Pergamon

Oral Oncol, Eur J Cancer, Vol. 32B, No. 3, pp. 150–153, 1996

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

0964-1955(95)00065-8

Molecular Changes in Oral Cancer May Reflect Aetiology and Ethnic Origin

I.C. Paterson, J.W. Eveson and S.S. Prime

Division of Oral Medicine, Pathology and Microbiology, Department of Oral and Dental Science, Lower Maudlin Street, Bristol, BS1 2LY, U.K.

Oral cancer, although uncommon in the Western world, accounts for up to 40% of all malignancies in parts of India and South East Asia. Recognised aetiological agents of oral cancer include tobacco and alcohol. This paper reviews the spectrum of molecular changes found in oral squamous cell carcinomas from Western (U.K., U.S.A., Australia) and Eastern (India, S.E. Asia) countries. *p53* mutations are common in tumours from the West (47%) but are infrequent in the East (7%). Tumours from India and South East Asia are characterised by the involvement of *ras* oncogenes, including mutation, loss of heterozygosity (H-*ras*) and amplification (K- and N-*ras*), events which are uncommon in the West. The possibility that these genetic differences reflect aetiology and/or ethnic origin is discussed. Copyright © 1996 Elsevier Science Ltd

Keywords: oral cancer, tobacco, *ras* mutation, *p53* mutation

Oral Oncol, Eur J Cancer, Vol. 32B, No. 3, pp. 150–153, 1996.

The aetiology of oral cancer, specifically cancer of the tongue (ICD9 141) and mouth (ICD 143–145), is well established and predominantly involves the use of tobacco and alcohol. The prevalence of the disease in different parts of the world reflects different forms of exposure to these aetiological agents. In Europe, U.S.A., Australia, China and Japan, cigarette, cigar and pipe smoking are the main forms of tobacco use and the effect of tobacco is known, not only to be dose and time dependent but, also, to act synergistically with the intake of alcohol (spirits) to multiply disease risk [1, 2]. By contrast, in India, Sri Lanka, Papua New Guinea and South East Asia, tobacco chewing is prevalent, usually in the form of a betel quid that consists of the leaf of the betel vine (*Piper betle*) wrapped around areca nut, lime and tobacco; any combination of these constituents may be used. Spices such as cardamom, cloves, aniseed and turmeric, or extracts of *Acacia catechu* or *A. suma*, may also be incorporated for additional flavour [3]. Other forms of tobacco use which are also potent causes of oral cancer include the use of nass (an aqueous or oily mixture of tobacco, ash and lime), smoking of bedi (cheap cigarettes in which tobacco is rolled in a temburni leaf) and reverse smoking in which the lighted end of a cigarette or cheroot is held within the mouth [4]. In addition, factors such as dietary deficiencies, in particular vitamins A and C, iron and certain trace elements [5, 6], are thought to predispose to oral cancer.

In Europe/U.S.A., approximately 1% of all cancers are oral in origin, compared to some 40% in parts of India and Sri Lanka, figures that translate to around 400,000 new cases each year worldwide [7]. Tumour development in Europe/U.S.A. is more common in males than in females but in India there is a more equal sex distribution [8]. Oral cancer is a disease that is more common with advancing age, usually demonstrating a sharply rising incidence rate after approximately 40–50 years of age; it is disturbing to note that in the West the incidence of oral cancer has recently started to increase [9], particularly in young male cohorts [10].

Oral cancer is predominantly squamous cell carcinoma and, in the main, is a well-differentiated tumour. In India and South East Asia, oral carcinomas are commonly preceded by a premalignant lesion such as leukoplakia or erythroplasia. By contrast, in the West there is a much looser association between oral premalignant lesions and the development of squamous cell carcinoma; the majority of oral carcinomas arise in epithelium which is clinically normal and, similarly, the majority of leukoplakias do not form carcinomas [11]. When oral carcinoma arises in Western countries, it is typically found in a trough formed by the lateral border of the tongue, floor of mouth and lingual aspect of the lower alveolus, possibly due to the pooling of carcinogens [12]. The tumours are usually endophytic and may be deeply penetrating. Oral cancer associated with tobacco chewing, however, develops at the site of application of the bolus and is thus common in the buccal sulcus and the buccal mucosa. These tumours, in contrast, are usually exophytic and may be very large at presentation. Treatment modalities in the West include predominantly surgery, radiotherapy and chemotherapy whilst, in India,

Correspondence to I.C. Paterson.
Received 1 June 1995; provisionally accepted 9 Aug. 1995; revised manuscript received 12 Oct. 1995.

Table 1. Comparison of the genetic alterations detected in malignant tumours of the oral cavity from Western and Eastern countries

	WEST			EAST		
Genetic aberration*	Frequency†	(%)	References	Frequency	(%)	References
p53 mutation	131/275	47.6	[14–27]	5/68	7.3	[22, 28]
H-*ras* mutation	10/162	6.1	[29–33]	20/57	35	[36]
K-*ras* mutation	0/52	0	[32–34]	6/90	6.6	[35, 36]
N-*ras* mutation	0/40	0	[32, 33]	0/57	0	[36]
EGF-R amplification	6/96	6.2	[34, 37–40]	19/66	29	[42]
C-*myc* amplification	13/87	14.9	[37, 38, 40, 41, 43, 44]	25/125	20	[45, 46]
N-*myc* amplification	0/13	0	[40]	36/125	28.8	[45, 46]
L-*myc* amplification	0/13	0	[40]	0/23	0	[45]
H-*ras* amplification	0/79	0	[37, 38, 40, 41, 43]	0/23	0	[45]
K-*ras* amplification	0/18	0	[37, 40]	4/23	17.3	[45]
N-*ras* amplification	0/13	0	[40]	35/125	28.0	[45, 46]
LOH H-*ras*‡	0/7	0	[47]	7/23	30.4	[45]

*Only gene mutations and amplification confirmed by DNA sequencing, SSCP or Southern analysis, respectively, were included.
†The occurrence of a particular genetic alteration is presented as the number of positive samples (nominator) expressed in terms of the total number of specimens (denominator) reported in the cited references.
‡Loss of heterozygosity at the H-*ras* locus.

radiotherapy is the major method of control [8]. In the West, there has been little or no change in 5 year relative survival rates for oral cancer since 1950, although localised lesions have a more favourable prognosis than more extensive ones, and survival rates tend to be better in women than men [13].

This paper is not intended to be a comprehensive review of the molecular biology of human oral carcinogenesis, but summarises the spectrum of molecular changes found in oral squamous cell carcinomas from Europe and the U.S.A. versus India and South East Asia, where data is available to make comparisons (Table 1). Many studies have discussed "head and neck" cancer as a group, sometimes including such disease sites as lip, mouth, maxillary sinuses, pharynx, larynx, salivary glands and skin. Because such analyses are no more logical than discussing "abdominal cancer", the data in this review is taken specifically from primary tumours of the tongue and oral cavity *only*; other lesions of the head and neck have been excluded. We are aware that other authors have noted differences in the frequency of *ras* mutations between Eastern and Western populations but their findings have been reported anecdotally and do not include other genetic anomalies. The purpose of the present review, therefore, is to collate existing information regarding the molecular changes seen in oral squamous cell carcinomas from patients with diverse (East versus West) ethnic backgrounds.

The most striking difference is seen in the prevalence of *p53* mutations which are infrequent in tumours from the Indian subcontinent (7%) but are common in carcinomas from Europe and the U.S.A. (47%). The *p53* gene is an important, early target for mutation in oral tumour development [16] and not only occurs at multiple mucosal sites [18, 48] but provides a selective advantage for the clonal expansion of preneoplastic and neoplastic cells [49]. The prevalence of *p53* mutations described here (47% in oral cancer from the West) differs significantly from that reported recently (81%), presumably using a wider data base [50]. Nevertheless, even if the data in the present review are an underestimate, the figures emphasize the marked differences that exist between East and West populations regarding the prevalence of *p53* mutations in oral cancer. The *p53* gene in tumours from the West was mutated at a range of codons from exons 4 to 9 (Fig. 1A) and particular

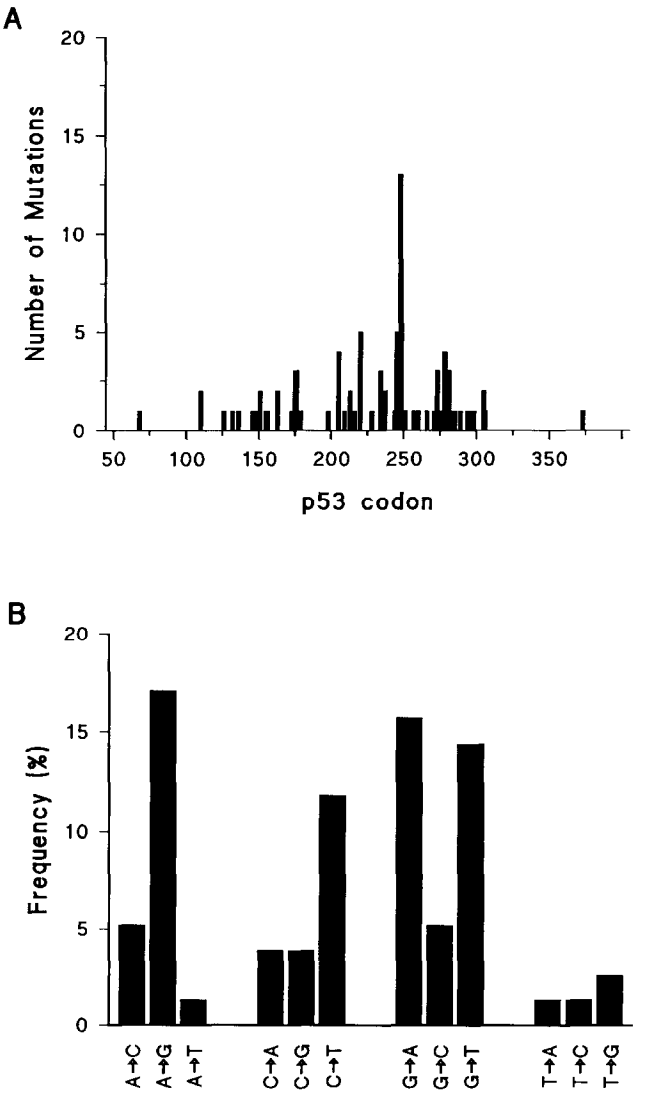

Fig. 1. Distribution of *p53* mutations (A) and the frequency of transitions/transversions (B) detected in human oral squamous tumours from the Western world [14–27].

"hot spots" were present between codons 205 and 248. When the spectrum of *p53* mutations was examined in these same tumours, G→A, G→T, C→T and A→G mutations were common (Fig. 1B), data that substantiates previous observations in primary oral cancer [50]. Interestingly, different patterns of *p53* mutations are seen in laryngeal and pharyngeal primary tumours [50] which could be due to differences in carcinogen exposure (soluble tobacco-specific N-nitrosamines in oral cancers versus tobacco combustion products in laryngeal primaries). *p53* mutations produce a more stable protein than the wild-type product [51] and existing data indicate that some 35–54% of oral squamous cell carcinomas are positive immunocytochemically [52, 53]. The prevalence of *p53* mutations as demonstrated by DNA sequencing and SSCP, therefore, is comparable to the immunocytochemical reactivity of oral tumours. The relationship between positive *p53* immunoreactivity and smoking/drinking in oral carcinomas from Europe, however, remains equivocal [53–55].

In the present review, multiple genetic anomalies characterised tumours from India and South East Asia with a preponderance of Ha-*ras* mutations (35%), loss of heterozygosity of Ha-*ras* (30%), N-*ras* amplification (28%) and N-*myc* amplification (29%) (Table 1). The multiplicity of genetic lesions in tumours from India has been noted previously [46]. It is probable that such genetic lesions, in the absence of *p53* mutations, provide the necessary selective growth advantage but, also, it cannot be excluded that normal *p53* function may be interrupted by changes in genes that participate in *p53*-mediated pathways. Interestingly, changes in both *ras* and *myc* are not prevalent in tumours from Europe/U.S.A. but this observation is necessarily limited by the paucity of data currently available, whether in terms of the number of samples examined or the fact that few studies have reported molecular changes in oral as distinct from "head and neck" cancer. Furthermore, the fact that multiple genetic lesions have not been described in Europe/U.S.A. may simply reflect that the range of such genetic anomalies has not been investigated in any one tumour. It is also possible that Western tumours contain different genetic defects. For example, amplification of the 11q13 amplicon (approximately 20% of tumours; [37–40, 43]), loss of heterozygosity and mutations in the *MTS1/p16* gene [56] and alterations in Ha-*ras* expression [57] have been noted in Western tumours; these parameter have not been investigated in Eastern tumours.

A number of factors could account for the variation in gene mutations between Europe/U.S.A. and India/South East Asia. Environmental exposure to carcinogens is clearly relevant and both cigarette smoke [58] and betel quid [59] contain a complex mixture of tumour initiators, promoters, complete carcinogens and cocarcinogens. Cancer susceptibility factors, such as DNA repair mechanisms and germline polymorphisms in genes involved in carcinogen metabolism, may also reflect ethnic features of populations and are likely to make a significant contribution to tumour development. However, whether Caucasians chewing betel quid would accumulate similar genetic defects as their Asian counterparts remains to be determined. Furthermore, the cellular microenvironment, either epithelial or stromal, may influence tumour progression. Tobacco chewing, for example, can lead to fibro-elastic changes in the underlying lamina propria and result in the development of submucous fibrosis, an established premalignant condition of oral squamous cell carcinoma [60].

The molecular biology of oral cancer, and particularly the emerging field of molecular epidemiology, is an area necessitating further investigation. The data reported in this review emphasise the importance of identifying gene mutations that are specific to an ethnic group, and/or aetiological factors, before correlations are made between molecular and clinical characteristics of tumours. Future studies are likely to concentrate on the biological significance of these genetic alterations.

1. Wynder EL, Bross IJ, Feldman RM. A study of the etiological factors in cancer of the mouth. *Cancer* 1957, **10**, 1300–1323.
2. Graham S. Dentition, diet, tobacco and alcohol in the epidemiology of oral cancer. *J Natl Cancer Inst* 1977, **59**, 1611–1618.
3. Thomas S, Kearsley J. Betel quid and oral cancer: a review. *Oral Oncol, Eur J Cancer* 1993, **29B**, 251–255.
4. World Health Organisation Meeting—Control of oral cancer in developing countries. *Bull WHO* 1984, **62**, 817–830.
5. Stitch HF, Rosin MP, Harnby AP, Sankaranarayanan R, Nair MK. Remission of oral leukoplakias and micronuclei in tobacco/betel quid chewers treated with beta-carotene and with beta-carotene plus vitamin A. *Int J Cancer* 1988, **421**, 195–199.
6. La vecchia C, Franceschi S, Levi F, Lucchini F, Negri E. Diet and human oral carcinoma in Europe. *Oral Oncol, Eur J Cancer* 1993, **29B**, 17–22.
7. Cancer Research Campaign Factsheet 14. 1993. Oral Cancer 14.1–14.5.
8. Sankaranarayanan R. Oral cancer in India: an epidemiologic and clinical review. *Oral Surg Oral Med Oral Pathol* 1990, **69**, 325–330.
9. Boyle P, Macfarlane GJ, Maisonneuve P, Zheng T, Scully C, Tedesco B. Epidemiology of mouth cancer in 1989: a review. *J R Soc Med* 1990, **83**, 724–730.
10. Macfarlane GL, Boyle P, Scully C. Oral cancer in Scotland: changing incidence and mortality. *Br Med J* 1992, **305**, 1121–1123.
11. MacDonald DG. Premalignant lesions of oral epithelium. In Dolby AE, ed. *Oral Mucosa in Health and Disease*. Oxford, Blackwell Scientific Publications, 1975, 335–369.
12. Moore C, Catlin D. Anatomic origins and locations of oral cancer. *Am J Surg* 1967, **114**, 510–513.
13. Smith CJ. Epidemiology and aetiology. In Langdon JD, Henk JM, eds. *Malignant Tumours of the Mouth, Jaws and Salivary Glands*. London, Edward Arnold, 1995, 1–13.
14. Brachman DG, Graves D, Vokes E, *et al.* Occurrence of p53 gene deletions and human papilloma virus infection in human head and neck cancer. *Cancer Res* 1992, **52**, 4832–4836.
15. Burns JE, Baird MC, Clark LJ, *et al.* Gene mutations and increased levels of p53 protein in human squamous cell carcinomas and their cell lines. *Br J Cancer* 1993, **67**, 1274–1284.
16. Boyle JO, Hakim J, Koch W, *et al.* The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993, **53**, 4477–4480.
17. Caamano J, Zhang SY, Rosvold EA, Bauer B, Klein-Szanto AJP. p53 alterations in human squamous cell carcinomas and carcinoma cell lines. *Am J Pathol* 1993, **142**, 1131–1139.
18. Chung KY, Mukhopadhyay T, Kim J, *et al.* Discordant p53 gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. *Cancer Res* 1993, **53**, 1675–1683.
19. Sakai E, Rikimaru K, Ueda M, *et al.* The p53 tumor-suppressor gene and *ras* oncogene mutations in oral squamous cell carcinoma. *Int J Cancer* 1992, **52**, 867–872.
20. Somers KD, Merrick MA, Lopez ME, Incognito LS, Schechter GL, Casey G. Frequent p53 mutations in head and neck cancer. *Cancer Res* 1992, **52**, 5997–6000.
21. Soo Kim M, Li S-L, Bertolami CN, Cherrick HM, Park N-H. State of p53, Rb and DCC tumor suppressor genes in human oral cancer cell lines. *Anticancer Res* 1993, **13**, 1405–1414.
22. Thomas S, Brennan J, Martel G, *et al.* Mutations in the conserved regions of p53 are infrequent in betel-associated oral cancers from Papua New Guinea. *Cancer Res* 1994, **54**, 3588–3593.
23. Xu L, Chen Y-T, Huvos AG, *et al.* Overexpression of p53 protein

in squamous cell carcinomas of head and neck without apparent gene mutations. *Diag Mol Pathol* 1994, **3(2)**, 83–92.
24. Yeudall WA, Paterson IC, Patel V, Prime SS. Presence of human papillomavirus sequences in tumour-derived human oral keratinocytes expressing mutant p53 protein. *Oral Oncol, Eur J Cancer* 1995, **31B**, 136–143.
25. Zariwala M, Schmid S, Pfaltz M, Ohgaki H, Kleihues P, Schafer R. p53 gene mutations in oropharyngeal carcinomas: a comparison of solitary and multiple primary tumours and lymph-node metastases. *Int J Cancer* 1994, **56**, 807–811.
26. Chang Y-S, Lin Y-J, Tsai C-N, *et al.* Detection of mutations in the p53 gene in human head and neck carcinomas by single strand conformation polymorphism analysis. *Cancer Letts* 1992, **67**, 167–174.
27. Sakai E, Tsuchida N. Most human squamous cell carcinomas in the oral cavity contain mutated p53 tumor-suppressor genes. *Oncogene* 1992, 7, 927–933.
28. Ranasinghe A, Macgeoch C, Dyer S, Spurr N, Johnson NW. Some oral carcinomas from Sri Lankan betel/tobacco chewers overexpress p53 oncoprotein but lack mutations in exons 5–9. *Anticancer Res* 1993, **13**, 2065–2068.
29. Anderson JA, Irish JC, McLachlin CM, Ngan BY. H-*ras* oncogene mutation and human papillomavirus infection in oral carcinomas. *Arch Otolaryngol Head Neck Surg* 1994, **120**, 755–760.
30. Clark LJ, Edington K, Swan IRC, *et al.* The absence of Harvey *ras* mutations during development and progression of squamous cell carcinomas of the head and neck. *Br J Cancer* 1993, **68**, 617–620.
31. Warnakulasuriya KAAS, Chang SE, Johnson NW. Point mutations in the Ha-*ras* oncogene are detectable in formalin-fixed tissues of oral squamous cell carcinoma, but are *infrequent* in British cases. *J Oral Pathol Med* 1992, **21**, 225–229.
32. Yeudall WA, Torrance LK, Elsegood KA, Speight P, Scully C, Prime SS. *ras* gene point mutation is a rare event in premalignant tissues and malignant cells and tissue from oral mucosal lesions. *Oral Oncol, Eur J Cancer* 1993, **29B**, 63–67.
33. Chang SE, Bhatia P, Johnson NW, *et al. Ras* mutations in United Kingdom examples of oral malignancies are infrequent. *Int J Cancer* 1991, **48**, 409–412.
34. Irish JC, Bernstein A. Oncogenes in head and neck cancer. *Laryngoscope* 1993, **103**, 42–52.
35. Kuo MYP, Jeng JH, Chiang CP, Hahn LJ. Mutations of Ki-*ras* oncogene codon 12 in betel quid chewing-related human oral squamous cell carcinoma in Taiwan. *J Oral Pathol Med* 1994, **23**, 70–74.
36. Saranath D, Chang SE, Bhoite LT, *et al.* High frequency mutation in codons 12 and 61 of H-*ras* oncogene in chewing tobacco-related human oral carcinoma in India. *Br J Cancer* 1991, **63**, 573–578.
37. Somers KD, Cartwright SL, Schechter GL. Amplification of the *int*-2 gene in human head and neck squamous cell carcinomas. *Oncogene* 1990, **5**, 915–920.
38. Leonard JH, Kearsley JH, Chenevix-Trench G, Hayward NK. Analysis of gene amplification in head and neck squamous cell carcinomas. *Int J Cancer* 1991, **48**, 511–515.
39. Ishitoya J, Toriyama M, Oguchi N, *et al.* Gene amplification and overexpression of EGF receptor in squamous cell carcinomas of the head and neck. *Br J Cancer* 1989, **59**, 559–562.
40. Merritt WD, Weissler MC, Turk BF, Gilmer TM. Oncogene amplification in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 1990, **116**, 1394–1398.
41. Volling P, Jungehulsing M, Stutzer H, Diehl V, Tesch H. Analysis of proto-oncogenes in squamous cell carcinomas of the head and neck (SCCHN). *Int J Oncology* 1993 **3**, 671–677.
42. Saranath D, Panchal RG, Nair R, Mehta AR, Sanghavi VD, Deo MG. Amplification and overexpression of epidermal growth factor receptor gene in human oropharyngeal cancer. *Oral Oncol, Eur J Cancer* 1992, **28**, 139–143.
43. Berenson JR, Yang J, Mickel R. Frequent amplification of the *bcl*-1 locus in head and neck squamous cell carcinomas. *Oncogene* 1989, **4**, 1111–1116.
44. Paterson IC, Patel V, Sandy JR, Prime SS, Yeudall WA. Effects of TGF-β1 on growth-regulatory genes in tumour-derived human oral keratinocytes. *Br J Cancer* 1995, **72**, 922–927.
45. Saranath D, Panchal RG, Nair R, *et al.* Oncogene amplification in squamous cell carcinoma of the oral cavity. *Jpn J Cancer Res* 1989, **80**, 430–437.
46. Saranath D, Bhoite LT, Deo MG. Molecular lesions in human oral cancer: the Indian scene. *Oral Oncol, Eur J Cancer* 1993, **29B**, 107–112.
47. Kiaris H, Spandidos DA, Jones AS, Field JK. Loss of heterozygosity and microsatellite instability of the H-*ras* gene in cancer of the head and neck. *Int J Oncol* 1994, **5**, 579–582.
48. Nees M, Homann N, Discher H, *et al.* Expression of mutated p 53 occurs in tumor-distant epithelia of head and neck cancer patients: a possible molecular basis for the development of multiple tumors. *Cancer Res* 1993, **53**, 4189–4196.
49. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell* 1992, **70**, 523–526.
50. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994, **54**, 4855–4878.
51. Lane DP. p53 and human cancers. *Br Med Bull* 1994, **50**, 582–599.
52. Warnakulasuriya KAAS, Johnson NW. Expression of p53 mutant nuclear phosphoprotein in oral carcinoma and potentially malignant oral lesions. *J Oral Pathol Med* 1992, **21**, 404–408.
53. Ogden GR, Kiddie RA, Lunny DP, Lane DP. Assessment of p53 protein expression in normal, benign and malignant oral mucosa. *J Pathol* 1992, **166**, 389–394.
54. Field JK, Spandidos DA, Stell PM. Overexpression of p53 gene in head and neck cancer, linked with heavy smoking and drinking. *Lancet* 1992, **339**, 502–503.
55. Matthews JB, Scully C, Jovanovic A, Van der Waal I, Yeudall WA, Prime SS. Relationship of tobacco/alcohol use to p53 expression in patients with lingual squamous cell carcinomas. *Oral Oncol, Eur J Cancer* 1993, **29B**, 285–289.
56. Yeudall WA, Crawford RY, Ensley JF, Robbins, KC. MTS1/CDK4I is altered in cell lines derived from primary and metastatic oral squamous cell carcinoma. *Carcinogenesis* 1994, **15**, 2683–2686.
57. Kiaris H, Spandidos DA, Jones AS, Vaughan ED, Field JK. Mutations, expression and genomic instability of the H-*ras* proto-oncogene in squamous cell carcinomas of the head and neck. *Br J Cancer* 1995, **72**, 123–128.
58. DeMarini DM. Genotoxicity of tobacco smoke and tobacco smoke condensate. *Mutat Res* 1983, **114**, 59–89.
59. Nishikawa A, Prokopczyk B, Rivenson A, Zang E, Hoffman D. A study of betel quid carcinogenesis—VIII. Carcinogenicity of 3-(methylnitrosamino) propionaldehyde in F344 rats. *Carcinogenesis* 1992, **13**, 369–372.
60. Paymaster JC. Cancer of the buccal mucosa: a clinical study of 650 cases in Indian patients. *Cancer* 1956, **9**, 431–435.