P53 immunohistochemical staining patterns in benign, premalignant and malignant lesions of the oral cavity: A study of 68 cases

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Abstract
Objectives: The p53 tumor suppressor gene is a frequent target for mutations in a variety of tumors. The mutated gene is more stable than the normal gene and can be detected by immunohistochemical methods. The aim of the presented study is: 1) to determine the expression of p53 immunohistochemically in a spectrum of benign, premalignant lesions and in various histological grades of oral squamous cell carcinoma (SCC), 2) to verify if any correlation exists, between the localization and intensity of p53 staining pattern, and the degree of dysplasia.

Methods: p53 expression was studied in 68 cases of oral lesions using immunohistochemistry (Dako cytomation). The location and intensity of staining was noted. Suprabasal positivity was considered as abnormal.

Results: All the benign lesions and low grade dysplasia were negative or showed only basal positivity. Suprabasal positivity increased from premalignant lesions (50%) to oral carcinomas (73.8%). In the premalignant category, intensity of p53 staining increased with increasing grades of dysplasia. In the malignant category, intensity of staining was stronger in the poorly differentiated tumors (68.4% vs 47.8%), while positivity was higher in well differentiated tumors (78.3% vs 68.4%). A small percentage of malignant tumors (21.4%) were negative. In 31% cases of SCC, the epithelium adjacent to the tumor which showed just hyperplasia/mild dysplasia on light microscopy, revealed suprabasal positivity.

Conclusions: Expression of p53 above the basal layer is an early event in oral carcinogenesis. Stronger staining (increasing grades of dysplasia) had greater risk of progressing into malignancy. The p53 positivity is an early indicator of a developing carcinoma preceding morphological tissue alterations.

INTRODUCTION
Oral cancer is one of the ten most common cancers in the world and accounts for about 15-20% of all the cancers detected in India. Despite advances in surgery, radiotherapy and chemotherapy over the past 20-30 years, no significant improvement in the prognosis for oral cancer has been observed. This could change if the cancer can be detected at an early stage. The immunohistochemical studies in order to determine the tumor-associated antigenic constituents, commonly referred to as “tumor markers”, has received considerable attention [1]. Cell markers associated with malignant transformation within oral mucosa have been investigated with the aim of identifying a marker of malignancy. To date, the p53 protein appears potentially to be such a marker [2].

The p53 tumor suppressor gene was first described in 1979 as a protein that binds SV40 virus large T antigen [3]. The gene exists on chromosome 17p and behaves as a tumor suppressor. However, mutation of the gene
can inactivate this tumor suppressor activity. The p53 gene is a frequent target for mutation. In normal cells, the p53 protein has a very short half-life and cannot be detected immunohistochemically. In contrast, the mutant forms are more stable and thus have an extended half-life and can be detected using immunohistochemical techniques [2].

MATERIALS AND METHODS

A total of 68 cases of oral lesions received in the department of Pathology, Kasturba Medical College, Manipal over a period of three years from January 2003 to December 2006 were studied.

The histopathology sections were stained with Hematoxylin and Eosin (H&E) and for p53 (Dako cytomation LSAB2 system-HRP). Antigen retrieval for immunohistochemistry (IHC) was done using microwave technique at 98°C for 10mins.

The stained slides were interpreted independently by two pathologists. Assessment of antigen expressing cells was performed by using light microscope at 5X, 10X and 40X magnifications. The criteria used to define p53 positive cells were: granular brown staining within the nucleus of the epithelial cells. Staining profiles were categorized into three groups according to the location of the positive cells in different layers of the epithelium (i.e. basal, suprabasal) as:

- 0: Negative – no expression of p53 in any epithelial cell nucleus
- 1: Nuclear staining confined to basal cell layer
- 2: Clear suprabasal nuclear staining

Intensity of staining was scored as:

- 0: Weak
- 1: Moderate
- 2: Strong

The highest score in the several regions of a section analyzed was taken as final. H&E stained sections were then evaluated, independently without knowing the previous histopathological diagnosis or the p53 staining pattern (blind study). Subsequently the two were

In the present study, an attempt has been made: 1) To determine the expression of p53 immunohistochemically in a spectrum of benign, premalignant lesions and in various histological grades of oral squamous cell carcinoma, 2) To verify if any correlation exists, between the localization and intensity of p53 staining pattern, and the degree of dysplasia, compared to establish a relationship between the p53 stained areas and the respective histopathological diagnoses.

RESULTS

A total of 68 cases of oral lesions were studied for p53 expression. The age group ranged from 20-80yrs with a mean age of 59.8 years and male: female ratio of 3:1.

The commonest location was the tongue (49%) and the least common was the floor of the mouth (6%). The gross appearances included: patch, ulcer, proliferative and ulceroproliferative growths. 53% of the patients were chronic smokers, 58% were chronic tobacco users and 37% were chronic alcoholics.

The various histopathologic diagnoses offered and the number of cases is as depicted in Table 1.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Squamous cell carcinoma</td>
<td>42</td>
</tr>
<tr>
<td>Verrucous carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>19</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>16</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>3</td>
</tr>
<tr>
<td>II. Pre-malignant lesions</td>
<td>16</td>
</tr>
<tr>
<td>III. Benign lesions</td>
<td>10</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>68</td>
</tr>
</tbody>
</table>

In the benign category, 7 out of 9 cases of hyperplasia showed basal p53 positivity whereas the only case of squamous papilloma was negative. None of the cases showed suprabasal positivity (Figure 1).
Among the premalignant lesions: 2/3 (66.7%) cases of severe dysplasia, all 3 (100%) cases of moderate dysplasia and 3/7 (42.85%) cases of verrucous hyperplasia showed weak to strong suprabasal p53 positivity (Figures 2 and 3). The intensity of suprabasal p53 staining was stronger with increasing grade of dysplasia. As for mild dysplasia, 2/3 (66.7%) were p53 negative and the remaining case showed only basal p53 positivity.

The p53 staining pattern in the benign and premalignant cases is as shown in Table 2.

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Negative</th>
<th>Basal</th>
<th>Number of cases.</th>
<th>Suprabasal positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign (n=10)</td>
<td>3</td>
<td>7</td>
<td>-</td>
<td>W 1 M 1 S 1</td>
</tr>
<tr>
<td>Premalignant lesions</td>
<td></td>
<td></td>
<td></td>
<td>W 1 M 1 S 1</td>
</tr>
<tr>
<td>Mild dysplasia (n=3)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>W 1 M 1 S 1</td>
</tr>
<tr>
<td>Moderate dysplasia (n=3)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>W 1 M 1 S 1</td>
</tr>
<tr>
<td>Severe dysplasia (n=3)</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>W 1 M 1 S 1</td>
</tr>
<tr>
<td>Verrucous hyperplasia (n=7)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>W 1 M 1 S 1</td>
</tr>
</tbody>
</table>

W- weak, M- moderate, S- strong
The intensity of suprabasal p53 staining ranged from weak to strong in case of well differentiated carcinomas, with 47.4% cases showing strong positivity. Moderately differentiated and poorly differentiated carcinomas showed strong suprabasal positivity in 68.8% and 66.7% of cases respectively (Figures 4 to 6). The p53 staining pattern in malignant cases is as shown in Table 3.

A small percentage of malignant tumors (9/42 cases, 21.4%) were negative in the invasive component, although the overlying non-malignant epithelium showed suprabasal p53 positivity (Figure 7), indicating that negative staining did not necessarily mean that there was no abnormality in the p53 gene.

In 13/42 (31%) cases of SCC, the epithelium adjacent to the tumor which showed only hyperplastic/ mildly dysplastic changes on routine H&E sections showed strong suprabasal p53 positivity. This could be a pointer that immunohistochemical staining with p53 may be a better indicator of a premalignant change at the molecular level.

The p53 staining pattern and number of cases showing abnormal suprabasal p53 staining with intensity of staining is depicted in Table 4.

One of the other observations in this study was the false positive staining due to cytoplasmic melanin pigmentation (Figure 8).

Table 3. The p53 staining pattern in various grades of squamous cell carcinoma (n=42)

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Negative N (%)</th>
<th>Basal N (%)</th>
<th>Suprabasal positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verrucous carcinoma (n=4)</td>
<td>0</td>
<td>0</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Well differentiated SCC (n=19)</td>
<td>4 (21.1)</td>
<td>1 (5.2)</td>
<td>14 (73.7)</td>
</tr>
<tr>
<td>Moderately differentiated SCC (n=16)</td>
<td>4 (25)</td>
<td>1 (6.2)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td>Poorly differentiated SCC (n=3)</td>
<td>1 (3.3)</td>
<td>0</td>
<td>2 (66.7)</td>
</tr>
</tbody>
</table>

W- weak, M- moderate, S- strong

Table 4. The p53 staining pattern with intensity of abnormal suprabasal staining in benign, premalignant and malignant lesions (n=68)

<table>
<thead>
<tr>
<th>Intensity of suprabasal positivity</th>
<th>Negative N (%)</th>
<th>Basal N (%)</th>
<th>Suprabasal N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>3 (30)</td>
<td>7 (70)</td>
<td>0</td>
</tr>
<tr>
<td>Premalignant</td>
<td>4 (25)</td>
<td>4 (25)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Malignant</td>
<td>9 (21.4)</td>
<td>2 (4.8)</td>
<td>31 (73.8)</td>
</tr>
</tbody>
</table>

W- weak, M- moderate, S- strong

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**Figure 3.** a) Mild to focal moderate mucosal dysplasia (H&E, x200), b) Strong focal suprabasal positivity (p53, x200).
Figure 4. a) Moderate dysplasia with area of doubtful invasion (H&E, x100), b) Strong diffuse positivity in invading islands (p53, x100).

Figure 5. a) Well differentiated squamous cell carcinoma (H&E, x100), b) Strong suprabasal positivity with negative staining in central keratinized areas (p53, x100).

Figure 6. a) Moderately differentiated squamous cell carcinoma (H&E, x400), b) Strong diffuse p53 positivity (p53, x400).
DISCUSSION

The growth of normal cells is controlled by a tightly regulated process with altered growth and tumor development arising due to the accumulation of genetic mutations, which affect these processes. The genes involved in this process include the oncogenes, sequences which suppress tumor growth and those which control DNA repair and apoptosis [4].

Several chromosomal areas, which are very likely to harbor tumor suppressor genes for oral cancer, have been identified and include 2q, 3p, 4q, 7q, 8p, 9p, 9q, 11q, 13q, 17p, 18q, and 21q, where ‘p’ represents the short arm of the chromosome and ‘q’ the long arm. The p53 tumor suppressor gene, is located at locus 17p13.1 [4].

The p53 gene behaves as a multifunctional transcription factor involved in: control of cell cycles, programmed cell death, senescence, differentiation and development, transcription, DNA replication, DNA repair and maintenance of genomic stability [5]. In view of these activities, p53 has been rightfully called a “GUARDIAN OF THE GENOME”. With homozygous loss of p53, DNA damage goes unrepaired, mutations become fixed in dividing cells and the cell turns onto a one-way street leading to malignant transformation [6].

In the normal cells, the p53 protein has a very short half-life and cannot be detected immunohistochemically. In contrast, the mutant forms are more stable and thus have an extended half-life and can be detected using immunohistochemical techniques [2].

Mutations in the evolutionary conserved codons of p53 tumor suppressor gene are common in diverse types of human cancers. The p53 mutational spectrum differs among various cancers. Analysis of these mutations can
provide clues to the etiology of these diverse tumors and to the functions of specific regions of p53 [7].

The gene p53 has a wide variety of effects in oral epithelium and oral cancer: a) It affects many proteins and pathways, b) is acted on by other proteins, and c) its mutations can have profound effects. These events are central to apoptosis and failure of apoptosis in oral tissues. These complex pathways provide many opportunities for the development of new understanding and new treatments for oral cancer [8].

Review of the literature uncovers important insights regarding role of p53 in oral carcinogenesis. The p53 expression in oral carcinomas as described in the literature ranges from 34 to 80% [2, 9, 10-13]. Kerns et al first studied p53 expression in paraffin embedded tissues using monoclonal antibody Pas1801 and observed identical staining in fresh frozen and paraffin embedded tissue [14]. Warnakulasuriya KAAS et al suggested that p53 gene mutations were commonly involved in oral cancer but were neither sufficient nor necessary for the development of malignancy [15]. Ogden et al concluded that p53 expression was not found in any normal oral mucosal lesions [2]. Field et al recorded a correlation between the patients with smoking history and positive p53 staining [9]. Somers et al demonstrated a high frequency of p53 mutations in squamous cell carcinoma of head and neck with preferential G to T transversions clustered at codons 245 and 248 [16].

Boyle et al found that the incidence of p53 mutations in noninvasive lesions was 19% and increased to 43% in invasive carcinomas. These data suggested that p53 mutation can precede invasion in primary head and neck cancer [11]. Caomano et al noticed that, of the five very advanced primary tumors of head and neck, four showed intense p53 immunostain. These observations supported the evidence that alteration in this tumor suppressor gene could be related to late events in tumor progression [17].

Piffko et al found that p53 positive tumor cells were accumulated at the periphery of the invading margins, while the more differentiated and keratinizing central areas were negative. There was also no significant correlation between p53 reactivity and histopathological grade of the tumors [18]. In the present study, intensity of staining was stronger in the more in the well differentiated tumors. Cruz et al observed that expression of p53 above the basal cell layer was an early event in oral carcinogenesis and an indicator of a developing carcinoma preceding morphological tissue alterations [19]. Presented study further confirms this observation.

Zarovnaya et al attempted to distinguish benign mucosa with varying degrees of pseudoepitheliomatous hyperplasia (PEH), inflammation and suboptimal orientation from invasive squamous cell carcinoma in mucosal biopsy specimens from head and neck utilizing immunohistochemical stains for p53, MMP-1, E-adhesion and collagen IV. The invasive SCC showed nuclear reactivity for p53, with staining of nuclei throughout the full thickness of the epithelium (suprabasal) rather than just the basal areas as in benign tissues. They referred to this pattern as an invasive staining pattern as opposed to the orderly basal pattern [20]. Kerdpon et al observed that the proportion of cases with positive p53 expression increased from hyperplasia (36%) to dysplasia (85%) to oral SCC (95%). These results may indicate an involvement of p53 in neoplastic transformation as well as in proliferative events [21]. In the present study, p53 expression significantly increased from oral premalignant lesions (50%) to SCC (73.8%). An interesting observation in this study was p53 negativity in the invasive component in a small percentage (21.4%) of malignant tumors. This finding suggests that negative p53 does not rule out malignancy and that morphology is the gold standard for diagnosing malignancy. However p53 can serve as a useful adjunct to morphology for early detection of potentially malignant lesions.

CONCLUSION

Abnormal, suprabasal p53 expression was seen in 50% of the oral premalignant lesions and 73.8% of oral carcinomas. None of the benign lesions showed suprabasal p53 positivity. In 13/42 (31%) of cases of squamous cell carcinomas, the epithelium adjacent to the tumor which showed only hyperplastic/mildly dysplastic changes on routine H&E, showed strong suprabasal p53 positivity. This could be a pointer that immunohistochemical staining with p53 may be a better indicator of a premalignant change at the molecular level.

All the benign lesions were negative or showed only basal positivity for p53. On contrary, 50% of premalignant lesions showed suprabasal p53 positivity. As for the malignant lesions, 73.7% of well differentiated, 68.8% of moderately differentiated, 66.7% of poorly differentiated carcinomas and 100% verrucous carcinomas showed suprabasal p53 positivity.

However we found that 9/42 (21.4%) cases of oral squamous cell carcinomas were p53 negative. Thus we have to keep in mind that negative p53 staining does not automatically rule out malignancy.
The intensity of suprabasal p53 staining progressively increased with increasing grade of dysplasia. However mild dysplasia showed negative staining. In the malignant category, intensity of staining was stronger in the less differentiated tumors (68.4% vs 47.8%), whereas positivity was more in the well differentiated tumors (78.3% vs 68.4%).

Thus the authors wish to emphasize that expression of p53 above the basal layer is an early event in oral carcinogenesis and an indicator of a developing carcinoma preceding morphological tissue alterations. However, since immunochemistry cannot always detect changes in p53 expression in premalignant/malignant lesions, it is strongly recommended that p53 immunohistochemistry be used in conjunction with routine histology to increase the sensitivity of detection of cases that eventually progress to malignancy.

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REFERENCES


