Prognostic significance of apoptotic index and bcl-2 and p53 expression in epithelial ovarian carcinoma

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Summary

Purpose of the study: To determine if bcl-2 and p53 expression, and apoptotic index (AI) were associated with patient outcome in epithelial carcinomas of the ovary (EOC) and therefore useful as prognostic factors to predict survival.

Methods: A total of 50 women with epithelial carcinomas of the ovary were retrospectively analyzed. The archival paraffin-embedded material of of these cases were evaluated for expression of p53 and bcl-2 by immunohistochemical techniques. Apoptotic cells were detected with an in situ hybridisation method.

Results: A total of 33 (66%) of 50 cases showed positive immunoreactivity for the p53 antibody. Twenty-four of the 50 cases showed positive bcl-2 protein expression. Median value for AI was found to be 2.48. No statistically significant association was found between bcl-2 and p53 expression and clinicopathologic features. Univariate survival analysis of AI failed to reveal any effect on prognosis in the study population.

Conclusion: We found neither p53 nor bcl-2 immunoreactivity to be of prognostic significance in patients with EOC. In addition, AI was not found to be an independent prognostic factor.

Key words: Apoptosis; Protein p53; Protein bcl-2; Apoptotic index; Ovarian cancer.

Introduction

Ovarian cancer is still the most fatal of gynecologic malignancies [1]. However little is known about the molecular events leading to the development of ovarian cancer. Malignancies usually possess aberrations in more than a single pathway. Either increased proliferation or decreased death might result in an augmentation of cell numbers [2]. Most of our knowledge concerning oncogenic events has concentrated on mechanisms of increased cell growth and proliferation. However, tissue growth not only depends on cell proliferation, but also on the rate of physiologically occurring cell death which is called apoptosis [3]. The new interest in apoptosis has touched many fields, but none more so than cancer biology. Apoptosis is defined as eliminating cells with DNA damage or growth de-regulation that could become precursors of malignant clones. In this way it provides growth arrest and permits DNA repair mechanisms to preserve the genetic integrity of tissues [4]. Thus, it is conceivable that neoplastic growth may also be caused or promoted by factors inhibiting cell death. Different endogenous regulators of the apoptotic process have been described, among which the p53 tumor suppressor gene and the bcl-2 family of apoptosis regulators are the most prominent [5].

The p53 tumor supressor gene product plays a role in DNA damage recognition, DNA repair, cell cycle regulation and most particularly in triggering apoptosis after genetic injury [6]. In the presence of DNA damage, it serves to arrest cell division at the G_1 phase, thus protecting against the propagation of genetic errors [7]. The level of wild-type p53 present in nontransformed cells is low because of the short half-life of native proteins. Mutations in the p53 gene are common, and constitute the basis for detection by immunohistochemistry [8]. The resultant proteins all have significantly increased half-lives compared to wild-type proteins, resulting in accumulation of enough protein products for of immunohistochemical detection.

The bcl-2 gene is located at chromosome 18q21 and its by-product is characterized as a protein inhibiting programmed cell death, apoptosis [9, 10, 11]. The bcl-2 gene was first identified in 1984 while studying the t(14; 18) chromosome translocations that occur frequently in B-cell leukemia and non-Hodgkin's follicular lymphoma [12]. Many members of the bcl-2 family have been isolated with antagonistic function, and regulation of cell death by members of this gene family may be achieved through competing dimerisation [13]. The mechanism of action of the bcl-2 protein has not been fully defined but may involve oxidative phosphorylation and/or mitochondrial electron and metabolite transport [6, 14]. Interestingly, expression of bcl-2, the major inhibitor of apoptosis, has been shown to be connected with parameters of favorable prognosis and prolonged survival in breast and recently, also in ovarian cancer [15, 16, 17].

In this study, we aimed to determine if bcl-2 and p53 expression, and apoptotic index (AI) which might be an indicator of apoptosis were associated with patient outcome in epithelial carcinomas of the ovary and therefore useful as prognostic factors to predict survival.

Material and Methods

The study population included a total of 50 patients with the diagnosis of EOC who received treatment at the Department of Obstetrics and Gynecology the Ankara University School of

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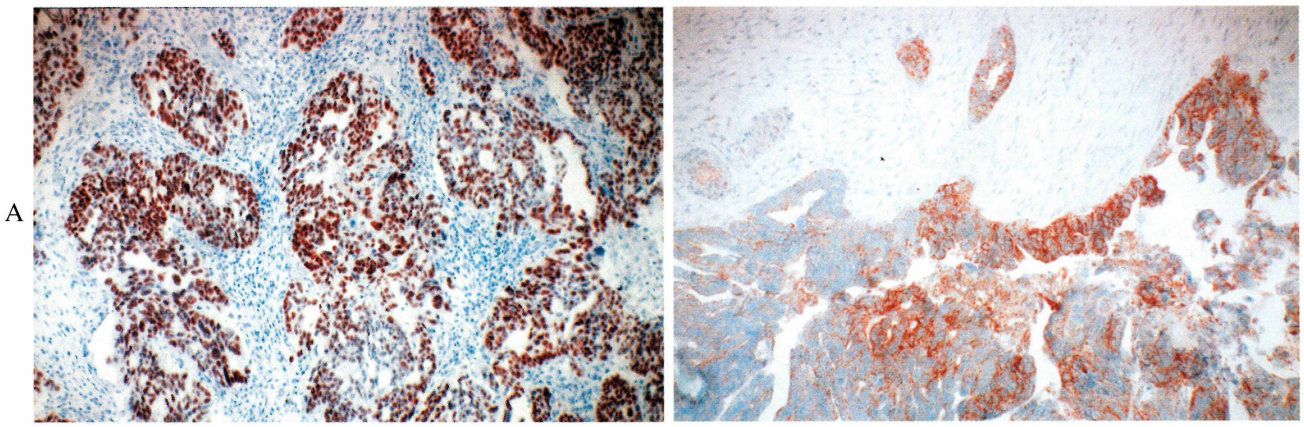

Figure 1. — Immunohistochemical analysis showing (A) nuclear staining for p53 and (B) cytoplasmic staining for bcl-2 (x 200).

Medicine, in the period from 1990 to 1997. All the cases underwent optimal debulking staging surgery, and then received platinum-based chemotherapy. Patients who were given any treatment before the primary surgery were excluded. Retrospective reviews from patient files were performed to obtain necessary information on the primary tumor, type of surgery, adjuvant teratment, and survival. The tumor staging was done on the basis of the FIGO criteria [18]. Patients with stage I and II, and with stage III and IV were classified into an early-stage group and advanced-stage group, respectively. The archival paraffin-embedded material of ovarian carcinomas was re-examined and histologically reclassified in accordance with the WHO classification for histologic subtype and grade [19]. A detailed description of the clinicopathologic data of the patients is given in Table 1.

Table 1. — *Patient characteristics (n = 50).*

Characteristics	N	%
Age (years)		
Median	53.5	
Range	25 - 71	
Follow-up (month)		
Median	45.12	
Range	10 -93	
Surgical Staging		
I + II	14	28
III + IV	36	72
Histological Type		
Serous	34	68
Mucinous + Endometrioid	16	32
Histological Grade		
1	15	30
2	26	52
3	9	18
p53 expression		
negative (≤ %10)	17	34
positive (> %10)	33	66
bcl-2 expression		
negative (≤ %10)	26	52
positive (> %10)	24	48
Apoptotic Index (AI)		
Median	2.48	
Range	0-10	
Survival		
Alive	39	78
Deceased	11	22
Total	50	100

Immunohistochemical staining for p53 and bcl-2 was performed on 6-micron tissue sections by a peroxidase-labelled streptavidin-biotin technique as previously described by Henriksen [20]. Based on the literature, immunoreactivity for p53 and bcl-2 antibodies was evaluated according to the percentage of positive cells: positivity in less than 10% of the tumor cells was accepted as negative [21, 22]. Examples of immunohistochemical staining patterns for p53 and bcl-2 are shown in Figures 1A and 1B.

Apoptotic cells were detected by an in situ hybridisation method (Apopdotec®/ Cell Death Assay System, Enzo). We counted at least 1,000 tumor cells in ten randomly chosen fields per case, and the number of apoptotic cells per 1000 tumor cells was designated as the AI on stained sections. Since 2.48 represented the mean value of all samples under study, tumors were classified into two groups: low AI, ≤ 2.48; high AI, > 2.48).

Statistical analysis

The Statistical Package for Social Science (SPSS, Inc, Chicago, IL) was used for the statistical analysis. Chi-square tests were used to assess the association between AI and p53 protein expression, bcl-2 protein expression, and various clinicopathologic parameters. Univariate survival analyses were based on the Kaplan-Meier method. Comparisons between the survival curves were analyzed using the log-rank test; p values < 0.05 were considered statistically significant.

Results

Fifty cases with EOC were enrolled in this study and patient characteristics are summarized in Table 1. The age of patients varied between 25 and 71 years, with a median of 53 years. During a median follow-up of 45 months (range 10 to 93 months), 11 deaths occurred. The median survival rate was 75 months and three years survival probability was found as 82%.

A total of 33 cases (66%) of 50 cases showed positive immunoreactivity with the p53 antibody. We examined possible associations between p53 positivity and other disease parameters (Table 2). There was no significant association with disease stage and histologic subtypes. Although p53 positivity was mostly seen in high-grade tumor, it did not reach a statistical significance ($p > 0.05$).

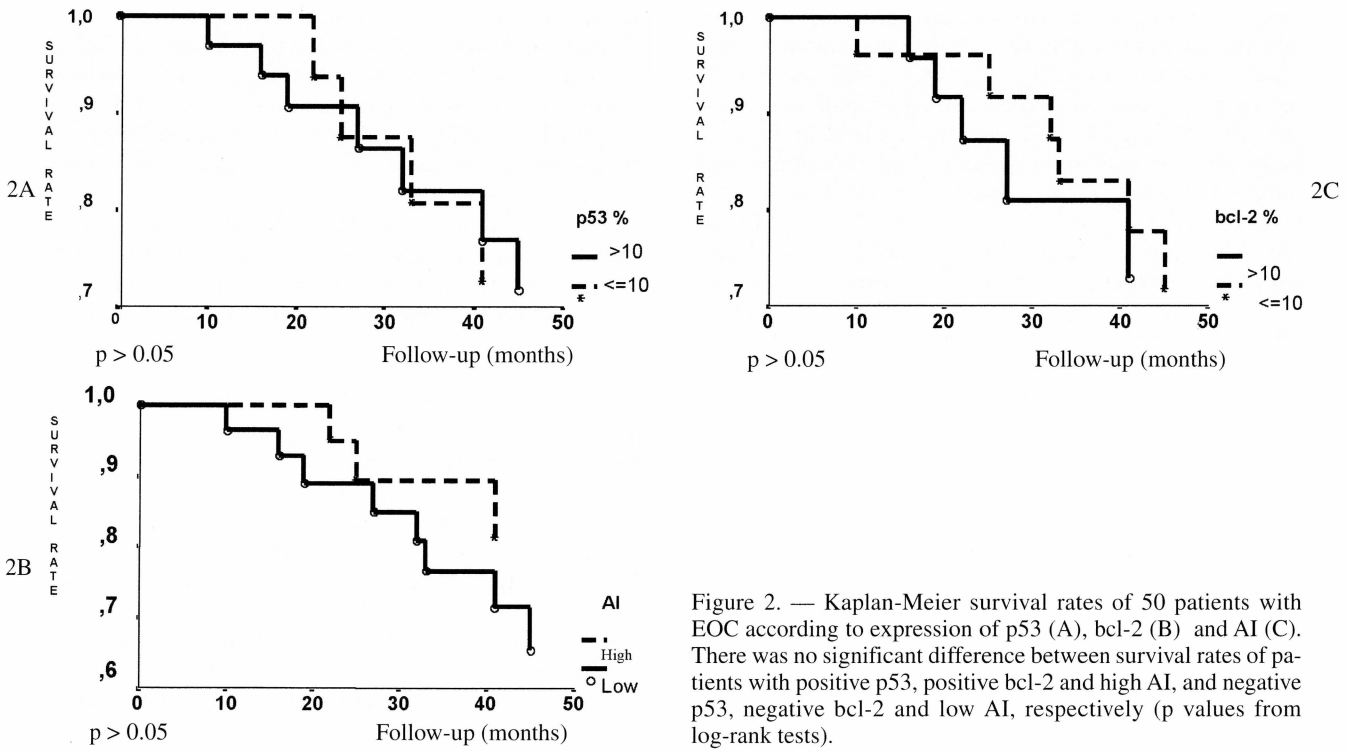

Figure 2. — Kaplan-Meier survival rates of 50 patients with EOC according to expression of p53 (A), bcl-2 (B) and AI (C). There was no significant difference between survival rates of patients with positive p53, positive bcl-2 and high AI, and negative p53, negative bcl-2 and low AI, respectively (p values from log-rank tests).

The median survival was found to be 68 months and 75 months in p53 negative and positive cases respectively which was not statistically significant ($p > 0.05$). In univariate analysis, p53 positivity was not found to be predictive of survival (Figure 2A).

Twenty-four of the 50 cases showed positive bcl-2 protein expression. No statistically significant association was found between bcl-2 expression and surgical staging, histological subtypes, grades and patient ages (Table 2). The median survival was 68 months for bcl-2 negative cases, and was 75 months for bcl-2 positive cases. This was not statistically significant ($p > 0.05$). Also in univariate analysis, bcl-2 was not found to be predictive of survival (Figure 2B).

The AI (apoptotic cells/1,000 tumor cells) was evaluated in EOC cases, all surgically resected. In general, the

Table 2. — *Correlations between p53, bcl-2 protein expression and AI, and other clinicopathologic parameters in 50 patients with EOC.*

Characteristics	p53 negative (≤ %10)		p53 positive (> %10)		p	bcl-2 negative (≤ %10)		bcl-2 positive (> %10)		p	AI low (≤2,48)		AI high (>2,48)		p
	n/17 case	%	n/33 case	%		n/26 case	%	n/24 case	%		n/28 case	%	n/22 case	%	
Surgical Staging															
I+II	5	29	9	27	>0.05	7	27	7	29	>0.05	10	35.7	4	18.2	>0.05
III+IV	12	71	24	73		19	73	17	71		18	64.3	18	81.8	
Histologic Grade															
1	7	41	8	24	>0.05	6	23	9	38	>0.05	6	21.4	9	40.9	>0.05
2+3	10	59	25	76		20	77	15	62		22	78.6	13	59.1	
Histologic Type															
Serous	13	76	21	64	>0.05	19	73	15	63	>0.05	17	60.7	17	77.3	>0.05
Mucinous + Endometrioid	4	24	12	36		7	27	9	37		11	39.3	5	22.7	
Age															
≤ 55	11	65	16	48	>0.05	13	50	14	58	>0.05	17	60.7	10	45.5	>0.05
> 55	6	35	17	52		13	50	10	42		11	39.3	12	54.5	
Survival															
Alive	13	76	26	79	>0.05	20	77	19	79	>0.05	20	71.4	19	86.4	>0.05
Exitus	4	24	7	21		6	23	5	21		8	28.6	3	13.6	

number of apoptotic cells detected was low. Median value for AI was found to be 2.48, and patients were classified into groups according to this e.g. cases with low AI and high AI. Associations between clinicopathologic features and AI in EOC cases are summarized in Table 2. Although most patients with early stage carcinomas had low AI, there was no statistically significant association between surgical stage and AI ($p > 0.05$). In addition, when we evaluated the relationship between AI and age, histologic subtypes and tumor grade, there was no statistically significant association ($p > 0.05$). Median survival was 71 months in the low AI group, and 72 months in the high AI group which was not statistically significant ($p > 0.05$). Univariate survival analysis of the apoptotic index failed to reveal any effect on prognosis in the study population ($p > 0.05$) (Figure 2C).

p53 protein expression was found to be positive in 20 of the cases with low AI and 13 of the 22 cases with high AI; bcl-2 expression was positive in 13 of the 28 cases with low AI and 11 of the 22 cases with high AI. The apoptotic index was not correlated with bcl-2 expression nor with p53 protein accumulation ($p > 0.05$).

Discussion

Ovarian cancer is the leading cause of death from gynecologic cancer in the Western world. Despite many advances in the medical and surgical treatment of women with a variety of cancers, increases in long-term survival of patients with ovarian cancer have been minimal [23]. A better understanding of the biology of this disease will provide better therapeutic results. Some of the molecular mechanisms underlying the neoplastic growth of EOC have been elucidated during recent years and apoptosis, the physiologically occurring cell death which is clearly different from necrosis, plays an important role [24, 25]. The regulation of apoptosis is central to morphogenesis during fetal development, and to maintenance of tissue homeostasis during adulthood. Moreover, it seems to be of importance for neoplastic transformation in some organs [26]. Both bcl-2 and p-53 have been implicated in the regulation of this critical process [27]. To this purpose, we examined the possible prognostic significance of expression of both p53 and bcl-2 proteins and AI in a cohort of uniformly treated patients with EOC.

Mutational inactivation of the p53 tumor suppressor gene occurs in approximately 50% of ovarian cancers resulting in the synthesis of mutant proteins which are less rapidly degraded than wild-type p53 [23, 28, 29]. A close correlation between the presence of p53 immunoreactivity in EOC and mutations in the p53 gene or allele loss at 17p has been shown previously [30-33]. Several authors have reported that p53 expression analyzed immunohistochemically is present in 40% to 60% of ovarian carcinomas [34-36]. The proportion of p53 expression (66%) found in this study is in accordance with previous studies. In different studies, advanced-stage disease patients were much more prevalent than patients with early-stage disease [36-38]. In addition, in the study of Bosari *et al.* it was reported that p53 immunoreactivity was more prevalent in less differentiated tumors [36]. However, in our investigation there were no statistically significant differences in the distribution of p53 accumulation among different disease stages, histologic types, and grades.

Currently, in carcinomas of the breast, stomach, colon-rectum, bladder, prostate and lung, p53 protein expression is considered to be an independent prognostic factor [39, 40]; however, only a few studies have compared p53 alterations in ovarian cancer with clinical outcome. Some of these studies reported that p53 immunostaining in ovarian carcinomas was associated with significantly shorter overall survival [6, 41-43]. However, p53 immunoreactivity was not correlated with survival in various other studies [32, 44-46]. Marks *et al.* investigated p53 expression immunohistochemically in patients with epithelial ovarian cancer and found intense staining in 50% of the patients [15]. They compared p53-positive cases and p53-negative cases with respect to several clinicopathologic parameters. No statistically significant association with survival was found, although the median survival of advanced stage p53-positive cases was somewhat shorter than comparable patients with normal p53 expression. Our findings are in accordance with those reported by Marks *et al.*; we could not demonstrate any significant association between p53 expression and length of survival. Although p53 abnormalities, as detected immunohistochemically, occur commonly in patients with EOC, p53 expression did not yield independent prognostic information in our study. Thus, there is no well established concensus on the prognostic value of p53 immunoreactivity in the literature yet. Only prospective studies will permit a better definition of the prognostic role of p53 in ovarian carcinoma patients.

The prevalence of bcl-2 positivity in our study was found to be 48% which is consistent with the present data [6, 15]. Some investigators demonstrated a positive correlation between bcl-2 expression and lower degree of malignancy [17] and earlier tumor stages [16]. However we could not find a statistically significant association between bcl-2 expression and tumor stage, histologic subtypes and grade.

It is well known that bcl-2 is one of the regulators of apoptosis, and plays a role in tumor development and progress by prolonging the survival of malignant cells. However, most of the studies found a paradoxical correlation between bcl-2 expression and favorable predictive factors as well as prolonged survival in breast cancer, lung cancer and also in ovarian cancer [6, 16, 17, 47]. Although, there is no clear biologic explanation for this finding, a plausible explanation is that it is the overall balance between proapoptotic proteins such as Bax, Bak, and Bad and antiapoptotic proteins such as bcl-2, Bcl-X_L, and Mcl-1 that relates to cell survival after DNA damage and less likely the expression of a single one of these proteins [5, 48]. The determination of bcl-2 expression at only one point in time is likely to give limited

information, considering the highly dynamic nature of the regulation of the bcl-2 family of genes [49]. Furthermore, bcl-2 has been shown to delay entry of cells into the S phase, and the resulting lower tendency of proliferation may help to explain the favorable prognostic significance of this factor [50]. In our study, we also confirmed that bcl-2 expression was not associated with a poor prognosis although it did not reveal an independent prognostic factor in univariate analysis. However, more extended studies may provide clues for a better understanding of its role in tumor progression and effect on final outcome.

Cancer research has traditionally focused on cell proliferation. However, apoptosis has increasingly attracted the attention of oncologists researching carcinogenesis, cancer development, and cancer therapy [51]. Studies with various malignant tumors such non-Hodgkins lymphoma, breast cancer, bladder cancer and prostate cancer indicate that frequent apoptosis is a poor prognostic sign [52, 53]. Little is known about the significance of apoptosis in ovarian carcinoma. To the best of our knowledge, the present study is the second report considering the prognostic value of AI in epithelial ovarian carcinoma patients. In the first study, Yamasaki *et al.* reported that counting apoptosis could be useful for predicting patient survival in ovarian carcinoma, although AI was not found to be an independent prognostic factor [54]. However, in our study, AI had no impact on patient survival or prognosis. In addition, it was not revealed as an independent prognostic factor in univariate analysis.

Although in the study of Diebold *et al.*, apoptosis was particularly prominent in high grade tumors [17], in the preliminary study done by Yamasaki *et al.*, no significant correlation was found between AI and other clinicopathologic factors, such as age, clinical stage, lymph node metastasis, tumor size, and histology of the tumor [54]. Supporting the previous study, we could not show a significant correlation between AI and other clinicopathologic features including age, stage, grade, and tumor type. In addition, we could not find any correlation between expression of p53 and bcl-2 proteins and AI. Making the situation more complex, recent studies report different findings about the relationship between AI and bcl-2 and p53 protein expression. Baretton at al reported that bcl-2 expression correlated with a low AI in colorectal adenomas and carcinomas, while p53 did not [55]. Diebold *et al.* could not find any correlation between bcl-2 or p53, and apoptosis in ovarian carcinomas, a finding similar to ours [17]. Thus, our findings suggest that the regulation of apoptosis in neoplastic tissue appears to be a complex process.

Since apoptosis is a rapid phenomenon it remains visible histologically for only a few hours. In this study, the number of a apoptotic cells was small. Therefore, we believe that AI may not be the real indicator of an apoptotic process. Thus, although it is well known that apoptosis plays an important role in ovarian carcinomas, counting the apoptotic cells is not useful in predicting patient survival.

Conclusion

We found neither p53 nor bcl-2 immunoreactivity to be of prognostic significance in patients with EOC. In addition, AI was not found to be an independent prognostic factor. These results suggest that the regulation of apoptosis in neoplastic tissue is a highly complex process, and apparently can not simply be reduced to an assesment of the amount of bcl-2 and p53 proteins. Elucidation of molecular mechanisms underlying apoptosis may identify new targets for anticancer agents whose effects are not tightly linked to proliferative status. Since our limited sample size may limit the generalizability of these findings, further investigations will be required to elucidate the multiple genes involved in ovarian carcinogenesis and to understand their biological and clinical significance.

References

[1] Kristensen G., Trope C.: "Epithelial ovarian carcinoma". *Lancet*, 1997, *349*, 113.
[2] Stanley J. Korsmeyer.: "Bcl-2 initiates a new category of oncogenes: regulators of cell death". *Blood*, 1992, *80 (4)*, 879.
[3] Kerr J. F. R., Winterford C. M., Harmon B. V.: "Apoptosis: its significance in cancer and cancer therapy". *Cancer*, 1994, *73*, 2013.
[4] Bellamy C. O. C.: "P53 and apoptosis". *Brit. Med. Bull.*, 1996, *53 (3)*, 522.
[5] White E.: "Life, death, and pursuit of apoptosis". *Genes. Dev.*, 1996, *10*, 1.
[6] Herod J. J., Eliopoulos A. G., Warwick J., Niedobitek G., Young L. S., Kerr D. J.: "The prognostic significance of Bcl-2 and p53 expression in ovarian carcinoma". *Cancer Res.*, 1996, *56 (9)*, 2178.
[7] Livingstone L. R., White A., Sprouse J.: "Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53". *Cell*, 1992, *70*, 923.
[8] Hollstein M., Sidransky D., Vogelstein B., Harris C. C.: "p53 mutations in human cancers". *Science*, 1991, *253*, 49.
[9] Cleary M. L., Smith S. D., Sklar J.: "Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(l4; 18) translocation". *Cell*, 1986, *47*, 19.
[10] Vaux D. L., Cory 5, Adams J. M.: "Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells". *Nature*, 1988, *335*, 440.
[11] Pezzella F., Tse A. G. D.: "Expression of the bcl-2 oncogene protein is not specific for the 14;18 chromosomal translocation". *Am. J. Pathol.*, 1990, *137*, 225.
[12] Tsujimoto Y., Finger L. R., Yunis J.: "Cloning of the chromosome brake point of neoplastic B cells with the (14, 18) chromosome translocation". *Science*, 1989, *226*, 1097.
[13] Jiang M. C., Yang-Yen H. F., Lin J. K.: "Differential regulation of p53, c-myc, bcl-2 and bax protein expression during apoptosis induced by widly divergent stimuli in human hepatoblastoma cells". *Human Cell*, 1996, *9 (3)*, 223.
[14] Hockenbery D., Nunez G., Milliman C., Schreiber R. D., Korsmeyer S. J.: "Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death". *Nature*, 1990, *348*, 334.
[15] Marx D., Binder C., Meden H., Lenthe T., Ziemek T., Hiddemann T. *et al.*: "Differential expression of apoptosis associated genes bax and bcl-2 in ovarian cancer". *Anticancer Res.*, 1997, *17*, 2233.
[16] Henriksen R., Wilander E., Oberg K.: "Expression and prognostic significance of Bcl-2 in ovarian tumours". *Br. J. Cancer*, 1995, *72 (5)*, 1324.
[17] Diebold J., Baretton G., Felchner M., Meier W., Dopfer K., Schmidt M. *et al.*: "bcl-2 expression, p53 accumulation, and apoptosis in ovarian carcinomas". *Am. J. Clin. Pathol.*, 1996, *105 (3)*, 341.
[18] Cancer Committee of the International Federation of Gynecology and Obstetrics: Staging Announcement: FIGO Cancer Committee. *Gynecol. Oncol.*, 1986, *25*, 383.

[19] Serov S. F., Scully R. E., Sobin L. H.: International Histologic Classification and Staging of Tumors (vol 9) Histologic Typing of Ovarian Tumors. Geneva, Switzerland, World Health Organization, 1973.
[20] Henriksen R., Strang P., Wilander E., Backstrom T., Tribukait B., Oberg K.: "p53 expression in epithelial ovarian neoplasms: Relationship to clinical and pathological parameters, Ki-67 expression and flow cytometry". *Gynecol. Oncol.*, 1994, *53*, 301.
[21] Soini Y., Paakko P.: "Extent of apoptosis in relation to p53 and bcl-2 expression in germ cell tumors". *Hum. Pathol.*, 1996, *27*, 1221.
[22] Anttila M. A., Ji H., Juhola M. T., Saarikoski S. V., Syrjanen K. J.: "The prognostic significance of p53 expression quantitated by computerized image analysis in epithelial ovarian cancer". *Int. J. Gynecol. Pathol.*, 1999, *18 (1)*, 42.
[23] Wen W. H., Reles A., Runnebaum I. B., Sullivan-Halley J., Bernstein L., Jones L.A. *et al.*: "p53 mutations and expression in ovarian cancers: correlation with overall survival". *Int. J. Gynecol. Pathol.*, 1999, *18 (1)*, 29.
[24] Cummings M. C., Winterford C. M., Walker N. I.: "Apoptosis". *Am. J. Surgical Pathol.*, 1997, *21 (1)*, 88.
[25] Sato S., Kigawa J., Minagawa Y., Okada M., Shimada M., Takahashi M.: "Chemosensitivity and p53-dependent apoptosis in epithelial ovarian carcinoma". *Cancer*, 1999, *86*, 1307.
[26] Reed I. C.: "Bcl-2 and the regulation of programmed cell death". *J. Cell. Biol.*, 1994, *124*, 1.
[27] Stewart B. W.: "Mechanisms of apoptosis: integration of genetic, biochemical, and cellular indicators". *J. Natl. Cancer Inst.*, 1994, *86*, 1286.
[28] Vogl F. D., Stickeler E., Weyermann M., Kohler T., Grill H. J., Negri G. *et al.*: "p53 autoantibodies in patients with primary ovarian cancer are associated with higher age, advanced stage and a higher proportion of p53-positive tumor cells". *Oncology*, 1999, *57 (4)*, 324.
[29] Milner B. J., Allan L. A., Eccles D. M., Kitchener H. C., Leonard R. C., Kelly K. F. *et al.*: "p53 mutation is a common genetic event in ovarian carcinoma". *Cancer Res.*, 1993, *53 (9)*, 2128.
[30] Okamoto A., Sameshima Y., Yokoyama S.: "Frequent allelic losses and mutations of the p53 gene in human ovarian cancer". *Cancer, Res.*, 1991, *51*, 5171.
[31] Kihana T., Tsuda H., Teshima S., Okada S., Matsuura S., Hirohashi S.: "High incidence of p53 gene mutation in human ovarian cancer and its association with nuclear accumulation of p53 protein and tumor DNA aneuploidy". *Jpn. J. Cancer Res.*, 1992, *83 (9)*, 978.
[32] Marks J. R., Davidoff A. M., Kerns B. J., Humphrey P. A., Pence J. C., Dodge R. K. *et al.*: "Overexpression and mutation of p53 in epithelial ovarian cancer". *Cancer Res.*, 1991, *51 (11)*, 2979.
[33] Eccles D. M., Brett L., Lessells A., Gruber L., Lane D., Steel C. M. *et al.*: "Overexpression of the p53 protein and allele loss at 17p13 in ovarian carcinoma". *Br. J. Cancer*, 1992, *65 (1)*, 40.
[34] Kohler M. F., Kerns B. J., Humphrey P. A., Marks J. R., Bast R. C. Jr., Berchuck A.: "Mutation and overexpression of p53 in early-stage epithelial ovarian cancer". *Obstet. Gynecol.*, 1993, *81*, 643.
[35] Kupryjanczyk J., Bell D. A., Yandell D. W., Scully R. E., Thor A. D.: "p53 expression in ovarian borderline tumors and stage I carcinomas". *Am. J. Clin. Pathol.*, 1994, *102 (5)*, 671.
[36] Bosari S., Viale G., Radaelli U., Bossi P., Bonoldi E., Coggi G.: "p53 accumulation in ovarian carcinomas and its prognostic implications". *Hum. Pathol.*, 1993, *24 (11)*, 1175.
[37] Livingstone L. R., White A., Sprouse J.: "Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53". *Cell*, 1992, *70*, 923.
[38] Yamaguchi A., Kurosaka Y., Fushida S.: "Expression of p53 protein in colorectal cancer and its relationship to short-term prognosis". *Cancer*, 1992, *70*, 2778.
[39] Chang F., Syrjanen S., Syrjanen K.: "Implications of the p53 tumor suppressor gene in clinical oncology". *J. Clin. Oncol.*, 1995, *13*, 1009.
[40] Dowell S. P., Hall P. A.: "The clinical relevance of the p53 tumour suppressor gene". *Cytopathology*, 1994, *5 (3)*, 133.
[41] Hartmann L. C., Podratz K. C., Keeney G. L., Kamel N. A., Edmonson J. H., Grill J. P. *et al.*: "Prognostic significance of p53 immunostaining in epithelial ovarian cancer". *J. Clin. Oncol.*, 1994, *12 (1)*, 64.
[42] Levesque M. A., Katsaros D., Yu H., Zola P., Sismondi P., Giardina G. *et al.*: "Mutant p53 protein overexpression is associated with poor outcome in patients with well or moderately differentiated ovarian carcinoma". *Cancer*, 1995, *75 (6)*, 1327.
[43] Klemi P. J., Pylkkanen L., Kiilholma P., Kurvinen K., Joensuu H.: "p53 protein detected by immunohistochemistry as a prognostic factor in patients with epithelial ovarian carcinoma". *Cancer*, 1995, *76 (7)*, 1201.
[44] Niwa K., Itoh M., Murase T., Itoh N., Mori H., Tamaya T.: "Alteration of p53 gene in ovarian carcinoma: clinicopathological correlation and prognostic significance". *Br. J. Cancer*, 1994, *70*, 1191.
[45] Sheridan E., Silcocks P., Smith J., Hancock B. W., Goyns M. H.: "P53 mutation in a series of epithelial ovarian cancers from the U.K., and its prognostic significance". *Eur. J. Cancer*, 1994, *30 A (11)*, 1701.
[46] Reles A., Schmider A., Press M. F., Schonborn I., Friedmann W., Huber-Schumacher S. *et al.*: "Immunostaining of p53 protein in ovarian carcinoma: correlation with histopathological data and clinical outcome". *J. Cancer Res. Clin. Oncol.*, 1996, *122 (8)*, 489.
[47] Kiberu S. W., Pringle J. H., Murphy P., Lauder I.: "Correlation between apoptosis, proliferation and bcl-2 expression in malignant non-Hodgkin's lymphoma". *J. Clin. Pathol. Mol. Pathol.*, 1996, *49*, 268.
[48] Hickman J.: "Apoptosis and chemotherapy resistance". *Eur. J. Cancer*, 1996, *32*, 921.
[49] Reed J.: "Bcl-2 family proteins: Regulators of apoptosis and chemoresistance in hematologic malignancies". *Semin. Hematol.*, 1997, *34*, 9.
[50] Brady H. J., Gil-Gomez G., Kirberg J., Berns A. J.: "Bax alpha perturbs T cell development and affects cell cycle entry of T cells". *EMBO J.*, 1996, *15 (24)*, 6991.
[51] Thompson C. B.: "Apoptosis in the pathogenesis and treatment of disease". *Science*, 1995, *267*, 1456.
[52] Leoncini L., Del vecchio M. T., Kraft R., Cottier H.: "Correlations between apoptotic and proliferative indices in malignant non-Hodgkin's lymphomas". *Am. J. Pathol.*, 1993, *142*, 755.
[53] Chyle V., Pollack A., Terry N. H., Meyn R. E.: "Apoptosis and downstaging after preoperative radiotherapy for muscle-invasive bladder cancer". *Int. J. Radiat. Oncol. Biol. Phys.*, 1996, *35*, 281.
[54] Yamasaki F., Tokunaga O., Sugimori H.: "Apoptotic index in ovarian carcinoma: correlation with clinicopathologic factors and prognosis". *Gynecol. Oncol.*, 1997, *66 (3)*, 439.
[55] Baretton G. B., Diebold J., Christoforis G., Dopfer K.: "Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas". *Cancer*, 1996, *77*, 255.

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