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bcl-2 Protein Expression Correlates with Recurrence and Survival in Early Stage Head and Neck Cancer Treated by Radiotherapy

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Abstract

Inherent cellular radioresistance plays a critical role in the failure of radiation therapy (RT). The proto-oncogene *bcl-2* encodes a protein that inhibits apoptosis, a common mechanism of cell death induced by several genotoxic agents, including γ-radiation. Thus, it is likely that *bcl-2* gene expression could be involved in the complex mechanisms of radioresistance in human tumors with some prognostic implications. In this study, we analyzed the predictive relevance of *bcl-2* expression on 5-year disease-free and overall survival in patients with early stage squamous cell carcinoma of the head and neck (SCCHN) primary treated with RT. The expression of *bcl-2* was analyzed by immunohistochemistry on paraffin-embedded sections from 71 consecutive stage I–II SCCHN patients treated with curative RT. We detected bcl-2 protein in 21% of SCCHN studied. A suggestive association was observed between tobacco exposure and bcl-2 protein expression ($P < 0.1$); this association was stronger in those patients who failed primary RT ($P = 0.03$). Moreover, we documented a higher rate of *bcl-2* immunoreactive tumors in postirradiated biopsies from relapsed patients than in preirradiated ones ($P = 0.03$). In both univariate and multivariate analyses, *bcl-2* expression was the most important indicator for disease-free survival ($P = 0.08$ and $P = 0.01$, respectively) and overall survival ($P = 0.004$ and $P = 0.05$) within 5 years of RT. The present study indicates that the proto-oncogene *bcl-2* is abnormally expressed in some SCCHN, and its expression may prove to be a useful tool in selecting patients for conventional RT with clear prognostic implications.

Introduction

Carcinoma of the head and neck is one of the most common cancers, with global incidence of 500,000 cases per year (1). The majority of these tumors are squamous cell carcinomas associated with tobacco and alcohol use. Surgery and radiation, singly or in combination, are the cornerstones of curative treatment in head and neck cancer, but despite new therapeutic approaches and strategies, survival rates for these tumors have not changed significantly in the last 30 years (2).

Early stage (stage I–II) cancers of the head and neck can usually be treated effectively with both RT[2] and conservation surgery. RT is commonly used because of its minimal cosmetic effect and for the possibility of preservation of near-normal voice in laryngeal cancer. However, even those patients with persistent or recurrent disease after RT can often be successfully treated with surgery, nevertheless radiation failures frequently require extended salvage surgery with an increased rate of complications (3, 4) and are associated with a higher risk of distant metastases (5).

A major impediment to successful RT is the failure of some tumor types to respond to either form of treatment and the appearance of resistant cell populations upon relapse of an originally responsive malignancy. Consequently, understanding the molecular basis of cellular radioresistance with identification of markers predictive of responsiveness to irradiation would be helpful in selecting the proper treatment for head and neck cancer patients. Those likely to be resistant to RT may be candidates for conservation surgery, thus, optimizing treatments and improving survival.

In the search for novel, potentially useful prognostic or predictive markers, the expression of bcl-2 protein is of particular interest. *bcl-2* is a recently recognized mitochondrial oncogene which regulates cell death (6, 7). This proto-oncogene was discovered as a result of its involvement in the 14;18 translocation common in human follicular lymphomas (8). Unlike most other oncogenes, *bcl-2* contributes to neoplastic cell growth not by accelerating rates of cellular proliferation but rather by prolonging cell survival through inhibition of cell death. After the initial extensive studies in hematopoietic tumors, alterations of bcl-2 protein expression have been reported more recently in several solid tumors, including adenocarcinoma of the prostate (9) and breast (10), squamous cell carcinoma of the lung (11, 12), neuroblastomas (13), melanomas (14), and colon cancer (15).

Although the tumor-specific action of most anticancer agents has been attributed to their debilitating effects on actively proliferating cells, an increasing body of evidence suggests that anticancer agents, including radiation, induce apoptosis (16). More recently, gene transfer experiments have demonstrated that *bcl-2* can provide protection against apoptosis induced by numerous genotoxic insults, including γ-radiation (17, 18), in lymphoid (19) and nonlymphoid tumor cells (20) *in vitro*. Thus, it is likely that *bcl-2* gene expression, rendering tumor cells resistant to apoptosis induced by DNA-damaging agents, could

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[2] The abbreviations used are: RT, radiation therapy; SCCHN, squamous cell carcinoma of the head and neck.

Table 1 Clinical characteristics of 71 SCCHN patients primary treated with RT

No. of patients	71
Sex (M[a]/F)	67/4
Age at RT (yr)	
Range	42–82
Mean	63.5
Median	62
Site	
Larynx	50 (70.4%)
Oral cavity	12 (16.9%)
Pharynx	9 (12.7%)
T stage	
T_1	56 (78.9%)
T_2	15 (21.1%)
Tumor differentiation	
Well	29 (40.8%)
Moderate	28 (39.4%)
Poor	14 (19.8%)
Alcohol consumption[b]	
No	16 (26.2%)
Moderate	38 (62.3%)
Heavy	7 (11.5%)
Smoking history[c]	
No	6 (9.4%)
Moderate (1–40 pack-yr)	25 (39.1%)
Heavy (>40 pack-yr)	33 (51.5%)
RT dosage (Gy)	
Range	52–70
Mean	63.8
Median	64

[a] M, male; F, female.
[b] Ten missing values.
[c] Seven missing values.

Table 2 Clinical characteristics of 71 SCCHN patients treated with RT according to bcl-2 protein expression[a]

	bcl-2 positive	bcl-2 negative	*P*
No. of patients	15 (21.1%)	56 (78.9%)	
Sex (M[b]/F)	15/0	52/4	0.572
Age at RT (yr)			
Range	44–79	42–82	
Mean	61.7	64.0	
Median	60	63	0.383
Site			
Larynx	10 (20.0%)	40 (80.0%)	0.484
Oral cavity	4 (33.3%)	8 (66.7%)	
Pharynx	1 (11.1%)	8 (88.9%)	
T stage			
T_1	11 (19.6%)	45 (80.4%)	0.721
T_2	4 (26.7%)	11 (73.3%)	
Distant metastases			
Yes	2 (33.3%)	4 (66.7%)	0.600
No	13 (20.0%)	52 (80.0%)	
Tumor differentiation			
Well	6 (20.7%)	23 (79.3%)	1.000
Moderate	6 (21.4%)	22 (78.6%)	
Poor	3 (21.4%)	11 (78.6%)	
Alcohol consumption[c]			
No	3 (18.8%)	13 (81.2%)	0.826
Moderate	10 (26.3%)	28 (73.7%)	
Heavy	2 (28.6%)	5 (71.4%)	
Smoking history (pack-yr)[d]			
Range	18.75–135	0–160	
Mean	53.4	36.8	
Median	47.5	35.5	0.098
RT dosage (Gy)			
Range	60–70	52–70	
Mean	64.5	63.6	
Median	64	64	0.541

[a] Statistical analyses were performed using Fisher's exact test and the rank sum test as appropriate.
[b] M, male; F, female.
[c] Ten missing values.
[d] Seven missing values.

be involved in the complex mechanisms of *in vivo* resistance to several anticancer therapies in human tumors.

To test this hypothesis and to evaluate the possible involvement of the *bcl-2* proto-oncogene as a prognostic factor in head and neck cancer, we evaluated bcl-2 protein expression in a series of 71 consecutive patients with SCCHN, who received primary RT with curative intent and for whom detailed information on long-term follow-up was available.

Materials and Methods

Patients. Seventy-one consecutive, unselected patients with head and neck squamous cell carcinoma diagnosed at the Institute of Otolaryngology Head and Neck Surgery of Florence University and who had undergone primary RT from January 1986 to December 1989 were studied. Archival materials from original untreated tumors and, for those patients who experienced radiation failures, from relapsed malignancies were identified retrospectively through a systematic search in the files of the Surgical Pathology Division of the Institute of Anatomic Pathology of the University of Florence. All histological specimens stained with hematoxylin and eosin were reviewed by one pathologist (S. B.) to confirm original diagnosis and to classify tumors as well-, moderately, or poorly differentiated squamous cell carcinomas.

This study included 71 patients (67 males and 4 females) with stage I–II head and neck cancers. Clinical characteristics of these patients are summarized in Table 1. Categorized by tumor site, these included 50 (70%) laryngeal cancers, 12 (17%) oral cavity cancers, and 9 (13%) pharyngeal cancers.

Criteria for selecting treatment of early stage head and neck cancer with RT over to conservation surgery were mostly subjective. Generally, patients with stage I or II disease undergo either surgery or RT with curative intent. With regard to laryngeal cancers, our institution has in the past preferred treatment of T_{1a} glottic carcinomas with conservation surgery, whereas for patients with glottic cancer involving the anterior commissure or both vocal cords (T_{1b}) RT has been favored. Moreover, because of the high rate of occult neck disease in oral cavity and pharyngeal cancers, *en block* surgery of both the primary and neck was the treatment choice for stage I–II patients. This is reflected in the composition of the present group which shows an overrepresentation of T_{1b} glottic carcinomas (38 patients) compared to a few cases of T_{1a} glottic cancer (10 patients), as well as of overall laryngeal cancers (70%) compared to oral cavity and pharyngeal cancers (30%).

All patients were irradiated with ^{60}Co or a 4/5 MeV linear accelerator. The daily dose ranged from 1.8 to 2.0 Gy. Patients received doses in the range of >52–70 Gy (mean, 63.8 Gy) to

Table 3 Univariate Cox proportional hazards analysis of 5-year disease-free and overall survival in 71 SCCHN patients primary treated with RT

		5-year disease-free survival			5-year overall survival		
	Score	HR[a] (95% Cl)	χ^{2b}	*P*	HR[a] (95% Cl)	χ^{2b}	*P*
bcl-2 Expression							
Negative[c]	0						
Positive	1	2.01 (0.92–4.41)	3.07	0.080	4.56 (1.64–12.67)	8.49	0.004
Sex							
Male[c]	0						
Female	1	1.03 (0.25–4.34)	0.002	0.965	1.23 (0.16–9.49)	0.04	0.840
Age (yr)							
<60[c]	0						
≥60	1	1.01 (0.49–2.09)	0.002	0.965	0.80 (0.29–2.23)	0.17	0.681
T stage							
T_1[c]	0						
T_2	1	0.80 (0.47–1.39)	0.59	0.443	0.61 (0.28–1.31)	1.57	0.210
Site							
Larynx[c]	0						
Oral cavity	1	1.49 (0.60–3.70)	0.74	0.388	3.23 (1.07–9.71)	4.34	0.037
Pharynx	2	0.73 (0.22–2.44)	0.26	0.607	0.79 (0.10–6.31)	0.05	0.826
Tumor differentiation							
Well[c]	0						
Moderate	1	0.78 (0.37–1.67)	0.39	0.530	2.05 (0.65–6.43)	1.52	0.218
Poor	2	0.36 (0.10–1.23)	2.67	0.102	0.90 (0.17–4.65)	0.02	0.902
RT dosage (Gy)							
<64[c]	0						
≥64	1	1.24 (0.53–2.89)	0.25	0.617	0.97 (0.30–3.05)	0.002	0.963

[a] Unadjusted HR of relapsing or dying.
[b] Computed using Wald's statistic.
[c] Reference category.

the primary and 50–58 (mean, 54.2) to the neck. In all patients, treatment was initiated 3–6 weeks after the initial diagnosis and completed in 4–6 weeks, with breaks not exceeding 1 week.

During follow-up after primary RT, 30 patients (42%) examined in the study relapsed in varying degrees: persistent (6 cases), local (16 cases), regional (1 case), locoregional (3 cases), or distant (4 cases; disease-free interval: mean, 14.2 months; median, 13.5 months; range, 0–37). Surgical salvage was attempted in all but one patient with local or locoregional radiation failure.

The criteria for distinguishing recurrence from second primary cancer was: (*a*) site of recurrence rigorously the same as that of the original malignancy, and (*b*) time of relapse not exceeding 3 years from initial RT.

For all patients who experienced local radiation failures or persistence (overall 25 cases), a biopsy of the relapsed malignancy was available for histopatological and immunohistochemical analysis.

Tobacco exposure and alcohol consumption was documented retrospectively from clinical records. For those who smoked cigarettes, pack-year history was calculated by multiplying the number of packs consumed per day by the numbers of years exposed. The smoking pattern was also classified as nonsmoker, moderate smoker (under 40 pack-years), or heavy smoker (over 40 pack-years); patients who had stopped smoking for more than 5 years previously were excluded from the smoking analysis.

Alcohol consumption was coded initially in three categories: (*a*) rare to no alcohol consumption; (*b*) consumption of up to 1 liter/day wine; and (*c*) consumption of more than 1 liter/day wine plus superalcoholic beverage.

All information was obtained from the clinical records of the Institute of Otolaryngology and from the Radiotherapic Unit of the Institute of Radiology of Florence University where the patients were referred for initial treatment. All patients after initial diagnosis and treatment were followed up regularly by radiotherapists and otolaryngologysts at 1–3-month intervals. Radiographical studies including chest roentgenogram and computer tomography of the head and neck were carried out every 6 months, or earlier whenever clinically indicated.

Immunohistochemical Analysis. *bcl-2* immunoreactivity was assessed on paraffin-embedded sections using monoclonal antibody 124 (DAKO, Glostrup, Denmark) as described by others (10). Slides were incubated with the antibody at a 1:200 dilution for 1 h at room temperature and processed using the highly sensitive streptavidin-biotin immunohistochemical method. Negative controls were obtained by omitting the primary antibody.

bcl-2 expression was categorized as follows: negative (−), if no staining was seen in tumor cells or if only a weak positive and heterogeneous staining was observed in <30% of the tumor cells, or positive (+), if staining was observed in >30% of the cells according to others (10).

Statistical Analysis. Statistical tests were performed using Egret (Statistics and Epidemiology Research Corporation, Seattle, WA) and StatXact (Cytel Software Corporation, Cambridge, MA). The pattern of associations between bcl-2 protein expression and clinical parameters was determined using the Fisher's exact test, and differences between medians were assayed using the rank sum test. The role of each possible prognostic factor (univariate analysis) and their joint effect (multivariate analysis) was explored using the Cox proportional

hazards survival analysis. The final results of these analyses are the HRs and their CIs (95%); the Wald statistic was used to test the hypothesis: HR = 1.0.

All patients had a 5-year minimum follow-up. The date of initial RT was considered the starting day of observation; patients who died of other causes without evidence of disease or who were unavailable for follow-up were either censored at the time of death or last follow-up. The disease-free and overall survival curves were calculated according to the Kaplan-Meier method (21). Differences in survival between cases showing negative and positive *bcl-2* immunoreactivity were assessed using the log rank test. P values <0.05 were considered significant.

Results

Of the 71 patients studied, 15 (21.1%) were found to have cytoplasmic staining for the bcl-2 protein.

Clinical characteristics of these patients who underwent primary RT for SCCHN according to bcl-2 protein expression are presented in Table 2. There was no association of bcl-2 expression with age ($P = 0.383$), sex ($P = 0.572$), T stage ($P = 0.721$), tumor site ($P = 0.484$), distant metastases ($P = 0.600$), tumor differentiation ($P = 1.000$), or radiation doses ($P = 0.541$).

The analysis of bcl-2 protein expression in relation to alcohol consumption did not show any statistically significant difference ($P = 0.829$). Conversely, we found that patients with bcl-2 protein expression were heavier smokers (median, 47.5; mean, 53.4; range, 18.75–135 pack-years) than those with bcl-2-negative cancers (median, 35.5; mean, 36.8; range, 0–160 pack-years), although this difference is only suggestive ($P = 0.098$). Moreover, 80% (12/15) of patients with *bcl-2*-positive tumors smoked cigarettes and drank alcohol, while 2 patients only smoked and 1 only drank ($P = 0.537$).

On the basis of tumor response to RT, we compared the clinical characteristics of patients who failed (30 cases) primary RT according to bcl-2 protein expression. Nine of 30 patients with recurred disease had *bcl-2*-positive tumors while 21 patients (70%) had *bcl-2*-negative tumors. In the relapsed patient group, patients with oral cavity cancers who experienced a radiation failure showed a higher rate of bcl-2 protein expression than those with relapsed laryngeal and pharyngeal tumors ($P = 0.09$). Moreover, patients with *bcl-2*-positive recurred cancers were found to be heavier smokers than those with *bcl-2*-negative tumors (median, 47.5; mean, 55.1; range, 18.75–135 versus median, 34; mean, 30.4; range, 0–60 pack-years, $P = 0.03$). There were no significant differences in terms of age, sex, tumor stage and differentiation, RT dosage, or disease-free intervals between relapsed patients with or without *bcl-2*-positive tumors (data not shown).

The immunohistochemical analysis of tumor biopsies obtained from unresponsive or recurred cancers (25 cases) showed an increased rate of *bcl-2* immunoreactivity. In fact, 15 (60%) of 25 of postirradiated cancers showed *bcl-2* immunostaining compared to 9 (30%) of 30 of the preirradiated samples ($P = 0.03$). Among these preirradiated *bcl-2*-positive tumors, only one lost its immunoreactivity in postirradiated tumor biopsy, while the remaining cases showed an analogous or increased *bcl-2* immunoreactivity in both pre- and post-RT tumor specimens.

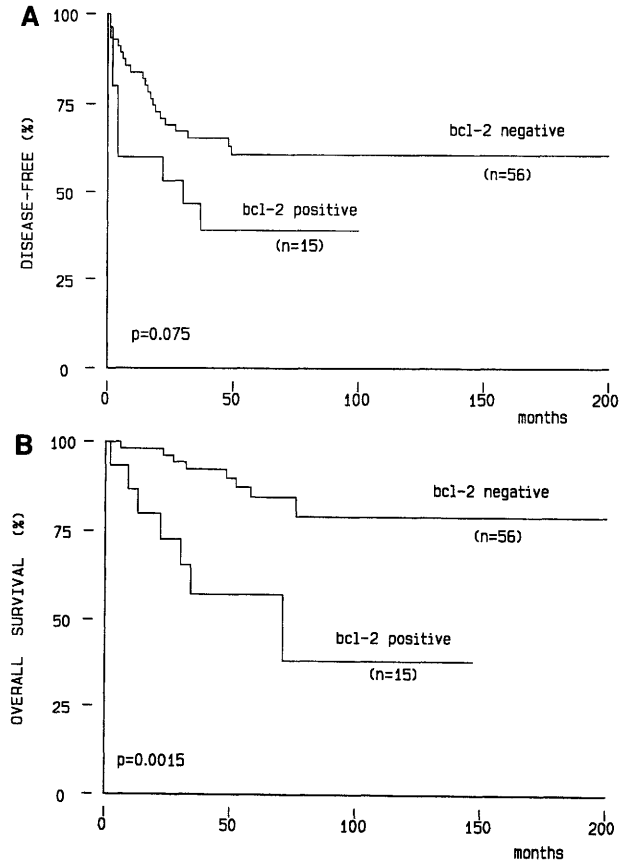

Fig. 1 Probability of disease-free survival (*A*) and overall survival (*B*) as a function of bcl-2 protein expression in 71 stage I–II SCCHN patients treated with RT.

Univariate analysis (Table 3) showed that high bcl-2 protein expression (>30% of positive cells) was a statistically significant indicator of disease-free and overall survival. In particular, disease-free and overall survival curves using the Kaplan-Meier method showed a worse prognosis for patients whose tumors had more than 30% of *bcl-2*-positive cells than for those with lower or no positivity ($P = 0.07$ and $P = 0.001$, respectively; Fig. 1).

In this study, univariate analysis also showed that well-differentiated tumors tend to have a higher risk of recurrence after RT than poorly differentiated cancers ($P = 0.102$). Moreover, patients with oral cavity cancer had a worse prognosis than those with laryngeal cancers ($P = 0.027$). Cox's regression multivariate analysis confirmed that the only significant prognostic factor in predicting both disease-free and overall survivals in our series was the bcl-2 protein expression ($P = 0.019$ and ($P = 0.005$, respectively) Table 4. Moreover, in multivariate analysis tumor grade was found to be a prognostic factor in predicting recurrence, being well-differentiated tumors at highest risk ($P = 0.08$), while tumor stage and site were found to add predictive information on the overall survival in our series ($P = 0.04$ and $P = 0.07$, respectively).

Table 4 Multivariate Cox proportional hazards analysis of 5-year disease-free and overall survival in 71 SCCHN patients primary treated with RT

		5-year disease-free survival			5-year overall survival		
	Score	HR[a] (95% CI)	χ^{2b}	*P*	HR[a] (95% CI)	χ^{2b}	*P*
bcl-2 Expression							
Negative[c]	0						
Positive	1	2.98 (1.20–7.41)	5.55	0.019	6.20 (1.74–22.11)	7.91	0.005
Sex							
Male[c]	0						
Female	1	0.62 (0.11–3.46)	0.29	0.589	0.36 (0.03–4.23)	0.65	0.419
Age (yr)							
<60[c]	0						
≥60	1	1.27 (0.54–2.95)	0.30	0.579	1.43 (0.42–4.87)	0.33	0.564
T stage							
T_1[c]	0						
T_2	1	0.70 (0.40–1.22)	1.50	0.219	0.45 (0.20–0.98)	4.00	0.045
Site							
Larynx[c]	0						
Oral cavity	1	1.17 (0.41–3.28)	0.30	0.764	3.38 (0.90–12.56)	3.30	0.069
Pharynx	2	1.19 (0.29–4.88)	0.06	0.804	1.62 (0.15–17.39)	0.16	0.689
Tumor differentiation							
Well[c]	0						
Moderate	1	0.91 (0.40–2.07)	0.05	0.831	2.11 (0.60–7.42)	1.17	0.243
Poor	2	0.29 (0.07–1.16)	3.03	0.082	0.60 (0.09–4.00)	0.28	0.598
RT dosage (Gy)							
<64[c]	0						
≥64	1	0.91 (0.36–2.26)	0.04	0.843	0.72 (0.20–2.49)	0.27	0.601

[a] Adjusted HR of relapsing or dying.
[b] Computed using Wald's statistic.
[c] Reference category.

Discussion

A major factor in the failure of RT is inherent or induced cellular radioresistance. Tumors that do not respond to irradiation or recur locally after RT contain more radioresistant cells than do tumors that have not received RT (22, 23).

In recent years, the understanding of the process of programmed cell death or apoptosis has suggested that anticancer agents can induce apoptosis of target cells (16). Thus, because apoptosis requires a genetic program, alterations in apoptotic pathways could produce tumor resistance to anticancer therapies.

Studies suggest that the *bcl-2* proto-oncogene is an essential component of the apoptotic program induced by anticancer agents in oncogenically transformed cells because of its activity as a negative regulator of mammalian cell death (24).

The detection of bcl-2 protein in basal cells but not in more superficial, differentiated cells from several epithelial tissues, including nasopharynx, skin, lung, and intestine (11, 15, 25, 26), suggests that the *bcl-2* gene could be responsible for the survival of stem cells while preventing the overaccumulation of differentiated cells (6). Thus, the *bcl-2* gene aberration could potentially be involved in growth and development of epithelial tumors with some prognostic implications (9–15).

In this study, we first report that dysregulation of *bcl-2* gene expression, although at a low rate (*i.e.*, 20%), can also occur in SCCHN.

The expression of bcl-2 protein in our series was unrelated to sex, age, tumor grade, size, or site. On the contrary, we found that *bcl-2* expression seems to be associated with smoking history, as measured in pack-years. This association was suggestive in our SCCHN patients ($P = 0.098$), and became statistically significant if evaluated considering only those patients who failed primary RT ($P = 0.03$). These findings further support our original report on a possible correlation between cigarette smoking and bcl-2 protein expression evaluated in a different series of head and neck patients which also included more advanced lesions (stage III–IV; Ref. 27).

Our study demonstrates by univariate and multivariate Cox regression analysis that bcl-2 protein expression appears to be associated with less responsive and more aggressive tumors in early head and neck cancer primary treated with RT. In fact, in SCCHN patients the estimated recurrence and death hazards were higher for *bcl-2*-positive cancers compared to *bcl-2*-negative ones; the log rank test showed that bcl-2 protein expression was closely associated with a higher risk of recurrence and poor survival ($P = 0.07$ and $P = 0.001$, respectively).

Although in our series we demonstrate a suggestive but not statistically significant difference in the rate of bcl-2 protein expression in tumor tissues from patients who did or did not fail primary curative RT (9/30, *i.e.*, 30% versus 6/41, *i.e.*, 14.6%; $P = 0.15$), multivariate Cox analyses for both 5-year disease-free and overall survival clearly showed the prognostic value of *bcl-2* immunostaining in early SCCHN ($P = 0.019$ and $P = 0.005$, respectively). Moreover, with regard to a possible role of the *bcl-2* proto-oncogene in radioresistance and modulation of tumor response to RT *in vivo*, we demonstrated an increased *bcl-2* immunoreactivity in posttherapy biopsies from patients with relapsed malignancies. In fact, among 25 patients who

experienced a local recurrence or persistence of the tumor after curative RT, 15 (60%) were found to have *bcl-2*-positive recurred cancers. Moreover, all but one RT-recurred patient with original *bcl-2*-positive tumors showed an analogous or an increased immunoreactivity in posttherapy tumor biopsies. The increased rate of *bcl-2* expression in post-RT tumor biopsies is in accordance with similar findings reported by Castle *et al.* (28) and more recently by Krajewski *et al.* (29) in human neuroblastomas after primary chemotherapy. These authors demonstrated that more than 80% of specimens obtained from residual tumors in patients affected by neuroblastomas after chemotherapy expressed bcl-2 protein, suggesting that bcl-2 could be involved in chemotherapy-induced apoptosis in neuroblastoma (20). These data in accordance with the negative prognostic value of bcl-2 protein expression in our series could indicate the role of *bcl-2* proto-oncogene in controlling radiation-induced apoptosis and tumor response to RT in SCCHN.

In accordance with a common tobacco-induced carcinogenesis among squamous cell carcinomas of the upper and lower aerodigestive tracts, the rate of bcl-2 protein expression detected in this patient series (21%) is similar to the 25% of *bcl-2* positivity reported by Pezzella *et al.* (11) in 80 consecutive squamous carcinomas of the lung. Although this study did not investigate a possible correlation between tobacco exposure and *bcl-2* immunoreactivity, the data reported here regarding a possible influence of tobacco smoke on bcl-2 protein expression in squamous cell carcinoma of the head and neck seem to suggest the possible involvement of the *bcl-2* proto-oncogene in both lung and head and neck tobacco-related carcinogenesis.

In contrast to our data, in lung cancers *bcl-2* expression appears to be related to less aggressive tumor behavior (11, 12). This apparently unexpected discrepancy could be explained on the basis of the different therapeutic strategies utilized in the two patient series. In fact, lung cancer patients underwent surgery as treatment choice whereas our SCCHN patients had primary curative RT. Since tumor growth could be the result of a balance between cell proliferation and programmed cell death (30), it is likely that early activation of the *bcl-2* proto-oncogene during carcinogenesis by inhibiting cell death could lead to selection of less proliferative and indolent tumors, in which prolonged survivals opposed to the high proliferation rate is the key mechanism in determining tumor growth. Thus, less proliferative *bcl-2*-positive tumors are likely to be more resistant to conventional therapies involving an apoptotic-induced cytotoxicity such as radiotherapy and chemotherapy, while the same tumors would be more responsive to conventional surgery.

We conclude that *bcl-2* is abnormally expressed in some head and neck cancers, and its evaluation would be useful in selecting patients with early SCCHN for radiation therapy. Further investigations are needed to better clarify the role of *bcl-2* in the biological response to RT in head and neck cancer and its possible relation to tobacco smoke.

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