Review Article

Variants of Oral Squamous Cell Carcinoma with Emphasis on Histopathological Features, Biomarkers

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Abstract

Squamous cell carcinoma (SCC) is a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges. The epidermoid carcinoma is the most common malignant neoplasm of the oral cavity which also certain histopathological variations. The present review article focuses on histopathological variations seen in SCC along with brief emphasis on the biomarkers and prognostic factors which help in accurate diagnosis of SCC.

Keywords: Biomarkers, epidermoid carcinoma, histopathological variants, oral squamous cell carcinoma, squamous cell carcinoma variants

INTRODUCTION

Squamous cell carcinoma is defined as "a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges" (Pindborg JJ *et al*, 1997).The epidermoid carcinoma is the most common malignant neoplasm of the oral cavity.[1] This review article covers all the histopathological variants of squamous cell carcinoma.

SPINDLE CELL CARCINOMA

Histologic features

a. Spindle cell carcinoma is a bimorphic or biphasic tumor which, although almost always ulcerated, will show foci of surface epidermoid carcinoma or epithelial dysplasia of surface mucosa, usually just at the periphery and limited [Figure 1][1,2]
b. Proliferation and "dropping-off" of basal cells to spindle cell elements is a common phenomenon
c. The tissue patterns-either fasciculated, myxomatous, or streaming
d. Fasiculated form-elongated with elliptical nuclei, although pleomorphic cells are also common
e. The number of mitoses may vary from few to many
f. Giant cells, both benign-appearing of the foreign-body type and the bizarre, pleomorphic, atypical cells may be found
g. Finally, an inflammatory cell infiltrate is often present
h. Osteoid formation within the tumor component is sometimes seen
i. Microscopic invasion of subjacent structures is evident
j. Spindle cells may not be obviously epithelial appearance, they react for cytokeratins. These are best demonstrated with a pan–keratin antibody such as MNF 116
k. The tumor cells are negative for Smooth muscle actin (SMA), actin, desmin, S100 protein and vimentin.

ADENOID SQUAMOUS CELL CARCINOMA

Histologic features [Figure 1b]

• There is a proliferation of surface dysplastic epithelium into the connective tissue
• The lateral or deep extensions of this epithelium show the characteristic solid and tubular ductal structures which

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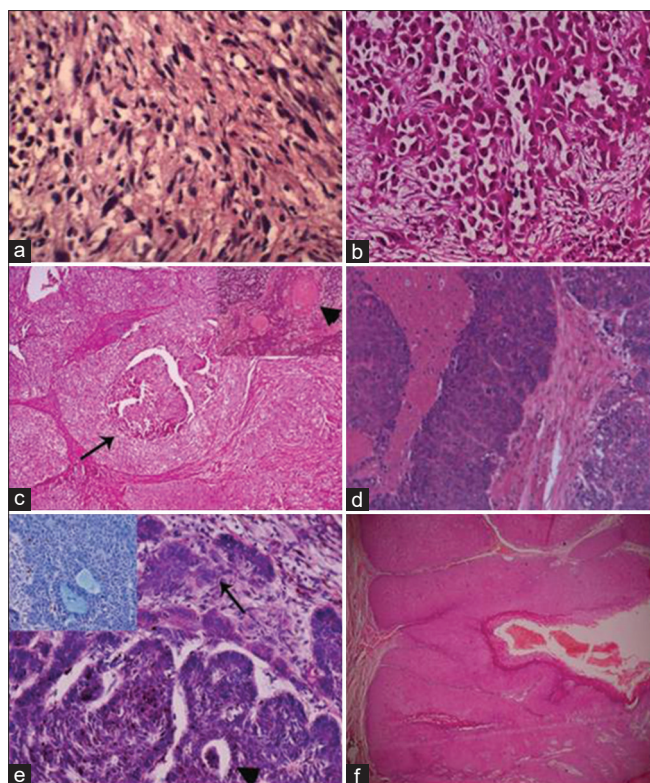
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Figure 1: (a- f) Histopathology of variants of squamous cell carcinoma

typify the lesion

- These duct-like structures are lined by a layer of cuboidal cells and often contain or enclose acantholytic or dyskeratotic cells
- Degenerative cells in the central zone may stain for periodic acid–Schiff (PAS)
- Acantholytic lesions in particular mimic the range of adenoid and alveolar neoplasms and may easily be considered metastatic or even sarcomatous.

Basaloid Squamous Cell Carcinoma

Histologic features [Figure 1c and d][2,3]

- Basaloid squamous carcinoma has two microscopic components
- The first is a superficial well-differentiated or moderately differentiated squamous cell carcinoma (SCC), often with surface ulceration, multifocal origin, and areas of carcinoma *in situ*
- The second, deeper component is an invasive basaloid epithelium arranged in islands, cords, and gland-like lobules. This deeper tumor often shows palisading of peripheral cells, necrosis of central regions, and occasional squamous differentiation
- The interface between the two components is typically sharp and distinct, but the transition from squamous to basaloid cells may occasionally be seen
- Basaloid cells and islands of cells often are surrounded by mucoid stroma

- Microcystic spaces filled with PAS-positive basal lamina material may be interspersed among the tumor islands
- Small deposits of PAS-positive hyaline material are often encountered towards the periphery of the tumor islands giving a false impression of adenocarcinoma
- Ocal prickle cells and keratin may be found in large tumors.

Adenosquamous Carcinoma

Histologic features [Figure 1e][2,3]

a. Adenosquamous carcinoma appears as an admixture of a surface SCC and an underlying ductal adenocarcinoma
b. The glandular component tends to be most prominent in deeper portions of the tumor
c. Intracytoplasmic mucin is noted by mucicarmine staining in most cases, making differentiation from mucoepidermoid carcinoma difficult but helping to distinguish adenosquamous carcinoma from forms of SCC that exhibit a pseudo glandular pattern of degeneration*
d. Both squamous and glandular components immunoreact with antibodies directed against high molecular-weight cytokeratin s (KLI)
e. Frozen section Intraclass correlation coefficient (ICC) using antibodies to individual keratin proteins will confirm the dual composition of these tumors, cell populations containing simple keratins being interspersed with those showing the stratified epithelial keratins.

Verrucous Carcinoma

Histological features [Figures 1f and 2a, b][1,3]

A. Marked epithelial proliferation with downgrowth of the epithelium into the connective tissue but usually without a pattern of true invasion
B. The epithelium is well differentiated and shows little mitotic activity, pleomorphism, or hyperchromatism
C. Characteristically, cleft-like spaces lined by a thick layer of parakeratin extend from the surface deeply into the lesion
D. Parakeratin plugging also occurs extending into the epithelium. The parakeratin lining the clefts with the parakeratin plugging is the hallmark of verrucous carcinoma
E. Even though the lesion may be very extensive, the basement membrane will often appear intact
F. When the lesions become infected, focal intraepithelial abscesses are often seen
G. Significant chronic inflammatory cell infiltration in the underlying connective tissue may or may not be present
H. The most conspicuous feature is the highly acantholytic epithelium with broad, bulbous rete processes described as elephant foot profile
I. There is no infiltration of the submucosa and the basement membrane remaining intact.
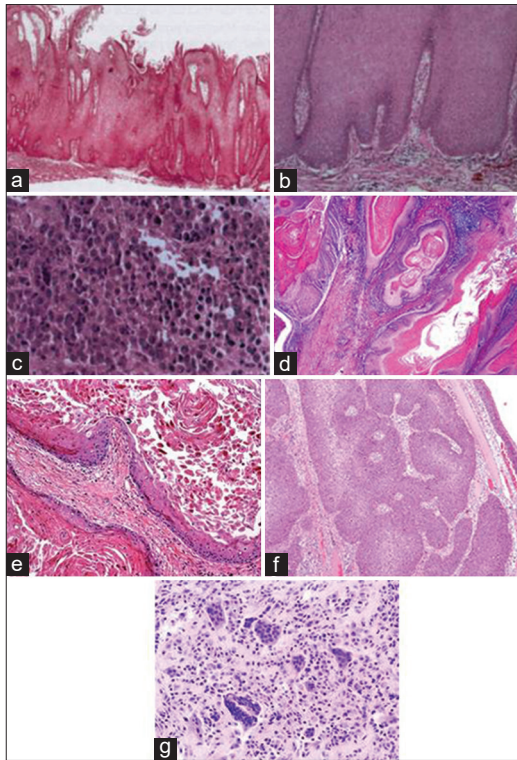
Figure 2: (a – g) Histopathology of variants of squamous cell carcinoma

Figure 3: Biomarkers for squamous cell carcinoma

NASOPHARYNGEAL CARCINOMA

Histologic features [Figure 2c]

1. Three types of nasopharyngeal carcinoma are recognized nonhistological appearance
   - Well differentiated SCC
   - Nonkeratinizing carcinoma
   - Undifferentiated carcinoma
2. Serum antibodies to Epstein–Barr virus antigens can be used to confirm the diagnosis and monitor the progress of the disease
3. The differentiated diagnosis among malignancies relies heavily on ICC to demonstrate cytokeratin in the tumor cells. An antibody to CD 45 (leukocyte common antigen) should be included to exclude lymphoma and anti– desmin and anti–myoglobin to exclude rhabdomyosarcoma diagnosis of which may be suggested by the apparent presence of syncytial structures
4. Treatment for the undifferentiated type carries the best prognosis as it is the most radiosensitive, the keratinizing type being the least so
5. Surgery is rarely an option due to inaccessibility.

CARCINOMA CUNICULATUM

Histologic features [Figure 2d and e][2]

A. Keratinizing, endophytic, and variably exophytic epithelial mass with a cohesive rete pattern invaginated into the tissues
B. If bone is invaded, the epithelium extends directly to bone at the deep invasive surface and is separated from it by only a thin layer of tissue and numerous osteoclast
C. Overall appearance– well-differentiated para or ortho keratinizing epithelium with frequent keratin pearls and clearly defined basal and prickle cell
D. A particular and diagnosis characteristic is the presence of deep and often complex branching keratin-filled crypts– named cuniculus (Rabbit warren) – lined by well-differentiated stratified squamous epithelium
E. Microabscess formation is common
F. Mitoses are frequently seen in basal and parabasal layers
G. Prominent host response of plasma cells and lymphocytes with the variable number of neutrophils and eosinophils
H. Patterns of spread are variations on direct invasion through bone especially but also through soft tissue
I. Regional lymph nodes are likely to show reactive enlargement but no metastatic spread.

PAPILLARY SQUAMOUS CARCINOMA

Histologic features [Figure 2f][2]

- There is a papillomatous surface and dysplasia with a papilloma-like structure of branching fronds with fibrous cores but no surface keratinization
- Rete pattern differentiates the lesion from Verrucous carcinoma
- Some authors consider exophytic lesion a papillary carcinoma or some need an invasive component

- Dysplastic lesions show the histological atypia found in other oral dysplastic lesions and have a primarily exophytic architecture of projections on papilloma like vascular cores of connective tissue
- When the invasion is found, the exophytic dysplastic component is generally considered to represent a preexisting state and the lesion should be diagnosed as conventional SCC.

Giant Cell (Pleomorphic) Carcinoma

Giant cell carcinoma identical to that recognized in the lung may occur in the upper respiratory tract or oropharynx and occasionally in the mouth ususally posteriorly. Malignant multinucleated giant cells of osteoclast-like size lie in the dyscohesive sheets of pleomorphic mononuclear cells. No squamous differentiation has been evident there may be a spindle cells component.

Immunocytochemistry may be required to confidently exclude melanoma, lymphoma, and sarcoma. These pleomorphic carcinomas can be very infiltrative and carry a poor prognosis. Metastatic pleomorphic carcinoma must be excluded before the diagnosis can be finalized and although lung, bladder, thyroid, and pancreas are the most likely primarily sites, similar carcinomas may arise almost anywhere[2] [Figure 2g].

Pseudocarcinomatous Hyperplasia and Lesions[2]

Pseudocarcinomatous hyperplasia is seen in a number of oral lesions and on occasion causes extreme difficulty in diagnosis. The oral lesions classically associated with pseudocarcinomatous hyperplasia are the Granular cell tumor and Necrotising sialometaplasia. The most common causes of oral pesudocarcinomatous hyperplasia are: chronic inflammation and trauma from teeth or dentures.

Discoid lupus erythematosus is accompanied by rete hyperplasia which amounts to pseudocarcinomatous hyperplasia and any atypia. A florid rete hyperplasia is seen in oral blastomycosis which usually affects the gingiva, with features identical to the skin lesion. Sometimes a localized area of pseudocarcinomatous hyperplasia results from low-grade chronic trauma. In the mouth, the causes of trauma are mucosa overlying superficially buried tooth roots, denture trauma, foci of infection such as small sequestra, and the margins of chronic traumatic ulcers.

Occasional localized lesions without apparent cause are reported in the literature as primary pseudocarcinomatous hyperplasia: These may be an intraoral equivalent of the keratoacanthoma or inverted papilloma.

Carcinogen Metabolizing Enzyme[4]

1. Alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde, which is cytotoxic and results in the production of free radicals and DNA hydroxylated bases; ADH type 3 genotypes appear predisposed to oral SCC

2. Cytochrome p450 can activate many environmental procarcinogens. Ethanol is also metabolized, to some extent, by cytochrome p450 IIEI to acetaldehyde. Mutations in some tumor suppressor genes may be related to cytochrome p450 genotypes and predispose to oral SCC
3. Glutathione S transferase (GST) genotypes may have impaired activity; for example, the null genotype of GSTM1 has a decreased capacity to detoxify tobacco carcinogens. Some GSTM1 and GSTP1 polymorphic genotypes and GSTM1 and GSTT1 null genotypes have been shown to predispose to oral SCC
4. N-acetyltransferases NAT1 and NAT2 acetylate procarcinogens. N-acetyl transferase NAT1*10 genotypes may be a genetic determinant of oral SCC, at least in some populations
5. DNA repair genes are clearly involved in the pathogenesis of some rare cancers such as are seen in xeroderma pigmentosum but, more recently, evidence of defective DNA repair has also been found to underlie some oral SCC. Immune defects may predispose to oral squamous cell carcinoma (OSCC), especially lip cancer.

Prognostic Factors in Oral Squamous Cell Carcinoma

Clinical parameters[5,6]

Age and sex

Age and sex were reportedly not associated with the survival rate in OSCC. Few cohort studies imply a lower survival rate in men, especially in the early stage of tongue tumors and patients younger than 50.

Tobacco and alcohol

Most authors stated a higher survival rate in nonsmokers and nondrinkers, but there is no difference between ever smokers and current smokers.

Socioeconomic status

Socioeconomic conditions. Apparently, the outcome is somewhat worse for patients with lower socioeconomic status and education, most likely because of poorer oral hygiene and more difficult access to medical care.

Anatomic site

Vascular and lymphatic networks, which vary between different anatomic sites, may influence tumor evolution and the outcome. Higher metastatic disease rates for SCC at the base rather than at the oral tongue have been reported. Some anatomic sites are linked with poorer outcomes owing to the rich lymphatic drainage and the local extension being hard to evaluate and manage, such as the superior gingivolabial sulcus.

Tumor thickness

The risk of nodal metastases and mortality rates vary directly with the thickness of the primary tumor. P O-charoenrat *et al* (2003). found that tumor thickness above 5 mm is a strong

predictor of occult nodal metastases and should indicate an elective neck dissection. There is evidence that tumor thickness may exercise more influence on the survival rates than factors such as clinical and pathologic staging.

Tumor staging

Staging of OSCC is performed with the use of its variant pathological tumor-node-metastasis, which are based on clinical and pathologic evaluation of tumor size and lymph node involvement. Most authors believe that the most significant factors influencing survival rate is tumor staging "by assessing the primary tumor size and cervical lymph node status." It should be noted that TNM staging alone cannot predict prognosis.

Loco-regional recurrence

Loco-regional recurrence (LRR) rate in OSCC has been reported variable according to different researches, but it factors of survival rate. Type of treatment, the presence of lymphovascular permeation, and observation of malignant cells microscopically in muscles, excluding "the extrinsic muscles of the tongue, pterygoid, and master muscles" can affect LRR.

Lymph node metastasis

Several studies have indicated that the involvement of cervical lymph nodes in OSCC patients decreases significantly survival rates than those with disease-free nodes.

Diagnostic delays

It seems highly likely that diagnostic delays raise the probability of higher tumor growth and spread, consequently aggravating the prognosis. Another possible theory is that patients with more hostile tumors develop symptoms earlier, seeking medical attention sooner; nevertheless, these patients still have to face a more grievous outcome, because these malignancies display a more aggressive biologic behavior.

Miscellaneous

Comorbid conditions may worsen disease outcomes as a consequence of increased organic stress afflicting the patient. Survival rates are lower in patients with concomitant OSCC and disorders such as congestive heart failure, arrhythmias, peripheral vascular disease, pulmonary and renal diseases, and other cancers, either treated or untreated. Depressed host immune status seems to play an adverse role in the survival of patients with oral cancer. Patients under immunosuppressive therapy following solid organ transplant who developed OSCC fare worse than individuals with a less depressed immune system.

Histological parameters[6,7]

Histologic grading

The histologic grading is used to predict the clinical behavior of OSCC for many decades, but its prognostic value is still controversial. Different histologic grading systems such as Broder's, Anneroth's, Bryne's, and Jakobsson's are used. The degree of cell differentiation, keratinization, and pattern of invasion correlate with survival rate among OSCC patients.

Perineural invasion

Perineural invasion correlates with larger tumor size, higher depth of tumor invasion, risk of nodal metastasis and lower 5-year survival rates in patient with OSCC.

Surgical margins

Pathologic positive margin has been proven to be an adverse prognostic factor for OSCC patients, which apparently correlates with local recurrence and overall survival.

Extracapsular spread and depth of invasion

Extracapsular spread is defined as an extranodal extension of metastatic deposit outside the lymph node capsule. The tumor thickness <10 mm is an independent prognostic factor for increasing overall survival and disease-specific survival.

Angiogenesis

Malignancies have the ability to induce the growth of new blood vessels, which is important for tumor progression, aggressiveness, and the ability to metastasize. It is a highly regulated and complex process. Most authors assess tumor angiogenesis by counting the number of blood vessels (microvessel density, microvessel density) in tissue sections. Marked angiogenesis correlated well with the risk of nodal metastases and should probably imply a more aggressive postoperative adjuvant therapy. Moreover, OSCC angiogenesis correlates with T and N parameters and is an independent predictor of tumor recurrence and a reliable prognosticator. The vascular endothelial growth factor plays a decisive role in the development of blood vessels; it is a key component in tumor angiogenesis.

Invasive tumor front

The invasive tumor front (ITF) has been defined as the most progressed, three to six tumor cell layers or detached tumor cell groups at the advancing edge of OSCC. Loss of cohesion and marked anaplasia is usually observed at the ITF in comparison with other parts of the tumor. The ITF can be used as a prognostic indicator as it is believed to indicate the tumors' invasive and metastatic capacity.

The tumor host interface or invasive front is the site for molecular events such as increased proliferation, neoangiogenesis, and loss of adhesion molecules. The ITF is composed of aggressive cells with maximal cellular atypia. The biological aggressiveness of a tumor is well defined at the ITF. Regarding the difference in opinion among pathologists in the grading of OSCC, the differentiation at the ITF is considered.

The prognosis of the tumor is reflected best in the ITF compared with any other region of the tumor. The ITF must be considered as a part of staging systems to be more accurately assess OSCC in terms of treatment planning and recurrence.

Genetic alterations and molecular biomarkers of oral squamous cell carcinoma[5,6]

Genetic alterations

OSCC, like most other malignancies, arises from the accumulation of a number of discrete genetic events that

lead to invasive cancer. These changes occur in genes that encode for proteins, which control the cell cycle, cell survival, cell migration, and angiogenesis. Significant alteration in OSCC, most frequently in chromosome 9, chromosome 17 gene as well as 3P, 13q21 and 18q21 significant correlation between hypermethylation of TP73, PIK3R5 and CELSR3,42 downregulation of MYC,43 SMAD3/TGFBR2 genes mutation, amplification of cyclin D 1 genes and survival rate in OSCC patients have been reported [Figure 3].

Molecular biomarkers

As previously mentioned, many different proteins, which are genes products, control cell cycle and cell proliferation. Dysregulation and pathological imbalance of these proteins may lead to tumorigenesis. These proteins can be detected by various methods such as immunohistochemistry staining, polymerase chain reaction (PCR), reverse transcriptase PCR, proteomics analysis, and Western blot.

Tumor expression of cyclooxygenase-2

Recently, a strong correlation was found in OSCC between high cyclooxygenase-2 (COX-2) expression and higher lymph node involvement, higher recurrence rates, and shorter disease-free survival. Marked COX-2 expression was found in the cytoplasm of cells of the tumor invasive front and also in the cells of the surrounding stroma and vessels, indicating a putative role in tumor invasion and development of metastases. COX-2 overexpression in OSCC was associated with higher radioresistance; tumor cells treated *in vitro* with a COX-2 inhibitor showed a better response to radiotherapy.

MUC 4

Mucins are membrane-bound or membrane-secreted glycoproteins expressed in epithelial cells. They contain a polymorphic central domain that comprises a variable number of tandem repeats, a hallmark of the mucin family. To date, 21 different mucins have been found in humans, and these are functionally classified into two major categories: secreted (MUC2, MUC5AC, MUC5B, MUC6-MUC8, and MUC19) and membrane-bound (MUC1, MUC3, MUC4, MUC12, MUC13, MUC15-MUC17, MUC20, and MUC21). These mucins serve to protect normal epithelial tissue throughout the body. In addition, mucins are involved in the differentiation and renewal of the epithelium and modulation of cell adhesion, immune response, and cell signaling. MUC4 is a membrane-bound mucin encoded by a gene on chromosome 3q29.6 The largest and most distinct feature of MUC4 is its extracellular tandem repeat domain. MUC4 expression is related to aggressive tumor behavior and a poor outcome in patients with intrahepatic cholangiocarcinoma, invasive ductal carcinoma of the pancreas, extrahepatic bile duct carcinoma, and lung adenocarcinoma.

The dysfunction of these regulations of MUC4 expression such as epigenetic alterations and alternative splicing might be a candidate for a molecular marker and a therapeutic target of OSCC in the future. The expression of MUC4 is a novel independent poor prognostic factor in OSCC. Therefore, MUC4 is a useful marker for choosing therapy and for predicting the poor outcome of patients with OSCC. Patients with OSCC who show positive MUC4 expression should be followed-up carefully. In addition, MUC4 might be a strong candidate to target of future therapy in OSCC.

Miscellaneous

Uridine phosphorylase (UPase) is an enzyme that catalyzes the phosphorolysis of uridine to uracil. Its expression and activity are increased in solid tumors, including head and neck tumors. Positive UPase marking correlates well with lymphatic metastases, but not with tumor size or location, histologic differentiation, or global survival. Protein S100A4 (involved in the mobility of cancer cells) has been associated with strong tumor invasiveness and a higher probability of nodal metastasis. The expression of the glucose transporter Glut-1 was studied in OSCC, as well as tumor glucose metabolism. High levels of both parameters correlated with significantly shorter survival, which is consistent with the notion that tumors with aggressive biologic behavior present higher metabolic levels.

Lymphocytic infiltration surrounding invasive carcinomas is composed mainly of T lymphocytes and represents a true immune response. The prognosis seems better for marked infiltrations than for smaller ones. A strong association between OSCC autofluorescence (emitted by pigments of the porphyrin group) and categories T and N has been found; therefore, autofluorescence may be an indicator of tumor progression and, eventually, prognosis.

HUMAN PAPILLOMAVIRUS

There is escalating evidence of a causal association between human papillomavirus (HPV) and OSCC, 82–93 with several studies showing that HPV is associated with increased risk of oral cancer, independently of exposure to tobacco and alcohol. This association is valid for high-risk HPV, which comprises subtypes 16, 18, 33, and 35. HPV-16 may be responsible for more than 80% of HPV-positive OSCC. The virus can be detected in tissues or cells using several methods, namely, biochemical, immunologic, microscopic, and molecular. PCR is considered the most sensitive assay.

p53 remains the most commonly mutated gene in many common human cancers, but in a high proportion of cases lacking mutations, its function is compromised by other mechanisms. Regarding HPV, this may occur via interaction of p53 with protein E6 encoded by the oncogenic HPV types, mainly HPV-16 and HPV-18, which results in increased ubiquitin-dependent proteolysis of p53. The expression of wild-type p53 in HPV-positive tumors contrasts with the higher frequency of p53 mutation in HPV-negative cases, and this fact seems to be associated with a better prognosis in patients with HPV-positive OSSC.

The presence of HPV DNA in a significant fraction of OSCC raised the question of whether HPV tumor status affects the outcome of oral cancer. A large study conducted has shown that patients with HPV-positive tumors had a 60% reduction in risk of death from cancer and significantly improved disease-specific survival when compared patients with HPV-negative Tumors. These findings support the theory that HPV-positive OSSC may represent a distinct molecular, biologic, and clinical identity, supposedly associated in causal terms with HPV infection and possibly carrying better prognosis than HPV-negative cases.

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Conflicts of interest

There are no conflicts of interest.

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