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European Journal of Cancer 37 (2001) 2247–2256

European Journal of Cancer

www.ejconline.com

A role for *c-myc* in DNA damage-induced apoptosis in a human *TP53*-mutant small-cell lung cancer cell line

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Received 19 January 2001; received in revised form 1 June 2001; accepted 2 August 2001

Abstract

Based on the role of p53 in the control of apoptosis following DNA damage, the status of the *TP53* gene has been implicated as a major determinant of tumour responsiveness to cytotoxic therapies. In spite of the high frequency of *TP53* mutations, small-cell lung cancer (SCLC) is recognised as one of the most chemoresponsive solid tumours. Since the relevance of the *TP53* gene status in the modulation of tumour responsiveness is dependent on the molecular/biological context, in the present study, we have examined the relationship between chemosensitivity and susceptibility to apoptosis of a *TP53*-mutant human SCLC cell line. The cell line, in spite of *TP53* mutation, retained an efficient response to genotoxic stress as documented by cells ability to modulate the p53 protein, arrest in the G1 and G2 phases of the cell cycle and its marked susceptibility to apoptosis following treatment with DNA damaging agents. Exposure to DNA-damaging agents caused an increase of c-Myc, a DNA damage-responsive transcription factor. An analysis of damage-induced apoptosis in the presence of an anti-Fas/CD95 inhibitory antibody indicated that Fas/CD95 was not required for the apoptotic response. The results support an implication of *c-myc* in sensitising cells to apoptosis, since inhibition of c-Myc expression with an antisense oligodeoxynucleotide (AS-ODN) almost abolished the drug-induced apoptotic response. In conclusion, the present results support a role for *c-myc* in the induction of apoptosis by genotoxic stress in the absence of a functional p53 and provide new insights into the mechanisms that may influence apoptosis in *TP53*-mutant cells. Elucidation of this pathway and of the possible cooperation with p53-dependent mechanisms may provide a basis for therapeutic intervention.

Keywords: Apoptosis; *c-myc*; *TP53*-mutation; Small-cell lung cancer; DNA damage

1. Introduction

Since p53 is a critical participant in the cellular response to DNA damage, the protein has been implicated as a determinant of the chemosensitivity of tumour cells [1]. Several studies support the view that the loss of wild-type p53 function in tumour cells is associated with resistance to DNA-damaging cytotoxic agents [2–4]. p53 has multiple functions (including cell-cycle progression control, DNA repair and activation of apoptosis) that may have contradictory effects on the cellular outcome following DNA damage. Despite a major role for p53 in the activation of the apoptotic pathway, the finding that *TP53*-null cell systems (in particular, lymphoid and leukaemic cells) undergo rapid apoptosis following cytotoxic treatment indicated the presence of p53-independent pathways [5,6]. However, p53-deficient cells may be relatively resistant, thus requiring relatively higher doses of genotoxic agents for the initiation of the apoptosis. It is likely that the relevance of the *TP53* gene status in the modulation of chemosensitivity is dependent on the biological background. For example, small-cell lung carcinoma (SCLC) is recognised as a chemoresponsive solid tumour in spite of a high frequency of *TP53* mutations.

In the present study, in an attempt to study the cellular basis of p53-independent apoptosis, we used a human cell line (derived from a SCLC) [7], that contains

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0959-8049/01/$ - see front matter
PII: S0959-8049(01)00268-4

a mutant *TP53* (one allele is deleted and the other has a mutation at codon 245 Gly→Asp). This is a hotspot mutation frequently observed in lung cancer [8,9]. Mutation at this codon was found in Li–Fraumeni patients and has been associated with p53 inactivation [10].

2. Materials and methods

2.1. Drugs and chemicals

Doxorubicin (DX) (Pharmacia UpJohn, Milan, Italy) was dissolved in water and diluted in 0.9% NaCl solution before use. A 15-mer anti-sense[sense]aligodeoxynucleotide (AS) [S]ODN) (5′-AACGTTGAGGGGCAT-3′) complementary to the translation initiation region of the *c-myc* mRNA was used. The scrambled sequence [S]ODN containing the 'G-quartet' motif (5′-AAGCATACGGGGTGT-3′) was used as a control [11].

2.2. Cell cultures and treatments

POGB, a SCLC cell line, was derived from a human lung tumour biopsy; it was maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Bio-Whittaker, Verviers, Belgium) supplemented with 10% fetal calf serum (Mascia Brunelli, Milan, Italy). A detailed characterisation of the cell line has been reported elsewhere [7]. According to Carney [12], the cell line can be classified as type 3, because cells grow as loosely adherent aggregates. Cells were seeded (2×10^4 cells/cm^2) and 48 h later they were either irradiated with a ^{137}Cs source delivering 0.13 Gy/s or treated with DX. At 24, 48 and 72 h after treatment, floating and adherent cells were collected, washed in phosphate-buffered solution (PBS) and counted to evaluate the cytotoxic effect of the treatment. In combined treatments, different concentrations (0.25 and 0.5 μg/ml) of an anti-Fas ZB4 antibody (MBL, Nagoya, Japan) were added to the cells 1 h before exposure to DX (200 ng/ml). In experiments performed in the presence of *c-myc* AS-ODN, the ODNs were added at 100 μg/ml immediately before DNA-damaging treatment and added at 50 μg/ml every 24 h after the beginning of treatment. This concentration was found to be optimal without non-specific effects in a previous paper [11] and no effects of the scrambled oligonucleotide were observed.

2.3. Apoptosis and cell-cycle analysis

Treated and untreated cells were fixed in 70% (v/v) ice-cold ethanol and samples were stored at −20 °C until analysis (2–5 days). Cells, rehydrated in PBS, were stained in propidium iodide (PI) solution (20 μg/ml in PBS containing 66 units/ml RNAse A). Using these samples, apoptosis was determined morphologically (chromatin condensation and DNA fragmentation) by fluorescence microscopy on at least 100 cells in two different smears from each sample. The percentage of apoptotic cells was calculated with reference to the cell number of the whole population (floating + adherent cells). Moreover, apoptosis was also determined by annexin and Tdt-mediated dUTP-biotin nick-end labelling (TUNEL) reaction by using a fluorescent activated cell sorter (FACScan) flow cytometer equipped with an argon laser for fluorescence excitation at 488 nm (Becton Dickinson, Mountain View, CA, USA). For the annexin reaction, cells (1×10^5) were suspended in 195 μl of binding buffer (10 mM Hepes–NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and 5 μl of annexin (Bender System, Vienna, Austria) was added to each sample and incubated for 10 min. After washing, cells were resuspended in binding buffer containing PI (1 μg/ml) and analysed by FACScan. In Fig. 2, the annexin fluorescence is reported in the abscissa and the PI fluorescence in the ordinate. For the TUNEL reaction, cells (5×10^5) were fixed in 4% (w/v) paraformaldehyde for 45 min at room temperature. After rinsing with PBS, cells were permeabilised in a solution of 0.1% (v/v) Triton X-100 in 0.1% (w/v) sodium citrate for 2 min on ice. Samples, washed in PBS, were then incubated in the TUNEL reaction mix (Boehringer Mannheim, Mannheim, Germany) for 1 h at 37°C in the dark and, after rinsing with PBS, analysed by FACScan. The number of TUNEL positive cells (in the ordinate) as a function of forward scatter (in the abscissa) is reported in the figure.

Cell-cycle distribution was investigated by flow cytometry on adherent cells fixed and stained with PI as above. In FACScan analysis 10 000 cells were measured in each sample. The percentage of cells in the different regions was calculated by means of the LYSIS II software (Becton Dickinson). For bromo-deoxyuridine (BrdU) incorporation, cells were pulse-labelled with BrdU (10 μM for 1 h) at 72 h of treatment. Then cells were collected, fixed in 70% (v/v) ice-cold ethanol and exposed to 1 N HCl for 30 min and to 0.1 M sodium tetraborate for 10 min. After incubation in the presence of a BrdU antibody (Becton Dickinson) ((3 μg/ml) in PBT solution (PBS + 1%(w/v) bovine serum albumin + 0.2% (v/v) Tween 20) and then with a anti-mouse-fluorescein isothiocyanate (FITC) antibody (Sigma, St. Louis, MO, USA) diluted 1:50 in PBT solution, cells were counterstained with PI and analysed by flow cytometry. The green FITC-fluorescence, expressed on a logarithmic scale, indicated the BrdU incorporation, and the PI-red fluorescence, on a linear scale, was the index of DNA content.

2.4. Western blot analysis

Cell lysates from treated and control cells were prepared as previously described with minor modifications [2]. Samples (80 μg/lane) were fractionated by sodium

dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and blotted on nitrocellulose sheets. Blots were preblocked for 1 h at room temperature in PBS containing 5% (w/v) dried non-fat milk. Filters were incubated overnight at 4 °C with monoclonal antibodies to p53 (DO-7, Dako, Glostrup, Denmark), Bcl-2 (Dako), topoisomerase IIα (Dako) cyclin A (Santa Cruz Biotechnology, Santa Cruz, CA, USA), p21/WAF-1 (Neomarkers, Union City, CA, USA) and c-Myc (Oncogene Science, Uniondale, NY, USA) or with rabbit antibodies to Bax (Pharmingen, San Diego, CA, USA). A rabbit anti-actin antibody (Sigma) was used as a control for loading. Antibody binding to the nitrocellulose blots was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Cologno Monzese, Italy). Band intensity was quantified using a PhosphoImager dedicated software.

2.5. Immunofluorescence determination of Fas/CD95 and c-Myc expression

Ethanol or paraformaldehyde-fixed cells were washed in PBS, preincubated for 15 min in PBT solution (PBS + 1% bovine serum albumin + 0.2% Tween 20) to block unspecific antibody binding, and incubated for 60 min at room temperature in 100 μl of specific monoclonal antibody in PBT solution (2.5 μg/ml anti-Fas ZB4 antibody, 5 μg/ml anti-c-Myc Ab-1). Thereafter, cells were washed twice with PBT and incubated for 60 min with a secondary FITC-conjugated antibody (Sigma) diluted 1:100 in PBT solution. Negative controls consisted of cells incubated only with the secondary antibody. After washing, cells were resuspended in PBS, and cell fluorescence was measured with a FACScan flow cytometer and evaluated by means of LYSIS II software (Becton Dickinson).

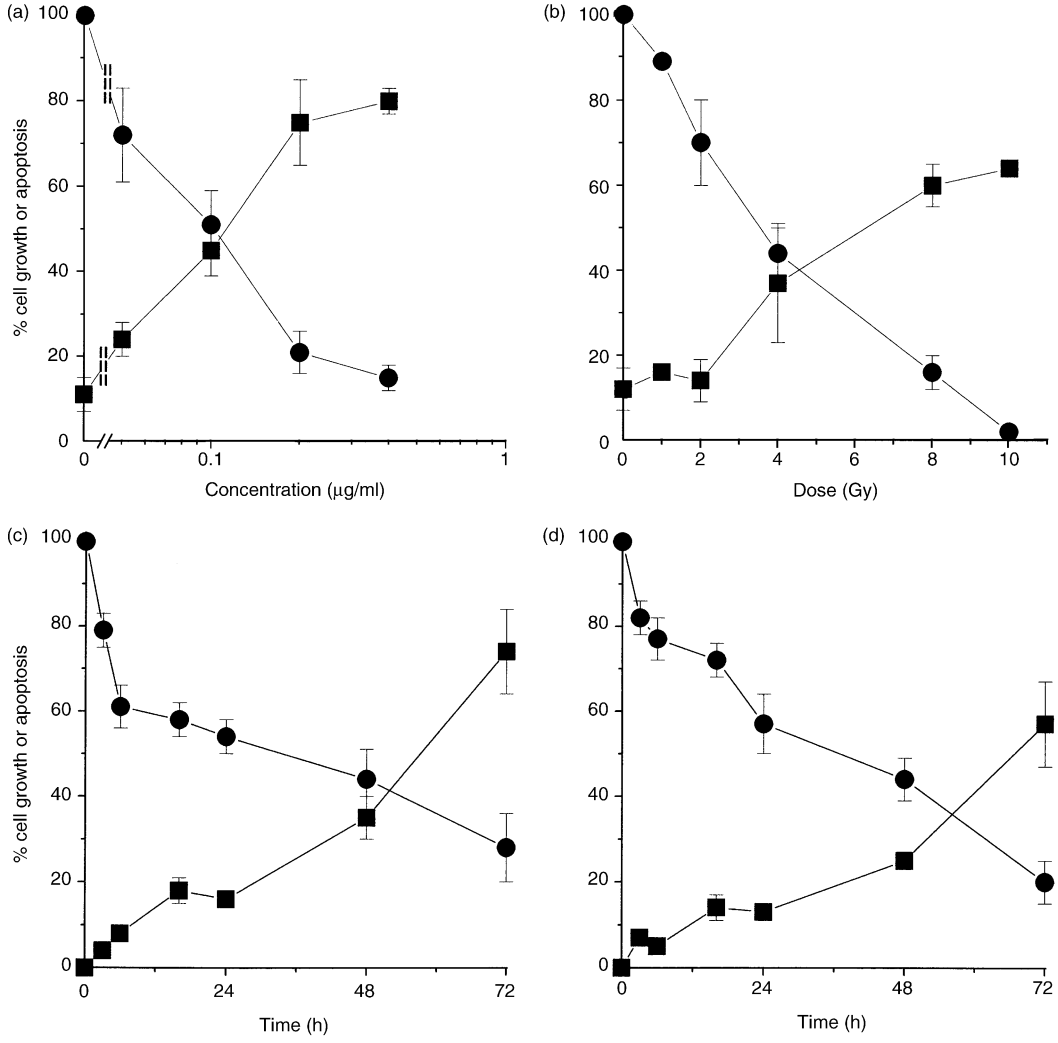

Fig. 1. Dose-dependent effect of (a) doxorubicin (DX) or (b) γ-ray at 72 h and time-course of (c) DX 200 ng/ml or (d) γ-ray 8 Gy on POGB cell growth (circles) or apoptosis (squares). Growing cells were treated with different doses of DX or γ-rays. Cell viability and apoptosis were detected at different times after the beginning of treatment by cell counting of fresh cells or of propidium iodide stained fixed cells. Values were derived from at least three independent experiments (mean ±standard deviation (S.D.)).

3. Results

3.1. Cytotoxicity and apoptosis

The relationship between cytotoxicity and apoptosis in the POGB cells was studied for two DNA-damaging agents which are known to produce different types of DNA lesions (i.e. DNA strand breaks caused by ionising radiation-induced reactive radicals and topoisomerase II-mediated DNA cleavage induced by DX). With both agents, the cytotoxic effects were dose-dependent as shown by dose–response curves (Fig. 1a and b). Following 72 h of exposure, the IC_{50} for DX was 0.1 ± 0.05 μg/ml and the ID_{50} for ionising radiation was 3.4 ± 0.4 Gy. Both cytotoxicity and drug-induced apoptosis were time-dependent effects (Fig. 1c and d). Annexin (Fig. 2a and b) and TUNEL (Fig. 2c) reactions further supported the presence of a time-dependent apoptosis. Values obtained by flow cytometric analysis were similar to those obtained by morphological evaluation. Dose–response curves clearly documented a relationship between an antiproliferative effect and apoptosis.

3.2. Cell-cycle perturbations

The effect of DNA-damaging treatment on cell cycle distribution was examined in adherent POGB cells as propidium iodide fluorescence (Fig. 3a) and BrdU incorporation (Fig. 3b). Studies were performed under conditions (from 3 to 5 days after cell seeding) where no appreciable modifications in the cell-cycle distribution of the control cells could be observed. In cells exposed to highly cytotoxic doses/concentrations (ID_{80} for γ-rays and IC_{80} for DX), perturbations of the cell cycle were evident already after 24 h of exposure. DX (200 ng/ml) induced a marked increase in accumulation of cells in

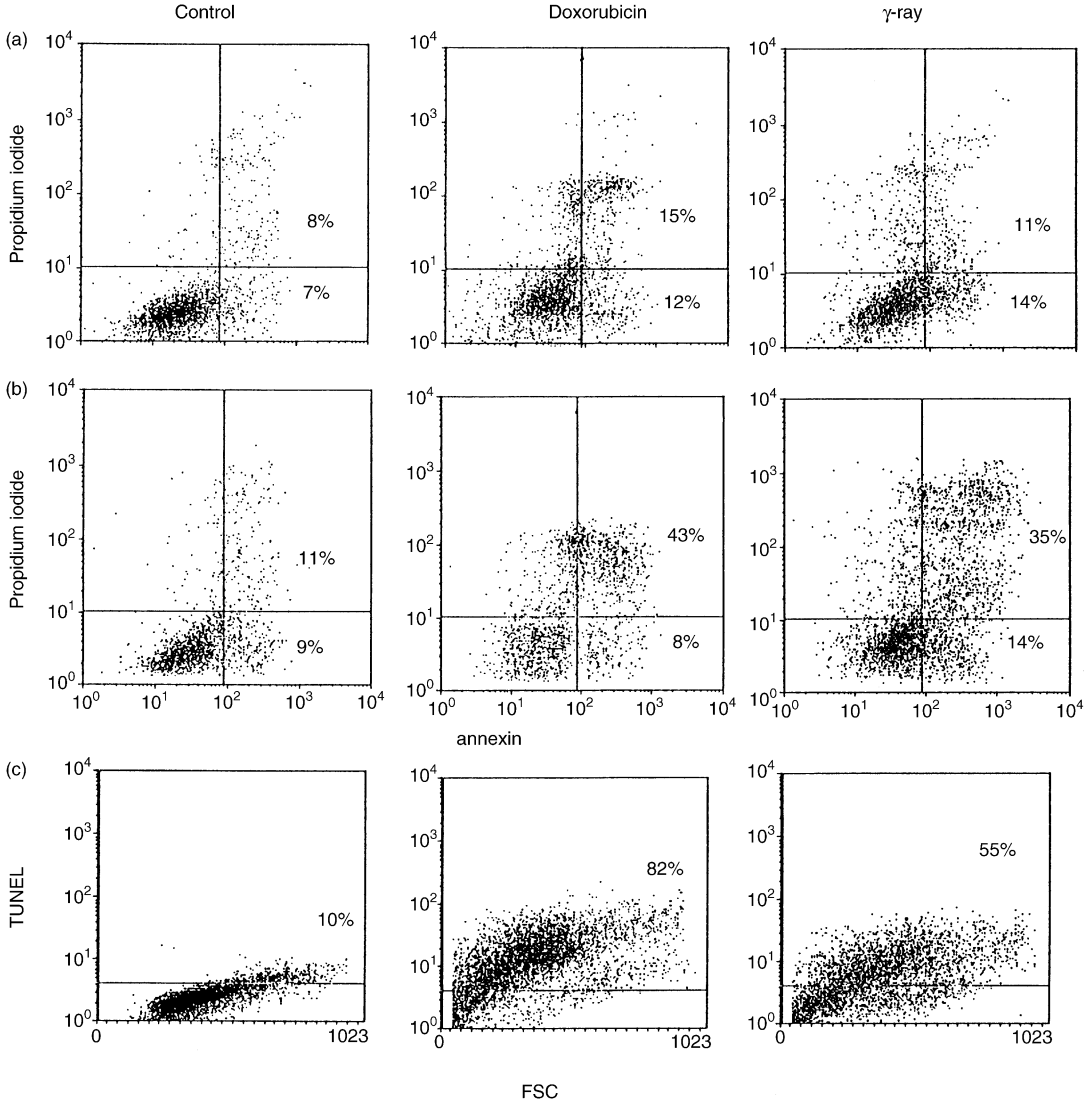

Fig. 2. Flow cytometric analysis of annexin ((a) 24 h; (b) 48 h) and Tdt-mediated dUTP biotin nick-end labelling (TUNEL) (c) 72 h after DX (200 ng/ml) or γ-ray (8 Gy) treatment. Dot-plots are from one representative experiment. FSC, forward scatter.

the G2/M phase, which was persistent up to 72 h, and a reduction of cells in the G1 phase. An equitoxic dose of ionising radiation (8 Gy) induced a different cell cycle perturbation. From 24 to 72 h after radiation exposure, the percentage of cells in G2/M phase was significantly higher than in the untreated control. However, for up to 48 h, relevant fractions of irradiated cells were in the G1 phase and in active DNA synthesis (Fig. 3b). At 72 h, the percentage of DNA synthesising cells decreased and G1 and G2 arrests were evident.

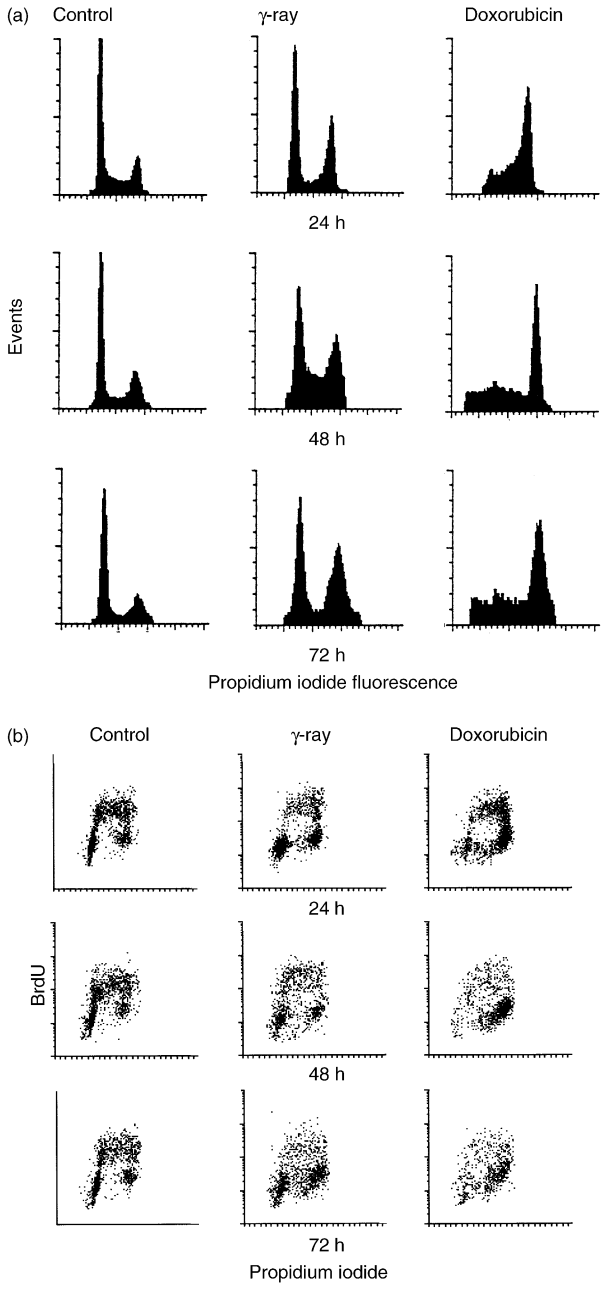

Fig. 3. Time-course of the cell cycle distribution of POGB cells after doxorubicin (DX) (200 ng/ml) and γ-ray (8 Gy) treatment. (a) Fixed cells were stained with propidium iodide solution and analysed with a fluorescent activated cell sorter (FACS)can flow cytometer. (b) Cells were exposed to bromodeoxyuridine (BrdU) for 1 h, collected, fixed and incubated with an anti-BrdU antibody and then with an anti-mouse-fluorescein isothiocyanate (FITC) antibody and counterstained with propidium iodide. Fluorescence was determined by FACScan analysis. Propidium iodide staining (DNA content) is plotted on the abscissa and FITC fluorescence (BrdU incorporation) is plotted on the ordinate. Profiles are from one experiment that is representative of two.

3.3. Expression of p53, $p21^{WAF1/Cip1}$ and apoptosis-related genes

As expected for a mutant protein, p53 was expressed at high levels in control untreated POGB cells. The expression level of p53 in POGB cells treated with DX or γ-rays was investigated by western blot 24 and 48 h after treatment (Fig. 4a). Both DNA damaging agents determined a marked increase in the protein expression level.

The expression of other genes implicated in the regulation of apoptosis was determined after exposure to DX or γ-rays (Fig. 4a). A slight phosphorylation of the anti-apoptotic protein Bcl-2 was detected 24 and 48 h after treatment. The pro-apoptotic protein Bax increased 24 h after treatments.

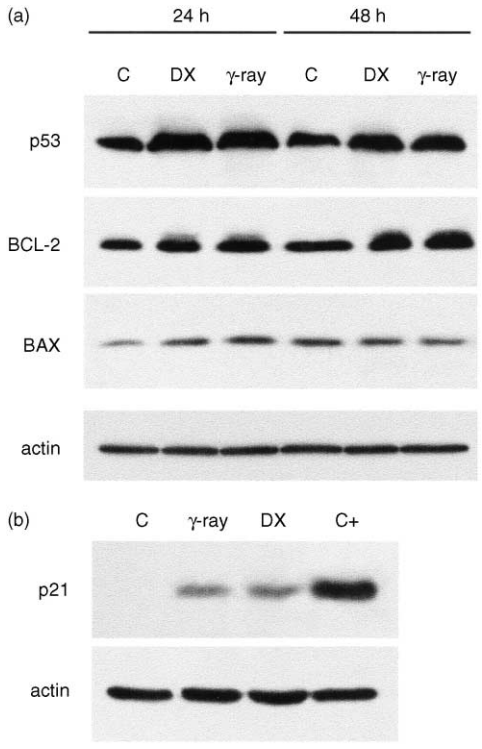

Fig. 4. Western blot analysis of p53, Bcl-2 and Bax protein levels in control, 200 ng/ml DX or 8 Gy-treated cells (a) at 24 and 48 h after the beginning of treatment and (b) of $p21^{WAF1/Cip1}$ at 24 h after the beginning of treatment. IGROV-1 cells were used as a positive control. 80 μg of total protein was loaded in each lane and fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). An anti-actin antibody was used as a loading control. One experiment representative of three is reported.

No appreciable modulation of other apoptosis-related proteins including Bak, Bcl-x_L, Bcl-x_S and Bag-1 was observed following treatment (data not shown).

The analysis of $p21^{WAF1/Cip1}$ indicated a lack of expression in the untreated and treated cells (Fig. 4b).

3.4. c-Myc expression and modulation

To examine the possible contribution of *c-myc* to the cellular response to DNA damage in POGB cells, we performed a flow cytometric analysis of its expression in POGB cells (Fig. 5). Under conditions of exponential growth, continuous exposure to DX produced a marked increase in c-Myc expression, which was maintained for at least 48 h, when apoptosis was pronounced. Ionising radiation caused an increase in c-Myc expression with a peak at 16 h, and a subsequent decrease to the levels of the controls. The different time-course of c-Myc modulation could be related to the modality of treatment with the two DNA-damaging agents, because, in contrast to irradiation, DX was maintained in the culture medium.

3.5. Effect of inhibition of c-Myc expression

To better define the role of *c-myc* in the cellular response to DNA damage, we investigated the effects of a specific AS-ODN [11]. Western blot analysis of cells at 24 h of treatment, indicated that pretreatment with the *c-myc* AS-ODN (which was maintained in the culture throughout the experiment) caused, in comparison to samples not treated with AS-ODN (Fig. 6 lanes A–C; densitometric analysis of the c-Myc/actin ratio gave values of 0.21, 0.18 and 0.33 in the control cells, γ-irradiated and DX-treated cells, respectively), a reduction in the c-Myc expression in controls as well as in cells exposed to γ-rays or DX (Fig. 6, lanes A′–C′; densitometer analysis of c-Myc/actin gave values of 0.086, 0.089 and 0.078 in control cells, γ-irradiated and DX-treated cells, respectively). The reduction induced by AS-ODN was more marked in the DX-treated cells (lane C′) where, in the absence of the AS-ODN, a slight increase in the expression of c-Myc (lane C) was observed.

To assess the specificity of the AS-ODN used, the levels of cyclin A, a *c-myc*-target gene [13], were analysed. In cells not pretreated with the *c-myc* AS-ODN, an increase in cyclin A expression was induced by ionising radiation (lane B) and, to a higher extent, by DX-treatment (lane C) (the ratios of densitometric evaluation of cyclin A/actin were 1.81 and 2.93, respectively, and 1.3 in the control samples). Treatment with the *c-myc* AS-ODN resulted in a reduction of cyclin A expression in all of the samples which was more marked in the treated cells (lanes A′–C′) (the ratios of densitometric evaluation of cyclin A/actin were 1.37 for ionising treatment, 0.69 for DX and 0.91 in control samples).

Moreover, the exposure of POGB cells to the *c-myc* AS-ODN determined a reduction in the cytotoxic effect of the DNA damaging agents (Table 1). This effect was consistent with a marked decrease in the percentage of apoptotic cells. The AS-ODN alone had no effect on cell proliferation and on the apoptosis levels of the untreated cells. A lack of significant effects of *c-myc* AS-ODN itself may be consistent with a low basal expression of the protein compared with other cell lines (data

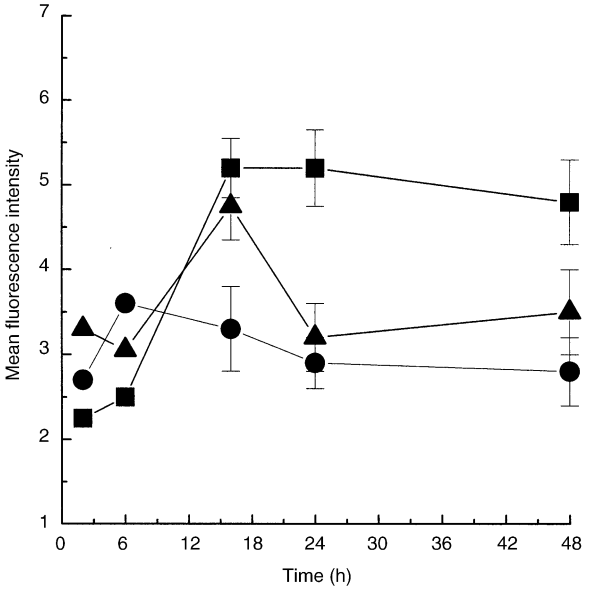

Fig. 5. Time course of modulation of c-Myc expression in doxorubicin (DX) and ionising radiation-treated cells. POGB cells were treated with DX (200 ng/ml) (■), γ-rays (8 Gy) (▲) or untreated (●). Fixed cells were incubated with anti-c-Myc antibody and then with a fluorescein isothiocyanate (FITC) secondary antibody. Mean fluorescence intensity (arbitrary units), representative of the c-Myc levels, was determined by a fluorescent activated cell sorter (FACS)can flow cytometer.

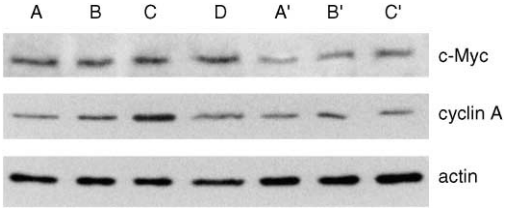

Fig. 6. p64 c-Myc and cyclin A expression in POGB cells either control, or after 24 h of treatment with 8 Gy or with 200 ng/ml DX in the absence of the *c-myc* antisense (A, B and C, respectively) or in the presence of *c-myc* antisense (A′, B′ and C′, respectively). In lane D, cells were exposed to a scrambled sequence used as a control. 80 μg of total protein was loaded in each lane and fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). An anti-actin antibody was used as a loading control. One experiment representative of three is reported.

Table 1
Effect of *c-myc*-antisense oligodeoxynucleotide (ODN) on apoptosis induction by DNA-damaging agents[a]

	Cell growth (%)	% Apoptotic cells[b]
Control	100	11±2
Anti-*c-myc*-ODN	108±15	10±1
Ionising radiation	34±10	43±5
Ionising radiation + anti-*c-myc*-ODN	48±10	22±4
Doxorubicin	24±4	60±8
Doxorubicin + anti-*c-myc*-ODN	76±2	8±1

[a] Results are the mean of two independent experiments ± standard deviation (S.D.). All determinations were performed 72 h after the beginning of treatment. *C-myc*-antisense was added to the cell culture immediately before the DNA-damaging treatments.

[b] Apoptotic cells were detected by fluorescence microscopy on propidium iodide-stained cells.

not shown). The effect of *c-myc* was not mediated by topoisomerase II α as shown by the lack of modulation of the levels of this enzyme in samples treated with the DNA damaging agents in the presence or absence of the *c-myc* AS-ODN (Fig. 7).

3.6. Interaction between the anti-Fas/CD95 antibody and doxorubicin activity

Since Fas/CD95 has been implicated in c-Myc-mediated apoptosis [13], we studied the effect of the anti-Fas/CD95 antibody on the induction of apoptosis in DX-treated cells. Fas/CD95, detected by flow cytometric analysis, was expressed to an extent comparable to that of the HL-60 cell line (Fig. 8a), which is known to express the receptor [14], and was enhanced by radiation and doxorubicin exposure (Fig. 8b). However, treatment with an anti-Fas/CD95 antibody, capable of inhibiting the signalling through the receptor, did not modify the level of basal apoptosis or that induced by DX (Fig. 9). Such results rule out the Fas/CD95-mediated signalling as an obligate step in DX-induced apoptosis of POGB cells.

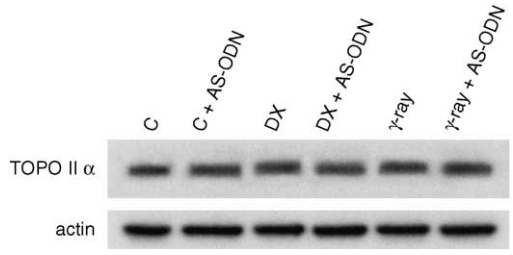

Fig. 7. Western blot analysis of topoisomerase II α levels after 24 h of treatment with the DNA-damaging agents in the absence or in the presence of the *c-myc* antisense. 80 μg of total protein was loaded in each lane and fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Anti-actin antibody was used as a loading control. One experiment is used that is representative of two.

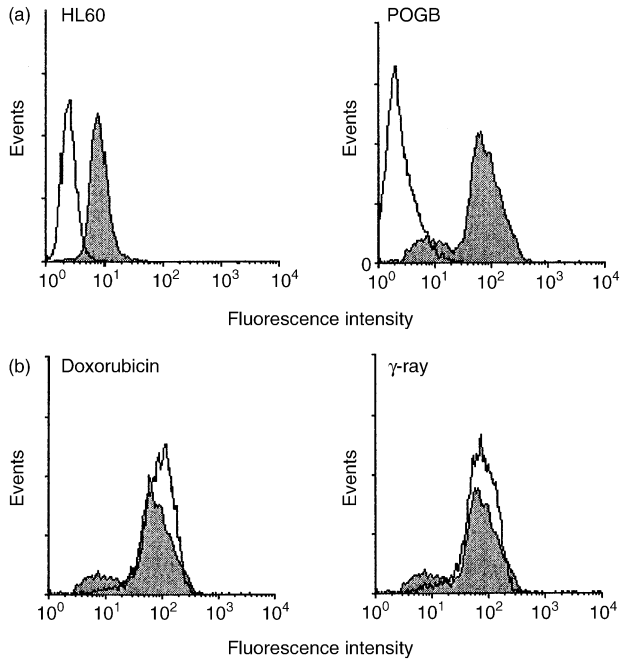

Fig. 8. Fas/CD95 expression and modulation in POGB cells. (a) Fas/CD95 expression in POGB cells and HL60 used as positive control (grey profiles); empty profiles: blank. (b) Fas/CD95 expression in POGB cells at 24h of treatment with doxorubicin (200 ng/ml) or γ-ray (8 Gy) (empty profiles); grey profiles: control untreated cells. Ethanol-fixed cells were incubated with anti-Fas/CD95 antibody ZB4 (2.5 μg/ml) and a fluorescein isothiocyanate (FITC) secondary antibody. Fluorescence was analysed with a fluorescent activated cell sorter (FACS)can flow cytometer. The blank was incubated only with the FITC secondary antibody.

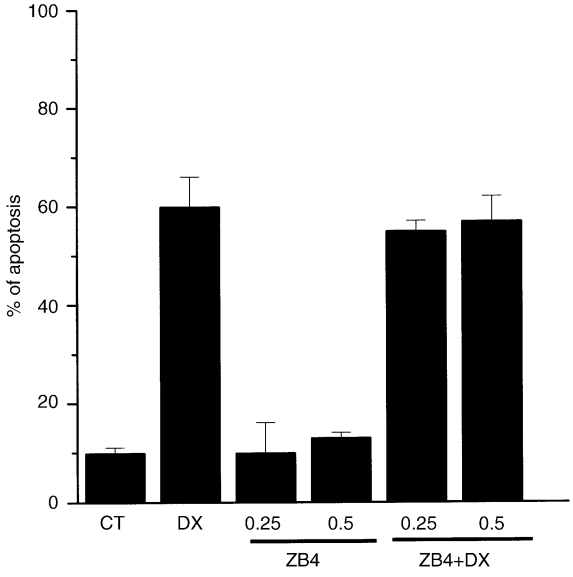

Fig. 9. Effect of Fas/CD95 antibody ZB4 on doxorubicin (DX)-induced apoptosis. Cells were exposed to anti-Fas ZB4 (0.25, 0.5 μg/ml) 1 h before DX-treatment (200 ng/ml). After 72 h of exposure, cells were fixed and stained with propidium iodide to determine the amount of apoptotic cells. CT, control.

4. Discussion

Several lines of evidence support the possibility that susceptibility to apoptosis contributes to the response of tumour cells to most currently used cytotoxic agents. Thus, alterations that impair susceptibility to apoptosis should produce drug resistance. A major pathway of cell death caused by exposure of tumour cells to cytotoxic agents involves activation of p53-dependent apoptosis [1,3,4]. Mutations of the *TP53* gene are detectable in 80% of human lung cancers [15]; in particular, codon 245 is a mutational hotspot in lung cancer and mutation at this site has been related to tumour progression [9,10]. Thus, inactivation of *TP53* raises the possibility that a defective apoptotic pathway may contribute to the natural resistance of lung tumours.

In this study, a SCLC cell line carrying a mutant *TP53* gene (codon 245) was used to investigate the mechanisms that underlie the *TP53*-independent apoptotic pathway. The results indicated that massive apoptosis can be induced by cytotoxic levels of DNA damage produced by physical or chemical agents. The high basal level of Bcl-2 expression is consistent with p53 inactivation because p53 can inhibit *bcl-2* gene expression [16]. Treatment-associated Bcl-2 phosphorylation, likely resulting in loss of its anti-apoptotic function (17), could favour apoptosis induction. POGB cells retained an efficient DNA-damage response as documented by modulation of genotoxic stress-responsive transcription factors (p53 and c-Myc) and by the efficiency of DNA damage checkpoints (i.e. arrest at G1 and G2 phase).

Among the transcriptional factors involved in the cellular response to DNA damage [18], we examined the role of c-Myc, because it has been implicated in the regulation of apoptosis [13,19]. An increase in c-Myc expression was observed following DNA damage caused by agents known to induce different types of DNA lesions. Induction of c-Myc expression, more persistent in DX-treated cells compared with irradiated cells (Fig. 5), was likely related to the nature and duration of the cytotoxic stress (i.e. continuous exposure) and could account for a different cell-cycle perturbation (arrest in G1 after ionising radiation and in G2 after DX treatment; Fig. 3) and a more dramatic effect on apoptosis. A critical role for c-Myc as a determinant of the ability of POGB cells to trigger apoptosis was strongly supported by the inhibitory effects of the *c-myc* antisense ODN on the apoptotic response induced by the genotoxic treatment.

The mechanisms underlying the c-Myc-dependent response seem to vary among cells of different tumour types. A Fas/CD95-mediated apoptosis has been reported in lymphoblastic leukaemia cell lines (HL-60 and U-937) after anthracycline and ionising radiation treatment [14,20]. Moreover, a role for the Fas/Fas ligand signalling cascade in Myc-mediated apoptosis was demonstrated in fibroblasts [21]. In our cell system, the Fas/CD95-mediated pathway was not involved in the apoptotic response to DNA damage in spite of a detectable upregulation. Our observation is consistent with a similar finding in other lung tumour cells [22] and in other cell systems [23].

It is conceivable that in chemosensitive cells the expression of a functional wild-type p53 lowers the threshold at which drug-induced DNA damage triggers apoptosis, and p53-dependent and-independent pathways could cooperate in determining chemosensitivity to DNA-damaging agents [24]. Our interpretation is consistent with experiments indicating that the introduction of a wild-type *TP53* gene conferred chemosensitisation to POGB cells (data not shown). A similar finding has been reported for a human lung carcinoma cell line characterised by *TP53* deletion [25].

Based on the available information, a plausible explanation for the DNA damage-induced apoptosis in tumour cells with a different biological background is that various pathways are activated by transcriptional factors (including p53 and c-myc) [6,13] responsive to DNA damage and may participate in determining the cellular outcome. Relevant to such an interpretation is the observation that active E2F proteins released following c-Myc activation can cooperate with p53 to stimulate apoptosis [26,27]. c-Myc may induce apoptosis by both p53-dependent and p53-independent mechanisms [13]. The relative susceptibility to drug-induced apoptosis may be dependent on the expression of multiple factors activated by the triggering signals if the rest of the apoptotic pathway remains intact. Like p53, c-Myc can also have multiple functions, including promotion of cell proliferation and induction of apoptosis [19,26]. The detailed mechanisms involved in c-Myc-induced apoptosis remain unknown. In cell systems retaining an efficient cell-cycle control (like POGB), it is possible that cell death results from a conflict between c-Myc-mediated growth-promoting signals and growth-arresting signals (required for DNA repair) [26]. Additional factors may participate in determining the cellular response to DNA damage in POGB cells. Among the studied apoptosis-related proteins a slight upregulation of the pro-apoptotic Bax was observed. Although this type of modulation has been related to p53-dependent activation of the *bax* promoter, a p53-independent role of Bax function has been documented [28]. A pathway involving Bax is also supported by the phosphorylation of Bcl-2 following doxorubicin and γ-ray treatment which has been associated with inactivation of its anti-apoptotic function [17]. Moreover, it has recently been documented that Bax is a transcriptional target of c-Myc and mediates c-Myc induced apoptosis [29].

In conclusion, inactivation of p53 function in POGB cells, as a consequence of gene mutation, was not associated with loss of the cell's ability to undergo apopto-

sis, since the cell line retained an apoptotic response following cytotoxic lesions. The relative resistance toward apoptosis induced by DNA-damaging agents (requiring high doses for induction) may reflect the lack of cooperation between p53 and other DNA-damage-responsive factors in triggering apoptosis in response to genotoxic signals. It is proposed that a number of transcriptional factors are involved in the response to DNA damage as determinants of cell susceptibility to apoptosis. The evidence that DNA damage-induced apoptosis in SCLC cells may occur through a p53-independent pathway is consistent with the responsiveness of SCLC to cytotoxic therapy, in spite of the high frequency of *TP53* mutations (around 80%) [15]. The observation that in this biological context apoptosis induction required relatively high doses of cytotoxic agents may support the clinical interest of high-dose therapy for this lung tumour histotype. Although the general relevance of the described cellular response for possible therapeutic intervention remains to be documented in other cell systems, the observation that a large number of SCLC show overexpression of c-Myc [30,31] may be consistent with a potential role for a c-Myc-mediated apoptotic pathway in this tumour type.

Acknowledgements

The authors wish to thank Laura Zanesi and Mario Azzini for editorial assistance. The study was partially supported by the Consiglio Nazionale delle Ricerche (Finalized Project ACRO), by the Ministero della Sanita', and by the Associazione Italiana per la Ricerca sul Cancro.

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