

# Genetic alterations in head and neck squamous cell carcinomas

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## Abstract

The genetic alterations observed in head and neck cancer are mainly due to oncogene activation (gain of function mutations) and tumor suppressor gene inactivation (loss of function mutations), leading to deregulation of cell proliferation and death. These genetic alterations include gene amplification and overexpression of oncogenes such as *myc*, *erbB-2*, *EGFR* and cyclinD1 and mutations, deletions and hypermethylation leading to p16 and *TP53* tumor suppressor gene inactivation. In addition, loss of heterozygosity in several chromosomal regions is frequently observed, suggesting that other tumor suppressor genes not yet identified could be involved in the tumorigenic process of head and neck cancers. The exact temporal sequence of the genetic alterations during head and neck squamous cell carcinoma (HNSCC) development and progression has not yet been defined and their diagnostic or prognostic significance is controversial. Advances in the understanding of the molecular basis of head and neck cancer should help in the identification of new markers that could be used for the diagnosis, prognosis and treatment of the disease.

## Key words

- Genetic alterations
- Oncogene
- Tumor suppressor gene
- Head and neck cancer

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## Introduction

Squamous cell carcinoma of the head and neck is an epithelial malignant disease arising from the mucosa of the upper aerodigestive tract (oral cavity, larynx, oropharynx and hypopharynx). The disease is characterized by local tumor aggressiveness, early recurrence and high frequency of second primary tumors. Synchronous or metachronous multiple lesions occurring in the same patient have led to the field cancerization hypothesis (1). According to which prolonged exposure of the epithelium to certain carcinogenic agents (tobacco and alcohol) could induce cellular alterations and tumor forma-

tion at multiple anatomical sites. Second primary tumors arise mainly in regions exposed to tobacco, including esophagus, lung and other areas of the upper aerodigestive tract (1). In fact, epidemiological data implicate tobacco smoking and alcohol consumption as the major risk factor for the disease (2).

The relative risk of developing cancer in individuals exposed to tobacco varies from person to person, depending in part on genetic components related to cancer susceptibility. There have been few data showing evidence of inheritance in head and neck squamous cell carcinoma (HNSCC) (3); however, common genes with low penetrance related to cancer susceptibility might account

for some of these tumors. The status of genes encoding drug-detoxifying enzymes (expression or form variation) determines how cells activate and detoxify chemical carcinogens. The active metabolites of chemical carcinogens covalently bind to the DNA and form adducts, which lead to mutations. Several studies have demonstrated variable levels of DNA adduct formation depending on the genotype of certain enzymes, such as *CYP1A1* (cytochrome p4501A1), *NATs* (N-acetyltransferases) and *GSTM1* (glutathione-S-transferase) and the risk to develop several types of tumors (4). An association between the genotype pattern of the enzymes *CYP1A1* (5), *NAT2* (6), *GSTM1* and *GSTM3* (7) and increased susceptibility to the development of HNSCC has been recently shown. These results indicate that these genetic polymorphisms could predict the levels of DNA adducts formed by certain carcinogens and could, therefore, predict the individual response to the exposure of these compounds and could be used to assess the risk and to

prevent the disease.

Human papilloma virus (HPV) infection has also been implicated in the etiology of HNSCC tumors. There is a prevalence of HPVs type 16 and 18 among HPV-positive head and neck patients. HPV infection has been found in 18 to 35% of benign and pre-invasive lesions; in invasive carcinomas, HPV positivity is higher in tumors from the oral cavity (59%), pharynx (43%) and larynx (33%) (8). In spite of the evident role played by HPV infection in HNSCC etiology its clinical implications or prognostic and diagnostic value have not yet been established.

## Genetic alterations

### Oncogene activation

Several cytogenetic and molecular studies have investigated the occurrence of genetic alterations in HNSCC, demonstrating that oncogene activation and tumor suppressor gene inactivation are involved in the development of the disease (Table 1). Genetic alterations, mainly gene amplification, mutation and overexpression, leading to oncogene activation have been observed in HNSCC; however, these studies are preliminary and involve a small number of patients and short follow-up. Data about the relationship of these genetic changes with disease progression and patient outcome have been inconclusive.

*ras* mutations have been identified in less than 5% of the head and neck tumors from Western world patients (9). A high incidence (35%) of Ha-*ras* mutations has been reported in oral squamous cell carcinomas from India (10). In this case betel quid chewing and reverse smoking are probably initiating agents (11). Whereas activating *ras* mutations appear to be an infrequent event in head and neck tumors from the Western world, studies using immunohistological staining and RT-PCR have demonstrated that overexpression c-Ha-*ras* is a common event

Table 1 - Summary of the genetic alterations observed in head and neck squamous cell carcinomas.

Oncogene activation	
ras	Mutation and overexpression
11q13 (int-2/bcl-1/PRAD-1)	Amplification
Cyclin D1 (PRAD-1)	Amplification and overexpression
myc, EGFR, TGF $\beta$	Overexpression
Loss of heterozygosity (>25%)	
1p, 1q	Loss of heterozygosity of specific chromosomal regions (candidate tumor suppressor genes)
3p, 4q	
5p, 5q	
6p, 6q	
8p, 9p	
11q, 13q	
14q, 17p	
18q	
Tumor suppressor gene inactivation	
TP53	Mutations and deletions
p16	Deletions, hypermethylation and mutations

in these tumors (11,12). However, the exact mechanism accounting for *ras* overexpression is unknown and its association with existing prognostic factors is not yet clear.

Amplification and overexpression of the oncogenes *myc*, *erbB-2* and epidermal growth factor receptor (*EGFR*) have been observed in HNSCC, and are correlated with a poor prognosis. The members of the *myc* gene family (*c-myc*, *N-myc*, *L-myc*) encode for a 62-kDa protein with transactivational activity (13). The *myc* proteins (p62<sup>myc</sup>) are able to dimerize with a second protein, *max*. Overexpression of *myc* leads to increased amounts of *myc-max* heterodimers and reduced *max-mad* heterodimer formation (13), changing the regulation of many genes and contributing to malignant transformation. The frequency of *c-myc* amplification and overexpression reported in the literature varies from 9 to 48% (14,15). Using immunohistochemistry, Field et al. (14) observed *c-myc* amplification in 48% of the tumors analyzed. Although no association has been found between the *c-myc* amplification and sex, gender, tumor size, clinical stage or differentiation, these investigators observed that patients with tumors with high levels of *c-myc* showed shortened overall survival. In addition, Porter et al. (16) demonstrated *c-myc* amplification in 22% of tumors from the nasopharynx and observed that the prognosis for patients with tumors with *c-myc* amplification was worse compared with patients with tumors without *c-myc* amplification.

*c-erbB-2* gene (also named *neu* or *Her-2*) encodes for a 185-kDa protein with tyrosine kinase activity, homologous to the *EGFR* (17). *c-erbB-2* amplification and overexpression have been observed in several human tumors (18,19). In head and neck carcinomas, the frequencies of *erbB-2* amplification and overexpression range from 0 to 41% (20-22). The prognostic value of these genetic alterations observed in *erbB-2* oncogene is controversial. Field et al. (14) and

Craven et al. (21) reported a lack of correlation between *erbB-2* overexpression and clinicopathological characteristics or patient survival. However, Press et al. (22) observed that patients with salivary gland tumors with *erbB-2* overexpression have a poor prognosis.

The *EGFR* gene is frequently amplified and overexpressed in cell lines and primary head and neck tumors. A good correlation has been found between the presence of *EGFR* genetic alterations and poor prognosis, particularly in head and neck tumors of the larynx. Amplification and overexpression of *EGFR* have been detected in 61% of larynx tumors and the elevated expression was associated with a poor prognosis (23). In addition, *EGFR* overexpression has been found primarily in poorly differentiated larynx tumors and in tumors from patients with short disease-free interval and overall survival (24,25).

Amplification of the 11q13 region is frequently observed in head and neck tumors. Several putative oncogenes have been identified on 11q13, including *bcl-1*, *int-2*, *hst-1*, *EMS-1* and cyclin D1/*PRAD-1*. Amplification and coamplification of *bcl-1*, *int-2* and *hst-1* have been found in 30-52% of the head and neck tumors (26,27). The *int-2* (*FGF3*) and *hst-1* (*FGF4*) oncogenes are members of the fibroblast growth factor (*FGF*) associated with the induction of cell proliferation and angiogenesis. These genes are considered good candidates to play an important role in the genesis of HNSCC (11,28). A high frequency of *hst-1/FGF4* and *bcl-1* amplification has been observed in tumors of the hypopharynx and in lymph node-positive patients (27). However, amplification of these genes is not followed by overexpression, suggesting that other genes located in the 11q13 region could be involved in the tumorigenic process of the head and neck.

Cyclin D1/*PRAD-1* and *EMS-1* genes are also considered to be possible targets of 11q13 amplification. The *EMS-1* gene shows

homology with the *v-src* substrate and may play a role in cytoskeleton organization and cell adhesion (29). *EMS-1* amplification and overexpression were observed in a high proportion of cell lineages derived from head and neck carcinomas (30).

Amplification and overexpression of cyclin D1 have been consistently associated with the tumorigenic process of the head and neck (31). Cyclin D1 is encoded by the *CCND1/PRAD-1* gene (32) and is among the major regulators of the G1 restriction point of the cell cycle (33). Cyclin D1 is able to form a complex with cyclin-dependent kinases (CDK4 and CDK6), which phosphorylate *RB* releasing E2F. Cyclin D1 overexpression has been described in 39-64% of primary HNSCC (34,35). In a recent study of HNSCC, overexpression of cyclin D1 has been correlated with lymph node metastases and advanced clinical stage (36). In addition, cyclin D1 immunoreactivity is more frequent in tumors of patients with shortened disease-free interval and overall survival (34,35).

#### **Tumor suppressor gene inactivation**

Deletion of specific chromosomal regions is one of the most common genetic events observed in solid tumors and the deleted regions are believed to contain potential tumor suppressor genes. High frequencies of loss of heterozygosity (LOH) involving chromosomal regions 3p, 5q, 8p, 9p, 9q, 11p and 17p have been reported in HNSCC (37). The highest LOH frequencies occur on chromosomal arms 3p, 9p and 17p (37,38), which harbor unknown and already cloned tumor suppressor genes, such as *VHL* (3p), p16 (9p) and *TP53* (17p). The occurrence of LOH in these regions has been associated with the development and progression of the disease (39,40).

Genetic alterations involving the tumor suppressor genes p16 and *TP53* are frequently observed in head and neck tumors. Inactiva-

tion of both of these genes appears to be involved in the early stages of the disease. However, the diagnostic and prognostic value of these genes remains unclear at present.

The p16 tumor suppressor gene located on chromosome 9p21 encodes a 16-kDa protein that belongs to an important group of cyclin-dependent kinase inhibitors (CDKIs) which includes p15<sup>ink4b</sup>, p21<sup>waf1</sup> and p27<sup>kip1</sup>. These genes regulate the G1 phase of the cell cycle in a negative way. The p16 gene product binds to CDK4 and CDK6 inhibiting their association with cyclin D1. The inhibition of the cyclin D1/CDK4/6 complex activity prevents pRB phosphorylation and the release of E2F, leading to inhibition of the cell cycle in the G1-S transition (41). Genetic abnormalities inactivating the p16 gene might confer cell growth advantages contributing to the tumorigenic process.

High frequencies of loss of heterozygosity on the short arm of chromosome 9 (9p21-22), where the tumor suppressor gene p16 is located, have been reported in head and neck tumors, including dysplasia and carcinoma *in situ* (39,42), suggesting the involvement of this region in the early stages of the disease.

Genetic alterations leading to p16 inactivation have been detected in 7 to 67% of head and neck tumors. Point mutations, small deletions and insertions were found in a small subgroup of HNSCC (43,44). Homozygous deletions and hypermethylation have been found in 20-67% of the HNSCC and are considered to be the major genetic mechanisms for p16 inactivation (43,44). Hypermethylation of the 5' CpG island of p16 has been associated with gene silencing (43,45). Absent or reduced expression of p16 has been observed in a significant proportion of carcinomas (44) and pre-invasive lesions (46). The high incidence of p16 inactivation in head and neck tumors indicates that this gene plays an important role in the development of the disease. At present, however, the diagnostic or prognostic value of these find-

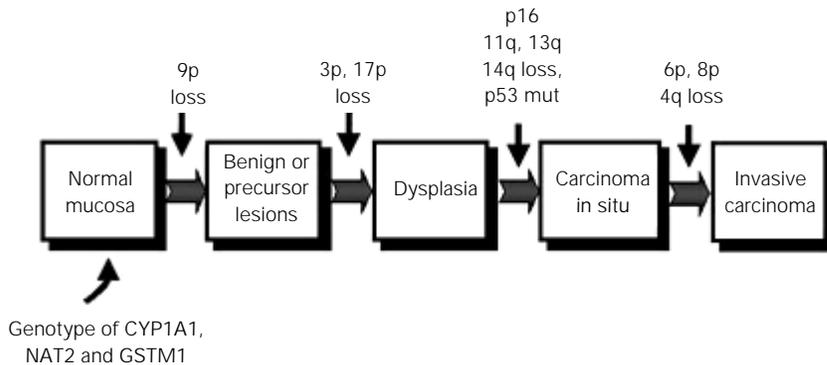


Figure 1 - Molecular model proposed for tumorigenesis in head and neck squamous cell carcinomas (Califano et al. (39), with modifications). The status of genes encoding enzymes (such as CYP1A1, NAT2 and GSTM1) associated with the metabolism and detoxification of chemical carcinogens might increase the relative risk to develop head and neck cancer. Genetic alterations involving several chromosomal regions are required for normal cells to progress from benign or precursor lesions to dysplasia, to carcinoma in situ and to invasive carcinoma.

ings remains to be determined.

Genetic alterations leading to loss of normal function of the *TP53* tumor suppressor gene is believed to contribute to the development of the majority of human cancers (47,48). The *TP53* gene is located on chromosome 17p13.1 and consists of 11 exons coding for a nuclear phosphoprotein which can bind to specific DNA sequences acting as a transcription factor (49). Most *TP53* gene mutations are missense mutations that tend to cluster within exons 5 to 8, spanning the evolutionarily conserved region of the protein (48). This highly conserved region encompasses amino acids 102 to 292 containing the DNA-binding domain of the protein (50). Many of these point mutations may alter the p53 wild-type conformation, resulting in protein stabilization and accumulation in cells (51,52), which allow their detection by immunohistochemistry. p53 biological activities are complex and have not been completely elucidated. However, a number of findings indicate that p53 plays an important role in cell-cycle control (both G1/S and G2/M checkpoints) and in the induction of apoptosis in several different physiological conditions (49).

The *TP53* gene is probably the most widely studied gene in head and neck cancer. Immunohistochemical studies have shown accumulation of p53 in 50 to 100% of cell lines derived from head and neck tumors (53,54). In primary tumors, p53 immunoreactivity was observed in 11 to 79% of cases (see Ref. 55, for a recent review). Molecular

studies show that approximately 50% of head and neck tumors harbor *TP53* mutations (56-58). These mutations were distributed among exons 4 to 8 spanning the evolutionarily conserved region of the protein (58).

The exact role of the *TP53* genetic alterations in different stages of the tumorigenic process is not completely established. In head and neck tumors p53 immunoreactivity has been observed in normal mucosa of patients with head and neck carcinoma (59) and mutations have been observed in non-invasive lesions (57), suggesting that *TP53* inactivation is involved in the early stages of the disease.

*TP53* mutations are a common alteration in tobacco-associated cancers (47,48). In head and neck cancer, positive p53 immunostaining is more frequently observed in tumors from patients with a smoking history (11,60). In addition, p53 overexpression is more prevalent in normal mucosa from smoking patients than in normal mucosa from non-smoking patients (54) and G:C→T:A transversions, which are thought to be induced by benzo-pyrene found in tobacco smoke, are more frequently observed in head and neck tumors from patients with a history of tobacco consumption (47,59).

The exact sequence of the genetic events in head and neck cancer has not yet been established. However, based on the detection of loss of heterozygosity in 87 head and neck tumors including benign and pre-invasive lesions, Califano et al. (39) proposed a preliminary progression model for HNSCC

(Figure 1; proposed model with modifications). According to this model, loss of heterozygosity in chromosomal region 9p is already observed in benign and precursor lesions; deletions in chromosomal regions 3p and 17p are observed in dysplasia; deletions in the 11q, 13q and 14q regions are associated with carcinoma *in situ*, and deletions involving chromosomal regions 6p, 8p and 4q are associated with invasive lesions. These investigators point out that it is the accumulation and not necessarily the order

of the genetic events that determines tumor progression.

The study of the genetic alterations associated with the carcinogenic process of the head and neck including continued analysis of new genetic alterations and their temporal sequencing during initiation, promotion and progression will allow us to identify new diagnostic and prognostic factors and provide a promising basis for the application of more rational and efficient treatments.

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