

REVIEW ARTICLE

Role of human papillomavirus and tumor suppressor genes in oral cancer

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ABSTRACT

The incidence of oral cancer remains high and is associated with many deaths in both Western and Asian countries. Several risk factors for the development of oral cancer are now well known, including smoking, drinking and consumption of smokeless tobacco products. Genetic predisposition to oral cancer has been found in certain cases, but its components are not yet entirely clear. In accordance with the multi-step theory of carcinogenesis, the natural history of oral cancer seems to gradually evolve through transitional precursor lesions from normal epithelium to a full-blown metastatic phenotype. A number of genomic lesions accompany this transformation and a wealth of related results has appeared in recent literature and is being summarized here. Furthermore, several key genes have been implicated, especially well-known tumor suppressors such as the cyclin-dependent kinase inhibitors, TP53 and RB1 and oncogenes such as the cyclin family, epidermal growth factor receptor and RAS. Viral infections, particularly oncogenic human papillomavirus subtypes and Epstein-Barr virus, can have a tumorigenic effect on oral epithelia and their role is discussed, along with potential therapeutic interventions. A brief explanatory theoretical model of oral carcinogenesis is provided and potential avenues for further research are highlighted.

Key words: Epstein-Barr virus, oncogenes, oral cancer, tumor suppressor genes, human papillomavirus

INTRODUCTION

Oral cancer refers to a subgroup of head and neck malignancies that develop at the lips, tongue, salivary glands, gingiva, floor of the mouth, oropharynx, buccal surfaces and other intra-oral locations, according to the International Classification of Diseases (ICD version 9, categories: 140–146, 149). Nevertheless, the term is synonymous to squamous cell carcinoma (SCC) of oral mucosal origin that accounts for more than 90% of all malignant presentations at the aforementioned anatomical sites.^[1] More than 300,000 new cases worldwide are being diagnosed with oral SCC (OSCC) annually. Approximately, 30,000 new cases are recorded annually in the US and 40,000 new cases are recorded in the Europe. Oral cancer is estimated by the WHO to be the eighth

most common cancer worldwide.^[2] However, the incidence of oral cancer has a significant local variation and is increasing in some parts of the world. In India and other Asian countries, oral and oropharyngeal carcinomas (OCs) 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.^[3]

RISK FACTORS

The most important risk factor for the development of oral cancer in the Western countries is the consumption of

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tobacco and alcohol.^[4] 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, pan masala and betel quid is responsible for a considerable percentage of oral cancer cases.^[5] Several studies have reported a significant familial component in the development of oral cancer. The estimates of risk in first-degree relatives of oral cancer patients vary widely and have been reported to be 1.1, 9.25, 10.3, 5.11 or 3.812 in various studies, although it should be noted that some of these studies refer to head and neck cancer in general.^[6] Oral cancer patients whose relatives have upper respiratory and digestive tract tumors are also more likely (odds ratio 3.8) to develop a second primary tumor, an important cause of treatment failure. Familial aggregation of oral cancer, possibly with an autosomal dominant mode of inheritance, was reported in a very small percentage of oral cancer patients, but further details are lacking.^[7]

The familial risk for oral cancer could be acquired as a result of imitating high-risk habits within the family, such as smoking and drinking, or as a genetic trait.^[8] Polymorphic variation of genes in the xenobiotic metabolism pathways may be implicated, such as in CYP1A1 or the genes coding for glutathione S-transferase-M1 and N-acetyltransferase-2. Individuals that carry the fast-metabolizing alcohol dehydrogenase type 3 allele 18 may be particularly vulnerable to the effects of chronic alcohol consumption and could be at increased risk to develop oral cancer although newer evidence does not support this association. A recent review has highlighted the necessity for larger and more extensive studies to resolve this issue. Finally, the single nucleotide polymorphism A/G870 in the CCND1 gene that encodes cyclin D has been associated with oral cancer susceptibility.^[9] The AA genotype may increase risk (odds ratio 1.77) for head and neck cancer or oral cancer (odds ratio 2.38). Intriguingly, in another study, it was the GG wild-type genotype, instead of the AA genotype, that was associated with increased susceptibility to oral cancer (GG genotype, odds ratio 3.37).

STAGING AND PROGNOSIS

Staging of oral cancer is conventionally performed with the use of the “tumor, node, metastasis” (TNM) classification system and its variant (pathological TNM), which are, respectively, based on a clinical and pathological assessment of tumor size and lymph node involvement.^[10] However, traditional staging is often inadequate and does not always provide accurate prognostic information. New tumor characteristics, such as locoregional control, extent of recurrence, maximum tumor thickness, differentiation grade and mode of invasion, are being utilized to refine prognosis and allow the selection of appropriate treatment. The multi-step model of carcinogenesis is widely accepted and requires the step-wise transition from

pre-malignant lesions to the metastatic tumor phenotype. A variety of alterations accumulates to potentiate this transition and gradually increase malignancy. A similar progression has been shown to occur in oral cancer from benign hyperplasia, to dysplasia, to carcinoma *in situ* and advanced cancer with accompanying genomic alterations.^[11]

THERAPY

The therapy of oral cancer is not always satisfactory. Early Stage (I and II) oral cancer may be curable by surgery or radiation therapy alone, but advanced cancers (Stages III and IV) are generally treated by surgery followed by radiation therapy.^[12] Using multimodal protocols that combine surgery with preoperative or postoperative radiotherapy and/or adjuvant chemotherapy the 2-year and 5-year survival rates for advanced cancers were as low as 20% and 12%, respectively.^[13] In fact, survival of advanced-stage patients rarely exceeds 30 months, even for those that initially achieve complete clinical remission. Furthermore, most oral SCCs exhibit limited responsiveness to common cytotoxic drugs, due to mechanisms that either block the transport of these agents into the cells or interfere with their intracellular molecular targets.^[14] Fortunately, new sensitive kits for early tumor detection are being developed many of which are based on the molecular analysis of exfoliative cytology or saliva. Clearly, a better understanding of the molecular profile of oral cancer should facilitate the development of more efficient targeted therapies. Growth receptors are known to induce different cellular responses in response to the binding of specific ligands that represent external stimuli. The ErbB family of receptors and the epidermal growth factor receptor in particular (EGFR, also known as ErbB1 or Her-1) has received attention due to its inherent ability to stimulate the proliferation of epithelial cells. The usefulness of gefitinib (“Iressa”), a recently developed EGFR inhibitor, has been evaluated in oral cancer cell lines, oral cancer xenografts in mice and patients with advanced head and neck cancer with mixed results, but large-scale human studies of its efficiency in oral or head and neck cancer are lacking. A novel monoclonal antibody against Her-2 (trastuzumab) may serve as targeted adjuvant therapy for a subgroup of patients in the future, but extensive trials are required to justify its use in oral cancer. Anti-EGFR monoclonal antibodies (mAbs) and EGFR-tyrosine kinase inhibitors have been studied the most and are furthest along in clinical development. Monoclonal antibodies prevent ligands from binding to the EGFR extracellular domain, inhibiting receptor activation and facilitating receptor degradation. Another monoclonal antibody targeting strategy is conjugating anti-EGFR mAbs with toxins, which mediated indirect action by the immune system to selectively attack tumor cells overexpressing EGFR. Hence, EGFR-targeted therapy will play a major role in the treatment of oral cancer in future.^[15]

GENOMIC ALTERATIONS

Theory of field cancerization

The aggregation of genomic alterations during phenotypic progression is assumed to happen in a wide population of cells, a heterogeneous “field of genetically altered cells” that is expected to give rise to precursor lesions. This theory attempts to explain the frequent local recurrence and the emergence of the second primary tumors in oral cancer.^[16] According to a recent adaptation of this concept, the genetically altered cells will gradually proliferate and expand into a noninvasive field that is vulnerable to further genomic damage. This field, despite being macroscopically undetectable, is fertile ground for the evolution of premalignant lesions and eventually invasive cancer. Although local excision can completely remove an oral carcinoma, the field may persist and the patient can be at risk for the subsequent appearance of a second tumor from the same field. The exact molecular characteristics of a susceptible genetically altered field are not clearly defined, but key tumor suppressors such as TP53, CDKN2A and the pRb pathway are likely to be compromised from its early stages.^[14]

Oncogenes

Oncogenes are genes that are able to increase malignant potential. Many of the major oncogenes that are implicated in other cancer types also contribute to oral cancer. A large number of these genes promote unscheduled, aberrant proliferation, override the G–S, G–M and M checkpoints of the cell cycle, prevent apoptosis and enable cellular survival under unfavorable conditions.

Growth receptors are known to induce different cellular responses in response to the binding of specific ligands that represent external stimuli. The ErbB family of receptors and the EGFR in particular (EGFR, also known as ErbB1 or Her-1) has received attention due to its inherent ability to stimulate the proliferation of epithelial cells. Amplification of EGFR is found in a considerable percentage of oral tumors and also in premalignant lesions. Although several studies demonstrate the association between EGFR overexpression and tumor grade or stage there are few studies that determine its practical clinical usefulness. EGFR overexpression was reported to be an independent prognostic marker of survival in betel quid chewers and a component of a prognostically significant molecular profile. Other members of the ErbB family are also able to exert transforming effects. ErbB2 (also known as Her-2 or Neu) amplification has been found in oral cancer specimens, nondysplastic oral leukoplakia and patient sera. Notably, ErbB2 overexpression seems to be more frequent in oral cancer than in head and neck cancer. High levels of ErbB2 may be associated with worse prognosis. Cyclin D single nucleotide polymorphism has been associated with susceptibility to oral cancer. Cyclin A overexpression, which is closely associated with the presence of S-phase cells,

has also been observed immunohistochemically and was most prevalent in advanced tumors.

Similarly, cyclin B was overexpressed in 37% of tongue tumors and in oral cancer in general. Angiogenesis, the formation of new vessels from preexisting ones, is a crucial step in tumor growth, progression and metastasis. Regulation of angiogenesis *in vivo* is complex and is controlled by a variety of factors. Among them, vascular endothelial growth factor (VEGF) is considered to play a dominant role. It has been well established that VEGF promotes the progression of OSCC by up-regulating microvessel density. Its enhanced expression in oral malignant tumors may be triggered by a hypoxic stimulus.^[17]

Tumor suppressors

Tumor suppressors are genes that prevent cells from acquiring malignant characteristics. Tumor suppressor genes are usually entrusted with the regulation of discrete checkpoints during cell cycle progression and with the monitoring of DNA replication and mitosis. Cellular stress and a variety of insults can activate tumor suppressor pathways to arrest the cell cycle. The retinoblastoma protein and its associated molecular network are frequent and early targets in many tumor types. When in a hypo-phosphorylated state, the retinoblastoma protein and the other pocket protein family members p107 and p130 bind and inactivate the E2F transcription factors which are essential for cell cycle progression from G to S phase. Lack of immunohistochemical pRb expression was found in approximately 70% of oral tumors and 64% of premalignant lesions. Similarly, in a later study, about half of oral cancer specimens did not express pRb and 20% of those that did express pRb only contained the inactive, phosphorylated form. Most importantly, 84% of premalignant lesions and 90% of OSCCs show altered expression of at least one of the components of the pRb network.^[16] The cyclin-dependent kinase inhibitors, in particular, are known targets in oral cancer, most likely due to their ability to prevent pRb phosphorylation. The CDKN2A locus that encodes p16INK4A is located in 9p21, one of the most vulnerable areas of the genome in oral cancer, as discussed above. Indeed, lack of immunohistochemical p16 expression can be found in up to 83% of oral tumors and up to 60% of premalignant lesions. The predominant mode of inactivation is an allelic imbalance, but point mutations and promoter methylation also occur with lower frequency. The alternative CDKN2A transcript, p14ARF, is also commonly suppressed, but down-regulation of other INK4 family members, like p15INK4B, is less frequent. The prognostic significance of p16INK4A levels is uncertain, although a study has reported favorable prognosis for patients overexpressing p16INK4A. The deregulation of the p53 tumor suppressor network is observed in many tumor types, including oral cancer. In fact, the activation of the DNA damage response is one of the earliest findings in the natural history of cancer. The p53 protein is able to enforce cell cycle arrest or apoptosis under

replication stress, thus halting the proliferation of potentially malignant cells. As mentioned above, loss-of-heterozygosity in the 17p13 region that hosts the TP53 gene is very common in oral cancer. Immunohistochemical evaluation for p53 is positive in up to 57% of oral tumors but is also positive in distant, macroscopically normal areas, in accordance with the theory of “field cancerization.” The initial alterations seem to occur at the basal cell layer under the influence of smoke, alcohol and or other carcinogens and may involve deactivation of TP53 and other key tumor suppressors. The transition of normal epithelium to invasive cancer is—more often than not—progressive and is accompanied by “multiple hits” which promote proliferation, angiogenesis, local invasion and eventually, distant metastatic spread.^[18]

VIRAL INFECTIONS

Viral etiopathogenesis in oral cancer

The role of oncogenic viruses in human cancer is an emerging area of research. Viruses are capable of hijacking host cellular apparatus and modifying DNA and the chromosomal structures and inducing proliferative changes in the cells. Human papillomavirus (HPV) and herpes simplex virus (HSV) have been established in recent years as causative agents of OC. HPV has been identified in approximately 23.5% of OC cases.^[19] The most commonly detected HPV in head and neck SCC (HNSCC) is HPV-16, which has been demonstrated in 90–95% of all HPV-positive HNSCC cases, followed by HPV-18, HPV-31 and HPV-33. The prognostic significance of HPV in pre-cancerous oral lesion is not clear. However, few studies have found improved disease-specific survival and better prognosis for HPV-positive OC. HSV-1 or “oral herpes” is commonly associated with sores around the mouth and lip and has been suggested to be a causative agent of OC. Epidemiological studies showed higher level of immunoglobulin G and immunoglobulin M antibodies to OC patients compared to control subjects. It is also been reported that oncogenic relationship between HSV-1 and OSCC exists. A population-based study showed HSV-1 to enhance the development of OSCC in HPV infected patients and individuals with a history of cigarette smoking. Risk of oral cavity and pharyngeal cancer is two-fold higher among human immunodeficiency virus (HIV) patients indicating a link between HIV and OSCC. Epstein–Barr virus (EBV), human herpesvirus-8 and cytomegalovirus have also been reported as risk factors of OSCC in different studies.^[7]

Human papillomavirus

A plethora of viral agents has been linked to human tumors. Among these, HPV holds a prominent position. To date, more than one hundred HPV types have been identified and are referred to as “low” or “high risk” according to their oncogenic potential. Two products, in particular, of the early genomic region of high-risk HPVs are capable of

forming specific complexes with vital cell-cycle regulators: E6, which binds to p53 and induces its degradation and E7, which interacts with pRb and blocks its downstream activity. Functional deregulation of these key oncosuppressors results in uncontrolled DNA replication and apoptotic impairment and explains the increased tumorigenic ability of high-risk types.^[11]

Epstein–Barr virus

The EBV is a member of the herpes virus family. Even though its contribution to the malignant transformation of B lymphocytes has been well established, the influence of EBV in the pathogenesis of OSCC remains elusive. It has been reported that EBV is more frequently detected in oral lesions such as oral lichen planus and OSCC in comparison with healthy oral epithelium.^[20] In another study, latent membrane protein-1, the principal oncoprotein of the virus, has been found in many EBV-positive OSCCs, which means that this latent infection may play a role in the malignant transformation of the oral mucosa. However, these findings are not universal and several studies have reported the lack of a conclusive relation between EBV and oral cancer or premalignant lesions. Considerable skepticism is justified, in view of the variability between studies that employ different detection methods and refer to different patient populations.^[5]

Hepatitis C virus

Oral verrucous and SCCs have been reported in HCV-infected patients, while HCV infection has been found to be more prevalent in patients with oral lichen planus.^[3] However, 1–2% of the patients with oral lichen planus (OLP) develop SCC of the oral cavity, which implies the existence of common pathogenic mechanisms among them. Finally, HCV-RNA strands were detected in OLP tissues and there is evidence to indicate that HCV may occasionally replicate in oral lichen tissue and contribute to mucosal damage.^[9]

A brief explanatory theoretical model of oral carcinogenesis is provided in Figure 1.^[21]

CONCLUSION

The study of oral cancer is particularly challenging. Oral cancer is an important cause of morbidity and mortality, especially in developing countries and its prevalence may rise in the foreseeable future. Advances in diagnosis and treatment have slowly accumulated, but a sound understanding of underlying cell biology is likely to enable further, much-needed progress.

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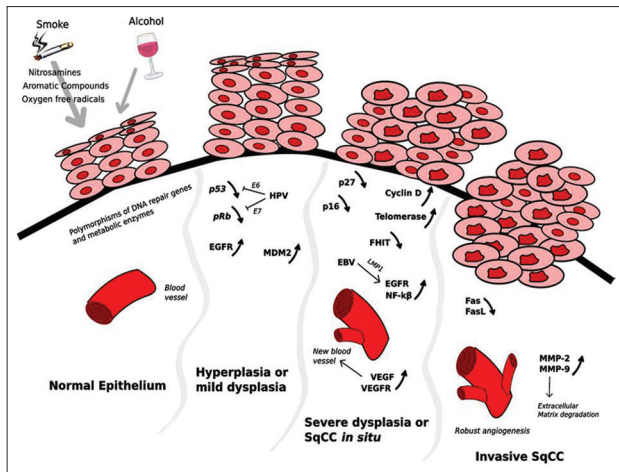


Figure 1: Theoretical model of carcinogenesis in the oral cavity based on the "multiple hit" hypothesis. The majority of the molecular genetic lesions that accompany the histological transition from normal to cancerous epithelium persist during later stages, but they are presented in the stage of their appearance. (Modified from: Tsantoulis PK *et al.* *Advances in the biology of oral cancer.* *Oral Oncol* 2007)

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Conflicts of interest

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