

# Genetic and molecular alterations associated with oral squamous cell cancer (Review)

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**Abstract.** The development of oral squamous cell cancer (OSCC) is a multistep process involving the accumulation of multiple genetic alterations modulated by genetic predisposition and environmental influences such as tobacco and alcohol use, chronic inflammation, and viral infections. All of these factors can lead to a wide range of genetic and molecular alterations that can be detected using a range of molecular studies. The alterations mostly affect two large groups of genes: oncogenes and tumor suppressor genes, which can be either inactivated or overexpressed through mutations, loss of heterozygosity, deletions, or epigenetic modifications such as methylation. Other molecules that are closely associated with tumor pathogenesis and prognosis also exist and warrant further study. Important advances in molecular biology are helping to shed light on oral cancer and thus aiding in the early diagnosis and development of new personalized treatment approaches. The purpose of the review is to explore the genetic and molecular alterations associated with OSCC.

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## 1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. Accounting for between 90 and 95% of all malignant lesions of the mouth, OSCC

has become practically synonymous with oral cancer. Based on statistics from June 24, 2008, the American Cancer Society reported that 1,437,181 new cancer cases and 565 650 cancer-related deaths were expected in the United States in 2008 (1). The estimated number of cases of oral cavity cancers was 22,900, equivalent to 3% all cancer cases.

Carcinogenesis is a multistep process modulated by both environmental and genetic factors (Fig. 1). As early as 1988, Boyd and Reade (2) described the different mechanisms involved in carcinogenesis of the oral mucosa and distinguished between 2 major groups: chemical mechanisms, physical mechanisms, and viral mechanisms (2). More than a decade later, Hanahan and Weinberg (3) described 6 hallmarks of cancer: acquisition of growth signaling autonomy (oncogenes), growth-inhibitory signals (tumor suppressor genes), evasion of apoptosis, cellular immortalization, angiogenesis, and finally, invasion and metastasis.

## 2. Differential gene expression in oral squamous cell carcinoma

Numerous methods are available for directly analyzing tumor DNA, either directly in the tumor tissue itself or in previously prepared tissue. The most common methods are *in situ* hybridization, Southern and Northern blot analysis, polymerase chain reaction (PCR), and automatic DNA sequencing. Microarray technology, for its part, is particularly useful for establishing general gene expression patterns and for screening for differential gene expression. Array results, however, need to be validated using an alternative method such as Northern blot analysis or quantitative real-time (RT) PCR, used to evaluate product accumulation during the log phase of the reaction. Quantitative RT-PCR, currently considered the most reliable and reproducible gene quantification method available, is the most widely used technique for validating gene expression results obtained using microarray technology (5-7). To determine gene expression in OSCC, many researchers have combined DNA microarray hybridization with other molecular methods (such as quantitative RT-PCR) that measure differential gene expression.

A large number of studies have used DNA microarray technology to profile gene expression patterns in head and neck cancer and in OSCC in particular (8-29). The majority of these studies have concluded that there is a possible association between different genes and squamous cell

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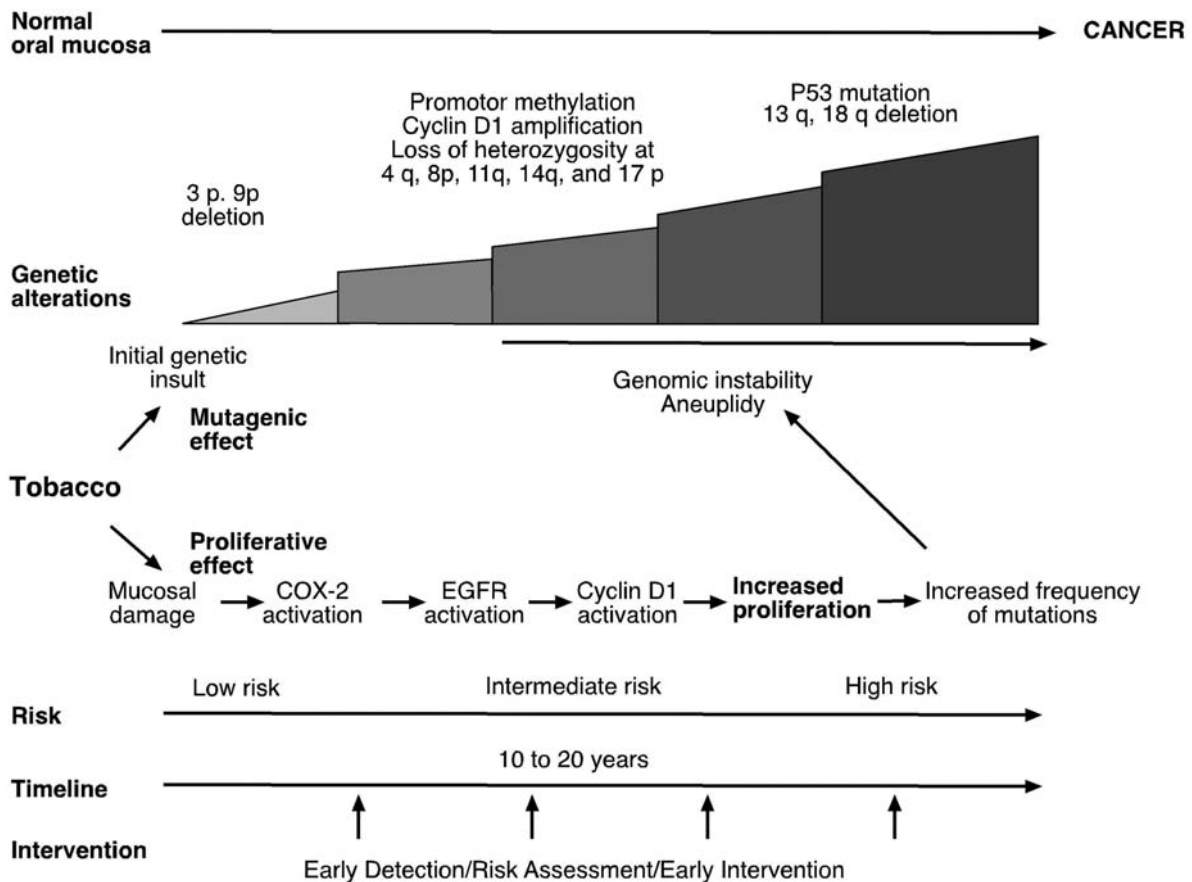


Figure 1. Chronic exposure to carcinogens such as tobacco causes genetic changes in the epithelial cells of the oral mucosa. The accumulation of genetic insults leads to genomic instability, the development of premalignant lesions, and ultimately invasive carcinoma. Carcinogens also have parallel indirect effects such as the induction of proliferative activity through, for example, the epidermal growth factor receptor (EGFR). COX, cyclooxygenase; RAR, retinoic acid receptor. Obtained from (18).

carcinoma. Many of the genes implicated have been previously described and are well known, but others have an unknown biological function and have yet to be analyzed in depth. Based on the results of these microarray studies, these new genes can now be investigated individually to determine their nature and function.

In a relatively recent study, our research group studied gene expression profiles in 5 patients with OSCC using the Atlas Glass Human 3.8 I Microarray (Fig. 2) (30). This microarray consists of 3,888 spots, including 3,757 oligonucleotides, 9 house-keeping genes, and controls. The genes, all well known and characterized, have a wide range of biological functions. Statically significant differences were found between tumor tissue and normal tissue for 426 genes, 322 (75.58%) of which were overexpressed and 104 (24.41%) of which were under-expressed. Interestingly, while the genes found to be differentially expressed varied to some extent between analyses, the biological functions they encoded were identical (Table I) (30).

### 3. Genetic and epigenetic anomalies in OSCC

To facilitate comprehension, we have classified the genetic and epigenetic anomalies associated with OSCC according to the type of structure affected (chromosome, allele, onco-

gene, tumor suppressor gene, or nucleotide) and the type of anomaly (polymorphism, point mutation, deletion, and other alterations).

There are reports of frequent chromosomal aberrations (deletions) at 2q21-24, 2q33-35, and 2q37 which affect numerous tumor suppressor genes including *LRP1B*, *CASP8*, *CASP10*, *BARD1*, *ILKAP*, *PPP1R7*, and *ING5* (31). One recent study reported that the loss of alleles 3p14 and 9p21 occurs early on in the development of OSCC tumors and can even occur in simple keratosis (32). Polysomy 3, for its part, is more common than polysomy 9 and is characteristic of dysplasia and *in situ* carcinoma (33).

A high frequency of loss of heterozygosity (LOH) at chromosomal loci 13q and 17p has been described in premalignant oral lesions and early carcinomas (34). The affected regions harbor important genes whose suppressor function in the development of tumors is probably severely altered by this LOH. Chromosome 9 appears to be one of the regions that is altered most often and earliest in tumor development; allelic losses at 9p21, for example, have been described in the majority of premalignant oral lesions and early carcinomas (Fig. 3) (35).

The 9p21 region harbors genes that code for the cyclin-dependent kinase inhibitors p16 and p14, two important regulators of cell proliferation. Several regions of chromo-

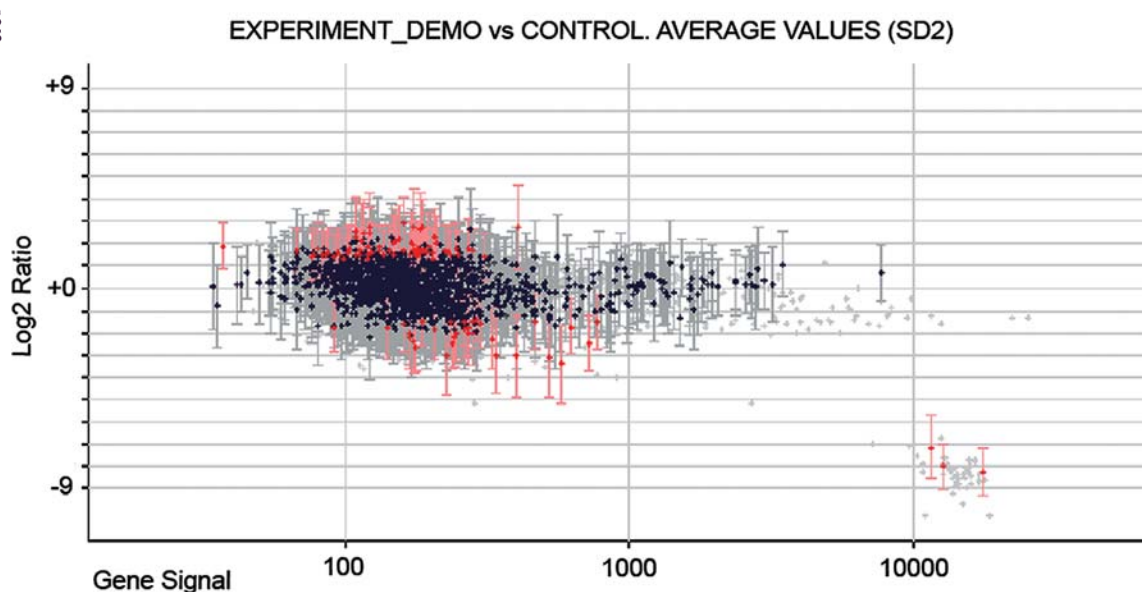


Figure 2. Log<sub>2</sub> graph showing Cy3/Cy5 ratio (after normalization of data using the Lowess method) and signal intensity of each microarray spot. The red dots (426 genes and 86 controls) correspond to significantly differentially expressed genes ( $P=0.05$ , t-test) in tumor tissue compared to normal tissue. The vertical bars show the standard deviation. Obtained from (29).

Table I. Number and percentages of genes in each functional category showing significant differences in expression (t-tests,  $p<0.05$ ) between normal and tumoral tissue.

Function	Total no.	% Total	No. up-regulated	% up-regulated	No. down-regulated	% down-regulated
Cell surface antigens	8	1.87	5	1.55	3	2.88
Transcription factors	52	12.20	33	10.24	19	18.26
Cell cycle proteins	3	0.70	3	0.93	0	0
Cell adhesion receptors/proteins	22	5.16	19	5.90	3	2.88
Immune system proteins	8	1.87	5	1.55	3	2.88
Extracellular transport/carrier proteins	17	3.99	11	3.41	6	5.76
Oncogenes and tumor suppressors	11	2.58	8	2.48	3	2.88
Stress response proteins	14	3.28	10	3.10	4	3.84
Membrane channels and transporters	27	6.33	19	5.90	8	7.69
Extracellular matrix proteins	8	1.87	5	1.55	3	2.88
Trafficking/targeting proteins	39	9.15	28	8.69	11	10.57
Metabolism proteins	80	18.77	68	21.11	12	11.53
Post-translational modification/protein folding	18	4.22	13	4.03	5	4.80
Translation	26	6.10	17	5.27	9	8.65
Apoptosis associated proteins	6	1.40	4	1.24	2	1.92
RNA processing, turnover, and transport	11	2.58	11	3.41	0	0
DNA binding and chromatin proteins	5	1.17	4	1.24	1	0.96
Cell receptors	38	8.92	33	10.24	3	2.88
Cell signaling, extracellular communication proteins	19	4.46	14	4.34	5	4.80
Intracellular transducers/effectors/modulators	53	12.44	36	11.18	17	16.34
Protein turnover	22	5.16	15	4.65	7	6.73
Cytoskeleton/motility proteins	25	5.86	22	6.83	3	2.88
DNA synthesis, recombination, and repair	11	2.58	5	1.55	6	5.76
Functionally unclassified	17	3.99	14	4.34	3	2.88

Obtained from (30).

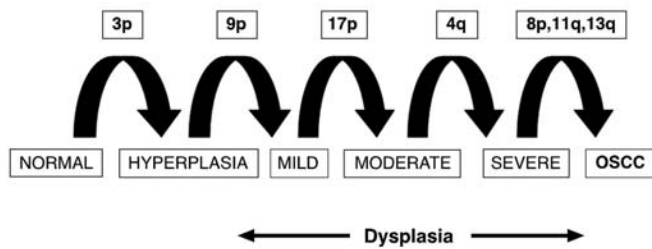


Figure 3. Molecular model of oral carcinogenesis. The diagram shows the genetic progression from dysplasia to oral squamous cell carcinoma (OSCC), through changes in the p or q arm of chromosomes 3, 4, 8, 9, 11, 13, and 17. Adapted from (64).

some 3, in particular 3p25, 3p21, and 3p13-14, commonly harbor chromosomal aberrations in oral cancer, although it is not yet known which genes are affected (36). Other aberrations such as allelic losses at 5q21-22, 22q13, 4q, 11q, 18q, and 21q, are often found in association with advanced tumor stages and poorly differentiated carcinomas (37).

Recent studies have identified allelic polymorphisms in the genes *HLA* and *MICA* (major histocompatibility complex-class-I-chain-related gene A) (38). In one of these studies, HLA-B35 and HLA-B40 were strongly associated with tumor metastasis (39).

Anomalies in certain oncogenes such as *ErbB1* (*Her-1*), *ErbB2*, and *N-, K- and H-ras* have not been found to play a key role in oral cancer (40-43). When altered, genes that code for the synthesis of cyclin proteins, may act as oncogenes. Overexpression of *CCND1*, for example, can induce overexpression of the cyclin D1 protein, which has been associated with poor prognosis in early-stage oral tumors (44-46).

Tumor suppressor gene anomalies are also found in malignant oral lesions. Most oral carcinomas are characterized by aberrant expression of at least one of the members of the retinoblastoma (pRb) family of growth suppressor proteins. *CDKN2A*, for example, which encodes the protein p16, is located at locus 9p21, one of the most vulnerable areas of the human genome in oral cancer, while p14, the alternative transcript of the same gene, is frequently deleted in malignant oral lesions. One of the most important tumor suppressor genes in humans is *TP53* (47). The functions of this gene and its molecular system have been found to be suppressed in numerous tumors, constituting one of the earliest findings in the natural history of oral cancer (48,49).

Three single nucleotide polymorphisms detected in the promoter region of the *DNMT3B* gene-*C46359T* [-149C>T], -238T>C, and -579>T-might play a causative role in several cancers, including OSCC (50).

In contrast, in OSCC, the base excision repair pathway, which comprises the genes *MUTYH*, *OGG1*, and *MTH1* and which repairs mutations that involve 8-oxoguanine, has been seen to play a very small or possibly even non-existent role in tumor development (51). Similar findings have been reported following argyrophilic nucleolar organizer region (AgNOR) analyses, although the results have been disputed (18). According to Teresa *et al* and Costa *et al* AgNOR and Ki-67 analyses can be used to determine proliferative states

of epithelial cells in oral cancer (39,52), and Schwartz *et al* have shown levels of proliferating cell nuclear antigen to be increased in an experimental model (53).

Our research group found a very interesting association between OSCC and overexpression of *ATP6VIC1* 54, which seems to be the main gene involved in the regulation of V-ATPase enzymes and the acidity of solid oral tumors (54).

The most common epigenetic alteration is DNA methylation. Imai *et al* (55) recently demonstrated that Ras association family genes (RASSF) were altered in OSCC. In particular, they found *RASSF2* to be methylated in 26% of OSCC tumors analyzed (55).

#### 4. Other molecules associated with OSCC

Other molecules that have been associated with OSCC are cyclo-oxygenase 2 (COX-2), which has been found in high levels in dysplastic lesions (56); the human trophoblast cell-surface antigen (TROP2), which appears to be associated with shorter survival (57); and the epithelial adhesion molecule (EpCAM), which has been associated with tumor size, regional lymph node metastasis, histologic differentiation, and an invasive pattern (31). The connective tissue growth factor, CCN2 (also known as CTGF), was recently associated with head and neck squamous cell cancer; findings included high levels of messenger RNA (mRNA) and its protein in stromal fibroblasts, tumor cells, and vascular endothelial cells (58). Overexpression of *MMP-2* and *MMP-9* has been associated with the invasive potential of tumors and levels of alcohol, leading several authors to hypothesize that alcohol might play a role in oral carcinogenesis through the stimulation of these genes (37).

It has been shown that molecular analysis of saliva can be used to study genomic DNA expression but not mRNA expression (59). Other studies have detected the soluble fragment of cytokeratin 19, Cyfra 21-1, in patients with OSCC, although further studies are required to determine the true diagnostic and prognostic value of this marker (60,61). Recent studies have shown that the determination of proteins and oxidated DNA levels in saliva might indicate high levels of reactive oxygen species, which appear to be involved in the development of OSCC (62).

Other recent findings in this area include the observation of an association between oral cancer and a set of new molecules called advanced glycation endproducts (AGEs) and their receptors (RAGEs) (63). According to the authors of the study, RAGE expression decreases with an increase in OSCC differentiation. RAGEs, for example, are expressed in 100% of normal epithelial cells but in 0% of poorly differentiated OSCC cells.

#### 5. Conclusions

Oral carcinogenesis is a multifactorial process involving numerous genetic processes that can alter the function of oncogenes, tumor suppressor genes, and other related molecules. The resulting anomalies can increase the production of growth factors and the number of cell surface receptors, and/or increase transcription or intracellular messenger factor levels. These changes can, in turn, cause a loss of tumor



activity and give rise to a phenotype capable of promoting cellular proliferation, weakening cell cohesion, and causing local infiltration and metastasis.

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