Microorganisms and cancer of the oral cavity

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Abstract

Numerous microorganisms, such as bacteria, viruses and fungi, inhabit the oral cavity, and it has been pointed out that poor oral hygiene and chronic periodontitis increase the risk of oral squamous cell carcinoma (OSCC). However, the molecular mechanism whereby the risk of OSCC is increased under such conditions has not yet been clarified. Microbes in the oral cavity may elicit both innate and acquired immune responses, resulting in the establishment of uncontrolled inflammation such as chronic periodontitis. Further microbial attack and various host-derived factors may subsequently contribute to events such as genetic and epigenetic alterations, inhibition of apoptosis, increased cell growth, promotion of invasion and metastasis, and lymphangiogenesis, thus linking the chronic inflammation to OSCC. Here we review the various factors involved in promoting the development and progression of OSCC. The facts suggest the importance of early prevention and treatment of chronic periodontitis to maintain oral health and prevent serious diseases such as OSCC.

Introduction

Most malignant neoplasms of the oral cavity are squamous cell carcinomas (SCCs) [1], and many factors are involved in the development of oral squamous cell carcinoma (OSCC) [2]. Although the primary causes of OSCC are tobacco and alcohol [1], the malignancy can occur even in individuals who do not smoke and drink [3], suggesting that other factors may also play a role. It has been pointed out that poor oral hygiene [4-8] and chronic periodontitis [9-12] may increase the risk of oral cancer. Numerous microorganisms, inhabiting in the oral region, such as bacteria, viruses and fungi, may affect the development and progression of OSCC. In this article, we describe the association of oral microorganisms and host-derived factors with OSCC, and also emphasize the importance of early prevention and treatment of chronic periodontitis for maintaining oral health and preventing lethal diseases such as OSCC.

Dental caries and cancer

Dental caries, one of the two major common diseases of the oral cavity, is characterized by destruction of enamel, dentin and cementum by commensal gram-positive bacteria. As the condition progresses, pulpitis and apical periodontitis develop jointly as sequelae. In extreme cases, patients may develop osteomyelitis and periostitis of the jaw, maxillary sinusitis, or Ludwig's angina, leading to potentially fatal sepsis or systemic inflammatory response syndrome (SIRS) (Figure 1). Therefore, early prevention and treatment of dental caries are clearly very important.

Tezal *et al.* [13] have reported an inverse association between dental caries and head and neck squamous cell carcinoma (HNSCC) and showed an inverse association, although the reason for this remains unknown and further studies are needed.

Association between periodontal diseases and cancer

Periodontal disease is the other major common disease of the oral cavity. Not only does it lead to destruction of periodontal tissue accompanied by tooth loss, but also it is associated with systemic conditions such as cardiovascular disease, adverse pregnancy outcomes, rheumatoid arthritis, diabetes, lung diseases, and cancer development and progression [9-12,14-24] (Figure 2). Therefore, treatment and prevention of periodontal disease are also a major concern.

There is a close association between periodontal disease and HNSCC [9-12]. It has been reported that each millimeter of alveolar bone loss is associated with a 5.23-fold increase in the risk of tongue cancer [9] and with more than a 4-fold increase in the risk of HNSCCs such as oropharyngeal and laryngeal carcinomas [10]. This association between periodontal disease and HNSCC is remarkable even in nonsmokers, making periodontal disease an independent risk factor for HNSCC [10]. In addition, it has been indicated that patients with periodontitis are more likely to develop poorly differentiated OSCC than those without [10], showing the association of periodontal disease with the development as well as progression of oral cancer.
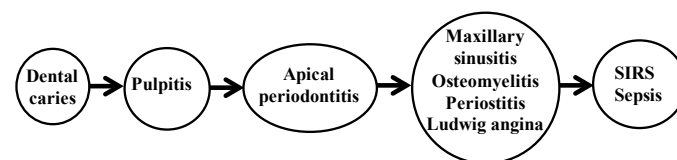

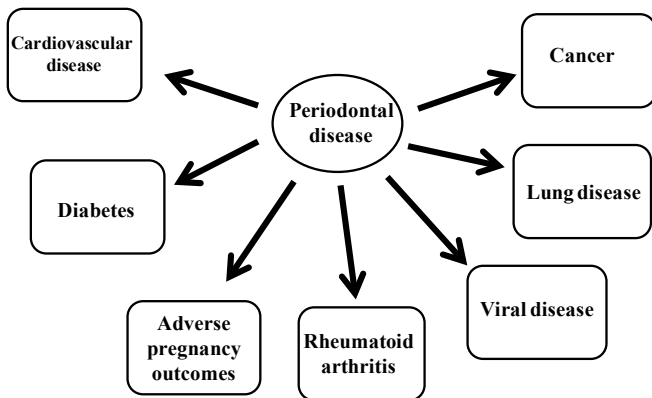**Figure 1.** Dental caries and its sequelae.

Figure 2. Systemic diseases associated with periodontal disease.

In addition to oral cancer, it has been shown that periodontal diseases are a risk factor for cancers of the oropharynx, larynx, lung, esophagus, pancreas, colon, rectum, kidney, and even hematopoietic organs [9-11,15-18,21-24].

However, all these findings have been revealed by epidemiological studies, and the actual mechanism responsible for the relationship between periodontal disease and the development and progression of human cancer remains to be determined.

Involvement of oral bacteria in the development and progression of oral cancer

Numerous microorganisms, such as bacteria, viruses and fungi, inhabit the oral region, and may be involved in the development and progression of oral cancer. Nagy *et al.* [25] reported that anaerobic bacterial species such as *Veillonella*, *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Actinomyces* and *Clostridium*, and the aerobic species *Haemophilus*, *Enterobacteriaceae* and *Streptococcus* are detected much more frequently in oral cancer tissue than in normal oral mucosa. *Candida albicans* is also found in oral cancer tissue. Therefore, the question arises as to whether these microorganisms play a role in oral carcinogenesis.

It is well known that *Helicobacter pylori* is associated with chronic atrophic gastritis and the development of gastric cancer [26,27]. It has been reported that ectopic expression of activation-induced cytidine deaminase (AID), a gene-editing enzyme, in gastric mucosal epithelium due to infection with *H. pylori* which harbors a gene family called 'cag' pathogenicity island (cagPAI) might be a mechanism of mutational accumulation in gastric carcinogenesis [28]. Oral epithelial dysplasia and squamous cell carcinoma are also often associated with chronic inflammation. We have found ectopic expression of AID in oral moderate epithelial dysplasia and squamous cell carcinoma, and our in vitro study revealed that the expression of AID is enhanced by TNF-α *via* NF-κB activation in an oral cancer cell line (Figure 3) [29].

IL-8 plays an important role in the induction of inflammation and cell proliferation, and its secretion is induced in chronic gastritis by resulting from *H. pylori* infection. Chemokines including IL-8, and their receptors are considered to be related to the development and progression of various cancers [30]. Do certain oral bacteria play a role in the oral region similar to that of *H. pylori* in the stomach? It has been described that the enhanced expression of IL-8 in oral cancer tissue and the higher concentration of serum IL-8 in oral cancer patients relate to the prognosis [31]. Furthermore, IL-8 increases the number of CD163 positive M2 macrophages in the invasive front of oral cancer [31].

Fibroblasts are known to express hepatocyte growth factor (HGF) by infection of oral bacteria and inflammatory cytokines. C-MET is a receptor of HGF and dysregulation of its signaling is observed in oral carcinogenesis [32]. In addition, *Actinobacillus actinomycetemcomitans*, *Escherichi coli* LPS and TNF-α upregulate the production of CCL20 in oral cancer cell lines, suggesting that CCL20 contributes to the oral immunoresponse to bacterial infection and is involved in the growth of OSCC [33]. These findings indicate that oral bacteria may be related directly or indirectly to the promotion of cell proliferation.

Oral bacteria are detected much more frequently on the surface of primary oral cancer tissue and in the metastatic lymph nodes, in comparison with normal oral mucosa and non-metastatic lymph nodes [34,35]. Oral bacteria may invade through areas of tissue surface destruction due to oral cancer and flow into the cervical lymph nodes, suggesting a contribution of oral bacteria to progression of oral cancer. In fact, *P. gingivalis* is able to invade gingival epithelial cells and survive within them [36,37]. *P. gingivalis* is known to prevent apoptosis of gingival epithelial cells through various mechanisms, thus allowing itself to survive [38-41]. Furthermore, it has been reported that *P. gingivalis* promotes invasion of oral cancer through induction of proMMP9 and its activation [42]. It also stimulates secretion of IL-6 and IL-8 from oral epithelial cells [43] and inhibits the secretion of CXCL9, 10 and 11, resulting in promotion of angiogenesis and tumor growth [44]. Gallimidi *et al.* [45] have reported that chronic infection of *P. gingivalis* and *F. nucleatum* promotes chemically induced OSCC in mice, with augmentation of the IL-6-STAT3 signaling pathway. We have shown that butyric acid produced by *P. gingivalis*, known to be an inhibitor of histone deacetylase (HDAC), increases the expression of podoplanin in oral cancer cell lines and promotes cell migration [46] (Figure 4). Butyric acid may induce epigenetic changes in periodontal and surrounding tissues. Recently, Planello *et al.* [47] reported a significant overlap between altered DNA methylation patterns in chronic periodontitis and OSCC.

Streptococcus thermophiles and *Streptococcus mitis* hidden in deep periodontal pockets exhibit alcohol dehydrogenase activity and produce acetaldehyde, a known carcinogen derived from alcohol, suggesting one mechanism whereby pathogenesis of oral cancer may occur [48].

These findings suggest that periodontal disease is associated with a number of mechanisms of oral cancer development and progression, such as genetic and epigenetic alterations, inhibition of apoptosis,
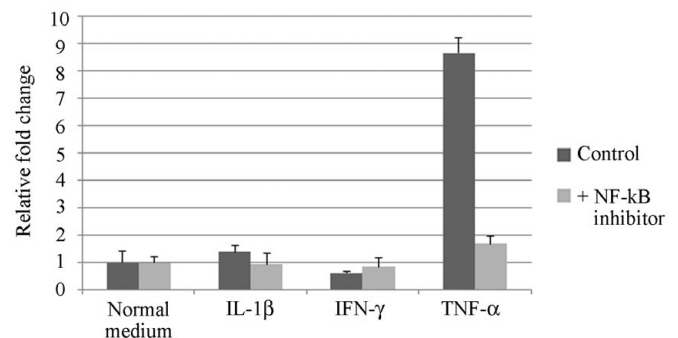

Figure 3. Effect of NF-kB activation by inflammatory cytokines on AID mRNA expression in HSC-2 cells[29]. After incubation of HSC-2 cells with inflammatory cytokines (interleukin-1β, IL-1β; interferon-γ, IFN-γ; tumor necrosis factor-α, TNF-α) with or without NF-kB inhibitor, RNAs were isolated from the cells. Real-time RT-PCR shows that NF-kB inhibitor attenuated AID mRNA expression stimulated by TNF-α. Each column and bar represents the mean ± SD of duplicate cultures.

increased cell proliferation, promotion of invasion and metastasis, and angiogenesis.

Involvement of viruses in the development and progression of oral cancer

Various viruses such as Epstein-Barr virus (EBV), cytomegalovirus, herpes simplex virus type 1 and human papilloma virus (HPV) are known to reside in the oral cavity [49-53].

Patients with HPV-positive tongue cancer have more significant alveolar bone loss than HPV-negative patients [54], and chronic periodontitis tends to be more common in HPV-positive patients with primary SCC of the pharynx, larynx and mouth [55]. As is the case for cervical cancer, HPV has long been considered to be involved in OSCC [56-58]. However, recent studies [59-62] have revealed that HPV is much more commonly associated with cancers of the pharynx, larynx and tonsil, than with oral cancer.

Latent EBV infection is common in adults. In the oral regions, EBV is detectable in normal gingival epithelium and significantly detected in periodontal disease [63]. EBV-associated tumors are divided into those of the epithelial and lymphatic systems. Among epithelial tumors, nasopharyngeal carcinoma is the most common tumor associated with EBV [64]. EBV has also been associated with cancers of stomach, salivary gland, and breast [65-67]. We examined EBV latent infection genes and their expression in normal and dysplastic oral epithelium as well as in squamous cell carcinoma, and showed an association of EBV with the dysplasia-carcinoma sequence [68] (Figure 5). Although EBV is also associated with Burkitt's lymphoma and Hodgkin's lymphoma [69], EBV latent infection genes and their expression have been detected in immunodeficiency-related lymphoproliferative disorders (LPDs) such as methotrexate (MTX) and age-related LPDs [70-73]. LPDs of the oral cavity occur as intractable ulcers and are associated with severe periodontal disease [71-73] (Figure 6).

Hepatitis C virus (HCV) is known to induce hepatitis, cirrhosis, and hepatocellular carcinoma. It has been reported that HCV infection is related to the occurrence of oral lichen planus and oral cancer, although the pathogenetic mechanism is unknown [74-76].
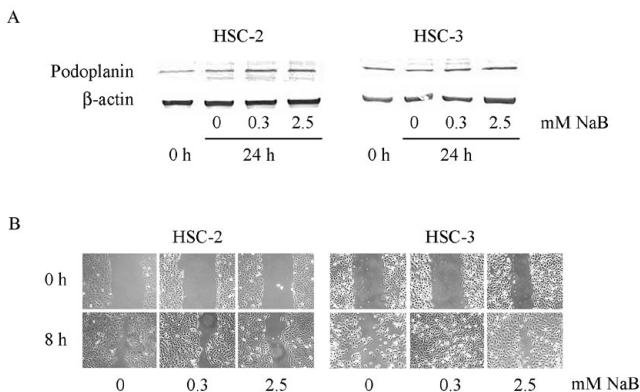

Figure 4. Effect of sodium butyrate (NaB) on podoplanin expression and oral cancer cell migration [46].A: Podoplanin and β–actin expression studied by Western blot analysis after incubation of HSC-2 and HSC-3 cell lines with 0.3 or 2.5 mMNaB for 24 h. B: Scratch assay to study the effect of NaB on cell migration. The HSC-2 and HSC-3 cell lines were incubated in 12-well tissue culture slides with 0.3 or 2.5 mMNaB for 8 h. These findings indicate that NaB promotes podoplanin expression and migration of oral cancer cells.
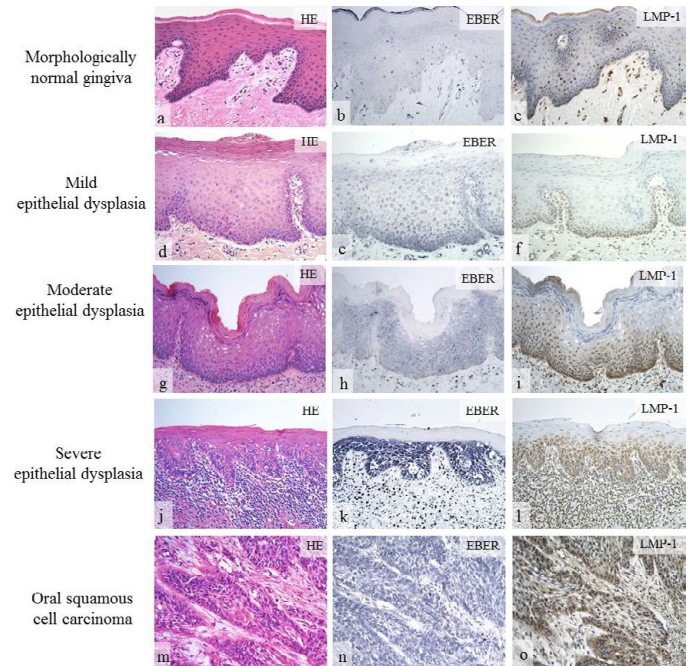

Figure 5. Expression of EBV latent infection gene in oral lesions [68]. In morphologically normal epithelium, expression of EBER and LMP-1 was negative or weakly positive (b, c). Expression of EBER and LMP-1 was greater in severe epithelial dysplasia (k, l) than in mild (e, f) to moderate (h, i) epithelial dysplasia. In oral squamous cell carcinoma, expression of EBER and LMP-1 was significantly positive (n, o).
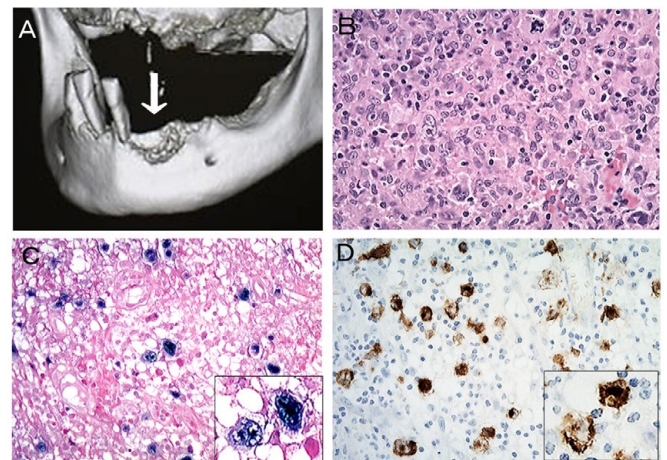

Figure 6. Age-related lymphoproliferative disorder [72]. 3D-CT view showing irregular bone resorption surface, the so-called "moth-eaten" appearance, and remaining-teeth with severe periodontitis are noted (a). Bizarre giant cells with Hodgkin and Reed-Sternberg-like appearance are evident in areas of granulomatous proliferation (b). The bizarre giant cells are positive for EBER (c) and LMP-1 (d).

Interaction of oral bacteria and viruses in the development and progression of oral cancer

It has been accepted that oral bacteria, typically *P. gingivalis*, and viruses interact, because the etiology of cancer due to bacteria alone cannot adequately explain a number of clinical aspects. It is known that a bacterial metabolite, butyric acid, inhibits the catalytic action of HDAC and induces transcription of silenced genes including HIV-1 provirus, indicating that butyric acid-producing bacteria could be involved in AIDS progression by reactivating the latent HIV provirus and may contribute to the prevention of the AIDS development and

transmission by eliminating such bacterial infection [77]. In addition to HIV-oral bacterial association, it has been reported that more EBV DNA is found in deeper periodontal pockets of in Japanese patients with chronic periodontitis [51]. A molecular study has revealed that *P. gingivalis* induces EBV reactivation via epigenetic change, and that butyric acid is responsible for this effect [50]. These findings suggest that periodontal disease is a risk factor for HIV or EBV reactivation in infected individuals [50,77].

However, it is not well understood how the interaction of oral bacteria and viruses is involved in the development and progression of cancer. One plausible possibility is the activation of a natural immune sensor, the toll-like receptor (TLR). TLR-2, -4 and -9 are activated by oral bacteria, whereas TLR-3, -7, -8, and -9 are activated by viruses [78]. The function of TLRs is complicated; they contribute to not only the promotion but also the prevention of oral carcinogenesis [78-90]. A number of pathways are activated by microorganisms of the oral cavity, resulting in expression of various genes and secretion of many mediators into the extracellular environment. When periodontal tissue is exposed to continuous microbial stimuli or attack, innate as well as acquired immune responses are induced, and production of inflammatory mediators causes destructive changes, resulting in chronic periodontitis. Further chronic exposure to microbial and host-derived products modifies the microenvironment of the oral cavity and other distant tissues, linking uncontrolled inflammation to the development and progression of cancer characterized by various events, such as genetic and epigenetic alterations, enhanced cell proliferation, inhibition of apoptosis, lymphangiogenesis, and promotion of invasion and metastasis.

Conclusion

Poor oral hygiene and chronic periodontitis increase the risk of OSCC. Microbial infections in the oral cavity may induce innate and acquired immune responses, resulting in the establishment of uncontrolled chronic inflammation manifested as chronic periodontitis. Further microbial attack and various host-derived factors may contribute to events, such as genetic and epigenetic alterations, inhibition of apoptosis, increased cell growth, promotion of invasion and metastasis, and lymphangiogenesis, linking the chronic inflammation to OSCC. Therefore, early prevention and treatment of chronic periodontitis are extremely important for maintenance of oral health and prevention of potentially lethal diseases, such as OSCC.

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