Immunocytochemical analysis of AE1/AE3, CK 14, Ki-67 and p53 expression in benign, premalignant and malignant oral tissue to establish putative markers for progression of oral carcinoma

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ABSTRACT

Squamous cell carcinoma (SCC) is the most common form of oral malignancy and is often preceded by premalignant lesions, some of which are more likely to progress to carcinoma than others. In this study, a panel of monoclonal antibodies (AE1/AE3, cytokeratin [CK] 14, Ki-67 and p53) is applied to 10 cases of human oral tissue in each of six categories to establish staining patterns indicative of which lesions are more likely to progress to malignancy. The six tissue categories are normal tissue; abnormal benign lesions; mild, moderate and severe dysplasia; and SCC. A statistical analysis of Ki-67 and p53 immunoexpression is performed. The results showed that AE1/AE3 and CK 14 expression was reduced as a late event in oral carcinogenesis, particularly in poorly differentiated SCC. Expression of Ki-67 and p53 proved to be a weak but statistically significant predictor of malignant progression in oral tissue.

KEY WORDS: Dysplasia. Immunohistochemistry. Mouth neoplasms. Neoplasms, squamous cell.

Introduction

Carcinoma of the oral cavity shows a strong association with the habitual use of tobacco and is particularly prevalent in countries where extensive, persistent smoking or chewing of tobacco-related products is a common component of society.[1,2] In 1999 in England and Wales, 1670 deaths were attributed to carcinoma of the lip, mouth or pharynx.[3] Government statistics for England and Wales also demonstrate that the incidence of carcinoma of the lip, mouth or pharynx increases over the age of 40 years in either sex.[3]

Squamous cell carcinoma (SCC) is the most common form of malignancy that affects the oral cavity, representing approximately 90% of all oral malignancies, and is frequently preceded by premalignant lesions of variable histopathological severity.[4–7] Such lesions display evidence of abnormal cellular changes collectively known as dysplasia.[5–8]

Dysplastic cellular changes are assigned a histopathological grade (mild, moderate or severe) depending on the extent of individual cellular and architectural alterations present microscopically.[5–8] In mildly dysplastic lesions changes are confined to the lower third of the epithelium, moderate dysplasia extends into the lower two-thirds of the epithelium, and severely dysplastic lesions extend throughout the entire thickness of the epithelium.[8] Some dysplastic lesions may regress but others continue to increase in histopathological severity and eventually develop into malignancy.[5–9]

Much research has attempted to establish why some premalignant lesions show a greater tendency to progress to carcinoma than others, so that lesions which require closer clinical monitoring can be identified and treated.[9–14]

In this study, a panel of monoclonal antibodies is applied to 60 cases of human tissue from various sites within the oral cavity. The panel of antibodies is designed to include differentiation, proliferation and cell cycle regulatory markers, in order to provide information about different aspects of development within normal, abnormal benign, premalignant and malignant oral tissue.

Cytokeratins (CK) constitute classes I and II of the five classes of intermediate filament proteins that form the cytoskeleton of most eukaryotic cells.[15,16] The profile of CK types expressed by epithelial cells varies at different stages of differentiation, thus cytokeratins can act as markers of epithelial differentiation and cell type.[14–20] Expression of CKs can be altered in premalignant lesions and in carcinoma.[14–20]

The AE1/AE3 antibody used in this investigation (Dako M3515) is a cocktail of specific antibodies for human CKs. The AE1 monoclonal component recognises type A acidic cytokeratins 10, 13, 14, 15, 16 and 19, whereas the monoclonal AE3 clone recognises type B neutral/basic CKs 1, 2, 3, 4, 5, 6, 7 and 8. Therefore, the AE1/AE3 antibody can be

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Table 1. Location of immunocytochemical staining observed with the AE1/AE3, CK14, Ki-67 and p53 antibodies.

Tissue category	Antibodies																			
	AE1/AE3 Staining location					CK14 Staining location					Ki-67 Staining location					p53 Staining location				
	BL	1/3	2/3	3/3	TI	BL	1/3	2/3	3/3	TI	BL	1/3	2/3	3/3	TI	BL	1/3	2/3	3/3	TI
Normal	0	0	0	10	n/a	10	0	0	10	n/a	7	3	0	0	n/a	8	0	0	0	n/a
ABL	1	0	0	10	n/a	10	0	0	10	n/a	6	4	0	0	n/a	8	2	0	0	n/a
Mild dysplasia	2	0	0	10	n/a	10	0	0	10	n/a	3	5	2	0	n/a	2	5	0	0	n/a
Moderate dysplasia	1	0	0	10	n/a	10	0	0	10	n/a	1	3	6	0	n/a	0	2	4	0	n/a
Severe dysplasia	5	0	0	10	n/a	10	0	0	10	n/a	10	0	0	10	n/a	1	0	0	7	n/a
SCC	5	0	0	10	8	10	0	0	10	10	10	0	0	10	10	1	0	2	3	8

BL: positive cell staining within basal layer of epithelium.
1/3: positive cell staining within lower third of oral epithelium.
2/3: positive cell staining within lower two thirds of oral epithelium .
3/3: positive cell staining within entire thickness of oral epithelium.
TI: positive cell staining within malignant tumour islands.

Table 2. Immunocytochemical staining intensities observed with the AE1/AE3, CK14, Ki-67 and p53 antibodies.

Tissue category	Staining intensity of antibodies															
	AE1/AE3				CK14				Ki-67				p53			
	0	+	++	+++	0	+	++	+++	0	+	++	+++	0	+	++	+++
Normal tissue	0	0	10	0	0	0	0	10	0	10	0	0	2	8	0	0
ABL	0	0	9	1	0	0	1	9	0	6	4	0	1	6	3	0
Mild dysplasia	0	2	6	2	0	0	8	2	0	4	6	0	3	1	5	1
Moderate dysplasia	0	2	7	1	0	0	0	10	0	2	6	2	4	2	1	3
Severe dysplasia	0	1	4	5	0	0	2	8	0	2	4	4	2	3	2	3
SCC	0	5	4	1	0	3	3	4	0	0	6	4	2	0	3	5

0: negative (no positive antibody staining).
+: weak (AE1/AE3, CK14 = antibody staining sparse/patchy) (Ki-67,p53 = average of 1-50 positive cells).
++: moderate (AE1/AE3, CK14 = antibody staining quite strong, but with patchy/negative/weaker staining areas) (Ki-67, p53 = average of 51-150 positive cells).
+++: intense (AE1/AE3, CK14 = antibody staining consistently strong) (Ki-67, p53 = average exceeding 150 positive cells).

used as a marker for squamous cells and in distinguishing carcinomas from tumours of non-epithelial origin.[17]

The basal layer of stratified squamous epithelium, such as that found within the oral cavity, expresses CK 5 and CK 14.[14–20] Monoclonal CK 14 antibody stains the basal layer of stratified squamous epithelium and therefore also can be used to distinguish tumour cells of squamous origin and highlight malignant invasion of the basal layer.[14–20]

Proliferating cells begin to express the non-histone nuclear protein Ki-67 during S phase of the cell cycle, with levels increasing during the G_2 phase and peaking during the mitotic phase.[13,21] The level of Ki-67 antigen decreases during the G1 phase. No Ki-67 antigen is detectable during the G_O quiescent phase.[13]

Proliferating cells can be detected immunocytochemically using monoclonal Ki-67 antibody.[11,13,21] Increased cellular proliferation is indicative of a disturbance of normal cell growth and is frequently associated with premalignant lesions and malignancy.[11,13,21] Assessment of Ki-67 expression in premalignant lesions of the oral cavity may provide a marker of abnormal growth and identify those lesions which may possess a greater potential to malignant transformation and progression than others.[13,21]

The *p53* tumour suppressor gene has an essential role in the regulation of cell proliferation and is the most frequently mutated gene in many cancers, including oral carcinoma.[11– 13,22,23] Mutated p53 protein has a prolonged half-life in comparison to the wild-type form and accumulates in the nuclei of cells, making it possible to detect immunocytochemically with monoclonal p53 antibody.[11–13,22,23] Increased expression of p53 within cells can often indicate malignant transformation and an underlying disruption of cell-cycle regulation.[11–13,22,23]

The present investigation has two aims. First, to perform a qualitative assessment of differences in immunostaining patterns between and within the diagnostic categories, using the monoclonal antibody panel previously described, in order to identify lesions that are more likely to progress to

Table 3. Summary of multiple regression analysis: p53 and Ki-67 antibodies.

Multiple regression coefficient (R)	*P* value (p53 and Ki-67)
0.639	<0.001

Statistically significant difference: $P<0.05$
Independent variables: p53 and Ki-67 staining
Dependant variable: histopathological grade.

Table 4. One-way ANOVA: p53 and Ki-67 antibodies.

p53	Ki-67
0.007	0.000

Significant: $P<0.05$

malignancy. Second, to quantify the mean number of cells that stain with the Ki-67 and p53 antibodies in all cases and to identify statistically significant differences in the mean number of positive cells between diagnostic categories. This may indicate a role for these two antibodies in the prediction of malignant progression in oral tissue, and to assist in the development of a statistical model to aid accurate diagnosis of oral lesions.

Materials and methods

Selection of appropriate tissues

Ten representative cases from each of the following categories were selected from specimens received by the histopathology department at Charing Cross Hospital, London, between 2000 and 2002: normal oral tissue; oral tissue showing benign lesions (squamous papillomas) or inflammatory changes (including oral lichen planus); oral tissue displaying evidence of mild/moderate/severe dysplasia; and invasive oral SCC.

All tissues used in this investigation were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Haematoxylin and eosin (H&E)-stained sections were reviewed to confirm the presence of the relevant histopathological features. Known positive control tissues were used for each antibody. For each tissue section treated with one of the four antibodies, a corresponding negative control section was included, replacing the primary antibody with Tris-buffered saline (TBS; pH 7.6) in the immunocytochemical technique used.

Ethical approval for this investigation was granted by the Riverside Research Ethics Committee, London.

Immunocytochemical analysis

After removal of paraffin wax in xylene and immersion in 99% industrial methylated spirit, tissue sections were immersed in a 2% solution of 30% hydrogen peroxide in 300 mL methanol for 10 min to block endogenous peroxidase activity. Antigen retrieval for sections to which the AE1/AE3 monoclonal antibody was applied involved immersion in 0.01g protease in 100 mL preheated TBS (pH 7.6) for 5 min at 37°C. Antigen retrieval for those sections treated with the p53, Ki-67 and CK14 monoclonal antibodies involved immersion in 1400 mL 0.01 mol/L sodium citrate buffer (pH 6.0) for 90 sec at full pressure in a pressure cooker. After antigen retrieval, all sections were rinsed in distilled water and TBS (pH 7.6). All sections were then treated with 200 µL 10% horse serum (Serotec) for 10 min to minimise non-specific background staining.

The primary antibodies used were as follows: Novocastra NCL p53-D07 mouse monoclonal antibody (diluted 1 in 200 in TBS [pH 7.6]); Novocastra NCL Ki-67-MM1 mouse monoclonal antibody (diluted 1 in 200); Novocastra NCL LL002 (CK 14-specific) mouse monoclonal antibody (diluted 1 in 50); DakoCytomation mouse monoclonal AE1/AE3 antibody (M3515; diluted 1 in 150).

Sections were incubated with the primary antibodies for 60 min at room temperature and then washed (x2) with TBS (pH 7.6). A 1 in 400 dilution (in TBS) of Vectastain Elite PK-6100 universal BA-2000 biotinylated horse anti-mouse IgG antibody solution was added (200 mL per slide) for 40 min. Each slide was washed (x2) with TBS (pH 7.6). A 1 in 100 dilution (in TBS) of Vectastain Elite avidin-biotin complex was added (200 µL per slide) for 40 min and each slide was washed (x2) with TBS (pH 7.6). Vector Laboratories' peroxidase substrate (DAB SK-4100) was used per the manufacturer's instructions and applied to the sections for 6 min. The sections were immersed in running tap water for 2 min and then counterstained in Harris's haematoxylin, dehydrated, cleared and mounted in DPX.

Qualitative/statistical analysis of immunocytochemical staining

The immunomorphology achieved with the AE1/AE3 and CK 14 antibodies resulted in an inability to count individual positively stained cells, therefore quantitative data for statistical analysis could not be generated for these antibodies. Analysis of the location and intensity of immunostaining seen with the AE1/AE3, CK14, Ki-67 and p53 antibodies was performed and is shown in Tables 1 and 2. In addition, a quantitative analysis of Ki-67 and p53 staining was performed.

Using a light microscope at x100 magnification with a Weibel 1 mm^3 eyepiece graticule, the number Ki-67/p53-positive cell nuclei in five representative microscopic fields from each tissue section was recorded. The same five fields were selected from the sections in order to achieve accurate comparisons of the immunocytochemical staining. The mean average of the positive cell counts from the five microscopic fields was calculated for each case, and a statistical analysis of the data performed.

A multiple regression model was used to establish any correlation between tissue category and observed and predicted numbers of Ki-67/p53-positive cells to see if the results could be interpreted as statistically significant predictors of disease progression for oral SCC (Table 3). One-way ANOVA was performed to calculate any statistically significant differences in the mean number of Ki-67/p53-positive cells, both within and between the tissue

categories (Table 4).

Bonferroni analysis (post-hoc test) was used to establish which categories showed statistically significant differences in the mean numbers of Ki-67/p53-positive cells (Table 5). All statistical tests were performed using SPSS for Windows (version 10; Microsoft, California).

Results

The results of this study are shown in Tables 1 to 5 and Figures 1A–D and 2A–D.

Discussion

The aims of implementing the panel of antibodies used in this study were to develop qualitative and quantitative strategies to assist the accurate classification/diagnosis of oral lesions and also to predict which lesions were more likely to progress to malignancy.

AE1/AE3 immunostaining in the basal layer was negative in the majority of cases except in severe dysplasia where 50% of cases showed a positively stained basal layer. This phenomenon of negative basal layer staining is difficult to explain. Archival material was used in this study and therefore it is possible that antigenicity may have been lost over time, making immunostaining with certain antibodies less effective. The lack of basal layer staining in the benign category may be explained by the presence of underlying inflammatory conditions such as oral lichen planus (Fig. 1A). One of the characteristic features of oral lichen planus is the degeneration of the basal layer, which may affect cytokeratin expression.[8,10,24,25]

Cytokeratin 5 and CK 14 form the CK network in the basal layer of stratified squamous epithelium.[14–20,24,25] Reduced expression of one of this pair of CKs could result in the reduced detection of the other.[14,15] The presence of CK 14 was confirmed by the immunostaining results, showing expression in the basal layer in all cases studied. Therefore, the frequent lack of basal layer staining could represent a reduction in CK 5 expression at an earlier stage in malignant progression, reducing the detection by AE1/AE3 antibody of CK 14 expression.[14,20,24,25]

Some normal oral tissue was taken from sites adjacent to oral SCC resection margins. The 'field cancerisation' hypothesis states that there are multiple sites within the mucosa of the upper digestive tract of patients with head and neck carcinoma that have acquired the same carcinogen-induced mutations as the primary tumour, which may have the potential to develop into additional primary tumours at a later stage.[26] This concept may have relevance to the present study. Areas of histologically normal oral tissue adjacent to a malignancy may have acquired genetic alterations seen in the surrounding tumour cells.[26]

The work of Vaidya[14] shows a reduction in CK 5 expression in cases of mild and moderate oral dysplasia, indicating an increased potential for progression to malignancy.[14] In the present study, the cases of mild, moderate and severe dysplasia that showed no AE1/AE3 staining in the basal layer

Fig. 1. Immunostaining patterns observed in benign and premalignant oral lesions: A) AE1/AE3 staining in a benign oral lesion, demonstrating lack of staining in the epithelial basal layer; B) CK 14 staining in entire epithelium of mildly dysplastic oral tissue; C) p53 staining in area of moderate dysplasia with adjacent normal oral epithelium; and D) Ki-67 staining in severely dysplastic oral epithelium (original magnifications x100).

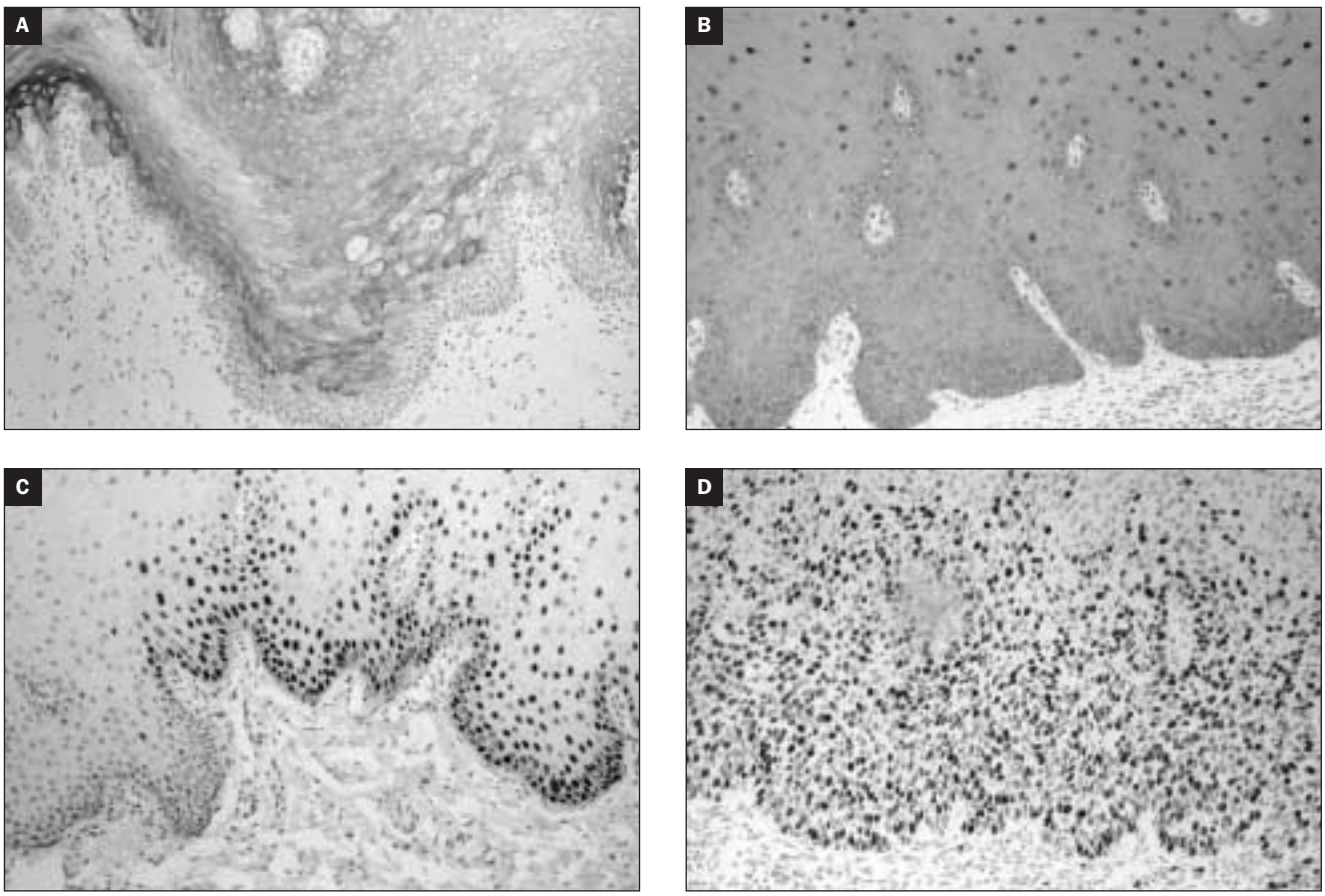
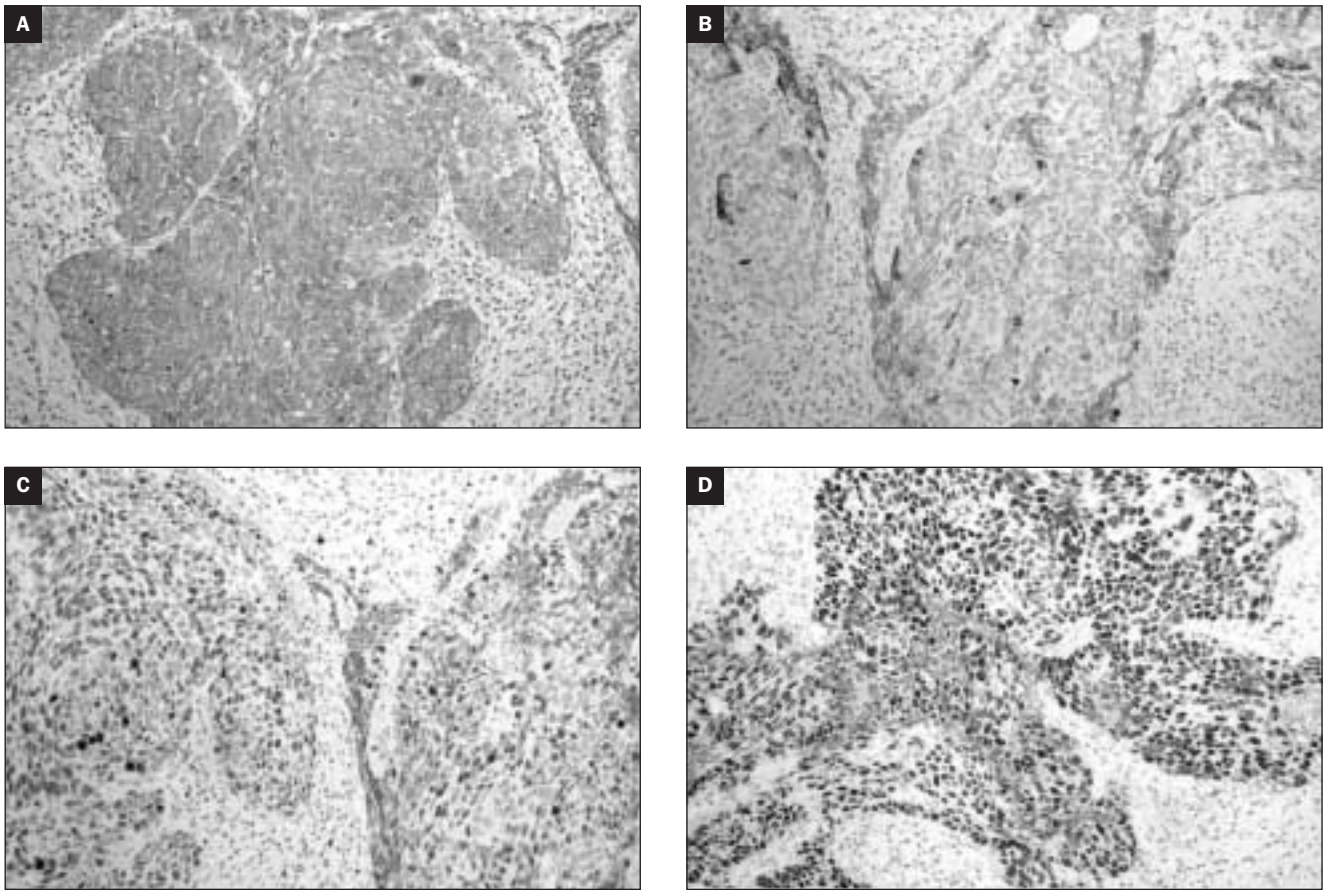


Fig. 2. Immunostaining patterns observed in poorly differentiated oral squamous cell carcinoma: A) weak AE1/AE3 staining in malignant tumour islands; B) CK 14 expression is weak in the tumour cells; C) strong p53 staining throughout epithelium and tumour deposits; and D) strong Ki-67 staining throughout epithelium and tumour (original magnifications x100).



may be lesions that are more likely to progress to malignancy.

The AE1/AE3 antibody staining was mostly of moderate intensity in the majority of the diagnostic categories except SCC (Table 2). AE1/AE3 immunostaining was generally weaker in moderately and poorly differentiated oral SCC than in well-differentiated oral SCC, reflecting increased disruption of cytokeratin expression in less-well differentiated tumours (Fig. 2A).[14–20] The AE1/AE3 antibody proved unhelpful in assessing malignant invasion of the basal layer in any of the oral tissue categories.

In all the tissue categories studied, CK 14 immunostaining was present throughout the entire thickness of the epithelium, including the basal layer. In all cases except oral SCC, this staining was mostly of moderate or strong intensity. All cases of oral SCC expressed CK 14 in the tumour islands, although the intensity varied. Both cases of well-differentiated SCC showed moderate to strong CK 14 immunostaining. Of the four cases of moderately differentiated SCC, two showed strong staining and two showed moderate staining. Of four cases of poorly differentiated SCC, only one displayed strong staining, while the other three showed sparse areas of very weak CK 14 expression. This suggests a reduction in CK14 expression in oral SCC, particularly in poorly differentiated tumours (Fig. 2B).

These results suggest that disruption of CK 14 expression is a late event in oral carcinogenesis, and they support the work of Marley.[15] In an investigation by Schulz,[25] loss of CK 5, 6, 11, 14, 15 and 16 was reported in three cases of poorly differentiated oral carcinoma, supporting a correlation between the loss of CK 14 expression and increasingly undifferentiated oral carcinomas.[25] In the present study, CK 14 proved a useful diagnostic tool for assessing basal layer degeneration in abnormal benign oral lesions and in demonstrating progression to invasion in premalignant oral tissue (Fig. 2B).

Ki-67 staining in the normal tissue category was mostly limited to the basal layer; however, some Ki-67-positive cells were seen in the lower third of the epithelium in three cases. This may be explained by the field cancerisation hypothesis[26] or may indicate a proliferative response to underlying infection or chemical stress.[11] In the benign oral lesions, a slight increase in the number of stained cells was seen. This increased proliferation may be due to inflammation (e.g., non-specific inflammation or associated with lichen planus) or reflect a benign process (e.g., squamous papilloma).[8–10]

The majority of mildly dysplastic cases exhibited Ki-67 staining in the lower third of the epithelium only, and this is consistent with the diagnosis. Cases in this category in which staining was confined to the basal layer may represent benign lesions that have been diagnosed incorrectly or lesions that are less likely to progress. Two cases in this category showed staining in the lower two-thirds of the epithelium, which is consistent with a diagnosis

Table 5. Bonferroni analysis as a post-hoc test: p53 and Ki-67 antibodies..

Category (I)		Category (II)	Mean difference (I – II)	p53 (*P*)	Mean difference (I – II)	Ki-67 (*P*)
Normal	*vs*	Benign	-17.8	1.000	-28.020	1.000
		Mild	-60.3	1.000	-34.360	1.000
		Moderate	-56.7	1.000	-59.240	0.486
		Severe	-112.2	0.086	-98.280	**0.009**
		SCC	-134.8	**0.016**	-134.220	**0.000**
Benign	*vs*	Normal	17.8	1.000	28.020	1.000
		Mild	-42.5	1.000	-6.340	1.000
		Moderate	-38.9	1.000	-31.220	1.000
		Severe	-94.4	0.284	-70.260	0.178
		SCC	-117.0	0.061	-106.200	**0.004**
Mild	*vs*	Normal	60.3	1.000	34.360	1.000
		Benign	42.5	1.000	6.340	1.000
		Moderate	3.6	1.000	-24.880	1.000
		Severe	-51.9	1.000	-63.920	0.321
		SCC	-74.5	0.921	-99.860	**0.008**
Moderate	*vs*	Normal	56.7	1.000	59.240	0.486
		Benign	38.9	1.000	31.220	1.000
		Mild	-3.6	1.000	24.880	1.000
		Severe	-55.5	1.000	-39.040	1.000
		SCC	-78.1	0.754	-74.980	0.112
Severe	*vs*	Normal	112.2	0.086	98.280	**0.009**
		Benign	94.4	0.284	70.260	0.178
		Mild	51.9	1.000	63.920	0.321
		Moderate	55.5	1.000	39.040	1.000
		SCC	-2.6	1.000	-35.940	1.000
SCC	*vs*	Normal	134.8	0.016	134.220	**0.000**
		Benign	117.0	0.061	106.200	0.004
		Mild	74.5	0.924	99.860	0.008
		Moderate	78.1	0.754	74.980	0.112
		Severe	22.6	1.000	35.940	1.000

$P < 0.05$ was accepted as statistically significant and indicated in bold type.

of moderate dysplasia, and may indicate lesions that are more likely to progress to malignancy.

The moderately dysplastic cases showed variability in staining pattern and in the number of positive cells present. Some exhibited Ki-67 staining in the lower two-thirds of the epithelium, which is consistent with the morphological diagnosis, and may identify lesions that are more likely to become malignant. Four cases in this category showed Ki-67 staining in either the lower third of the epithelium or in the basal layer only, suggesting more likely diagnoses of mild dysplasia and benign oral lesion, respectively.

All cases of severe dysplasia exhibited Ki-67 staining throughout the entire epithelium (Fig 1D). All cases of oral SCC showed strong Ki-67 staining throughout the epithelium and tumour deposits, irrespective of the level of differentiation (Fig 2D).

Weak p53 antibody staining was observed within the basal layer in the majority of normal cases. An increase in p53-positive cells was observed in the majority of abnormal benign oral lesions (when compared to normal oral tissue), and this correlated with the pattern of Ki-67 staining. In half of the mildly dysplastic cases examined, p53 expression correlated with the histological diagnosis, and to some extent with the Ki-67 staining also. This indicates a disruption to normal cell proliferation.

In two cases, p53 (and Ki-67) immunostaining was confined to the basal layer, suggesting a diagnosis of abnormal benign lesion rather than mild dysplasia. In four cases diagnosed as moderately dysplastic, p53 immunostaining correlated with histological diagnosis and Ki-67 staining (Fig. 1C). In two of the moderately dysplastic cases, p53 and Ki-67 staining patterns were more in keeping with mild dysplasia. Seven cases of severe dysplasia showed p53-positive cells present throughout the entire thickness of the epithelium, correlating with the diagnosis and the Ki-67 staining.

p53 immunostaining was present in all but two of the oral SCC cases, and the pattern varied between staining in the basal layer, lower two-thirds or full thickness of the epithelium and tumour islands (Fig. 2C). In all diagnostic

categories but the abnormal benign lesions, some cases showed a lack of p53 staining.

Cases which were both p53- and Ki-67-positive may distinguish a subset of lesions with a greater potential to progress to malignancy, or, in the oral SCC cases, a more aggressive tumour type.[11] Conversely, p53-negative cases with weak proliferative activity may indicate lesions that are less likely to progress to malignancy.[11–13]

The underlying mechanism by which p53 protein is stabilised is of great importance when interpreting immunocytochemical staining using p53 antibodies.[26–34] Although there is strong correlation between *p53* gene mutation and the resultant stabilisation/accumulation of the p53 protein within cells, other factors may also bring about this effect.[33] Infection with certain types of human papillomavirus can result in stabilisation and inactivation of wild-type p53 protein.[34] Cellular proteins such as mdm-2 participate in the autoregulation of p53 function, and over-expression of mdm-2 can cause cellular transformation due to the associated inactivation of p53.[33]

Genotoxic damage, smoking and ultraviolet light can induce the production of wild-type p53 and also affect the stability of mutant protein forms.[26–33] Unfortunately, information about smoking (and alcohol consumption) was not generally available for the cases used in the present study. The p53 staining observed in the normal oral tissue cases may represent an accumulation of wild-type p53 protein in response to such genotoxic insults, or perhaps supports the concept of field cancerisation and reflects early mutations of the *p53* gene.[26–33]

Variations in staining intensity seen with the p53 antibody in the SCC category may reflect the normal functioning of wild-type p53 protein in response to an increased frequency of genetic errors in the tumour cells, or may reflect genuine mutation of the *p53* gene.[33] Nonsense *p53* mutations may go undetected immunocytochemically because they produce only a slight increase in p53 protein levels.[30,31]

Expression of high levels of mutated p53 protein may go undetected immunocytochemically, giving rise to false-negative results, due to variability in antibody clones and the inability of detection systems to identify mutated (and possibly wild-type) forms of p53 protein.[32,33] Thus, a negative immunocytochemical result does not necessarily rule out the presence of an underlying *p53* mutation, and a positive result may simply reflect a natural cellular response.[28–33]

In order to assess whether or not quantitative data from Ki-67- and p53-positive cell counts could be used in a statistical model to predict disease progression, a multiple regression analysis was performed (Table 3). This showed a weak but statistically significant correlation between observed and predicted values for the mean number of Ki-67/p53-positive cells and histopathological grade, with Ki-67 staining being the statistically stronger predictor (Table 3).

One-way ANOVA showed a statistically significant difference between mean Ki-67-positive cell counts in the six tissue categories (Table 4). Subsequent Bonferroni analysis showed that these significant differences occurred between the normal and severely dysplastic groups, the normal and SCC groups, the benign and SCC groups, and the mildly dysplastic and SCC groups (Table 5).

One-way ANOVA showed a weak statistically significant difference between mean p53-positive cell counts in the six tissue categories (Table 4). Subsequent Bonferroni analysis showed that the significant difference occurred between the normal and SCC groups only (Table 5). To some extent these findings support the work of Pignataro.[13] However, the statistical analysis may have been impaired by the cases in which no p53 immunostaining was demonstrated and from which no quantitative data could be obtained.

Conclusions

The four antibodies used in this study constitute a satisfactory diagnostic panel for assessing benign and premalignant oral lesions as they provided information about epithelial differentiation, proliferation, and cell-cycle regulation. AE1/AE3 antibody is not a useful marker in assessing tumour invasion as it does not stain the basal layer in all cases. It is a satisfactory marker of squamous epithelium and has a useful application in distinguishing between SCC and malignancies of non-epithelial origin. Both AE1/AE3 and CK 14 expression is reduced in some cases of oral SCC, particularly when the tumour is poorly differentiated.

Ki-67 and p53 staining appear to be weak but statistically significant indicators of malignant disease progression in oral tissues. Ki-67 antibodies may be useful in the development of a quantitative system for more accurate classification of oral lesions. The value of p53 antibodies in such a quantitative system is limited due to variability in the expression, detection and accurate interpretation of results. □

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