IMMUNOHISTOCHEMICAL EVALUATION OF bcl-2 ONCOPROTEIN IN ORAL DYSPLASIA AND CARCINOMA

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Abstract

The proto-oncogene bcl-2 is associated with follicular lymphoma involving translocation t (14; 18) (q32; q21) and is also over expressed in various neoplasms. Investigations into the sequential over expression of this oncoprotein in precancerous and cancerous lesions of the oral mucosa are lacking, and the possible role of bcl-2 in oral tumour progression remains unknown.

Objectives: Objectives of the study were to evaluate the expression of bcl-2 oncoprotein in severe epithelial dysplasia and different grades of Oral squamous cell carcinoma.

Methods : Immunohistochemical analysis were carried out in 10 cases of severe epithelial dysplasia & 10 cases each of well differentiated, moderately differentiated and poorly differentiated oral squamous cell carcinomas.

Results & Conclusion: - Over expression of this oncoprotein was seen in severe epithelial dysplasia involving entire thickness of the epithelium with its down regulation in differentiating carcinomas, suggesting the role of this oncoprotein in early stages of tumour progression.

Introduction

Oral cancer is one of the most formidable health problems in terms of morbidity and mortality facing the mankind today. It is the sixth most common cancer worldwide accounting for 4% of all malignancies in men and 2% in women[1]. Despite the advances in the detection and management of cancers, survival rates have remained poor with a 5 year mortality rate of 50%[1,2].

Recent studies on cancer have shown that the process of carcinogenesis may involve not only increased cell proliferation but also decreased apoptosis or increased cell survival[3]. Normally the elimination of genetically damaged cells by apoptosis precludes the development of tumors. On the contrary, an imbalance of the apoptotic pathway contributes to the immortalization of replicating cells, thus favoring the accumulation of sequential genetic damage that normally would induce cell death[4,5].

Deregulation of oncogenes and tumor suppressor genes have been associated with tumor development and progression[5]. Some proto-oncogenes are known to prevent apoptosis. The bcl-2 is an important member having a relevant role in tumor development by inhibiting apoptosis.

The term bcl-2 is an acronym for B cell lymphoma/leukemia – 2 gene[6]. The human bcl-2 protein is an intracellular, integral membrane protein, with a molecular mass of ~26Kda[6]. They are found in the nuclear envelope, outer mitochondrial membrane, plasma membrane and endoplasmic reticulum[6,7]. This was first discovered in B cell lymphomas with a chromosomal translocation t (14:18) (q32:q21) and is associated with the development of lymphomas[7]. This cytogenetic abnormality places the bcl-2 gene from chromosome 18 into juxtaposition with the transcriptionally active immunoglobulin heavy-chain locus on chromosome 14, resulting in inappropriately high levels of bcl-2 gene expression[6]. The protein encoded by this gene contributes to malignant cell expansion primarily by prolonging cell survival rather than by increasing the rate of cellular proliferation[7]. The specific mechanism by which bcl-2 protein extends cell survival remains enigmatic, but high levels of bcl-2 protein have been shown to delay or block programmed cell death (apoptosis) in a variety of circumstances. Currently, three models are used to explain bcl-2 function. These models describe bcl-2 proteins as transmembrane ion channels or

as proteins that modulate activation of caspases or as inhibitors of cytochrome c export from mitochondria[8].

Bcl-2 is expressed in cells in proliferating zones and cells with long life spans and is down regulated in terminally differentiated cells[9]. Topographically, bcl-2 gene expression has been identified in basal cells of normal oral mucosa, but not in superficial cells[8,9]. Alterations in bcl-2 expression play a role in cellular differentiation and development of tumors. Studies have shown that this oncoprotein has a role in early stages of tumor progression as it is up regulated in sequentially progressing epithelial dysplasia and down regulated in differentiating carcinomas[8, 10].

In the present study an attempt has been made to evaluate the expression of bcl-2 oncoprotein in oral dysplasia and carcinoma in order to determine whether this oncoprotein has a key role in early stages of tumor progression and whether this can be used as a marker for early detection and elucidation of oral cancer.

Materials and methods.

The material for the study includes 40 formalin fixed paraffin embedded tissue blocks of oral squamous cell carcinoma. Among the 40 blocks, 10 blocks each from histologically diagnosed well, moderately and poorly differentiated oral squamous cell carcinomas and severe epithelial dysplasia were considered for immunohistochemical staining for bcl-2.

The bcl-2 immunohistochemical kit [Super Sensitive Polymer-HRP Detection System-A Biotin Free Detection System (QD400-60K Super Sensitive Polymer-HRP Detection kit HRP/DAB)] was obtained from Biogenex Laboratories.

Scoring

The bcl-2 stained sections were studied in detail and the tissue sections were graded as follows. On light microscopic examination, only those cells exhibiting cytoplasmic staining for bcl-2 were counted as positive.

Three high power microscopic fields were randomly selected in the positively stained area in each slide. A total of 100 cells were counted in each field and the percentage of cells immunoreactive for bcl-2 oncoprotein was calculated. Each slide was scored according to the grading system by Singh[9] et al which categorizes more than 50% of cells positive as +++ (3+); 25 – 50% positive as ++(2+); 10 – 24% positive cells as +(1+); 5 – 9% positive as +/- and fewer than 5% positive as negative (-). The statistical analysis was performed by using Fischer's exact test and a probability of $p < 0.05$ was considered as significant.

Results and Observations

Out of 10 cases of severe epithelial dysplasia, 8 cases (80%) exhibited intense reactivity that involved nearly the entire thickness of epithelium and 2 cases (20%) exhibited moderate reactivity.(Fig-1)

All grades of SCC showed heterogeneous immunoreactivity based on the degree of terminal cell differentiation (keratinization). Generally, peripheral cells of differentiating epithelial islands were intensely stained with decreasing immunoreactivity towards the centre.

Among the 10 cases of well differentiated SCC, 3 cases (30%) exhibited moderate positivity and 7 cases (70%) exhibited weak positivity.(Fig-2)

Of the 10 cases of moderately differentiated SCC, 8 cases (80%) exhibited moderate positivity and 2 cases (20%) exhibited intense immunoreactivity.(Fig-3)

However in 10 cases of poorly differentiated SCC, 4 cases (40%) exhibited moderate positivity and the remaining 6 cases (60%) exhibited intense reactivity.(fig-4)

Correlation between histopathologic diagnoses and degrees of bcl-2 expression in severe epithelial dysplasia and different grades of oral SCC were tabulated. (Table I).

Comparison was made between each group of well, moderately and poorly differentiated SCC's for the degree of bcl-2 expression using Fischer's exact test.

While comparing the degree of bcl-2 expression in well and moderately differentiated SCC, it was found to be statistically significant ($p<0.05$) (Table II).

While comparing the degree of bcl-2 expression in well and poorly differentiated SCC, it was also found to be statistically significant. ($p<0.05$) (Table III).

Finally, on comparing the degree of bcl-2 expression in moderately and poorly differentiated SCC, it was also found to be statistically significant (Table IV).

TABLE I: CORRELATION BETWEEN HISTOPATHOLOGIC DIAGNOSIS AND DEGREE OF bcl-2 EXPRESSION IN ORAL DYSPLASIA AND CARCINOMA

	Degree of bcl-2 Expression				
Diagnosis (No.of cases)	+++	++	+	±	-
Severe dysplasia (10)	8	2	0	0	0
Poorly differentiated (10)	6	4	0	0	0
Mod. differentiated (10)	0	8	2	0	0
Well differentiated (10)	0	3	7	0	0

TABLE II: COMPARISON BETWEEN DEGREE OF bcl-2 EXPRESSION IN WELL AND MODERATELY DIFFERENTIATED ORAL SCC

Diagnosis	Degree of bcl-2 Expression	
	++	+
Well differentiated SCC	3	7
Mod. differentiated SCC	8	2

TABLE III: COMPARISON BETWEEN DEGREE OF bcl-2 EXPRESSION IN WELL AND POORLY DIFFERENTIATED ORAL SCC

Diagnosis	Degree of bcl-2 Expression	
	+++	++/+
Well differentiated SCC	0	10
Poorly differentiated SCC	6	4

TABLE IV: COMPARISON BETWEEN DEGREE OF bcl-2 EXPRESSION IN MODERATELY AND POORLY DIFFERENTIATED ORAL SCC

Diagnosis	Degree of bcl-2 Expression	
	+++	++/+
Moderately differentiated SCC	0	10
Poorly differentiated SCC	6	4

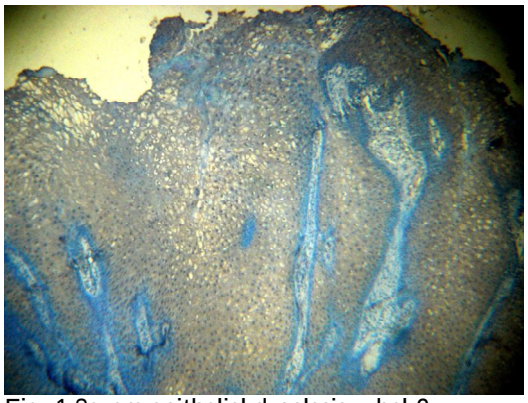
Fig -1 Severe epithelial dysplasia – bcl-2

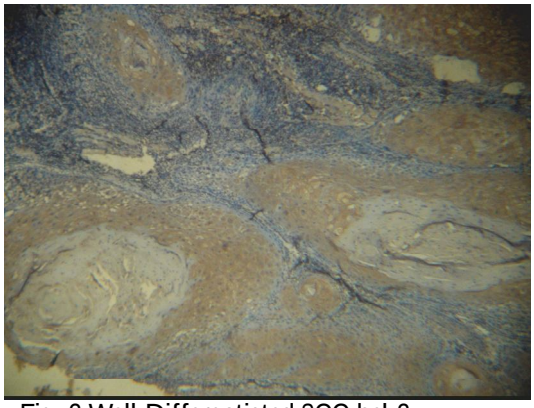
Fig -2 Well Differentiated SCC bcl-2

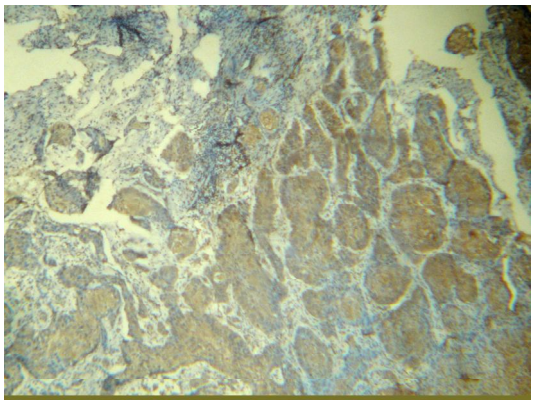
Fig -3 Moderately Differentiated SCC bcl-2

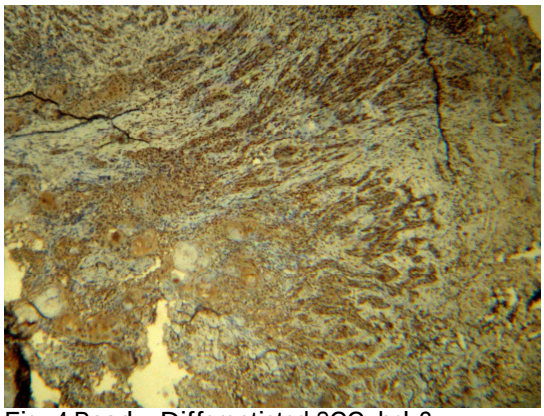
Fig -4 Poorly Differentiated SCC bcl-2

Discussion

This study demonstrates deregulation of bcl-2 oncoprotein expression in 10 cases each from severe epithelial dysplasia and varying grades of oral SCC. It was found that bcl-2 was over expressed in cells with histopathologic features of epithelial dysplasia and in peripheral cells of the islands of differentiating squamous cell carcinomas. Moderately and poorly differentiated SCC also over expressed this oncoprotein with a diffuse and intense staining pattern. But, there was a decrease in bcl-2 immunoreactivity in terminally differentiated cells (keratinizing cells).

In the 10 cases of severe epithelial dysplasia, 8 cases (80%) showed intense immunoreactivity and 2 cases (20%) exhibited moderate reactivity. There was a marked immunoreactivity that nearly involved the entire thickness of the epithelium with diminishing reaction to the superficial layers. This finding was in accordance with the findings of Singh[9] et al, Bronner[11] et al , Lai Fa Shu[12] et al & Birchall[13] et al. This may be due to the inhibition of apoptosis and offer an advantage to the rapidly growing tumor by slowing down the cell death rate[14]. This enhancement of bcl-2 expression in epithelial dysplasia may be a factor in early stages of carcinogenesis. Our observations support the hypothesis that the enhanced bcl-2 expression which prevents apoptosis may be a common early event and play an important role in the development of some tumors of epithelial origin.

Many studies have shown that transformation rate of oral dysplasia to invasive carcinoma is approximately 13.8%[15]. The genetic events associated with the evolution of epithelial dysplasia to invasive carcinoma have not been well characterized, but alteration of oncogenes and tumor suppressor genes have been reported[15]. In severe epithelial dysplasia there was

intense bcl-2 immunoreactivity involving almost the entire thickness of the epithelium. Studies of bcl-2 expression in dysplastic lesions at other sites have also shown an up regulation of this protein[16]. They had proposed that this up regulation permits prolonged cell survival and a selective growth advantage to the dysplastic epithelial cells and then will exist the potential for emergence of a neoplastic clone of cells susceptible to a further mutagenic event[2].

Attempts were also made to assess the expression of bcl-2 oncoprotein in various grades of oral SCC and found over expression was most common in poorly differentiated group where 6/10 cases (60%) exhibited intense immunoreactivity and the remaining 4 cases (40%) showed only moderate staining intensity. By contrast, in well-differentiated group, 7/10 cases (70%) exhibited weak immunoreactivity and 3/10 cases (30%) showed moderate intensity. Moreover, in differentiated tumors, it was found that the peripheral cells of the tumor islands showed marked over expression with decreasing intensity towards the centre (Keratinizing areas). These findings were also consistent with the earlier studies[2,17]

The over expression pattern in moderately differentiated squamous cell carcinomas falls in between well and poorly differentiated groups in which 8/10 cases (80%) showed moderate staining intensity and 2/10 cases (20%) showed intense immunoreactivity. These findings are consistent with certain tumors at other sites such as colorectal neoplasia[18], small cell lung carcinoma[19], etc where bcl-2 expression is linked to poorer tumor differentiation. This further emphasizes the over expression of bcl-2 in poorly differentiated carcinomas and down regulation in well differentiated carcinomas.

In the present study, the bcl-2 expression in poorly differentiated OSCC was intense and this finding is in accordance with earlier findings. We hypothesize that this up regulation may reflect the lost ability of malignant keratinocytes for terminal differentiation and suggest that those cells over expressing bcl-2 have a stem cell phenotype. A predominance of bcl-2 has an ant apoptotic effect on the cells and favours a stem cell phenotype.

In moderately differentiated group, the bcl-2 expression was of moderate intensity falling between well and poorly differentiated groups which are in agreement with the findings by Martin C Jackel[20]et al, Leahy[14] et al, & Ravi[17] et al.

In this study, a direct correlation was found between bcl-2 immunoreactivity and tumor differentiation. That is, it is up regulated in poorly differentiated carcinoma and sequentially down regulated in moderately and well differentiated squamous cell carcinomas. This down regulation of bcl-2 in differentiating carcinomas indicates that this oncoprotein may play a key role in progression of oral neoplasia.

This study demonstrates bcl-2 oncoprotein over expression in all phases of oral neoplasia, and we conclude that it may play a key role in the early stages of oral tumor genesis. In addition, bcl-2 expression appears to be inversely related to the degree of epithelial differentiation.

Conclusion

This study suggests that the bcl-2 protein participates in the control of terminal differentiation of normal oral keratinocytes by protecting their stem cells from apoptosis. Also, the results of the current study highlight the possibility that bcl-2 may be effectively employed to characterize oral SCC better and to possibly predict its biologic behavior.

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