Review Article

Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis

Yin-Ju Chen,[1] Joseph Tung-Chieh Chang,[2,7] Chun-Ta Liao,[3,7] Hung-Ming Wang,[4,7] Tzu-Chen Yen,[5,7] Ching-Chi Chiu,[1] Ya-Ching Lu,[1] Hsiao-Fang Li[1] and Ann-Joy Cheng[6,7,8]

[1]Graduate Institute of Biomedical Science, Chang Gung University, 259 Wen-Hwa 1st Road, Taoyuan 333; [2]Department of Radiation Oncology, Chang Gung Memorial Hospital, 5 Fu-Shin Road, Taoyuan 333; [3]Department of Otorhinolaryngology, Head Neck Surgery, Chang Gung Memorial Hospital, 5 Fu-Shin Road, Taoyuan 333; [4]Department of Medical Oncology, Chang Gung Memorial Hospital, 5 Fu-Shin Road, Taoyuan 333; [5]Molecular Imaging Center and Department of Nuclear Medicine, Chang Gung Memorial Hospital, 5 Fu-Shin Road, Taoyuan 333; [6]Department of Medical Biotechnology, Chang Gung University, 259 Wen-Hwa 1st Road, Taoyuan 333; [7]Taipei Chang Gung Oncology Group, Chang Gung Memorial Hospital and Chang Gung University, Taoyuan 333, Taiwan

(Received February 29, 2008/Revised April 11, 2008/Accepted April 15, 2008/Online publication July 29, 2008)

Head and neck cancer (HNC) is one of the 10 most frequent cancers worldwide, with an estimated over 500 000 new cases being diagnosed annually. The overall 5-year survival rate in patients with HNC is one of the lowest among common malignant neoplasms and has not significantly changed during the last two decades. Oral cavity squamous cell carcinoma (OSCC) shares part of HNC and has been reported to be increasing in the betel quid chewing area in recent years. During 2006, OSCC has become the sixth most common type of cancer in Taiwan, and it is also the fourth most common type of cancer among men. It follows that this type of cancer wreaks a high social and personal cost. Environmental carcinogens such as betel quid chewing, tobacco smoking and alcohol drinking have been identified as major risk factors for head and neck cancer. There is growing interest in understanding the relationship between genetic susceptibility and the prevalent environmental carcinogens for HNC prevention. Within this review, we discuss the molecular and cellular aspects of HNC carcinogenesis in Taiwan, an endemic betel quid chewing area. Knowledge of molecular carcinogenesis of HNC may provide critical clues for diagnosis, prognosis, individualization of therapy and molecular therapeutics. (*Cancer Sci* 2008; 99: 1507–1514)

Head and neck cancer (HNC) is one of the 10 most frequent cancers worldwide,[1] with an estimated over 500 000 new cases being diagnosed annually.[1] Squamous cell carcinoma represents more than 95% of all head and neck cancers. Therefore the HNC problem primarily concerns the diagnosis, biology and management of squamous cell carcinoma.[1]

According to the 9th revision of the International Classification of Diseases (ICD-9), the term HNC relates to malignant neoplasms of the lip (ICD140), tongue (ICD141), gum (ICD143), floor of the mouth (ICD 144), bucca and other unspecified parts of mouth (ICD145), oropharynx (ICD146), hypophrynx (ICD148) and other head and neck sites (ICD149). The World Health Organization (WHO) estimated that the global incidence rate for cancer of the head and neck in 2000 was 14.27 per 100 000. However, the prevalence of HNC differs greatly in different parts of the world. Epidemiologic studies have shown a wide variation of incidence between worldwide areas. HNC is highly prevalent in South-east Asia, comprising 35–40% of all malignancies in India, compared with approximately 9% in Taiwan and 2–4% in Western countries.[2,3] Also, the tumor sites of HNC are discrete from various regions. Cancers of tongue and buccal mucosa constitute the majority of HNC in India and Taiwan.[2–5] In contrast, the Western registries show cancers of the mouth floor are the most frequent, with cancer of gum or tongue being rare.[2,3] The differences may be attributed to certain environmental exposures prevalent in this population, as well as to genetic factors.

Within this paper, we review the Taiwanese studies on the epidemiology of HNC, features of premalignant lesions, potential carcinogens associated with susceptibility and prognosis and molecular mechanisms of tumorigenesis.

Descriptive epidemiology of head and neck cancer in Taiwan

The incidence of HNC is one of the highest in the world, and this malignancy has been one of the 10 leading causes of cancer deaths in Taiwan (Table 1). All the epidemiology data are from the Cancer Registry Annual Report of Taiwan, Health and National Health Insurance Annual Statistics Information Service, Department of Health, Executive Yuan, ROC Taiwan (http://www.doh.gov.tw/statistic/index.htm). In 1982, the incidence rate of HNC was 5.12 per 100 000 people in males and 1.54 per 100 000 people in females (Fig. 1). In 1991, the incidence rate of HNC had not much changed, with 6.02 and 1.51 per 100 000 people in males and females, respectively. However, in 2003, the incidence rate of HNC significantly increased to 35.08 and 3.56 per 100 000 people in males and females, an alarming 5.82-fold increase in men and 2.35-fold increase in woman in a decade (Fig. 1). In 2006, oral cavity squamous cell carcinoma (OSCC) had become the 6th most common cancer in Taiwan and the 4th most common cancer in Taiwanese men (Table 1). Similarly, mortality rate also increased significantly, from 4.25 per 100 000 in 1995 to 9.6 per 100 000 in 2006, a 2.26-fold increase in the past decade (Fig. 2). This unfavorable trend reflects the increased mortality rate from HNC mostly in men. Overall, mortality rates for HNC in males increased 2.33-fold, from 7.6 per 100 000 in 1995 to 17.7 in 2006 (Fig. 2). Given the magnitude of the HNC problem in Taiwan and its profound adverse impact on public health, there is a need for intervention and the initiation of preventive actions.

There has been a trend toward lower age at diagnosis of HNC over time. From 1989 to 1993, the peak of incident rate was for people aged 50–59 years, but this shifted to ages 40–49 years between 1993 and 2000 (Fig. 3). A similar trend was also found in the mortality rate. During the period between 1991 and 1994,

[8]To whom correspondence should be addressed. E-mail: ajchen@mail.cgu.edu.tw

doi: 10.1111/j.1349-7006.2008.00863.x

Table 1. The ten most common cancers in Taiwan, 2006

Rank	All incidence	Mortality	Male incidence	Male mortality
1	Lung	Liver	Liver	Liver
2	Liver	Lung	Lung	Lung
3	Colon–rectum	Colon–rectum	Colon–rectum	Colon–rectum
4	Breast (F)	Stomach	Oral cavity	Oral cavity
5	Stomach	Oral cavity	Stomach	Stomach
6	Oral cavity (F)	Breast (F)	Esophagus	Esophagus
7	Prostate	Esophagus	Pancreas	Prostate
8	Cervix	Pancreas	Prostate	Pancreas
9	Esophagus	Non-Hodgkin lumphoma	Non-Hodgkin lumphoma	Non-Hodgkin lumphoma
10	Pancreas	Gallbladder	Nasopharynx	Nasopharynx

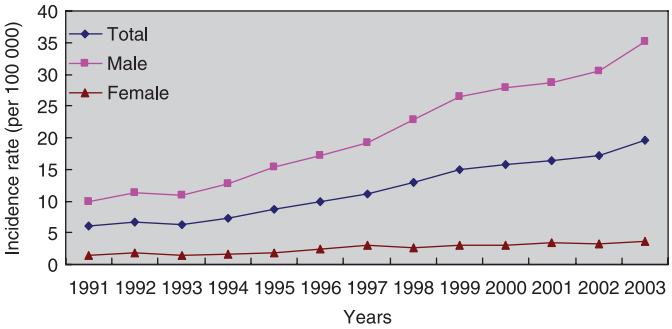

Fig. 1. The incidence rate (per 100 000) of head and neck cancer in Taiwan by sex between 1991 and 2003.

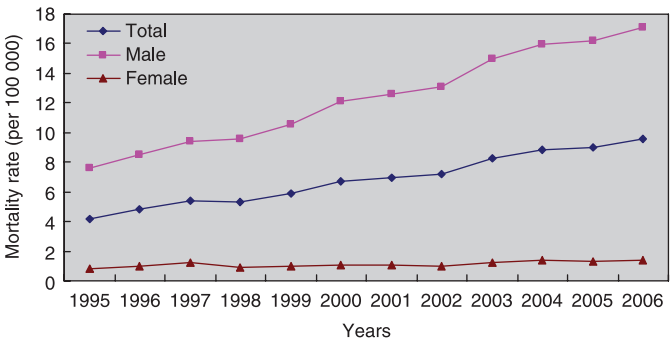

Fig. 2. The mortality rate (per 100 000) of head and neck cancer in Taiwan by sex between 1995 and 2006.

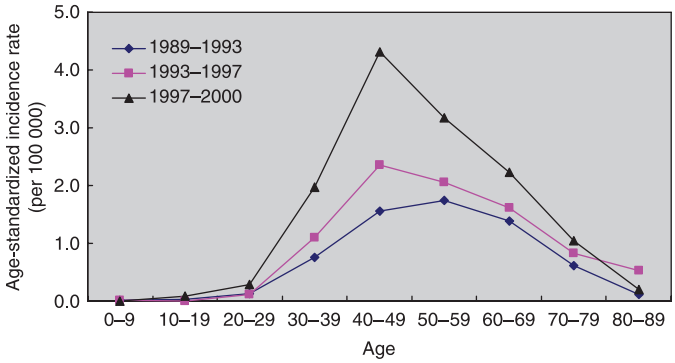

Fig. 3. The age-standardized incidence rate (per 100 000) of oral cavity cancer in Taiwan between 1989 and 2000.

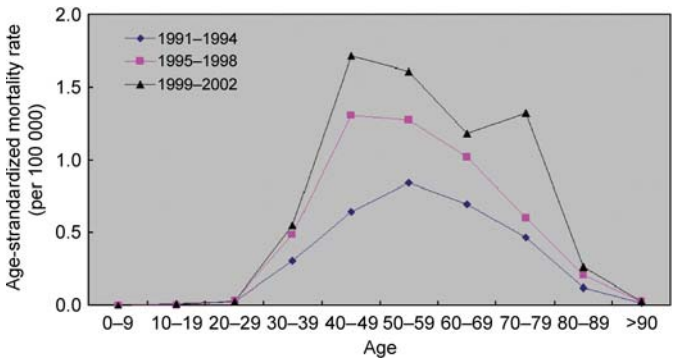

Fig. 4. The age-standardized mortality rate (per 100 000) of oral cavity cancer in Taiwan between 1991 and 2002.

mortality rate peaked at age 50–59 years, but shifted to age 40–49 years between 1999 and 2002 (Fig. 4). (http://www.doh.gov.tw/statistic/index.htm). These data are consistent with other regional reports from northern and southern Taiwan.[5–7] Moreover, patients with HNC are generally younger than those with other forms of cancer. In 2006, the median age at death from HNC was 54 years compared with 69 years in other forms of cancer.

Gender differences in HNC have been described, with a marked male predominance. A study analyzing 703 OSCC patients between 1985 and 1996 in southern Taiwan found a 51:1 male-to-female ratio.[7] Our previous studies in oral cavity cancer patients demonstrated that male cases were far more common than females, comprising 90–93% men and 7–10% women.[4,5,8] This gender difference may be explained by the lower proportion of betel quid chewing habits in females. Concerns about the disfiguring effects of areca quid chewing (including red staining of lips and teeth and foul-smelling breath) are frequently reported by females, which may account for sex differences in HNC prevalence.

The overall 5-year survival rate in patients with HNC is one of the lowest among common malignant neoplasms and has not significantly changed during the last two decades.[1] Cancer tumor stage (Table 2) is the major determinant of survival rate. The 5-year survival rates of oral cavity cancer patients in stages I, II, III and IV are 72–90%, 39–85%, 27–70% and 12–50%, respectively.[5,8,9] Survival rates for HNC are significantly influenced by tumor size, lymph node involvement, distant metastasis, tumor differentiation and betel quid chewing.[4,5] Chewing betel quid independently contributes to the risk of HNC, and the estimated prevalence of betel quid chewing in Taiwanese patients with HNC is approximately 85%.[4,5,8] Approximately 50% of

doi: 10.1111/j.1349-7006.2008.00863.x

Table 2. Clinical staging of oral cavity cancer based on American Joint Committee on Cancer (AJCC) system. (Greene FL *et al.* ed. AJCC Cancer Staging Manual, 6th edn, 2002, Springer). The assessment of the primary tumor is based on inspection and palpation of the oral cavity and neck. Additional studies may include computed axial tomography (CT) or magnetic resonance imaging (MRI). Clinical stage is grouped based on the status of primary tumor (T), regional lymph nodes (N) and distant metastasis (M)

Definition of primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4	Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth or skin of face
T4a	Tumor invades adjacent structures
T4b	Tumor invades masticator space, pterygoid plates, or skull base

Definition of regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, or in multiple ipsilateral or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

Definition of distant metastasis (M)	
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Clinical stage grouping			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1–3	N1	M0
Stage IVA	T4a	N0–2	M0
	T1–3	N2	M0
Stage IVB	T4b	any N	M0
	any T	N3	M0
Stage IVC	any T	any N	M1

patients who were betel quid chewers are also alcohol drinkers and tobacco smokers.[4,5,8]

With regard to the anatomical localization of oral cavity cancers, approximately 30–40% of all cases occur in the tongue or in the buccal mucosa. Altogether, lesions at these sites account for approximately 70% of all oral cavity malignancies.[5,7,9]

Premalignant lesions carry a high risk to progress towards malignant transformation

Premalignant lesions of the head and neck – including oral leukoplakia, erythroplakia, squamous papilloma and submucous fibrosis – carry a high risk to progress towards malignant transformation. Oral leukoplakia is a whitish patch or plaque that cannot be characterized clinically or pathologically as any other disease. It originates from non-specific reactions of the epithelium as a consequence of various exogenous and endogenous stimuli. Submucous fibrosis is a disease that produces changes similar to those of scleroderma but is limited to oral tissue. It presents as a whitish yellow discoloration with a chronic, insidious biological course. Oral squamous papilloma, characterized by a warty appearance, is composed of papillary and verrucous growths of benign epithelium and minor amounts of supporting connective tissue.

Studies have shown that between 1 and 18% of oral premalignant lesions will develop into oral cancer.[10] As regards the age distribution of patients with premalignant lesions, oral submucous fibrosis is predominant in young patients whereas leukoplakia is more commonly found in older individuals. Patients with premalignant lesions have a high risk of HNC development. In a study of 1458 patients with premalignant lesions, 3.02% developed clinical evidence of carcinoma over a mean follow-up period of 42.6 months.[11] Specifically, 1.87% of patients with dysplastic lesion and 3.55% of those with hyperkeratosis/epithelial hyperplasia progressed to malignant transformation.[11] In another study of 1046 patients with oral leukoplakia, the prevalence rate of carcinoma was 12.9%.[12] The relative risks for the presence of malignancy in leukoplakias on the tongue and floor of mouth with non-homogeneous appearance were 2.72- and 28.13-fold higher, respectively, compared with those on buccal mucosa with homogeneous surface.[12] The average age of patients with leukoplakia was lower in patients engaged in the oral habits of alcohol drinking, betel quid chewing and cigarette smoking compared with those without any oral habit.[12] These results

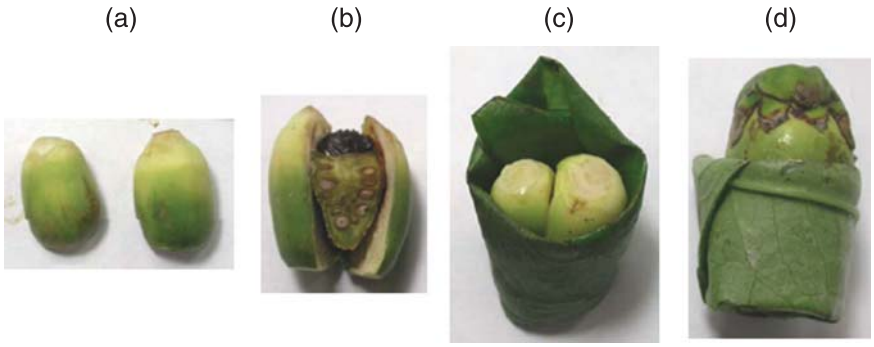

Fig. 5. Areca fruit and three major types of betel quid used in Taiwan, with (a) unripe areca fruit (b) Ching-a (c) Shuang-zi-sing and (d) Bao-hyeo.

clearly show that some leukoplakias may contain a malignant component. Although premalignant lesions with certain features are more prone to carcinomas, no clinical attributes may bring certitude. Therefore, all oral leukoplakias should be submitted to microscopic analysis before any definite treatment. Long-term follow-up of patients with premalignant oral lesions is highly recommended.[11,12]

Betel quid chewing, alcohol drinking and tobacco smoking can promote HNC

The oral habits of betel quid chewing, alcohol drinking and tobacco smoking have been documented as risk factors for HNC.[13–15] The 1985 International Agency for Research on Cancer (IARC) monograph on betel quid reported that there was sufficient evidence for carcinogenicity to humans for betel quid containing tobacco (Group 1 carcinogen), but reported inadequate evidence for carcinogenicity to humans for betel quid without tobacco (Group 3 carcinogen).[16] However, case-control studies from South-east Asia have reported that betel quid use, specifically without tobacco, may act as a risk factor for HNC.[17,18] Therefore, the recent 2003 IARC monograph declared chewing of betel quid, by itself, to be a Group 1 carcinogen and the areca nut to be, correspondingly, a Group 1 carcinogen.[19] The practice of betel quid chewing is widespread in Taiwan, especially for the indigenous people and blue-collar workers, with total estimated two million habitual users (10% of population).[20] Notably, the consumption of betel quid has gradually increased since 1970.[21] Aside from the psychoactive effect and facilitating social interactions between chewers, several phenomena may explain the increase of betel usage. In the 1970s, when Taiwan's economy was booming, to increase the labor-intensive productivity, bosses often handed out betel quids freely to the workers to enhance euphoric mood and reduce tension. During the 1980s, during the democracy movement, despite knowledge of the harmful effects, betel quid chewing became a symbol of Taiwanese identity and more people took up the habit. Gradually, people developed a tolerance to betel and demand increased. However, as evidence mounted identifying betel quid chewing as the major cause of oral cancer, cumulative antibetel actions were launched by the Taiwan government and betel consumption become steady after 2000.[21]

Risks of betel quid vary with the type of quid. Chewers using betel inflorescence in the quid are at highest risk, whereas those using betel leaves are at the lowest.[22] Unlike chewers from most countries in South-east Asia, which use mature betel fruit, the Taiwanese chewer commonly uses fresh, unripe betel fruit with slaked lime as an essential ingredient.[23,24] There are three major types of betel quid used in Taiwan, locally called 'Ching-a', 'Shuang-zi-sing' and 'Bao-hyeo' (Fig. 5). Ching-a is commonly used in urban areas. It is made by putting a piece of inflorescence of *Piper betle* Linnaeus with red slaked lime paste into a slit-open unripe betel nut (Fig. 5b). Shuang-zi-sing is made by wrapping two pieces of half unripe betel nut and red slaked lime paste with a piece of betel leaf (Fig. 5c). Bao-hyeo is made by wrapping an unripe betel nut and white slaked lime paste with a piece of betel leaf (Fig. 5d). Tobacco is never added in any type of chewing quid. The habit of chewing tobacco alone or pipe smoking is rare in the general population of Taiwan. However, cigarette smoking is also common, especially with areca/betel quid chewers, but people with both chewing and smoking habits rarely simultaneously use.

The prevalence of oral precancerous lesions (leukoplakia, submucous fibrosis and verrucous lesions) has been associated with different life styles relating to quid chewing, tobacco smoking and alcohol drinking.[25] A population-based study of 320 individuals has shown that the odds ratio for chewing areca/betel quid and having at least one oral precancerous lesion was 8.21.[24] Reported odds ratios for oral leukoplakia in betel quid chewers and tobacco smokers were 17.4 and 3.2, respectively, in a case-control study of 435 subjects.[26] A dose–response effect for duration and amount of chewing habit on HNC risk was especially observed, with the prevalence of oral lesions increasing as the years of chewing or daily consumption increased.[25]

Tobacco and betel quid may act synergistically as a carcinogen.[27] A hospital-based case-control study involving 307 participants has reported that betel chewing and smoking increase the risk of oral cancer to 28- and 18-fold, respectively. A cumulative effect from betel quid chewing, alcohol drinking and tobacco smoking has been observed, with a 123-fold increased risk of oral cancer when the three risk factors are present.[8] In modelling the effect of betel quid chewing on HNC risk, it has been shown that betel quid chewers had a lower median age of onset (approximately 6–12 years earlier) compared with non-chewers.[6] Betel quid chewing has also been associated with cancer prognosis in a dose- and time-dependent fashion. In a study of 378 HNC patients, the 5-year survival rate of chewers was significantly lower than that of non-chewers.[28] In contrast, no significant difference was seen in 5-year survival between smokers and non-smokers or between alcohol users and non-users.[28] Similarly, the risk of death has been reported to be 31.4-fold higher in heavy betel quid users (duration >30 years, daily consumption >30 quids) compared with those who chewed betel quid to a milder extent (duration <10 years, daily consumption <15 quids).[29]

Betel-quid-associated molecular pathology

The composition of betel quid differs geographically; the areca quid used in Taiwan contains areca nut, lime and Piper betel inflorescence.[23,24] Piper betel inflorescence contains high concentrations of hydroxychavicol and safrole, whereas arecoline, a major areca nut alkaloid, is considered to be the most important carcinogen in the areca nut. Areca nut extract (ANE)

doi: 10.1111/j.1349-7006.2008.00863.x

is highly cytotoxic and genotoxic to cultured human oral mucosal epithelial cells and fibroblasts. Exposure of human keratinocytes to ANE results in apoptosis, generation of reactive oxygen species, genetic damage and micronuclei formation.[30] The same study has found that 24-h treatment with ANE induced mutations at the hypoxanthine phosphoribisyltransferase (HPRT) locus in human keratinocytes.[30] Increased intracellular levels of reactive oxygen species and 8-hydroxyguanosine in cells exposed to ANE have been also reported.[30]

Salivary concentration of arecoline during betel quid chewing has been detected to be in the millimolar concentration range.[31] Arecoline has been shown to induce structural chromosomal aberration, sister chromatid exchange and micronuclei formation in different cell types.[32,33] Studies in human oral cancer cells have shown that exposure to arecoline or ANE results in growth arrest in the late S and G2/M phases.[34] Moreover, it has been shown that arecoline induced a significant elevation of p21waf1 and a decline of cdc2 and cyclin B1 in gingival keratinocytes.[34]

Piper betel inflorescence, which contains safrole, is a unique ingredient of betel quid in Taiwan. Safrole–DNA adducts have been suggested to play an important role in oral carcinogenesis. Accordingly, a high frequency of safrole-like DNA adducts has been reported in betel-quid-associated oral squamous cell carcinomas and non-cancerous matched tissue, in contrast to the absence of such adducts in all of non-betel-associated oral cancers.[35]

Hydroxychavicol, a phenolic component of betel leaf, has been found in human saliva at a 4.6 mM concentration after betel quid chewing.[36] Hydroxychavicol may induce the formation of single-strand DNA breaks and 8-hydroxydeoxyguanosine – a marker of oxidative DNA damage – in cultured cells.[37,38] Moreover, COX-2 expression and PGE_2 production have been shown to be significantly enhanced by hydroxychavicol in human normal oral keratinocytes.[39] Another study has shown that hydroxychavicol has the capacity to modulate cigarette carcinogen benzo[a]pyrene-mediated toxic effects by induction of dihydrodiol dehydrogenase (DDH) and HPRT gene mutation.[40] A further report has provided evidence that alkaline saliva generated by chewing betel quid may play a role in cigarette-related nicotine-induced DNA damage and reactive oxygen species may be involved in generating this DNA damage.[41] These findings provide a molecular explanation for the synergistic effect of betel quid chewing and tobacco smoking in the development of HNC in Taiwan.

Molecules changes during the process of head and neck carcinogenesis

Under normal physiological conditions, gene expression is highly regulated to maintain cell homeostasis. The process of carcinogenesis involves gain of oncogene activity and loss of tumor suppressor gene function. Differential expression of these critical genes and other genes controlled by them contributes to malignant transformation. The identification of these genes is essential for understanding HNC molecular carcinogenesis.

The entry and progression of a cell through the cell cycle is controlled by changes in the levels and activities of several cyclins, cyclin-dependent kinases (CDK) and their inhibitors. Disruption of the G1–S checkpoints leads to uncontrolled cell growth, resulting in the development of cancer. Immunohistochemical studies have shown that overexpression of cyclin A protein is associated with tumor progression and patient prognosis in oral squamous cell carcinoma.[42] Notably, overexpression of cyclin A is significantly associated with increased tumor size, advanced tumor and lymph node involvement. Similarly cyclin D1, which is required for transition from G1 to S phase, was found to be hyperexpressed in oral squamous cell carcinomas. Specifically, tumors containing more cyclin D1-positive cells had significantly shorter survival rates than those of tumors containing a lower number of cyclin D1-positive cells.[43] An altered expression of the cdk inhibitor p27Kip1, which is capable of blocking cell cycle progression from the G1 to the S phase, has been reported in HNC specimens. A previous immunohistochemical study has shown that the loss of p27Kip1 protein expression is a common event and may play a crucial role in the pathogenesis of oral cancer in Taiwan.[44] It has been also observed that patients with low or absent p27Kip1 protein expression had poor prognosis.[44]

Besides cyclins and their inhibitors, recent studies have focused on the potential role played by telomerase in HNC. Telomerase expression has been shown to be closely associated with cellular immortality and cancer, probably because it maintains telomere length and chromosome stability. Studies have shown that cancer severity and prognosis correlate with the expression of telomerase.[3] MDM2 (murine double minute gene 2) overexpression has been also suggested to play a role in human tumorigenesis via inhibition of the p53 tumor suppressor protein. A high degree of MDM2 overexpression has been reported in oral squamous cell carcinomas in Taiwan, but no significant association was found between the MDM2 immunostaining and clinical staging or primary tumor status.[45]

Alterations of apoptotic signal transduction proteins, such as c-Jun, have been reported in HNC. c-Jun is unique in its ability to positively regulate cell proliferation through the repression of tumor suppressor gene expression and function.[46] A 3-fold increase in c-Jun expression has been described in oral mucosal fibroblasts after exposure to ANE or arecoline.[47] It has been also reported that 60% of oral cavity cancer patients in Taiwan has a positive c-Jun immunostaining that correlates with shorter overall survival rates.[48] Rac1, a member of the Ras superfamily of small guanosine triphosphatases (GTPases) that act as molecular switches to control cytoskeletal rearrangements and cell growth, has been found to be hyperexpressed in a high fraction of HNC.[49] The inhibitor of apoptosis (IAP) proteins, a family of antiapoptotic regulators that block cell death in response to diverse stimuli through interactions with inducers and effectors of apoptosis, have been found to be expressed in potentially malignant and malignant oral lesions, but not in normal oral mucosa [50]. Moreover, high levels of survivin – a recently identified protein that suppresses programmed cell death and regulates cell division – have emerged as an important indicator of poor prognosis in HNC.[50]

Cell adhesion molecules and cell surface receptors have been implicated in the pathogenesis of HNC in Taiwan. It was recently shown that expression of epidermal growth factor receptor (EGFR; c-erbB-1) and its family protein Her-2 (c-erbB-2) are increased in oral cancer by 3.5-fold and 1.5-fold, respectively.[51] EGFR hyperexpression has been found to be associated with clinical stage, extracapsular spread and poor prognosis.[51] Hepatocyte growth factor (HGF) (scatter factor) and its receptor, the c-met proto-oncogene product (c-met), have also been implicated in HNC progression.[52] Specifically, c-met expression was significantly associated with T status, N status and clinical staging of oral cancer, whereas the hepatocyte growth factor (HGF) in the tumor invasion front was significantly correlated with N status and clinical staging.[52]

CD44 is a transmembrane adhesion molecule postulated to play a role in tumor aggression. The overexpression of CD44 has been shown to be associated with metastasis and poor prognosis in several human malignancies, but the CD44 splice isoform CD44v7–8 has been shown to act as a tumor suppressor factor. Accordingly, a higher degree of CD44v7–8 staining has been associated with a better prognosis in HNC.[53] Desmoglein 3 (DSG3), a calcium-binding transmembrane glycoprotein component of desmosomes in epithelial cells, has been found to

be overexpressed in HNC specimens.[54] Notably, correlations were seen between DSG3 expression and T stage, N stage, overall stage, tumor depth and extracapsular spread.[54]

In recent years, advances in comprehensive genomic and proteomic technologies are providing researchers with an unprecedented opportunity for high-throughput molecular analysis of HNC. A recent proteomic study has identified a total of 41 proteins overexpressed in oral cancer, including alphaB-crystallin, tropomyosin 2, myosin light chain 1, heat shock protein 27, stratifin, thioredoxin-dependent peroxide reductase, flavin reductase, vimentin, rho GDP-dissociation inhibitor 2, glutathione S-transferase Pi and manganese superoxide dismutase.[55] Real-time quantitative reverse transciptase–polymerase chain reaction (RT-PCR) was used to validate selected proteomic data, confirming that αB-crystallin, heat shock protein 27 and manganese superoxide dismutase are significantly overexpressed in this malignancy.[55] Using cDNA microarray analysis, Tsai and colleagues identified 84 genes dysregulated in oral cancer associated with betel quid chewing.[56] Four genes, including *caspase-1*, *STAT-1*, *COX-2* (up-regulated) and *pleiotrophin* (down-regulated), were validated by further analyses.[56] Other authors have used fluorescent differential display analysis to identify gene expression profiles of HNC.[57] As a result, NPM, CDK1, NDRG1, HMGCR, EF1A, NAC and DSG3 were found to be up-regulated in HNC, whereas CHES1 was down-regulated[57]. Hyperexpression of CDK1 and NDRG1 was also associated with poorly differentiated tumors. Expression of CDK1 and NDRG1, as well as of CDK1 and CHES1, was significantly correlated, suggesting that these genes share a very close regulatory relationship or interact synergistically in oncogenesis.[57]

Although the full significance of dysregulation of gene expression in HNC is not entirely known, these discoveries hold promise with respect to improved diagnosis and treatment. Notably, differential display screening initially identified DSG3 as overexpressed in HNC.[57] Further studies identified the same molecule as associated with disease severity.[54] Consistent with the clinical data, inhibition of DSG3 by RNA interference (RNAi) significantly reduced cell growth and colony formation in three HNC cell lines.[54] Moreover, an *in vivo* xenograft study showed that administration of DSG3-RNAi plasmid significantly inhibited tumor growth in mice.[54] These findings suggest that DSG3 may be a potential molecular target in the development of adjuvant therapy for HNC.

Mutations and polymorphisms associated with HNC in Taiwan

Single nucleotide polymorphisms (SNP), point mutations, deletions and deregulation of DNA methylation have been suggested to play a role in HNC carcinogenesis. Research considering genetic alterations jointly with environmental exposures could be relevant for a better understanding of HNC in the betel quid chewing area. Specifically, somatic mutations induced by betel quid or other environmental carcinogens may be involved in the tumorigenesis of this carcinoma. On the other hand, genetic polymorphism may play a significant role in person-to-person variability in cancer susceptibility, raising the intriguing possibility that some individuals could be predisposed to HNC development. However, there is shortage of literature regarding the association of SNP and betel quid chewing.

The tumor suppressor protein *p53* is a key molecule in regulating expression of genes that mediate cell cycle arrest, and nucleotide variations in the *p53* gene have been extensively studied in HNC. Loss of heterozygosity (LOH) affecting *p53* exon 4 has been found in 42–74% of tumor samples, whereas LOH at the *p53* intron 6 was detected in 50% of specimens in Taiwan.[58,59] Interestingly, 84.4% of patients informative for *p53* gene exon 4 had a history of both habitual cigarette smoking and betel quid chewing.[51] The contribution of the *p53* Arg72Pro polymorphism in the development of HNC has been also investigated. A report has shown that the combined susceptible genotype homozygous Pro/Pro and heterozygous Arg/Pro was associated with a higher risk of HNC compared with the Arg/Arg genotype.[60] Previous studies have also screened the conserved midregions of the *p53* gene (exons 5–9) for mutations. Missense or nonsense mutations at codons 161, 175, 177, 222, 255, 266, 273, 277 and 282 were found in approximately 20% of oral cancers in Taiwan.[61,62] Therefore, the incidence of mutations in conserved exons of *p53* in the betel quid chewing area is significantly different from that reported (46%) for worldwide oral squamous cell carcinoma related primarily to tobacco consumption.[61,62]

The tumor suppressor *p16/MTS1 (CDKN2)* gene, on chromosome 9p21, codes for a cyclin-dependent kinase inhibitor and is frequently inactivated in many human cancers. A study has examined the presence of mutations, deletions and the methylation status of *p16/MTS1* in oral cancer associated with betel quid chewing in Taiwanese patients.[63] The authors identified mutations in exon 2 and at the intron 1/exon 2 splice site that disrupted the encoded protein.[63] Several base transitions were identified, including codon 51 $GTC^{Val}\rightarrow GCC^{Ala}$, codon 101 $GGG^{Gly}\rightarrow GGA^{Gly}$, codon 102 $GCG^{Ala}\rightarrow GTG^{Val}$ and a nonsense mutation 80 $CGA^{Arg}\rightarrow TGA$ that resulted in a premature stop codon.[63] Interestingly, methylation of the *p16/MTS1* promoter region occurred preferentially in carcinomas of the tongue (54%) compared with other sites (22%).[63]

Other authors have investigated the role of adenomatous polyposis coli (*APC*) tumor suppressor gene during oral carcinogenesis. This gene plays an important role in various cellular functions including regulation of β-catenin levels, cell migration and adhesion and cell cycle control. Five missense mutations (codon 1352 $GTT^{Val}\rightarrow GCT^{Ala}$, codon 1367 $CAG^{Glu}\rightarrow CGG^{Arg}$, codon 1382 $GTT^{Val}\rightarrow GCT^{Ala}$, codon 1402 $GCC^{Ala}\rightarrow ACC^{Thr}$ and codon 1652 $TCC^{Ser}\rightarrow TTC^{Phe}$) and a single nucleotide deletion at codon 1593 resulting in a premature stop codon in the *APC* gene have been identified in HNC patients.[64] Notably, a significant correlation was observed between these mutations in the *APC* gene and the patients' tobacco/betel quid consumption.[64]

Genetic mutations or deletions in other cancer-related genes have been associated with susceptibility to HNC in the betel quid chewing area. A biallelic polymorphism – adenine (A) to guanine (G) transition at position +49 of exon 1 of the *CTLA4* (Cytotoxic T lymphocyte associated antigen 4) gene – has been associated with HNC. Specifically, the homozygous AA genotype was found to be associated with a lower age at onset and poor prognosis.[65] Genetic polymorphisms in the promoter region of the tumor necrosis factor-α (*TNF-α*) gene are involved in the regulation of expression levels and have been associated with various malignant conditions. Two polymorphisms in the promoter region of the *TNF-α* gene (–308 G/A and –238 G/A) have been investigated for their role in susceptibility to HNC.[66] Results revealed that the frequency of the –308 TNFG allele genotype was higher in patients with oral cancer, and that of TNFA/G was lower; additionally, the frequency of the –238 TNFG/A allele genotype was lower in the patient group.[66] Angiogenesis, the formation of new blood vessels from endothelial precursors, is a prerequisite for the growth and progression of solid malignancies, and the vascular endothelial growth factor (*VEGF*) gene has been investigated in relation to HNC. A study has shown that the distribution of *VEGF* 5′-UTR –460 polymorphism was significantly different between HNC patients and controls; specifically, carriage of the *VEGF* –460T allele was associated with HNC.[67] Moreover, known genetic polymorphisms for a number of detoxification enzymes (null genotype in either *GSTM1* or *GSTT1* genes, *CYP2E1* c1/c2 or c2/c2 genotype)

doi: 10.1111/j.1349-7006.2008.00863.x

have been linked to HNC among individuals who did not chew betel quid.[68] In light of the possible contribution of factors associated with thrombosis and inflammation to carcinogenesis, the angiotensin-converting enzyme (*ACE*) gene insertion/deletion (I/D) polymorphism has been recently investigated in relation to the presence of oral precancerous lesions in Taiwanese subjects who chew betel quid.[69] Results showed that the DD genotype independently predicted the presence of oral premalignant lesions.[69] Finally, mutations in mitochondrial DNA (mtDNA) have been suggested to play an important role in the development of HNC. Specifically, a study has found that betel quid chewing significantly enhanced the accumulation of mtDNA deletions in human oral tissues.[70]

Virus associated with head and neck cancer in Taiwan

Human papillomavirus (HPV), the causal agent of cervical cancer, has been suggested to play a role in the etiology of cancer of the oral cavity and oropharynx. More than 120 different types of HPV exist, with approximately 40 types associated with lesions of the genital tract.[71] The most important oncogenic types are HPV type 16 (HPV-16) and HPV-18.[72] In many advanced preneoplastic cervical lesions and most derived carcinomas, HPV genomes are found to be integrated into the host cell chromosomes.[73,74] Following integration, the early HPV oncoproteins E6 and E7 are responsible for the malignant phenotype, mainly through inactivation of tumor suppressor proteins such as p53 and pRB.[75,76]

Besides cervical cancer, HPV infections have been linked to several other malignancies, including HNC.[77,78] An epidemiologic survey in Taiwan has shown that HPV16, HPV18, betel quid chewing and tobacco smoking were statistically significant risk factors for oral squamous cell carcinoma, whereas HPV6 and HPV11 were not.[79] Multivariate analysis identified HPV16 and betel quid chewing as independent predictors of oral cancer.[79] Another study has shown that the positive rates of all HPV types and of high-risk HPV types were significantly higher in oral cancer samples compared with normal mucosa.[80] Moreover, rates of HPV infections were significantly higher in non-oral habits-associated oral cancer samples.[80] In light of these findings, the authors concluded that HPV infections may play an oncogenic role in oral cancer patients without cancer-associated oral habits.[80]

Concluding remarks

In Taiwan, HNC incidence and mortality has increased over the past two decades. During 2006, OSCC has become the sixth most common type of cancer in this country, and it is also the fourth most common type of cancer among men. It follows that this type of cancer wreaks a high social and personal cost. The high incidence of HNC in the Taiwanese population has been attributed to certain oral habits prevalent in this population (betel quid chewing, tobacco smoking and alcohol drinking), as well as to genetic factors. The study of molecular carcinogenesis in an endemic betel quid chewing area not only provides a useful means to improve our understanding of HNC pathology at the molecular level but may also allow specific targeting of advice and therapy to high-risk individuals (i.e. those with a high-risk genotype in a high-risk environment).

References

1 Clayman GL, Lippman SM, Laramore GE, Hong WK. Head and neck cancer. In: Jolland JF, Frei E, Bast RC, Kufe DW, Morton DL, Weichselbaum RR, eds. *Cancer Medicine*, 4th edn, chapter 105. Philadelphia, USA: Williams & Wilkins 1997; 1645–710.
2 Control of oral cancer in developing countries. A WHO meeting. *Bull World Health Organ* 1984; **62**: 817–30.
3 Daftary DK, Murti PR, Bhonsle RR, Gupta PC, Mehta FS, Pindborg JJ. Risk factors and risk markers for oral cancers in high risk areas of the world. In: Johnson NW, ed. *Oral Cancer: Detection of Patients and Lesions at Risk*. Cambridge: Cambridge University Press, 1991; 29–63.
4 Liao CT, Chang JT, Wang HM *et al*. Telomerase as an independent prognostic factor in head and neck squamous cell carcinoma. *Head Neck* 2004; **26**: 504–12.
5 Liao CT, Chang JT, Wang HM *et al*. Analysis of risk factors predictive of local tumor control in oral cavity cancer. *Ann Surg Oncol* 2008; **15**: 915–22.
6 Su CC, Yang HF, Huang SJ, Lian Ie B. Distinctive features of oral cancer in Changhua County: high incidence, buccal mucosa preponderance, and a close relation to betel quid chewing habit. *J Formos Med Assoc* 2007; **106**: 225–33.
7 Chen YK, Huang HC, Lin LM, Lin CC. Primary oral squamous cell carcinoma. an analysis of 703 cases in southern Taiwan. *Oral Oncol* 1999; **35**: 173–9.
8 Liao CT, Kang CJ, Chang JT *et al*. Survival of second and multiple primary tumors in patients with oral cavity squamous cell carcinoma in the betel quid chewing area. *Oral Oncol* 2007; **43**: 811–19.
9 Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. *J Oral Pathol Med* 1995; **24**: 450–3.
10 Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. *Crit Rev Oral Biol Medical* 2003; **14**: 47–62.
11 Hsue SS, Wang WC, Chen CH, Lin CC, Chen YK, Lin LM. Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. *J Oral Pathol Med* 2007; **36**: 25–9.
12 Lee JJ, Hung HC, Cheng SJ *et al*. Carcinoma and dysplasia in oral leukoplakias in Taiwan: prevalence and risk factors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; **101**: 472–80.
13 Choi SY, Kahyo H. Effect of cigarette smoking and alcohol consumption in the aetiology of cancer of the oral cavity, pharynx and larynx. *Int J Epidemiol* 1991; **20**: 878–85.
14 Mashberg A, Boffetta P, Winkelman R, Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer* 1993; **72**: 1369–75.
15 Menvielle G, Luce D, Goldberg P, Leclerc A. Smoking, alcohol drinking, occupational exposures and social inequalities in hypopharyngeal and laryngeal cancer. *Int J Epidemiol* 2004; **33**: 799–806.
16 IARC. Betel-quid and areca-nut chewing. *IARC Monogr Eval Carcinog Risks Hum* 1985; **37**: 141–291.
17 Sankaranarayanan R, Duffy SW, Padmakumary G, Day NE, Krishan Nair M. Risk factors for cancer of the buccal and labial mucosa in Kerala, southern India. *J Epidemiol Community Health* 1990; **44**: 286–92.
18 Sankaranarayanan R, Duffy SW, Padmakumary G, Day NE, Padmanabhan TK. Tobacco chewing, alcohol and nasal snuff in cancer of the gingiva in Kerala, India. *Br J Cancer* 1989; **60**: 638–43.
19 IARC. Betel-quid and areca nut chewing and some related nitrosamines. *IARC Monogr Eval Carcinog Risks Hum* 2003; **85**: 11–18.
20 Ko YC, Chiang TA, Chang SJ, Hsieh SF. Prevalence of betel quid chewing habit in Taiwan and related sociodemographic factors. *J Oral Pathol Med* 1992; **21**: 261–4.
21 Wang A. Betel nut culture turns over a new leaf. *Taiwan Rev* 2008; **58** (2): (http://taiwanreivew.nat.gov.tw/).
22 Lee KW, Kuo WR, Tsai SM *et al*. Different impact from betel quid, alcohol and cigarette: risk factors for pharyngeal and laryngeal cancer. *Int J Cancer* 2005; **117**: 831–6.
23 Yang YH, Chen CH, Chang JS, Lin CC, Cheng TC, Shieh TY. Incidence rates of oral cancer and oral pre-cancerous lesions in a 6-year follow-up study of a Taiwanese aboriginal community. *J Oral Pathol Med* 2005; **34**: 596–601.
24 Yang YH, Lee HY, Tung S, Shieh TY. Epidemiological survey of oral submucous fibrosis and leukoplakia in aborigines of Taiwan. *J Oral Pathol Med* 2001; **30**: 213–19.
25 Chung CH, Yang YH, Wang TY, Shieh TY, Warnakulasuriya S. Oral precancerous disorders associated with areca quid chewing, smoking, and alcohol drinking in southern Taiwan. *J Oral Pathol Med* 2005; **34**: 460–6.
26 Shiu MN, Chen TH, Chang SH, Hahn LJ. Risk factors for leukoplakia and malignant transformation to oral carcinoma: a leukoplakia cohort in Taiwan. *Br J Cancer* 2000; **82**: 1871–4.
27 Lee CH, Ko YC, Huang HL *et al*. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. *Br J Cancer* 2003; **88**: 366–72.
28 Lo WL, Kao SY, Chi LY, Wong YK, Chang RC. Outcomes of oral squamous cell carcinoma in Taiwan after surgical therapy: factors affecting survival. *J Oral Maxillofac Surg* 2003; **61**: 751–8.

29 Lee JJ, Jeng JH, Wang HM *et al*. Univariate and multivariate analysis of prognostic significance of betel quid chewing in squamous cell carcinoma of buccal mucosa in Taiwan. *J Surg Oncol* 2005; **91**: 41–7.
30 Lai KC, Lee TC. Genetic damage in cultured human keratinocytes stressed by long-term exposure to areca nut extracts. *Mutat Res* 2006; **599**: 66–75.
31 Sundqvist K, Liu Y, Nair J, Bartsch H, Arvidson K, Grafstrom RC. Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. *Cancer Res* 1989; **49**: 5294–8.
32 Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. *Oral Oncol* 2001; **37**: 477–92.
33 Shirname LP, Menon MM, Nair J, Bhide SV. Correlation of mutagenicity and tumorigenicity of betel quid and its ingredients. *Nutr Cancer* 1983; **5**: 87–91.
34 Lee PH, Chang MC, Chang WH *et al*. Prolonged exposure to arecoline arrested human KB epithelial cell growth: regulatory mechanisms of cell cycle and apoptosis. *Toxicology* 2006; **220**: 81–9.
35 Chen CL, Chi CW, Chang KW, Liu TY. Safrole-like DNA adducts in oral tissue from oral cancer patients with a betel quid chewing history. *Carcinogenesis* 1999; **20**: 2331–4.
36 Wang CK, Hwang LS. Phenolic compounds of betel quid chewing juice. *Food Sci (in Chinese)* 1992; **20**: 458–71.
37 Lee-Chen SF, Chen CL, Ho LY *et al*. Role of oxidative DNA damage in hydroxychavicol-induced genotoxicity. *Mutagenesis* 1996; **11**: 519–23.
38 Chen CL, Chi CW, Liu TY. Enhanced hydroxychavicol-induced cytotoxic effects in glutathione-depleted HepG2 cells. *Cancer Lett* 2000; **155**: 29–35.
39 Tang DW, Lin SC, Chang KW, Chi CW, Chang CS, Liu TY. Elevated expression of cyclooxygenase (COX)-2 in oral squamous cell carcinoma – evidence for COX-2 induction by areca quid ingredients in oral keratinocytes. *J Oral Pathol Med* 2003; **32**: 522–9.
40 Tang DW, Chang KW, Chi CW, Liu TY. Hydroxychavicol modulates benzo[a]pyrene-induced genotoxicity through induction of dihydrodiol dehydrogenase. *Toxicol Lett* 2004; **152**: 235–43.
41 Wu HJ, Chi CW, Liu TY. Effects of pH on nicotine-induced DNA damage and oxidative stress. *J Toxicol Environ Health* 2005; **68**: 1511–23.
42 Chen HM, Yen-Ping KM, Lin KH, Lin CY, Chiang CP. Expression of cyclin A is related to progression of oral squamous cell carcinoma in Taiwan. *Oral Oncol* 2003; **39**: 476–82.
43 Kuo MY, Lin CY, Hahn LJ, Cheng SJ, Chiang CP. Expression of cyclin D1 is correlated with poor prognosis in patients with areca quid chewing-related oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 1999; **28**: 165–9.
44 Kuo MY, Hsu HY, Kok SH *et al*. Prognostic role of p27 (Kip1) expression in oral squamous cell carcinoma in Taiwan. *Oral Oncol* 2002; **38**: 172–8.
45 Huang JS, Ho TJ, Chiang CP, Kok SH, Kuo YS, Kuo MY. MDM2 expression in areca quid chewing-associated oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 2001; **30**: 53–8.
46 Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002; **4**: E131–6.
47 Ho TJ, Chiang CP, Hong CY, Kok SH, Kuo YS, Yen-Ping Kuo M. Induction of the c-jun protooncogene expression by areca nut extract and arecoline on oral mucosal fibroblasts. *Oral Oncol* 2000; **36**: 432–6.
48 Kuo RC, Lin CY, Kuo MY. Prognostic role of c-Jun activation in patients with areca quid chewing-related oral squamous cell carcinomas in Taiwan. *J Formos Med Assoc* 2006; **105**: 229–34.
49 Liu SY, Yen CY, Yang SC, Chiang WF, Chang KW. Overexpression of Rac-1 small GTPase binding protein in oral squamous cell carcinoma. *J Oral Maxillofac Surg* 2004; **62**: 702–7.
50 Lin CY, Hung HC, Kuo RC, Chiang CP, Kuo MY. Survivin expression predicts poorer prognosis in patients with areca quid chewing-related oral squamous cell carcinoma in Taiwan. *Oral Oncol* 2005; **41**: 645–54.
51 Chen IH, Chang JT, Liao CT, Wang HM, Hsieh LL, Cheng AJ. Prognostic significance of EGFR and Her-2 in oral cavity cancer in betel quid prevalent area cancer prognosis. *Br J Cancer* 2003; **89**: 681–6.
52 Chen YS, Wang JT, Chang YF *et al*. Expression of hepatocyte growth factor and c-met protein is significantly associated with the progression of oral squamous cell carcinoma in Taiwan. *J Oral Pathol Med* 2004; **33**: 209–17.
53 Kuo MY, Cheng SJ, Chen HM, Kok SH, Hahn LJ, Chiang CP. Expression of CD44s, CD44v5, CD44v6 and CD44v7–8 in betel quid chewing-associated oral premalignant lesions and squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 1998; **27**: 428–33.
54 Chen YJ, Chang JT, Lee L *et al*. DSG3 is overexpressed in head neck cancer and is a potential molecular target for inhibition of oncogenesis. *Oncogene* 2007; **26**: 467–76.
55 Lo WY, Tsai MH, Tsai Y *et al*. Identification of over-expressed proteins in oral squamous cell carcinoma (OSCC) patients by clinical proteomic analysis. *Clin Chim Acta* 2007; **376**: 101–7.
56 Tsai WC, Tsai ST, Ko JY *et al*. The mRNA profile of genes in betel quid chewing oral cancer patients. *Oral Oncol* 2004; **40**: 418–26.
57 Chang JT, Wang HM, Chang KW *et al*. Identification of differentially expressed genes in oral squamous cell carcinoma (OSCC): overexpression of NPM, CDK1 and NDRG1 and underexpression of CHES1. *Int J Cancer* 2005; **114**: 942–9.
58 Hsieh LL, Huang TH, Chen IH *et al*. p53 polymorphisms associated with mutations in and loss of heterozygosity of the p53 gene in male oral squamous cell carcinomas in Taiwan. *Br J Cancer* 2005; **92**: 30–5.
59 Huang MF, Chang YC, Liao PS, Huang TH, Tsay CH, Chou MY. Loss of heterozygosity of p53 gene of oral cancer detected by exfoliative cytology. *Oral Oncol* 1999; **35**: 296–301.
60 Twu CW, Jiang RS, Shu CH, Lin JC. Association of p53 codon 72 polymorphism with risk of hypopharyngeal squamous cell carcinoma in Taiwan. *J Formos Med Assoc* 2006; **105**: 99–104.
61 Wong YK, Liu TY, Chang KW *et al*. p53 alterations in betel quid- and tobacco-associated oral squamous cell carcinomas from Taiwan. *J Oral Pathol Med* 1998; **27**: 243–8.
62 Kuo MY, Huang JS, Hsu HC *et al*. Infrequent p53 mutations in patients with areca quid chewing-associated oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 1999; **28**: 221–5.
63 Lin SC, Chang KW, Chang CS *et al*. Alterations of p16/MTS1 gene in oral squamous cell carcinomas from Taiwanese. *J Oral Pathol Med* 2000; **29**: 159–66.
64 Kok SH, Lee JJ, Hsu HC, Chiang CP, Kuo YS, Kuo MY. Mutations of the adenomatous polyposis coli gene in areca quid and tobacco-associated oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 2002; **31**: 395–401.
65 Wong YK, Chang KW, Cheng CY, Liu CJ. Association of CTLA-4 gene polymorphism with oral squamous cell carcinoma. *J Oral Pathol Med* 2006; **35**: 51–4.
66 Liu CJ, Wong YK, Chang KW, Chang HC, Liu HF, Lee YJ. Tumor necrosis factor-alpha promoter polymorphism is associated with susceptibility to oral squamous cell carcinoma. *J Oral Pathol Med* 2005; **34**: 608–12.
67 Ku KT, Wan L, Peng HC, Tsai MH, Tsai CH, Tsai FJ. Vascular endothelial growth factor gene-460 C/T polymorphism is a biomarker for oral cancer. *Oral Oncol* 2005; **41**: 497–502.
68 Hung HC, Chuang J, Chien YC *et al*. Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 901–5.
69 Chung FM, Yang YH, Chen CH, Lin CC, Shieh TY. Angiotensin-converting enzyme gene insertion/deletion polymorphism is associated with risk of oral precancerous lesion in betel quid chewers. *Br J Cancer* 2005; **93**: 602–6.
70 Lee HC, Yin PH, Yu TN *et al*. Accumulation of mitochondrial DNA deletions in human oral tissues – effects of betel quid chewing and oral cancer. *Mutat Res* 2001; **493**: 67–74.
71 Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kuhndel K, Liebert UG. PCR detection of human papillomavirus of the mucosa. comparison between MY09/11 and GP5$^+$/6$^+$ primer sets. *J Clin Virol* 2004; **30**: 302–8.
72 Bosch FX, Manos MM, Munoz N *et al*. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995; **87**: 796–802.
73 Durst M, Croce CM, Gissmann L, Schwarz E, Huebner K. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proc Natl Acad Sci* 1987; **84**: 1070–4.
74 Riou GF, Bourhis J, Le MG. The c-myc proto-oncogene in invasive carcinomas of the uterine cervix: clinical relevance of overexpression in early stages of the cancer. *Anticancer Res* 1990; **10**: 1225–31.
75 Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16, E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989; **243**: 934–7.
76 Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990; **63**: 1129–36.
77 Brandwein M, Zeitlin J, Nuovo GJ *et al*. HPV detection using 'hot start' polymerase chain reaction in patients with oral cancer: a clinicopathological study of 64 patients. *Mod Pathol* 1994; **7**: 720–7.
78 Young SK, Min KW. In situ DNA hybridization analysis of oral papillomas, leukoplakias, and carcinomas for human papillomavirus. *Oral Surg Oral Med Oral Pathol* 1991; **71**: 726–9.
79 Chen PC, Kuo C, Pan CC, Chou MY. Risk of oral cancer associated with human papillomavirus infection, betel quid chewing, and cigarette smoking in Taiwan – an integrated molecular and epidemiological study of 58 cases. *J Oral Pathol Med* 2002; **31**: 317–22.
80 Chang JY, Lin MC, Chiang CP. High-risk human papillomaviruses may have an important role in non-oral habits-associated oral squamous cell carcinomas in Taiwan. *Am J Clin Pathol* 2003; **120**: 909–16.

doi: 10.1111/j.1349-7006.2008.00863.x