Journal of
Oral Pathology & Medicine

doi: 10.1111/jop.12020

J Oral Pathol Med (2013) 42: 339–344

wileyonlinelibrary.com/journal/jop

Relationship between anti-apoptotic proteins survivin and Bcl-2, and response to treatment in patients undergoing post-operative RT for laryngeal cancer: a pilot study

Gino Marioni[1], Alberto Staffieri[1], Marco Lionello[1], Giulia Tealdo[1], Claudia Staffieri[2], Luciano Giacomelli[3], Maria Luisa Friso[4], Roberto Stramare[5], Giancarlo Ottaviano[1], Stella Blandamura[3]

[1]Department of Neurosciences, Otolaryngology Section, University of Padova, Padova, Italy; [2]Department of Neurosciences, Otolaryngology Section, Treviso Branch, University of Padova, Treviso, Italy; [3]Department of Medicine, University of Padova, Padova, Italy; [4]Radiotherapy and Nuclear Medicine Unit, Istituto Oncologico Veneto IOV IRCCS, Padova, Italy; [5]Department of Medicine, Institute of Radiology, University of Padova, Padova, Italy

BACKGROUND: Clinicopathological research has focused on identifying molecular and biological prognostic factors for laryngeal carcinoma (LSCC) treated with post-operative radiotherapy (RT). The aim of this study was to assess the prognostic importance of anti-apoptotic proteins survivin and B-cell lymphoma-2 (Bcl-2) in a series of patients with LSCC who had primary surgery followed by RT.

METHODS: Thirty-three consecutive patients who underwent primary surgery followed by RT were considered. Survivin nuclear and cytoplasmic expressions and Bcl-2 expression were determined immunohistochemically.

RESULTS: The loco-regional recurrence rate was significantly higher among LSCC patients with a nuclear survivin expression >10.0% ($P = 0.029$), and their disease-free survival (DFS) was shorter than in cases whose nuclear survivin expression was $\leq$ 10.0% ($P = 0.002$). DFS was significantly shorter in cases with a Bcl-2 expression >2.0% than in those whose Bcl-2 expression was $\leq$ 2.0% ($P = 0.035$).

CONCLUSIONS: Nuclear survivin expression and Bcl-2 expression warrant further investigation as potential predictive biomarkers to enable individualized treatments (e.g. post-operative chemo-radiotherapy instead of RT alone for patients whose LSCCs strongly express nuclear survivin or/and Bcl-2). This preliminary evidence justifies the design of new studies on the association of agents targeting survivin and Bcl-2 with conventional chemotherapeutic agents and RT for advanced LSCC.

J Oral Pathol Med (2013) **42**: 339–344

Correspondence: Gino Marioni, MD, Department of Neurosciences, Otolaryngology Section, Via Giustiniani 2, 35128 Padova, Italy. Tel: +49 049 8212029, Fax: +39 049 8213113, E-mail: gino.marioni@unipd.it
Accepted for publication September 27, 2012

Keywords: Bcl-2; laryngeal carcinoma; post-operative radiotherapy; prognosis; survivin

Introduction

A fairly recent review on laryngeal squamous cell carcinoma (LSCC) concluded that post-operative radiotherapy (RT) with or without chemotherapy is indicated in cases with one or more of the following adverse features: close/positive surgical margins; pT4 disease; subglottic extension of primary carcinoma; perineural/lymphatic/vascular invasion; multiple positive nodes (three or more metastatic lymph nodes); extracapsular spread or perineural involvement; N3 nodes (1). In 2012, Strojan and coworkers (2) analyzed the indications for RT after neck dissection, concluding that the only well-characterized indication for adjuvant irradiation of the dissected neck is extracapsular extension, whereas the number of nodes with cancer necessitating a combined approach remains to be seen. It is crucial to identify factors that can help establish which patients might benefit from post-operative RT to fine-adjust combined treatment strategies. The TNM system is widely accepted as a prognostic system for LSCC and used worldwide, but its weaknesses in terms of prognostic accuracy are well recognized. Clinicopathological research has necessarily focused on identifying molecular and biological prognostic factors (irrespective of TNM stage) for LSCCs treated with post-operative RT.

Apoptosis is a natural and well-studied process by means of which multicellular organisms shed aging or damaged cells. It is governed by a fine balance between proapoptotic and antiapoptotic factors, and the capacity to evade apoptosis is one of the essential changes in cell physiology that dictate the growth of malignant cells. Survivin is the smallest mammalian member of the family of inhibitor of apoptosis protein (IAP) genes. A single-copy survivin gene

is located on the human chromosome 17q25 and comprises three introns and four exons (3). The survivin gene is expressed as a 16.5-kDa protein with 142 amino acids. Five splice variants of human survivin have been described: survivin wild-type, survivin-2a, survivin-2b, survivin-3b, and survivin-DEx3 (3). Survivin activity is regulated by multiple mechanisms at the transcriptional, translational, and post-translational levels (4). Significant survivin overexpression has been demonstrated in tumors of the lung, breast, colon, stomach, esophagus, pancreas, liver, uterus, and ovaries, in Hodgkin's disease, non-Hodgkin's lymphoma, leukemia, neuroblastoma, pheochromocytoma, soft tissue sarcomas, brain tumors, and melanoma (5). Survivin protein expression correlates strongly with the more aggressive forms of malignancies, and there have also been investigations into its expression and biological role in head and neck carcinoma in recent years (3). The family of B-cell lymphoma-2 proteins (Bcl-2) governs whether a cell continues to live or must die through the mitochondrial apoptotic pathway (6). Anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-$_{XL}$, Bcl$_W$, Bfl-1, and Mcl-2) are pivotal regulators of apoptotic cell death (6–8). Bcl-2 is implicated in cancer development and progression and in protecting cells against a variety of cytotoxic insults, including cytokine deprivation, irradiation, and chemotherapeutic drugs. Numerous studies have demonstrated Bcl-2 overexpression in solid tumors, including melanoma and breast, colorectal, prostate, and small cell lung cancer, as well as cancers of hematological origin (7).

The aim of the present study was to assess the prognostic importance of the anti-apoptotic proteins survivin and Bcl-2 in a series of patients with LSCC who underwent primary surgery followed by RT.

Materials and methods

Patients

The fulfillment of the present investigation was approved by the internal committee of the Section. The study concerned 33 consecutive patients who had primary surgery followed by RT for LSCC at Padova University according to currently accepted protocols (1). The considered patients underwent post-operative RT for one or more of the following adverse features: pT4 disease [nine cases], subglottic extension of primary carcinoma [seven cases], lymphatic and/or vascular invasion [five cases]; three or more metastatic lymph nodes [13 cases], extracapsular spread [seven cases]; N3 nodes [one case]. None of the patients in this series received post-operative RT for positive surgical margins.

Preoperatively, all patients were investigated with upper aerodigestive tract endoscopy, neck ultrasonography (with or without fine needle aspiration cytology), and microlaryngoscopy with laryngeal biopsy. Head and neck contrast-enhanced computerized tomography (CT) and/or magnetic resonance imaging, chest X-rays, and liver ultrasonography were also performed.

The patients' main clinicopathological characteristics (based also on the 7th edition of the TNM Classification of Malignant Tumors (9) are given in detail in Table 1. The only two cN0 patients who underwent no neck dissection developed no neck lymph node metastases during a follow-up of 32 and 67 months. No distant metastases (M) were detected at diagnosis in this series.

Table 1 Clinicopathological characteristics of 33 patients with LSCC given postoperative RT

	No. cases
Male	27
Female	6
pT1	1
pT2	9
pT3	14
pT4	9
N0 [cN0 (2 cases) + pN0 (14 cases)]	16
N+*	17
Stage I	1
Stage II	6
Stage III	5
Stage IV	21
G1	4
G2	18
G3	11
Without locoregional recurrence	17
With locoregional recurrence	16

*Pathologically positive (N2 in 16 cases, N3 in 1 case).

The general follow-up schedule (adjustable to patients' individual characteristics) was as follows: (i) once a month for the 1st year after treatment; (ii) every 2 months in the 2nd year; (iii) every 3 months in the 3rd year; (iv) every 4 months in the 4th year; (v) every 6 months in the 5th year; and (vi) every 12 months thereafter. Neck ultrasonography and chest X-rays were also performed at least yearly. Contrast-enhanced CT of the neck, total body positron emission tomography (PET–CT), chest CT, and liver ultrasonography were also performed as necessary. The mean follow-up was 43.0 ± 30.1 months (median, 34 months).

Radiotherapy

All patients were treated with external beam radiotherapy using a-6 MV photon beam delivered from a linear accelerator. The patients were immobilized with a thermoplastic mask, and CT images were acquired in the treatment position to enable three-dimensional treatment planning. The RT technique adopted depended on the target volume. In most cases, the dose was delivered using two parallel-opposed fields up to 40 Gy, and then the fields were shielded to cover the spine and matched with electron beams (8–10 MeV) on the spinal chains. In selected cases, the dose was delivered using multiple beams to cover the whole planning target volume. Conventional fractionation was used, that is, 1.8–2 Gy/fraction, once a day, with a total dose ranging from 50 to 70 Gy (median, 60 Gy). The median overall treatment time was 30 days.

Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections using a fully automated system (Bond-maX; Leica, Newcastle Upon Tyne, UK). In brief, 4-micron-thick sections were cut from each paraffin-embedded block. The sections were

deparaffinized in Bond Dewax Solution (Leica) at 72°C, rinsed in ethanol, and rehydrated in distilled water. Antigens were retrieved by heating sections for 30 min at 99°C in Bond Epitope Retrieval Solution 1 (Leica). Endogenous peroxidase was blocked with 3% hydrogen peroxide before 30 min of incubation with mouse monoclonal anti-survivin (clone D-8; Santa Cruz Biotechnology, Santa Cruz, CA; 1:50 diluted) and mouse monoclonal anti-human BCL2 (clone 124; Dako, Glostrup, Denmark; 1:200 diluted). Specimens were then washed with phosphate-buffered saline (pH, 7.0) and incubated with Bond Polymer Refine Detection Kit (Leica) according to the manufacturer's protocols. Staining was visualized with 3,3 -diaminobenzidine (DAB), and the slides were counterstained with Mayer's hematoxylin. The sections were then dehydrated, cleared, and mounted. Samples from formalin-fixed, paraffin-embedded human breast tumor (survivin), and tonsil (Bcl-2) were used as positive controls, and serum without the primary antibody as a negative control.

Sections were scanned by the pathologist (SB), and survivin and Bcl-2 expressions in carcinoma cells were measured as the percentage of stained cells (among at least 600 cells counted at 200 magnification in 20 carcinoma fields with no signs of necrosis or hemorrhage).

Statistical analysis

The statistical tests applied were Fisher's exact test, the Mann–Whitney *U*-test, and the non-parametric test for trend, as appropriate. Spearman's rank correlation was used to assess the relationship between survivin nuclear expression, survivin cytoplasmic expression, and Bcl-2 expression. The risk ratios for recurrent disease were also calculated. The log-rank test and the Kaplan–Meier product limit estimator were also used to compare disease-free survival (DFS) times stratified by the different parameters analyzed. The receiver operating curve (ROC) approach (failure vs. parameter) was used to establish the analytically best-fitting cutoff for binarizing the continuous variables (survivin nuclear and cytoplasmic expressions, and Bcl-2 expression) according to the highest level of the positive likelihood ratio. The best performance has an area under the ROC curve (AUC) of 1.0. A P-value <0.05 was considered significant. The STATA^{TM} 8.1 (Stata Corp, College Station, TX, USA) statistical package was used for all analyses.

Results

Clinical outcome

Seventeen of the 33 patients with LSCC experienced no disease relapse after post-operative RT, while 16 developed loco-regional recurrences. Fisher's exact test ruled out any significant differences in the distributions for pT (P = 1.0), lymph node status (N0/N+) (P = 0.30), stage grouping (P = 0.22), and grade (P = 1.0), in the two subgroups of patients with and without loco-regional carcinoma recurrences. The log-rank test showed no significant differences in DFS (in months) when patients were stratified by pT (P = 0.80), lymph node status (P = 0.12), stage grouping (P = 0.33), or grade (P = 0.44).

Survivin and Bcl-2 immunohistochemical expression and clinicopathological features (Table 2)

As previously reported (10), epithelial cells from normal laryngeal mucosa showed weak immunohistochemical staining for survivin in scattered groups of cells in the basal and parabasal layers. A nuclear reaction predominated in most of the primary LSCC specimens considered (Fig. 1A) (28 of 33 samples showed nuclear survivin expression >0%). In 15 of 33 cases, a cytoplasmic survivin expression >0% was found. Only two cases showed cytoplasmic survivin staining alone. The mean immunohistochemical expression of nuclear survivin was 10.7 ± 9.5% (median, 10%), and for cytoplasmic survivin, it was 12.6 ± 26.2% (median, 0%).

Statistical analysis failed to disclose any significant differences in nuclear survivin expression when patients

Table 2 Immunohistochemical expression of nuclear and cytoplasmic survivin and Bcl-2

	Mean nuclear survivin expression (%) ± SD (%) [median (%)]	*Mean cytoplasmic survivin expression (%) ± SD (%) [median (%)]*	*Mean Bcl-2 expression (%) ± SD (%) [median (%)]*
T1	–	–	–
T2	8.7 ± 8.5 [5.0]	14.1 ± 30.6 [2.0]	16.0 ± 21.9 [2.0]
T3	11.9 ± 10.9 [10.0]	6.0 ± 15.4 [0.0]	11.2 ± 21.7 [1.0]
T4	11.8 ± 7.6 [10.0]	13.9 ± 25.1 [0.0]	4.4 ± 8.3 [0.0]
N0 [cN0 (2 cases) + pN0 (14 cases)]	7.6 ± 7.0 [6.5]	13.6 ± 29.4 [0.0]	6.4 ± 13.1 [0.0]
N+	13.5 ± 10.6 [10.0]	11.7 ± 22.8 [0.0]	14.1 ± 23.0 [2.0]
Stage I	–	–	–
Stage II	10.0 ± 9.1 [10.0]	20.8 ± 35.6 [7.5]	13.7 ± 17.7 [6.0]
Stage III	5.4 ± 4.1 [5.0]	0.4 ± 0.8 [0.0]	0.0 ± 0.0 [0.0]
Stage IV	12.5 ± 10.0 [10.0]	10.0 ± 20.9 [0.0]	12.3 ± 21.3 [2.0]
G1	18.0 ± 10.9 [17.5]	3.0 ± 4.1 [1.0]	1.3 ± 2.2 [0.0]
G2	7.2 ± 6.2 [6.5]	17.2 ± 29.7 [2.5]	15.2 ± 23.3 [2.0]
G3	13.7 ± 10.7 [11.0]	8.5 ± 22.8 [0.0]	5.6 ± 11.5 [0.0]
Without locoregional recurrence	7.1 ± 7.1 [5.0]	10.2 ± 25.6 [0.0]	6.0 ± 14.5 [0.0]
With locoregional recurrence	14.5 ± 10.2 [13.0]	15.1 ± 26.6 [5.0]	14.9 ± 22.3 [3.5]

SD, standard deviation.

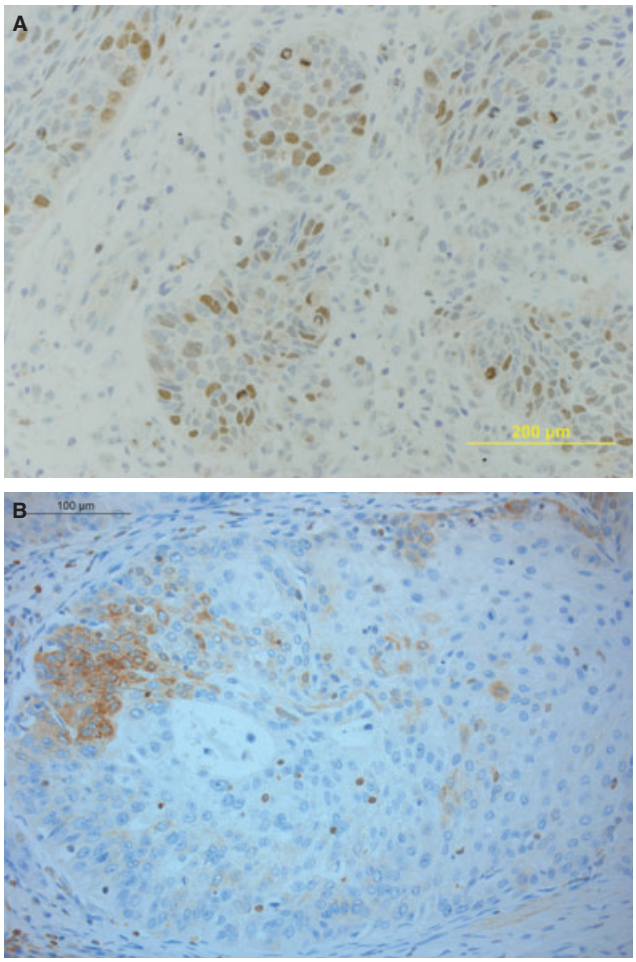

Figure 1 (A) Nuclear survivin expression in laryngeal carcinoma cells (original magnification 200×); (B) Bcl-2 expression in LSCC (original magnification 200×).

were distributed by pT (non-parametric test for trend, $P = 0.23$), lymph node status (Mann–Whitney *U*-test, $P = 0.10$), stage grouping (non-parametric test for trend, $P = 0.59$), or grade (non-parametric test for trend, $P = 0.82$).

Statistical analysis likewise ruled out any significant differences in cytoplasmic survivin expression when patients were stratified by pT (non-parametric test for trend, $P = 0.36$), lymph node status (Mann–Whitney *U*-test, $P = 0.95$), stage grouping (non-parametric test for trend, $P = 0.76$), or grade (test for trend, $P = 0.54$).

The normal laryngeal squamous epithelium was Bcl-2-negative or showed only weak, focal Bcl-2 positivity in the basal layer. In laryngeal carcinoma cells, Bcl-2 reactivity (Fig. 1B) was cytoplasmic, of both membranous and granular type, and mostly in the perinuclear area. Fifteen of 33 cases showed Bcl-2 expression >0%. The mean Bcl-2 expression was 10.3% ± 19.2% (median, 0%).

Statistical analysis revealed no significant differences in Bcl-2 expression when patients were distributed by pT (non-parametric test for trend, $P = 0.26$), N (Mann–Whitney *U*-test, $P = 0.15$), stage (non-parametric test for trend, $P = 0.62$), or grade (non-parametric test for trend, $P = 0.54$).

Survivin and Bcl-2 immunohistochemical expression and prognosis in LSCC

The ROC approach was used to find the analytically best-fitting nuclear survivin expression cutoff for prognostic purposes, and the value calculated was 10.0% (AUC = 0.72, sensitivity 75.0%, specificity 64.0%). The loco-regional recurrence rate was significantly higher among LSCC patients with a nuclear survivin expression >10.0% (12 cases) (Mann–Whitney *U*-test, $P = 0.029$), and their DFS was shorter than in cases whose nuclear survivin expression was $\leq 10.0\%$ (log-rank test, $P = 0.002$) (Fig. 2A). The risk ratio of recurrence during the follow-up considered was 5.1 times higher for patients with a nuclear survivin expression >10.0% (CI 95% 1.67–15.96, Fisher's exact test $P = 0.0019$).

The Mann–Whitney *U*-test and log-rank test ruled out any significant association between cytoplasmic survivin expression (cutoff 2.0% calculated using the ROC approach, AUC = 0.66, sensitivity 62.0%, specificity 70.6%) and disease recurrence rate ($P = 0.069$) or DFS ($P = 0.065$).

The ROC approach was also used to identify the analytically best-fitting Bcl-2 expression cutoff for prognostic purposes, which was calculated to be 2.0% (AUC = 0.66; sensitivity 62.5%; specificity 70.5%). The Mann–Whitney *U*-test ruled out any significant difference in the recurrence rate for the subgroup of LSCCs with a Bcl-2 expression >2.0% (12 cases) ($P = 0.069$). The risk ratio of recurrent disease was 3.4 times higher for patients with a Bcl-2 expression >2.0% (95% CI, 1.11–10.42; Fisher's

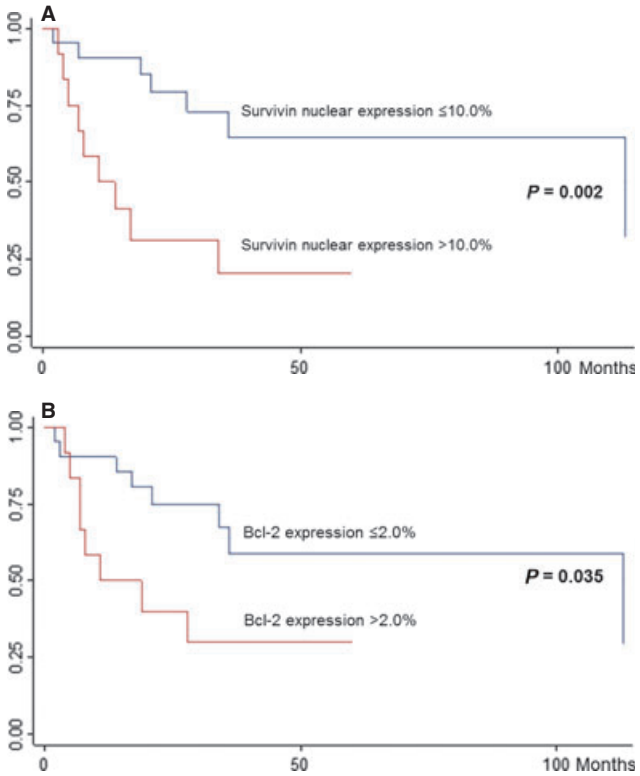

Figure 2 Disease-free survival in LSCC patients estimated from nuclear survivin expression (A), and Bcl-2 (B) levels; time (abscissa) calculated in months.

exact test $P = 0.019$). The log-rank test showed that DFS was significantly shorter in patients with a Bcl-2 expression >2.0% than in those whose Bcl-2 expression was $\leq 2.0\%$ ($P = 0.035$) (Fig. 2B).

Statistical relationship between survivin and Bcl-2 in LSCC

Spearman's rank correlation test found no significant associations between the expression levels of nuclear and cytoplasmic survivin ($P = 0.32$), nuclear survivin and Bcl-2 ($P = 0.75$), or cytoplasmic survivin and Bcl-2 ($P = 0.82$).

Discussion

The ability to evade apoptosis is a fundamental cause of treatment resistance in various human cancers. Numerous molecular defects at several points along the apoptotic pathway can lead to a resistance to apoptosis, including defects in the deregulation of death receptors, negative regulation of post-receptor signaling, an anti-apoptotic imbalance of the intramitochondrial Bcl-2 proteins, and upregulation of IAPs such as survivin (10, 11). The anticancer activity of most cytotoxic therapies, including RT, relies on their ability to activate cell death programs like apoptosis in cancer cells. A better understanding of the molecular events responsible for the defects encountered in the apoptosis signaling network may therefore offer novel prospects of tackling radioresistance in human cancers (12).

Much of the research on the potential of molecular profiles as prognostic markers in the setting of RT for head and neck cancer refers to series of patients given RT alone, while very few studies have focused on the predictive value of molecular markers in post-operative RT for cases of LSCC (13–16). The present report is the first to investigate the potential prognostic role of the apoptosis-related proteins survivin and Bcl-2 in patients with LSCC after post-operative RT.

The expression of the IAP survivin and its prognostic role in LSCC have been discussed in only a handful of studies in the English-language medical literature (17–21). Marioni et al. (10) very recently investigated survivin expression using immunohistochemistry, and the expression of the wild-type, survivin-2B, and survivin-DEx3 splice variants by reverse transcription and quantitative real-time PCR (used for the first time at this anatomical site) in 86 consecutive LSCCs. A significant association emerged between higher nuclear survivin expression levels and disease recurrence, and the DFS was shorter in patients whose nuclear survivin expression was >7.0% than in cases with levels $\leq 7.0\%$. Quantitative real-time PCR showed that wild-type survivin was definitely the splice variant most frequently detected in LSCC tissues. The significant relationship between the levels of wild-type survivin transcription and survivin protein expression presumably indicates that survivin is primarily localized in the nucleus of LSCC cells. In the present series of 33 patients with LSCC given post-operative RT, the recurrence rate was significantly higher among patients whose nuclear survivin expression was >10.0% ($P = 0.029$), and their DFS was shorter ($P = 0.002$). The risk ratio of disease recurrence was 5.1 times higher for patients with a nuclear survivin expression >10.0%. On the strength of these results, nuclear survivin expression might serve as a new marker for predicting response to post-operative RT in specimens of primary tumor before starting any RT. Nuclear survivin expression consequently warrants further investigation for its potential as a predictive biomarker to enable individualized treatments (e.g. post-operative chemo-radiotherapy instead of RT alone in patients whose LSCCs strongly express nuclear survivin).

Bcl-2-related radioresistance has been hypothesized in LSCC and investigated in series of patients who had RT alone. Condon et al. (22) compared Bcl-2 immunohistochemical expression in pre-RT biopsies of radioresistant (eight cases) and radiosensitive (13 cases) LSCCs. They found a significant correlation between Bcl-2 overexpression and radioresistance and suggested that overexpression of the anti-apoptotic protein Bcl-2 enabled carcinoma cells with RT-induced DNA damage to continue proliferating. Nix et al. (23) analyzed the Bcl-2 and survivin expressions in the biopsies of 124 patients (62 radioresistant and 62 radiosensitive) who had curative RT alone for early stage (T1-T2, N0) LSCC. Multivariate regression analysis using treatment failure as the dependent factor indicated that Bcl-2 expression was an independent variable. In the present study, statistical analysis showed a trend toward a significantly higher recurrence rate for LSCCs with a Bcl-2 expression >2.0% ($P = 0.069$); the patients concerned had a risk ratio of recurrence of 3.4. The log-rank test showed that DFS was significantly shorter in patients with LSCC whose Bcl-2 expression was >2.0% ($P = 0.035$).

The results of our pilot study on the role of anti-apoptotic proteins in patients with LSCC treated with primary surgery followed by RT point to a survivin and Bcl-2 radioresistance mechanism in LSCC. These observations now need to be tested in larger-scale prospective trials. If our preliminary findings are confirmed, they could prompt the design of new treatment strategies for advanced LSCC, including agents targeting survivin and Bcl-2 in association with conventional therapeutic agents and RT. Despite the marked improvement in our understanding of its biology, the number of agents targeting survivin in clinical practice is still small. Unlike proteins expressed on the cell surface or those with a catalytic activity, survivin is a challenging target for classical small-molecule approaches. The methods currently in the most advanced stages of clinical evaluation (Phase I or II) are antisense oligonucleotides targeting survivin mRNA, survivin transcription inhibitors, and immunotherapeutic strategies (4). The anti-apoptotic members of the Bcl-2 family are also attractive tumor-associated antigens for the purposes of targeted therapy. Several different strategies have been used in an attempt to overcome the cytoprotective effects of Bcl-2 in cancer, including shutting off gene transcription; inducing mRNA degradation with antisense oligonucleotides; and attacking Bcl-2 directly with small-molecule drugs (7). Orally bioavailable inhibitors of the Bcl-2 family capable of specifically inhibiting protein–protein interactions between BH3 and Bcl-2 at low nanomolar concentrations have the potential to mark a significant development in cancer therapy (6).

References

1. Marioni G, Marchese-Ragona R, Cartei G, Marchese F, Staffieri A. Current opinion in diagnosis and treatment of laryngeal carcinoma. *Cancer Treat Rev* 2006; **32**: 504–15.
2. Strojan P, Ferlito A, Langendijk JA, Silver CE. Indications for radiotherapy after neck dissection. *Head Neck* 2012; **34**: 113–9.
3. Marioni G, D'Alessandro E, Bertolin A, Staffieri A. Survivin multifaceted activity in head and neck carcinoma. Current evidence and future therapeutic challenges. *Acta Otolaryngol (Stockh)* 2010; **130**: 4–9.
4. Church DN, Talbot DC. Survivin in solid tumors: rationale for development of inhibitors. *Curr Oncol Rep* 2012; **14**: 120–8.
5. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003; **22**: 8581–9.
6. Azmi AS, Wang Z, Philip PA, Mohammad RM, Sarkar FH. Emerging Bcl-2 inhibitors for the treatment of cancer. *Expert Opin Emerg Drugs* 2011; **16**: 59–70.
7. Straten P, Andersen MH. The anti-apoptotic members of the Bcl-2 family are attractive tumor-associated antigens. *Oncotarget* 2010; **1**: 239–45.
8. Kelly PN, Strasser A. The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. *Cell Death Differ* 2011; **18**: 1414–24.
9. Sobin LH, Gospodarowicz MK, Wittekind C, eds. *TNM classification of malignant tumors*, 7th edn. Oxford: Wiley-Blackwell, 2009.
10. Marioni G, Agostini M, Bedin C, et al. Survivin and laryngeal carcinoma prognosis: nuclear localization and expression of splice variants. *Histopathology* 2012; **61**: 247–56.
11. Samm N, Werner K, Rückert F, Detlev Saeger H, Grützmann R, Pilarsky C. The role of apoptosis in the pathology of pancreatic cancer. *Cancers* 2011; **3**: 1–16.
12. Fulda S. Inhibitor of apoptosis (IAP) proteins as therapeutic targets for radiosensitization of human cancers. *Cancer Treatment Rev*, 2012; **38**: 760–6.
13. Lera J, Lara PC, Perez S, Cabrera JL, Santana C. Tumor proliferation, p53 expression, and apoptosis in laryngeal carcinoma: relation to the results of radiotherapy. *Cancer* 1998; **83**: 2493–501.
14. Jiang H, Yang BB. p53, epidermal growth factor receptor and proliferating cell nuclear antigen in laryngeal squamous cell carcinoma are not predictive markers for the effect of adjuvant radiotherapy. *Acta Otolaryngol (Stockh)* 2009; **129**: 101–7.
15. Marioni G, Blandamura S, Loreggian L, et al. Laryngeal carcinoma prognosis after postoperative radiotherapy correlates with CD105 expression, but not with angiogenin or EGFR expression. *Eur Arch Otorhinolaryngol* 2011; **268**: 1779–87.
16. Lionello M, Blandamura S, Loreggian L, et al. High mTOR expression is associated with a worse oncological outcome in laryngeal carcinoma treated with postoperative radiotherapy: a pilot study. *J Oral Pathol Med* 2012; **41**: 136–40.
17. Dong Y, Sui L, Watanabe Y, Sugimoto K, Tokuda M. Survivin expression in laryngeal squamous cell carcinomas and its prognostic implications. *Anticancer Res* 2002; **22**: 2377–83.
18. Pizem J, Cör A, Gale N. Survivin expression is a negative prognostic marker in laryngeal squamous cell carcinoma and is associated with p53 accumulation. *Histopathology* 2004; **45**: 180–6.
19. Marioni G, Bertolin A, Giacomelli L, et al. Expression of the apoptosis inhibitor protein survivin in primary, laryngeal carcinoma and cervical lymph node metastases. *Anticancer Res* 2006; **26**: 3813–8.
20. Zhao H, Ren J, Zhuo X, Ye H, Zou J, Liu S. Prognostic significance of survivin and CD44v6 in laryngeal cancer surgical margins. *J Cancer Res Clin Oncol* 2008; **134**: 1051–8.
21. García-Fernández E, De Diego JI, Collantes-Bellido E, et al. Aurora B kinase expression in laryngeal squamous cell carcinoma and its prognostic implications. *Histopathology* 2011; **58**: 368–76.
22. Condon LT, Ashman JN, Ell SR, Stafford ND, Greenman J, Cawkwell L. Overexpression of Bcl-2 in squamous cell carcinoma of the larynx: a marker of radioresistance. *Int J Cancer* 2002; **100**: 472–5.
23. Nix P, Cawkwell L, Patmore H, Greenman J, Stafford N. Bcl-2 expression predicts radiotherapy failure in laryngeal cancer. *Br J Cancer* 2005; **92**: 2185–9.

Acknowledgements

This study was supported in part by grant No. 60A07-3774/11 (G. Marioni) from the University of Padova, Italy. The authors thank Rocco Cappellesso, MD, Department of Medicine, University of Padova, Italy for his photographic contribution, and Frances Coburn for correcting the English version of this paper.

Conflict of interest

None to declare.