Pergamon

Oral Oncol, Eur J Cancer Vol. 32B, No. 6, pp. 394–400, 1996

Printed in Great Britain
0964-1955/96 $15.00+0.00

PII: S0964-1955(96)00033-4

Differential Expression of *bcl*-2 and *bax* in Squamous Cell Carcinomas of the Oral Cavity

R.C.K. Jordan,[1,2,3] G.C. Catzavelos,[2] A.W. Barrett[3] and P.M. Speight[3]

[1]Department of Dentistry; [2]Department of Laboratory Medicine, Sunnybrook Health Science Centre, Toronto, Canada; and [3]Department of Oral Pathology, Eastman Dental Institute, London, U.K.

The *bcl*-2 oncogene is a member of a family of genes encoding for proteins which regulate apoptosis (programmed cell death). Recent evidence suggests that the *bcl*-2 protein is regulated by a homologous protein *bax* which counteracts its effects and promotes apoptosis. Overexpression of *bcl*-2 has been reported in a number of human cancers, although correlations with tumour differentiation and clinical outcome are conflicting and depend on tumour type and site. We studied *bcl*-2 and *bax* protein expression in adjacent serial sections of 30 squamous cell carcinomas of the oral cavity and correlated this with tumour differentiation. Examination of normal epithelium showed *bcl*-2 expression confined to basal keratinocytes and dendritic cells. The *bax* immunostaining was seen throughout the thickness of the epithelium but was most intense in the suprabasal cells. Overall, moderate or marked immunostaining for *bcl*-2 was identified in 18/30 (60%) carcinomas and for *bax* in 19/30 (63%) tumours. The *bcl*-2 immunoreactivity was strongest in the poorly differentiated carcinomas where 6/7 (86%) showed strong staining. By contrast, *bax* immunoreactivity was strongest in the well-differentiated carcinomas with 8/11 (72%) staining strongly. In the well-differentiated tumour islands, there was inverse topographic distribution of *bcl*-2 and *bax*, with both proteins showing a pattern that recapitulated normal epithelium. Upregulation of *bcl*-2 protein was identified in dysplastic epithelium adjacent to invasive tumour and in many cases there was reduced *bax* immunostaining. These results suggest that alterations of *bcl*-2 and *bax* may play a role in the development of squamous cell carcinoma. Furthermore, disturbances of protein expression in dysplastic epithelium suggest a role in the early stages of epithelial carcinogenesis. Copyright © 1996 Elsevier Science Ltd

Key words: *bcl*-2, *bax*, apoptosis, carcinoma, tumour differentiation

Oral Oncol, Eur J Cancer, Vol. 32B, No. 6, pp. 394–400, 1996

INTRODUCTION

Oral cancer is the sixth most common cancer worldwide, accounting for 4% of all malignancies in men and 2% in women. Despite advances in the detection and management of cancers at other sites, survival rates for oral cancer have remained poor. Overall, less than 50% of those with oral cancer will be alive in 5 years [1].

The study of oncogenic events which play a role in the development of oral cancer have concentrated primarily on the mechanisms which increase cell growth and proliferation. In particular, many studies have identified alterations in dominantly acting oncogenes that may uncouple normal growth controls and contribute to carcinogenesis [2]. More recently, a great deal of attention has been directed at studying tumour suppressor genes which normally regulate cell growth and proliferation in a negative fashion. For example, mutations of the p53 gene are the most common reported genetic abnormality in human cancer. In the oral cavity, mutant p53 expression has been reported in up to 50% of oral carcinomas and is associated particularly with tobacco smoking [3, 4].

By contrast, few studies have addressed the role of apoptosis in the natural history of oral cancer. Under physiological conditions, cell death is an important mechanism to achieve cellular homeostasis, but when dysregulated potentially can contribute to a number of diseases. A group of genes now have been identified which regulate apoptosis. The first of this group, the *bcl*-2 gene, was identified originally in molecular analysis of the t(14;18) chromosome translocation in follicular B-cell lymphomas [5, 6]. This

Correspondence to Dr Richard Jordan at Department of Dentistry, H-126, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5.
Received 1 Mar. 1996; provisionally accepted 17 Apr. 1996; revised manuscript received 20 May 1996.

Table 1. *Summary of results of* bcl-2 *and* bax *immunohistochemistry staining on squamous cell carcinomas*

Case number	Patient age	Sex	Site*	Grade†	Stage	*bcl*-2 score	*bax* score
1	49	M	FOM	Well	T4N0	3	1
2	50	F	FOM	Well	T2N0	2	3
3	61	M	FOM	Well	T4N0	3	1
4	72	M	FOM	Well	T3N0	0	3
5	80	M	FOM	Well	T4N0	1	3
6	60	M	Gingiva	Well	T2N1	3	0
7	50	M	Palate	Well	T4N0	0	3
8	48	F	Retromolar	Well	T2N0	1	3
9	47	F	Retromolar	Well	T2N0	1	3
10	36	F	Tongue	Well	T2N0	2	3
11	66	M	Tongue	Well	T4N0	1	3
12	40	F	FOM	Moderate	T4N0	3	1
13	53	M	FOM	Moderate	T2N1	0	3
14	65	F	FOM	Moderate	T2N0	2	3
15	68	F	FOM	Moderate	T3N1	1	2
16	48	M	Gingiva	Moderate	T4N1	3	1
17	64	M	Gingiva	Moderate	T3N0	1	3
18	81	M	Gingiva	Moderate	T4N1	2	3
19	33	F	Tongue	Moderate	T1N0	1	3
20	47	F	Tongue	Moderate	T4N1	1	3
21	57	M	Tongue	Moderate	T3N0	2	0
22	59	M	Tongue	Moderate	T2N1	1	3
23	67	F	Tongue	Moderate	T1N0	2	3
24	73	F	Cheek	Poor	T2N0	3	1
25	58	M	FOM	Poor	T4N0	3	3
26	56	M	Gingiva	Poor	T2N0	3	1
27	66	M	Gingiva	Poor	T2N0	3	1
28	60	M	Tonsil	Poor	T4N0	3	0
29	47	M	Tongue	Poor	T3N1	3	1
30	49	F	Tongue	Poor	T2N0	2	3

* FOM, floor of mouth.

† Grade refers to differentiation of the tumour.

translocation is found in over 85% of follicular lymphomas and one-third of diffuse lymphomas [7, 8]. It results in the apposition of the *bcl*-2 gene on chromosome 18 upstream to the immunoglobulin heavy chain gene (JH) on chromosome 14 where it comes under the effects of JH promoters [9]. The increased *bcl*-2 protein expression has been shown to protect B cells from programmed cell death [10]. Topographically, *bcl*-2 gene expression also has been identified in a number of other non-haematopoietic tissues such as oral mucosa, skin and intestinal epithelium where it is detectable in the basal cells but not in the more superficial cells [11, 12]. A number of reports have demonstrated overexpression of *bcl*-2 in lung, thyroid, nasopharyngeal and breast carcinomas [13–16]. In some tissues, such as the thyroid gland, this overexpression has been linked to better tumour differentiation [13]. In non-small cell lung cancer, *bcl*-2 protein has been associated with improved 5-year survival and clinical outcome [17]. In patients with non-Hodgkin's lymphoma, prostate cancer and acute myelogeneous leukaemia, *bcl*-2 overexpression or *bcl*-2 gene rearrangements have been associated with poor response to therapy [18–20].

A number of other proteins have been described which also regulate apoptosis. The product of the *bax* gene encodes for a protein sharing homology with *bcl*-2 [21]. The most common transcript of this gene is a 21 kD protein which forms heterodimers with *bcl*-2 protein and can counteract its death repressor activity [22]. The cellular distribution of both *bcl*-2 and *bax* have been described *in vivo*. In many normal tissues, there is an inverse distribution of both proteins within differing cell types consistent with the role of *bax* as a dominant inhibitor of *bcl*-2 [21]. To date, few studies have examined the relationship between *bcl*-2 and *bax* in human tumours. This paper describes alterations in *bcl*-2 and *bax* proteins in squamous cell carcinomas of the head and neck and suggests that they may play a role in the cellular differentiation and development of these tumours.

MATERIALS AND METHODS

Case selection

Thirty squamous cell carcinomas of the oral cavity were obtained from the surgical pathology files of the Department of Oral Pathology, Eastman Dental Institute. Cases were screened initially using haematoxylin and eosin tissue sections for adequacy of both lesions and perilesional tissues. All specimens had been diagnosed independently and graded by two pathologists based on established histological criteria [23]. Clinical details were obtained from the pathology requisition forms and consultation with the relevant clinician where necessary.

Control tissues consisted of biopsies of routinely processed fibroepithelial polyps from 10 patients retrospectively selected from the biopsy service. Biopsies of normal lymph node and tonsil were used as positive controls for immunohistochemistry for all experiments.

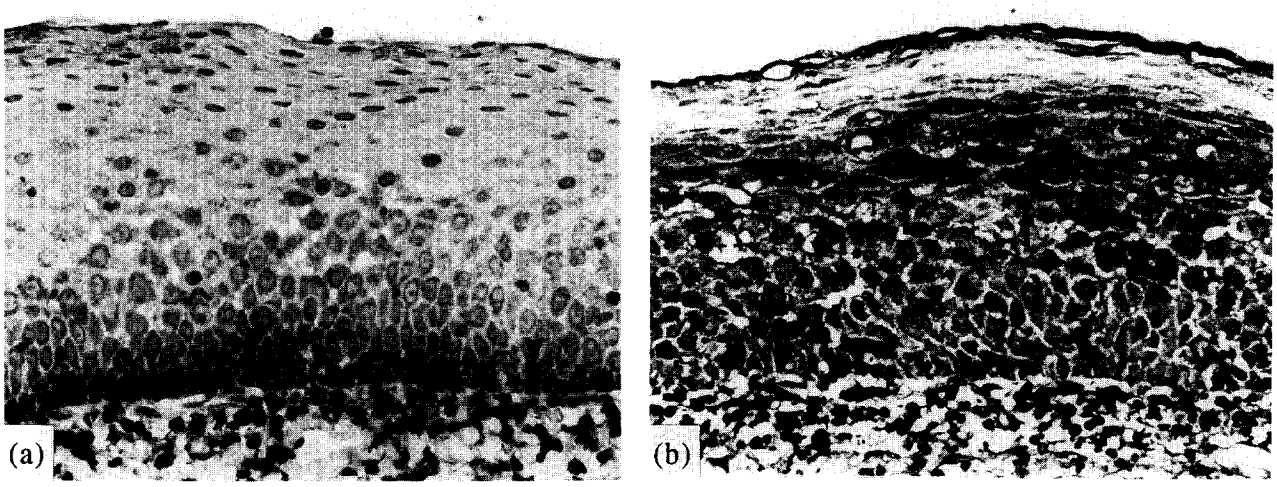

Fig. 1. (a) Normal oral mucosa showing *bcl*-2 expression as cytoplasmic reaction product in basal keratinocytes (arrowhead). Lymphocytes below the epithelium also show *bcl*-2 expression. (b) The *bax* staining was present throughout the epithelium, but was strongest in the suprabasal epithelial cells (arrow) (ABC ×120).

Immunohistochemistry

All tissues had been fixed in formalin and routinely processed to paraffin. Serial sections of 5 μm were cut and mounted on glass slides coated with 2% APES in dry acetone. Sections were de-waxed in xylene and rehydrated in graded ethanols. Endogenous peroxidase activity was blocked by immersion in 0.5% methanolic peroxide for 15 min followed by two washes in 1× tris-buffered saline (TBS) for 5 min each. For *bcl*-2, immunoreactivity of the target antigen was enhanced using pressurised heat antigen retrieval (pressure-cooking) [24]. The sections were placed in a pressure-cooker containing 0.6 M sodium citrate buffer and heated to 130°C for 2 min. The sections were then removed, quenched in de-ionised water and rinsed in TBS. For *bax* immunostaining, non-specific binding of the secondary antibody was blocked by incubation with a 1:5 concentration of normal goat serum in TBS for 10 min. The sections were then incubated with either an anti-*bcl*-2 monoclonal antibody (Dakopatts, Denmark) at a concentration of 1:100 or an anti-*bax* polyclonal antibody (Santa Cruz, U.S.A.) at a concentration of 1:200. All incubations were carried out for 1 h at room temperature. This was followed by two washes in TBS and then incubated with a biotinylated secondary antibody, either rabbit anti-mouse for *bcl*-2 or goat anti-rabbit for *bax*, diluted 1:200 in TBS for 30 min. The sections were washed twice in TBS followed by the application of preformed avidin–biotin complex (Dakopatts, Denmark) for 30 min [25]. The bound complexes were visualised by the application of a 0.05% solution of 3-3′-diammobenzadine (DAB; Sigma, U.S.A.), pH 7.6, containing 0.3% hydrogen peroxide as a substrate. Following incubation, the sections were washed and then lightly counterstained in haematoxylin, dehydrated and cover-slipped.

Quantification of immunohistochemistry

Sections stained by immunohistochemistry were scored independently by two examiners in a blinded fashion. Sections were examined at ×40 objective to assess the proportion of cells which had cytoplasmic reaction product with confirmation of positive staining done at ×100 objective. The percentage of positive cells were scored according to the method of Nakagawa *et al.* [26] as follows: 3 = strong staining, more than 50% of cells staining; 2 = moderate staining, between 25 and 50% of cells staining; 1 = week staining, between 5 and 25% of cells staining; 0 = negative, less than 5% of cells staining.

RESULTS

The 30 oral squamous cell carcinomas were obtained from 12 women and 18 men (Table 1). The mean age at time of biopsy was 57 years (range 33–81 years). There were 10 tumours from the floor of the mouth, nine from the tongue, six from the gingiva, two from the retromolar area and one each from the palate, buccal mucosa and tonsil. The tumours were graded into three groups comprising 11 well, 12 moderately and seven poorly differentiated carcinomas.

The distribution of *bcl*-2 and *bax* staining in normal tissues was similar to that previously described [21]. Within lymph nodes, most interfollicular and almost all mantle zone lymphocytes showed strong cytoplasmic immunostaining for *bcl*-2 protein with only a few positive cells seen within the lymphoid follicles. By contrast, *bax* staining was less specific, with more background staining generally seen. In lymph nodes, *bax* staining was strongest within germinal centre cells, but cytoplasmic reactivity was also identified in some interfollicular lymphocytes. In normal epithelium, immunostaining for *bcl*-2 protein was identified in basal keratinocytes and in dendritic cells adjacent to the basement membrane. There was no staining of the suprabasal epithelial cells. There was diffuse *bax* immunostaining in all layers of normal epithelium, but this was strongest in the suprabasal epithelial cells (Fig. 1a, b).

In the tumour group, *bcl*-2 and *bax* staining were seen within the epithelial tumour cells. Overall, 18/30 (60%) carcinomas showed moderate or strong *bcl*-2 expression. For *bax* protein, 19/30 (63%) carcinomas showed moderate or strong immunoreactivity. No relationship was identified between the size or stage of the primary tumours and immunoreactivity of either *bcl*-2 or *bax* proteins.

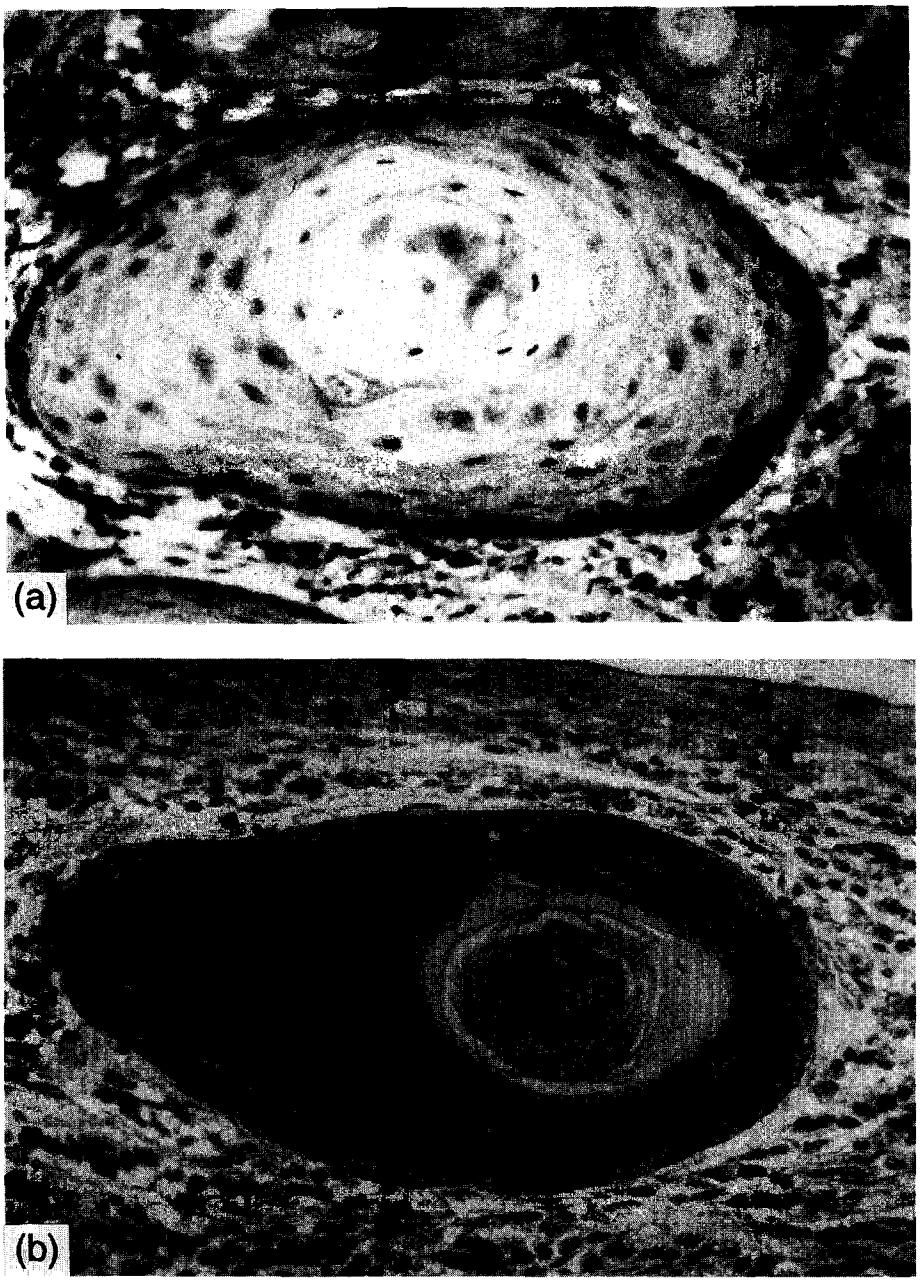

Fig. 2. (a) A well-differentiated squamous cell carcinoma showing *bcl*-2 expression in peripheral cells of a tumour island. Normal lymphocytes also show strong immunostaining. (b) The *bax* protein is expressed in all layers of a tumour island (ABC ×90).

Of the 11 well-differentiated carcinomas, only three (27%) cases showed strong *bcl*-2 staining. By contrast, 8/11 (72%) well-differentiated carcinomas showed strong staining for *bax* protein. The topographical distribution of *bcl*-2 and *bax* immunoreactivity in well-differentiated carcinomas paralleled that seen in normal mucosa with *bcl*-2 staining most prominent in the peripheral cells of the tumour islands. By contrast, *bax* staining generally was restricted to malignant epithelial cells within the centre of tumour islands (Fig. 2a, b)

In the moderately differentiated carcinomas, only 2/12 (17%) cases showed strong *bcl*-2 staining, compared with 8/12 (67%) of tumours using *bax* antibody. In this group, there appeared to be little topographical relationship between the two proteins unless foci of better differentiated tumour islands were present. In these areas, there was an inverse pattern of staining similar to that observed in the well-differentiated carcinomas (Fig. 3a, b)

Of the seven poorly differentiated carcinomas, six (86%) showed strong *bcl*-2 staining. By contrast, strong *bax* immunoreactivity was identified in only two (29%) cases with the remainder of tumours showing only weak or no *bax* staining. No topographical relationship was identified between *bcl*-2 and *bax* expression in this group of tumours.

Dysplastic epithelium adjacent to the carcinomas showed increased *bcl*-2 protein. In contrast to normal epithelium, where *bcl*-2 expression was restricted to basal keratinocytes, in dysplastic epithelium, full thickness immunoreactivity was often seen. In addition, there was evidence of reduced

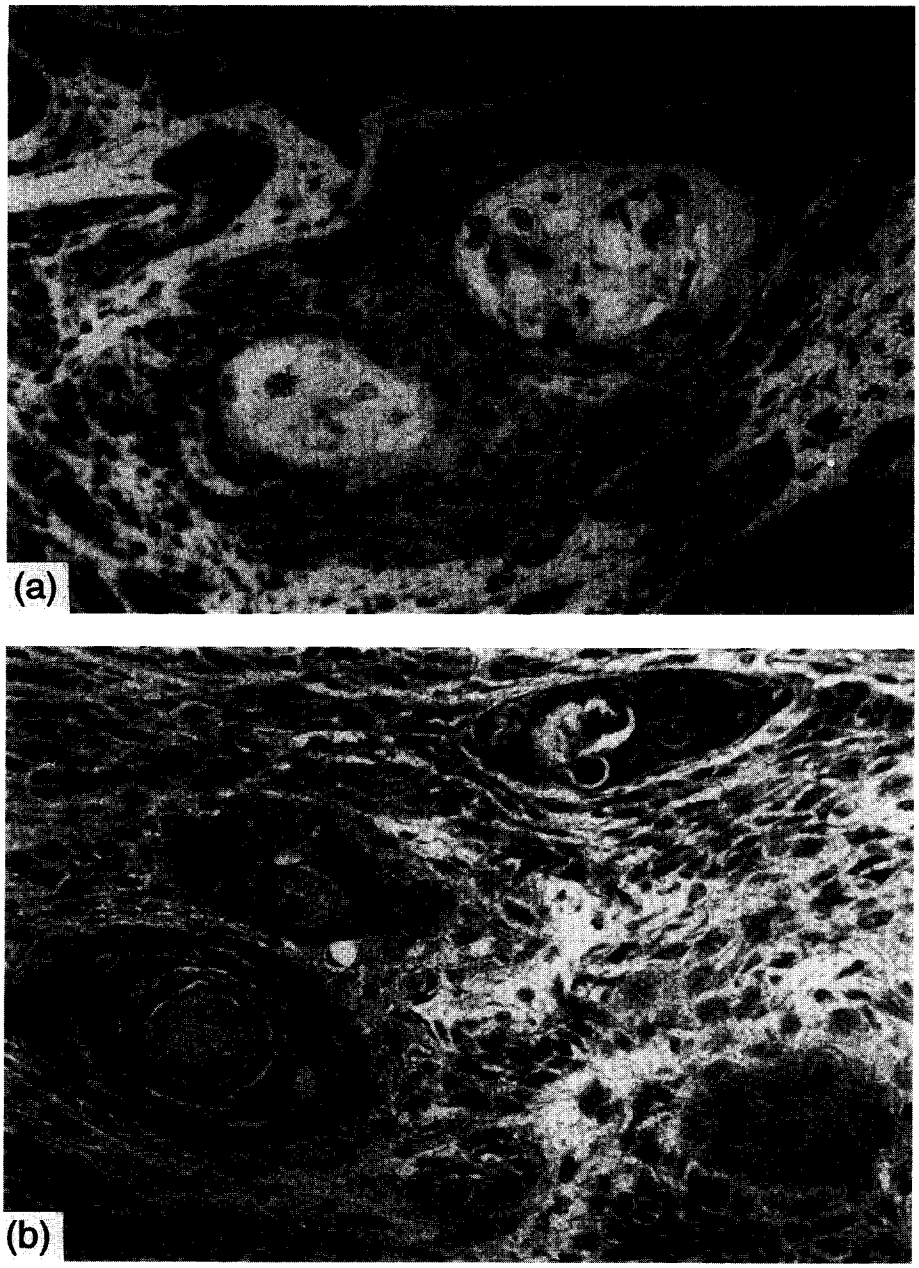

Fig. 3. (a) A moderately differentiated squamous cell carcinoma showing diffuse cytoplasmic expression of *bcl*-2 protein. (b) Cytoplasmic *bax* expression is restricted to more central cells where tumour cells show keratin whorl formation (ABC ×75).

expression of *bax* protein in a number of dysplastic epithelial lesions adjacent to the invasive carcinomas (Fig. 4a, b).

DISCUSSION

The discovery of the *bcl*-2 gene heralded the introduction of a new class of genes which regulate cellular growth through the inhibition of apoptosis. Recently, understanding of the functional role of *bcl*-2 as a cell death repressor has expanded with the discovery that it forms heterodimers with the protein product of the *bax* gene. Overexpression of *bax* counteracts the death repressor activity of *bcl*-2 and it has been suggested that the ratio of *bcl*-2 to *bax* determines survival or death of cells when apoptosis is stimulated [22]. The importance of this family of genes in carcinogenesis is uncertain, but it is thought that the protein product of *bcl*-2 promotes cell survival by inhibiting apoptosis and thereby conferring a survival or growth advantage to the neoplastic cells [27].

Overexpression of *bcl*-2 has been reported in a number of tumours including those arising in breast [15], lung [28], thyroid [13] and skin [26, 29]. This study expands the range of tumours expressing *bcl*-2 by demonstrating protein expression in squamous cell carcinomas of the oral cavity. Overexpression of *bcl*-2 has also been reported in squamous cell carcinomas at other sites including those arising in the skin and lung [17, 26]. The aetiological factors and histological features of non-small cell lung and oral carcinomas are similar and the expression of *bcl*-2 by both tumours is not unexpected. However, moderate or strong *bcl*-2 protein expression was identified in 60% of the tumours in this

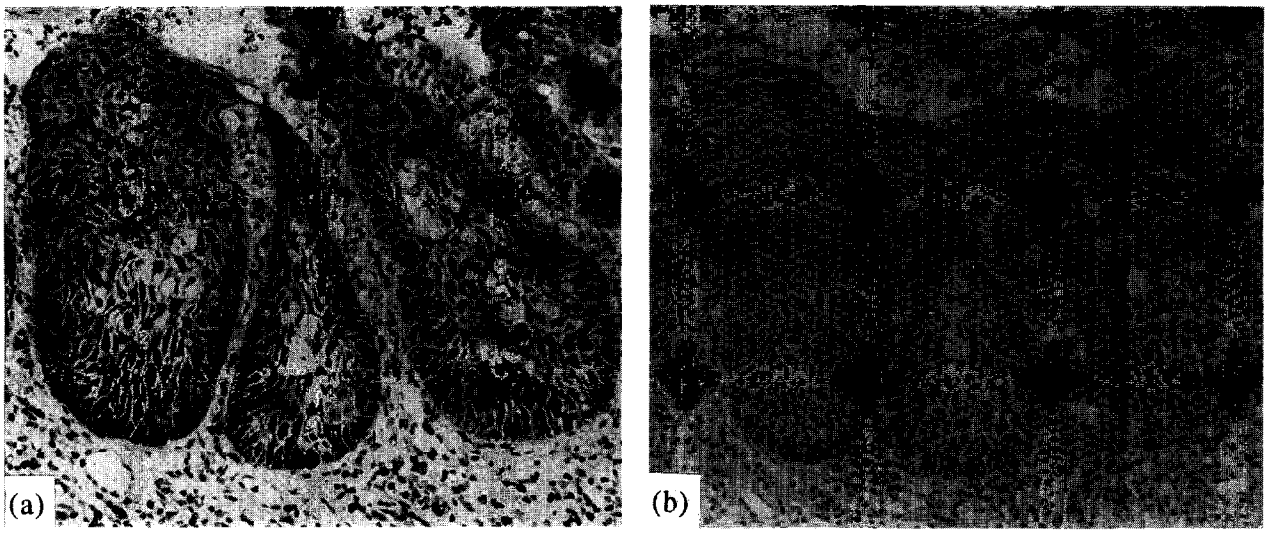

Fig. 4. (a) Oral mucosa with severe epithelial dysplasia showing increased *bcl*-2 expression throughout the thickness of the epithelium. This is in contrast to normal epithelium where *bcl*-2 expession was restricted to basal keratinocytes. (b) In comparison with normal mucosa, where there was full thickness *bax* expression, in the dysplastic epithelium there is reduced expression of *bax* protein (ABC ×75).

series in comparison with studies of non-small cell carcinoma, where the prevalence of immunoreactivity was lower [30]. Differences in quantification of positive cells may only partially account for the differences in expression level and genuinely may reflect the biological difference between tumours at varying sites.

The *bcl*-2 overexpression was most common in the poorly differentiated group of squamous cell carcinomas, where 6/7 (86%) tumours showed strong staining, in contrast to only 3/11 (27%) in the well-differentiated group. By contrast, *bax* immunoreactivity was more common in the well-differentiated carcinomas with 8/11 (72%) showing strong staining, as compared with only 2/7 (28%) of the poorly differentiated tumours. These findings are consistent with certain tumours at other sites, such as neuroblastoma, where *bcl*-2 expression is linked to poorer tumour differentiation [31]. However, the lack of correlation between *bcl*-2 expression and tumour size or clinical stage in this study may reflect the small study population and suggests that analysis of a larger cohort is needed to determine if overexpression has prognostic significance.

The inverse expression pattern of *bcl*-2 and *bax* in normal epithelium is consistent with the fact that *bcl*-2 is lacking in terminally differentiated cells capable of apoptosis such as suprabasal keratinocytes [32]. In the well-differentiated carcinomas, *bcl*-2 and *bax* expression recapitulated the pattern seen in normal epithelium with *bcl*-2 expression strongest in the peripheral cells of the epithelial islands and reduced with keratin production. In the poorly differentiated group, the increased *bcl*-2 expression may reflect the lost ability of malignant keratinocytes for terminal differentiation and suggests that those cells overexpressing *bcl*-2 have a stem cell phenotype. This is further supported by the reduced expression of *bax* in the poorly differentiated tumours and suggests that in epithelial carcinomas the ratio of *bcl*-2 and *bax* may contribute to the phenotype of the tumour cell population. Hence, a predominance of *bcl*-2 has an anti-apoptotic effect on the cells and favours a stem cell phenotype. By contrast, a predominance of *bax* promotes apoptosis and favours a terminal differentiation pathway for the neoplastic keratinocytes.

Oral carcinoma is thought to develop in antecedent mucosal epithelial dysplasia. Similar to epithelial dysplasia at other sites, three well-defined groups are recognised, each with a varying risk of transformation to carcinoma [33]. The genetic events associated with the evolution of epithelial dysplasia to carcinoma have not been well characterised, but alterations of oncogenes and tumor-supressor genes have been reported in precancerous lesions [34–36]. The finding of upregulated *bcl*-2 protein in dysplasia adjacent to invasive carcinoma suggests that this change is an early event in epithelial carcinogenesis and is consistent with reports of early upregulation of *bcl*-2 in dysplastic lesions at other sites [37, 38]. These results suggest that an alternative mechanism involving genes that regulate apoptosis may be important in epithelial carcinogenesis. According to this model, increased expression of *bcl*-2 and/or downregulation of *bax* would permit prolonged cell survival and a selective growth advantage to the dysplastic epithelial cells. The potential for the emergence of a neoplastic clone of cells susceptible to a further mutagenic event involving an oncogene or tumour-suppressor then would exist. Currently, studies are being undertaken to examine the expression of *bcl*-2 and *bax* in a large series of dysplastic epithelial lesions.

In conclusion, this pilot study shows an altered expression of *bcl*-2 and *bax* proteins in oral squamous cell carcinomas. The *bcl*-2 protein was most frequently overexpressed in poorly differentiated carcinomas and *bax* in the well-differentiated tumours. Furthermore, the identification of strong *bcl*-2 overexpression in dysplastic lesions adjacent to the invasive tumours raises the possibility that *bcl*-2 dysregulation is an important early event in carcinogenesis of mucosal epithelium.

1. Boyle P, Macfarlane GJ, Maisonneuve P, Zheng T, Scully C, Tedesco B. Epidemiology of mouth cancer in 1989: a review. *J R Soc Med* 1990, **83**, 724–730.

2. Field JK. Oncogenes and tumour-suppressor genes in squamous cell carcinoma of the head and neck. *Oral Oncol, Eur J Cancer* 1992, **28B,** 67–76.
3. Field JK, Spandidos DA, Stell PM. Overexpression of the p53 gene in head and neck cancer, linked with heavy smoking and drinking. *Lancet* 1992, **339,** 502–503.
4. Brennan JA, Mao L, Hruban RH, *et al.* Molecular assessment of histopathological staging in squamous cell carcinoma of the head and neck. *N Engl J Med* 1995, **332,** 429–435.
5. Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM. The t(14;18) chromosome translocations involved in B-cell neoplasms results from mistakes in VDJ joining. *Science* 1985, **299,** 1390–1393.
6. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. *Cell* 1986, **47,** 19–28.
7. Pezzella F, Ralfkiaer E, Gatter KC, Mason DY. The 14;18 translocation European cases of follicular lymphoma: comparison of Southern blotting and polymerase chain reaction. *Br J Haematol* 1990, **76,** 58–64.
8. Aisenberg AC, Wilkes BM, Jacobson JO. The bcl-2 gene is rearranged in many diffuse B-cell lymphomas. *Blood* 1988, **71,** 969–972.
9. Tsujimoto Y, Croce CM. Analysis of the structure, transcripts and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc Natl Acad Sci USA* 1986, **83,** 5214–5218.
10. Hockenbery DM, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 1990, **348,** 334–336.
11. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. *bcl-2* protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc Natl Acad Sci USA* 1991, **88,** 6961–6965.
12. Lu Q, Poulsom R, Wong L, Hanby AM. bcl-2 Expression in adult and embryonic non-haematopoietic tissues. *J Pathol* 1993, **169,** 431–437.
13. Pilotti S, Collini P, Cattoretti G, Del Bo R, Pierotti MA. bcl-2 protein expression in carcinomas originating from the follicular epithelium of the thyroid gland. *J Pathol* 1994, **172,** 337–342.
14. Ikegaki N, Katsumata M, Minna J, Tsujimoto Y. Expression of bcl-2 in small cell lung carcinoma cells. *Cancer Res* 1994, **54,** 6–8.
15. Leek RD, Kaklamanis L, Pezzella F, Gatter KC, Harris AL. bcl-2 in normal human breast and carcinoma, association with oestrogen receptor-positive, epidermal growth factor receptor-negative tumours and in situ cancer. *Br J Cancer* 1004, **69,** 135–139.
16. Lu QL, Elia G, Lucas S, Thomas JA. bcl-2 proto-oncogene expression in Epstein–Barr virus-associated nasopharyngeal carcinoma. *Int J Cancer* 1993, **53,** 29–35.
17. Pezzella F, Turley H, Kuzu I, *et al.* bcl-2 protein in non-small-cell lung carcinoma. *N Engl J Med* 1993, **329,** 690–694.
18. Yunis JJ, Mayer MG, Arensen MA, Aeppli DP, Oken MM, Frizzera G. bcl-2 and other genomic alterations in the prognosis of large cell lymphomas. *N Engl J Med* 1989, **320,** 1047–1054.
19. McDonnell TJ, Troncoso P, Brisbay SM, *et al.* Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res* 1992, **52,** 6940–6944.
20. Campos L, Roualult J, Sabido O, *et al.* High expression of bcl-2 in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood* 1993, **18,** 3091–3096.
21. Krajewski S, Krajewska M, Shabaik A, Miyashita T, Wang HG, Reed JC. Immunohistochemical determination of *in vivo* distribution of bax, a dominant inhibitor of bcl-2. *Am J Pathol* 1994, **145,** 1323–1336.
22. Oltvai ZN, Milliman CL, Korsmeyer SJ. bcl-2 heterodimerizes *in vivo* with a conserved homolog, bax, that accelerates programmed cell death. *Cell* 1993, **74,** 609–619.
23. Wahl PN, Cohen B, Luthra UK, Torloni H *Histological Typing of Oral and Oropharyngeal Tumours.* Geneva, WHO 1971, 17–18.
24. Norton AJ, Jordan S, Yeomans P. Brief, high-temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 1994, **173,** 371–379.
25. Hsu SM, Raine L, Fanger H. Use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981, **29,** 577–580.
26. Nakagawa K, Yamamura K, Maeda S, Ichihashi M. *bcl-2* expression in epidermal keratinocytic diseases. *Cancer* 1994, **74,** 1720–1724.
27. Korsmeyer SJ. bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood* 1992, **80,** 879–886.
28. Pezzella F, Tse AGD, Cordel JL, Pulford KAF, Gatter KC, Mason DY. Expression of the bcl-2 oncogene protein is not specific for the 14;18 chromosomal translocation. *Am J Pathol* 1990, **137,** 225–232.
29. Ramsay JA, From L, Kahn HJ. *bcl-2* protein expression in melanocytic neoplasms of the skin. *Modern Pathol* 1995, **2,** 150–154.
30. Pezzella F, Turley H, Kuzu I, *et al.* bcl-2 protein in non-small-cell lung. *N Engl J Med* 1993, **329,** 690–694.
31. Castle VP, Heidelberger KP, Bromberg J, Ou XG, Dole M, Nunez G. Expression of the apoptosis-suppressing protein *bcl-2,* in neuroblastoma is associated with unfavorable histology and N-*myc* amplification. *Am J Pathol* 1993, **143,** 1543–1550.
32. McCall CA, Cohen JJ. Programmed cell death in terminally differentiating keratinocytes: role of endogenous endonuclease. *J Invest Dermatol* 1991, **97,** 111–114.
33. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol* 1995, **79,** 321–329.
34. Voravud N, Shin DM, Ro JY, Lee JS, Hong WK, Hittelman WN. Increased polysomies of chromosomes 7 and 17 during head and neck multistage tumorigenesis. *Cancer Res* 1993, **53,** 2874–2883.
35. Ogden GR, Kiddie RA, Lunny DP, Lane DP. Assessment of p53 protein expression in normal, benign, and malignant oral mucosa. *J Pathol* 1992, **166,** 389–394.
36. Huang WY, Coltrera M, Schubert M, Morton T, Truelove E. Histopathologic evaluation of proliferating cell nuclear antigen (PC10) in oral epithelial hyperplasias and premalignant lesions. *Oral Surg Oral Med Oral Pathol* 1994, **78,** 748–754.
37. Bronner MP, Culin C, Reed JC, Furth EE. The *bcl-2* proto-oncogene and the gastrointestinal epithelial tumor progression model. *Am J Pathol* 1995, **146,** 20–26.
38. Lauwers GY, Scott GV, Hendricks J. Immunohistochemical evidence of aberrant *bcl-2* protein expression in gastric epithelial dysplasia. *Cancer* 1994, **73,** 2900–2904.