CMLS, Cell. Mol. Life Sci. 59 (2002) 1406–1412
1420-682X/02/081406-07

CMLS Cellular and Molecular Life Sciences

Research Article

Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer

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Received 3 April 2002; received after revision 6 June 2002; accepted 20 June 2002

Abstract. Stable transfection of human ovarian carcinoma cells with survivin cDNA caused a four- to sixfold increase in cell resistance to taxotere and taxol (two-sided Student's *t* test, $p < 0.05$), with a concomitant reduction in the apoptotic response to taxol, but did not affect cell sensitivity to cisplatin or oxaliplatin. Such findings were indirectly supported by similar observations obtained with clinical tumours. In fact, high levels of survivin protein expression (>30% positive cells), detected by immunohistochemistry in 90/124 (73%) advanced ovarian carcinomas, were significantly associated with clinical resistance to a taxol/platinum-based regimen but unrelated to tumour shrinkage following cisplatin-including combinations (non-taxol based). In the 95 patients receiving a taxol/platinum-based regimen, survivin overexpression correlated with a lower clinical or pathologic complete remission rate than absent/low protein expression (43 vs 75%, $p = 0.0058$ by logistic regression adjusted for tumour stage, histological grade and p53 expression). Conversely, in the 29 cases treated with cisplatin-containing regimens (not taxol based), survivin expression was unrelated to tumour response. Cellular studies and clinical data suggest a direct link between survivin expression and tumour cell susceptibility to taxol.

Key words. Survivin; apoptosis; ovarian cancer; taxol; cisplatin.

Introduction

In advanced ovarian carcinoma, taxol has proved to be an important chemotherapy agent. However, its utility is limited by the development of drug resistance, partly related to defects in the apoptotic pathway. The search for pro- or anti-apoptosis markers associated with drug sensitivity/resistance has identified molecular predictors of clinical outcome following taxol- and platinum-including regimens, and a complete response (CR) has been found to be associated with BAX overexpression [1] and mutant TP53 [2] in two independent studies.

Recently, the anti-apoptotic protein survivin has been described as being selectively expressed in the most common human neoplasms [3] and related to clinical progression [4]. Survivin is a structurally unique member of the IAP (inhibitors of apoptosis proteins) family that is potentially involved in both control of cell division and inhibition of apoptosis [5]. Specifically, its anti-apoptotic function seems to be related to an ability to directly/indirectly inhibit caspases [6], although the precise role of survivin in the modulation of the caspase cascade has not been fully elucidated [7]. Expression of the protein is regulated in a highly cell cycle-dependent manner, with a marked increase in the G_2M phase [8]. During this phase, survivin associates with and is phosphorylated by $p34^{cdc2}$ on Thr34 [9]. Such a phosphorylated form seems to be responsible for the anti-apoptotic function of the protein.

Survivin binds polymerized microtubules [10], and a putative tubulin-binding domain has been identified by mu-

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tational analysis in the extended survivin C-terminal α helix [11]. Moreover, forced expression of survivin was able to counteract apoptosis induced by taxol in NIH3T3 fibroblasts [10]. Therefore, the available evidence suggests that the protein plays a role in determining the cellular response to taxanes through mechanisms likely different from those involving BAX or TP53.

To investigate whether survivin is involved in regulating cell sensitivity to taxanes and platinum compounds (i.e. drugs used in the standard regimens for the treatment of ovarian cancer), in the present study we planned a parallel investigation on cell lines and clinical specimens from ovarian carcinomas.

Material and methods

Cell lines and transfection procedures

Two human ovarian carcinoma cell lines (IGROV-1 and OAW42) were used in the study. Transfectants were prepared by lipofection. IGROV-1 and OAW42 at 70% confluency were incubated with serum-free medium containing 20 µl lipofectin and 3 µg plasmid DNA for 5 h.

DNA consisted of survivin expression plasmid that was made by cloning the 1619 bp full-length human survivin cDNA (kindly provided by Dr. D. Altieri, Yale University School of Medicine) into the *Eco*RI site of the mammalian pCI Neo expression vector (Promega, Milan, Italy) under the control of the CMV promoter. Empty pCI Neo vector was also used as a negative control. Stable transfectants were selected in the presence of G418 (400 µg/ml for IGROV-1 cells, 1 mg/ml for OAW42 cells). Polyclonalpopulations of transfected cells were maintained in selective medium.

RNA isolation and RT-PCR

Total RNA from exponentially growing cells was purified using the RNeasy Midi kit (Qiagen, Valencia, Calif.) and then reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Gaithersburg, Md.) and Oligo(dT)12–18 primers (Gibco BRL). Incubation was performed at 37 °C for 1 h and 95 °C for 10 min. cDNA was then amplified by PCR using Taq TaKaRa DNA polymerase (M-Medical-Genenco, Firenze, Italy) and specific primers designed on the basis of the nucleotide sequence of the survivin gene: the sense primer was 5′-ATTTGAATCGCGGGACCCGTTG-3′ and the anti-sense primer was 5′-TGGCTCGTTCT CAGTGGGGCAGT-3′. Cycling conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 66 °C for 30 s, 72 °C for 40 s, and, finally, 72 °C for 5 min. Identical PCR conditions were used to amplify the β-actin cDNA, which was used as an internal control: the sense primer used was 5′-GATATCGCCGCGCTCGTCGTC-3′ and the anti-sense primer was 5′-GCACAGCCTGGATAGCAACGTA CATG-3′. Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide and photographed with an ImageMaster system (Amersham Pharmacia Biotech, Uppsala, Sweden).

Immunoblotting

Total cellular lysate was separated on a 15% SDS-PAGE gel and transferred to polyvinyldendifluoride (Immobilion, Millipore Corporation, Bedford, Mass.). Filters were probed with the primary antibodies anti-human survivin (Novus Biologicals, Littleton, Co.) and anti-human actin (Sigma, St. Louis, Mo.) and then incubated with the secondary anti-rabbit IgG horseradish peroxidase-linked whole antibody (Amersham Pharmacia Biotech). Bound antibodies were detected using an enhanced chemoluminescence western blotting detection system (Amersham Pharmacia Biotech).

For assessment of the Thr34-phosphorylated form of survivin, 100 µl of precleared, detergent-solubilized cell extracts was immunoprecipitated with the anti-human survivin antibody (20 µg/ml) for 16 h at 4 °C, with precipitation of the immune complex by addition of 50 µl of a 50:50 protein A slurry. After separation by SDS gel electrophoresis, samples were transferred to nitrocellulose and incubated with the antibody (0.1 µg/ml) to Thr34-phosphorylated survivin (kindly provided by Dr. D. Altieri, Yale University) [9] followed by secondary anti-rabbit antibody and chemoluminescence.

Cell growth inhibition assay

After harvesting in the logarithmic growth phase, cells were seeded in six-well plates and treated with varying doses of drugs. Exposure to cisplatin (Pharmacia Upjohn, Uppsala, Sweden) and oxaliplatin (Sanofi-Synthelabo, Milan, Italy) was limited to 1 h, whereas for taxol (Sigma) and taxotere (Rhone-Poulenc Rorer, Vitry sur Seine, France), it was prolonged for up to 24 h, in accordance with the specific action mechanisms of the drugs. Such treatment times have already been used in several previously published papers dealing with the in vitro activity of the drugs [12–14]. After drug removal, cells were incubated in fresh medium for an additional 72 h at 37 °C in a 5% CO_2-humidified atmosphere. The cells were then trypsinized and counted in a particle counter (Coulter Counter; Coulter Electronics, Luton, UK). Each experimental sample was run in triplicate. The results were expressed as the number of adherent cells in treated samples compared with control samples. In vitro drug activity was expressed in terms of concentrations able to inhibit cell proliferation by 50% (IC_{50}) and 80% (IC_{80}).

Tdt-mediated dUTP nick-end labelling (TUNEL) analysis

At different intervals after a 24-h exposure to taxol (IC_{80}), floating and adherent cells were fixed in 4% para-

formaldehyde for 45 min at room temperature. After rinsing with phosphate-buffered solution (PBS), cells were permeabilized in a solution of 0.1% Triton X-100 in sodium citrate 0.1% for 2 min in ice. Samples washed with 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, they were suspended in PBS and analysed by a FACScan cytofluorimeter (Becton Dickinson, Sunnivale, Calif.). Results were expressed as the percentage of TUNEL-positive cells on the overall cell population.

Patient population and case material

The clinical component of this retrospective study was represented by 124 previously untreated patients with histologically confirmed invasive advanced ovarian cancers, stages III and IV according to the FIGO classification, who underwent exploratory laparotomy in different institutions of northern Italy (Istituto Nazionale Tumori, Milan, 81 cases; University Department of Obstetrics and Gynaecology, Milan, 31 cases; Ospedale San Gerardo, Monza, 12 cases) from October 1989 to December 1999. To enter this study, cases had to meet the following criteria: (i) treatment with paclitaxel-based or cisplatinum-containing combinations (non-paclitaxel based) at standard doses; (ii) adequate sampling of the primary tumour before chemotherapy; (iii) adequate tumour staging and assessable clinical response following cytoreductive chemotherapy. Following surgery, all patients received postoperative chemotherapy, consisting of high-dose cisplatin (160 mg/m^2 per course for four to five courses) and glutathione as chemoprotector [15] for 29 cases, or four to six courses of therapy including a platinum compound (total dose ≥ 400 mg/m^2) and taxol (total dose ≥ 700 mg/m^2) for 95 cases. The distribution of pathobiological features was superimposable in the two treatment subsets. Written informed consent was obtained from each patient for clinical treatment and for using their tumour for research upon institutional review board approval.

Clinical response was assessed after completion of first-line chemotherapy. CR was defined as the complete disappearance of clinically detectable tumour assessed by clinical, gynaecological and surgical examinations (although the last approach was not mandatory), abdominal or pelvic ultrasonography and radiologic investigations, and as the absence of detectable serum levels of CA 125, according to the World Health Organization criteria.

Immunohistochemistry

The immunohistochemical evaluation of survivin expression was carried out according to Adida et al. [16] with the standard alkaline phosphatase technique using a commercial kit (DAKO, Copenhagen, Denmark) after antigen retrieval by pressure cooking. Tissue sections (4 µm thick) from tumour specimens fixed in 10% buffered formalin and paraffin embedded were deparaffinized in xylene for 5 h and washed twice in ethanol. Slides were then placed in a pressure cooker containing 1.5 l of 9 mM sodium citrate, pH 6, which had been brought to the boil, and heating was continued for 6 min. For survivin staining, the primary antibody (kindly provided by Dr. D. Altieri) was applied at a concentration of 0.5 µg/ml in PBS, pH 7.0, containing 0.5% bovine serum albumin and 5% normal goat serum, and incubated overnight at 4°C. For each sample, the omission of primary antibody was used as a negative control, whereas the positive control for each immunohistochemical run was a human ovarian carcinoma cell line expressing survivin (IGROV-1) [17].

The accumulation of p53 was evaluated by immunohistochemistry with the ABC immunoperoxidase system (Vectastain ABC Kit; Vector Laboratories, Burlingame, Calif.) as previously described [18] on 4-µm-thick tissue sections from tumour specimens fixed in 10% buffered formalin and paraffin embedded. The primary antibody (PAb1801; Oncogene Science, Manhasset, N. Y.) was applied for 1 h at room temperature at a 1:50 dilution. For each sample, the omission of primary antibody was used as a negative control, whereas the positive control for each immunohistochemical run was represented by a set of ovarian surface epithelial-stromal tumours with high levels of p53 accumulation.

For each sample, survivin or p53 immunoreactivity was independently evaluated under the microscope by two observers, by scoring a total of 1000–3000 tumour cells on consecutive areas from different specimens of the same tumour lesion. Only cells completely and darkly stained (at a cytoplasmic level for survivin, or at a nuclear level for p53) were regarded as positive. Positivity was expressed as the percentage ratio between stained cells and total number of tumour cells.

Statistical analysis

Differences between IC_{50} values of individual drugs in the different cell lines were statistically assessed by Student's *t* test for paired samples. In clinical specimens, the association between survivin expression and the other pathobiological variables was assessed by the χ^2 test, continuity adjusted when appropriate. The relationship between the investigated variables and clinical response was assessed in univariate and multivariate analyses by logistic regression models. All p values were two sided. Statistical analysis was performed by SAS (SAS Institute, Cary, N. C.) software packages.

Results and discussion

To investigate the role of survivin expression on cell sensitivity to taxanes and platinum compounds, we stably transfected two ovarian cancer cell lines (IGROV-1 and

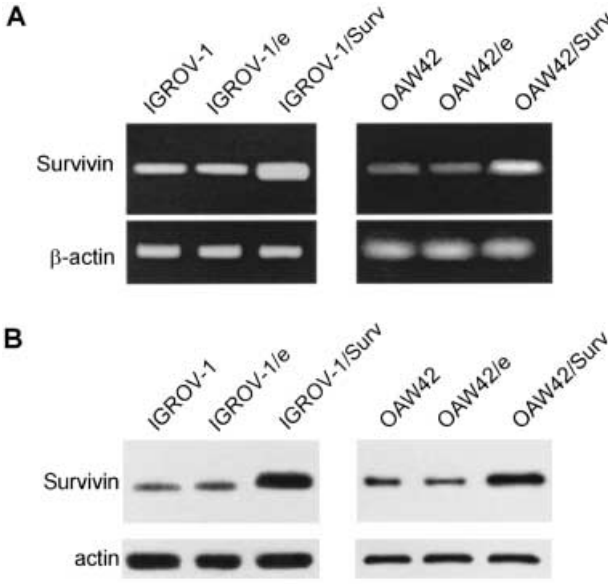

Figure 1. Representative RT-PCR (*A*) and Western blotting (*B*) experiments illustrating survivin mRNA and protein expression in parental (IGROV-1, OAW42), empty-vector transfectant (IGROV-1/e, OAW42/e) and survivin transfectant (IGROV-1/Surv, OAW42/Surv) cells.

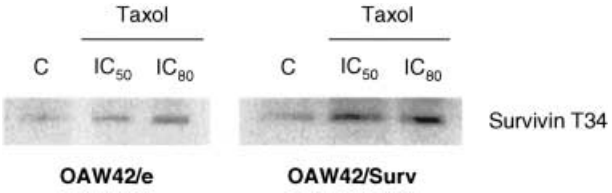

Figure 2. A representative Western blotting experiment illustrating the phosphorylation of survivin on Thr34 evaluated before and after a 24-h exposure to taxol (IC_{50} and IC_{80}) in OAW42/e and OAW42/Surv cells. Survivin was immunoprecipitated using the anti-human survivin antibody and analysed with the antibody to phosphorylated Thr34 (Survivin T34). C, control cells.

OAW42) with the pCI Neo expression vector carrying the full-length human survivin cDNA. Survivin overexpression was monitored by RT-PCR and Western blot analysis. A marked increase in mRNA level was observed in polyclonal populations of survivin-transfected cells (IGROV-1/Surv and OAW42/Surv) compared to parental and empty-vector transfected cells (IGROV-1/e, OAW42/e) (fig. 1A). Survivin levels were consistently increased in both survivin-transfected cell systems (around fivefold in IGROV-1/Surv, threefold in OAW42/Surv) (fig. 1B).

To assess drug sensitivity profiles, cells were treated with different concentrations of taxanes (taxol and taxotere) for 24 h, or platinum derivatives (cisplatin and oxaliplatin) for 1 h, and the effect on cell growth was evaluated after a 72-h incubation in drug-free medium. Survivin overexpression did not modify the sensitivity of cells to cisplatin (table 1). Conversely, survivin overexpression significantly decreased the susceptibility of both cell systems to taxanes (two-tailed Student's *t* test for paired samples, $p < 0.05$). Specifically, an increase of about sixfold in taxol IC_{50} was observed in survivin-transfected clones compared to corresponding empty-vector transfectant cells. The extent of resistance to taxotere following survivin overexpression was slightly lower than that observed for taxol, with about fourfold increases in IGROV-1/Surv and OAW42/Surv cells, compared to corresponding mock-transfected cells (table 1).

Since taxol has been previously demonstrated to be able to induce phosphorylation of apoptosis-related proteins such as bcl-2 [19], we evaluated whether taxol treatment modulated the extent of survivin phosphorylation on Thr34 in OAW42/e and OAW42/Surv cell lines (fig. 2). Results from Western blotting experiments using a phospho-specific antibody showed a very modest accumulation of the Thr34-phosphorylated form of survivin in OAW42/e cells 72 h after taxol treatment. Conversely, a more marked increase in the extent of survivin phosphorylation was detected after taxol exposure in OAW42/Surv cells overexpressing the protein. Such results suggest that increased accumulation of the anti-apoptotic phosphorylated form of survivin in OAW42/Surv cells could be implicated in their resistance to the cytotoxic effect of taxol.

To evaluate whether a different ability to undergo programmed cell death could account for the different sensitivity to taxol observed in our cell lines, we monitored the apoptotic response of cells at different intervals after taxol (IC_{80}) exposure by TUNEL analysis. Marked differ-

Table 1. Sensitivity of ovarian cancer cells to taxanes and platinum compounds as a function of survivin expression.

Cell line	IC_{50} (μM)			
	cisplatin	oxaliplatin	taxol	taxotere
IGROV-1/e	5.26 ± 0.5	7.18 ± 0.7	0.059 ± 0.07*	0.0046 ± 0.002*
IGROV-1/Surv	6.60 ± 1.5	6.29 ± 1.0	0.39 ± 0.1*	0.017 ± 0.001*
OAW42/e	12.44 ± 3.8	13.90 ± 2.3	0.004 ± 0.001*	0.0022 ± 0.001*
OAW42/Surv	10.00 ± 1.7	12.13 ± 0.9	0.027 ± 0.001*	0.0092 ± 0.002*

Data represent the mean values ± SD from three independent experiments. Different superscripts, *$p < 0.05$, two-tailed Student's *t* test for paired samples.

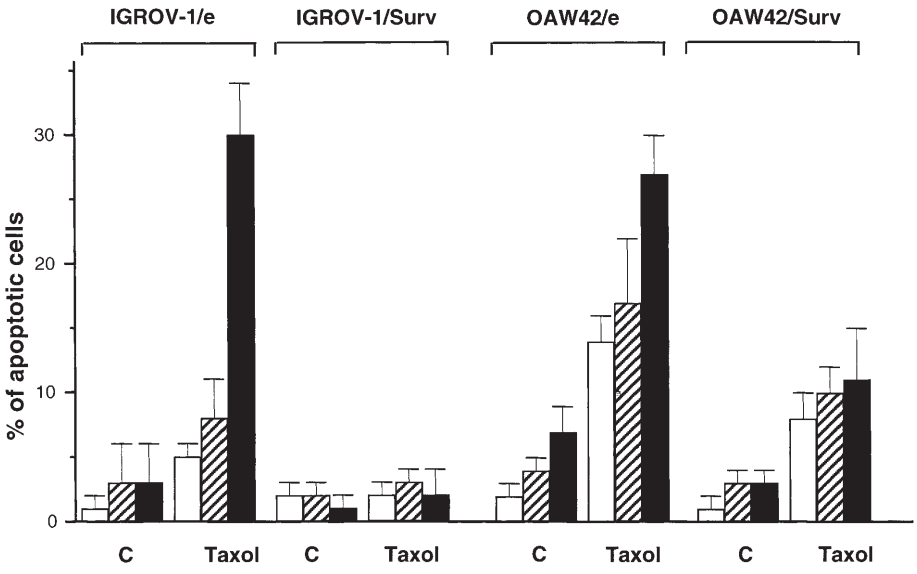

Figure 3. TUNEL analysis of taxol-induced apoptosis in the different ovarian cancer cell lines at different intervals (24 h, empty column; 48 h, hatched column; 72 h, solid column) after a 24-h exposure to taxol (IC_{80}). Data represent the mean values ± SD from three independent experiments. C, control cells; Taxol, taxol-treated cells.

ences were observed among the different cell lines as a function of the extent of survivin expression (fig. 3). Specifically, the empty-vector-transfected clones showed a time-dependent induction of TUNEL-positive cells, with maximum percentages of apoptotic cells around 30% of the overall population at 72 h after treatment. Conversely, DNA fragmentation was much less relevant in both survivin-transfected clones (around 5% and 10% of cells in IGROV-1/Surv and OAW42/Surv, respectively). This finding is in keeping with previously reported results [8] demonstrating that forced survivin overexpression efficiently inhibited taxol-induced apoptosis in NIH3T3 human fibroblasts. Moreover, a possible link between susceptibility to taxol and survivin expression was recently suggested by Mesri et al. [20], who reported that infection with a replication-deficient adenovirus encoding a Thr34 → Ala mutant of survivin enhanced taxol-induced cell death in cell lines of different tumour histotypes.

In vitro results obtained in the present study which indicate lack of a correlation between survivin expression and sensitivity of ovarian cancer cells to cisplatin are apparently in contradiction with previous findings obtained in experimental models of a different tumour type, i.e. malignant melanoma. Specifically, Grossman et al. [21] showed that transfection of YUSAC2 melanoma cells with a phosphorylation-defective mutant (Thr34 → Ala) of survivin increased their sensitivity to cisplatin, as demonstrated by the more than double sub-G_1 apoptotic cell fraction compared to control cells. Similarly, we recently found that transfection of JR8 melanoma cells with hammerhead ribozymes targeting different portions of survivin mRNA induced a significant increase in the apoptotic response to cisplatin of these cells as indicated by a threefold higher caspase-3 catalytic activity than parental cells [22]. Such an apparent discrepancy among diverse cell systems can be tentatively explained at least in part by considering that other genes that contribute to the overall anti-proliferative effect of this anti-cancer agent, some of which are also involved in the control of survivin expression and function (as in the case of p53 [23] and $p34^{cdc2}$ [9]), are differently expressed or modulated by drug treatment in the different cellular contexts.

To investigate the association between survivin expression and clinical response, and to test in the clinical setting the results obtained from preclinical models, we determined by immunohistochemistry survivin expression in 124 histologically confirmed invasive advanced ovarian cancers (table 2). Most of the cases were diagnosed as serous (69%), grade 3 (79%) and stage III (83%), and 51% exhibited p53 protein accumulation by immunohistochemistry, in keeping with previous results in this tumour type [2, 18, 24]. Survivin was detectable (in >30% tumour cells) in 90 cases, with a similar rate in the two treatment groups (79 and 71% in cases treated with cisplatin- or taxol-based regimens, respectively), and its expression appeared unrelated to histology, differentiation grade, FIGO stage or p53 accumulation. Pathological or clinical CR following taxol-including regimens was inversely associated with survivin expression considered as a continuous variable ($p = 0.005$ by logistic regression adjusted for FIGO stage, histological grade and p53 accumulation). CR was achieved by 21/28 cases exhibiting absent/low survivin expression, and by 29 of the 67 cases

Table 2. Clinicopathological and biological characteristics of ovarian cancers from patients subjected to a taxol-based regimen.

	Total (95 cases)	Number of survivin-overexpressed cases	Clinical or pathologic complete response					
			number	%	univariate analysis		multivariate analysis	
					$\chi^{2\,a}$	p value	$\chi^{2\,a}$	p value
Differentiation grade								
G1–G2	16	12 (75.0%)	6/16	37.5	1.741	0.19	0.317	0.69
G3	76	53 (69.7%)	42/76	55.3				
Unspecified	3	2 (66.7%)						
FIGO stage								
III	78	57 (73.1%)	45/78	57.7	5.143	0.023	5.458	0.019
IV	16	10 (62.5%)	4/16	25.0				
Unspecified	1	1						
p53 accumulation								
Absent	49	38 (77.6%)	19/49	38.8	7.318	0.0068	3.082	0.07
Present	43	28 (65.1%)	29/43	67.4				
Not assessable	3	1 (33.3%)						
Survivin expression								
Absent/low	28	–	21/28	75.0	7.416	0.0063	7.615	0.0058
High	67	–	29/67	43.3				

[a] By logistic regression model.

overexpressing survivin (p = 0.0058 by logistic regression), regardless of the other covariates (table 2). Worth mentioning in this treatment subset is that survivin expression provided predictive information on clinical response independent of p53 accumulation, which has been already identified as a molecular predictor of tumour shrinkage in taxol/platinum-based chemotherapy [2]. Conversely, in cases treated with cisplatin-based chemotherapy, the CR rate appeared independent of survivin expression, with 3 CRs in 6 of the no/weakly and 8 in 23 of the overexpressing cases. Such findings, indicating for the first time an inverse relationship between survivin expression and sensitivity to taxol in clinical tumours, support a role for survivin as a determinant of the response of ovarian cancers to taxanes. Moreover, the outcome of the study might have implications for the proposition of innovative approaches using survivin as a cellular target to modulate responsiveness of ovarian cancers to standard treatments including taxanes.

Acknowledgements. The authors thank Dr. Francesco Raspagliesi, Department of Surgery, Istituto Nazionale Tumori, Dr. G. Scarfone, Department of Obstetrics and Gynaecology, University of Milan, and Dr. F. Vecchione, Department of Obstetrics and Gynaecology, Ospedale San Gerardo, Monza. The study was supported in part by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC), Consiglio Nazionale delle Ricerche (CNR) and Ministero della Salute.

1 Tai Y. T., Lee S., Niloff E., Weisman C., Strobel T. and Cannistra S. A. (1998) BAX protein expression and clinical outcome in epithelial ovarian cancer. J. Clin. Oncol. **16:** 2583–2590
2 Lavarino C., Pilotti S., Oggionni M., Gatti L., Perego P., Bresciani G. et al. (2000) P53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. J. Clin. Oncol. **18:** 3936–3945
3 Ambrosini G., Adida C. and Altieri D. C. (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat. Med. **3:** 917–921
4 Altieri D. C. and Marchisio C. (1999) Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. Lab. Invest. **79:** 1327–1333
5 LaCasse E. C., Baird S., Korneluk R. G. and MacKenzie A. E. (1998) The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene **17:** 3257–3259
6 Shin B. C., Sung B. J., Kim H. J., Ha N. C., Hwang J. I., Chung C. W. et al. (2001) An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry **40:** 1117–1123
7 Reed J. C. (2001) The survivin saga goes in vivo. J. Clin. Invest. **108:** 965–969
8 Li F. and Altieri D. C. (1999) The cancer anti-apoptosis mouse survivin gene: characterization of locus and transcriptional requirements of basal and cell cycle-dependent expression. Cancer Res **59:** 3143–3151
9 O'Connor D. S., Grossman D., Plescia J., Li F., Zhang H., Villa A. et al. (2000) Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc. Natl. Acad. Sci. USA **97:** 13103–13107
10 Li F., Ambrosini G., Chu E. Y., Plescia J., Tognin S., Marchisio P. C. et al. (1998) Control of apoptosis and mitotic spindle checkpoint by survivin. Nature **396:** 580–584
11 Verdecia M. A., Huang H., Dutil E., Kaider D. A., Hunter T. and Noel J. P. (2000) Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. Nat. Struct. Biol. **7:** 602–608
12 Zaffaroni N., Silvestrini R., Orlandi L., Bearzatto A., Gornati D. and Villa R. (1998) Induction of apoptosis by taxol and cisplatin and effect on cell cycle-related proteins in cisplatin-sensitive and -resistant human ovarian cancer cells. Br. J. Cancer **77:** 1378–1385
13 Perego P., Caserini L., Gatti L., Carenini N., Romanelli S., Supino R. et al. (1999) A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Mol. Pharmacol. **55:** 528–534

14 Orlandi L., Colella G., Bearzatto A., Abolafio G., Manzotti C., Daidone M. G. et al. (2001) Effects of a novel trinuclear platinum complex in cisplatin-sensitive and cisplatin-resistant human ovarian cancer cells: interference with cell cyle progression and induction of apoptosis. Eur. J Cancer **37:** 649–659
15 Di Re F., Bohm S., Oriana S., Spatti G. B., Pirovano C., Tedeschi M. et al. (1993) High-dose cisplatin and cyclophosphamide with glutathione in the treatment of advanced ovarian cancer. Ann. Oncol. **4:** 5–61
16 Adida C., Crotty P. L., McGrath J., Berrebi D., Diebold J. and Altieri D. C. (1998) Developmentally regulated expression of the novel anti-apoptosis gene survivin in human and mouse differentiation. Am. J. Pathol. **152:** 43–49
17 Tamm I., Wang Y., Sausville E., Scudiero D. A., Vigna N., Oltersdorf T. et al. (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by fas (CD95), bax and anticancer drugs. Cancer Res. **58:** 5315–5320
18 Silvestrini R., Daidone M. G., Veneroni S., Benini E., Scarfone G., Zanaboni F. et al. (1998) The clinical predictivity of biomarkers of stage III–IV epithelial ovarian cancers in a prospective randomized treatment protocol. Cancer **82:** 159–167
19 Haldar S., Chintpally J. and Croce C. M. (1996) Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res **56:** 1169–1174
20 Mesri M., Wall N. R., Li J., Kim R. W. and Altieri D. C. (2001) Cancer gene therapy using a survivin mutant adenovirus. J. Clin. Invest. **108:** 981–990
21 Grossman D., Kim P. J., Schechner J. S. and Altieri D. C. (2001) Inhibition of melanoma tumor growth in vivo by survivin targeting. Proc. Natl. Acad. Sci. USA **98:** 635–640
22 Pennati M., Colella G., Folini M., Citti L., Daidone M. G. and Zaffaroni N. (2002) Ribozyme-mediated attenuation of survivin expression sensitizes human melanoma cells to cisplatin-induced apoptosis. J. Clin. Invest. **109:** 285–286
23 Mirza A., McGuirk M., Hockenberry T. N., Wu Q., Ashar H., Black S. et al. (2002) Human survivin is negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway. Oncogene **21:** 2613–2622
24 Righetti S. C., Della Torre G., Pilotti S., Menard S., Ottone F., Colnaghi M. I. et al. (1996) A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. Cancer Res. **56:** 689–693