Prognostic Value of Apoptosis-Regulating Protein Expression in Anal Squamous Cell Carcinoma

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ABSTRACT

Purpose: This study evaluated the prognostic value of pro and antiapoptotic protein expression, as well as that of spontaneous apoptosis, in anal carcinoma patients treated by radiotherapy (RT) with or without chemotherapy.

Environmental Design: Ninety-eight patients with available pretreatment biopsy specimens were studied. Patients had been treated with split-course RT: 30–40 Gy fractionated external beam, followed by a 20–22-Gy boost using interstitial or external RT. Fifty-one patients also received concomitant mitomycin-C and 5-fluorouracil. Median follow-up for surviving patients was 124 months. Tissue sections were examined immunohistochemically for expression of proapoptotic proteins (Bax, p53), antiapoptotic proteins (Bcl-2, Mcl-1), and spontaneous apoptosis (M30). Except for M30, staining of less than 5% of tumor cells was considered negative. Protein expression was correlated with local tumor control (LC) and disease-free survival (DFS) outcomes. The Kaplan-Meier method was used for the monovariate analysis and the Cox proportional hazard models for the multivariate analysis.

Results: For LC, beside advanced T- and N-categories and longer overall treatment time (OTT), lack of Bcl-2 expression was associated with poorer 5-year outcome (62% versus 84%, P = 0.009). For DFS, advanced T- and N-categories, longer OTT, and the lack of Bcl-2 expression correlated significantly with lower rates. In the multivariate analysis, N-category (P = 0.0026), OTT (P = 0.04), Bcl-2 (P = 0.0015), and M30 (P = 0.035) expressions were significant factors for LC. For DFS, age (P = 0.049) and N-category (P < 0.0001), as well as expression of Bcl-2 (P = 0.001), p53 (P = 0.003), and M30 (P = 0.03), were found to be independent significant variables. Patients with Bcl-2(-)/p53(-) tumors had a significantly higher 5-year LC compared with patients whose tumors were Bcl-2(−)/p53(+) (93% versus 53%, P = 0.004).

Conclusions: Bcl-2 and particularly the combination of p53 and Bcl-2 expression may prove to be useful predictors of tumor response to RT or radiochemotherapy in anal carcinomas. Patients having tumors that are Bcl-2(−) and p53(+) may require intensified radiochemotherapy or adoption of an alternative therapeutic approach.

INTRODUCTION

The treatment of anal carcinoma has changed radically during the past two decades, moving away from radical surgery toward sphincter-conserving treatment based on radiotherapy (RT) and chemotherapy. Whereas conservation approaches yield a high rate of control, local failure occurs in up to 30% of patients treated with curative intent (1–3). Locally recurrent anal cancer cannot only hamper patients’ survival but also seriously impair their quality of life, because many such patients will die with symptomatic local tumor progression (4–5). Identifying pretreatment factors that predict tumor response to radiochemotherapy would thus be of great interest. Patients whose outcomes are likely to be unfavorable with current approaches might be selected for alternative strategies, either by intensifying the radiochemotherapy schedules, by adding innovative biological agents, or by integrating surgery into the treatment program.

Apoptosis and apoptosis-related proteins are among the biological factors in epithelial cancers that have been most intensively studied, and data on their putative predictive and/or prognostic value are continuously emerging. Apoptotic cell death contributes to the cytotoxic effect of most chemotherapeutic drugs (6) and RT (7), although for the latter the extent of this contribution has been subject for debate (8). Apoptosis is defined by distinct morphological and biochemical changes mediated by a family of cysteine proteases or caspases that are activated by different apoptotic stimuli. Two different activation pathways have been defined (9), namely the extrinsic and the intrinsic pathways, with an established cross-talk between the two pathways. The extrinsic pathway is initiated by transmembrane receptors (CD95, TNF and TRAIL receptor) that activate the caspase cascade sequence (activator, then effector caspases) whereas the intrinsic pathway requires the release of mitochondrial proteins and cytochrome c after disruption of the mitochondrial membrane. The proapoptotic (e.g., Bax, Bcl-Xs, Bak, Bad) and antiapoptotic (e.g., Bcl-2, Bcl-Xl, Mcl-1) Bcl-2 family proteins are key elements in regulating the permeabilization of the mitochondrial membrane (10). After intrinsic stresses (e.g., DNA damage, hypoxia, oncogenes) the intrinsic apoptotic pathway can be initiated, and as a sensor of cellular stress, p53 plays a major role in initiating this pathway (6).

Because dysregulation of the intrinsic apoptotic pathway is common in cancer cells and anticancer drugs and ionizing radiation activate the intrinsic apoptotic pathway, there has been intense interest in defining the precise role of the different apoptosis-related proteins as well as in assessing the predictive/
prognostic value of their altered expression. Whereas studies have yielded conflicting results, the spontaneous pretreatment apoptotic index (AI) was proposed as a possible predictor of outcome in cervix and lung carcinomas (11–13). Furthermore, the expression of apoptosis-related proteins such as the Bcl-2 family and p53 has been correlated with patient outcome after RT or chemotherapy in different solid tumors (14–18). Whereas p53 overexpression was reported to be a poor prognostic indicator after chemoradiotherapy for anal carcinoma (19, 20), information regarding the prognostic significance of other apoptotic-related proteins and AI is lacking. The purpose of the present study was to assess the potential prognostic and/or predictive value of the expression of the proapoptotic proteins Bax and p53 and the antiapoptotic proteins Bcl-2 and Mcl-1, as well as the spontaneous apoptosis in a large series of anal carcinomas treated by RT with or without chemotherapy in one institution.

PATIENTS AND METHODS

Patients and Samples. From January 1976 to November 1998, 196 patients were treated by RT with or without chemotherapy with curative intent for anal carcinomas at the University Hospital of Geneva. The selection of patients for this study was based only on the availability of adequate pretreatment paraffin blocks. We were able to collect 100 adequate embedded biopsy specimens, whereas for different reasons (e.g., unavailability of specimens from outside pathology laboratories, insufficiency of the remaining material), we were unable to collect the remainder. After the exclusion of two patients treated with brachytherapy alone, the study group consisted of 98 patients. All of the pretreatment biopsies were reviewed by an experienced pathologist (M-A. B.). All of the tumors were classified according to the 1997 staging system of the Union Internationale Contre le Cancer (UICC). Pretreatment characteristics of the patients are shown in Table 1.

Treatment and Follow-Up. Treatment details have been published previously (2). For the present series, excepting one patient, RT was delivered in a split course, the first sequence consisting of wide-field external-beam RT and the second of a small-volume boost. The latter consisted either of interstitial low-dose-rate $^{192}$Ir brachythrapy (68 patients) or of external-beam RT (30 patients). The initial treatment was carried out using megavoltage photons ($^{60}$Co or 6–18 MV X-rays) to doses of 30 Gy in 10 fractions or 40 Gy in 20 fractions. The median boost doses were 22 Gy for brachytherapy and 20 Gy in 10 fractions for external-beam RT. The median overall treatment time (OTT) was 72 days (range, 40–261 days).

Concomitant chemotherapy was administered to 51 patients (52%). Combined treatment was reserved initially for patients with advanced stages and gradually extended to include almost all patients, except for selected patients with very favorable tumors or those in poor general condition. Generally chemotherapy started on day 1 and consisted of one cycle of mitomycin-C (10 mg/m^2 i.v. bolus) and a 5-day continuous infusion of 5-fluorouracil (600–800 mg/m^2/day). Eighteen patients received a second course of the same chemotherapy during the boost treatment.

Follow-up information was available for all patients except one, who was lost for follow-up at 30 months without evidence of disease. Information was collected from the medical records for recent patients, whereas for earlier patients, information was provided by their private physician or by contacting the patients themselves. Median follow-up for surviving patients was 124 months (range, 30–266 months).

Immunohistochemistry. Immunohistochemical studies were performed on pretreatment biopsies, after review of all of the slides to confirm the diagnosis of a squamous cell carcinoma (either of large cell/keratinizing, basaloid, or mixed type). Formalin-fixed and paraffin-embedded tissue blocks were cut at 4 μm and were mounted on silane-coated glass slides. Immunohistochemical stains were performed applying a standard ABC technique for Bcl-2 (clone 124; DAKO; 1:100), p53 (clone DO7; DAKO; 1:200), and M30 (clone M30; Boehringer Mannheim). Bax (polyclonal rabbit antihuman Bax; DAKO; 1:500), and Mcl-1 (polyclonal rabbit antihuman Mcl-1; DAKO; 1:500) were incubated overnight at 4°C and then were developed using a two-step amplifying detection system (EnVision; Dako). In all cases, heat-induced antigen retrieval was applied before incubation with primary antibodies.

Evaluation of immunohistochemical stains was done by one of the authors (M-A. B.), blinded to patient outcome. Results were assessed using a semiquantitative score based on the estimated percentage of positive cells: 0 = 0%; 1 = <5%; 2 = 5–50%; 3 = 50–90%; and 4 = >90%. Positive expression of p53, Bcl-2, Bax, and Mcl-1 proteins was considered if >5% of the malignant cells were stained (score, 2–4), whereas staining of <5% of the cells was considered as negative expression (score, 0–1). Spontaneous apoptosis was evaluated by the monoclonal antibody M30, which recognizes a neo-epitope liberated after cleavage of CK18 by caspase, thus signaling an early event in the apoptotic cascade (21). Any tumor containing M30-positive cells was considered as positive. Tumors were then stratified into two groups according to the absence or presence of expression of the protein considered.

Statistical Evaluation. The main end points for this study were local control (LC) and disease-free survival (DFS).
Tumor persistence or recurrence in the anorectal area or the perineal skin was considered as an event in determining the LC rate, whereas the DFS rate additionally took into account regional nodal recurrences as well as distant metastases. Actuarial LC, overall, and DFS rates were calculated by the product-limit method. The time interval for the above-mentioned end points was calculated from the first day of RT until the date of an event or of the last follow-up. The log-rank test was used to assess the correlation of these end points with the expression of the apoptosis-related proteins, the clinical factors [age, tumor (T) stage, and nodal (N) stage], and therapeutic variables (the addition of chemotherapy and the OTT). The multivariate analysis was carried out with the Cox proportional hazard models. Fisher’s exact test (two-tailed) was used to evaluate differences in proportions. A difference with a P of <0.05 was considered significant.

RESULTS

Overall Results. At last follow-up, 50 patients were alive, 47 had died, and 1 was lost to follow-up. Thirty-four patients presented with one or more events. Twenty-seven patients presented with persistent or recurrent local disease, 13 with regional disease (4 alone), and 7 with distant metastases (3 alone). At 5 years, actuarial LC was 71% (95% confidence interval, 62–80%), actuarial DFS was 64% (95% confidence interval, 54–74%), and overall survival was 64% (95% confidence interval, 54–73%).

Immunostaining and Clinicopathological Correlations.
The distribution of the percentage of cells stained for the different apoptotic-related proteins is given in Table 2. Apoptotic tumor cells, as assessed by the M30 staining, were identified in 12 specimens (12%). In nine cases, <5% of cells were stained, and in three cases, between 5 and 50% were stained. Positive expression was found in 57% of tumors for p53, in 71% for Bax, in 42% for Bcl-2, and in 64% for Mcl-1.

The proportion of tumor expressing Bcl-2 was significantly higher in nonkeratinizing small cell histological type, compared with large cell keratinizing type (51 versus 31%; P = 0.009). In females, tumors had a significantly higher proportion of Bcl-2 expression than in males (47 versus 29%; P = 0.05). No difference was noted in protein expressions according to the median age nor with tumor classification (T_{1-2} versus T_{3-4}). Although nonsignificant, tumors associated with lymph node involvement had a lower proportion of Bax expression (58 versus 76%; P = 0.12). According to outcome, the locally controlled group had a significantly higher proportion of Bcl-2(+) tumors compared with noncontrolled group (50 versus 22%; P = 0.02) and had a lower proportion of tumors with apoptotic cells (M30+; 6 (8%) of 71 versus 6 (22%) of 27; P = 0.08). Patients presenting with any event (local, regional, or distant failure) had a significantly higher proportion of tumors with p53+ (70 versus 50%; P = 0.056) as well as a lower proportion of tumors with Bcl-2(-) (30 versus 48%, P = 0.086). Tumors expressing p53 also had a tendency to express Bcl-2 (48 versus 33%; P = 0.15); otherwise, no significant correlation in the expression was observed among the other proteins.

Univariate Analysis. The results of the univariate analysis for LC and DFS using different clinical and therapeutic variables, as well as the expression of the different proteins studied, are given in Table 3. For LC, besides advanced T- and N-categories and longer OTT, the lack of Bcl-2 expression was correlated significantly with lower DFS rates. A tendency to a significant difference was noted in protein expressions according to the time interval for the above-mentioned end points (5-yr DFS 72% versus 64%; P = 0.08). Patients presenting with any event (local, regional, or distant failure) had a significantly higher proportion of tumors with p53+ (70 versus 50%; P = 0.056) as well as a lower proportion of tumors with Bcl-2(-) (30 versus 48%, P = 0.086). Tumors expressing p53 also had a tendency to express Bcl-2 (48 versus 33%; P = 0.15); otherwise, no significant correlation in the expression was observed among the other proteins.

Multivariate Analysis. Factors significantly influencing LC and/or DFS in univariate analysis were included in the Cox models as well as factors showing a tendency to a significant effect (P < 0.1). In this first model, N-category (P = 0.0026), OTT (P = 0.04), Bcl-2 (P = 0.0015), and M30 (P = 0.035) expressions retained their significance for LC. For DFS, age

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution percentage of cells stained for the apoptosis-related proteins</th>
</tr>
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<tbody>
<tr>
<td>Proteins</td>
<td>0%</td>
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<tr>
<td>M30</td>
<td>86</td>
</tr>
<tr>
<td>p53</td>
<td>1</td>
</tr>
<tr>
<td>Bax</td>
<td>5</td>
</tr>
<tr>
<td>BCL-2</td>
<td>40</td>
</tr>
<tr>
<td>MCL-1</td>
<td>11</td>
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<table>
<thead>
<tr>
<th>Table 3</th>
<th>Univariate analysis of clinical, biological, and therapeutic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>5-yr local control</td>
</tr>
<tr>
<td>Age, ≤68/≥68 yr</td>
<td>48/50</td>
</tr>
<tr>
<td>T category (UICC 1997), T_{1-2}/T_{3-4}</td>
<td>60/38</td>
</tr>
<tr>
<td>N category, N_0/N_1</td>
<td>74/24</td>
</tr>
<tr>
<td>Chemotherapy, No/yes</td>
<td>47/51</td>
</tr>
<tr>
<td>Overall treatment time, ≤72/&gt;72 days</td>
<td>50/48</td>
</tr>
</tbody>
</table>

Immunostaining

Bcl-2 absent/present | 57/41 | 62/84 | 0.009 | 56/75 | 0.04 |
Mcl-1 absent/present | 35/63 | 67/73 | 0.85 | 60/66 | 0.66 |
p53 absent/present | 42/56 | 78/66 | 0.22 | 75/55 | 0.067 |
Bax absent/present | 28/70 | 64/74 | 0.25 | 61/65 | 0.58 |
M30 absent/present | 86/12 | 75/46 | 0.057 | 67/37 | 0.07 |

* DFS, disease-free survival; UICC, International Union against Cancer; T, tumor; N, node.
(P = 0.049) and N-category (P < 0.0001), as well as expression of Bcl-2 (P = 0.001), p53 (P = 0.003), and M30 (P = 0.035) were found to be independent significant variables. The relative risks (RRs) associated with these factors are listed in Table 4.

Taking into account the possible linkage between the different apoptotic-related proteins and to assess the relevance of Bcl-2 expression when the other proteins are in the model, we constructed a second model in which each nonselected protein variable was additionally included. In this second model, N-category (P = 0.0005), OTT (P = 0.033), and Bcl-2 (P = 0.0003), as well as p53 (P = 0.03), were found to be significant variables for LC. For DFS, the second model showed no additional significant variables compared with the first model, and all of the significant variables of the first model retained their significance.

**Subgroup Analysis.** To better define subgroups with wide differences in outcome, we tried to group patients according to the most significant variables. The best discriminative combination was that of Bcl-2 and p53. Thus, patients with Bcl-2(+) and p53(−) tumors had a significantly higher 5-year LC (93%) compared with the group having Bcl-2(−) and p53+(53%), whereas the group with other combinations had intermediate rates (71–79%; P = 0.004). A similar result was observed when studying DFS, with the respective rates of 93% for Bcl-2(+)/p53−, 45% for Bcl-2(−)/p53+; and 66–67% for the intermediate groups (P = 0.01; Fig. 2). In the group of patients with Bcl-2(+) and Bax+ tumors, the 5-year LC was 86%, compared with 58% in patients with Bcl-2-/Bax− tumors (P = 0.01), whereas for DFS, the difference was less significant (P = 0.046). In addition, patients with tumors expressing both Bcl-2 and Mcl-1 had a higher LC rate compared with those with tumors lacking both proteins (87 versus 59%; P = 0.009), whereas intermediate groups had a 64–79% LC rate. For DFS, the rates were 83% for patients with tumors expressing both Bcl-2 and Mcl-1 and were 55–62% for the other groups (tumors lacking one or both proteins; P = 0.039). No clear correlation was observed when assessing the other combinations.

**DISCUSSION**

There is an increasing body of evidence demonstrating the implication of apoptosis and/or its regulating proteins in the

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**Table 4** Cox proportional hazards model (first model) for local control (LC) and Disease-Free Survival (DFS)

<table>
<thead>
<tr>
<th>Variables</th>
<th>LC</th>
<th>DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRa</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, ≤68/&gt;68 yr</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>T-category, T1,T3–4</td>
<td>0.5</td>
<td>0.22–1.13</td>
</tr>
<tr>
<td>N-category, N0,N1–3</td>
<td>0.22</td>
<td>0.11–0.63</td>
</tr>
<tr>
<td>OTT, ≤72/&gt;72 days</td>
<td>0.43</td>
<td>0.18–1.04</td>
</tr>
<tr>
<td>Bcl-2, absent/present</td>
<td>4.66</td>
<td>1.8–12.0</td>
</tr>
<tr>
<td>p53, absent/present</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>M30, absent/present</td>
<td>0.36</td>
<td>0.15–0.93</td>
</tr>
</tbody>
</table>

*a* RR, relative risk; CI, confidence interval; NA, nonapplicable; OTT, overall treatment time; T, tumor; N, node.
outcome of tumors treated by RT and chemotherapy (7, 21–25).
In the present study, we investigated the value of the expression of the proapoptotic proteins Bax and p53 and of the antiapoptotic proteins Bcl-2 and Mcl-1, as well as the value of spontaneous apoptosis regarding the outcome in patients who have had nonsurgical treatment for anal cancer. With the exception of p53, to our knowledge, this is the first study that addresses the question of prognostic significance of these proteins in this disease. Although rare, anal carcinoma is one of the few cancers treated primarily by RT and chemotherapy. Identification of factors that can reliably predict a favorable treatment outcome would, thus, have paramount clinical importance in this disease. Moreover, biological predictive factors shown to be reliable in anal carcinoma might prove to be of value in other cancers that are treated primarily with radiochemotherapy.

The possible relationship between apoptosis and proliferation in human tumors (26), evidence for a relationship between apoptosis with hypoxia (27), and differences in the extent of apoptosis from tumor to tumor, all suggest that spontaneous apoptosis may reflect inherent biological characteristics that may determine individual tumor aggressiveness. In the present series, spontaneous apoptosis was detected in 12% of the tumors, in most cases with less than 5% of cells staining for M30 monoclonal antibody. No significant association was found between positivity for M30 and T-N-categories, histological type, or the apoptosis-regulating proteins studied. Patients having tumors with spontaneous apoptosis had a lower LC and DFS, and in the multivariate analysis, this parameter was found to be an independent adverse factor. However, the subgroup of patients having M30+ tumors was rather small, which may limit the interpretation of the result obtained. A number of investigators have tried to correlate the spontaneous AI to the outcome after RT, but findings have been rather conflicting. In cervix cancers, high AI was associated with good outcome in adenocarcinomas (28), whereas in squamous cell carcinomas, the converse was found (11, 12). In non-small cell lung cancer, high AI was associated with favorable outcome for squamous cell carcinomas but with unfavorable outcome in the other histological types (13). The same discrepancy was found when studying radiation-induced apoptosis, for which conflicting results were reported from one tumor to another. These findings suggest that AI may reflect different biological parameters that can have different significance from one histological tumor type to another (28).

We found that anal carcinomas express p53 in 57% of cases, which is consistent with rates of 48–55% found in other studies using the same definition (>5% staining) of expression [Bonin et al. (19) and Wong et al. (20)]. This provides further evidence for the possible implication of altered p53 function in the carcinogenesis of these cancers. Mutation in the p53 gene is a common genetic alteration in human cancers (29) and is believed to promote tumor cell growth. In anal carcinomas, mutant p53 protein has been detected in up to 58% of specimens (30). Because mutant p53 has been demonstrated to have a longer half-life than does the wild-type p53 and, consequently, is more readily detected (31), overexpression of p53 is often used as a surrogate indicator of alteration of its function. Indeed, in a series of 78 head and neck carcinomas, a significant correlation between p53 mutation and protein overexpression was reported ($P < 0.0001$; Ref. 32). In the present series, patients having p53+ tumors had a lower LC ($P = 0.22$) and DFS rate ($P = 0.067$). In the multivariate analysis, p53+ was found to be an adverse independent factor for LC (second model: RR, 0.38; $P = 0.03$) and particularly for DFS (RR, 0.29; $P = 0.003$). Similar findings were reported recently by Wong et al. (20) in a series of 58 patients in whom p53 expression was a significant adverse factor for DFS ($P = 0.01$). In another series of 64 patients, a trend in lower rate of locoregional control was also reported in patients with tumors overexpressing p53 (52 versus 72%; $P = 0.13$; Ref. 19).

Fig. 2 Actuarial DFS according to the combined expression of Bcl-2 and p53.
Apart from their ability to bind and inhibit the antiapoptotic action of Bcl-2 or Bcl-X\textsubscript{L}, proapoptotic proteins such as Bax can also mediate cell death via a direct effect on mitochondrial function (33). In the present series, 71% of tumors expressed Bax protein. This high rate is similar to the rate of 83% (>10% of cell staining) reported in cervical carcinomas (14). Besides a trend to lower expression in tumors with positive lymph nodes, no significant association was found with the other clinical, pathological, or biological factors. Although favorable outcomes have been reported in some human cancers associated with positive Bax expression (34, 35), the present study found no correlation between Bax expression and outcome as assessed by LC and DFS. This finding is in line with the results obtained in cervical carcinomas (14, 36). It is possible that the level of Bax expression induced during RT might be of importance, as reported by Harima et al. (37) in a series of cervical carcinomas, in which higher levels of induced expression were associated with better ultimate LC.

The main findings in the present study were that 42% of anal tumors expressed Bcl-2, and this expression correlated significantly with better LC and DFS. Indeed, in the multivariate analysis, the lack of expression was found to be an independent negative factor for both LC (RR, 4.66; \(P = 0.0015\)) and DFS (RR, 4.11; \(P = 0.001\)). Several studies have advanced the hypothesis that Bcl-2 protein blocks radiation- and chemotherapy-induced apoptosis (25, 38), thus conferring treatment resistance to cells overexpressing Bcl-2. Although some clinical studies found a negative impact of Bcl-2 overexpression, particularly in hematological malignancies (17, 39, 40), others found overexpression to correlate with strongly favorable clinical outcome (15, 41–43), particularly in a solid tumor that shares common pathogenesis with anal carcinomas, namely cervix carcinomas (14, 36). This counterintuitive result may be due to several reasons. First, the possible molecular alterations leading to Bcl-2 overexpression are poorly characterized in solid tumors, compared with hematological malignancies, for which the causes have been intensively explored (40, 43). Furthermore, the molecular alteration leading to the aberrant protein level may have different significance in different tumor systems and/or circumstances. Supporting this idea is the observation that overexpression is generally an adverse prognostic factor in hematological malignancies, whereas for solid tumors the converse seems to occur. Moreover, it has been recently shown that the antiapoptotic function of Bcl-2 was level-dependent, because at low expression, it inhibits the proapoptotic function of Fas, whereas overexpression quickly and drastically induced apoptosis in U251 glioblastoma cells (44). Also, overexpression of the Bcl-2 protein was reported to increase the half-life of the proapoptotic protein Bax (45). These observations, along with the finding that Bcl-2 may have a role as an antiangiogenesis factor in non-small cell lung cancer (46), may explain in part the biological effect behind our finding.

The Mcl-1 has a structural and functional (interaction with Bax) homology with Bcl-2, but its expression and regulation are reported to be different (47). A high level of Mcl-1 was shown to confer resistance in hematological malignancies (48). In the present series, 64% of tumors expressed Mcl-1. Except for a trend to higher expression in anal margin carcinomas (\(P = 0.16\)), there was no significant correlation of the expression of Mcl-1 and the clinical, pathological, or biological factors studied. Also we found no significant correlation between Mcl-1 expression and patient outcome (LC and DFS). This finding is in agreement with the lack of prognostic significance of Mcl-1 in cervical carcinoma (36) and in ovarian carcinoma when using a multivariate analysis (16).

Because of the importance of dynamic interplay of the different apoptosis-related proteins in determining the apoptotic threshold in individual cells, tumor response to a given therapy is unlikely to depend on the expression of one apoptosis-related protein. Thus, we combined expression of the different proteins to see whether any pattern might be found to be an important indicator of outcome. We found that the best discriminative combination was that of Bcl-2 and p53 expressions. Indeed, patients with Bcl-2(+) and p53− tumors had a significantly higher 5-year LC, compared with the group having Bcl-2(−) and p53+ (\textit{93 versus 53%; \(P = 0.004\)}). A similar result was observed when studying DFS, with the respective rates of 93% (Bcl-2+/p53−) and 45% (Bcl-2−/p53+; \(P = 0.01\)). The prognostic importance of this particular pattern was reported in rectal carcinoma after surgery in 160 patients, in whom the poorest prognosis was observed in the p53+/Bcl-2− subgroup (15). The same results were also reported in ovarian carcinomas after surgery and chemotherapy (49) and in bladder carcinomas (50).

In conclusion, pretreatment expression of apoptosis-related proteins seem to correlate with patient outcome. Although Bcl-2 protein is purported to block apoptosis and, consequently, to decrease radiochemosensitivity, in the present series, Bcl-2 expression was associated with better LC and DFS rate. This finding is in line with the results observed in other solid tumors, indicating the complex role that this protein can play \textit{in vivo}. The best prognostic indication was obtained when combining expression data from Bcl-2 and p53, p53+ tumors without Bcl-2 expression having a poor prognosis. Thus, this subgroup of patients might be candidates for innovative treatment approaches.

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