Oral Oncogenesis and Chemoprevention

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Summary

Despite considerable progress in the prevention, detection, and treatment of several cancers, most oral squamous cell carcinomas are detected in the advanced stages. In addition, the multistep process of oral oncogenesis involving accumulation of genetic alterations is complex and not yet fully understood. This short review focuses the current understanding oral oncogenesis, biomarkers, chemoprevention and rodent models for oral cancer for understanding and fighting oral cancer development.

Keywords: Oral cavity; Squamous cell carcinoma; Oncogenesis; Animal model; Chemoprevention

Abbreviations: OSCC: Oral Squamous Cell Carcinoma; ADH3: Alcohol Dehydrogenase Type 3; HPV: Human Papilloma Virus; IL: Interleukin; EGFR: Epidermal Growth Factor Receptor; MMP: Matrix Metalloproteinase; MN: Micronucleus; miRNAs: MicroRNAs; 4-NQO: 4-nitroquinoline 1-oxide; NORs: Nucleolar Organizing Regions; COX: Cyclooxygenase

Introduction

Head and neck cancers, including oral carcinoma, is the sixth most common human cancer, representing 3% of all types of cancer [1]. Forty-eight percentages of head and neck cancers develop in the oral cavity covered by stratified squamous epithelium and 90% of oral cancers are histopathologically squamous cell carcinoma [2,3]. They are preceded by precancerous lesions. Oral squamous cell carcinoma (OSCC) occurs within a field of precancerized oral epithelium. Additional acquired genetic alterations of precancerous squamous cells may result in OSCC development [4]. There are more than 300,000 new cases of OSCC annually [5]: 35,000, 40,000 and 10,915 new OSCCs are recorded annually in the US [6], EU [7] and Japan [8]. Intraoral cancers most commonly occur in the tongue. They locate on the tongue, floor of the mouth, lips, salivary glands and the oropharynx. Precancerous lesions often are derived from the same initial clone [4,8].

Oral Oncogenesis

Oral oncogenesis possesses complex and multiple processes that start when squamous epithelial cells acquire several genetic alterations [11]. Recently, high-throughput approaches have been introduced for searching oral cancer biomarkers in saliva and serum, called “biofluids” [12,13]. Natural history of oral cancer and sequential genetic changes are illustrated in Figure 1.

The theories of the “field cancerization [14,15]” or “field precancerization [4]” may explain potential development of cancers at multiple sites that covered by the same type of epithelium, such as head and neck (squamous epithelium) and urological tissues (urothelium). Oral cancers develop over many years and at multiple sites, neoplastic transformation occurs together with oncogenes mutation throughout the oral cavity during this period [16]. Inactivation in tumor suppressor genes, such as p53, caused by smoking is an increased risk for OSCC [17]. The continual presence of mutations may also produce changes in DNA repair and apoptosis. These changes are known to increase the susceptibility for future transformation. Recent genetic analysis has revealed that cancers developing at distant sites within the oral cavity often are derived from the same initial clone [4,8].

Risk factors

The most important risk factors for OSCC development include the consumption of tobacco and alcohol [18,19]. Although drinking and smoking are independent risk factors [20], they synergistically affect oral oncogenesis as risk factors. In Asian countries, the use of smokeless tobacco products, such as gutkha and betel quid, is responsible for a considerable percentage of OSCC patients [5]. Polymorphism of CYP1A1 in the xenobiotic metabolism pathways or the genes coding for glutathione S-transferase and N-acetyltransferase may be involved in OSCC development [21-23]. People who carry alcohol dehydrogenase type 3 (ADH3) allele may be at increased risk to develop oral cancer [24,25]. The use of smokeless tobacco is another cause of oral cancer [26-29]. In South and Southeast Asia, where OSCC accounts for up to quarter of all malignancies [30-32], betel-quid chewing is an important risk factor.
risk factor [33-35]. Betel nut could promote hepatocarcinogenesis as well as tongue carcinogenesis [36].

Human papilloma virus (HPV), especially HPV type 16 [37], may be an etiologic factor among persons who are non-smokers or non-drinkers [38-40]. Recently there has been an increasing body of evidence to suggest a possible relationship between infection/inflammation and oral cancer development [41,42]. Recent reports have suggested that patients with inflammatory bowel disease belong to the high-risk group of developing oral cancer and premalignancy, a phenomenon amplified by the increasing HPV prevalence [43,44].

Histopathologically preneoplastic lesions for OSCC include leukoplakia, erythroplakia, nicotine stomatitis and tobacco pouch keratosis, lichen planus and submucous fibrosis [45]. In 1877, Dr. Schlimmer first used the term “leukoplakia” to describe a white lesion of the tongue representing syphilitic glossitis [46]. Based on the definition of the World Health Organization, leukoplakia is a white patch or plaque that cannot be characterized clinically or pathologically as any other disease [47]. Leukoplakia is observed most frequently in over middle-aged men, with an increasing prevalence with age [48]. The most common sites of leukoplakia include the buccal mucosa and lower lip, but dysplastic lesions occur in the floor of mouth, lateral tongue, and lower lip [49].

Dr. Queyrat first used the term “erythroplasia” to describe red precancerous lesions in the penis [50]. The lesion shows a clinically and histopathologically similar process that occurs on the oral mucosa [51]. Oral erythroplakia occurs most frequently in older men and the floor of mouth, lateral tongue, retromolar pad, and soft palate are the most common sites of involvement [51]. Some lesions may be intermixed with white areas (erythroleukoplakia).

Nicotine stomatitis most frequently related to pipe smoking is a thickened, hyperkeratotic alteration of the palatal mucosa [47].

**Biomarkers**

Alterations in biomarkers will help us to detect the genetic and molecular changes during the process of oral oncogenesis [52]. They are useful for screening, risk assessment, prediction of recurrence, and therapeutic efficacy of oral cancers. Biomarkers listed in Table 1 are roughly classified as genomic, proteomic, and metabolomics [33,53-62].

| Potential biomarkers are the oncogenes (epidermal growth factor receptor (EGFR) and cyclin D1), the tumor-suppressor genes (p53 and p21), and the apoptotic effector (Bcl-2) [59]. A salivary biomarker, cluster of differentiation factor 34, is able to identify recurrence of OSCC. Integrin α3 and integrin β4, are the genomic biomarkers for estimating invasion and dissemination of OSCC [56]. Other salivary biomarkers for oral cancer include mRNA and proteins for interleukin (IL)-8, CD44, matrix metalloproteinase (MMP)-1, MMP-3, Cyfra 21-1 and ZNF510, in spite of no sufficient scientific evidence to predict and diagnose of oral cancer [55].

Genomic instability can be estimated by micronucleus (MN) assay. It is known that genotoxic/carcinogenic chemicals are potent clastogenic and mutagenic agents to induce chromatid/chromosomal aberrations, which result in MN. Since gradual increase in number of MN from normal oral mucosa through oral premalignancy to OSCC is observed, MS is suggestive of a biomarker for neoplastic progression. When MN scoring is used in exfoliative oral cytology, MN could be an earlier biomarker to identify preneoplastic oral lesions. MN counting can also be used for screening of high-risk population of OSCC [65]. MicroRNAs (miRNAs) belonging to class of small non-coding RNAs regulate numerous biological processes by targeting messenger RNAs. Overexpression of oncogenic miRNA may reduce protein products of tumor-suppressor genes. On the other hand, loss of tumor-suppressor miRNA expression may cause elevated levels of oncogenic protein.

**Table 1: Potential biomarkers for oral carcinogenesis.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Biomarkers</th>
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<tbody>
<tr>
<td>Genomic</td>
<td>Micronuclei</td>
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<tr>
<td>Oncogenic</td>
<td>Modified oncogenes’ expression</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>EGFR expression</td>
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<tr>
<td>Differentiation</td>
<td>Cytokeratins</td>
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<tr>
<td>Oxidative stress</td>
<td>Glutathione S-transferase</td>
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<tr>
<td>Apoptosis</td>
<td>Bcl-2 family</td>
</tr>
<tr>
<td>Immunologic and/or Inflammatory</td>
<td>Cytokines</td>
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High-risk HPV-type infection causes aberrant expression of cellular oncogenic and tumor suppressive miRNAs [53]. Increases in expression of multiple miRNAs in the biofluids could be non-invasive biomarkers for detecting an early stage of oral oncogenesis [13].

**Animal model**

A variety of animals has been used for the study of tumor growth, the process of carcinogenesis and the prevention/treatment research [64-67]. The most widely used animal models for oral carcinogenesis are the hamster cheek pouch model [66] and the 4-nitroquinoline 1-oxide (4-NQO)-induced oral (tongue) carcinogenesis model [68]. The continual development of transgenic or knockout mice has improved our understanding of the role of specific genes in tumor growth [69-71]. In the hamster model, a complete carcinogen, 7,12-dimethylbenz(a)anthracene (0.5%), is applied to the hamster cheek pouch (3 times/week for 16 weeks) to produce invasive OSCC. The 4-NQO models for the study of oral carcinogenesis include those in rats and mice. 4-NQO is a water soluble carcinogen, 4-NQO. The carcinogen is supplied in the water (20 ppm) or by painting for rodents to induce oral cancer. Administration with 4-NQO in drinking water (20 ppm) for 8 weeks in rats and mice produces tongue squamous cell neoplasms within 32 weeks [72]. 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 [36,65,73-75]. Increases in the tissue level (oral mucosa) of polyamine synthesis and the number of nuclear organizing regions (NORs) of oral preneoplastic and neoplastic squamous cells have been noted [65], suggesting that polyamine levels and counting NQOs are potential biomarkers of oral oncogenesis.

**Chemoprevention of Oral Cancer**

Cancer chemoprevention is the use of natural or synthetic substances to delay or reverse malignant progression in certain tissues with risk to develop invaded cancer [9,10]. Recently, researchers focus development of agents targeted to specific steps in oncogenesis. Examples of molecularly targeted agents include inhibitors of cyclooxygenase (COX)-2 and EGFR [76,77]. Cyclooxygenase pathway is a good target for oral cancer prevention, since COX-2 is overexpressed in head and neck squamous carcinoma [78]. The receptor tyrosine kinase EGFR is also a promising molecular target for intervention against oral...
oncogenesis [77]. EGFR is overexpressed in oral preneoplastic and
cancerous lesions and associated with worse prognosis in patients
with OSCC [79]. EGFR inhibitors, alone or in combination with
chemotherapy and radiotherapy, have been effective against head
and neck squamous carcinoma in clinical trials [80].

Other potential chemopreventive agents against oral cancer
development include protochatechuic acid [81], green tea polyphenols
[82,83], metformin [84], flavonoids [85], polyphenols [86], non-
steroidal anti-inflammatory drugs (NSAIDs) [87], peroxisome
proliferator-activated receptor (PPAR)γ [88], and Bowman-Birk
Inhibitor [89].

Although chemoprevention studies have currently matured, we
should keep in mind the risk of chemoprevention due to long-term use of
potential chemopreventive agent(s) [90].

Conclusion
Field cancerization or field precancerization can be applied to oral
oncogenesis. Animal models of oral oncogenesis are widely used for
development of diagnostic and prognostic biomarkers, understanding
multistep oncogenesis, establishment of cancer chemoprevention and
development of effective therapeutic methods. Determining high and
low-risk populations by reliable biomarkers help us to understand the
mechanisms and prevention of oral oncogenesis. The biomarkers that
are modified during oncogenesis could be used for the detection and
prevention of early premalignant changes. Cancer chemoprevention
has an impact on the reduction of oral malignancy.

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