

phosphorylation of I $\kappa$ B $\alpha$  by a specific kinase released NF- $\kappa$ B moves to the nucleus and combines with an element in the areas of I $\kappa$ B promoter of target genes, including those antiapoptosis.

Protocatechuic acid also inhibited hepatocyte apoptosis induced by TNF- $\alpha$  in *in vivo* studies of rodents.<sup>38,69,70</sup> Sepsis induced by bacterial lipopolysaccharide administration causes the release of free radicals and various cytokines—mediators of inflammation—which leads to a substantial damage in a variety of tissues. Exponential liver damage in the course of endotoxin shock is hepatocyte apoptosis, which triggers the binding of proinflammatory cytokines TNF- $\alpha$  and TNF receptor 1 (p55). Protocatechuic acid isopropyl ester decreased the level of TNF- $\alpha$  and increased the level of anti-inflammation of interleukin-10.<sup>38</sup> This process was accompanied by increased expression of iNOS in hepatocytes, increased production of iNOS, and increased levels of nitrogen compounds in the blood. Protocatechuic acid and its derivatives, such as isopropyl ester of protocatechuic acid, are demonstrated to counter these phenomena.<sup>37,69</sup>

It is important to evaluate the toxicity of chemopreventives for use in humans with a high risk of cancer.<sup>1,2</sup> In our preliminary study for determining the maximum tolerated dose of protocatechuic acid, the value was more than 10 g protocatechuic acid/kg basal diet (10,000 ppm). Rats fed protocatechuic acid-containing diet at this dose for 6 weeks did not show clinical signs or histopathological changes for toxicity, weight gain retardation, or abnormalities of chemical profiles.<sup>22</sup> The lowest dose of protocatechuic acid that effectively acted as a cancer chemopreventive agent was 500 ppm. Phenolic compounds are ubiquitous in edible vegetables, fruits, and nuts. It is estimated that an average of 1–2 g/d of phenolic compounds is consumed by humans.<sup>71</sup> Protocatechuic acid is a widely distributed phenolic acid. Several kinds of fruits, vegetables, and plants, such as citrus fruit and fennel, contain a small amount of protocatechuic acid. The lowest level of protocatechuic acid found to be effective in inhibiting tumorigenesis is almost four times greater than that consumed by humans, assuming that 10 g lettuce and/or strawberries, which contain 10–40 mg protocatechuic acid/100 g, are consumed daily. Further research is needed to better understand the underlying mechanisms of chemopreventive action and the pharmacokinetic absorption, distribution, metabolism, and excretion (ADME) data of protocatechuic acid.

#### 4. Conclusion

Protocatechuic acid is one of the biologically active substances isolated from a number of popular medicinal plants growing in different parts of the world.<sup>9,16,18,19,41,57</sup> Research conducted over the past several years indicates that it may be used in conventional medicine to prevent cardiovascular diseases and cancer.<sup>13–15,21</sup> The mechanism of the preventive action of protocatechuic acid is based on its antioxidant properties, that is, inhibition of the generation of free radicals, and their ability to scavenge and increase the catalytic activity of endogenous enzymes involved in the neutralization of free radicals. It is important that the impact of protocatechuic acid on the activity of enzymes involved in Phase I and II biotransformation of carcinogens and, possibly, direct blocking of specific binding sites of carcinogens with DNA molecule. Other aspects regarding the impact on the activity of cyclooxygenase-2, iNOS, inflammatory cytokines, and the proteins regulating cell cycle process are poorly understood. Our inflammation-associated colon carcinogenesis model (Tanaka model)<sup>72,73</sup> is useful in investigating the effects of protocatechuic acid and other compounds, which are candidate chemopreventive agents, on the expression of these molecules and proteins during carcinogenesis.<sup>74–76</sup> The safety of protocatechuic acid in humans should be considered based on the

reports that the chemical increased oxidative stress and the number of certain types of tumors in experimental animals.<sup>32,47–49</sup> However, both effects have been observed using only very high doses of the chemical. Daily consumption of protocatechuic acid was not clearly defined, but we should take into account the fact that its content in food rarely reaches 2–10 mg/g; hence, it is probably much less than 1 mg/kg. The dietary dose of protocatechuic acid is, therefore, at least 100–500 times smaller than those that caused side effects, which is described in several reports.<sup>9,11,49</sup> However, protocatechuic acid may be one of the active metabolites of plant phenolic compounds with more complex structural construction, such as anthocyanins, and therefore, its concentration in the human body may be higher than that in the acid content in the products consumed.<sup>44</sup> When considering the development of chemoprevention strategy with protocatechuic acid in humans, we should take into account its content both in the daily diet and in other natural or synthetic antioxidant compounds consumed, which may increase the effects of protocatechuic acid under certain conditions.

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## Review Article

# Understanding Carcinogenesis for Fighting Oral Cancer

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Oral cancer is one of the major global threats to public health. Oral cancer development is a tobacco-related multistep and multifocal process involving field cancerization and carcinogenesis. The rationale for molecular-targeted prevention of oral cancer is promising. Biomarkers of genomic instability, including aneuploidy and allelic imbalance, are able to measure the cancer risk of oral premalignancies. Understanding of the biology of oral carcinogenesis will give us important advances for detecting high-risk patients, monitoring preventive interventions, assessing cancer risk, and pharmacogenomics. In addition, novel chemopreventive agents based on molecular mechanisms and targets against oral cancers will be derived from research using appropriate animal carcinogenesis models. New approaches, such as interventions with molecular-targeted agents and agent combinations in high-risk oral individuals, are undoubtedly needed to reduce the devastating worldwide consequences of oral malignancy.

## 1. Introduction

Head and neck cancer 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 carcinoma [2]. They are sometimes preceded by precancerous lesions, such as leukoplakia and erythroplakia. More than 300,000 new cases worldwide are being diagnosed with oral squamous cell carcinoma annually [3]. Approximately 35,000 new cases are recorded annually in the US [2], 40,000 new cases in the EU, and 10915 new cases in Japan [4]. The most common site for intraoral carcinoma is the tongue, which accounts for around 40% of all cases in the oral cavity proper. Tongue cancers most frequently occur on the posteriolateral border and ventral surfaces of the tongue. The floor of the mouth is the second most common intraoral location. Less common sites include the gingival, buccal mucosa, labial mucosa, and hard plate.

The incidence of oral cancer has significant local variation. In India and other Asian countries, oral and pharyngeal carcinomas comprise up to half of all malignancies, with this particularly high prevalence being attributed to the influence

of carcinogens and region-specific epidemiological factors, especially tobacco and betel quid chewing. 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 years old with tongue cancer over past 30 years. However, few data have been published on the etiology and natural history of this increase [5]. Oral malignancy including tongue cancer is associated with severe morbidity and less than 50% long-term survival despite advances in treatment (surgery, radiation, and chemotherapy) of oral cancer. The survival of the patients remains very low, mainly due to their high risk of developing a second primary cancer. Thus, early detection and prevention of oral cancer and premalignancy are quite important [6–10]. This paper will focus on our understanding of oral carcinogenesis for preventing and early detection of oral malignancy.

## 2. Oral Carcinogenesis

Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by

several genetic alterations. The use of several molecular biology techniques to diagnose oral precancerous lesions and cancer may markedly improve the early detection of alterations that are invisible under the microscope. This would identify patients at a high risk of developing oral cancer [11]. Natural history of oral cancer and sequence of genetic alterations are illustrated in Figure 1. There are approaches to understanding of the molecular basis of oral cancer [12–14]. They include microarray technology, methylation microarrays, gene expression microarrays, array comparative genomic hybridization, proteomics, mitochondrial arrays, and micro-RNA arrays [15]. To date, high-throughput approaches are being used to search for oral cancer biomarkers in biofluids (saliva and serum) [15].

“Field cancerization” refers to the potential development of cancer at multiple sites [16, 17]. This has been observed during the development of cancer in the tissues covered with squamous epithelium (head and neck tumor) and transitional epithelium (urothelial carcinoma). It is evident that oral cancer, like carcinomas in other tissues, develops over many years, and during this period, there are multiple sites of neoplastic transformation occurring throughout the oral cavity. Mutations of this gene have been observed in various sites of premalignant leukoplakia and carcinoma in the same oral cavity [18]. A reduction in tumor suppressor activity by the gene and the development of mutations in *p53* have been associated with smoking and an increased risk for oral carcinoma development [19]. Therefore, multifocal presentations and mutational expressions of tumor suppressor genes may be the consequence of long-term (e.g., 20–40 years) exposure to various environmental and exogenous factors. The continual presence of mutations may also signify changes in DNA repair and apoptosis, thereby increasing the susceptibility for future transformation. Mutational adaptations that modify the survivability of particular clones of transforming cells may also further enhance the level of resistance to therapeutic control. Recent genetic analysis has revealed that cancers developing at distant sites within the oral cavity often are derived from the same initial clone [20]. The multiplicity of the oral carcinogenesis process makes it difficult to interrupt the progression to cancer through surgical removal of a premalignant lesion.

### 3. Risk Factors of Oral Cancer

The most important risk factor for the development of oral cancer in the Western countries is the consumption of tobacco [21] and alcohol [22]. Although drinking and smoking are independent risk factors, they have a synergistic effect and greatly increase risk together. In Asian countries, the use of smokeless tobacco products such as gutkha and betel quid [5, 23] is responsible for a considerable percentage of oral cancer cases. Several studies have reported a significant familial component in the development of oral cancer. The estimates of risk in the first degree relatives of oral cancer patients vary widely and have been reported to be 1.1 [24] ~ 3.8 [25] although some of these refer to head and neck cancer in general. Familial aggregation of

oral cancer, possibly with an autosomal dominant mode of inheritance, was reported in a very small percentage of oral cancer patients [26]. Polymorphic variation of genes in the xenobiotic metabolism pathways, such as in *CYP1A1* or the genes coding for glutathione S-transferase-M1 [27, 28] and *N*-acetyltransferase-2 [29] may be implicated. Individuals that carry the fast-metabolizing alcohol dehydrogenase type 3 (*ADH3*) allele [30] may be particularly vulnerable to the effects of chronic alcohol consumption and could be at increased risk to develop oral cancer [31].

Human papilloma virus (HPV), particularly HPV type 16, may be an etiologic factor, especially among persons who do not smoke or drink alcohol [32, 33]. Ang et al. [34] reported that tumor HPV status is a strong and independent prognostic factor for survival among patients with oropharyngeal cancer. They also noted that the risk of death significantly increased with each additional pack year of tobacco smoking. Although the idea that bacterial infections could lead to oral cancer has not been well regarded, there recently has been an increasing body of evidence to suggest a possible relationship between microorganisms and oral cancer development. The most notable example is that of the common pathogenic bacterium *Helicobacter pylori* and its association with gastric cancer. The mouth comprises a variety of different surfaces that are home to a huge diversity of microorganisms, including more than 750 distinct taxa of bacteria, suggesting that the oral squamous epithelium is constantly exposed to a variety of microbial challenges, on both cellular and molecular levels. In this context, we should draw attention to how they may relate to oral cancer development [35, 36].

There are clinically apparent oral premalignant lesions of oral cancer. They include leukoplakia, erythroplakia, nicotine stomatitis and tobacco pouch keratosis, lichen planus, and submucous fibrosis [37]. The term “leukoplakia” first used by Schwimmer in 1877 [38] to describe a white lesion of the tongue probably represented a syphilitic glossitis. The definition of leukoplakia has often been confusing and controversial. Some clinicians now avoid using this term. As defined by the World Health Organization, leukoplakia is “a white patch or plaque that cannot be characterized clinically or pathologically as any other disease [39]”. As such, leukoplakia should be used only as a clinical term. The term has no specific histopathological connotation and should never be used as a microscopic diagnosis. In the evaluation of the patient, leukoplakia is a clinical diagnosis of exclusion. Sometimes, a white patch is initially believed to represent leukoplakia, but the biopsy reveals another specific diagnosis. In such cases, the lesion should no longer be categorized as a leukoplakia. Leukoplakia is seen most frequently in middle-aged and older men, with an increasing prevalence with age [40]. Fewer than 1% of men below the age of 30 have leukoplakia, but the prevalence increases to an alarming 8% in men over the age of 70 [40]. The prevalence in women past the age of 70 is approximately 2%. The most common sites are the buccal mucosa, alveolar mucosa, and lower lip. However, lesions in the floor of mouth, lateral tongue, and lower lip are most likely to show dysplastic or malignant changes [41].

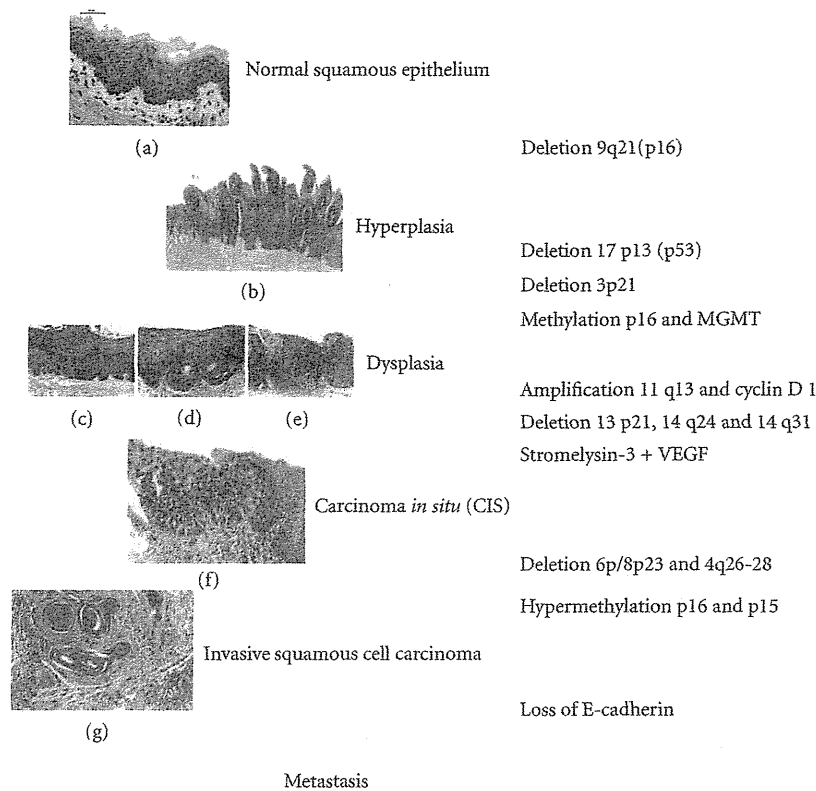


FIGURE 1: Natural history and genetic alterations of oral carcinogenesis. (a), Normal oral mucosa, (b) papillary hyperplasia, (c) mild dysplasia, (d) moderate dysplasia, (e) severe dysplasia, (f) carcinoma *in situ*, and (g) invasive squamous cell carcinoma (well differentiated).

The term “erythroplasia” originally used by Queyrat [42] to describe a red, precancerous lesion of the penis is used for a clinically and histopathologically similar process that occurs on the oral mucosa. Similar to the definition for leukoplakia, erythroplakia is a clinical term that refers to a red patch that cannot be defined clinically or pathologically as any other condition [39]. This definition excludes inflammatory conditions that may result in a red clinical appearance. Oral erythroplakia occurs most frequently in older men and appears as a red macule or plaque with a soft, velvety texture. The floor of mouth, lateral tongue, retromolar pad, and soft palate are the most common sites of involvement. Often the lesion is well demarcated, but some examples may gradually blend into the surrounding mucosa. Some lesions may be intermixed with white areas (erythroleukoplakia). Erythroplakia is often asymptomatic although some patients may complain of a sore, burning sensation.

Nicotine stomatitis is a thickened, hyperkeratotic alteration of the palatal mucosa that is most frequently related to pipe smoking, but milder examples can also develop secondary to cigar smoking or, rarely, from cigarette smoking [39]. The palatal mucosa becomes thickened and hyperkeratotic, sometimes developing a fissured surface. The surface often develops popular elevations with red centers, which represent the inflamed openings of the minor salivary gland ducts.

Detection and diagnosis of oral neoplasia has traditionally relied heavily on the clinical experience of the examiners and their ability to recognize often subtle morphologic changes. However, some early malignant lesions are clinically indistinguishable from benign lesions, and some patients develop carcinomas in the absence of clinically identifiable oral premalignant lesions. Furthermore, it can be difficult even for experts to determine which oral premalignant lesions are at significant risk to progress to invasive carcinoma. Therefore, an accurate, objective, and noninvasive method to help identify premalignant lesions and to distinguish those at risk of malignant conversion is needed.

#### 4. Biomarkers of Oral Cancer

Biomarkers help in the evaluation of prevention or use of therapies and the detection of the earliest stages of oral mucosal malignant transformation. Biomarkers reveal the genetic and molecular changes related to early, intermediate, and late end points in the process of oral carcinogenesis [43]. These biomarkers will refine our ability to enhance the prognosis, diagnosis, and treatment of oral carcinomas [44]. Genetic and molecular biomarkers will also determine the effectiveness and safety of chemopreventive agents. Chemopreventive agents are chemicals of natural or synthetic

origin. Unlike other drugs, which do not prevent disease, chemopreventive agents reduce the incidence of diseases such as cancer before clinical symptoms occur. This development is critical for the understanding of early oral mucosal transformation. Biomarkers will also reduce the number of patients and the time for long-term follow up required to define a significant clinical response to a chemopreventive agent [45, 46]. The markers may, therefore, clarify the types, doses, frequencies, and regimens to achieve the maximum level of benefit from chemopreventive agents. Decreasing the cost of the clinical trials is another factor that drives the development of biomarkers.

Biomarkers have been categorized following the recommendation by the Committee on Biological Markers of the National Research Council/National Academy of Sciences [47]. They fall into broad groups that detect exposure, progression, susceptibility to carcinogens, and/or the responses by the target cellular populations [46].

A distinct advantage to oral cancer studies is their anatomical access to the developing premalignant and malignant lesions. One could readily analyze biopsies of the primary lesion as well as apparently normal mucosal sites to determine the levels of DNA adducts and oral cancer risk. DNA adduct studies and cytogenetic analyses may also provide evidence for altered structure and function of susceptibility sites in the DNA following DNA-binding studies of nuclear proteins such as p53. Some researchers have focused on microscopic cytogenetic and somatic mutation changes as early biologic markers. One of the markers used to define chromosomal aberrations is the staining for micronuclei in exfoliated buccal mucosal cells [48]. Micronuclei have also been used to evaluate the reversal of leukoplakia and the effectiveness of retinoids, carotenoids, and vitamin E [49, 50]. Other methods include the determination of aneuploidy, and the assessment of losses and gains of genetic material particularly associated with somatic and sex chromosomes. Other sites of chromosomal aberrations are found in sister chromatid exchanges, and allele typic variations designated by losses on chromosomes 3, 4, 5, 6, 8, 9, 11, 13, 17, and 19.

Some molecular biomarkers with potential diagnostic relevance include DNA content and chromosome polysomy, loss of heterozygosity, nucleolar organizer regions, histoblood group antigens, proliferation markers, increased epidermal growth factor receptor (EGFR), and decreased expression of retinoic acid receptor- $\beta$ , p16, and p53 [51, 52]. Although a reliable, validated marker panel for providing clinically useful prognostic information in oral premalignant lesions patients has not yet been established, the advent of high throughput genomic and proteomic analysis techniques may soon yield major advances toward a prognostically relevant molecular classification system (Table 1).

## 5. Animal Models for Oral Carcinogenesis

A variety of animals has been used for the study of tumor growth, the process of carcinogenesis and the prevention/treatment research [8, 53–56]. The continual development of transgenic or knockout mice has improved our

TABLE 1: Potential biomarkers for oral carcinogenesis.

Category	Measures
Genomic biomarker	Micronuclei, DNA adduct, DNA content, and chromosomal aberration (polymorphism, allelic loss, gain, and amplification)
Oncogenic biomarker	Oncogenic expression, modified tumor suppressor genes, and <i>Src</i> genes
Proliferation biomarker	Nuclear and cyclin-related antigens, mitotic frequency, ornithine decarboxylase (ODC), and polyamines
Differentiation biomarker	Cytokeratins, transglutaminase Type I, and transcription factor (AP)-1
Oxidative stress biomarker	Glutathione S-transferase, stress proteins (HSPs), and Superoxide dismutase
Apoptosis biomarker	Bcl-2 family, chromatin condensation factors, caspases, and nucleosome formation
Immunologic biomarker	Cytokines

understanding of the role of specific genes in tumor growth. The most widely used animal models for oral carcinogenesis are the hamster cheek pouch model [54, 57] and the 4-nitroquinoline 1-oxide- (4-NQO-) induced oral (tongue) carcinogenesis model [8, 53, 58, 59].

In the former model, a complete carcinogen, 7,12-dimethylbenz(*a*)anthracene (DMBA, 0.5%), is applied to the hamster cheek pouch three times a week for 16 weeks. By week 16, all animals exhibit invasive oral squamous cell carcinoma. Many different studies have been conducted with the hamster buccal pouch model, and they have provided an array of changes that are analogous to those observed in human invasive oral carcinoma [54, 57]. These include a mutation in codon 61 of *Ha-ras*, which manifested in an A  $\rightarrow$  T transversion in the second position of codon 61, resulting in an amino acid change from glycine to leucine. The expression of *c-Ki-ras* in malignant tumors of the pouch, but not in the normal oral mucosa, has also been observed at very early stages of tumor development [57]. Although the hamster oral tumor model appears to parallel several changes observed in human oral cancer, the hamster still has several areas of uniqueness which must be considered in any evaluations of results from oral carcinogenesis studies. The hamster cheek pouch provides a relatively large surface area of oral mucosa for the development of invasive carcinoma, while the human does not possess this type of mucosal structure. In contrast to humans, mice, or rats, the hamster cheek pouch lacks lymphatic drainage, which allows various drugs or molecules to accumulate in the pouch. The Syrian hamster population was also derived from a small breeding pair that resulted in a restricted polymorphism for the antigen recognition region (Ia region) and some of the major histocompatibility K and D regions [60]. In addition, the number of T-cells in the hamster spleen exhibits a lower number/gram weight of the organ as compared with the mouse or human [60]. The hamster may also respond to



antigenic tumor sources with a natural killer macrophage or granulocyte cytotoxicity rather than a T cell response [60].

The latter animal models for the study of oral carcinogenesis include those in rats and mice using the water-soluble carcinogen, 4-NQO. The carcinogen is either supplied in the water (20 ppm) for the rats [58, 61–74] or by painting for the mice [75]. Administration with 4-NQO in drinking water (20 ppm) for 8 weeks in rats and mice produces tongue lesions including squamous cell neoplasms (Figure 2) within 32 weeks [71], while topical application of the carcinogen to the mouse palates for up to 16 weeks, just like the hamster model develops palate tumors within 49 weeks [75]. Since the most common site for intraoral carcinoma is the tongue and the drinking water administering of 4-NQO is a simple and easy method, the 4-NQO-induced tongue carcinogenesis model is quite useful for investigating oral carcinogenesis and identifying cancer chemopreventive agents [58, 61–74, 76–84]. In the rat model, with the progression of oral carcinogenesis, increased levels of polyamine synthesis have been noted as well as nucleolar organizing regions (NORs) [58]. The mouse model with 4-NQO has demonstrated some molecular mimicry of human oral cancers, as is true of the hamster model [75]. A number of chemical carcinogens including coal tar, 20-methylcholanthrene, DMBA, and 4-NQO have been used in experimental oral carcinogenesis. However, 4-NQO is the preferred carcinogen apart from DMBA in the development of experimental oral carcinogenesis. 4-NQO is a water-soluble carcinogen, which induces tumors predominantly in the oral cavity. It produces all the stages of oral carcinogenesis and several lines of evidences suggest that similar histological as well as molecular changes are observed in the human system. There are several review articles to collate the information available on mechanisms of action of 4-NQO, and studies have been carried out for the development of biomarkers and chemopreventive agents using 4-NQO animal models [8–10, 53, 58, 59, 61–68, 70–74].

The complexity and variety of biochemical changes can increase tumor development is the  $p53^{-/-}$  mice [85]. Unfortunately, this model and other genetic mouse models have not been exploited for studying the relationships among chemical oral carcinogenesis, specific genetic defects, and chemoprevention. Genetically altered mouse and rat models have been developed for evaluating molecular-targeted prevention and treatment of oral carcinoma [56]. We have developed *rasH2* transgenic mouse carcinogenesis model [86] and human *c-Ha-ras* proto-oncogene transgenic rat model [87] for chemoprevention studies on oral (tongue) carcinogenesis.

## 6. Chemoprevention

Chemoprevention is the use of natural or synthetic substances to halt, delay, or reverse malignant progression in tissues at risk to develop invasive cancer [8–10]. Retinoids are the most extensively studied agents for chemoprevention of oral cancer [88]. 13-*cis*-retinoic acid given for only 3 months

produced a clinical response rate of 67% versus 10% for placebo. However, toxicities were considerable, and a very high rate of relapse within 3 months of stopping treatment was reported. Subsequent studies with retinoids in patients with oral premalignant lesions have confirmed clinical and pathologic response rates though toxicities remain a concern [89]. However, translational studies showed that molecular abnormalities persisted in some patients with complete clinical and pathologic response to retinoid therapy [90], suggesting that cancer development may be delayed rather than prevented by these agents. Other agents that have been assessed in clinical trials for chemoprevention activity in oral leukoplakia patients include vitamin E [44], Bowman-Birk inhibitor concentrate (BBIC) derived from soybeans [91], curcumin [92], and green tea polyphenol epigallocatechin-3-gallate. Small clinical trials using oral BBIC revealed no significant toxicity and a 32% response rate [91].

Attention is focused now on the development of agents targeted to specific steps in the molecular progression from normal to oral premalignancy to invasive carcinoma. Examples of molecularly targeted agents that have shown promise *in vitro*, in animal models, or in early clinical trials include cyclooxygenase (COX)-2 inhibitors and epidermal growth factor receptor EGFR inhibitors [93–95]. Data from several sources suggest that the cyclooxygenase pathway is a good target for oral cancer prevention. COX-2 is overexpressed in head and neck squamous carcinoma [96], and COX-2 inhibitors prevented oral cancer development in animal models [97]. A randomized placebo-controlled trial of the COX-2 inhibitor ketorolac administered as an oral rinse in oral leukoplakia patients revealed that the treatment was well tolerated but did not result in greater clinical response than placebo [98]. However, analysis of the results of this trial are confounded somewhat by the high response rate (32%) in the placebo arm and difficulty in determining whether topical delivery of the agent allowed penetration to the damaged cells. The future of COX-2 inhibitors as chemoprevention agents will also depend on the determination of the extent of risk for cardiac toxicities associated with this class of agents. The EGFR is also a promising molecular target for intervention in oral malignant progression [93–95]. EGFR is a receptor tyrosine kinase that is overexpressed in oral dysplasia and invasive cancer and associated with worse prognosis in patients with head and neck squamous carcinoma [99, 100]. EGFR inhibitors, alone or in combination with chemotherapy and radiotherapy, have shown activity against head and neck squamous carcinoma in clinical trials, and toxicities were generally well tolerated [101]. Evidence has suggested that combination therapy targeting COX-2 and EGFR may be efficacious [95, 102]. Although chemoprevention appears to be a promising approach to managing oral premalignancy, prospective clinical trials using specific agents, and strong corollary translational and laboratory investigations, are needed to evaluate clinical, histologic, and molecular efficacy. In the future, it may be possible and necessary to individualize medical therapy to specific genetic abnormalities detected within the oral mucosa.



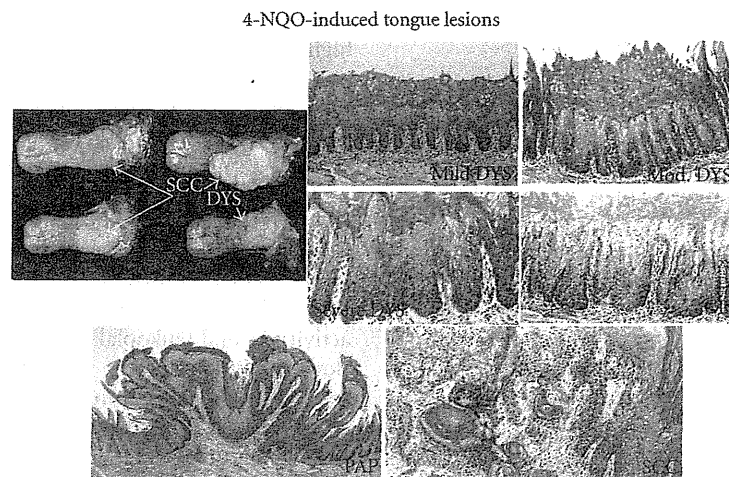


FIGURE 2: 4-NQO-induced tongue lesions in rats. 4-NQO, 4-nitroquinoline 1-oxide; DYS, dysplasia; PAP, papilloma; SCC, squamous cell carcinoma.

## 7. Conclusion

Human oral cancer being the sixth largest group of malignancies worldwide. Seventy percent of oral cancers appear from premalignant lesions. The process of oral cancer formation results from multiple sites of premalignant change in the oral cavity (field cancerization). Animal models are being widely used, aiming for the development of diagnostic and prognostic markers. The appearance of these premalignant lesions is one distinct feature of human oral cancer. At present, there is dearth of biomarkers to identify which of these lesions will turn into malignancy. Regional lymph node metastasis and locoregional recurrence are the major factors responsible for the limited survival of patients with oral cancer. Paucity of early diagnostic and prognostic markers is one of the contributory factors for higher mortality rates. Determining high- and low-risk populations by measuring reliable biomarkers help us to understand the dynamics and prevention of oral cancer development. The quantitation of genetic and molecular changes and the use of these changes as markers for the detection and prevention of early premalignant change require the harvesting of tissues and cells. Promising technologies are being rapidly developed to assist in localization of abnormal oral mucosa, in noninvasive and objective diagnosis and characterization of identified mucosal lesions, and in therapy of patients with oral cancer. Undoubtedly, the prevention or reduction in the smoking of tobacco products and alcohol consumption would have a profound influence on the incidence of oral cancer. Chemoprevention also has an impact on the development of malignant changes in the oral mucosa. Prevention through chemoprevention and/or the use of systemic medications has been an extensively studied strategy and continues to hold promise as a way of diminishing the morbidity and mortality associated with this malignancy.

## Abbreviations

BBIC: Bowman-Birk inhibitor concentrate  
 COX: Cyclooxygenase  
 DMBA: 7,12-dimethylbenz(*a*)anthracene  
 EGFR: Epidermal growth factor receptor  
 IL: Interleukin  
 4-NQO: 4-nitroquinoline 1-oxide.

## Conflict of Interests

The authors declared that there is no conflict of interests.

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## Review Article

# Oral Carcinogenesis and Oral Cancer Chemoprevention: A Review

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Oral cancer is one of the major global threats to public health. The development of oral cancer is a tobacco-related multistep and multifocal process involving field cancerization and carcinogenesis. The rationale for molecular-targeted prevention of oral cancer is promising. Biomarkers of genomic instability, including aneuploidy and allelic imbalance, are possible to measure the cancer risk of oral premalignancies. Understanding of the biology of oral carcinogenesis will yield important advances for detecting high-risk patients, monitoring preventive interventions, and assessing cancer risk and pharmacogenomics. In addition, novel chemopreventive agents based on molecular mechanisms and targets against oral cancers will be derived from studies using appropriate animal carcinogenesis models. New approaches, such as molecular-targeted agents and agent combinations in high-risk oral individuals, are undoubtedly needed to reduce the devastating worldwide consequences of oral malignancy.

## 1. Introduction

Head and neck cancer 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 carcinoma [2]. They are sometimes preceded by precancerous lesions, such as leukoplakia and erythroplakia. More than 300,000 new cases of oral squamous cell carcinoma are diagnosed annually [3]. Approximately 35,000 new cases are recorded annually in the US [2], 40,000 new cases are recorded in the EU and 10915 new cases in Japan [4]. The most common site for intraoral carcinoma is the tongue, which accounts for around 40% of all cases in the oral cavity proper. Tongue cancers most frequently occur on the posterior-lateral border and ventral surfaces of the tongue. The floor of the mouth is the second most common intraoral location. Less common sites include the gingival, buccal mucosa, labial mucosa, and hard plate.

The incidence of oral cancer has significant local variation. Oral and pharyngeal carcinomas account for up to half of all malignancies in India and other Asian countries, and this particularly high prevalence is attributed to the influence

of carcinogens and region-specific epidemiological factors, especially tobacco and chewing betel quid. An increase in the prevalence of oral cancer among young adults is a cause of special concern. There has been a 60% increase in the number of under 40 years olds with tongue cancer over past 30 years. However, little has been published on the etiology and natural history of this increase [5]. Oral malignancy, including tongue cancer, is associated with severe morbidity and long-term survival of less than 50% despite advances in the treatment (surgery, radiation, and chemotherapy) of oral cancer. The survival of the patients remains very low, mainly due to their high risk of developing a second primary cancer. Therefore, the early detection and prevention of oral cancer and premalignancy are quite important [6–10]. This article will focus on the current understanding of oral carcinogenesis for the early detection and prevention of oral malignancy.

## 2. Oral Carcinogenesis

Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by several genetic alterations. The use of several molecular

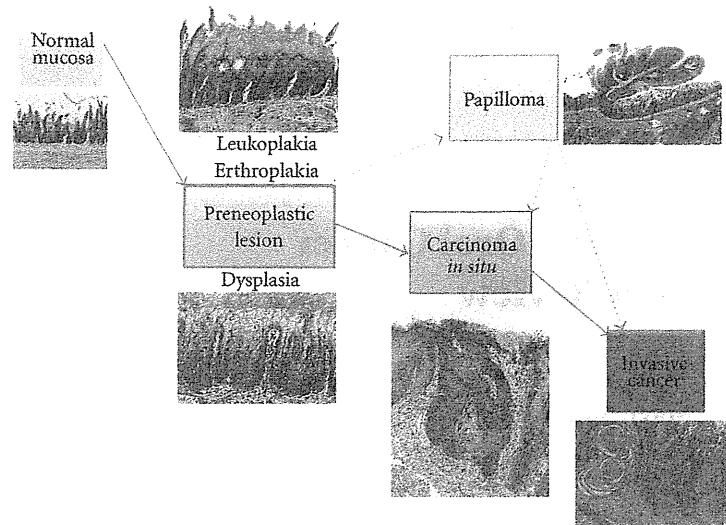


FIGURE 1: The natural history of oral carcinogenesis.

biology techniques to diagnose oral precancerous lesions and cancer may markedly improve the early detection of alterations that are invisible under the microscope. This would identify patients at a high risk of developing oral cancer [11]. The natural history of oral cancer and sequence of genetic alterations are illustrated in Figure 1. There are several approaches to understanding the molecular basis of oral cancer [12–14]. They include microarray technology, methylation microarrays, gene expression microarrays, array comparative genomic hybridization, proteomics, mitochondrial arrays, and micro-RNA arrays [15]. High-throughput approaches are currently being used to search for oral cancer biomarkers in biofluids, such as saliva and serum [15].

Field cancerization refers to the potential development of cancer at multiple sites [16, 17]. This has been observed during the development of cancer in the tissues covered with squamous epithelium (head and neck tumor) and transitional epithelium (urothelial carcinoma). It is evident that oral cancer, like carcinomas in other tissues, develops over many years, and during this period, there are multiple sites of neoplastic transformation occurring throughout the oral cavity. “Field cancerization” may also be defined by the expression of mutations in the exons of tumor suppressor genes. One such tumor suppressor gene is *p53*, and mutations of this gene have been observed in various sites of premalignant leukoplakia and carcinoma in the same oral cavity [18]. A reduction in tumor suppressor activity by the gene and the development of mutations in *p53* are associated with smoking and an increased risk for oral carcinoma development [19]. Therefore, multifocal presentations and mutational expressions of tumor suppressor genes may be the consequence of long-term (e.g., 20 ~ 40 years) exposure to various environmental and exogenous factors. The continual presence of mutations may also signify changes in DNA repair and apoptosis, thereby increasing the susceptibility to future transformation. Mutational adaptations that modify

the survivability of particular clones of transforming cells may also further enhance the level of resistance to therapeutic control. A recent genetic analysis revealed that cancers developing at distant sites within the oral cavity often are derived from the same initial clone [20]. The multiplicity of the oral carcinogenesis process makes it difficult to interrupt the progression to cancer through the surgical removal of a premalignant lesion.

### 3. Risk Factors of Oral Cancer

The most important risk factor for the development of oral cancer in the Western countries is the consumption of tobacco [21] and alcohol [22]. Although drinking and smoking are independent risk factors, they have a synergistic effect and greatly increase the risk together. The use of smokeless tobacco products such as gutkha and betel quid in Asian countries [5, 23] is responsible for a considerable percentage of oral cancer cases.

**3.1. Genetic.** Several studies have reported a significant familial component in the development of oral cancer. The estimates of risk in the first degree relatives of oral cancer patients vary widely and range from 1.1 [24] to 3.8 [25], although some of these cancers refer to head and neck cancer in general. Familial aggregation of oral cancer, possibly with an autosomal dominant mode of inheritance, is observed in a very small percentage of oral cancer patients [26]. Polymorphic variation of genes in the xenobiotic metabolism pathways such as in *CYP1A1* or the genes coding for glutathione S-transferase-M1 [27, 28] and *N*-acetyltransferase-2 [29] may be implicated. Individuals that carry the fast-metabolizing alcohol dehydrogenase type 3 (*ADH3*) allele [30] may be particularly vulnerable to the effects of chronic alcohol consumption and could be at increased risk to develop oral cancer [31]. The single



nucleotide polymorphism A/G870 in the *CCND1* gene that encodes Cyclin D is associated with susceptibility to oral cancer. The AA genotype [32] or the GG wild-type genotype [33] may increase risk for oral cancer.

**3.2. Inflammation.** Cytokines, including interleukins (ILs), tumor necrosis factors (TNFs), and certain growth factors, are an important group of proteins that regulate and mediate inflammation and angiogenesis. Tumor growth, invasion and metastasis are facilitated when there is a deregulation in their production. Genetic association studies suggest a putative correlation between functional DNA polymorphisms in cytokine genes and oral cancer [34]. Increased serum levels of proinflammatory cytokines, interleukin (IL)-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  as well as the anti-inflammatory cytokine, IL-10, are seen in patients with oral cancer in comparison to healthy controls. The anti-inflammatory cytokine IL-4 inhibits oral cancer invasion by the downregulation of matrix metalloproteinase-9.

**3.3. Infection.** Human papillomavirus (HPV), particularly HPV type 16, may be an etiologic factor, especially among persons who do not smoke or drink alcohol [35, 36]. Ang et al. [37] reported that tumor HPV status is a strong and independent prognostic factor for survival among patients with oropharyngeal cancer. They also noted that the risk of death significantly increased with each additional pack-year of tobacco smoking. Although the idea that bacterial infections could lead to oral cancer has been generally discounted, there is an increasing body of evidence to suggest a possible relationship between micro-organisms and the development of oral cancer. The most notable example is that of the common pathogenic bacterium *Helicobacter pylori* and its association with gastric cancer. The mouth contains a variety of different surfaces that are home to a huge diversity of micro-organisms, including more than 750 distinct *taxa* of bacteria, thus suggesting that the oral squamous epithelium is constantly exposed to a variety of microbial challenges, on both cellular and molecular levels. It is therefore important to consider how such factors may be related to oral cancer development [38, 39].

**3.4. Preneoplasia.** There are clinically apparent oral premalignant lesions of oral cancer. They include leukoplakia, erythroplakia, nicotine stomatitis and tobacco pouch keratosis, lichen planus, and submucous fibrosis, [40]. The term "leukoplakia" was first used by Schwimmer in 1877 [41] to describe a white lesion of the tongue that probably represented a syphilitic glossitis. The definition of leukoplakia has often been confusing and controversial. Some clinicians now avoid using this term. The World Health Organization defines leukoplakia as 'a white patch or plaque that cannot be characterized clinically or pathologically as any other disease [42]. Therefore, leukoplakia should be used only as a clinical term. The term has no specific histopathological connotation and should never be used as a microscopic diagnosis. Leukoplakia is a clinical diagnosis of exclusion. Sometimes a white patch is initially believed to

represent leukoplakia, but the biopsy reveals another specific diagnosis. These lesions should no longer be categorized as a leukoplakia. Leukoplakia is seen most frequently in middle-aged and older males, with an increasing prevalence with age [43]. Fewer than 1% of males below the age of 30 have leukoplakia, but the prevalence increases to an alarming 8% in men over the age of 70 [43]. The prevalence in females past the age of 70 is approximately 2%. The most common sites are the buccal mucosa, alveolar mucosa, and lower lip. However, lesions occurring on the floor of mouth, lateral tongue, and lower lip are most likely to show either dysplastic or malignant changes [44].

The term "erythroplasia" originally used by Queyrat [45] to describe a red, precancerous lesion of the penis is used for a clinically and histopathologically similar process that occurs on the oral mucosa. Similar to the definition for leukoplakia, erythroplakia is a clinical term that refers to a red patch that cannot be defined clinically or pathologically as any other condition [42]. This definition excludes inflammatory conditions that may result in a red clinical appearance. Oral erythroplakia occurs most frequently in older males and appears as a red macule or plaque with a soft, velvety texture. The floor of mouth, lateral tongue, retromolar pad, and soft palate are the most common sites of involvement. Often the lesion is well demarcated, but some examples may gradually blend into the surrounding mucosa. Some lesions may be intermixed with white areas (erythroleukoplakia). Erythroplakia is often asymptomatic, although some patients may complain of a sore, burning sensation.

**3.5. Tobacco.** Nicotine stomatitis is a thickened, hyperkeratotic alteration of the palatal mucosa that is most frequently related to pipe smoking, but milder examples can also develop secondary to cigar smoking or, rarely, from cigarette smoking [42]. The palatal mucosa becomes thickened and hyperkeratotic, sometimes developing a fissured surface. The surface often develops numerous elevations with red centers, which represent the inflamed openings of the minor salivary gland ducts.

Another specific tobacco-related oral mucosal alteration occurs in association with smokeless tobacco use, such as either snuff or chewing tobacco [40]. Such lesions typically occur in the buccal or labial vestibule where the tobacco is held, but they can also extend onto the adjacent gingiva and buccal mucosa. Early lesions show slight wrinkling that disappears when the tissues are stretched. Other lesions may appear as hyperkeratotic, granular patches. Advanced lesions exhibit greatly thickened zones of grayish white mucosa with well-developed folds and fissures. The degree of clinical alteration depends on the type and quantity of tobacco, the duration of tobacco usage, and host susceptibility. Smokeless tobacco keratosis shows microscopic hyperkeratosis and acanthosis of the mucosal epithelium. True epithelial dysplasia is uncommon, and when dysplasia is found, it tends to be mild [46].

**3.6. Mutations.** Genetic mutations often produce early phenotypic changes that may present as clinically apparent, recognizable lesions. An oral premalignant lesion is an area

of morphologically or genetically altered tissue that is more likely than normal tissue to develop cancer. The reported rates of malignant transformation of leukoplakia range from less than 1% to 18% [47, 48]. There is no accepted method to predict the risk of malignant progression of an individual oral premalignant lesions, but various factors, such as the location within the oral cavity, clinical appearance (homogeneous versus heterogeneous), and the presence of dysplasia are correlated with the risk of progression. The histological finding of dysplasia is strongly associated with an increased rate of invasive cancer development [47]. A velvety reddish mucosal lesion, known as erythroplakia, is associated with a higher rate of cancer development, occurs much less frequently, and is more difficult to detect clinically than oral leukoplakia. Virtually all erythroplakic lesions contain severe dysplasia, carcinoma *in situ*, or early invasive carcinoma at the time of presentation [49]. Formalized classification and staging systems for oral preneoplastic lesions have been proposed [50, 51], and their use is important to facilitate uniform reporting and comparisons of data.

Detection and diagnosis of oral neoplasia has traditionally relied heavily on the clinical experience of the examiners and their ability to recognize often subtle morphologic changes. However, some early malignant lesions are clinically indistinguishable from benign lesions, and some patients develop carcinomas in the absence of clinically identifiable oral premalignant lesions. Furthermore, it can be difficult, even for experts, to determine which oral premalignant lesions are at significant risk to progress to invasive carcinoma. Therefore, an accurate, objective, and noninvasive method to help identify premalignant lesions and to distinguish those at risk of malignant conversion is needed.

#### 4. Biomarkers of Oral Cancer

Biomarkers help in evaluating the preventive measures or therapies and the detection of the earliest stages of oral mucosal malignant transformation. Biomarkers reveal the genetic and molecular changes related to early, intermediate, and late end-points in the process of oral carcinogenesis. These biomarkers will refine the ability to enhance the prognosis, diagnosis, and treatment of oral carcinomas [52]. Genetic and molecular biomarkers will also determine the efficacy and safety of chemopreventive agents. Chemopreventive agents are chemicals of natural or synthetic origin. Unlike other drugs, which do not prevent disease, chemopreventive agents reduce the incidence of diseases such as cancer before clinical symptoms occur. This development is critical for the understanding of early oral mucosal transformation. Biomarkers will also reduce the number of patients and the time for long-term follow-up required to define a significant clinical response to a chemopreventive agent [53, 54]. The markers may therefore clarify the types, doses, frequencies, and regimens to achieve the maximum level of benefit from chemopreventive agents. Decreasing the cost of the clinical trials is another factor that drives the development of biomarkers.

Biomarkers have been categorized following the recommendation by the Committee on Biological Markers of the

National Research Council/National Academy of Sciences [55]. They fall into broad groups that detect exposure, progression, susceptibility to carcinogens, and/or the responses by the target cellular populations [54].

Oral cancer studies have a distinct advantage due the anatomical access to the developing premalignant and malignant lesions. One could readily analyze biopsies of the primary lesion as well as apparently normal mucosal sites to determine the levels of DNA adducts and oral cancer risk. DNA adduct studies and cytogenetic analyses may also provide evidence for altered structure and function of susceptibility sites in the DNA following DNA binding studies of nuclear proteins such as *p53*. Some studies have focused on microscopic cytogenetic and somatic mutation changes as early biologic markers. One of the markers used to define chromosomal aberrations is the staining for micronuclei in exfoliated buccal mucosal cells [56]. Micronuclei have also been used to evaluate the reversal of leukoplakia and the effectiveness of retinoids, carotenoids, and vitamin E [57, 58]. Other methods include the determination of aneuploidy and the assessment of losses and gains of genetic material particularly associated with somatic and sex chromosomes. Other sites of chromosomal aberrations are found in sister chromatid exchanges, and allele typic variations designated by losses on chromosomes 3, 4, 5, 6, 8, 9, 11, 13, 17, and 19.

Some molecular biomarkers with potential diagnostic relevance include DNA content and chromosome polysomy, loss of heterozygosity, nucleolar organizer regions, histoblood group antigens, proliferation markers, increased epidermal growth factor receptor (EGFR), and decreased expression of retinoic acid receptor- $\beta$ , *p16*, and *p53* [59, 60]. Although a reliable, validated marker panel for providing clinically useful prognostic information in oral premalignant lesions patients has not yet been established, the advent of high-throughput genomic and proteomic analysis techniques may soon yield major advances toward a prognostically relevant molecular classification system (Table 1).

#### 5. Animal Models for Oral Carcinogenesis

A variety of animals have been used for the study of tumor growth, the process of carcinogenesis, and the prevention/treatment research [8, 61–64]. The continual development of transgenic or knockout mice has improved our understanding of the role of specific genes in tumor growth. The most widely used animal models for oral carcinogenesis are the hamster cheek pouch model [62, 65] and the 4-nitroquinoline 1-oxide- (4-NQO-) induced oral (tongue) carcinogenesis model [8, 61, 66, 67].

DMBA is one of the widely used carcinogens in experimental oral carcinogenesis. Induction of SCC in cheek pouch of hamsters was first described with the aid of three polycyclic aromatic hydrocarbons, such as 7,12-dimethylbenz(*a*)-anthracene (DMBA), 20-methylcholanthrene (20-MC), and 3,4-benzpyrene [68]. A complete carcinogen, DMBA (0.5%), is applied to the hamster cheek pouch three times a week for 16 weeks. All animals exhibit invasive oral squamous cell carcinoma by week 16. Many studies have been conducted using the hamster buccal pouch model and thus elucidated

TABLE 1: Potential biomarkers for oral carcinogenesis.

Category of biomarkers	Measurements
Genomic	Micronuclei, DNA adduct, DNA content, Chromosomal aberration
Oncogenic	Oncogenic expression, Modified tumor suppressor genes, <i>Src</i> genes
Proliferation	Nuclear and cyclin related antigens, Mitotic frequency, Ornithine decarboxylase (ODC), Polyamines
Differentiation	Cytokeratins, Transglutaminase Type I, Transcription factor (AP)-1
Oxidative stress	Glutathione S-transferase, Stress proteins (HSPs), Superoxide dismutase
Apoptosis	Bcl-2 family, Chromatin condensation factors, Caspases, Mitochondrial pathway
Immunologic	Various cytokines

an array of changes that are analogous to those observed in human invasive oral carcinoma [62, 65]. These include a mutation in codon 61 of *Ha-ras*, which manifested in an A → T transversion in the second position of codon 61, thus resulting in an amino acid change from glycine to leucine. The expression of *c-Ki-ras* in malignant tumors of the pouch, but not in the normal oral mucosa, is also observed at the very early stages of tumor development [65]. Although the hamster oral tumor model appears to parallel several changes observed in human oral cancer, the hamster still has several areas of uniqueness which must be considered in any evaluation of results from oral carcinogenesis studies. The hamster cheek pouch provides a relatively large surface area of oral mucosa for the development of invasive carcinoma, while the human does not possess this type of mucosal structure. In contrast to humans, mice, or rats, the hamster cheek pouch lacks lymphatic drainage, which thus allows various drugs or molecules to accumulate in the pouch. The Syrian hamster population was also derived from a small breeding pair that resulted in a restricted polymorphism for the antigen recognition region (Ia region) and some of the major histocompatibility K and D regions [69]. In addition, the number of T-cells in the hamster spleen exhibits a lower number/gram weight of the organ in comparison to the mouse or human [69]. The hamster may also respond to antigenic tumor sources with a natural killer macrophage or granulocyte cytotoxicity rather than a T cell response [69]. DMBA and its solvent vehicle (acetone or benzene) are significant local irritants that cause severe inflammatory response, necrosis, and sloughing. Therefore, it is difficult to examine early squamous cell lesions [66, 70, 71]. Neoplasms induced by DMBA in the hamster cheek pouch possess many differences in histological features of differentiated SCC and do not closely resemble the lesions observed in human [72, 73].

The latter animal models for the study of oral carcinogenesis include those in rats and mice using the water soluble carcinogen, 4-NQO. The carcinogen is supplied either in the water (20 ppm) for the rats [66, 71, 74–86] or by painting for the mice [87]. The administration of 4-NQO in drinking water (20 ppm) for 8 weeks in rats and mice produces tongue lesions including squamous cell neoplasms within 32 weeks [83], while topical application of the carcinogen to the mouse palates for up to 16 weeks just like the

hamster model develops palate tumors within 49 weeks [87]. The 4-NQO-induced tongue carcinogenesis model is quite useful for investigating oral carcinogenesis and identifying cancer chemopreventive agents, because the most common site for intraoral carcinoma is the tongue and the administration drinking water containing of 4-NQO is a simple and easy method [66, 71, 74–86, 88–96]. Increased levels of polyamine synthesis, as well as nucleolar organizer regions (NORs) with the progression of oral carcinogenesis, have been noted in the rat model [66]. The mouse model with 4-NQO has demonstrated some molecular mimicry of human oral cancers, as is true of the hamster model [87]. A number of chemical carcinogens, including coal tar, 20-MC, DMBA, and 4-NQO, have been used in experimental oral carcinogenesis. However, 4-NQO is the preferred carcinogen apart from DMBA in the development of experimental oral carcinogenesis. 4-NQO is a water soluble carcinogen, which induces tumors predominantly in the oral cavity. It produces all the stages of oral carcinogenesis and several lines of evidences suggest that similar histological as well as molecular changes are observed in the human system. There are several review articles that collate the available information on the mechanisms of action of 4-NQO. In addition, studies have been conducted for the development of biomarkers and chemopreventive agents using 4-NQO animal models [8–10, 61, 66, 67, 74–86].

The complexity and variety of biochemical changes that can increase tumor development is demonstrated in the *p53<sup>-/-</sup>* mice [97]. Unfortunately, this model and other genetic mouse models have not been exploited for studying the relationships among chemical oral carcinogenesis, specific genetic defects, and chemoprevention. Genetically altered mouse and rat models have been developed to evaluate molecular-targeted prevention and treatment of oral carcinoma [64]. The *rasH2* transgenic mouse carcinogenesis model [98] and human *c-Ha-ras* proto-oncogene transgenic rat model [99] have been developed for chemoprevention studies on oral (tongue) carcinogenesis.

## 6. Chemoprevention

Chemoprevention is the use of natural or synthetic substances to halt, delay, or reverse malignant progression in tissues at risk for the development of invasive cancer [8–10].

Retinoids are the most extensively studied agents for chemoprevention of oral cancer [100]. Administration of 13-*cis*-retinoic acid for only 3 months yields a clinical response rate of 67% versus 10% for placebo. However, the toxicity is considerable, and there is a very high rate of relapse within 3 months of stopping treatment. Subsequent studies with retinoids in patients with oral premalignant lesions have confirmed clinical and pathologic response rates, though toxicities remain a concern [101]. However, translational studies show that molecular abnormalities persist in some patients with a complete clinical and pathologic response to retinoid therapy [102], suggesting that cancer development may be delayed rather than prevented by these agents. Other agents that have been assessed in clinical trials to evaluate the chemoprevention activity in oral leukoplakia patients include vitamin E [52], Bowman-Birk inhibitor concentrate (BBIC) derived from soybeans [103], curcumin [104], and green tea polyphenol epigallocatechin-3-gallate. Small clinical trials using oral BBIC have revealed no significant toxicity and a 32% response rate [103].

Attention is currently focused on the development of agents targeted to specific steps in the molecular progression from normal to oral premalignancy and to invasive carcinoma. Examples of molecularly targeted agents that have shown promise *in vitro*, in animal models, or in early clinical trials include cyclooxygenase- (COX-) 2 inhibitors and epidermal growth factor receptor (EGFR) inhibitors [105–107]. Data from several sources suggest that the cyclooxygenase pathway is a good target for oral cancer prevention. COX-2 is overexpressed in head and neck squamous carcinoma [108], and COX-2 inhibitors prevent oral cancer development in animal models [109]. A randomized placebo-controlled trial of the COX-2 inhibitor ketorolac administered as an oral rinse in oral leukoplakia patients revealed that the treatment is well tolerated but does not result in a greater clinical response than placebo [110]. However, an analysis of the results of this trial is somewhat confounded by the high response rate (32%) in the placebo arm and difficulty in determining whether topical delivery of the agent allowed penetration to the damaged cells. The future of COX-2 inhibitors as chemoprevention agents will also depend on determining the extent of risk for cardiac toxicities associated with this class of agents. The EGFR is also a promising molecular target for intervention in oral malignant progression [105–107]. EGFR is a receptor tyrosine kinase that is overexpressed in oral dysplasia and invasive cancer and associated with poor prognosis in patients with head and neck squamous carcinoma [111, 112]. EGFR inhibitors, alone or in combination with chemotherapy and radiotherapy, show activity against head and neck squamous carcinoma in clinical trials and are generally well tolerated [113]. Evidence suggests that combination therapy targeting COX-2 and EGFR may be efficacious [107, 114]. Although chemoprevention appears to be a promising approach to managing oral premalignancy, prospective clinical trials using specific agents, and strong corollary translational and laboratory investigations, are needed to evaluate clinical, histological, and molecular efficacy. It may be possible and

necessary to individualize medical therapy to specific genetic abnormalities detected within the oral mucosa.

## 7. Conclusion

Human oral cancer is the sixth largest group of malignancies worldwide. Seventy percent of oral cancers appear from premalignant lesions. The process of formation of oral cancer results from multiple sites of premalignant change in the oral cavity (field cancerization). Animal models are now being widely used for the development of diagnostic and prognostic markers. The appearance of these premalignant lesions is one distinct feature of human oral cancer. There is currently a dearth of biomarkers to identify which of these lesions will turn into malignancy. Regional lymph node metastasis and locoregional recurrence are the major factors responsible for the limited survival of patients with oral cancer. The paucity of early diagnostic and prognostic markers strongly contributes to the higher mortality rates. Determining high- and low-risk populations by measuring reliable biomarkers is expected to contribute to achieving a better understanding the dynamics and prevention of oral cancer development. The quantitation of genetic and molecular changes and the use of these changes as markers for the detection and prevention of early premalignant change require the harvesting of tissues and cells. Promising technologies are being rapidly developed to assist in the identification of an abnormal oral mucosa, noninvasive and objective diagnosis and the characterization of identified mucosal lesions, and in the therapies for patients with oral cancer. Undoubtedly, the prevention or reduction in the use of tobacco products and alcohol consumption would have a profound influence on the incidence of oral cancer. Chemoprevention also has an impact on the development of malignant changes in the oral mucosa. Prevention through chemoprevention and/or the use of systemic medications is an extensively studied strategy and continues to hold promise as a way of diminishing the morbidity and mortality associated with this malignancy.

## Abbreviations

BBIC:	Bowman-Birk inhibitor concentrate
COX:	Cyclooxygenase
DMBA:	7,12-dimethylbenz( <i>a</i> )anthracene
EGFR:	Epidermal growth factor receptor
HPV:	Human papillomavirus
IL:	Interleukin
20-MC:	20-methylcholanthrene
NORs:	Nucleolar organizer regions
4-NQO:	4-nitroquinoline 1-oxide
RAR:	Retinoid acid receptor
TNF:	Tumor necrosis factor.

## Conflict of Interests

The authors declared that there is no conflict of interest.

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