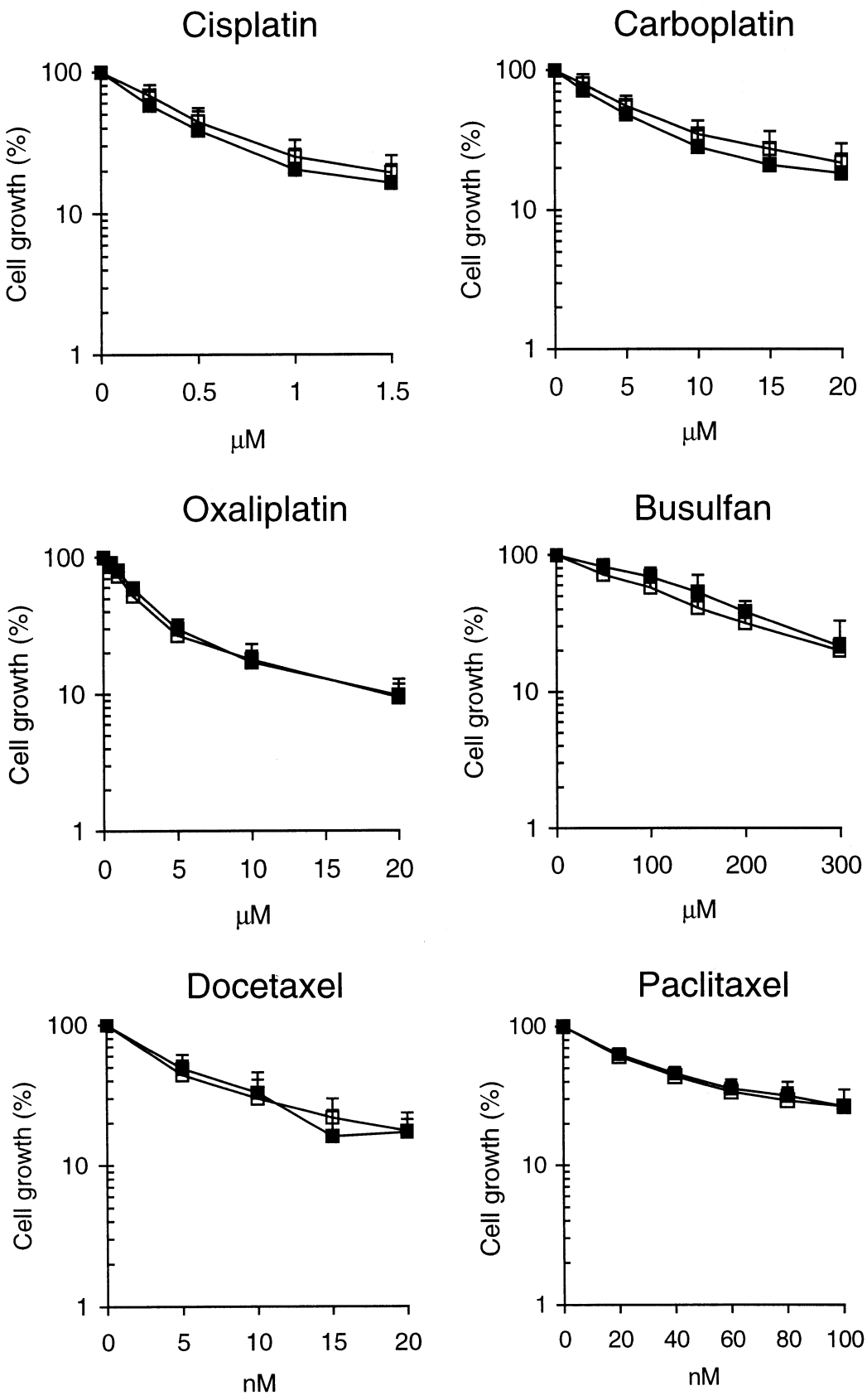

Figure 3. Growth inhibition in response to a continuous exposure to cisplatin, carboplatin, oxaliplatin, busulfan, docetaxel and paclitaxel in $atm^{+/+}/p53^{-/-}$ (filled squares) and $atm^{-/-}/p53^{-/-}$ (open squares) cell lines as determined by the MTT assay. Each point represents the mean of at least four independent experiments. Bars = SD.

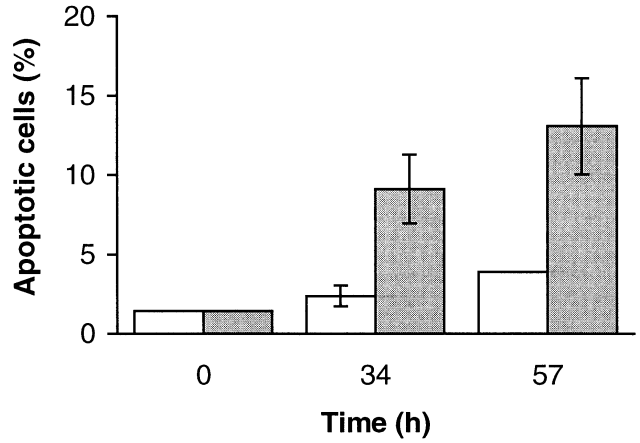

Figure 4. Effect of loss of ATM on the frequency of apoptotic cells in response to treatment with 25 nM epirubicin as a function of time. Open bars represent $atm^{-/-}/p53^{-/-}$ cells, whereas shaded bars represent $atm^{+/+}/p53^{-/-}$ cells. The data are the mean of two independent experiments. Bars = SD.

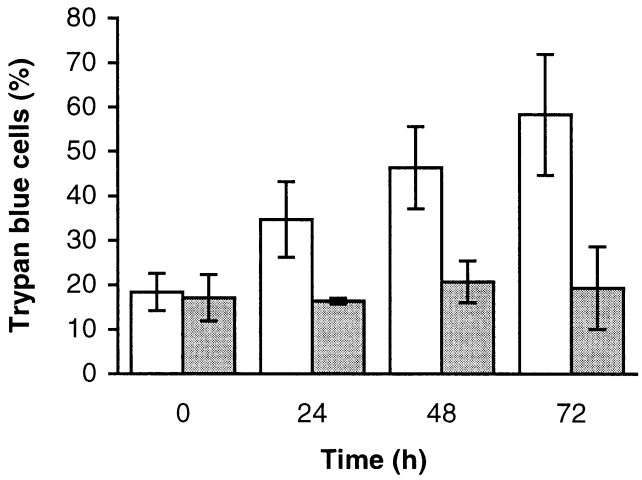

Figure 5. Effect of ATM deficiency on the frequency of Trypan Blue including $atm^{-/-}/p53^{-/-}$ (open bars) and $atm^{+/+}/p53^{-/-}$ (shaded bars) cells after treatment with 25 nM epirubicin. The data are the mean of two independent experiments. Bars = SD.

This study supports the concept that tumour-targeted functional inhibition of ATM increases the efficacy of some anticancer agents in the treatment of *p53*-deficient cancers, which comprise a large proportion of human tumours [1].

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Table 2. Percentage of ATM-proficient or -deficient *p53*-null cells in S phase in response to treatment with epirubicin determined by BrdU incorporation[a]

Time (h)	Epirubicin (10 nM)	
	$atm^{+/+}/p53^{-/-}$	$atm^{-/-}/p53^{-/-}$
0	29 ± 1	15 ± 3
24	7 ± 5	3 ± 1
48	0 ± 0	0 ± 0
72	0 ± 0	1 ± 1

[a]Values are the mean ± SD of two independent data sets.

Table 3. Percentage of ATM-deficient *p53*-null cells accumulated in the different cell cycle phases in response to treatment with epirubicin[a]

$atm^{-/-}/p53^{-/-}$	Epirubicin (10 nM)	
	G_1	G_2/M
Control	46 ± 10	37 ± 10
24 h	35 ± 5	47 ± 8
48 h	42 ± 6	31 ± 4
72 h	40 ± 4	28 ± 4

[a]Values are the mean ± SD of two independent data sets.

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