Cancer Chemother Pharmacol (2005) 56: 427–435
DOI 10.1007/s00280-004-0993-9

ORIGINAL ARTICLE

**Tania Dutta · Himani Sharma · Lalit Kumar
A. K. Dinda · Sunesh Kumar
Neerja Bhatla · Neeta Singh**

Neoadjuvant chemotherapy for epithelial ovarian cancer—role of apoptosis

Received: 26 May 2004 / Accepted: 26 November 2004 / Published online: 19 May 2005

Abstract *Background*: Ovarian cancer is one of the most frequently fatal gynecological cancers because most cases are diagnosed at an advanced stage. Loss of growth control and a marked resistance to apoptosis are considered major mechanisms driving tumor progression. Little is known about the effect of various treatment regimens on the distribution of molecular markers of apoptosis in epithelial ovarian cancer. The objective of this study was to compare the expression levels of both proapoptotic and antiapoptotic proteins p53, p73, Bcl-2, Bcl-XL and survivin in the ascitic cells and tumor samples of patients undergoing treatment with two different regimens. *Methods*: A total of 24 patients with untreated epithelial ovarian cancer were randomized into two groups of 12 each. Group 1 patients received three cycles of chemotherapy prior to surgery and three cycles after surgery and group 2 patients received six cycles of chemotherapy prior to surgery. The expression of apoptosis-related proteins was analyzed in ascitic fluid and tumor samples by Western blotting and immunohistochemistry. The apoptotic index was also determined in these samples by the TUNEL assay. *Results*: Significant decreases in antiapoptotic bcl-2 and survivin were seen, accompanied by increases in apoptotic index in tumors that had undergone chemotherapy as compared to the baseline ascites samples. No significant change in bcl-XL was observed. A significant decrease in proapoptotic p53 was also seen. No expression of p73 was observed in tumors or ascites. The findings were similar in groups 1 and 2 patients and were not statistically significantly different, perhaps due to the small sample size ($n = 12$) of each group. *Conclusions*: The above findings indicate that chemotherapy in ovarian carcinoma leads to an increase in apoptosis by a p53-independent pathway, which involves the downregulation of antiapoptotic Bcl-2 and survivin but not Bcl-XL. Furthermore, administering neoadjuvant chemotherapy (six cycles) as an alternative form of therapy for advanced epithelial ovarian cancer is more effective in inducing apoptosis than three cycles. However, the findings of this study need to be corroborated using a larger sample.

Keywords Ovarian carcinoma · Chemotherapy · Apoptosis · bcl-2 · Survivin and p53

T. Dutta · H. Sharma · N. Singh (✉)
Department of Biochemistry,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi, 110029, India
E-mail: singh_neeta@hotmail.com
Tel.: +91-11-26594945
Fax: +91-11-26588663

A. K. Dinda
Department of Pathology,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi, 110029, India

S. Kumar · N. Bhatla
Department of Obstetrics and Gynecology,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi, 110029, India

L. Kumar
Institute Rotary Cancer Hospital,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi, 110029, India

Introduction

Ovarian cancer is one of the most frequently fatal gynecological cancers. The symptoms usually do not become apparent until the tumor compresses or invades adjacent structures, ascites develops, or metastases become clinically evident. As a result, two-thirds of women with ovarian cancer have advanced disease (stage III or IV) at the time of diagnosis [1, 2]. Conventional treatment for advanced epithelial ovarian cancer (EOC) is surgery and postoperative combination chemotherapy, usually consisting of a platinum-based drug and a taxane to achieve prolonged progression-free and overall survival [3, 4]. Alternative strategies for the initial management of advanced EOC started in the late 1970s. One approach is known as "interval cytoreduction

surgery'' in which surgery is carried out after the patient has received three cycles of chemotherapy, and an additional three cycles of chemotherapy are administered after surgery [5]. Another approach is ''neoadjuvant chemotherapy'' in which six cycles of chemotherapy are given prior to surgery [5]. Since the chemotherapeutic drugs used in the treatment of EOC exert their action by inducing apoptosis, we looked at apoptosis and its related proteins in material from patients receiving these two treatment modalities.

A balance between pro- and antiapoptotic proteins regulates entry into apoptosis [6, 7]. A number of these have been identified such as the proapoptotic p53 and p73, and the antiapoptotic Bcl-2, Bcl-XL and survivin, a member of the inhibitor of apoptosis protein (IAP) family, which is altered in many cancers, including EOC. Hence, in this study we essentially investigated these apoptosis-related proteins as they are involved in DNA damage/chemotherapy-induced apoptosis through the mitochondria-mediated pathway.

p53 is often mutated in many cancers and is thus responsible for resistance to many chemotherapeutic drugs [8]. Previous reports suggest that ovarian cancer patients whose tumors have p53 mutations experience a lower chance of achieving a complete response following treatment with a platinum-based regimen when compared to patients without p53 mutations. Conversely, experimental and clinical data seem to show that paclitaxel enhances apoptosis through a p53-independent pathway that probably involves the Bax gene, whereas patients with wild-type p53 tumors have a good chance of responding to platinum-based drugs [6]. The p73 gene has sequence and functional similarities with its homologue p53. Therefore determining p53 and p73 status can be useful in predicting therapeutic response to specific drugs.

The antiapoptotic proteins Bcl-2 and Bcl-XL are overexpressed in most cancers conferring a selective growth advantage to tumors and providing protection against a number of chemotherapeutic drugs [9]. In vitro study on cell lines has shown that Bcl-2 protein has a strong influence on the response of ovarian cancer to cisplatin-based chemotherapy [10]. However, such associations have not been demonstrated clinically. We have evaluated the prognostic/predictive significance of these antiapoptotic proteins in ovarian cancer tissue. Survivin is undetectable in terminally differentiated adult tissues but is markedly expressed in most human cancers [11]. A previous report suggests that expression of survivin is associated with resistance to paclitaxel in human ovarian cancer [12].

Since dysregulation of apoptosis involves altered response or resistance to apoptosis-inducing stimuli, many antineoplastic therapies are directed towards induction of apoptosis. We therefore compared the expression levels of these apoptosis-related proteins in the ascitic cells and tumor samples of the patients undergoing two different treatment regimens.

Materials and methods

Sample collection

Ascitic fluid (200 ml) was collected from 24 untreated patients with newly diagnosed stage T3aN0M0 to T4N1M0 EOC from the Institute Rotary Cancer Hospital, AIIMS. The clinicopathological characteristics of the patients are listed in Table 1. Cells isolated from the ascitic fluid by differential centrifugation were stored at −70°C until further processing. The patients were then randomly allocated to two groups of 12 patients each. Since all the patients had stages III or IV disease and had ascites, they did not have up-front surgery. Moreover, the size of the tumor was large and inoperable. Hence, they were first given chemotherapy to debulk the tumor, after obtaining informed consent. Patients in group 1 were given three cycles of chemotherapy, were operated upon, and given another three cycles of chemotherapy after surgery. Patients in group 2 were given six cycles of chemotherapy after ascitic fluid tapping and were then operated upon. Chemotherapy included carboplatin (day 1) and paclitaxel (day 2) given intravenously. This was repeated after 3 weeks, and this constituted one cycle.

Table 1 Clinicopathological characteristics of patients and their apoptotic index (*Scac* serous cystadenocarcinoma, *ND* not determined)

Patient no.[a]	Age (years)	Stage	Histopathology	Apoptotic index (AU)	
				Baseline (ascites)	After treatment (tumor)
Group 1					
1	54	IIIc	Scac	3.2	4
2	43	IIIc	Scac	2	2.2
3	56	IV	Scac	3	2.6
4	43	IIIc	Scac	2.4	3
5	47	IIIc	Scac	4.2	5
6	43	IIIc	Scac	1.8	2.4
7	65	IIIc	Scac	2.8	3.9
8	57	IIIc	Scac	ND	ND
9	50	IIIc	Scac	1.2	3.2
10	50	IIIc	Scac	0.8	1
11	48	IIIc	Scac	1.4	0.9
12	50	IIIc	Scac	ND	ND
Group 2					
13	42	IV	Scac	1.1	2.1
14	50	IIIc	Scac	1.7	4.4
15	65	IIIc	Scac	ND	ND
16	53	IIIc	Scac	3.2	6
17	50	IV	Scac	1.9	3.6
18	50	IV	Scac	ND	ND
19	54	IIIc	Scac	2.8	3.7
20	40	IIIc	Scac	1.1	4.2
21	42	IIIc	Scac	ND	ND
22	56	IIIc	Scac	1.1	3.2
23	65	IIIc	Scac	ND	ND
24	42	IIIc	Scac	ND	ND

[a]Group 1 received three cycles of chemotherapy; group 2 received six cycles of chemotherapy

Samples of EOC obtained from patients after surgery were snap-frozen and stored at −70°C until further processing. Concurrent tumor sections were cut at 8 μm in a cryostat. These were used for histopathology, immunohistochemistry, Western blotting and the TUNEL assay. Histopathologically confirmed tumor tissue and ascites were taken for the study.

Immunohistochemical analysis

The expression of apoptosis-related proteins was determined by immunohistochemistry using appropriate antibodies as described previously [13]. Briefly, the endogenous peroxidases were first blocked with hydrogen peroxide in phosphate-buffered saline (PBS) (pH 7.4) containing 70% methanol and nonspecific binding blocked using 5% bovine serum albumin (BSA). The tumor tissue sections were then incubated with antibodies against Bcl-2, Bcl-XL and survivin (Santa Cruz Biotechnology, Santa Cruz, Calif.) at a dilution of 1:100 in 5% BSA for 48 h at 4°C. Immunodetection was done using 3,3′-diaminobenzidine (DAB) as chromogen and H_2O_2 as substrate, followed by light counterstaining with hematoxylin and examination under a microscope. Tonsil tissue served as a positive control for Bcl-2 and Bcl-XL and placental tissue was used as a positive control for survivin. The negative controls lacked the primary antibody. Protein expression was determined semiquantitatively. Scoring was done by microscopic examination of randomly selected fields containing at least 300 cells and expressed in arbitrary units (AU). Scoring of immunopositivity was done on the basis of percentage as well as intensity of staining as follows: (a) 1 AU, 5–10%; (b) 2 AU, 11–25%; (c) 3 AU, 26–50%; (d) 4 AU, >50%. Intensity of staining was scored as follows: (a) 1 AU, weak; (b) 2 AU, moderate; (c) 3 AU, intense. The final score was determined by adding up the scores of percentage and intensity of staining.

Western blot analysis

The samples were lysed in RIPA buffer containing protease and phosphatase inhibitors. Equal amounts of protein extracts were electrophoresed on 10–18% SDS-polyacrylamide gels and electrotransferred to nitrocellulose membrane, which was then incubated in 5% BSA for 3 h followed by overnight incubation with antibodies against rabbit Bcl-2, Bcl-XL and survivin, and mouse p53 and p73. The membrane was then washed twice with

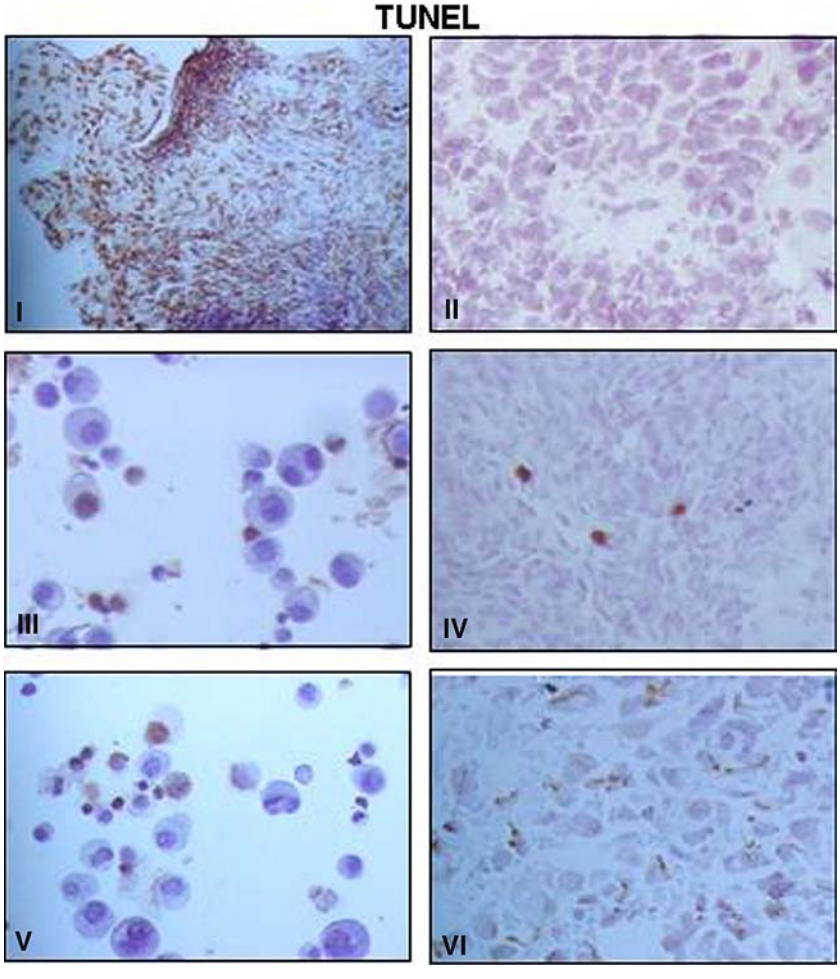

Fig. 1 TUNEL staining for apoptotic index: *I* positive control, *II* negative control, *III* ascitic cells (group 1), *IV* tumor (group 1), *V* ascitic cells (group 2), *VI* tumor (group 2)

Table 2 Expression of apoptosis-related proteins in ascites (baseline) and in tumors (after treatment) as assessed by Western blotting. The values shown are relative units (RU)

Patient no.[a]	Bcl-2		Bcl-XL		p53		Survivin		p73	
	Baseline (ascites)	After treatment (tumor)	Baseline (ascites)	After treatment (tumor)	Baseline (ascites)	After treatment (tumor)	Baseline (ascites)	After treatment (tumor)	Baseline (ascites)	After treatment (tumor)
Group 1										
1	0	0	2.74	0	0.443	0	4	3	0	0
2	4.32	4	1	1.73	0	0	0	0	0	0
3	9.86	9.9	4	5.6	9.8	0	4	3	0	0
4	0	0	0	0	0	0	2	1	0	0
5	2.1	1.47	2	1.78	3.77	2	3	3	0	0
6	5.96	6.25	4	2.96	0.44	2	4	0	0	0
7	9.6	0	3	0	9.21	4	3	0	0	0
8	3.8	4.5	2	1.72	0	0	0	0	0	0
9	0	0	1	2.27	6.4	5.7	1	0	0	0
10	1.65	1.5	0	0	0	0	4	2	0	0
11	7.97	6.25	4	5.6	0	0	1	0	0	0
12	0	0	3.2	0.76	0.44	0.32	3	2	0	0
Group 2										
13	8.4	6.25	2	0.72	0	4	4	4	0	0
14	2.4	1.12	0	0	8.4	9.2	4	1	0	0
15	4.1	2.3	4	1.73	3.2	0	2	1	0	0
16	3	0	2	5.6	4.2	2.1	0	0	0	0
17	7.46	6.53	9.91	1.6	1.54	0	0	0	0	0
18	5.1	4.17	4	2.85	4.2	0.67	4	2	0	0
19	9.44	7.56	4	3.3	2.92	0.78	0	0	0	0
20	2.26	0	3	1.92	6.3	3.7	2	0	0	0
21	1.8	0	3	2	1.7	0	4	1	0	0
22	0	0	3	3	1.9	0	2	1	1	0
23	1	0	2	2	1.4	1.2	0	0	0	0
24	3	2	3	3	3	4	4	3	0	0

[a]Group 1 received three cycles of chemotherapy; group 2 received six cycles of chemotherapy

Tris-buffered saline (TBS) containing Tween-20 and then with TBS alone for 10 min each time. Anti-rabbit and anti-mouse alkaline phosphatase-conjugated antibodies were then added to the blot and incubated for 2 h. After washing the membrane, color was developed using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate from Promega (Madison, Wis.). The bands were analyzed and quantitated using a BioRad scanning densitometer. Positive controls used for immunohistochemistry were also included. Protein expression was expressed as relative units (RU). One relative unit is the ratio of the density of a positive control to that of a negative control [13].

TUNEL assay

Apoptotic cells were visualized by the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) technique [13] using a Dead End colorimetric cell death detection kit (Promega). The tumor sections/ascitic cells fixed on glass slides were incubated with Proteinase K/Triton X-100 for 20 min at room temperature. TdT was used to catalyze the addition of biotin-conjugated dUTP to the 3′-OH ends of DNA fragments. The incorporated biotin was detected by streptavidin conjugated to horseradish peroxidase. The staining was done using DAB as chromogen and H_2O_2 as substrate. The sections were counterstained with hematoxylin. The apoptotic index was determined by microscopic examination of randomly selected fields containing at least 500 cells. The results are expressed as the percentage of apoptosis-positive cells in 500 cells.

Statistical analysis

Statistical analysis of the samples was done using SPSS version 10.0 software. A paired *t*-test was used for comparison of ascites and tumors. An unpaired *t*-test was used to compare the effect of three cycles of chemotherapy versus six cycles. Correlation was determined using Spearman's correlation coefficient.

Results

Effect of chemotherapy on apoptosis and its related proteins

To investigate the effect of chemotherapy on apoptosis and its related proapoptotic and antiapoptotic proteins, baseline samples (ascites, 24 samples) were compared with tumors surgically resected after treatment, including both groups of patients ($n = 24$). The mean baseline apoptotic index was 2.1 ± 0.23 AU whereas in tumors it

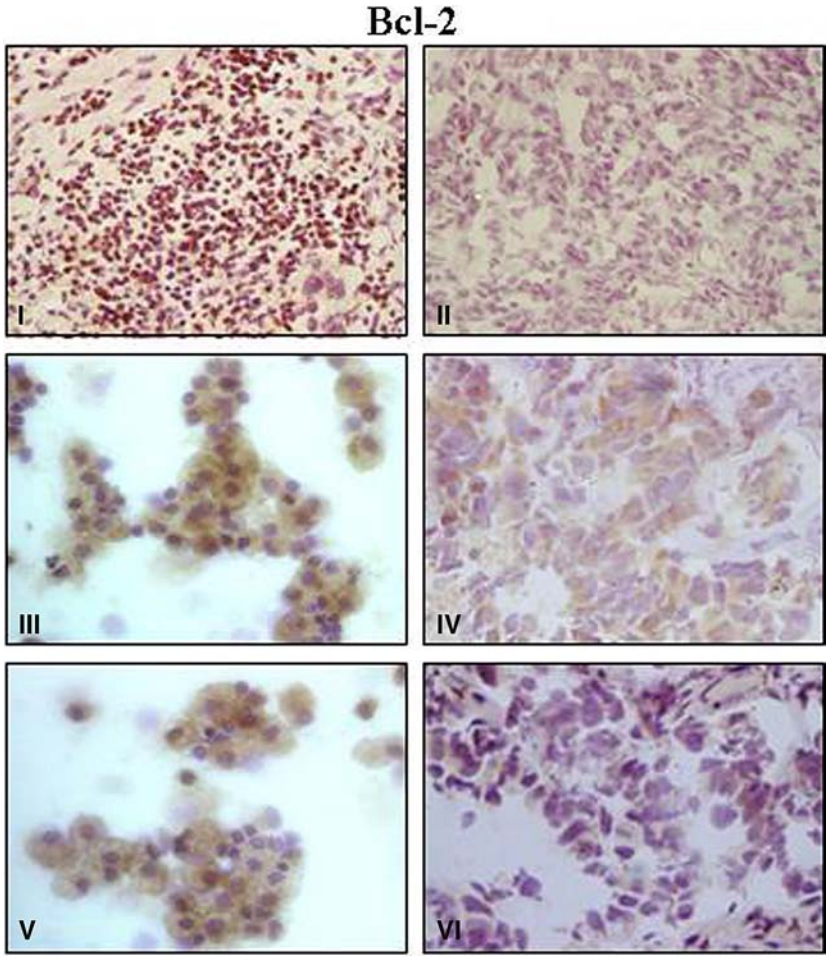

Fig. 2 Immunostaining for Bcl-2 protein expression: *I* positive control, *II* negative control, *III* ascitic cells (group 1), *IV* tumor (group 1), *V* ascitic cells (group 2), *VI* tumor (group 2)

was 3.26 ± 0.32 AU. This increase was statistically significant ($t = -4.417$, $P < 0.001$; Table 1 and Fig. 1).

The baseline Bcl-2 expression was 3.88 ± 0.68 RU, and in the tumor group it was 2.65 ± 0.62 RU (Table 2 and Fig. 2). This decrease was statistically significant ($t = 2.966$, $P = 0.007$). Similarly, survivin also showed a significant decrease in the chemotherapy-treated tumor (1.12 ± 0.26 RU) as compared to baseline (2.3 ± 0.34 RU; $t = 4.897$, $P < 0.001$; Table 2 and Fig. 3). However, there was no significant difference between baseline Bcl-XL expression (2.78 ± 0.41 RU) and tumor expression (2.08 ± 0.35 RU) (Table 2 and Fig. 4). There was a statistically significant decrease in p53 expression in tumor (1.65 ± 0.48 RU) as compared to baseline (2.88 ± 0.63 RU; $t = 2.305$, $P = 0.031$; Table 2 and Fig. 5). No expression of p73 was observed generally in either baseline samples or tumors.

Comparison between three cycles (group 1) versus six cycles (group 2) of chemotherapy

The mean apoptotic index was 2.82 ± 0.41 AU in tumors which had undergone three cycles of chemotherapy, but was 3.88 ± 0.45 AU in tumors that had undergone six cycles of chemotherapy (Table 3 and Fig. 1).

The tumor group which had undergone three cycles of chemotherapy showed a mean Bcl-2 expression of 2.82 ± 0.96 RU whereas the group which had undergone six cycles showed a value of 2.49 ± 0.83 RU (Table 3 and Fig. 2). The Survivin expression was 1.17 ± 0.39 RU in tumors that had undergone three cycles of chemotherapy and 1.08 ± 0.38 RU in those that had undergone six cycles of chemotherapy (Table 3 and Fig. 3). The mean Bcl-XL expression was 1.87 ± 0.58 RU in tumors that had undergone three cycles of chemotherapy and 2.3 ± 0.41 RU in tumors that had undergone six cycles of chemotherapy (Table 3 and Fig. 4). The mean p53 expression was 1.17 ± 0.55 RU in tumors that had undergone three cycles of chemotherapy and 2.1 ± 0.79 RU in tumors that had undergone six cycles of chemotherapy (Table 3 and Fig. 5). However, none of the above differences was found to be statistically significant perhaps due to the small sample size of 12 samples in each group.

Discussion

Apoptosis is a genetically regulated biological process that plays a major role in chemotherapy-induced tumor cell killing. There is a significant increase in apoptotic

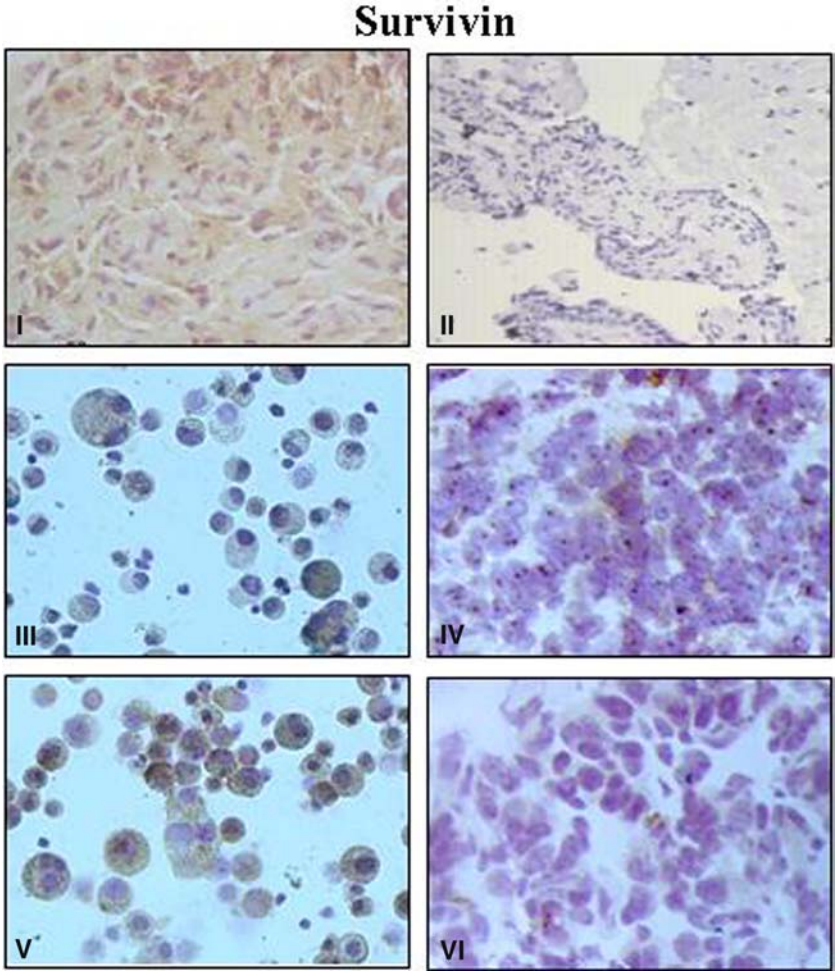

Fig. 3 Immunostaining for survivin protein expression: *I* positive control, *II* negative control, *III* ascitic cells (group 1), *IV* tumor (group 1), *V* ascitic cells (group 2), *VI* tumor (group 2)

index after chemotherapy, indicating that chemotherapeutic drugs induce cell killing in ovarian cancer by inducing apoptosis. The p53 gene is involved in the regulation of apoptosis. Missense point mutations in p53 are most common in EOC. p53 plays a critical role in cellular response to DNA damage and has been suggested to be a strong predictor of response to platinum-based chemotherapy in EOC [6]. Because taxanes can induce p53-independent apoptosis [14], we assessed the p53 status in ovarian carcinoma patients receiving paclitaxel and platinum-containing chemotherapy. Though a decrease in p53 was observed after treatment, there was no significant change in its expression in relation to the number of cycles of chemotherapy that were given. These results indicate that the chemotherapeutic drugs induced apoptosis by perhaps a p53-independent pathway and corroborate the results of an earlier study showing that paclitaxel cytotoxicity in ovarian cancer is mediated by a p53-independent pathway [15]. Similarly, p73 has been found to enhance the sensitivity of human lung and head and neck cancer cells to cisplatin-based therapy [16]. However, we did not observe p73 expression in any of the samples of EOC. Allelic loss on chromosome 1p36 is common in EOC and could be a reason for the absence of this protein [17]. Furthermore, some reports suggest that aberrant methylation of CpG islands is a major pathway leading to the inactivation of tumor suppressor genes and the development of cancer. DNA methylation at CpG islands in exon 1 of p73 may contribute to lack of expression of this protein [18]. Thus it appears that p73 protein expression may have been lost by one of the above mechanisms. Our findings corroborate those of a previous study in which no expression of p73 was found in ovarian cancer [19].

It is known that bcl-2 plays a role in the pathogenesis of ovarian cancer. Increased bcl-2 expression is associated with primary resistance to chemotherapy in human EOC [10] and hence could be a possible predictor of response to chemotherapy and prognosis in patients with advanced ovarian carcinoma [20]. The observed decrease in its expression after chemotherapy suggests that the increase in apoptosis seen in the chemotherapy-treated tumors could perhaps have been due to a decrease in antiapoptotic bcl-2 and survivin.

Bcl-XL, another antiapoptotic member of the Bcl-2 family of proteins, is overexpressed in ovarian tumors resulting in dysregulation of cell cycle checkpoints, and therefore inhibition of apoptosis. It has been shown that Bcl-XL inhibits chemotherapy-induced apoptosis of drugs such as cisplatin and paclitaxel in ovarian cancer

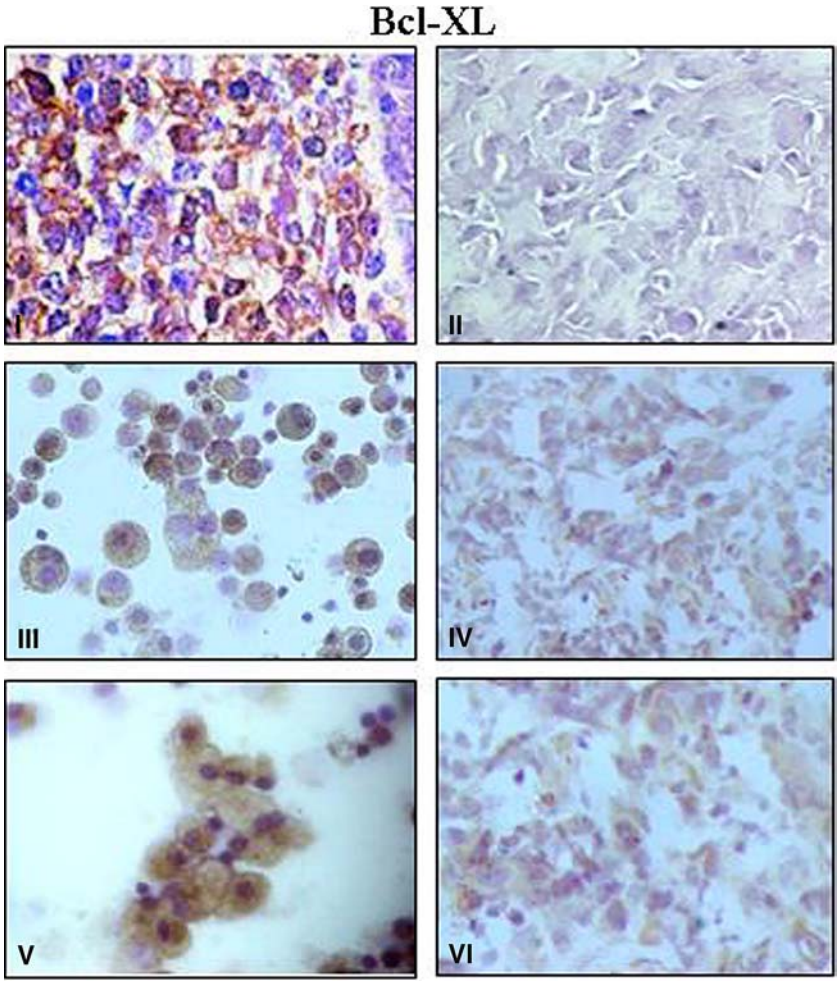

Fig. 4 Immunostaining for Bcl-XL protein expression: *I* positive control, *II* negative control, *III* ascitic cells (group 1), *IV* tumor (group 1), *V* ascitic cells (group 2), *VI* tumor (group 2)

[9]. However, we did not find any significant decrease in the expression of Bcl-XL after chemotherapy. A previous study has also shown similar findings to ours in which the administration of platinum drugs led to a reduction in Bcl-2 expression without affecting the levels of other antiapoptotic members of this family [21]. Survivin, another antiapoptotic protein has been found to be associated with microtubules and regulates entry into the G_2/M phase of the cell cycle. It binds to downstream caspases and inhibits apoptosis [22]. Paclitaxel acts on microtubules to arrest mitosis and prevent tumor progression [23]. It is also reported that survivin is associated with resistance to paclitaxel in human ovarian cancer [12]. The observed decrease in Bcl-2 and survivin expression after chemotherapy and the inverse correlation between survivin, Bcl-2 expression, and apoptotic index suggests that paclitaxel probably sensitizes the EOC cells by inducing apoptosis through the downregulation of antiapoptotic survivin.

The above findings therefore indicate that chemotherapy of ovarian carcinoma leads to an increase in apoptosis by a p53-independent pathway, which involves the downregulation of antiapoptotic Bcl-2 and survivin but not Bcl-XL. Furthermore, administering neoadjuvant chemotherapy (six cycles) as an alternative form of therapy for advanced epithelial ovarian cancer is more effective in inducing apoptosis by reducing expression of antiapoptotic genes Bcl-2 and survivin than three cycles. However, the findings of this study need to be corroborated using a larger sample.

References

1. Pettersson F (1991) Annual report on the results of treatment of gynecologic cancer. Int J Gynecol Obstet 36:238–277
2. Richardson GS, Scully RE, Nikrui N (1985) Common epithelial cancer of the ovary. N Engl J Med 312:415–424
3. Natarajan M, Saravanan SM, Elson DL (2003) Advanced ovarian carcinoma as a chronic disease: a case report and review. S D J Med 56:515–521
4. See HT, Kavanagh JJ, Hu W, Bast RC (2003) Targeted therapy for epithelial ovarian cancer: current status and future prospects. Int J Gynecol Cancer 13:701–734
5. Mazzeo F, Berliere M, Kerger J, Squifflet J, Duck L, D'Hondt V, Humblet Y, Donnez J, Machiels JP (2003) Neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy in patients with primarily unresectable, advanced-stage ovarian cancer. Gynecol Oncol 90:163–169
6. Gadducci A, Cosio S, Muraca S, Genazzani AR (2002) Molecular mechanisms of apoptosis and chemosensitivity to platinum and paclitaxel in ovarian cancer: biological data and clinical implications. Eur J Gynaecol Oncol 23:390–396

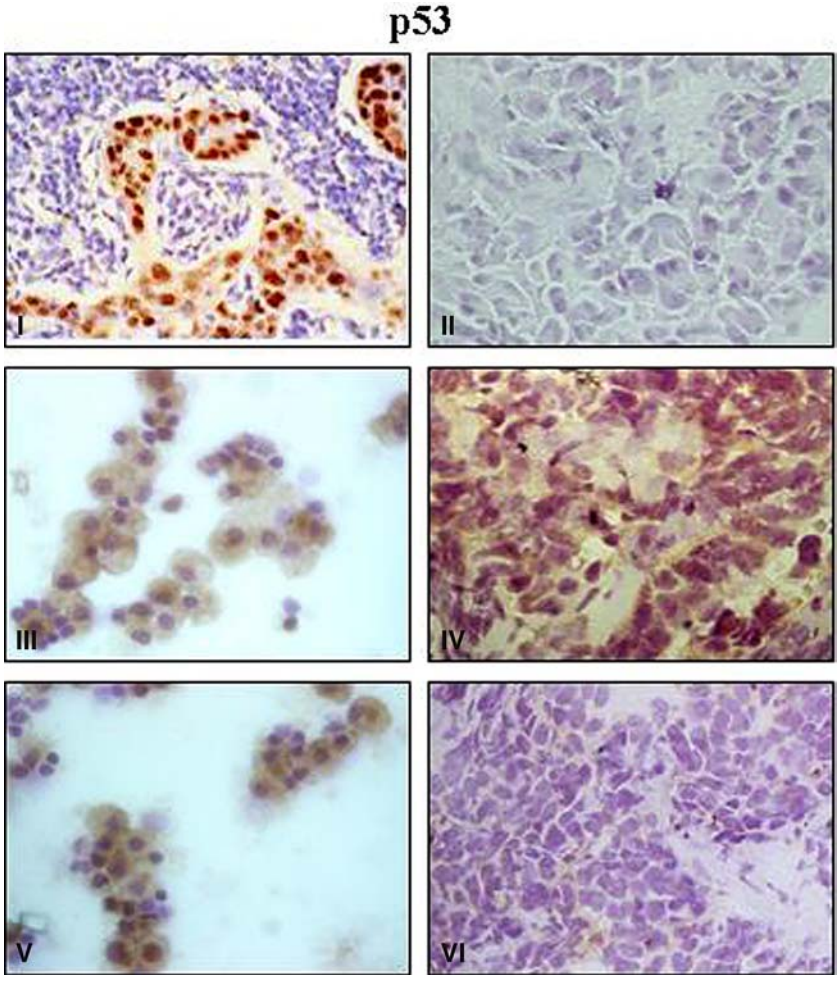

Fig. 5 Immunostaining for p53 protein expression: *I* positive control, *II* negative control, *III* ascitic cells (group 1), *IV* tumor (group 1), *V* ascitic cells (group 2), *VI* tumor (group 2)

Table 3 Apoptotic index (*AI*) and apoptosis-related proteins in ascites and tumors after chemotherapy. The results are presented as means ± SE of 24 samples for ascites and 12 samples each for groups 1 and 2

	Baseline (ascites)	Tumors (three cycles)	Tumors (six cycles)
AI (AU)	2.1 ± 0.23	2.82 ± 0.41	3.88 ± 0.45
Bcl-2 (RU)	3.88 ± 0.68	2.82 ± 0.96	2.49 ± 0.83
Bcl-XL(RU)	2.78 ± 0.41	1.87 ± 0.58	2.3 ± 0.41
p53 (RU)	2.88 ± 0.63	1.17 ± 0.55	2.1 ± 0.79
p73 (RU)	0	0	0
Survivin (RU)	2.3 ± 0.34	1.17 ± 0.39	1.08 ± 0.38

7. Strasser A, O'Connor L, Dixit VM (2000) Apoptosis signaling. Annu Rev Biochem 69:217–245
8. Daponte A, Guidozi F, Tiltman AJ, Marineanu A, Taylor L (1999) p53 as a prognostic factor for stage III serous adenocarcinoma of the ovary. Anticancer Res 19:2387–2389
9. Liu JR, Fletcher B, Page C, Hu C, Nunez G, Baker V (1998) Bcl-XL is expressed in ovarian carcinoma and modulates chemotherapy-induced apoptosis. Gynecol Oncol 70:398–403
10. Mano Y, Kikuchi Y, Yamamoto K, Kita T, Hirata J, Tode T, Ishii K, Nagata I (1999) Bcl-2 as a predictor of chemosensitivity and prognosis in primary epithelial ovarian cancer. Eur J Cancer 35:1214–1219
11. Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M, Tanigawa N (2000) Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res 6:127–134
12. Zaffaroni N, Pennati M, Colella G, Perego P, Supino R, Gatti L, Pilotti S, Zunino F, Daidone MG (2002) Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer. Cell Mol Life Sci 59:1406–1412
13. Sharma H, Sen S, Mathur M, Bahadur S, Singh N (2004) Combined evaluation of expression of telomerase, survivin, and anti-apoptotic Bcl-2 family members in relation to loss of differentiation and apoptosis in human head and neck cancers. Head Neck 26:733–740
14. Lavarino C, Pilotti S, Oggionni M, Gatti L, Perego P, Bresciani G, Pierotti MA, Scambia G, Ferrandina G, Fagotti A, Mangioni C, Lucchini V, Vecchione F, Bolis G, Scarfone G, Zunino F (2000) p53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. J Clin Oncol 18:3936–3945

15. Gadducci A, Cianci C, Cosio S, Carnino F, Fanucchi A, Buttitta F, Conte PF, Genazzani AR (2000) p53 status is neither a predictive nor a prognostic variable in patients with advanced ovarian cancer treated with a paclitaxel-based regimen. Anticancer Res 20:4793–4799
16. Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, Yulug I, Merlano M, Numico G, Comino A, Attard M, Reelfs O, Gusterson B, Bell AK, Heath V, Tavassoli M, Farrell PJ, Smith P, Lu X, Crook T (2003) p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 3:387–402
17. Alvarez AA, Lambers AR, Lancaster JM, Maxwell GL, Ali S, Gumbs C, Berchuck A, Futreal PA (2001) Allele loss on chromosome 1p36 in epithelial ovarian cancers. Gynecol Oncol 82:94–98
18. Chen CL, Ip SM, Cheng D, Wong LC, Ngan HY (2000) p73 gene expression in ovarian cancer tissues and cell lines. Clin Cancer Res 6:3910–3915
19. Codegoni AM, Bertoni F, Patregnani C, Marinetti E, D'Incalci M, Broggini M (1999) Allelic expression of p73 in human ovarian cancers. Ann Oncol 10:949–953
20. Kassim SK, Ali HS, Sallam MM, Fayed ST, Seada LS, abd-Elkawy E, Seada MA, Khalifa A (1999) Increased bcl-2 expression is associated with primary resistance to chemotherapy in human epithelial ovarian cancer. Clin Biochem 32:333–338
21. Beale PJ, Rogers P, Boxall F, Sharp SY, Kelland LR (2000) BCL-2 family protein expression and platinum drug resistance in ovarian carcinoma. Br J Cancer 82:436–440
22. Altieri DC (2003) Survivin and apoptosis control. Adv Cancer Res 88:31–52
23. Bhalla KN (2003) Microtubule-targeted anticancer agents and apoptosis. Oncogene 22:9075–9086