**Abstract.** Oral carcinogenesis is a multifactorial process involving numerous genetic events that alter normal functions of oncogenes and tumor suppressor genes. This may increase the production of growth factors or the number of receptors on the cell surface, and/or increase transcription factors or intracellular signal messengers. Together with the loss of tumor suppressor activity, these changes lead to a cell phenotype that can increase cell proliferation, with loss of cell cohesion, and infiltration of adjacent tissue thus causing distant metastasis. Molecular pathology is responsible for defining the molecular mechanisms that underlie the onset of oral precancer and cancer. The aim of this review is to describe recent advances in our understanding of the molecular control of the innumerable pathways related to these processes. These may lead to short- or medium term improvements in the diagnosis and prognosis of oral precancerous and cancerous lesions and to the development of novel therapeutic approaches to this disease.

Head and neck cancer (HNC) is the sixth most common human cancer (1), representing 3% of all types of cancer. They are located in the oral cavity in 48% of cases, and 90% of these are oral squamous cell carcinomas (OSCC) (2), which are sometimes preceded by precancerous lesions, e.g. leukoplakia, erythroplakia, actinic cheilitis and palatal keratosis associated with inverted smoking (3). An increase in oral cancer prevalence among young adults is a cause of special concern. There has been a 60% increase in the number of under 40-year-olds with tongue cancer over the past 30 years. However, few data have been published on the aetiology and natural history of oral cancer. Because more than half of patients have not been exposed to the carcinogens usually implicated in the disease, e.g. alcohol or tobacco, other risk factors have been sought and proposed. These include genetic predisposition, immunodeficiency, diet and viral infections, e.g. HPV (human papillomavirus) and HHV (human herpes virus). Moreover, it has been claimed that these cases show a higher aggressiveness and worse prognosis (4).

Oral precancerous lesions are usually histologically classified by the presence or absence of oral epithelial dysplasia (OED), categorised by the WHO (2003) as slight, moderate, severe hyperplasia or carcinoma in situ according to the presence and severity of cell atypia and other structural aspects of the epithelium. This proposal was incorporated into a new WHO classification of head and neck tumours published in 2005 (5). This histopathological grading of OED was designed to assist prediction of the malignant transformation of these lesions. Nevertheless, no objective methods are yet available to typify dysplastic lesions and allow consistent and reproducible results to be obtained (6), since not all oral lesions with OED progress to OSCC. Moreover, the utility of this subdivision of OED has been questioned, since wide inter- and intra observer variability has been observed in assessments of the presence/absence and degree of this condition (7). A binary OED classification method was recently described, dividing them between lesions at low and high risk of malignant transformation. This could be used as a complementary method to improve the sensitivity and specificity offered by the 2005 system in current use (6).

Oral carcinogenesis is a highly complex multifactorial process that takes place when epithelial cells are affected...
by several genetic alterations. The use of molecular biology techniques to diagnose oral precancerous and cancerous lesions may markedly improve the detection of alterations that are invisible under the microscope. This would aid identification of patients at a higher risk of developing oral cancer (8). Despite advances in the treatment of oral cancer, the survival of these patients remains very low, mainly due to their high risk of developing a second cancer. (1).

This review presents up-to-date evidence on molecular markers that have shown promising results in predicting the progression of oral precancerous disease and evaluating the efficacy of oral cancer treatments, with the potential to markedly increase the patient survival rate. Reference is also made to studies of head and neck cancer when it is not specified whether or not they are OSCCs.

**Cytogenetic and Epigenetic Alterations in Oral Cancer**

*Chromosomal instability*. The most widely used molecular approach to the loss of genetic material is the study of loss of heterozygosity (LOH) based on the detection of differences between normal maternal or paternal alleles in constitutional (normal) DNA and their homonyms in tumour tissue. When a multicellular organism inherits a mutation in an allele of a tumour suppressor gene, it is considered a heterozygote of that gene, *i.e.* it possesses a mutated (mutated allele) and a normal (normal allele) copy. When a somatic cell with this inherited mutation has a second mutation in the other allele of the suppressor gene, they are both mutated and there is a complete loss of normal protein function, a lack of suppressor function. This is a case of LOH, since both alleles are mutated and the cell or cells that derive from them will therefore be homozygotes (both alleles mutated).

Alterations in the length of short repetitive sequences of DNA (one or two nucleotides) are described by the term ‘microsatellite instability’ (MSI). MSI results from the functional failure of DNA repair genes, which are designated ‘mismatch repair’ (MMR) genes. MSI can be detected by comparing the selective amplification of microsatellite loci in DNA from paraffin-embedded tumour samples with that in DNA from normal tissue. Alterations in these regions have been used as clonality markers and to detect tumour cells among normal cells (9, 10).

Various researchers using microsatellite markers demonstrated that alterations in certain regions of chromosomes 3p, 9p, 17p and 18q are related to the development of HNC (11). Numerous studies have identified specific genetic alterations in OSCCs and oral precancerous lesions (Table 1). Bockmuhl et al., using comparative genomic hybridization, found deletions of chromosomes 3p, 5q and 9p with 3q gain in well-differentiated HNC versus deletions of 4q, 8p, 11q, 13q, 18q and 21q and gains in 1p, 11q, 13, 19 and 22q in poorly differentiated HNC, indicating a relationship with tumour progression (12).

Researchers using novel molecular techniques, *e.g.* microsatellite and restriction fragment length polymorphism (RFLP) tests, found the most common genetic change to be loss of the 9p21-22 chromosomal region (containing gene *p16*[^1]/*p14ARF*/*CDKN2A*[^2]*), an early event in 70% of all HNCs (1, 11, 13). *P16* inhibits CDK2 and CDK6, preventing initiation of the cell cycle and producing non-phosphorylation of pRb, causing cell cycle arrest in G1. El Naggar et al. found LOH in 9p21-p22 in 72% of HNCs in a preinvasive and/or invasive state (14).

Loss of the 3p region is another early cytogenetic change in oral carcinogenesis, with even benign hyperplastic lesions (the earliest precancerous lesions) showing loss of 9p21 or 3p in 30% of cases (13). Allelic losses in 13q and 8p are more frequent in OSCC than in OED (15).

Amplification of 11q13 has been observed in around one-third of head and neck cancers. This region contains the proto-oncogene cyclin *D1*/*PRAD1*/*CCD1*, whose function is to activate pRb by phosphorylation, facilitating progression of the cell cycle from the G1 (growth) to the S (synthesis) phase. Hence, D1 cyclin activation and *p16*[^1]*[^2]* inactivation have the same effect, *i.e.* an increase in pRb phosphorylation and progression from G1 to S in the cell cycle (13).

Loss of the 17p13 chromosomal region is considered a late effect in the progression of HNC. This region contains gene *p53*, which was mutated in 79% of 123 HNCs analysed by Balz et al. (16). Figure 1 depicts the genetic alterations most frequently observed during progression of these lesions.

A retrospective study by Mao et al. demonstrated that precancerous lesions with loss of 9p21 and 3p14 chromosomes are more prevalent in patients who subsequently develop a HNC (17). Another study of patients with benign or precancerous lesions with OED showed that those with losses of only 3p and 9p had a 3.8-fold higher risk of cancer progression, whereas those with losses of 4q, 8p, 11q and 17p had a 33-fold higher risk (18).

Gain of DNA content by aneuploidy may also be a predictor of cancer in precancerous lesions, since aneuploid oral leukoplakia lesions show higher rates of malignant progression to OSCC (13). A controversial study by Subdo et al. of 150 patients with dysplastic leukoplakias found that 70% were low-risk diploid lesions (3% progressed to OSCC), 13% were intermediate-risk tetraploid lesions (60% progressed to OSCC) and 17% were high-risk aneuploid lesions (84% progressed to OSCC). Nevertheless, these data must be verified by other research groups, since they were subsequently rejected by the editorial committee of the journal to which they were published (19).
Partridge et al. identified significantly higher allelic imbalances in TNM stage 4 patients than in patients with stages 1-3. The mortality rate was 25-fold higher in patients with allelic imbalance at one or more loci of 3p24-26, 3p21, 3p13 and 9p21 than in patients with heterozygosity at these loci (20).

Another study reported that intraoral OEDs at sites carrying a high risk of developing OSCC (e.g., floor of mouth, ventrolateral area of tongue and soft palate) had a higher LOH frequency and a loss pattern associated with an increased risk of malignant progression (21).

Epigenetic alterations. There is considerable interest in the role of epigenetic alterations in cancer, explaining their separate consideration in this review. These alterations affect or inactivate gene function by methylation of the promoter region, without changing the structure or sequence of the gene. Methylation is an epigenetic modification by which gene activity is controlled by addition of methyl groups (CH₃) to certain DNA cytosines (22). Most methylations occur in cytosines of CpG nucleotides (cytosine and guanine separated by a phosphate) and are present in a normal manner in promoter regions of certain genes. CpG islands act directly by inhibiting the binding of transcription factors to the DNA or by recruiting proteins that activate histone deacetylases (HDAC), which contain co-repressor complexes additional to DNA methylation. This phenomenon is the main epigenetic modification in humans and methylation pattern changes may play a very important role in carcinogenesis (23) because they are frequently related to the loss of gene expression. They also appear to be essential for the occurrence of the multiple genetic events required for tumour progression, since they can inactivate DNA-repair genes. Both hypermethylation (by suppressor gene inactivation) and hypomethylation (by inappropriate oncogene activation) can produce carcinogenesis (24).

Rosas et al. (23) used a methylation-specific polymerase chain reaction (PCR) to study methylation patterns of cyclin-dependent kinase 2A/p16 (CDK N 2A/p16 INK 4a), methylguanine-DNA methyltransferase (MGMT) and death-associated protein kinase (DAP-K) genes in saliva of HNC patients, detecting abnormal hypermethylation patterns in all three. They reported that this PCR technique offers effective and sensitive tumour DNA detection and is potentially useful to detect and follow up recurrences in these patients.

Apparently histologically normal tissue adjacent to tumours (25-27) and precancerous lesions (28) may also have high levels of methylation of some genes, suggesting that methylation is an early event in oral carcinogenesis (Figure 1). It has been proposed that a more sensitive evaluation of tumour resection margins might be achieved by studying methylation than by determining histological alterations (29).

Although research into gene methylation in oral cavity tumours is still relatively recent, four important genes have already been analysed in detail: CDKN2A/p16INK4a, CDH1 (Cadherin-1), MGMT and DAPK1 (22, 30).

Gene methylation panels may be useful in oral cancer screening. Ogi et al. detected promoter methylation in at least 1 out of 12 genes in 67 out of 96 OSCCs (31). Viswanathan et al. used 5 hypermethylated genes (p16INK4a, p15INK4b, human multIL homolog 1 (hMLH1), MGMT and E-cadherin) and detected OSCC in 38 out of 51 Indian patients (32). These studies show that a panel of specific markers can be designed.

Figure 1. Sequence of genetic and tumor/stroma alterations implicated in origin of oral cancer. [Modified from Califano, 1996 (11)].
to characterize and detect oral cavity tumours. In this context, E-cadherin hypermethylation has been associated with a more severe histological grade and worse survival rates (22).

Sánchez-Cespedes et al. were the first to use the methylation state as a clinical marker, using methylation-specific PCR to analyse 4 markers (CDKN2A, MGMT, DAPK and glutathione S-transferase Pi gene (GSTP1)) in sera from 95 patients with primary HNC. At least one gene was methylated in 52 out of 95 patients and 42% of serum samples analysed (21/50) showed the corresponding methylation pattern; DAPK promoter hypermethylation was observed in 27% of cases. The authors proposed that this serum analysis may be a viable method to determine tumour recurrence in treated patients (33).

Rosas et al. studied saliva samples from 30 patients with HNC, using methylation-specific PCR with the markers CDKN2A, MGMT and DAPK. At least one gene was methylated in 17 out of the 30 primary tumour samples, and 11 of the 17 corresponding saliva samples showed the same methylation pattern. There appears to be a higher probability of tumour detection in the saliva of patients with oral cavity tumours than in the saliva of those with tumours distant from the aerodigestive tract (23).

López et al. used methylation-specific PCR to analyse the methylation state of genes p16\(^{\text{NK4a}}\), p14\(^{\text{ARF}}\) and MGMT in saliva samples from 34 patients with oral leukoplakia lesions; methylation of p16\(^{\text{NK4a}}\), MGMT and p14\(^{\text{ARF}}\) was observed in 44%, 56% and 1% of samples, respectively. These findings demonstrate that methylation is an early event in oral carcinogenesis and that its study may be useful to detect precancerous lesions (Figure 1). Screening studies of general populations are required to assess its applicability as a diagnostic method for suspicious oral lesions (34).

Hypermethylation of the cell cycle-regulating gene promoters p16\(^{\text{NK4a}}\) and p15\(^{\text{NK4b}}\) was found to be common in OSCC (p16: 76%; p15: 30%), although no significant correlation was observed with clinico-pathological characteristics or with the prognosis (22, 33).

To date, the rapid histopathological study of fresh tissue has been considered adequate for the intraoperative assessment of surgical safety margins. Some researchers have investigated the molecular analysis of margins but none have addressed its feasibility. Goldenberg et al., using methylation-specific PCR, assessed margins during surgery by analysing the methylation of CDKN2A and MGMT. They concluded that this method can be intraoperatively applied and represents a new and feasible application of molecular analysis to the surgical treatment of oral cancer (35).

Hypermethylation can be reversed by means of agents such as 5-azacytidine (5AC). This treatment has proven to be clinically and biologically effective in malignant lung tumours and HNCs, associated with a decreased hypermethylation of p15\(^{\text{NK4b}}\), or p16\(^{\text{NK4a}}\) in the case of HNC. The main drawback is the toxicity of these agents (22). HDAC inhibitors have been used for haematological lesions and, in clinical trials, for solid tumours. In laboratory experiments, the combined application of 5AC and HDAC inhibitors has shown a promising synergistic effect (22).

### Table I. Common genetic alterations in Head and Neck Carcinoma (HNC) (Modified from Kim and Califano, 2004).

<table>
<thead>
<tr>
<th>Location and/or gene</th>
<th>Type of alteration and frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9p21-22/p16(^{\text{NK4a}})/p14(^{\text{ARF}})</td>
<td>Loss of heterozygosity (LOH) in 70%</td>
<td>Califano, 1996 (11); El Naggar, 1995 (14)</td>
</tr>
<tr>
<td>3p21/ RASSF1A</td>
<td>30% LOH in precancerous lesions</td>
<td>Kim and Califano, 2004 (13)</td>
</tr>
<tr>
<td>11q13/(PRAD-1/Cyclin D1/hst-1/int-2)</td>
<td>Amplification in &gt;50% of HNCs</td>
<td>Kim and Califano, 2004 (13); Balz, 2003 (16)</td>
</tr>
<tr>
<td>9p21/p15</td>
<td>Hypermethylation in 30% of OSCCs</td>
<td>Shaw, 2006 (22)</td>
</tr>
<tr>
<td>9p21/p16 (CDKN2A)</td>
<td>Hypermethylation in 76% of OSCCs</td>
<td>Sanchez-Cespedes, 2000 (33); Ha and Califano, 2006 (30)</td>
</tr>
<tr>
<td>EGFR</td>
<td>Alteration of pathway by overexpression or other mechanism in 90%</td>
<td>Grandis, 1993 (41)</td>
</tr>
<tr>
<td></td>
<td>Amplification in 30% of OSCCs</td>
<td>Scully, 1993 (42)</td>
</tr>
<tr>
<td>H-ras</td>
<td>Mutation in 55% of lip cancers</td>
<td>Milasin, 1994 (45)</td>
</tr>
<tr>
<td></td>
<td>Mutation in 35% of OSCCs</td>
<td>Kuo, 1994 (46)</td>
</tr>
<tr>
<td>17p13/p53</td>
<td>Mutation in 79% of HNCs</td>
<td>Balz, 2003 (16)</td>
</tr>
<tr>
<td></td>
<td>Overexpression in 47% of precancerous lesions</td>
<td>Warnafulusariya, 1998 (7)</td>
</tr>
<tr>
<td>19q34/DAPK</td>
<td>Hypermethylation in 27% of sera from HNC patients</td>
<td>Sanchez-Cespedes, 2000 (33)</td>
</tr>
<tr>
<td>10q26/MGMT</td>
<td>Hypermethylation in 56% of saliva samples</td>
<td>Lopez, 2003 (34)</td>
</tr>
<tr>
<td></td>
<td>Hypermethylation in 7-86% of primary tumours</td>
<td>Ha and Califano, 2006 (30)</td>
</tr>
</tbody>
</table>

HNC: Head and neck carcinoma; RASSF1A: Ras association domain family 1A.
and the basement membrane. Matrix metalloproteinases (MMPs) form a family of zinc dependent endopeptidases that appear to play a major role in ECM degradation and in tumour invasion and metastasis (13). MMP-2, MMP-9 and MMP-3 have all been found to be related to tumour invasion, a more aggressive growth and a worse prognosis. Expression of these genes could be used as tumour progression marker (1).

Stromelysin 3, MMP11, was initially reported due to its overexpression in primary breast carcinoma. Its expression in hyperplastic and dysplastic oral lesions suggests its association with phenotypic alterations acquired early during the malignant transformation of oral epithelium (13). Soni et al. recently demonstrated that expression of stromelysin 3 is an early event in oral mucosa (36). They immunohistochemically analysed stromelysin 3, erythroblastosis virus E26 oncogene homolog 1 (Ets-1) and vascular endothelial growth factor (VEGF) in 220 OSCCs, 90 precancerous lesions (59 hyperplasias and 31 dysplasias) and 81 normal oral mucosa samples. They found that the combined expression of stromelysin 3 and Ets-1 favours development of a precancerous state and that combined expression of stromelysin 3 and VEGF is related to progression from precancer to cancer (Figure 1). They concluded that VEGF expression is an adverse prognostic factor for patient survival in oral cancer (36).

E-cadherin is a 120 kDa transmembrane glycoprotein that participates in calcium-mediated cell adhesion. The loss or reduction of E-cadherin-mediated cell adhesion is an important step in the development of invasion and metastasis in numerous carcinomas, including OSCCs (37). Santos-García et al. demonstrated that loss of E-cadherin expression is an early event, being detected in moderate dysplasias, and is greater in carcinomas in situ and microinvasive carcinomas, suggesting that loss of epithelial cohesion may be an indicator of progression in these lesions (Figure 1). Thus, a marked and similar loss of expression was observed in OSCCs and lymph-node metastases, which increased with progression of the disease (38).

Another important mechanism for acquiring an invasive phenotype is anoikis resistance, defined as the ability to escape apoptosis by cell separation from the basement membrane, cell morphology changes and keratin accumulation. Swan et al. found that acquisition of anoikis resistance may be a prerequisite for the development of an invasive tumour phenotype and implies a worse prognosis in oral cancer (39).

Kupferman et al. recently used real-time RT-PCR to establish differences in the expression of genes S100P, KLK6 and CTNNALI between anoikis-resistant and anoikis-sensitive cell lines. The anoikis-resistant phenotype of OSCC has a distinct gene expression pattern, marked by chromosomal alterations that may contribute towards a differential expression of genes involved in various cellular functions. These mediators of anoikis resistance may serve as therapeutic target in cases of OSCC metastasis (40).

Oncogenes

Oncogenes are growth regulating and promoting genes that control transduction pathways of cell signals. Therefore, a mutation of these genes can lead to an overproduction or an increase in the function of the stimulatory proteins. Although isolated oncogenes are not able to transform normal epithelial cells, they appear to be important initiators of the process and are known to produce cell changes with the mutation of one copy of the gene (9).

Numerous oncogenes have been implicated in oral carcinogenesis. Abrupt expression of proto-oncogene epidermal growth factor receptor, (EGFR/c-erb 1) and members of gene families ras, c-myc, int-2/Fgf-3 (fibroblast growth factor-3), hst-1/HSTF1 (heparin-binding secretory transforming factor 1), PRAD-1 (parathyroid adenomatosis 1), and bcl-1(B-cell leukemialymphoma 1) is believed to contribute to cancer development (1).

Abrupt expression of transforming growth factor-α (TGF-α) is usually an early event in carcinogenesis. It is first observed in hyperplastic epithelium and then in OSCC, in the infiltrate of inflammatory cells around the infiltrating epithelium, especially eosinophils. TGF-α stimulates cell proliferation by autocrine and paracrine binding to EGFR (1). It is also believed to stimulate angiogenesis and has been described in ‘normal’ oral mucosa of patients that subsequently develop a second primary (41). EGFR, the biological receptor of EGF and TGF-α, is frequently overexpressed in oral cancer (1) and was shown to result from EGFR gene amplification in 30% of oral cancers (42).

Epidermal growth factor (EGF) influences cell division, migration, adhesion, differentiation and apoptosis via the tyrosine-kinase pathway. Elevated expression of EGF is a predictor of recurrence and a worse prognosis in HNC (13). Expression of its transmembrane receptor (EGFR) has been detected by immunohistochemistry (IHC) in 40-80% of OSCC cases and has been proposed to have prognostic value in these patients (43). Numerous studies have found that EGFR has a negative prognostic value, with its expression implying a higher likelihood of post-treatment recurrence and worse survival (41).

An ICH study by Ralhan et al. found a lower expression of retinoid receptors RARβ, γ and RXRα and of cell cycle regulators p16 and p21 in most of 244 OSCCs and 102 potentially malignant lesions than in normal oral mucosa samples. In the multivariate analysis, phenotype RAR α+/p21− (+, RAR α accumulation; −, loss of p21) was related to a lower survival rate, demonstrating the prognostic value of the expression of retinoid receptors and their interaction with cell cycle regulators in oral tumorigenesis (44).

Some studies showed that members of the ras oncogene family are overexpressed in oral cancer. Although loss of control of N-ras may be an early step in oral carcinogenesis, with
increased expression in dysplastic lesions, ras mutations are infrequent in Western patients and detected in fewer than 5% of oral cancers (1). In contrast, 55% of lip cancers have H-ras mutation (45), which is also present at 35% of oral cancers in Asian populations in association with betel nut chewing (46).

Transcription factors that activate other genes are also activated in oral cancer. We highlight c-myc, which participates in the regulation of cell proliferation. This gene is frequently overexpressed in OSCC as a result of gene amplification, which is itself usually related to poorly differentiated tumours (1). C-Myc induces cell proliferation and, in combination with p53, apoptosis. Nuclear protein pRb of the pRb gene interacts with the c-myc gene, hindering its transcription and thereby inhibiting cell proliferation.

The gene PRAD-1(cyclin D1/Bcl-1) is located in 11q13 and encodes cyclin D, which, together with the product of the pRb gene, controls cell cycle transition from the G1 to the S phase. This gene is amplified in 30-50% of HNCs. Amplification of PRAD-1 has been correlated with cytological grade, infiltrative growth pattern and metastasis (1).

Researchers using fluorescent in situ hybridization (FISH) with tissue microarray (TMA) found different oncogene amplification patterns according to the localization of HNCs, with more frequent amplification of CCND1 in pharyngeal carcinomas and of ZNF217 in oral and laryngeal carcinomas. They concluded that their methodology was effective to assess molecular changes and their relevance for HNC aetiology and progression (47).

For Kuo et al., the classification of OSCC by gene expression is a future possibility. Using a microarray that analysed 4,324 genes, they reported 210 genes possibly related to oral cancer. They identified several genes with altered expression (e.g. CKS1, TSPY, CBK, TLE4 and BCHE) that had not previously been related to OSCC, although they had been associated with other types of cancer (46, 48).

Méndez et al. reported 239 overexpressed and 75 underexpressed genes in OSCC. However, they found no difference in gene expression between incipient and advanced stages, or between tumours with and without metastasis (49). In the future, we shall be able to use these gene expression patterns to obtain a unique molecular ‘fingerprint’ for each tumour (50).

Finally, amplification of int-2, also known as fibroblast growth factor-3 (Fgf-3), which stimulates proliferation of mesenchymal, epithelial and neuroectodermal cells, has been described in premalignant dysplastic and hyperplastic lesions of the oral cavity. This suggests that int-2 may be amplified before development of OSCC (1).

**Tumour Suppressor Genes**

Although oncogenes alone are not able to cause oral cancer, they appear to be initiators of the process. The crucial event in the transformation from premalignancy to malignancy is inactivation of negative cell regulators (tumour suppressor genes). Tumour suppressor genes are frequently inactivated by point mutations, deletions and rearrangements in both copies of the gene (1, 51).

Among the most widely studied tumour suppressor genes are rb and p53, which express pRb (retinoblastoma protein) and p53 protein respectively. These proteins control the cell cycle and are involved in the inhibition of cell proliferation. Mutations in these proteins produce uncontrolled cell proliferation. P53 acts as transcription factor of cell cycle inhibitors such as p21/Waf1/Cip1/Sdi1 and prevents the cell from going beyond phase G1 of the cell cycle, permitting DNA repair. If this is not possible, p53 induces apoptosis of these cells to avoid the transmission of potentially carcinogenic information. The p53 gene is inactivated in approximately half of HNC cases but there is debate about the timing of this change in oral carcinogenesis. Aberrant expression of p53 has been reported in mild dysplasias by some authors but in association with advanced tumours by others (11). Overexpression of protein p53 and gene mutation have also been detected in OED adjacent to OSCC lesions (1, 11, 52).

A meta-analysis of results published in seven studies showed that a much higher percentage (47%) of premalignant oral lesions had p53 overexpression than underwent malignant transformation (7). However, another study proposed that p53 expression above the basal layer of the epithelium indicates carcinoma development, even in the absence of epithelial dysplasia (53, 54). Nonetheless, given that p53 positivity is not observed in all lesions that undergo malignant transformation, it is recommended to take into account conventional histological parameters or introduce new markers that are currently under study.

Lee et al. found a relationship between p53 positivity and a higher risk of oral cancer, especially when combined with chromosome polysomy and LOH in chromosome 3p or 9p (54). Partridge et al. looked for p53 mutations, allelic imbalance of protein p53 and expression of mdm2 in 45 cases of OSCC and found allelic imbalance in 23% and p53 mutation in 33% of cases. This discrepancy may suggest that normal and mutated p53 coexist in the same tumour and may be explained by the presence of oncogenic human papillomavirus (HPV) in 10-42% of OSCCs without p53 mutation (55).

Another suppressor gene is INK4-ARF, whose product, p16 protein, acts as powerful inhibitor of cyclin-dependent kinases (CDK4 and CDK6). Thus, inhibition of this factor, along with overexpression of cyclin D1, increases CDK4/6 activity and stimulates cell proliferation (56). Conversely, replacement of p26 in OSCC tumour cells had significant anti tumour effects in culture tissues and animal models (57).

Absence of pRb expression was observed in 66% of OSCCs and 64% of premalignant lesions, and absence of p16 expression in 63% of OSCCs and 59% of premalignant lesions (58).
Using IHC in 220 OSCC samples and 90 premalignant lesions (38 with dysplasia), Soni et al. found dysregulation of the p16/pRb/D1 cyclin pathway to be an initial event in the onset of epithelial dysplasia. Thus, 90% of OSCCs and 83% of premalignant lesions showed altered expression of some protein of the pRb pathway. Moreover, simultaneous alteration of the pRb pathway and p53 was related to malignant transformation and a worse prognosis (59).

**Viruses**

Oncogenic viruses represent another factor that may affect cell cycle regulation. There is increasing interest in the role of infection by HPV in HNC. A meta-analysis by Miller and Johnstone found the probability of detecting HPV to be 2 to 3-fold higher in precancerous oral mucosa and 4 to 5-fold higher in OSCC than in normal oral epithelium. They also reported a frequent association between high-risk HPVs (HPV 16 and 18) and OSCC lesions (60). A recent systematic review of 5,046 samples of head and neck squamous cell carcinoma (HNSCC) in 60 PCR studies showed a significantly higher prevalence of HPV in cancer of the oropharynx than in that of the oral cavity (35.6% vs. 23.5%). The most prevalent genotype at both sites was HPV 16, with a more frequent presence of HPV 18 in oral cavity than oropharyngeal lesions (61).

Oncogenic transformation by these HPVs depends on oncoproteins E6 and E7, which bind with wild-type p53 and pRb proteins and remove their ability to stimulate DNA-repair or apoptosis. This inactivation of tumour suppressor proteins represents a possible pathogenic mechanism (42).

Mishra et al. recently reported a higher expression of transcription factor NF-κB and its activation sequences in oral cancer that arises from OED lesions. Thus, HPV infection would activate NF-κB-p50/p65 complexes, which would in turn stimulate differentiation of oral neoplastic cells, improving the prognosis. However, the authors acknowledged that further research is required to confirm and explain this finding (62).

As occurred with the development of a vaccine to prevent HPV-related cervical cancer, animal experiments are under way to develop a similar vaccine for OSCC. Results to date suggest that L1-based immunization may prevent the development of OSCC associated with HPV (63).

**Conclusion**

Despite major advances in the molecular pathology of HNC and oral cancer, there remain numerous gaps in our knowledge of the molecular markers involved in oral carcinogenesis.

OSCCs appear to have a multifocal character, with only half of them developing on the same site as a previous leukoplakia. Complex molecular mechanisms are implicated and the identification of a single marker to predict outcomes in all oral premalignant lesions remains a difficult challenge. Among reported markers are those for oncogene activation, e.g., ras, EGFR, PRAD-1, int-2, and for inactivation of tumour suppressor genes, notably p53, pRb and INK4-ARF. Their study may contribute to the search for reliable molecular markers of the development of oral cancer.

Other molecular pathology approaches are emerging, including assessment of the hypermethylation of the promoter regions of certain genes (e.g., p16INK4a, MGMT, DAPK1) or tumour-stroma interactions, which could provide new and promising data. However, further research is essential to clarify the role and clinical significance of these molecular changes, allowing them to be used as effective diagnostic and prognostic markers to improve the management of these patients.

**References**


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