bcl-2 Expression, p53 Accumulation, and Apoptosis in Ovarian Carcinomas

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Because little is known about the importance of apoptosis and its regulation in epithelial ovarian cancer, the authors looked for bcl-2 expression and p53 accumulation by immunohistochemistry in 148 ovarian carcinomas of different histologic types and stages. The number of apoptotic cells was assessed \textit{in situ} by enzymatic detection of DNA fragmentation.

Strong bcl-2 expression correlated with low histologic grade \((P = .004)\) and was most often seen in endometrioid carcinomas \((P = .001)\), whereas p53 accumulation was predominantly found in serous and undifferentiated carcinomas \((P < .001)\) and high grade tumors \((P < .001)\). p53 accumulation was associated with advanced tumor stage \((P < .001)\) and the presence of residual disease after surgery \((P < .001)\). Apoptosis increased with histologic grade \((P = .012)\); apoptotic cells were sparse or absent in tumors of low malignant potential and mucinous carcinomas, but found in all other carcinoma types \((P = .001)\). Apoptosis, bcl-2 expression, and p53 protein accumulation were not correlated with each other. The analysis of the postoperative course of 110 patients showed that survival depended on histologic tumor type \((P = .0037)\), histologic grade \((P = .0143)\), FIGO (International Federation of Gynecology and Obstetrics) stage \((P = .0001)\), and the absence or presence of postoperative residual tumor mass \((P = .0001)\). p53 accumulation was also associated with adverse prognosis \((P = .0001)\). However, bcl-2 immunohistochemistry identified a subgroup of patients with p53 and bcl-2 positive carcinomas who had a statistically significantly better outcome than patients with p53 positive and bcl-2 negative tumors \((P = .0443)\). Regarding FIGO stage and p53 alone in a Cox model, p53 proved to contribute additional prognostic information both in FIGO stages I/II as well as in FIGO stages III/IV.

Thus, our observations point to different molecular alterations possibly underlying phenotypic diversity of ovarian carcinomas and provide clues for a better understanding of tumor progression in these neoplasms. Apoptosis plays a role in ovarian carcinomas, but seemingly is regulated in a different way than in nonneoplastic tissues. (Key words: Ovarian carcinomas; bcl-2; p53; Apoptosis; Immunohistochemistry; DNA fragmentation; Prognosis) Am J Clin Pathol 1996; 105:341–349.

Some of the molecular mechanisms underlying the neoplastic growth of epithelial ovarian cancers have been elucidated during the last years. Amplification of the protooncogenes erb-B2 and MYC in ovarian carcinomas were among the first described in human neoplasia. Recently, a number of studies have pointed to the importance of mutations of the tumor suppressor gene TP53 in ovarian carcinomas leading to a loss of function. Apart from these rather frequent alterations, RAS and FMS gene changes can be found in only some ovarian carcinomas.

All these changes may confer a growth advantage to the tumor cells by stimulating cell proliferation. However, tissue growth (ie, the rate of cell accumulation) is not only depending on cell proliferation, but also on the rate of physiologically occurring cell death. Thus, it is conceivable that neoplastic growth also may be caused or promoted by factors inhibiting cell death. The prototype of proteins involved in such a regulatory pathway is the bcl-2 protein. The bcl-2 gene located on chromosome 18 was discovered in follicular non-Hodgkin's lymphomas, where the t(14;18) translocation juxtaposes the bcl-2 gene to the immunoglobulin heavy chain gene on chromosome 14. In hematologic cell lines, the product of the bcl-2 gene was characterized as a protein inhibiting "programmed cell death" (apoptosis). However, apoptosis like cellular change can be regarded as a complex process obviously regulated by numerous factors. Apart from bcl-2, the best characterized regulators are TP53 and MYC. After its discovery in hematologic malignancies, it soon became clear that bcl-2 also plays a role in non-hematologic cell systems. Some studies on solid human tumors have shown that bcl-2 expression in non-small cell lung cancer is associated with better postoperative outcome.

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plasms, bcl-2 protein can be found in differentiated tu-
mors, but not in anaplastic carcinomas.\textsuperscript{29,30}

So far it is not known whether bcl-2 and its role in the
regulation of apoptotic cell death are of importance in
ovarian carcinomas. Therefore, we studied bcl-2 expres-
sion in a series of epithelial ovarian cancers. In addition,
we looked for accumulation of p53 protein in these cases
and assessed the amount of apoptotic cell death in situ
by an enzymatic detection method for DNA fragmen-
tation.\textsuperscript{31,32} Our special interest was whether a relationship
between bcl-2, p53, and cell death that seems to exist in
physiologic situations\textsuperscript{33} can also be demonstrated in
these neoplasms. Furthermore, we analyzed the postop-
erative outcome of the patients to look for possible prog-
nostic significance of these parameters.

\textbf{MATERIALS AND METHODS}

\textbf{Specimens}

Archival material from 148 sequential cases of ovarian
carcinomas and ovarian tumors of low malignant poten-
tial was studied including 69 serous (mean age ± SD: 64
± 10 years), 18 mucinous (53 ± 16 years), 15 endometri-
oid (60 ± 12 years), 5 clear cell (52 ± 7 years) and 11
undifferentiated carcinomas (62 ± 9) as well as 23 serous
(52 ± 15 years) and 7 mucinous tumors of low malignant
potential (57 ± 18 years). All patients were treated with
operation and (depending on tumor stage) with chemo-
therapy between 1985 and 1991 at the same institution
(Department of Gynecology of the University of Mu-
nich, Gro\sshadern). Carcinomas were graded as well,
moderately, or poorly differentiated (grade 1 to 3). Tu-
mors of low malignant potential were assigned a grade 0.
All FIGO stages were represented\textsuperscript{34}: FIGO I, 50 cases (Ia
16 cases, Ib 5 cases, Ic 29 cases); FIGO II, 21 cases (IIa 2
cases, IIb 14 cases, IIc 5 cases); FIGO III, 65 cases (IIia 5
cases, IIib 35 cases, IIIC 25 cases); and FIGO IV, 10 cases.
Regarding the small numbers in some subgroups and the
uneven distribution of cases only the four major groups
FIGO I to IV were used for analysis. Survival data with
maximal follow-up of 10 years were available for 130 pa-
tients. In addition, a number of benign ovarian cysts
were studied for comparison.

\textbf{Immunohistochemistry}

The paraffin-embedded specimens were sectioned at 2–
3 μm and mounted on SuperFrost/Plus microscope slides
(Menzel, Germany). After deparaffinization and rehydra-
tion, the tissue sections were placed in a Coplin jar of mi-
crowave compatible plastic (TPX; Brand, Germany) that
was filled with 10mM citrate buffer (pH 6.0). The jar was
heated in a Panasonic microwave oven (NN-4241) at 750
W for 5 minutes. Afterward, the fluid level was checked
and evaporated buffer was supplemented. An additional
two heating cycles of 5 minutes each followed in the same
way. Subsequently the plastic jar was allowed to cool for
about 1 hour at room temperature.

The monoclonal bcl-2 antibody (clone 124) was pur-
chased from Dianova (Hamburg, Germany). The lyo-
philisate was dissolved in Tris-buffer (pH 7.5) and the
antibody was used at a dilution of 1:60. The tissue sec-
tions were incubated with the primary antibody over-
night at 4 °C. For p53 immunohistochemistry, slides
were incubated with monoclonal antibody DO-1 (Dia-
nova, Hamburg, Germany) at a dilution of 1:35 for 60
minutes at room temperature.

The binding of primary antibodies was detected by use
of the alkaline phosphatase-anti-alkaline phosphatase
(APAAP) method. The sections were incubated with sec-
dary rabbit anti-mouse antibody (Dakopatts, Glostrup,
Denmark) at a dilution of 1:50 for 30 minutes at room tem-
perature and subsequently with the APAAP complex (Da-
kopatts) at 1:50 dilution for 30 minutes at room tem-
perature. To enhance the intensity of final staining, these two
steps were repeated.\textsuperscript{35} New Fuchsin-Naphthol As-Bi phos-
phate in Tris-HCl buffer (pH 8.8, containing 0.40 mg/mL
levamisole) was used as the alkaline phosphatase substrate
for 20 minutes at room temperature. Finally, the sections
were counterstained with hematoxylin for 30 seconds and
mounted in Aquatex (Merek, Darmstadt, Germany). Con-
trols were performed by replacing the primary monoclonal
antibodies with RPMI.

The immunohistochemical results for bcl-2 were
scored semiquantitatively on a scale with four grades: 0
= no immunoreaction; 1 = faint or equivocal immuno-
reaction; 2 = unequivocal, strong immunoreaction in
less than 30% of tumor cells; 3 = unequivocal strong immu-
noreaction in more than 30% of tumor cells. The re-
results of the p53 immunoreactivity were recorded as pos-
itive if distinct nuclear staining was seen at least focally
in the tumors. Cases with single positive cells were re-
garded as negative.

\textbf{Detection of Apoptotic Cells in Paraffin Sections}

DNA fragments of apoptotic cells were visualized by
an enzymatic reaction using the modified protocols of
Gavrieli and colleagues\textsuperscript{31} and Ansari and colleagues.\textsuperscript{32}

Paraffin sections were cut at 2–3 μm, adhered to pre-
treated silane coated slides and dried overnight at 56 °C.
After deparaffinization in xylene and 100%, 70%, 50%
ethanol, the sections were washed in double distilled wa-

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ter (DDW) two times for 5 minutes. Subsequently, incubation with proteinase K solution, containing 400 µL proteinase K stock solution (25 µg proteinase K in 2.5 mL DDW) in 200 mL Tris-HCl buffer (pH 7.0) followed for 15 minutes at room temperature.

After four washes in DDW for 2 minutes, the sections were incubated with terminal transferase reaction mixture under a coverslip for 1 hour at 37 °C. The terminal transferase reaction mixture for 15 slides consisted of 15 µL digoxigenin-dUTP, 2.7 µL dATP, 90 µL terminal transferase reaction buffer containing cacodylate, 30 µL CoCl₂, 3 µL terminal transferase (all from Boehringer, Mannheim, Germany) and 306.3 µL DDW. Around 25 µL per slide were applied.

After three washes in TB-buffer (300 mM sodium chloride and 30 mM sodium citrate in 1,000 mL DDW, pH 7.2) for 5 minutes and one wash in buffer 1 (100 mM Tris-HCl and 150 mM NaCl in 2,000 mL DDW, pH 7.5) for 5 minutes, the sections were immersed in buffer 2 for 30 minutes. For preparation of this buffer 0.1 g of blocking reagent 0.5% (Boehringer) were mixed for 1 hour at 60 °C with 200 mL of buffer 1.

Subsequently two washes in buffer 1 for 5 minutes were followed by incubation with alkaline phosphatase conjugated Fab fragments of polyclonal sheep anti-digoxigenin antibody (Boehringer) at a dilution of 1:2,000.

Afterward, three washes in buffer 1 for 5 minutes and one 5-minute washing step in a buffer containing 100 mM Tris-HCl, 100 mM NaCl and 50 mM MgCl₂ in 1,000 mL DDW, pH 9.5 (buffer 3) were followed by incubation with NBT solution, first for 30 minutes at room temperature in darkness and then overnight at 4 °C in darkness. For preparation of NBT solution 22.5 µL NBT stock solution containing 1 g NBT (Boehringer) in 9.33 mL 70% dimethylformamide-solution (Sigma, St. Louis, MO) were mixed with 17.5 µL bromochloroindolylphosphate (Boehringer), 5 mM Levamisole (Sigma) and 5,000 µL buffer 3. On the next day, after a wash in running tap water for 10 minutes, the slides were counterstained with nuclear fast red for 10 minutes. After two additional washes in DDW, the sections were mounted with Aquatex (Merck, Darmstadt, Germany).

As a positive control, sections of normal colonic mucosa or lymph node were used. Because the enzymatic reaction labels both apoptotic cells and areas of necrosis, only those labeled cells were regarded as positive that additionally showed other characteristics of apoptosis (eg, isolated localization within an intact cell complex without inflammatory reaction). The results were expressed as positive cells per 1,000 tumor cells.

FIG. 1. Strong bcl-2 expression in an ovarian follicle cyst (A) and an endometrioid ovarian carcinoma (FIGO I) (B). Bcl-2 antibody clone 124, APAAP; magnification ×375.

Statistical Analysis

The various parameters were compared by chi-square tests. For survival analyses Kaplan-Meier curves were calculated and log-rank tests performed. To study the relationship between FIGO stage and p53 accumulation, a Cox model was applied. For all calculations, SAS/STAT statistical software (SAS Institute, Cary, NC) was used. P values <.05 were considered statistically significant.

RESULTS

bcl-2 Expression

Strong bcl-2 immunoreactivity was seen in follicular ovarian cysts and some serous cystadenomas (Fig. 1A). The reaction product was always located in the cytoplasm. The results regarding carcinomas and tumors of
low malignant potential are given in Table 1. Unequivocal bcl-2 expression (bcl-2 score 2 and 3) was most often seen in endometrioid carcinomas (Fig. 1B); it was relatively frequent in undifferentiated, serous and clear cell carcinomas as well as in serous tumors of low malignant potential, but infrequent or absent in mucinous carcinomas and mucinous tumors of low malignant potential (P = .001). Low grade tumors were more likely to demonstrate strong bcl-2 immunoreactivity than high grade tumors (P = .004). Although the staining intensity was focally pronounced, significant intratumoral heterogeneity was seen only in tumors with heterogenous histologic differentiation. In these neoplasms, bcl-2 staining was a marker for endometrioid or serous differentiation, and mucinous areas were negative. There was no correlation between bcl-2 expression and FIGO stage (P = .35) (Table 2).

p53 Accumulation

All benign lesions were p53 negative by immunohistochemistry. The distribution of p53 positive cases among the various histologic tumor types can be seen in Table 1. p53 protein was particularly often demonstrated in serous and undifferentiated carcinomas (P < .001) and in high grade tumors (P < .001). In addition, p53 accumulation was associated with advanced tumor stage (FIGO II to IV) (P < .001) (Table 2) and with the presence of residual disease after surgery (P < .001). p53 accumulation did not correlate with bcl-2 expression (P = .35).

Apoptosis

In general, the number of apoptotic cells detected by the enzymatic assay was low (0-50 cells per 1,000 tumor cells). However, even within the different groups of carcinomas, the values varied considerably. For further analysis, the cases were assigned to four groups with <0.5%, 0.5-0.99%, 1.0-1.49%, and >1.5% detected cells. Despite wide scatter of the apoptotic indices, some correlations were revealed. The majority of the cases with high apoptotic indices (>1.0%) belonged to the groups of serous, endometrioid, clear cell, and undifferentiated carcinomas, whereas tumors of low malignant potential and mucinous carcinomas mostly showed low apoptotic indices (P = .001) (Fig. 2B). In addition, high tumor grade was associated with a high apoptotic index (P = .012) (Figs. 2A and 2C). Apoptosis neither correlate with bcl-2 expression (P = .23) nor with p53 protein accumulation (P = .26). Furthermore, there was no correlation between the apoptotic index and FIGO stage (P = .85).

Clinicopathologic Data and Survival Analysis

Advanced tumor stage, high tumor grade, as well as the serous and undifferentiated histologic types were all associated with the presence of residual disease after surgery (P < .001 for all parameters). Serous and mucinous tumors of low malignant potential as well as mucinous and endometrioid carcinomas more often presented at tumor stage FIGO I, whereas serous and undifferentiated carcinomas were mostly diagnosed at advanced tumor stages (P = .002). Furthermore, higher histologic grade was associated with advanced tumor stage at diagnosis (P = .005).

After exclusion of all patients in FIGO stage IV and those with multiple carcinomas, 110 patients remained for survival analysis. Log-rank testing showed that survival depended on histologic tumor type (P = .0037), histologic grade (P = .0143), FIGO stage (P = .0001), and the absence or presence of a postoperative residual tumor mass (P = .0001). Patients with mucinous and en-

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**TABLE 1. Bcl-2 Expression and p53 Accumulation in Relation to Histologic Type**

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Bcl-2 Expression</th>
<th>p53 Accumulation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n/n Cases %</td>
<td>n/n Cases %</td>
</tr>
<tr>
<td>Serous LMP</td>
<td>10/23 43.5</td>
<td>0/23 0.0</td>
</tr>
<tr>
<td>Mucinous LMP</td>
<td>0/7 0.0</td>
<td>0/7 0.0</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>40/69 58.0</td>
<td>36/69 52.2</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>5/18 27.8</td>
<td>5/18 27.8</td>
</tr>
<tr>
<td>Endometrioid carcinoma</td>
<td>13/15 86.7</td>
<td>3/15 20.0</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>3/5 60.0</td>
<td>1/5 20.0</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>8/11 72.7</td>
<td>8/11 72.7</td>
</tr>
<tr>
<td>Total</td>
<td>79/148 53.4</td>
<td>53/148 35.8</td>
</tr>
</tbody>
</table>

LMP = tumor of low malignant potential.
* Cases with bcl-2 score = 2 or 3.
† X² tests for comparison of bcl-2 expression and histologic type and for comparison of p53 accumulation and histologic type reveal P values of .001 (t) and <.001 (t).

**TABLE 2. Bcl-2 Expression and p53 Accumulation in Relation to FIGO Stage**

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>Bcl-2 Expression</th>
<th>p53 Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/n Cases %</td>
<td>n/n Cases %</td>
</tr>
<tr>
<td>I</td>
<td>25/50 50.0</td>
<td>6/50 12.0</td>
</tr>
<tr>
<td>II</td>
<td>15/21 71.4</td>
<td>11/21 52.4</td>
</tr>
<tr>
<td>III</td>
<td>33/65 44.5</td>
<td>31/65 47.7</td>
</tr>
<tr>
<td>IV</td>
<td>6/10 60.0</td>
<td>5/10 50.0</td>
</tr>
<tr>
<td>Total</td>
<td>79/146 54.1</td>
<td>53/146 36.3</td>
</tr>
</tbody>
</table>

* Cases with bcl-2 score = 2 or 3.
† X² tests for comparison of bcl-2 expression and FIGO stage and for comparison of p53 accumulation and FIGO stage reveal P values of .35 (t) and <.001 (t).
Bcl-2 in Ovarian Carcinomas

**RESULTS**

Diametrioid carcinomas had a better outcome than patients with serous and undifferentiated carcinomas. As expected the best prognosis was found for cases with tumors of low malignant potential.

p53 accumulation was associated with adverse prognosis ($P = .0001$, Fig. 4A). Survival analysis of the apoptotic index ($P = .59$) and of bcl-2 expression ($P = .88$) failed to reveal an effect on prognosis in the total study population. However, regarding those cases with p53 accumulation, bcl-2 immunohistochemistry identified a subgroup of patients with p53 and bcl-2 positive carcinomas who had a statistically significantly better prognosis than patients with p53 positive and bcl-2 negative tumors ($P = .0443$, Figs. 3 and 4B). After exclusion of p53 positive tumors of low malignant potential this relationship remained as a trend, but it was not statistically significant ($P = .06$).

In a next step, the analysis was restricted to patients without residual tumor. Sixty-four cases remained for evaluation. In this considerably smaller group, only FIGO stage ($P = .0006$) and p53 immunoreactivity ($P = .0005$) were of prognostic significance. p53 positive cases with strong bcl-2 expression still had a better outcome than patients with p53 positive but bcl-2 negative carcinomas, but the difference was not statistically significant. After further exclusion of all tumors of low malignant potential, only FIGO stage ($P = .0015$) and the result of p53 immunohistochemistry ($P = .0144$) were prognostically relevant for the remaining 43 cases. Regarding FIGO stage I carcinomas alone, the p53 status provided no prognostic information ($P = .27$).

To further analyze whether p53 accumulation contributed additional prognostic information over FIGO stage, a Cox model was calculated (Table 3). Both in FIGO stages I/II as well as in FIGO stages III/IV, the relative risk of death of disease proved to be statistically significantly greater for p53 positive than for p53 negative cases.

**DISCUSSION**

The prognosis of epithelial ovarian cancer is still among the worst of all gynecologic malignancies. However, there are remarkable differences in the postoperative outcome depending on FIGO stage, amount of residual disease, and, to a lesser extent, histologic type and grade. The molecular mechanisms that underlie these differences are only partially understood.

Our data confirm the pivotal role of alterations in the tumor suppressor gene TP53 for the biologic behavior of ovarian carcinomas. Differing from other human neoplasms, ovarian carcinomas can be reliably investi-
gated for TP53 mutations by analyzing its product p53 protein by means of immunohistochemistry. According to Kyprjanczik and colleagues, around 80% of mutations found in ovarian cancers are associated with an altered p53 protein detectable by immunohistochemistry because of its prolonged half-life time. In an estimated 10% of immunohistochemically p53 positive cases, an underlying mutation cannot be revealed. Thus, we can be quite sure that our data reflect TP53 mutations in the vast majority of cases studied. Furthermore, the number of p53 positive cases in our study is in agreement with data by Bosari and colleagues, who demonstrated p53 protein in 50% of serous, 35% of mucinous, and 39% of endometrioid carcinomas. In keeping with a report by Berchuck and colleagues, our observations emphasize that p53 accumulation is not characteristic at “borderline” lesions or early tumor stage.

In our study, the detection of p53 accumulation had a prognostic impact comparable to the FIGO stage in single parameter analysis even after exclusion of cases with favorable histologic type or residual disease after surgery. Analysis in a Cox model showed that p53 immunohistochemistry contributed additional prognostic information both in FIGO stages I/II as well as in FIGO stages III/IV. prognostic significance of p53 immunostaining has also been noted by other groups. This supports the view that tumor progression in epithelial ovarian cancer is governed by loss of function of TP53. Because TP53 is believed to have an important role in the protection of genome integrity, the inactivation of this factor could easily explain the acquisition of multiple secondary genetic changes conferring a growth advantage to the tumor cells and leading to more aggressive biologic behavior. Aneuploidy is a frequent finding in epithelial ovarian cancer and can be observed in around 75% of advanced stage cases.

bcl-2 plays a role in numerous human malignancies. Regarding the studies based on bcl-2 immunohistochemistry, the following observations were reported. Increased bcl-2 expression is a regular finding in low grade non-Hodgkin’s lymphomas that can be lost during transformation into high grade lymphomas. Simultaneous deregulation of both p53 and bcl-2 in large cell lymphomas is associated with poorer prognosis than p53 expression alone. However, in thyroid cancer, bcl-2 protein can easily be demonstrated in almost all differentiated carcinomas, whereas anaplastic carcinomas are bcl-2 negative and frequently positive for p53 protein. An inverse correlation between bcl-2 and p53 was also found in colorectal carcinomas and breast cancer. Non-small cell lung cancer patients with tumors showing bcl-2 expression have a statistically significantly better postoperative outcome in some studies, compared with patients with bcl-2 negative carcinomas. Thus, most observations suggest that bcl-2 is associated with less aggressive malignant behavior in a number of cancers. This view is in accordance with our data on ovarian cancer. Although there was no correlation to the stage of the disease or to survival in the whole study population, and although bcl-2 positive cases could be seen in all types of carcinomas, bcl-2 expression was more characteristic of low grade tumors and remarkably closely linked to the endometrioid type of ovarian carcinoma that is characterized by a relatively less adverse prognosis compared to serous and undifferentiated carcinomas.

Furthermore, regarding the p53 positive cases that had a particularly poor outcome, bcl-2 immunohistochemistry was able to recognize a subgroup of patients with sta-
**Fig. 4. Survival analysis by Kaplan-Meier curves for p53 positive and p53 negative cases (A) and for cases with p53 accumulation which are either bcl-2 positive or bcl-2 negative (B). P values from log-rank tests.**

The importance of such mechanisms is also suggested by the example of human lymphoid tissues which show a dissociation of bcl-2 mRNA and bcl-2 protein expression—in contrast to their neoplastic counterparts.\(^{50}\)

Considering both bcl-2 and p53, our data suggest different patterns of alterations for the various tumor types: (1) strong bcl-2 expression is dominant finding in endometrioid carcinomas and in serous tumors of low malignant potential; (2) serous and undifferentiated carcinomas express both proteins with high frequency; and (3) mucinous tumors of low malignant potential and mucinous carcinomas are characterized by absence or near absence of both bcl-2 and p53. Thus, our data that likely reflect molecular alterations may provide partial understanding for the different phenotypes of ovarian carcinoma. (The small number of clear cell carcinomas does not allow definite conclusions concerning this group of neoplasms.)

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.\(^{15,17}\) Both bcl-2 and p53 have been implicated in the regulation of this critical process—largely on the basis of observations in cell culture studies (summarized by Stewart\(^{17}\)). However, these findings seem to be only of limited value for our understanding of the situation in neoplastic tissues. We could not find any correlation between bcl-2 and p53 and the apoptotic index that we measured by means of enzymatic detection of DNA fragmentation. This method provides data that are in accord with conventional estimates of apoptosis.\(^{31,32}\) In general, the number of detected cells was small. This agrees perfectly with our knowledge on the rapidity of this process.\(^{15}\) Although we saw considerable variability in the extent of apoptotic cell death, there was a correlation to certain histologic types. Interestingly, apoptosis was particularly prominent in high grade tumors. As it can be speculated that these neoplasms may also possess high proliferative activity, our data suggest a high cellular turnover in these tumors, obviously with proliferation:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>FIGO I/II, p53 negative</td>
<td>1.0 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGO I/II, p53 positive</td>
<td>3.65</td>
<td>1.48-9.04</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>FIGO III/IV, p53 negative</td>
<td>3.76</td>
<td>1.77-8.01</td>
<td>&lt;0.0006</td>
</tr>
<tr>
<td>FIGO III/IV, p53 positive</td>
<td>7.38</td>
<td>3.45-15.76</td>
<td>&lt;0.0001</td>
</tr>
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CI = confidence interval.
exceeding cell death. This situation would favor the acquisition of additional genetic changes conferring further growth advantage to the cancer cells. Obviously, the regulation of apoptosis in neoplastic tissue seems to be a complex process that is probably differently regulated depending on tumor type. As has been stated by Stewart, this process apparently cannot simply be reduced to an assessment of the amount of \textit{bcl-2} protein or to the detection of wild type versus mutant p53. Nevertheless, future research will have to show whether apoptosis can be used as a target for novel approaches of anti-cancer therapy.

In summary, our observations provide data for a better understanding of differentiation diversity in ovarian carcinomas and possible steps of tumor progression. Apoptosis plays a role in this process and seemingly is regulated in a different way than in nonneoplastic situations. Apart from these insights into the biology of ovarian carcinomas, our data underscore that molecular pathologic alterations may be reflected in histologic phenotypes. Therefore, it is worthwhile to classify and stage ovarian carcinomas by application of conventional methods, because it provides highly relevant clinical information.

REFERENCES

Bcl-2 in Ovarian Carcinomas


