

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

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

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

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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,<sup>(1)</sup> with an estimated over 500 000 new cases being diagnosed annually.<sup>(1)</sup> 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.<sup>(1)</sup>

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), hypopharynx (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.<sup>(2,3)</sup> 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.<sup>(2–5)</sup> In contrast, the Western registries show cancers of the mouth floor are the most frequent, with cancer of gum or

tongue being rare.<sup>(2,3)</sup> 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

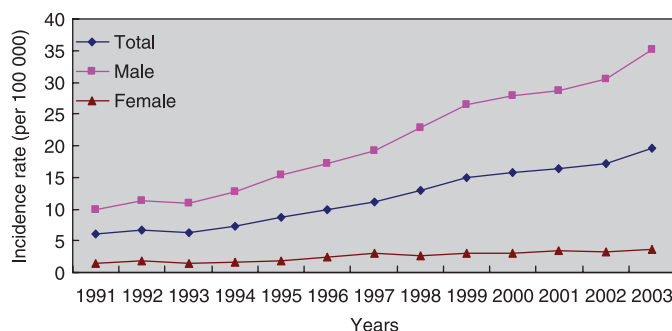
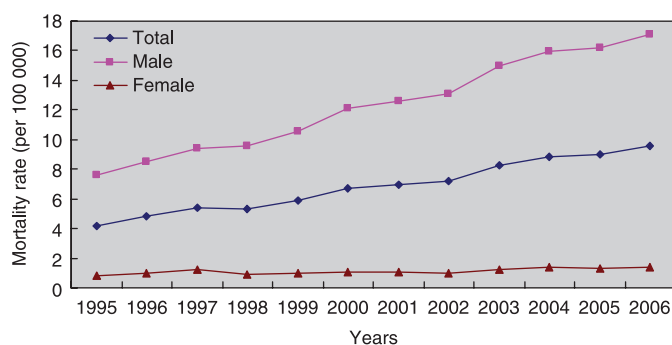
The incidence of HNC is one of the highest in the world, and this malignancy has been one of the 10 leading causes of cancer deaths in Taiwan (Table 1). All the epidemiology data are from the Cancer Registry Annual Report of Taiwan, Health and National Health Insurance Annual Statistics Information Service, 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,

<sup>8</sup>To whom correspondence should be addressed. E-mail: [ajchen@mail.cgu.edu.tw](mailto:ajchen@mail.cgu.edu.tw)

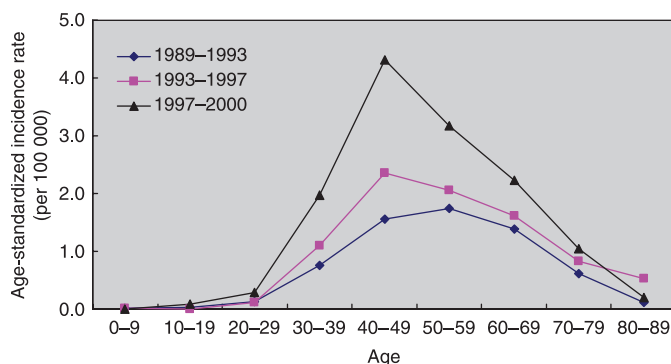
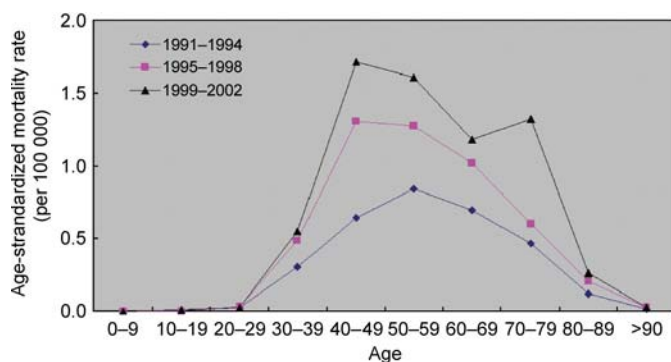
**Table 1.** The ten most common cancers in Taiwan, 2006

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 lymphoma	Non-Hodgkin lymphoma	Non-Hodgkin lymphoma
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.

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.<sup>(5–7)</sup> 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.<sup>(7)</sup> 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.<sup>(4,5,8)</sup> 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

**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.

(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.<sup>(1)</sup> 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.<sup>(5,8,9)</sup> Survival rates for HNC are significantly influenced by tumor size, lymph node involvement, distant metastasis, tumor differentiation and betel quid chewing.<sup>(4,5)</sup> 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%.<sup>(4,5,8)</sup> Approximately 50% of

**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 <i>in situ</i>		
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.<sup>(4,5,8)</sup>

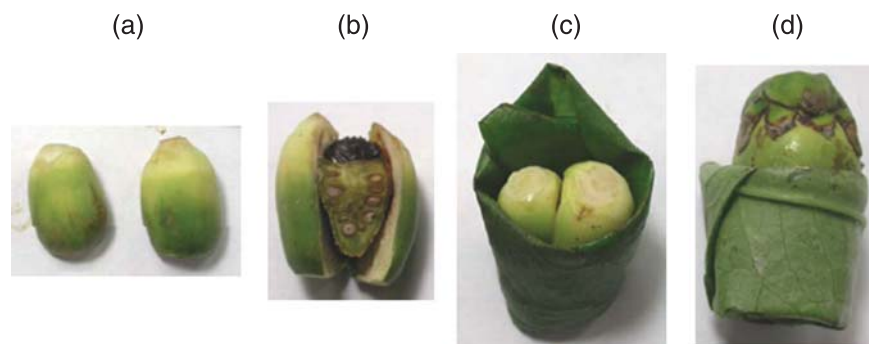
With regard to the anatomical localization of oral cavity cancers, approximately 30–40% of all cases occur in the tongue or in the buccal mucosa. Altogether, lesions at these sites account for approximately 70% of all oral cavity malignancies.<sup>(5,7,9)</sup>

### 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.<sup>(10)</sup> 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.<sup>(11)</sup> Specifically, 1.87% of patients with dysplastic lesion and 3.55% of those with hyperkeratosis/epithelial hyperplasia progressed to malignant transformation.<sup>(11)</sup> In another study of 1046 patients with oral leukoplakia, the prevalence rate of carcinoma was 12.9%.<sup>(12)</sup> 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.<sup>(12)</sup> 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.<sup>(12)</sup> 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.<sup>(11,12)</sup>

### **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.<sup>(13–15)</sup> 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).<sup>(16)</sup> 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.<sup>(17,18)</sup> 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.<sup>(19)</sup> 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).<sup>(20)</sup> Notably, the consumption of betel quid has gradually increased since 1970.<sup>(21)</sup> 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.<sup>(21)</sup>

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.<sup>(22)</sup> 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.<sup>(23,24)</sup> 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 inflores-

cence 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.<sup>(25)</sup> 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.<sup>(24)</sup> 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.<sup>(26)</sup> 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.<sup>(25)</sup>

Tobacco and betel quid may act synergistically as a carcinogen.<sup>(27)</sup> 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.<sup>(8)</sup> 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.<sup>(6)</sup> 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.<sup>(28)</sup> In contrast, no significant difference was seen in 5-year survival between smokers and non-smokers or between alcohol users and non-users.<sup>(28)</sup> 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).<sup>(29)</sup>

### **Betel-quid-associated molecular pathology**

The composition of betel quid differs geographically; the areca quid used in Taiwan contains areca nut, lime and *Piper betle* inflorescence.<sup>(23,24)</sup> *Piper betle* 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)

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.<sup>(30)</sup> The same study has found that 24-h treatment with ANE induced mutations at the hypoxanthine phosphoribosyltransferase (HPRT) locus in human keratinocytes.<sup>(30)</sup> Increased intracellular levels of reactive oxygen species and 8-hydroxyguanosine in cells exposed to ANE have been also reported.<sup>(30)</sup>

Salivary concentration of arecoline during betel quid chewing has been detected to be in the millimolar concentration range.<sup>(31)</sup> Arecoline has been shown to induce structural chromosomal aberration, sister chromatid exchange and micronuclei formation in different cell types.<sup>(32,33)</sup> 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.<sup>(34)</sup> Moreover, it has been shown that arecoline induced a significant elevation of p21waf1 and a decline of cdc2 and cyclin B1 in gingival keratinocytes.<sup>(34)</sup>

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.<sup>(35)</sup>

Hydroxychavicol, a phenolic component of betel leaf, has been found in human saliva at a 4.6 mM concentration after betel quid chewing.<sup>(36)</sup> Hydroxychavicol may induce the formation of single-strand DNA breaks and 8-hydroxydeoxyguanosine – a marker of oxidative DNA damage – in cultured cells.<sup>(37,38)</sup> Moreover, COX-2 expression and PGE<sub>2</sub> production have been shown to be significantly enhanced by hydroxychavicol in human normal oral keratinocytes.<sup>(39)</sup> 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.<sup>(40)</sup> 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.<sup>(41)</sup> 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.<sup>(42)</sup> 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.<sup>(43)</sup> 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.<sup>(44)</sup> It has been also observed that patients with low or absent p27Kip1 protein expression had poor prognosis.<sup>(44)</sup>

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.<sup>(3)</sup> 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.<sup>(45)</sup>

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.<sup>(46)</sup> A 3-fold increase in c-Jun expression has been described in oral mucosal fibroblasts after exposure to ANE or arecoline.<sup>(47)</sup> 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.<sup>(48)</sup> 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.<sup>(49)</sup> 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.<sup>(50)</sup> 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.<sup>(50)</sup>

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.<sup>(51)</sup> EGFR hyperexpression has been found to be associated with clinical stage, extracapsular spread and poor prognosis.<sup>(51)</sup> Hepatocyte growth factor (HGF) (scatter factor) and its receptor, the c-met proto-oncogene product (c-met), have also been implicated in HNC progression.<sup>(52)</sup> 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.<sup>(52)</sup>

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.<sup>(53)</sup> Desmoglein 3 (DSG3), a calcium-binding transmembrane glycoprotein component of desmosomes in epithelial cells, has been found to

be overexpressed in HNC specimens.<sup>(54)</sup> Notably, correlations were seen between DSG3 expression and T stage, N stage, overall stage, tumor depth and extracapsular spread.<sup>(54)</sup>

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.<sup>(55)</sup> Real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) was used to validate selected proteomic data, confirming that  $\alpha$ B-crystallin, heat shock protein 27 and manganese superoxide dismutase are significantly overexpressed in this malignancy.<sup>(55)</sup> Using cDNA microarray analysis, Tsai and colleagues identified 84 genes dysregulated in oral cancer associated with betel quid chewing.<sup>(56)</sup> Four genes, including *caspase-1*, *STAT-1*, *COX-2* (up-regulated) and *pleiotrophin* (down-regulated), were validated by further analyses.<sup>(56)</sup> Other authors have used fluorescent differential display analysis to identify gene expression profiles of HNC.<sup>(57)</sup> As a result, NPM, CDK1, NDRG1, HMGR, EF1A, NAC and DSG3 were found to be up-regulated in HNC, whereas CHES1 was down-regulated.<sup>(57)</sup> 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.<sup>(57)</sup>

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.<sup>(57)</sup> Further studies identified the same molecule as associated with disease severity.<sup>(54)</sup> Consistent with the clinical data, inhibition of DSG3 by RNA interference (RNAi) significantly reduced cell growth and colony formation in three HNC cell lines.<sup>(54)</sup> Moreover, an *in vivo* xenograft study showed that administration of DSG3-RNAi plasmid significantly inhibited tumor growth in mice.<sup>(54)</sup> 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.<sup>(58,59)</sup> Interestingly, 84.4% of patients informative for *p53*

gene exon 4 had a history of both habitual cigarette smoking and betel quid chewing.<sup>(51)</sup> 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.<sup>(60)</sup> 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.<sup>(61,62)</sup> 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.<sup>(61,62)</sup>

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.<sup>(63)</sup> The authors identified mutations in exon 2 and at the intron 1/exon 2 splice site that disrupted the encoded protein.<sup>(63)</sup> Several base transitions were identified, including codon 51 GTC<sup>Val</sup>→GCC<sup>Ala</sup>, codon 101 GGG<sup>Gly</sup>→GGA<sup>Gly</sup>, codon 102 GCG<sup>Ala</sup>→GTG<sup>Val</sup> and a nonsense mutation 80 CGA<sup>Arg</sup>→TGA that resulted in a premature stop codon.<sup>(63)</sup> Interestingly, methylation of the *p16/MTS1* promoter region occurred preferentially in carcinomas of the tongue (54%) compared with other sites (22%).<sup>(63)</sup>

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  $\beta$ -catenin levels, cell migration and adhesion and cell cycle control. Five missense mutations (codon 1352 GTT<sup>Val</sup>→GCT<sup>Ala</sup>, codon 1367 CAG<sup>Glu</sup>→CGG<sup>Arg</sup>, codon 1382 GTT<sup>Val</sup>→GCT<sup>Ala</sup>, codon 1402 GCC<sup>Ala</sup>→ACC<sup>Thr</sup> and codon 1652 TCC<sup>Ser</sup>→TTC<sup>Phe</sup>) and a single nucleotide deletion at codon 1593 resulting in a premature stop codon in the *APC* gene have been identified in HNC patients.<sup>(64)</sup> Notably, a significant correlation was observed between these mutations in the *APC* gene and the patients' tobacco/betel quid consumption.<sup>(64)</sup>

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.<sup>(65)</sup> Genetic polymorphisms in the promoter region of the tumor necrosis factor- $\alpha$  (*TNF- $\alpha$* ) 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- $\alpha$*  gene (–308 G/A and –238 G/A) have been investigated for their role in susceptibility to HNC.<sup>(66)</sup> 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.<sup>(66)</sup> 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.<sup>(67)</sup> 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)



have been linked to HNC among individuals who did not chew betel quid.<sup>(68)</sup> 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.<sup>(69)</sup> Results showed that the DD genotype independently predicted the presence of oral premalignant lesions.<sup>(69)</sup> 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.<sup>(70)</sup>

## 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.<sup>(71)</sup> The most important oncogenic types are HPV type 16 (HPV-16) and HPV-18.<sup>(72)</sup> In many advanced preneoplastic cervical lesions and most derived carcinomas, HPV genomes are found to be integrated into the host cell chromosomes.<sup>(73,74)</sup> 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.<sup>(75,76)</sup>

Besides cervical cancer, HPV infections have been linked to several other malignancies, including HNC.<sup>(77,78)</sup> 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.<sup>(79)</sup> Multivariate analysis identified HPV16 and betel quid chewing as independent predictors of oral cancer.<sup>(79)</sup> 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.<sup>(80)</sup> Moreover, rates of HPV infections were significantly higher in non-oral habits-associated oral cancer samples.<sup>(80)</sup> 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.<sup>(80)</sup>

## 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).

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