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Role of c-myc protein in hormone refractory prostate carcinoma: cellular response to paclitaxel

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Abstract

Amplification of the *c-MYC* proto-oncogene is a frequent alteration in hormone refractory prostate carcinomas (HRPC). In an attempt to investigate the role of c-myc in the cellular response to paclitaxel (PTX), we used two HRPC cell lines, DU145 and PC3, characterised by different levels of the protein and by different behaviour in response to taxane. In both cell lines, PTX-induced cell death was a caspase-mediated apoptosis. In DU145 cells, PTX induced an early apoptotic response associated with upregulation of c-myc restricted to the G2/M cell population. This event appeared delayed in the presence of c-myc antisense (AS-c-myc), suggesting an upstream regulation of the protein expression. In addition, the antisense approach provided evidence of an involvement of c-myc in the apoptotic response to the taxane. In contrast, in PC3 cells, the overexpressed c-myc was not modulated by drug-treatment and the addition of AS-c-myc did not affect the cell growth inhibition of PTX. In both cell lines, PTX-induced c-myc phosphorylation was concomitant with the mitotic arrest and not related to the modulation of the activation state of AKT and MAPK kinases. Our data indicate that the cellular response to PTX of HRPC cells can involve c-myc and suggest that its pro-apoptotic role is affected by the genetic background, thus supporting a complex and differentiated HRPC cell response to taxanes.

Keywords: c-myc; Prostatic neoplasms; Paclitaxel; Apoptosis; Oligodeoxynucleotide

1. Introduction

Hormone refractory prostate carcinoma (HRPC) is an aggressive and treatment-resistant disease. The pharmacological therapeutic approach for this disease has historically shown limited efficacy [1]. Recently, chemotherapy regimens involving agents that affect microtubule integrity have shown activity with tolerable adverse effects [2]. In particular, combination treatments including taxanes (paclitaxel or docetaxel) represent a promising treatment option for HRPC patients [3].

Taxanes bind microtubules and cause stabilisation of microtubule dynamics impairing mitosis and cell cycle progression [4]. Mitotic arrest is a constant aspect of the drug-induced cytotoxicity [5]. Nevertheless, the biological factors that determine the antiproliferative and apoptotic effects of taxanes in patient tumours have not been definitely elucidated [6]. Thus, a better knowledge of the molecular determinants of the cellular response to taxanes would be helpful to improve the therapeutic options for HRPC.

The acquisition of genetic mutations during the development of the hormone-refractory disease might favour the survival of prostate carcinoma cells in the absence of androgen [2]. The amplification of the *c-MYC* proto-oncogene has been identified as one of the most common genetic alterations in prostate cancers [7,8] and overexpression of c-myc protein is associated with the androgen refractory state [9]. The product of the *c-MYC* gene is a nuclear phospho-protein that plays an essential role in the

Abbreviations: AS-c-myc, c-myc antisense; HRPC, hormone refractory prostate carcinoma; ODN, oligodeoxynucleotide; PI, propidium iodide; PTX, paclitaxel; Scr-ODN, scrambled oligodeoxynucleotide; TUNEL, TdT-mediated dUTP nick-end labelling

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control of cell growth and cell fate (differentiation or apoptosis) [10–13]. C-myc protein overexpression has been reported to immortalise cells, to reduce their growth factor requirements and to promote cell cycle progression and genomic instability [12,14,15].

According to a role in the maintenance of the mitotic spindle integrity, overexpression of c-myc has been reported to promote endoreduplication and to disrupt the spindle checkpoint activated by taxanes [16,17]. In colon cancer cell lines, *c-MYC* amplification has been related to the modulation of the multiple effects of paclitaxel (PTX) [18].

In the present study, the role of c-myc in the cellular response to PTX was investigated in two prostate carcinoma cell lines, DU145 and PC3, characterised by different levels of *c-MYC* gene expression [19]. We previously reported that PTX showed a comparable antiproliferative activity on DU145 and PC3 cells. Nevertheless, the two prostate carcinoma cell lines underwent different cell fates after exposure to equitoxic concentrations of the drug as a consequence of different efficiency of cell cycle checkpoints activated by the spindle damage [20]. We examined the influence of c-myc on PTX-induced cell cycle progression and cell death by the antisense approach. Further, since the serine/threonine protein kinases of the MAPK family and AKT have been implicated in the phosphorylative events regulating c-myc stabilisation [21], as well as in the cellular response to the taxanes [22–24], we investigated drug-induced modulation of these kinases in the two HRPC cell lines.

2. Materials and methods

2.1. Cell culture and reagents

The DU145 cell line was kindly provided by Dr. Limonta P. (University of Milan, Italy). The PC3 cell line was purchased from Interlab Cell Line Collection, Genova, Italy. Both human androgen-independent prostate carcinoma cell lines were maintained in RPMI 1640 (Bio-Whittaker) supplemented with 10% foetal calf serum (Life Technologies). The doubling time of each cell line was calculated as 24 h.

PTX was kindly provided by Indena S.p.A. (Milan, Italy). Stock solutions (1 mg/ml) were prepared in dimethylsulfoxide and diluted in culture medium (final solvent concentration 0.007%). For experiments, cells were treated with 60 nM PTX corresponding to the IC_{80} of the drug in the two cell lines at 72 h [20].

The 15-mer antisense phosphorothioate oligodeoxynucleotide (AS-c-myc) and the phosphorothioate scrambled sequence (Scr-ODN) were provided by Inex Pharmaceuticals Corporation (Burnaby, British Columbia, Canada). The AS-c-myc was complementary to the translational initiation region of c-myc RNA whereas the Scr-ODN containing the 'G-quartet' motif was used as a control [25]. ODNs were dissolved in distilled water and diluted in culture medium to a final concentration of 100 μg/ml.

2.2. Antibodies

The following monoclonal antibodies were used: anti-PKBα/Akt from Trasduction Laboratories; anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) and anti-phospho-SAPK/JNK (Thr183/Tyr185) from New England Biolabs; anti-c-myc and anti-PARP from Calbiochem; anti-β-tubulin from Sigma. The rabbit polyclonal antibodies used were: anti-SAPK/JNK from New England Biolabs; anti-p44/42 MAP kinase from Upstate Biotechnology; anti-phospho-Akt (Ser473) and anti-phospho-c-Myc (Thr58/Ser62) from Cell Signalling; anti-actin from Sigma; anti-active caspase-3 from BD Pharmigen.

2.3. Western blotting

For biochemical analysis, cells were treated with 60 nM PTX for the indicated times. For caspase activation analysis, cells were exposed to PTX for 24 h and then incubated in drug-free medium before processing at the indicated times.

Then, whole cell extracts were prepared as previously reported [20]. Briefly, cells were washed with cold phosphate-buffered saline (PBS) buffer added with 0.1 mM sodium orthovanadate and lysed in SDS sample buffer [62.5 mM Tris–HCl (pH 6.8), 2% SDS] with 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml pepstatin, 12.5 μg/ml leupeptin, 100 KIU aprotinin, 1 mM sodium orthovanadate, 1 mM sodium molybdate and 25 mM sodium fluoride. After protein content determination by the BCA method (Pierce), samples were adjusted to a final concentration of 10% glycerol, 5% β-mercaptoethanol, 0.001% bromophenol blue. Equal amounts of protein were fractioned by SDS–PAGE and transferred on nitrocellulose membranes. Filters were incubated with primary antibodies and subsequently with anti-mouse or anti-rabbit horseradish peroxidase-coniugated secondary antibodies. Immunoreactive bands were visualised by chemioluminescence detection system from Amersham Biosciences or Pierce.

2.4. Biparametric analysis of c-myc expression

After 24 h of treatment with 60 nM PTX, DU145 cells were fixed in 1% paraformaldheyde, for 5 min, at room temperature. Cells were washed in PBS and preincubated in PBT solution (PBS + 1% bovine serum albumin + 0.2% Tween 20), for 15 min, to block unspecific antibody binding. Then, cells were incubated with 5 μg/ml anti-c-myc antibody at room temperature for 60 min and thereafter with a secondary FITC-conjugated antibody (Sigma) diluted 1:100 in PBT solution for 60 min. After washing,

cells were counterstained with a propidium iodide (PI) solution (20 μg/ml in PBS containing 66 U/ml RNase A) and analysed by FACScan flow cytometer (Becton Dickinson). The green FITC-fluorescence indicated c-myc expression, and the PI-red fluorescence was the index of DNA content.

2.5. Apoptosis assessment

The day after seeding, cells were treated with 60 nM PTX for 24 h. After 48 h from drug removal (72 h from the beginning of the treatment), floating and adherent cells were collected. When experiments were performed in the presence of the oligonucleotides, AS-c-myc or Scr-ODN (100 μg/ml) was added to the culture medium 30 min before PTX. The ODNs were added to the medium (50 μg/ml) every 24 h.

Apoptosis detection was performed by morphological analysis of cells stained with PI and by the TdT-mediated Nick-End Labeling (TUNEL) assay (Roche). For the first assay, cells were fixed in 70% ice-cold ethanol and stained with PI solution: 20 μg/ml PI in PBS containing 66 U/ml RNase A, for 18 h. The nuclear morphology of cells was analysed by a fluorescence microscope. At least 100 cells, in two different smears, were examined and the results were expressed as percentage of apoptotic cells over the cell number of the whole cell population. For the TUNEL assay, cells (5×10^5) were fixed in 4% paraformaldehyde and processed as previously described [20]. Apoptosis was detected by FACScan flow cytometer equipped with an argon laser.

2.6. Cell cycle analysis

For cell cycle distribution analysis, cells were exposed to PTX (60 nM), for 24 h. After 72 h from the beginning of the treatment, drug-containing medium with floating and loosely adherent cells were removed; then adherent cells were trypsinised. For experiments performed in the presence of ODNs, cells were treated as previously described in the apoptosis-assessment section. DNA content distribution was determined by flow cytometry on PI stained cells. At least 10,000 cells were evaluated in each sample. The percentage of cells in the different phases of the cell cycle was calculated by means of the LYSIS II software (Becton Dickinson).

3. Results

3.1. Expression and phosphorylation of c-myc

C-myc protein expression was examined in the two prostate carcinoma cell lines DU145 and PC3 by Western blot analysis (Fig. 1A). Differing basal levels of c-myc protein were detected in each cell line with PC3 cells characterised by higher basal expression compared with DU145 cells. Accordingly, the 8q24 region, where *c-MYC* is located, has been found over-represented in PC3 but not in the DU145 cell line [19]. In addition, treatment with PTX induced a time-dependent increase in c-myc levels in DU145 cells, whereas no modulation of the protein was observed in PC3 cells. The biparametric analysis of c-myc expression and DNA content by flow cytometry indicated that, in PTX-treated DU145 cells, the increase of c-myc expression was evident in cells in the G2/M phase of the cell cycle (Fig. 1B). In contrast, no difference in c-myc expression, in relation to cell cycle phases, was observed in PTX-treated PC3 cells (not shown).

Phosphorylation of c-myc has been associated with protein stabilisation [21] and described to occur as cells enter into mitosis [26]. We examined c-myc phosphorylation in the two cell lines after exposure to the taxane by the use of a phospho-specific antibody (Fig. 1A). In both cell lines, no c-myc phosphorylation was detected in untreated cells or following 6 h of treatment. C-myc phosphorylation was induced at a comparable level in the two cell lines following 18 h of drug-exposure. This effect was maintained in DU145 cells, whereas it was slightly reduced in PC3 cells after 24 h. The latter effect is in agreement with a more rapid mitotic slippage in PC3 cells compared with DU145 cells which was detectable after 24 h of treatment with PTX [20].

3.2. Modulation of signalling pathways by PTX

Since serine/threonine protein kinases of the MAPK family and AKT are involved in either c-myc phosphorylation/stabilisation [21] or in the cellular response to taxanes [22–24], we examined the state of activation of these kinases in DU145 and PC3 cells treated with PTX. Cells were exposed to PTX for 6, 18 and 24 h and analysed by Western blotting using phospho-specific antibodies (Fig. 2). In DU145 cells, phosphorylation of ERK kinases appeared partially inhibited after 18 and 24 h of treatment. Moreover, in this cell line, AKT phosphorylation was inhibited after 6 h of PTX-treatment and abrogated after prolonged exposure to the drug. No activation of ERK nor modulation of AKT kinases could be detected in PC3 cells in our experimental conditions. The JNK stress-kinases were affected by PTX in the two cell lines with a different timing. In fact, in DU145 cells the phosphorylation of JNK1/2 kinases was increased after 6 h of PTX-treatment, whereas in PC3 cells, the kinases appeared activated after 18 and 24 h of drug exposure.

3.3. Caspase activation

To determine whether apoptosis was responsible for taxane-induced cell death, the involvement of caspase-3 was investigated in our experimental models. The two cell lines were exposed to PTX for 24 h and the activation of

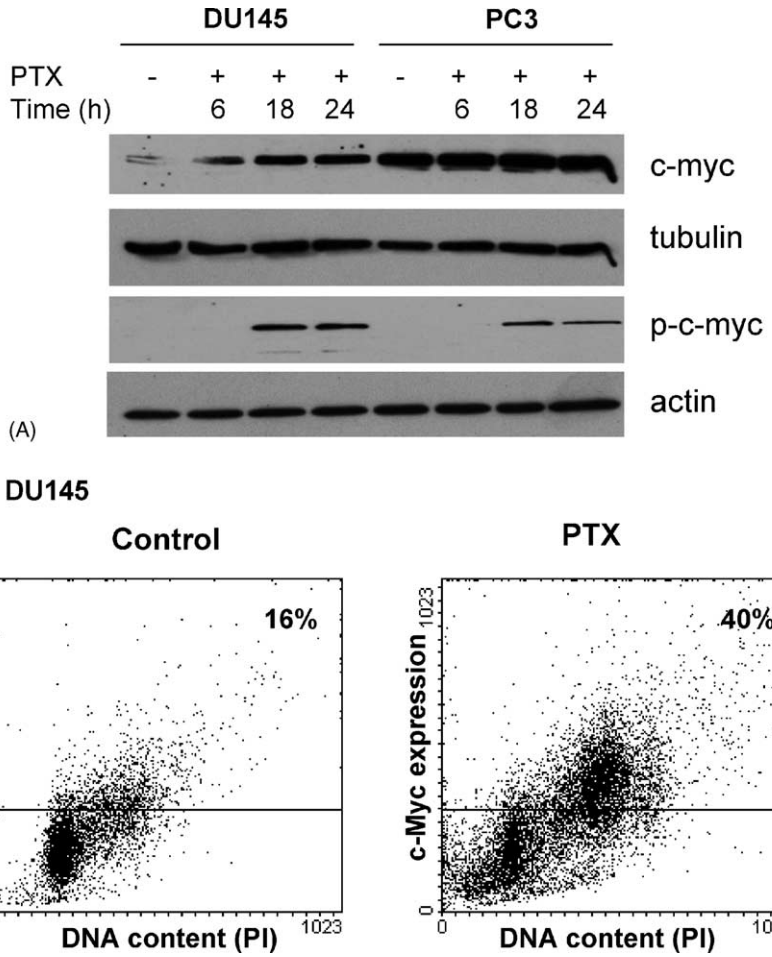

Fig. 1. Effect of PTX treatment on c-myc protein level in the two HRPC cell lines DU145 and PC3. (A) C-myc protein expression was examined in control (−) and (60 nM) PTX-treated cells (+) for the indicated times. Whole cell extracts were prepared and equal amounts of protein separated on SDS–PAGE. After protein transfer onto nitrocellulose, membranes were probed with antibodies recognising c-myc or the protein phosphorylated on Thr58 and Ser 62 residues (p-c-myc). Control for protein loading by tubulin and actin are shown. (B) Dual parameter analysis of c-myc expression and DNA content (PI staining) by flow cytometry of control and PTX-treated DU145 cells (60 nM for 24 h). The percentages of c-myc positive cells are reported.

caspase-3 was monitored by Western blot analysis at different times. In DU145 cells, the appearance of the proteolytic fragments of caspase-3 was evident at 48 h after the beginning of drug treatment, whereas, in PC3 cells, no caspase cleavage was observed at 48 h (not shown) and a faint band appeared at 72 h (Fig. 3). Accordingly, the cleavage of the caspase substrate PARP-1 followed the same time-course. Thus, caspase-3 activation confirmed an early apoptotic response in DU145 cells and a delayed modality of apoptotic cell death in PC3 cells triggered by taxane-treatment.

3.4. Effects of AS-c-myc

The role of c-myc in the cellular response to PTX was investigated in the two HRPC cell lines using an AS-c-myc. Cells were preincubated for 30 min with the AS-c-myc (100 μg/ml) or the Scr-ODN (100 μg/ml) before PTX treatment. The effect of the oligonucleotides on c-myc protein expression was analysed by Western blotting after 6, 18 and 24 h of exposure to the taxane. As shown in Fig. 4, in DU145 cells, PTX-induced upregulation of c-myc was delayed in the presence of the specific antisense, and was not affected by the Scr-ODN. In PC3 cells, c-myc expression was not modulated by the AS-c-myc (not shown).

PTX-induced apoptosis in the presence of the oligonucleotides was evaluated on floating and adherent cells in the two cell lines after 72 h from the beginning of drug-treatment. As shown in Table 1, pre-treatment with the AS-c-myc determined a significant reduction in the percentage of apoptotic cells in DU145 cells exposed to the taxane. In contrast, in PC3 cells, no change in the percentage of apoptotic cells was observed after treatment with PTX in the presence of the AS-c-myc. Even an increased concentration of ODNs (500 μg/ml) did not change the percentage of apoptosis in PC3 cells exposed to the taxane, although partially affecting cell growth (not shown).

To investigate the role of c-myc in cell cycle progression in the presence of taxanes, the effect of AS-c-myc (100 μg/ml) on PTX-induced cell cycle perturbations was examined on adherent cells. In PC3 cells, taxane treatment induced the appearance of cells with DNA content higher

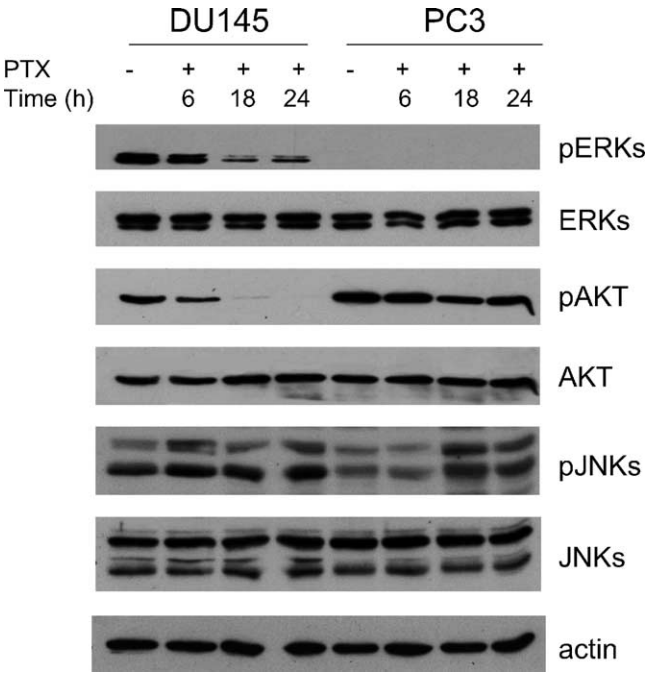

Fig. 2. Modulation of signalling pathways in DU145 and PC3 cells exposed to 60 nM PTX (+) or solvent (−) for 6, 18 and 24 h. The activation state of MAPK kinases (ERKs, JNKs) and AKT was analysed in whole cell lysates, after SDS–PAGE separation, by Western blotting using phospho-specific antibodies. Filters were then stripped and reprobed with antibodies directed against the respective proteins. Control for protein loading by actin is shown.

than 4 N, after 72 h (Fig. 5), as a consequence of a defective postmitotic checkpoint leading to DNA endoreduplication [20]. The addition of AS-c-myc determined a reduction of the cell population with 2 N DNA content and an increase in the percentage of cells with hypodiploid DNA. Such sub-G1 cell population could reflect the occurrence of unequal chromosome segregation [27] since no apoptotic cells could be detected by TUNEL staining (Table 1). The AS-c-myc induced a reduction of the 2 N DNA content peak also in PTX-treated DU145 cells without an increase of sub-G1 cell population (Fig. 5). In both cell lines, the Scr-ODN did not affect the cell cycle distribution. These findings supported a role of c-myc in the overtake of G1 postmitotic checkpoint in the two cell lines, and taxane-induced cell death only in DU145 cells.

Table 1
Effect of ODNs on HRPC cell growth and apoptosis

	DU145		PC3	
	Cell growth[a]	Apoptotic cells[b]	Cell growth[a]	Apoptotic cell[b]
Control	100	1	100	0
AS-c-myc	90 ± 7	0	94 ± 3	0
Scr-ODN	96 ± 7	0	92 ± 5	1
PTX	23 ± 8	45 ± 3	15 ± 2	6 ± 3
PTX + AS-c-myc	18 ± 12	10 ± 3	14 ± 1	4 ± 1
PTX + Scr-ODN	19 ± 4	31 ± 1	18 ± 3	3 ± 1

The percentage of viable and apoptotic cells was determined 72 h after the beginning of treatment by cell counting and TUNEL assay, respectively.
[a] Percentage of control ± standard deviation.
[b] Percentage of the total cell population ± standard deviation.

4. Discussion

Among the several alterations associated with the progression of HRPC, the overexpression of c-myc is fre-

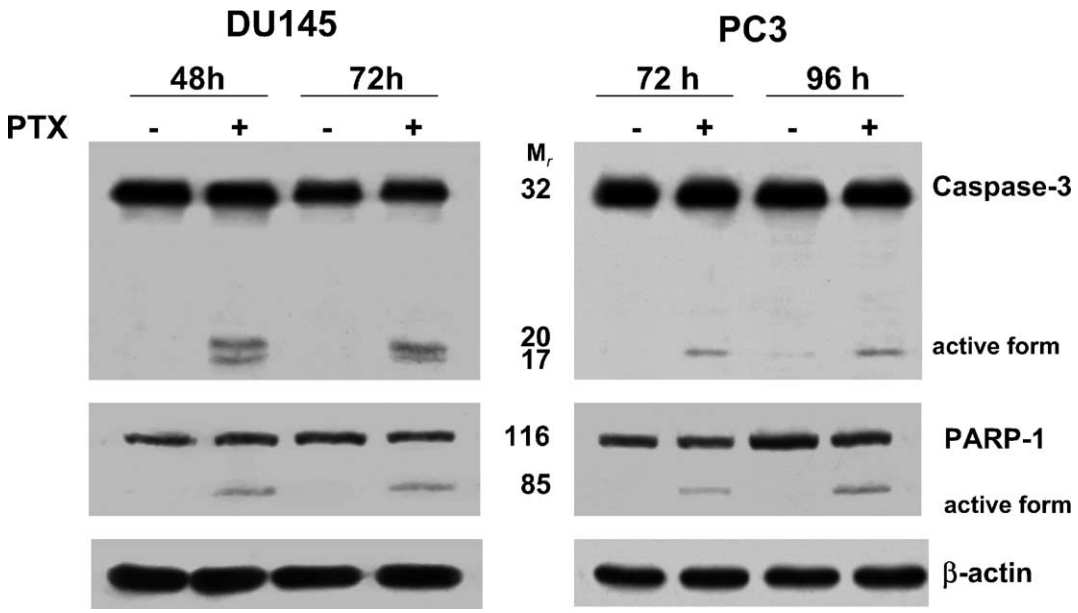

Fig. 3. Effect of PTX on caspase-3 and PARP-1 activation in the two HRPC cell lines. Cells were exposed to 60 nM PTX (+) or solvent (−) for 24 h, then incubated in drug-free medium and processed for whole cell extracts at the indicated times after the beginning of drug-treatment. Equal amounts of protein were separated on SDS–PAGE and transferred onto nitrocellulose. Membranes were probed with anti-caspase-3 and anti-PARP-1 antibodies to evidence protein cleavage. Control for protein loading by actin is shown.

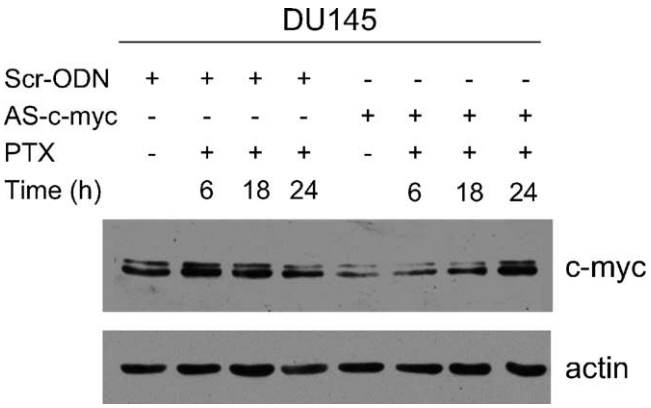

Fig. 4. Effects of AS-c-myc on c-myc protein levels in DU145 cells. Cells were exposed to AS-c-myc or Scr-ODN (100 μg/ml) 30 min before PTX (60 nM). After 6, 18 and 24 h of exposure to the taxane, cells were lysed and samples processed for Western blot analysis with anti-c-myc antibody. Correct protein loading was checked by anti-actin blot.

quently described [7,8]. In the present study, we investigated the role of c-myc in the cellular response to PTX of two HRPC cell lines, DU145 and PC3, displaying different expression levels of c-myc protein. The results showed that, in DU145 cells, PTX induced an upregulation of c-myc related to the apoptotic response to the taxane. In contrast, in PC3 cells, the overexpressed c-myc was not modulated by PTX treatment and was not involved in drug-induced cell death.

The two HRPC cell lines are characterised by a different genetic background affecting taxane-induced cell cycle checkpoints. We previously reported that this feature was related to a different modality to undergo cell death in response to PTX. In particular, DU145 cells, which are endowed with functional mitotic and post-mitotic checkpoints, rapidly died in response to the taxane. In contrast, PC3 cells underwent a rapid mitotic slippage and displayed a defective postmitotic checkpoint leading to a slow and less efficient cell death preceded by DNA endoreduplication [20]. Here, we show that, in both cell lines, PTX-induced cell death is a caspase-mediated apoptosis occurring with a different timing. Our findings support c-myc as an additional player involved in the different modality to undergo cell death. In DU145 cells, PTX-induced upregulation of c-myc was restricted to the cell population accumulated in the G2/M cell cycle phase. Phosphorylation of c-myc was previously described in cells entering into mitosis [26] and was associated with the stabilisation of the protein [21]. Our data do not support a relation between such phosphorylative events and PTX-induced c-myc upregulation. In fact, similar levels of c-myc phosphorylation were detected in the two cell lines and the time course of c-myc phosphorylation was consistent with the taxane-induced mitotic arrest and the more rapid mitotic slippage of PC3 cells. Moreover, the increase of c-myc expression in PTX-treated DU145 cells preceded c-myc phosphorylation, being already evident after 6h of treatment. Furthermore, in the presence of AS-c-myc, PTX-induced upregulation of c-myc was delayed in DU145 cells, thus suggesting an upstream modulation of protein expression.

We investigated the involvement of MAP kinases and AKT in the response of the two cell lines to PTX since these protein kinases have been implicated either in c-myc phosphorylation [21] or in taxane-induced cell death [22–24]. Indeed, our data suggest a correlation between PTX-induced modulation of these kinases and the cell fate rather than with the regulation of c-myc phosphorylation. The activation of ERKs or AKT kinases was inhibited by drug-treatment in DU145 cells thus suggesting that the switching off of pro-survival pathways in these cells could favour the apoptotic process. Accordingly, a protective role of ERK and AKT against taxane-induced apoptosis was described in other cell systems [23,24,28]. The JNK kinases were shown to be activated by PTX in a variety of human cell lines [22,29] being in some cases associated with PTX-induced mitotic arrest and Bcl-2 phosphorylation [30] or, independently of these events, with taxane-induced apoptosis [29]. In the two HRPC cell lines investigated in the present study, the different timing of JNK phosphorylation after PTX-treatment, with an early activation (6 h) in DU145 cells and a late activation (18 h) in PC3 cells, suggested a relation with the different kinetics of drug-induced cell death.

The different genetic background of the two HRPC cell lines could affect the role of c-myc in the cellular response to PTX. Specifically, the absence of a functional Rb in DU145 cells [31] might favour PTX-induced upregulation of c-myc, since its transcription is repressed in the presence of wild type Rb in prostate cancer cells [32]. In the PC3 cell line, the overexpression of Bcl-2 [33] might contribute to the delayed activation of the apoptotic process after PTX-treatment, either by inhibiting cytochrome c release from mitochondria [34] or by antagonising c-myc-mediated apoptotic cell death [35].

P53 and c-myc play an interdependent role in maintaining the genomic integrity after mitotic spindle disruption. In tumour cells, the combination of c-myc overexpression and loss of p53 function was shown to predispose diploid cells to the development of polyploidy due to the uncoupling of mitosis and the subsequent S-phase initiation, whereas the restoration of p53 function triggered a rapid apoptosis [16,17]. It is tempting to speculate that in the DU145 cell line the pro-apoptotic function of c-myc could be favoured by the expression of a mutated p53 [36] retaining the post-mitotic checkpoint control, as previously suggested [20]. In fact, in DU145 cells, PTX-induced apoptosis was reduced in the presence of AS-c-myc. In PC3 cells, the lack of p53 [36] and the overexpression of c-myc protein might co-operate to the occurrence of polyploidy after taxane-treatment due to the circumvention of the post-mitotic checkpoint. Such uncoupling of mitosis and the subsequent S-phase could be attenuated in PTX-treated PC3 cells in the presence of AS-c-myc. In

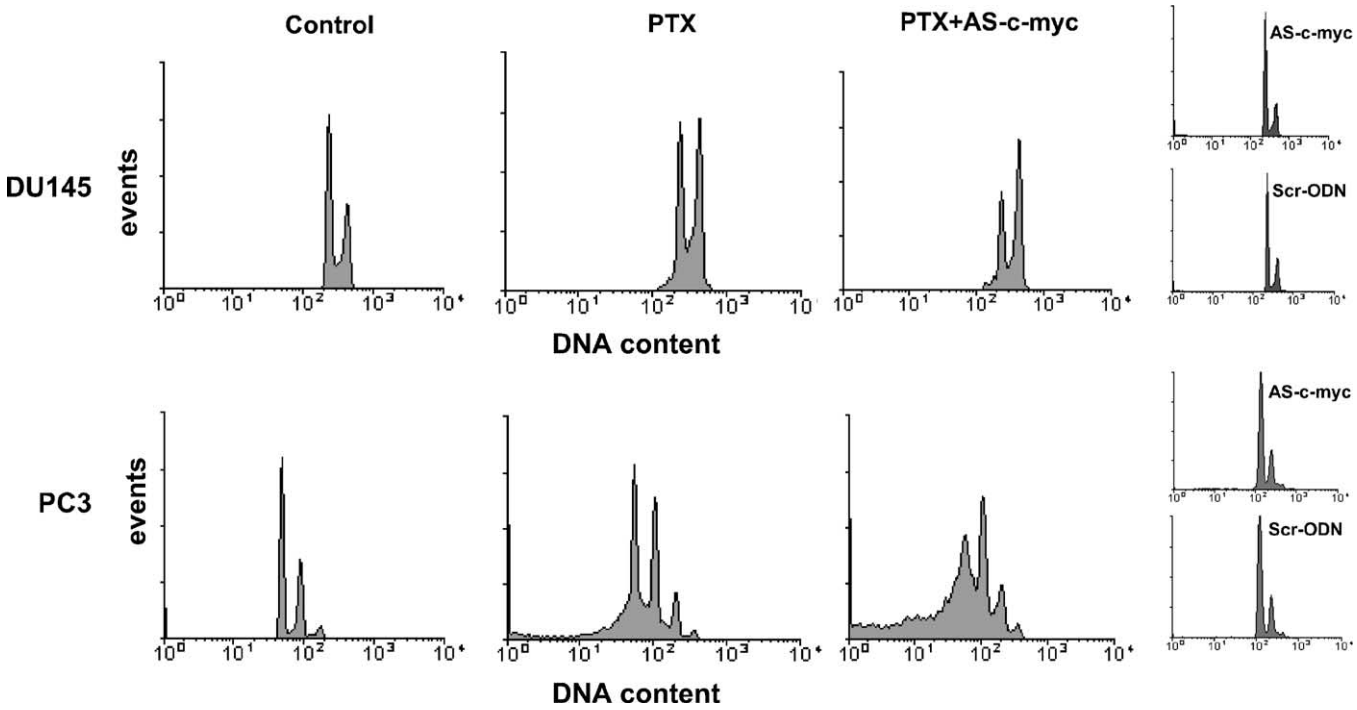

Fig. 5. Effect of AS-c-myc oligonucleotide on cell cycle distribution in DU145 and PC3 cells exposed to PTX (60 nM). The AS-c-myc (100 μg/ml) or the Scr-ODN (100 μg/ml) was added to cell culture medium 30 min before PTX. DNA content profiles were examined 72 h after the beginning of taxane treatment by FACScan analysis of PI-stained cells. Inserts: DNA profiles of DU145 and PC3 cells treated with AS-c-myc or Scr-ODN in the absence of PTX.

fact, the marked occurrence of non-apoptotic hypodiploid DNA in AS-c-myc/PTX-treated PC3 cells could be the result of incorrect cell division following chromosomal missegregation.

A super-imposable role of c-myc in DU145 and PC3 cells on cell cycle progression could be drawn by our data. In addition to the mitosis-associated c-myc phosphorylation above discussed, the analysis of DNA profiles showed, that in both cell lines, the co-treatment AS-c-myc/PTX for 72 h caused a reduction of the 2 N DNA peak as compared to the effect of PTX alone. Since PTX induces an early accumulation of 4 N DNA cells [20], it is conceivable that the AS-c-myc might cause a delay in cell cycle re-entering of these cells which are actually in a G1-like phase [5,20]. Accordingly, a lengthening of the G1 phase and a delay of the subsequent progression into the cell cycle were described in cells defective for c-myc function [37]. The delayed progression of the cell cycle could account for the lack of the AS-c-myc effect on cell growth inhibition by PTX. This delay could be regarded as a protective event against activation of apoptosis in cycling cells.

In conclusion, the present study shows that, in a favourable HRPC cellular context, c-myc can participate in mediating PTX-induced cell death. Our data support a complex and differentiated response of HRPC cells to PTX with the modality to undergo cell death and the involvement of specific players, including c-myc, likely influenced by the genetic background.

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