Oral Diseases (2007) 13, 126–133. doi:10.1111/j.1601-0825.2006.01349.x

http://www.blackwellmunksgaard.com

INVITED REVIEW

Molecular genetics of premalignant oral lesions

SK Mithani[1], WK Mydlarz[2], FL Grumbine[2], IM Smith[2], JA Califano[2]

Division of Plastic and Reconstructive Surgery, Departments of [1]Surgery and [2]Otolaryngology-Head and Neck Surgery, Johns Hopkins Medical Institutions, Baltimore, MD, USA

Oral squamous cell carcinoma (OSCC) is characterized by cellular and subcellular alterations that are associated with a progression towards dedifferentiation and growth. There are several histologically distinct lesions of the oral cavity which have malignant potential. These are leukoplakia, erythroplakia, lichen planus, and submucous fibrosis. These are characterized by a spectrum of chromosomal, genetic, and molecular alterations that they share with each other as well as with the malignant lesions that develop from them. In this review we summarize the investigation of the molecular genetics of each of these lesions and relate them to the alterations, which have been demonstrated in OSCC, to define their location on the continuum of changes, which lead to malignant transformation.
Oral Diseases (2007) **13**, 126–133

Keywords: oral; premalignant; erythroplakia; leukoplakia; lichen planus; submucous fibrosis; molecular genetics

Introduction

Oral Squamous cell carcinoma (OSCC) can be preceded by the appearance of lesions which have the potential either to develop into cancer, or portend the development of cancer in the oral cavity. There are several histologically distinct lesions of the oral mucosa which are characterized as having malignant potential. Among these are leukoplakia, erythroplakia, oral lichen planus (OLP), and submucous fibrosis. These lesions are found in association with and preceding OSCC. They are variable in their malignant potential as well as their genetic background.

The progression of OSCC has been described from a genetic perspective, with a distinct pattern and timing of genetic alterations along a continuum of malignant transformation. Premalignant lesions have been shown to demonstrate many of the genetic alterations present in OSCC (Califano *et al*, 2000; Ha *et al*, 2003; Ha and Califano, 2003). The degree and amount of similarity to OSCC found in premalignant lesions is, to some degree, dependent upon presence of atypia, however, individual lesions possess molecular genetic traits in common with OSCC even in the absence of histologically defined dysplasia.

The development of OSCC is generally predicated upon the development of multiple, clonal, genetic alteration, which lends a clonal population of cells a growth advantage over others. Due to the mechanism by which oral mucosa undergoes transformation in OSCC, i.e. chronic exposure to carcinogens in the form of tobacco, betel nut, with alcohol as a cocarcinogen, there are a wide variety of molecular alterations that have been associated with carcinogenesis. In addition to dysregulation of oncogenes and tumor suppressors, cytogenetic changes, epigenetic changes, and mitochondrial mutations have been implicated in development of OSCC. These alterations are reflected in varying degrees in premalignant lesions.

Leukoplakia

Leukoplakia is defined by WHO classification as, 'a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion' (Pindborg and Wahi, 1997). It is further characterized based upon the heterogeneity of the lesion, with increasing heterogeneity associated with increased likelihood of malignant transformation (Pindborg *et al*, 1963, 1968; Gupta *et al*, 1980; Schepman *et al*, 1998).

Leukoplakia is the most commonly diagnosed premalignant lesion in the oral cavity. It is also most associated with the development of OSCC (Cawson *et al*, 1996). Studies have demonstrated a co-incidence of leukoplakia at the time of diagnosis of OSCC of up to 60% (Gupta *et al*, 1980; Bouquot *et al*, 1988; Reibel, 2003). Patients who present with oral leukoplakia have up to a 36% incidence of subsequent OSCC development if the lesion demonstrates dysplastic features (Silverman *et al*, 1984; Lumerman *et al*, 1995;

Correspondence: Joseph Califano MD, Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins Medical Institutions, 601 N. Caroline Street, 6th Floor, Baltimore, MD 21287-0910, USA. Tel: +1 410 955 6420, Fax: +1 410 955 8510, E-mail: jcalifa@jhmi.edu
Received 28 September 2006; accepted 29 September 2006

Schepman *et al*, 1998). In the absence of dysplasia these lesions still possess a 15% incidence of cancer development (Silverman *et al*, 1984).

Cytogenetics

Early studies defining a progression model for cancers of the upper aerodigestive tract implicated loss of heterozygosity (LOH) as a mechanism by which genetic loci containing tumor suppressor genes are eliminated. Increased LOH was correlated to histopathologic progression in the upper aerodigestive tract (Califano *et al*, 1996). LOH of the chromosome arms 3p and 9p are associated with increased malignant potential in oral leukoplakia (Emilion *et al*, 1996; Mao, 1997; Zhang and Rosin, 2001). Fifty percent of leukoplakia contains allelic loss of either the 3p or 9p chromosome arms (Rosin *et al*, 2000; van der Riet, 1994). LOH at these loci alone renders a 3.8-fold increased risk of malignant transformation, while additional LOH at the 4q, 8p, 11q, 13q, and 17p loci are associated with 33-fold increased risk of malignant transformation (Rosin *et al*, 2000). Patterns of LOH in leukoplakia are reflected in the foci of early carcinomas, which are found within these lesions (Jiang *et al*, 2001).

Insertion or deletion of basepairs at microsatellites, termed microsatellite instability (MSI), is another cytogenetic feature shared between premalignant lesions and OSCC. The MSI is present in 55% of leukoplastic lesions (Partridge *et al*, 1998). There is a trend towards increasing MSI prevalence associated with histologic progression of premalignant lesions (Ha *et al*, 2002a). The underlying mechanisms for these findings are unclear given the absence of mismatch repair defects.

The presence of chromosomal aneuploidy in leukoplakia at loci containing putative oncogenes has been extremely controversial because of the high-profile retraction of several prominent studies (Sudbo *et al*, 2004; Curfman *et al*, 2006). Notwithstanding, this several studies have demonstrated the presence of aneuploidy in leukoplakia, although not with the prognostic value furthered in the discredited work. Studies have convincingly demonstrated a trend of increasing polysomy at several loci in the progression of upper aerodigestive tract tumors (Hittelman *et al*, 1993, 1996). Chromosome *in situ* hybridization has been used to detect replication of chromosomes in oral leukoplakia. These studies indicate that majority of leukoplakia lesions contain an abnormal number of chromosomes 7 and 17 (Lee *et al*, 1993) and lesions with a $>3\%$ proportion of cells with trisomy 9 had a significantly higher likelihood of progression to cancer (Lee *et al*, 2000).

Telomeres are tandem arrays of repeated TTAGGG hexamers found at the end of human chromosomes, which prevent degradation and fusion. Telomere length shortens with aging and progressive replication. The telomerase protein elongates telomeres in germline and fetal cells. Its expression is limited in somatic cells. Several studies have demonstrated that up to 75% of oral leukoplakia demonstrate telomerase activity (Mutirangura *et al*, 1996; Kannan *et al*, 1997; Chang *et al*, 1999; Miyoshi *et al*, 1999; Liao *et al*, 2000; Fujimoto *et al*, 2001). Several of these studies demonstrated a correlation of telomerase activity with degree of atypia and dysplastic changes (Mutirangura *et al*, 1996; Miyoshi *et al*, 1999). Telomerase mRNA expression levels have also been shown to correlate with advancing grade of dysplasia (Luzar *et al*, 2004), implying that transcriptional upregulation is responsible for increased activity in leukoplakia and OSCC progression.

p53 mutation

Mutation of p53 is one of the most common genetic events in tumorigenesis. It is frequently mutated and present in its inactivated form in OSCC (Nylander *et al*, 2000). Mutant p53 demonstrates a longer half life than wild type, and its mutant form is often detectable by molecular biology techniques (Reibel, 2003). p53 protein expression has been detected by immunohistochemistry (IHC) in 90% of oral leukoplakia, while it is absent in normal oral mucosa (Lippman *et al*, 1995). However, it is unclear whether this expression represents mutant p53 or stabilized wild type protein. Several studies have demonstrated that p53 detection by IHC alone or with other markers appears to be associated with greater risk for malignant progression (Nishioka *et al*, 1993; Kaur *et al*, 1994; Girod *et al*, 1995; Huang *et al*, 1997b; Ries *et al*, 1998; Scheifele *et al*, 2002; Kovesi and Szende, 2003). Meta-analysis concluded that 47% of premalignant lesions had p53 expression (Warnakulasuriya *et al*, 1998), but conclusion as to the role of this finding in carcinogenesis is unclear because of the inability to distinguish between functional p53 isoforms. Parabasal detection of expression of p53 by IHC has been shown to have a stronger correlation with progression to cancer in several studies (Lippman *et al*, 1995; Cruz *et al*, 1998, 2002a,b), lending credence to the potential application of p53 detection in IHC for stratification of risk of malignant transformation in oral leukoplakia.

The p53 functions as a powerful tumor suppressor gene making its mutation or expression alteration a powerful mechanism in carcinogenesis. Loss of function mutation, LOH, and gene promoter inactivation are mechanisms by which this can potentially occur. The LOH of the p53 (17p3) locus has been demonstrated specifically by restriction length fragment polymorphism in premalignant lesions, which have a higher risk for development of malignancy (Partridge *et al*, 1998). Single strand conformational polymorphism (SSCP) is a technique which can detect mutation of a gene by detection of aberrant hybridization to a wild type template. SSCP followed by sequencing demonstrated 11 p53 mutations in a set of brushings from 34 patients with oral leukoplakia (Lopez *et al*, 2004).

Mitochondrial alteration

Mitochondrial genomic mutations occur in response to oxidative damage and stress at a higher rate than the nuclear genome. Mitochondrial alteration has been demonstrated in a range of human tumors, including head and neck cancer (Sanchez-Cespedes *et al*, 2001), but the mechanism by which these mutations contribute

to carcinogenesis has not been demonstrated. It has been postulated to occur because of mitochondrial dysfunction in apoptosis or through reactive oxygen species generation, which perpetuates oxidative DNA damage (Gottlieb and Tomlinson, 2005). Mitochondrial microsatellite analysis of oral leukoplakia demonstrated alteration of the polycytosine tract of the diversity loop, the prevalence of which correlated to an increasing degree of atypia in lesions of leukoplakia (Ha *et al*, 2002b).

Increase in mitochondrial DNA copy number has been postulated to be a mechanism by which cells compensate for dysfunction caused by oxidative DNA damage. Increase in mitochondrial gene copy number has been shown to be present in leukoplakia in a ratio proportionate to the degree of histopathologic severity (Kim *et al*, 2004).

Epigenetic changes

Promoter hypermethylation is a well described mechanism by which tumor suppressor genes are transcriptionally inactivated in OSCC (Ha and Califano, 2006). Aberrant methylation of CpG-rich regions of the promoters prevent gene transcription by altering the structure of histone complexes. Several studies have addressed the occurrence of promoter hypermethylation in leukoplakia. The only study in the literature specifically addressing leukoplakia tissue methylation found RAR-β2 to be hypermethylated in 53% of lesions (Youssef *et al*, 2004). Oral rinses from patients with leukoplakia demonstrated 44% incidence of methylation of p16 and 56% incidence of methylation of O^6-methylguanine-DNA-methyltransferase (MGMT) (Lopez *et al*, 2003), two genes which have are methylated in OSCC. Studies are limited by the relatively large amount of DNA required to do methylation analysis. With advancements in the technical aspects of performing methylation analysis assays, further genes will undoubtedly be investigated leading to a more complete picture of the occurrence of methylation in leukoplakia and its role in early carcinogenesis.

Erythroplakia

Oral erythroplakia is a premalignant lesion of the oral mucosa that typically presents as a discrete, velvety red macule or plaque, <1.5 cm in diameter, on the floor of the mouth, the soft palate or the buccal mucosa (Reichart and Philipsen, 2005), 'which cannot be characterized clinically or pathologically as any other recognizable condition' (Kramer *et al*, 1978). Oral erythroplakia is most frequently diagnosed between the ages of 40 and 70 (Kumar *et al*, 2005). For reasons of a paucity of literature specifically devoted to oral erythroplakia, a definitive value for its prevalence is not readily available, however, data range from 0.02% to 0.83% (Shafer and Waldron, 1975; Lay *et al*, 1982; Zain *et al*, 1997; Hashibe *et al*, 2000). Chewing tobacco, alcohol intake, and chutta smoking are strong risk factors for the development of oral erythroplakia (Daftary and Shah, 1992; Hashibe *et al*, 2000). Reported values for the rate of malignant transformation to oral squamous-cell carcinoma range from 14.3% to 50.0% (Reichart and Philipsen, 2005). Additional evidence of the prognostic significance of oral erythroplakia is that previous studies have found 85–90% of asymptomatic oral squamous-cell carcinomas to be initially reported as erythroplakia (Mashberg and Meyers, 1976; Regezi and Sciubba, 1993).

As has been demonstrated with other premalignant oral lesions, the p53 tumor suppressor gene is frequently mutated in oral erythroplakia. A study by Qin *et al* found 11 of 24 oral erythroplakia lesions to contain a total of 12 p53 mutations that resulted in altered p53 protein sequence. These mutations were located in exons 6 (33%), 5 (25%), 8 (25%), and 9 (17%) of the p53 gene. Six mis-sense mutations, three single-base pair deletions, and three splice-site mutations were observed. Histologic examination of these same specimens did not reveal a significant difference between histologic grade and the frequency of p53 mutations, a finding that suggests that p53 might have been mutated early in oral squamous-cell cancer development when the initial lesion presented as erythroplakia (Qin *et al*, 1999).

Because of the histologic similarities between oral erythroplakia and leukoplakia, it is reasonable to expect that oral erythroplakia lesions might also possess similar subcellular changes as leukoplakia, including LOH, aneuploidy, and MSI. Polysomy of chromosomes 7 and 17 has been implicated in oral erythroplakia (Hittelman *et al*, 1993). Partridge *et al* examined oral erythroplakia and leukoplakia lesions for LOH and allelic gain at several chromosomal loci known to be altered in oral squamous-cell carcinoma. It can be inferred from this study that oral erythroplakia lesions may have LOH or allelic gain at 9p, 3p, within the Rb gene, p53 gene, or the DCC gene (Partridge *et al*, 1998). A more recent study also shows a trend of chromosomal aberrations and aneuploidy associated with oral erythroplakia, although these data are the subjects of a recent controversy (Sudbo *et al*, 2002).

Lichen planus

Oral lichen planus is a benign lesion with a characteristic white, lacy, reticular pattern that classically presents on the buccal mucosa. Papular, atrophic or erosive lesions constitute the major subtypes of OLP, and present infrequently. Erosive lesions, in particular, may be quite painful and result in multiple complications, such as secondary infections (predominantly candida species), as well as poor nutrition and dehydration because of pain (Katta, 2000).

Histopathology generally reveals characteristic bands of infiltrating lymphocytes at the epidermal–dermal junction and damage to the basal cells of the epidermis. The epidermis usually has a saw-toothed appearance secondary to wedge-shaped hypergranulosis and irregular acanthosis (Silverman *et al*, 1991; Buser *et al*, 1997).

Even though the World Health Organization (WHO) classifies OLP as a precancerous lesion, the premalignant or malignant potential of OLP continues to be the subject of an ongoing and controversial debate in

the literature. Mainly several retrospective studies and case series have reported a significant risk of malignant transformation from OLP to OSCC. The reported overall risk of malignant transformation ranges from 0.4% to 5.6%, or 0.04% to 1.74% per annum (van der Meij *et al*, 2003). Malignant transformation is most closely associated with cases of atrophic or erosive OLP (Silverman, 2000). Critics contend that these associations are either coincidental or evidence that a dysplastic epithelial lesion was misdiagnosed as OLP, especially as it is often difficult to differentiate one from the other. A recommendation was therefore made to differentiate carefully between OLP lesions with dysplasia, called lichenoid dysplasia, and simple epithelial dysplasia (Krutchkoff and Eisenberg, 1985). However, as some prospective studies on patients with OLP indicate rather high rates of malignant transformation to OSCC (Silverman *et al*, 1985, 1991), these lesions should be considered premalignant.

A number of scientific investigations are beginning to uncover some of the molecular genetic aberrations within OLP. One of the early molecular genetic studies of OLP looked at the frequency of LOH at nine loci located on chromosomes 3p, 9p, and 17 p, that occur frequently in most oral tumors. The OLP lesions contained minimal genetic deviation, while the various dysplastic and malignant OSCC lesions had steadily increasing frequencies of LOH on multiple chromosomes (Zhang *et al*, 1997). In a further study into the potential for genetic instability on chromosomes 9 and 17 in OLP, (Kim *et al*, 2001) OLP was compared with lichenoid dysplasia lesions that progressed to OSCC using chromosome *in situ* hybridization. There was a statistically significant difference in the degree of genetic instability between OLP and lichenoid dysplasia, with an increase in partial imbalance of chromosome 9 (Kim *et al*, 2001). This could suggest that monosomy of chromosome 9 may have a critical role in the malignant transformation of OLP.

Perturbations of p53 and c-erbB2 protein expression are seen in OLP lesions and may promote dysregulation of apoptosis and/or tyrosine kinase signaling. Expression of the p53 tumor suppressor gene has been detected with IHC in OLP, as well as malignant and metastatic OSCC, with concurrently increasing levels of proliferating cell nuclear antigen (PCNA) expression within these lesions (Girod *et al*, 1994). The increase in the number of p53 positive biopsies significantly correlates with the degree of dysplasia and loss of differentiation in OLP. Most OLP lesions have a characteristic pattern of p53 positive nuclei confined to the basal layer of the epithelium, and in one study, remarkably, nine out of 27 (33%) of the OLP lesions contained p53 mutations when screened for mutations in exons 5 through 8 (Ogmundsdottir *et al*, 2002).

Studies looking at c-erbB2 expression in serial OLP biopsies of progressive lesions and OSCC revealed a loss of expression of the protein, suggesting that loss of c-erbB2 function could potentially indicate a neoplastic transformation and be involved in carcinogenesis (Kilpi *et al*, 1996; Parise Junior *et al*, 2004). In addition, OLP keratinocytes harbor strong telomerase activity, which could be another indicator of their malignant potential (O'Flatharta *et al*, 2002).

Further investigation of the genetic alterations in OLP, is warranted. A more complete understanding of the genetic aberrations in this disease will help determine and better understand its malignant potential.

Submucous fibrosis

Oral submucous fibrosis (OSF) is a unique chronic disease seen almost exclusively in adult patients from south Asia, where its occurrence has a significant association with areca nut use, principally in betel quid (combined areca nut, betel leaf, tobacco, and slack lime) chewing (Sinor *et al*, 1990; Dave *et al*, 1992; Jeng *et al*, 2001). OSF is a chronic insidious disease characterized by irreversible generalized fibrosis of the oral soft tissues. The earliest clinical sign is blanching of the oral mucosal tissue, imparting a mottled, marble-like appearance. Mature lesions demonstrate palpable fibrous bands which are essential for the diagnosis. Ultimately, difficulties with mastication, speech, and swallowing develop secondary to the progressive oral fibrosis (Yusuf and Yong, 2002; Liu *et al*, 2004).

Early on, the histopathology consists mostly of chronic inflammatory cells with an eosinophilic component infiltrating the subepithelial connective tissues. Older lesions demonstrate a reduced vascularity, reduced numbers of inflammatory cells, and dense bundles and sheets of collagen deposited immediately beneath the epithelium. The diffuse hyalinization of the subepithelial stroma usually extends into the submucosal tissues, typically replacing the fatty and fibrovascular tissues (Jayanthi *et al*, 1992; Liu *et al*, 2004).

Oral submucous fibrosis has a propensity for malignant transformation, with as high as a fourth of all cases demonstrate epithelial dysplasia at the time of biopsy. The association of betel quid chewing, OSF, and oral OSCC is quite profound, especially in Taiwan and the Indian subcontinent where up to 80% of oral OSCC is associated with this habit. Epidemiologic studies have shown that the rate of malignant transformation ranges from 3% to 19% (Murti *et al*, 1985; Yusuf and Yong, 2002). The addition of tobacco to the majority of betel quid appears to increase further the risk of malignant transformation (Critchley and Unal, 2003). There is no effective treatment for OSF and the condition is irreversible once formed, hence patients should have regular, close follow up. It is prudent practice for physicians to biopsy the areas of severe disease repeatedly to monitor for malignancy (Zain *et al*, 1999).

Similarly to OLP, there are only a handful of published studies that looked at genetic aberrations in OSF, and their possible role in malignant transformation. Certain worrisome findings, such as polymorphisms of heterochromatic chromosome regions, sister-chromatid exchanges, and aberrant p53 expression and mutations may be involved in OSF carcinogenesis (Cox and Walker, 1996b).

One of the early genetic studies found a statistically significant increase in the size C-band heteromorphism patterns on chromosome 1 in OSF and OSCC patients when compared with healthy subjects (Dave *et al*, 1991). Another cytogenetic study found an increase in the frequency of sister-chromatid exchanges and chromosome aberrations in areca nut consumers with OSF and OSCC, further emphasizing the dangers of this habit, even without the concurrent use of smokeless tobacco (Dave *et al*, 1992).

Oral submucous fibrosis lesions also abnormally express and stain positive for p53, a finding that can once again be explained by specific clustered mutations in exon 5 of the p53 gene (Cox and Walker, 1996a; Chiba *et al*, 1998; Liao *et al*, 2001; Bathi and Prabhat, 2003). The PCNA growth fraction correlates significantly with that derived by Ki-67 labeling, but there was no correlation found between growth fraction and the extent of dysplasia. Once again p53 and PCNA expression was characteristically restricted to the basal epithelial layer, a potential common theme in premalignant lesions (Cox and Walker, 1996a). Furthermore, expression levels and mutation frequencies of p53 were progressively higher in OSCC than in OSF lesions, especially in betal quid chewers. Both groups had significantly higher expression levels and mutation frequencies when compared to normal mucosa, similar to OLP lesions (Chiba *et al*, 1998). Betal quid chewing could now be associated with chromosomal damage, as well as potential genetic mutations and aberrant protein expression.

Another tumor suppressor gene adenomatous polyposis coli (APC) could also potentially be involved in the malignant potential of OSF. Remarkably, in a small subset of patients with OSF, all the patients contained at least one mis-sense mutation, and 87% had an additional non-sense mutation that created a truncated protein and decreased expression levels of APC (Liao *et al*, 2001). Such alteration of the APC gene in OSF may imply a risk of progression to oral cancer, although additional work should be performed on a larger cohort of patients to characterize these alterations further. These are interesting results as LOH with mutation of APC has not been detected frequently in oral cancers, although several studies have described LOH in 25% of oral squamous cell cancers suggested a higher number, with 53.8% showing LOH (Largey *et al*, 1994; Huang *et al*, 1997a; Williams, 2000). Hence APC could play a role in the malignant transformation of OSF–OSCC, because of its functions in the regulation of the Wnt signaling pathway via its interaction with beta catenin (Sierra *et al*, 2006).

The growth of cancer is closely dependent on the balance between cell growth and cell death. The Bcl-2 family of proteins regulates apoptosis and bcl-2 and bax are two of its major mediators. In one report, patients with oral cancer and a variety of premalignant lesions including OSF, revealed aberrant expression of bcl-2 and bax exclusive of p53 expression. In contrast, up to 30% of OSCC demonstrated over expression of both proteins, and had a significant correlation between positive nodal status and expression of p53 and bcl-2 (Teni *et al*, 2002). Interestingly, only a few studies have been undertaken to investigate apoptosis in OSCC, but one study by Jordan *et al* demonstrated strong Bcl-2 immunoreactivity in poorly differentiated oral cancers and upregulation of expression in dysplastic epithelium adjacent to invasive tumor, whereas in contrast bax immunoreactivity was the strongest in well-differentiated OSCC (Jordan *et al*, 1996). These results suggest that alterations of the Bcl-2 family of proteins may play a role in the in the early stages of epithelial carcinogenesis and development of OSCC, and work needs to continue to evaluate these effects in early premalignant lesions such as OSF.

Conclusions

The distinction between benign and potentially malignant oral lesions is currently based upon histologic examination of biopsy specimens from suspicious lesions. In the absence of frank carcinoma or dysplasia, our ability to quantitate the risk associated with various clinically distinct oral lesions is limited. In fact, there is a significant overlap and subtlety in the diagnosis of these various lesions. The molecular studies that have been undertaken to this point serve as the basis by which we will be eventually able to augment not only clinical assessment and classification of oral lesions, but also predict their malignant potential more accurately. Considerable work remains to be performed in determination of the genetic alterations present, and the appropriate classification and risk stratification of oral lesions.

References

Bathi RJ, Prabhat (2003). p53 aberrations in oral sub mucous fibrosis and oral cancer detected by immunohistochemistry. *Indian J Dent Res* **14:** 214–219.

Bouquot JE, Weiland LH, Kurland LT (1988). Leukoplakia and carcinoma in situ synchronously associated with invasive oral/oropharyngeal carcinoma in Rochester, Minn., 1935–1984. *Oral Surg Oral Med Oral Pathol* **65:** 199–207.

Buser D, Rankin KV, Meier E, Plemons JM, Altermatt HJ, Rees TD (1997). Oral lichen planus. The clinical manifestations, histopathology, etiology, long-term course and patient data from the Stomatology Center at the Baylor College of Dentistry in Dallas, USA. *Schweiz Monatsschr Zahnmed* **107:** 294–306.

Califano J, van der Riet P, Westra W *et al* (1996). Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* **56:** 2488–2492.

Califano J, Westra WH, Meininger G, Corio R, Koch WM, Sidransky D (2000). Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* **6:** 347–352.

Cawson RA, Langdon JD, Eveson JW (1996). *Surgical pathology of the mouth and jaws*. Wright: Oxford, Boston.

Chang LY, Lin SC, Chang CS, Wong YK, Hu YC, Chang KW (1999). Telomerase activity and *in situ* telomerase RNA expression in oral carcinogenesis. *J Oral Pathol Med* **28:** 389–396.

Chiba I, Muthumala M, Yamazaki Y *et al* (1998). Characteristics of mutations in the p53 gene of oral squamous-cell carcinomas associated with betel-quid chewing in Sri Lanka. *Int J Cancer* **77:** 839–842.

Cox SC, Walker DM (1996a). Epithelial growth fraction and expression of p53 tumour suppressor gene in oral submucous fibrosis. *Aust Dent J* **41:** 91–96.

Cox SC, Walker DM (1996b). Oral submucous fibrosis. A review. *Aust Dent J* **41:** 294–299.

Critchley JA, Unal B (2003). Health effects associated with smokeless tobacco: a systematic review. *Thorax* **58:** 435–443.

Cruz IB, Snijders PJ, Meijer CJ *et al* (1998). p53 expression above the basal cell layer in oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. *J Pathol* **184:** 360–368.

Cruz I, Napier SS, van der Waal I *et al* (2002a). Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. *J Clin Pathol* **55:** 98–104.

Cruz I, Snijders PJ, Van Houten V, Vosjan M, Van der Waal I, Meijer CJ (2002b). Specific p53 immunostaining patterns are associated with smoking habits in patients with oral squamous cell carcinomas. *J Clin Pathol* **55:** 834–840.

Curfman GD, Morrissey S, Drazen JM (2006). Expression of concern: Sudbo J *et al* DNA content as a prognostic marker in patients with oral leukoplakia. *N Engl J Med* **1270:** 1278.

Daftary DK, ???? MP, Shah HT (1992). Reverse chutta smoking and palatal lesions. Control of tobacco-related cancers and oral diseases. In: Hi J, Gupta PC, Murti PR, eds. *International symposium 1990*. Oxford University Press: Bombay.

Dave BJ, Trivedi AH, Adhvaryu SG (1991). Variations in centromeric heterochromatin among patients with premalignant and malignant oral diseases. *Int J Cancer* **48:** 386–389.

Dave BJ, Trivedi AH, Adhvaryu SG (1992). Role of areca nut consumption in the cause of oral cancers. A cytogenetic assessment. *Cancer* **70:** 1017–1023.

Emilion G, Langdon JD, Speight P, Partridge M (1996). Frequent gene deletions in potentially malignant oral lesions. *Br J Cancer* **73:** 809–813.

Fujimoto R, Kamata N, Yokoyama K *et al* (2001). Expression of telomerase components in oral keratinocytes and squamous cell carcinomas. *Oral Oncol* **37:** 132–140.

Girod SC, Pape HD, Krueger GR (1994). p53 and PCNA expression in carcinogenesis of the oropharyngeal mucosa. *Eur J Cancer B Oral Oncol* **30B:** 419–423.

Girod SC, Cesarz D, Fischer U, Krueger GR (1995). Detection of p53 and MDM2 protein expression in head and neck carcinogenesis. *Anticancer Res* **15:** 1453–1457.

Gottlieb E, Tomlinson IP (2005). Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* **5:** 857–866.

Gupta PC, Mehta FS, Daftary DK *et al* (1980). Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dent Oral Epidemiol* **8:** 283–333.

Ha PK, Califano JA (2003). The molecular biology of mucosal field cancerization of the head and neck. *Crit Rev Oral Biol Med* **14:** 363–369.

Ha PK, Califano JA (2006). Promoter methylation and inactivation of tumour-suppressor genes in oral squamous-cell carcinoma. *Lancet Oncol* **7:** 77–82.

Ha PK, Pilkington TA, Westra WH, Sciubba J, Sidransky D, Califano JA (2002a). Progression of microsatellite instability from premalignant lesions to tumors of the head and neck. *Int J Cancer* **102:** 615–617.

Ha PK, Tong BC, Westra WH *et al* (2002b). Mitochondrial C-tract alteration in premalignant lesions of the head and neck: a marker for progression and clonal proliferation. *Clin Cancer Res* **8:** 2260–2265.

Ha PK, Benoit NE, Yochem R *et al* (2003). A transcriptional progression model for head and neck cancer. *Clin Cancer Res* **9:** 3058–3064.

Hashibe M, Mathew B, Kuruvilla B *et al* (2000). Chewing tobacco, alcohol, and the risk of erythroplakia. *Cancer Epidemiol Biomarkers Prev* **9:** 639–645.

Hittelman WN, Voravud N, Shin DM, Lee JS, Ro JY, Hong WK (1993). Early genetic changes during upper aerodigestive tract tumorigenesis. *J Cell Biochem Suppl* **17F:** 233–236.

Hittelman WN, Kim HJ, Lee JS *et al* (1996). Detection of chromosome instability of tissue fields at risk: *in situ* hybridization. *J Cell Biochem Suppl* **25:** 57–62.

Huang JS, Chiang CP, Kok SH, Kuo YS, Kuo MY (1997a). Loss of heterozygosity of APC and MCC genes in oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* **26:** 322–326.

Huang XF, Zhang WG, Song LJ, Fu HB (1997b). Immunohistochemical detection of PCNA and p53 protein in the premalignant lesions and squamous cell carcinomas of the oral mucosa. *Shanghai Kou Qiang Yi Xue* **6:** 73–74.

Jayanthi V, Probert CS, Sher KS, Mayberry JF (1992). Oral submucosal fibrosis–a preventable disease. *Gut* **33:** 4–6.

Jeng JH, Chang MC, Hahn LJ (2001). Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. *Oral Oncol* **37:** 477–492.

Jiang WW, Fujii H, Shirai T, Mega H, Takagi M (2001). Accumulative increase of loss of heterozygosity from leukoplakia to foci of early cancerization in leukoplakia of the oral cavity. *Cancer* **92:** 2349–2356.

Jordan RC, Catzavelos GC, Barrett AW, Speight PM (1996). Differential expression of bcl-2 and bax in squamous cell carcinomas of the oral cavity. *Eur J Cancer B Oral Oncol* **32B:** 394–400.

Kannan S, Tahara H, Yokozaki H *et al* (1997). Telomerase activity in premalignant and malignant lesions of human oral mucosa. *Cancer Epidemiol Biomarkers Prev* **6:** 413–420.

Katta R (2000). Lichen planus. *Am Fam Physician* **61:** 3319–3324, 3327–3328.

Kaur J, Srivastava A, Ralhan R (1994). Overexpression of p53 protein in betel- and tobacco-related human oral dysplasia and squamous-cell carcinoma in India. *Int J Cancer* **58:** 340–345.

Kilpi A, Rich AM, Konttinen YT, Reade PC (1996). Expression of c-erbB-2 protein in keratinocytes of oral mucosal lichen planus and subsequent squamous cell carcinoma. *Eur J Oral Sci* **104:** 278–284.

Kim J, Yook JI, Lee EH *et al* (2001). Evaluation of premalignant potential in oral lichen planus using interphase cytogenetics. *J Oral Pathol Med* **30:** 65–72.

Kim MM, Clinger JD, Masayesva BG *et al* (2004). Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clin Cancer Res* **10:** 8512–8515.

Kovesi G, Szende B (2003). Changes in apoptosis and mitotic index, p53 and Ki67 expression in various types of oral leukoplakia. *Oncology* **65:** 331–336.

Kramer IR, Lucas RB, Pindborg JJ, Sobin LH (1978). Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol* **46:** 518–539.

Krutchkoff DJ, Eisenberg E (1985). Lichenoid dysplasia: a distinct histopathologic entity. *Oral Surg Oral Med Oral Pathol* **60:** 308–315.

Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS (2005). *Robbins and Cotran pathologic basis of disease*. 7th edn. Elsevier Saunders: Philadelphia, PA.

Largey JS, Meltzer SJ, Sauk JJ, Hebert CA, Archibald DW (1994). Loss of heterozygosity involving the APC gene in oral squamous cell carcinomas. *Oral Surg Oral Med Oral Pathol* **77:** 260–263.

Lay KM, Sein K, Myint A, Ko SK, Pindborg JJ (1982). Epidemiologic study of 600 villagers of oral precancerous lesions in Bilugyun: preliminary report. *Community Dent Oral Epidemiol* **10:** 152–155.

Lee JS, Kim SY, Hong WK *et al* (1993). Detection of chromosomal polysomy in oral leukoplakia, a premalignant lesion. *J Natl Cancer Inst* **85:** 1951–1954.

Lee JJ, Hong WK, Hittelman WN *et al* (2000). Predicting cancer development in oral leukoplakia: 10 years of translational research. *Clin Cancer Res* **6:** 1702–1710.

Liao J, Mitsuyasu T, Yamane K, Ohishi M (2000). Telomerase activity in oral and maxillofacial tumors. *Oral Oncol* **36:** 347–352.

Liao PH, Lee TL, Yang LC, Yang SH, Chen SL, Chou MY (2001). Adenomatous polyposis coli gene mutation and decreased wild-type p53 protein expression in oral submucous fibrosis: a preliminary investigation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **92:** 202–207.

Lippman SM, Shin DM, Lee JJ *et al* (1995). p53 and retinoid chemoprevention of oral carcinogenesis. *Cancer Res* **55:** 16–19.

Liu CJ, Lee YJ, Chang KW, Shih YN, Liu HF, Dang CW (2004). Polymorphism of the MICA gene and risk for oral submucous fibrosis. *J Oral Pathol Med* **33:** 1–6.

Lopez M, Aguirre JM, Cuevas N *et al* (2003). Gene promoter hypermethylation in oral rinses of leukoplakia patients–a diagnostic and/or prognostic tool? *Eur J Cancer* **39:** 2306–2309.

Lopez M, Aguirre JM, Cuevas N *et al* (2004). Use of cytological specimens for p53 gene alteration detection in oral squamous cell carcinoma risk patients. *Clin Oncol* (*R Coll Radiol*) **16:** 366–370.

Lumerman H, Freedman P, Kerpel S (1995). Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **79:** 321–329.

Luzar B, Poljak M, Marin IJ, Eberlinc A, Klopcic U, Gale N (2004). Human telomerase catalytic subunit gene re-expression is an early event in oral carcinogenesis. *Histopathology* **45:** 13–19.

Mao L (1997). Leukoplakia: molecular understanding of premalignant lesions and implications for clinical management. *Mol Med Today* **3:** 442–448.

Mashberg A, Meyers H (1976). Anatomical site and size of 222 early asymptomatic oral squamous cell carcinomas: a continuing prospective study of oral cancer. II. *Cancer* **37:** 2149–2157.

van der Meij EH, Schepman KP, van der Waal I (2003). The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **96:** 164–171.

Miyoshi Y, Tsukinoki K, Imaizumi T *et al* (1999). Telomerase activity in oral cancer. *Oral Oncol* **35:** 283–289.

Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS (1985). Malignant transformation rate in oral submucous fibrosis over a 17-year period. *Community Dent Oral Epidemiol* **13:** 340–341.

Mutirangura A, Supiyaphun P, Trirekapan S *et al* (1996). Telomerase activity in oral leukoplakia and head and neck squamous cell carcinoma. *Cancer Res* **56:** 3530–3533.

Nishioka H, Hiasa Y, Hayashi I, Kitahori Y, Konishi N, Sugimura M (1993). Immunohistochemical detection of p53 oncoprotein in human oral squamous cell carcinomas and leukoplakias: comparison with proliferating cell nuclear antigen staining and correlation with clinicopathological findings. *Oncology* **50:** 426–429.

Nylander K, Dabelsteen E, Hall PA (2000). The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. *J Oral Pathol Med* **29:** 413–425.

O'Flatharta C, Leader M, Kay E *et al* (2002). Telomerase activity detected in oral lichen planus by RNA in situ hybridisation: not a marker for malignant transformation. *J Clin Pathol* **55:** 602–607.

Ogmundsdottir HM, Hilmarsdottir H, Astvaldsdottir A, Johannsson JH, Holbrook WP (2002). Oral lichen planus has a high rate of TP53 mutations. A study of oral mucosa in iceland. *Eur J Oral Sci* **110:** 192–198.

Parise O, Carvalho LV, Miguel RE, Kowalski LP (2004). Prognostic impact of p53, c-erbB-2 and epidermal growth factor receptor on head and neck carcinoma. *Sao Paulo Med J* **122:** 264–268.

Partridge M, Emilion G, Pateromichelakis S, A'Hern R, Phillips E, Langdon J (1998). Allelic imbalance at chromosomal loci implicated in the pathogenesis of oral precancer, cumulative loss and its relationship with progression to cancer. *Oral Oncol* **34:** 77–83.

Pindborg JJ, Wahi PN (1997). *Histological typing of cancer and precancer of the oral mucosa*. 2nd edn. Springer: Berlin; New York.

Pindborg JJ, Renstrup G, Poulsen HE, Silverman S Jr (1963). Studies in oral leukoplakias. V. Clinical and histologic signs of malignancy. *Acta Odontol Scand* **21:** 407–414.

Pindborg JJ, Jolst O, Renstrup G, Roed-Petersen B (1968). Studies in oral leukoplakia: a preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. *J Am Dent Assoc* **76:** 767–771.

Qin GZ, Park JY, Chen SY, Lazarus P (1999). A high prevalence of p53 mutations in pre-malignant oral erythroplakia. *Int J Cancer* **80:** 345–348.

Regezi JA, Sciubba JJ (1993). *Oral pathology: clinical-pathologic correlations*. 2nd edn. Saunders: Philadelphia.

Reibel J (2003). Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. *Crit Rev Oral Biol Med* **14:** 47–62.

Reichart PA, Philipsen HP (2005). Oral erythroplakia–a review. *Oral Oncol* **41:** 551–561.

Ries JC, Schreiner D, Steininger H, Girod SC (1998). p53 mutation and detection of p53 protein expression in oral leukoplakia and oral squamous cell carcinoma. *Anticancer Res* **18:** 2031–2036.

Rosin MP, Cheng X, Poh C *et al* (2000). Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* **6:** 357–362.

Sanchez-Cespedes M, Parrella P, Nomoto S *et al* (2001). Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. *Cancer Res* **61:** 7015–7019.

Scheifele C, Schlechte H, Bethke G, Reichart PA (2002). Detection of TP53-mutations in brush biopsies from oral leukoplakias. *Mund Kiefer Gesichtschir* **6:** 410–414.

Schepman KP, van der Meij EH, Smeele LE, van der Waal I (1998). Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. *Oral Oncol* **34:** 270–275.

Shafer WG, Waldron CA (1975). Erythroplakia of the oral cavity. *Cancer* **36:** 1021–1028.

Sierra J, Yoshida T, Joazeiro CA, Jones KA (2006). The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev* **20:** 586–600.

Silverman S Jr (2000). Oral lichen planus: a potentially premalignant lesion. *J Oral Maxillofac Surg* **58:** 1286–1288.

Silverman S Jr, Gorsky M, Lozada F (1984). Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* **53:** 563–568.

Silverman S Jr, Gorsky M, Lozada-Nur F (1985). A prospective follow-up study of 570 patients with oral lichen planus: persistence, remission, and malignant association. *Oral Surg Oral Med Oral Pathol* **60:** 30–34.

Silverman S Jr, Gorsky M, Lozada-Nur F, Giannotti K (1991). A prospective study of findings and management in 214 patients with oral lichen planus. *Oral Surg Oral Med Oral Pathol* **72:** 665–670.

Sinor PN, Gupta PC, Murti PR *et al* (1990). A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *J Oral Pathol Med* **19:** 94–98.

Sudbo J, Kildal W, Johannessen AC *et al* (2002). Gross genomic aberrations in precancers: clinical implications of a long-term follow-up study in oral erythroplakias. *J Clin Oncol* **20:** 456–462.

Sudbo J, Lippman SM, Lee JJ *et al* (2004). The influence of resection and aneuploidy on mortality in oral leukoplakia. *N Engl J Med* **350:** 1405–1413.

Teni T, Pawar S, Sanghvi V, Saranath D (2002). Expression of bcl-2 and bax in chewing tobacco-induced oral cancers and oral lesions from India. *Pathol Oncol Res* **8:** 109–114.

van der Riet P, Nawroz H, Hruban RH *et al* (1994). Frequent loss of chromosome 9_p21-22 early in head and neck cancer progression. *Cancer Res* **54:** 1156–1158.

Warnakulasuriya KA, Tavassoli M, Johnson NW (1998). Relationship of p53 overexpression to other cell cycle regulatory proteins in oral squamous cell carcinoma. *J Oral Pathol Med* **27:** 376–381.

Williams HK (2000). Molecular pathogenesis of oral squamous carcinoma. *Mol Pathol* **53:** 165–172.

Youssef EM, Lotan D, Issa JP *et al* (2004). Hypermethylation of the retinoic acid receptor-beta(2) gene in head and neck carcinogenesis. *Clin Cancer Res* **10:** 1733–1742.

Yusuf H, Yong SL (2002). Oral submucous fibrosis in a 12-year-old Bangladeshi boy: a case report and review of literature. *Int J Paediatr Dent* **12:** 271–276.

Zain RB, Ikeda N, Razak IA *et al* (1997). A national epidemiological survey of oral mucosal lesions in Malaysia. *Community Dent Oral Epidemiol* **25:** 377–383.

Zain RB, Ikeda N, Gupta PC *et al* (1999). Oral mucosal lesions associated with betel quid, areca nut and tobacco chewing habits: consensus from a workshop held in Kuala Lumpur, Malaysia, November 25–27, 1996. *J Oral Pathol Med* **28:** 1–4.

Zhang L, Rosin MP (2001). Loss of heterozygosity: a potential tool in management of oral premalignant lesions? *J Oral Pathol Med* **30:** 513–520.

Zhang L, Michelsen C, Cheng X, Zeng T, Priddy R, Rosin MP (1997). Molecular analysis of oral lichen planus. A premalignant lesion? *Am J Pathol* **151:** 323–327.