Pergamon

Oral Oncol, Eur J Cancer, Vol. 32B, No. 3, pp. 143–149, 1996

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

0964-1955(95)00095-X

Reviews

p53 Overexpression in Head and Neck Squamous Cell Carcinoma: Review of the Literature

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As a tumour suppressor gene, the inactivation of p53 induces the development of numerous human cancers. Mutations of p53 have been implicated in the pathogenesis of head and neck squamous cell carcinoma (HN-SCC) at a high incidence. In premalignant lesions and *in situ* carcinomas, p53 overexpression is not exclusively restricted to neoplastic cells, but frequently affects the normal appearing keratinocytes adjacent to p53 positive neoplasms or present in dysplastic areas. These results suggest that as contributors to the early phases of HN-SCC development, p53 alterations may be excellent biomarkers that indicate the predisposition of a particular oral cavity premalignant lesion toward malignancy. In most cases, the p53 overexpression status of a tumour metastasis is identical to that of a primary tumour, indicating that a p53 mutation precedes metastatic spread. In patients with multiple primary tumours, multiple foci of p53 overexpression are observed in epithelia distant from the tumour. So the expression of p53 in normal epithelium would indicate an increased risk for transformation to second or third primary cancers. Distinct p53 mutations in different primary tumours of the same patient indicate that these cancers arise as independent events; these results support the existence of multifocal polyclonal processes. Regardless of the aforementioned results that support p53 as a valid tumour biomarker, most studies have shown no relationship between the expression of p53 and clinical and histopathological parameters. The role played by p53 mutations in the progression and vital prognosis of HN-SCC has not yet been demonstrated.

Keywords: p53 tumour suppressor gene, immunohistochemistry, head and neck squamous cell carcinoma, oral precancer

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INTRODUCTION

Squamous cell carcinoma of the head and neck (HN-SCC) is the sixth most frequent cancer in the world. In the United States 44 000 new cases and nearly 11 000 deaths are estimated each year. The overall 5-year survival rate for patients with HN-SCC is among the lowest for major cancers and has not changed during the past two decades. The presence of metastatic SCC in cervical lymph nodes correlates with a 50% decrease in survival [1]. In Western countries, the two major risk factors for HN-SCC are the use of tobacco and consumption of alcohol, while in India and parts of south-east Asian countries, betel quid chewing appears to be the main cause [2, 3]. Accumulation of mutations, including those of proto-oncogenes and tumour supressor genes, play a key role in cancer development [4]. The p53 tumour-suppressor gene has been implicated in the pathogenesis of head and neck squamous cell carcinoma (HN-SCC) [5–13]. The wild-type (normal) p53, a 53 kD nuclear phosphoprotein, is essential for normal cell growth and the eventual suppression of the malignant phenotype. Inactivation of p53 induces the de-

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Received 22 Sep. 1995; provisionally accepted 6 Oct. 1995; revised manuscript received 13 Dec. 1995.

velopment of malignancy [14, 15]. Thus, normal p53 acts as a "molecular policeman" monitoring the integrity of the genome [14], usually residing in the cell nucleus. It is present at very low concentrations in all normal cells and tissues and has a very short half-life, so the wild-type protein is almost undetectable in conventional immunohistochemical assays. The wild-type p53 protein level strongly increases after DNA damage, and this is followed by a specific arrest of the cell cycle in the G1 phase. If DNA is damaged, wild-type p53 accumulates and switches off replication to allow extra time for DNA repair. If the DNA repair fails, wild-type p53 may trigger cell suicide by apoptosis. However, tumour cells containing mutated or inactivated p53 are unable to induce this cell cycle arrest [16]. Inactivation of p53 could increase on one hand the pool of proliferative cells and on the other the probability of their neoplastic transformation by inhibition of programmed cell death [17]. Thus, p53 acts as a tumour suppressor gene in the normal form, but as an oncogene in its mutant form [18].

REVIEW OF THE LITERATURE

The nature of mutations at the p53 locus in head and neck squamous cell carcinoma

Mutations of p53 are the most common genetic abnormalities found in human cancers, especially in the development of HN-SCC. Mutations in p53 have been shown to result from allelic loss, point mutation, deletion or rearrangement. The wild-type protein may also be inactivated by complex formation with mutant p53, viral or aberrant host-binding proteins [13]. These mutations result in either no expression of the wild-type p53 or overexpression of the mutant p53 protein [19, 20]. The p53 mutation causes both a loss of its tumour suppressor function and a gain of its oncogenic function by alteration of the repertory of genes controlled by p53 [14]. The loss of function of tumour suppressor genes corresponds to an increased risk of cancer [15]. Moreover, the protein expressed from the mutant p53 gene has been implicated in growth, deregulation and malignant progression of cancers [20]. A direct correlation between abnormal p53 protein expression and gene mutation has been shown in HN-SCC [6, 7, 21–24]. A point mutation stabilises the p53 protein and, together with a loss of the normal gene, may lead to accumulation of the mutant protein within the cell. This would remove the normal function of the p53 and at the same time transform the p53 into a dominantly acting oncogene [18]. Even in the absence of mutations, if allelic deletions are common, it is possible that cells harbouring such deletions may have a growth advantage over their normal counterparts, favouring malignant transformation [25].

Tumour-suppressor genes can often be inactivated, resulting in a loss of function. Thus, tumour-suppressor gene mutations are most commonly recessive [4]. Therefore, it only contributes to tumorigenesis when the wild-type allele is inactivated [15]. However, many of the mutant proteins can act as dominant negatives to inhibit the activity of the wild-type p53. As stated previously, this is achieved by formation of inactive hetero-oligomers between the mutant and the wild-type p53 proteins [16]. Commonly, one allele of a tumour-suppressor gene sustains a mutation (heterozygosity) which inactivates the function of its protein and then the second allele is lost via deletion or gene conversion, resulting either in a loss of heterozygosity or a reduction to homozygosity at the locus in the cells of the tumour [15, 18]. Largey *et al.* revealed that loss of heterozygosity in a p53 site was found in more than 70% of oral SCC [19]. Furthermore, in HN-SCC, allelic deletion of p53 occurs in virtually all cases that have a p53 point mutation [6, 22–24, 26, 27]. About 90% of the mutations at the p53 locus in human cancers are missense mutations that change the identity of an amino acid, resulting in a faulty or altered protein in the cell [4, 17]. Only 8.1% of the p53 mutations are deletions or insertions, 5.5% are nonsense mutations or frameshift mutations and 0.8% of these mutations are neutral and produce no amino acid changes [4]. In a study of HN-SCC, Boyle *et al.* found that the frequency of missense mutation was 72% of all mutations [8]. Point mutations which alter the p53 function are localised over a large region, especially in the hydrophobic midportion of the molecule between exons 5 and 8 [4, 17], while frameshift and nonsense mutations which truncate the protein are much more common outside this region. Many p53 mutations in HN-SCC have been described in the region between exons 5 and 8 [5, 7, 8, 21, 22, 26–32]. Mutations in HN-SCC were preferentially at the guanine nucleotides [5–8, 33]. Mutation modified guanine residue is a phenomenon that has been associated with the effect of carcinogens present in tobacco smoke [22]. Zariwala *et al.* found that 72% (13 out of 18) of the p53 mutations occurred at G:C base pairs [31]. Boyle *et al.* found that 64% of mutations were G→A transitions, G→T transversions or G→C transversions and only one patient with a G→A transition was a nonsmoker [8]. G→T transversion was detected in 33% of tumours with a mutation [23]. Greenblatt *et al.*, in a review of p53 mutations and cancer, showed that in 524 cases of HN-SCC, 31% of mutations were G:C→A:T transitions and 18% were G:C→T:A transversions and 11% were G:C→C:G transversions [2].

Yin *et al.* proposed a sequence of p53 alteration as follows: (a) p53 mutation; (b) deletion of wild-type allele; (c) increased dosage of the mutated gene by aneuploid increase in chromosome copies, and/or (d) p53 gene amplification. Alteration in the p53 gene is, therefore, a gradual process that spans many levels of tumour progression, and possibly involves four different molecular mechanisms [24].

Methodological variations for p53 detection by immunohistochemistry

Mutation stabilises the p53 protein and extends its half-life to such an extent that the ability to detect p53 with immunohistochemistry (IHC) in tumours is synonymous with the presence of a mutation [14]. Further studies by Lane *et al.* indicate that immunohistochemical results are often very striking, with all cells in the lesion staining intensely while an essentially negative background is observed in the normal stroma. These images, combined with the development of monoclonal and polyclonal antibodies to p53 that can be used on archival material, have generated a huge number of observations from a large variety of tumour types [34]. Missense mutations often increase the half-life and quantity of the p53 protein, allowing its detection by IHC [34]. Mutations which result in deletion or truncation of the protein (nonsense and frameshift) do not cause protein accumulation. However, IHC will be less sensitive in detecting mutations in tumour types with high proportions of these non-missense mutations [35]. Nylander *et al.* showed that non-sense mutations can be found in some p53-negative HN-SCC [36]. A lack of p53

protein expression by ICH in the tumour cells does not always mean therefore, that p53 is uninvolved. Tumours with no p53 oncoprotein staining may have lost the expression of both alleles [37] or may contain a level of p53 mutant that cannot be detected by the anti-p53 protein antibodies used in the various studies [38]. Other explanations can be that wild-type p53 protein may either bind to a papillomavirus which may degrade it [39] or form a complex with the mutant p53. HN-SCC cases lacking immunostaining for the p53 protein suggests that the p53 mutation is neither sufficient nor necessary for the development of HN-SCC [40], or that the tumour cells are in some way able to bypass the suppressive action of a normal p53 gene. It may be that, at least in some tumours, the p53 gene is normal [41]. The IHC approach would lead to underestimation of p53 mutation so that complementary methods, such as sequencing the gene following polymerase chain reaction, may be required [40]. The use of different antibodies which do not show 100% concordance may account for some discrepancies in the literature. Furthermore, the percentage of p53-positive cells in a specimen may be a subjective parameter, since it is the pathologist who determines, according to the intensity of the p53 reaction product, the cut-off point between p53-positive and -negative cases [25].

Immunoreactivity of p53 in premalignant oral lesions and in squamous cell carcinoma

The prevalance of p53-positive neoplasms determined by IHC shows significant discrepancies according to the study. p53 positivity ranged from 11 to 69% in oral SCC [11, 25, 40, 42–47], 34–79% in HN-SCC [5, 18, 21, 22, 30, 36, 38, 41, 48, 49] and 44–73% in larynx SCC [6, 11, 50, 51]. Few data on hypopharyngeal [11, 13], oropharyngeal [11] and oesophageal SCC [52, 53] have been reported.

The pattern of p53 immunoreactivity in HN-SCC is exclusively nuclear and correlates with the presence of mutant p53 [6, 30, 37, 40, 41, 45, 49, 53]. However, cytoplasmic reactivity is observed in a few cases [18, 54]. In SCC, p53 expression can be very heterogeneous within the same tumour sample. For example, in one tumour Shin *et al.* described an area without detectable p53 whereas another area of the tumour showed a high p53 expression [48]. Most authors have found no p53-positive staining in normal oral mucosa [18, 28, 44–46, 48, 50, 55] or in normal tissues adjacent to neoplasms [6, 13, 38, 40, 41, 49, 52]. However, Sauter *et al.* found in 5 out of 102 cases, p53-positive cells scattered throughout the basal layer in normal epithelium [56]. p53 immunostaining was also observed in the normal appearing keratinocytes adjacent to p53-positive carcinomas [44, 48, 51, 57]. Such positivity was never observed in tumour-negative cases [44, 51]. Sasano *et al.* observed 3 cases of scattered p53 immunoreactivity in parabasal and basal cells of morphologically normal oesophageal mucosa from sites distant from the carcinoma. He suggested that these cells were morphologically normal but were already in the process of malignant transformation in terms of abnormal cell proliferation [37]. p53-positive cells were also reported in dysplastic tissue adjacent to SCC [21, 37, 41, 45, 46, 50, 51, 53] and in premalignant lesions with mild, moderate or severe dysplasia [3, 12, 18, 30, 44, 45, 55, 56]. p53 protein expression was also observed in leucoplakias with and without dysplasia [8, 12, 33, 48, 55]. The p53 staining was observed in the dysplastic cells of the basal region of the squamous epithelium [30, 46, 53]. Shin *et al.* noted that if p53 overexpression was present, it was limited to the basal layer in normal epithelium adjacent to the tumour. The overexpression of p53 expanded into the parabasal and superficial layers in hyperplasia and dysplasia [48]. Other studies have shown that p53 overexpression is correlated with the severity of dysplasia, the level of p53 protein expression being higher in severe and moderate dysplasia [3, 12, 30, 45, 48, 56, 58]. Sauter *et al.* found 28% (18/62) p53 positivity in mild dysplasia, 45% (25/55) in moderate, 54% (15/28) in severe dysplasia and 50% (28/56) in carcinoma *in situ* [56]. In contrast, Regezi *et al.* found no clear relationship between the percentage of p53-positive cells and severity of atypia. This author detected p53 protein staining in 6 out of 13 patients with dysplasias, 3 of which progressed to p53-positive invasive carcinomas. In 4 p53-positive lesions out of 6 patients with *in situ* carcinomas, one advanced to a p53-positive carcinoma [57]. In all cases of p53 protein expression in invasive tumours, the adjacent carcinoma *in situ* or dysplasia areas also expressed p53 [41] and tumours with no p53 overexpression were invariably negative in the dysplastic areas as well as in the areas of non-invasive disease [41, 51]. In contrast, Nees *et al.* observed positive p53 staining in tumour-adjacent epithelia and in tumour-distant biopsies in patients with immunopositive but also immunonegative primary tumours [11]. Increased p53 overexpression in premalignant lesions was always observed in patients with p53-positive tumours. In only 2 out of 16 patients with p53-positive premalignant lesions was no p53 expression observed in their SCC [48].

It is of interest to note that Ogden *et al.* could identify p53-positive cells in oral smears from positive tumours. This suggests that p53 may prove to be a useful marker in the screening for oral cancer using exfoliative cytology [46].

Relationship between p53 expression and markers of cell proliferation

Since p53 regulates genomic stability and prevents cell cycle entry in response to DNA damage, the loss of p53 function may interfere with its growth-inhibiting capacity and would be expected to correlate with an increase in the proportion of cells exhibiting proliferation [2, 14]. Thus, numerous studies on the relationship between p53 expression and cell proliferation in SCC with immunohistochemical markers such as Ki-67 and PCNA have been conducted. Most authors found p53 to show a similar immunolocalisation as for PCNA or Ki-67, indicating that p53 levels increased in areas with proliferative activity [37, 41, 45, 49, 55, 59]. These authors have suggested the involvement of the mutated form of p53 in the alteration of the cell cycle regulation, conferring a proliferative advantage to the neoplasm [37, 45, 59]. Furthermore, Nakanishi *et al.* observed a positive immunoreaction in the periphery of tumour cells nests and in the dysplastic cells of the basal region of the squamous epithelium, both of which are considered to be areas of active cell division [30]. These authors concluded that a correlation between positive p53 staining and proliferative activity of these cells existed [11, 30]. Furthermore, immunoreactivity of p53 is strongly correlated with cell proliferation of malignant phenotype [37]. p53 oncoprotein and PCNA were detected at the periphery of nests of well-differentiated tumours [41, 45, 49], often located in the most invasive regions [45]. The positivity of Ki-67 as well as p53 protein tumour disappeared when tumour cells matured into

squamous pearls [41], whereas poorly differentiated tumours had a heterogeneous distribution throughout tumour nests [49]. Other authors have found no correlation between Ki-67 or PCNA expression and p53 positivity and p53 negativity [18, 32, 36, 41, 49, 59]. In such cases, cells may show increased proliferation without involving immunohistochemically detectable alterations in the p53 gene [41] which suggests that stabilisation of the p53 protein does not influence the proliferative advantage in carcinomas [59]. Nylander *et al.* found no correlation between cell proliferation and p53 overexpression [36].

Relationship between the expression of p53 in the primary tumour, recurrences and metastases in lymph nodes

Several studies have addressed the question of whether the primary tumour tissue, and the corresponding recurrences or metastatic lymph nodes show coordinated expression of p53. Dolcetti *et al.* showed that p53-negative metastases invariably derived from primary tumours with no p53 immunostaining [51]. The p53 overexpression status of tumour metastases was identical, in all cases, with that of the primary tumours [49]. Burns *et al.* showed that the primary and the corresponding lymph node metastasis had the same mutation in three SCCs of the tongue, suggesting that the mutant p53 genes continued to be important for SCC progression [60]. The high degree of congruence between primary and metastatic neoplasms indicated that p53 mutations typically preceded metastatic spread [31]. However, other authors found discordant staining between lymph node metastases and primary tumours [7, 11, 50, 52], showing that p53 was not associated with determinants of the malignant potential such as invasion or metastases [52]. Other authors have correlated mutated p53 with the invasiveness and metastasising ability of cancer cells. In those cases the prevalence of p53 expression was higher in patients with lymph node involvement than in those without lymph node metastases [18, 53]. Intensity of positive staining greater than in the primary tumour was observed in all lymph node metastases [53]. Girod *et al.* noted that the number of p53-positive tumours was highest in the group of recurrent SCC [12], whereas, neither the recurrence rate nor the time to recurrence were dependent on p53 positivity or negativity [47].

Relationship between the expression of p53 and the development of multiple tumours

Oral SCC originates in a multicentric fashion by a process of "field cancerisation", in which an area of the epithelium has been preconditioned by spontaneous alteration or by carcinogenic agents [61]. The prognosis for patients suffering from head and neck cancers is adversely affected by the very high occurrence of multiple primary and secondary tumours. Gallo and Bianchi showed p53-positive staining in 71% of multiple SCC. Fifty per cent showed positive staining of both first and second primary tumours, whereas 25% had positive labelling only for first primary cancer, 17% showed p53 expression only in the second primary cancer and only 8% showed no p53 immunoreactivity. These results show the discordance between the p53 gene mutations in primary cancers and the corresponding second primary cancers. Moreover, 10 out of 12 head and neck cancer patients with multiple cancers showed p53-positive staining in normal epithelium from different sites, even at a significant distance from the site of first and second primary malignancies [62]. These mucosal biopsies came mostly from patients with both first and second primary p53 immunoreactive tumours.

Therefore, the expression of p53 in normal epithelium from head and neck cancer patients would indicate an increased risk for second primary cancers. Nees *et al.* also showed p53 mutations in tumour-distant epithelia of 15 head and neck cancer patients with a single cancer, 5 of whom developed secondary or recurrent tumours. The presence of multiple foci of p53 overexpression in the mucosa at distances of up to 1 cm from the main lesion could indicate that these events are multifocal. The p53 positivity was limited to the basal and supra basal cells with a loss of positivity with differentiation [11]. The observation of p53 overexpression in normal epithelium adjacent to the tumour might support the conception of "field cancerisation", whereby the whole epithelium accumulates genetic damage over time and is at increased risk for developing multiple independent lesions that may become malignant [48]. The process of multiple tumour development in the head and neck region might be initiated by lateral movement of premalignant basal keratinocytes, thus favouring a monoclonal nature for the development of multiple primary, secondary, and recurrent tumours [21]. However, Nees *et al.* detected different genetic changes in p53 in different tumour-distant mucosal biopsies from the same patients and dramatic changes occurred in different regions within a single biopsy [11]. In one patient who had two separate tumours in the oral cavity, Boyle *et al.* showed distinct p53 mutations in each tumour [8]. These results strongly argue for a multifocal polyclonal process. This concept is strongly substantiated by data showing that the genetic lesions were discordant between the initial primary cancer and the second or third primary tumours, which suggested that these cancers arose as independent events [31, 62, 63]. The mutant p53 "signature" of a tumour could be used to distinguish between recurrent and second primary SCCs [63].

p53 mutation: an early or late event in head and neck squamous cell carcinoma development?

As stated above, numerous authors have observed that, in p53-positive cases, p53 overexpression is not exclusively restricted to neoplastic cells, but frequently affects also the normal appearing keratinocytes adjacent to p53-positive neoplasms as well as in dysplastic areas and *in situ* carcinomas. These results suggested that p53 abnormalities may contribute to the early phases of SCC development [3, 11, 18, 30, 33, 40, 44, 48, 50, 52, 55, 57] and that p53 may be involved in the first transformation stage [41]. Furthermore, Boyle *et al.* observed that the incidence of p53 mutations increased from early lesions (dysplasias) to invasive SCC [8]. However, other authors support a late role for p53 in the sequential expression of the malignant phenotype because p53-positive lymph node metastases may develop from p53-negative primary tumours [13] or because of intense p53 immunostaining found in very advanced primary tumours [22]. Furthermore, in mouse multistage SCC development, Kemp *et al.* showed that p53 loss influences only the later stages of progression to carcinoma [64].

Relationship between the expression of p53 and clinical and histopathological parameters

The relationship between p53 mutations and tumour grade and stage has been evaluated in many studies. Most of the

published data on oral, head and neck, oesophageal and laryngeal SCC have shown no positive relationship between p53 expression and histological grading of tumours [6, 13, 37, 38, 40, 43, 44, 47, 50, 52], TNM staging [6, 8, 24, 37, 38, 40, 42, 43, 47, 49, 51], size of the tumour [8, 37, 44], tumour site [6, 8, 25, 38, 40, 47], age [3, 6, 8, 25, 42, 43, 46] and sex [3, 8, 25, 43, 50]. However, other studies have demonstrated a positive correlation between p53 expression and high grades of malignancy [18, 37, 45, 46, 49]. In 40 highly differentiated tumours, p53 expression was detectable in 47% of tissue samples. In 45 tumours with a low grade of differentiation, 60% showed p53 expression [12]. Nishioka *et al.* and Shintani *et al.* found that the incidence of p53 positivity was higher in the poorly differentiated group [44, 45]. Despite the fact that p53 mutations were equally distributed throughout tumours of all stages, Zariwala *et al.* found a tendency toward a higher incidence of p53 positivity in highly malignant, poorly differentiated carcinomas [31]. In carcinomas forming large cancer nests with central keratinisation, p53-positive cells were observed predominantly in the periphery of these nests but not in central areas where cells are well differentiated [21, 37, 53]. Overexpression of p53 has been correlated with increased dedifferentiation [11] and with cellular atypia [41]. Furthermore, Ranasinghe *et al.* noted that the majority of oral SCC which did not show p53 overexpression (29/34) were well differentiated tumours [25]. For TNM staging, studies showed an increased tendency of p53 staining with advancing TNM [30], although the correlation was not statistically significant. Nylander *et al.* showed that the majority of p53-negative tumours (67%) were T1/T2 tumours, whereas 86% of the p53-positive tumours were T3/T4 tumours [36]. Furthermore, Langdon and Partridge observed a positive correlation between the intensity and the distribution of p53-positive cells and a high STNMP score [18]. In contrast, Nishioka *et al.* found higher levels of p53 staining in T1 but not in T4 tumours and in diffuse invasive SCC [45]. Frank *et al.* found that patients whose tumours stained strongly for p53 were significantly younger, were at a more advanced clinical stage and tended to have increased expression of epidermal growth factor receptors [13]. Moreover, p53 immunostaining intensity increased with the depth of cancer invasion [37, 49, 52, 53]. Furthermore, Nishioka *et al* found higher levels of p53 staining in diffuse invasive SCC [45]. Discrepancies according to the site were found: the percentage of p53 immunoreactivity tumours was highest in the oropharynx and the oral cavity, decreasing somewhat in the regions of the larynx and the hypopharynx [5, 11, 36].

Relationship between p53 expression and the progression and prognosis of tumours

Major interest exists on the role played by p53 mutations in the progression and prognosis of the tumour. Several authors have found a close correlation between p53 expression and survival time. Wang *et al.* showed that patients with oesophageal SCC in Linxian (China) surviving more than 10 years had a lower rate of expression for p53 than those surviving 3 years or less [53]. Shimaya *et al.* found the survival rate of patients with p53 expression in oesophageal SCC to be significantly lower than that of patients without p53 expression and that p53 expression was correlated with a worse disease outcome in patients with positive node involvement [52]. Field *et al.* have demonstrated a positive correlation between p53 overexpression and a very poor prognosis in patients at the end-stage of the disease [65]. Despite a small sample, Warnakulasuriya and Johnson found a striking difference in p53 overexpression status between patients who died early of the cancer and those who survived longer [59]. All patients demonstrating p53 overexpression died of recurrent disease within 5 years. Langdon and Partridge noted that 5 out of 8 patients with tumours which expressed p53 developed recurrences [18]. Furthermore, Brachman *et al.* found that 11 out of 15 (73%) patients with p53 mutations in HN-SCC had tumour recurrences compared to 6 out of 13 (46%) patients without p53 mutations [5]. Tumours with p53 gene mutations recurred at a median time of 6 months compared to a median time to recurrence of 17.4 months for those tumours without p53 mutations. Gluckman *et al.* showed that T1 lesions negative for p53 staining behaved less aggressively and were associated with better prognosis than those positive for p53 [66]. In contrast, Dolcetti *et al.* reported no p53 differences between tumours from patients with early relapse and those who had been disease-free for more than 5 years [51]. As well, in a study of 40 patients with lingual SCC, Matthews *et al.* showed that 50% (6 out of 12) of individuals with p53-positive tumours and 46.4% (13 out of 28) of individuals with p53-negative tumours remained alive and carcinoma free (4–11-year follow-up) [42]. Furthermore, Frank *et al.* found no significant association between p53 immunostaining and survival among patients with SCC of the hypopharynx [13]. Differences were not statistically significant, probably due to the small sample size. The median survival of patients who were positive for p53 was shorter than that for patients who were negative for p53. In experimental mice, the loss of p53 function or the absence of p53 greatly enhances progression from the premalignant to the malignant state during progression to SCC [64]. Burns *et al.* also showed that p53 mutations are required for tumour progression [60]. Anwar *et al.* found that the simultaneous overexpression of p53 and the presence of *RAS* mutation might be related to the progression stage of larynx SCC [50]. Huang *et al.* suggested the involvement of both p53 and the retinoblastoma gene (*Rb*) in the genesis and/or progression of most of the oesophagus SCC [27]. Only one study reports that patients having an overexpression of p53 in SCC of the tongue base had a greater mean survival than those who did not [43]. Other authors have suggested that p53 overexpression probably does not affect the overall survival [24, 36, 37, 47] and is not required for progression of HN-SCC toward a more malignant and metastatic phenotype [51, 52].

CONCLUSION

As a tumour suppressor gene the inactivation of p53 can induce the development of malignancy. Alterations of p53 may be excellent biomarkers as indicators of the predisposition of a particular oral premalignant lesion towards malignancy. Mutations of p53 are the most common genetic abnormalities found in human cancers, especially in the development of HN-SCC. The ability to detect p53 with IHC in tumours is almost synonymous with the presence of a mutation. Discrepancies observed in p53 IHC might be related to the antibodies used, but also to variations in fixation and IHC procedures. Therefore, immunostaining of wild-type p53 appears to be a valuable, if not absolutely reliable, adjunct in the study of HN-SCC and oral precancerous lesions.

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