BASIC SCIENCE REVIEW

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EXPLORING THE LINK BETWEEN MICROORGANISMS AND ORAL CANCER: A SYSTEMATIC REVIEW OF THE LITERATURE

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Abstract: The majority of cases of oral cancer have been related to tobacco use and heavy alcohol consumption. However, the incidence of oral cavity carcinoma appears to be increasing in many parts of the world in a manner that it is difficult to explain with traditional risk factors alone. Meanwhile, interest in the possible relationships between microorganisms and the different stages of cancer development has been rising and numerous mechanisms by which bacteria and yeast may initiate or promote carcinogenesis are currently under investigation. In particular, a persuasive body of evidence suggests a possible etiological role involving the metabolism and production of carcinogenic products, such as acetaldehyde. Other suggested mechanisms include the induction of chronic inflammation and direct interference with eukaryotic cell cycle and signaling pathways. This review aims to summarize the known associations between microbial infection and cancer and draw attention to how they may relate to oral carcinoma. © 2009 Wiley Periodicals, Inc. *Head Neck* **31:** 1228–1239, 2009

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METHODS OF LITERATURE SEARCH AND SELECTION CRITERIA

Systematic searches were carried out in the Medline and Embase databases, dated from their respective inceptions until September 2008. Only articles available in the English language were included. Results from the search terms "oral cancer/carcinoma/carcinogenesis" were combined with those from the terms "microbiology," "bacteria," and "*Candida*" initially yielding a total of 1424 articles. In addition, the bibliographies of relevant papers were searched for further studies. Because this was intended to be a summary of the literature, rather than a meta-analysis of quantitative or qualitative data, all articles (eg, reviews, case studies, experimental papers) that pertained to the occurrence, etiology, or development of cancer were included.

ORAL CANCER

Oral cancer is 1 of the 10 most prevalent cancers in the world[1] with more than 90% of the malignancies being squamous cell carcinomas (SCCs) originating from the oral mucosa.[2] Although surgical advances have improved the quality of life for patients, the overall mortality rates have not

changed since 50 years.[3] More worrying is that the incidence or oral cancer appears to be increasing in many parts of the world, including the United Kingdom.[4–7]

Traditionally, oral cancer has been a disease predominantly affecting men in their sixth or seventh decade.[5] In young adults the disease remains relatively uncommon, with only about 0.4% to 6% of cases occurring in patients under 45 years worldwide.[8–11] Although the highest incidence is still seen in patients aged 50 years or more,[12] there is, however, strong evidence that the incidence in the under-45 age group is on the increase.[5–7] Also, in recent years, the difference between male and female patient numbers has reduced dramatically, and there has been a noteworthy increase in incidence in women under 45 years.[13,14]

Several social habits and conditions have been associated with an increased risk of developing oral cancer, most particularly tobacco use and heavy alcohol consumption.[15] Notably, these 2 factors appear to have a synergistic effect, with the cancer risk increasingly multiplicatively in both alcohol-consumers who use tobacco and smokers who drink alcohol.[16] However, the precise role of any of the individual risk factors remains poorly understood. Oral squamous cell carcinoma (OSCC) is a multifactorial disease where no single clearly recognizable cause has been found.[17] Furthermore, although they are the chief clinical risk factors, smoking and drinking do not relate to all cases of oral cancer[18] and cannot explain the recent changes in incidence.[10,19]

Could the many bacteria that inhabit the human oral cavity play any role in the initiation and development of 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.[20,21] Thus, the oral epithelium is constantly exposed to a variety of microbial challenges, on both cellular and molecular levels.

Historically, the idea that bacterial infection could lead to cancer has not been well regarded. However, in recent years there has been an increasing body of evidence to suggest a possible relationship between microorganisms and the different stages of cancer development, which this article has attempted to discuss.

INFECTION AS A RISK OF CANCER

Much of the evidence linking specific microbial infection to carcinogenesis is epidemiological in nature. The most notable example is that of the common pathogenic bacterium *Helicobacter pylori* and its association with gastric cancer. In the first stage the normal mucosa becomes inflamed, and this gastritis can then lead to atrophy, intestinal metaplasia, dysplasia, and adenocarcinoma. Infection with *H. pylori* was found to correlate with the incidence of gastritis, indicating its involvement in the initiation and promotion of gastric adenocarcinoma in a subset of patients. This hypothesis was subsequently supported by numerous clinical and animal studies and, in 1994, *H. pylori* became the first species of bacteria to be classified by the World Health Organization International Agency for Research on Cancer (IARC) as a definite cause of cancer in humans.[22–24]

Since then, a growing number of possible associations between different types of bacteria and cancer have been reported. For instance, a prior cervical infection with *Chlamydia trachomatis* has been associated with an increased risk for the development of invasive cervical cancer.[25] *Chlamydophila* (formerly *Chlamydia*) *pneumoniae* infections have been linked with both malignant lymphoma and male lung cancer patients.[26,27] Patients with infectious endocarditis due to *Streptococcus bovis* seemingly have a greater risk of developing colonic carcinoma.[28,29] In addition, there is a significantly increased risk of developing carcinoma of the gallbladder in patients infected with *Salmonella typhi*.[30,31]

BACTERIA PRESENT WITHIN TUMORS

In additional to epidemiological data about specific bacterial species and types of cancer, the idea that bacteria can live and even thrive within human tumors has been recognized for more than 70 years. Since 1931 there have been countless reports of large numbers of viable bacteria present in various types of malignant tumor excised from patients.[32–34] More recently, using bacteria labeled with light-emitting proteins, several species have been shown to successfully survive and propagate within solid tumors in animal models.[35] Strains of *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium,* and *Vibrio cholerae* were all intravenously injected into live mice and seen, in real time, to enter the tumor tissues and replicate within them. Furthermore, the survival of the bacteria was site-specific; despite the bacteria becoming well-

distributed throughout the animals in the first few minutes following injection, within less than a week the labeled bacteria were replicating only inside the tumor tissue. Such selectivity was presumably because all bacteria were effectively shielded from the host immune system while being within the solid tumor.[35] It was suggested that this "tumor-finding" ability of the bacteria may offer new methods to detect cancer and to deliver gene therapy treatments specifically to tumor locations.[35,36]

CONDITIONS ASSOCIATED WITH ORAL CANCER

As mentioned previously, the oral cavity is home to a rich microflora comprising many different microbial species, each present in varying amounts. The composition and quantity of this microflora varies from person to person and can change throughout an individual's lifetime in response to a variety of factors.[37]

Poor oral hygiene can allow a person's microflora to overgrow and the balance of species to change.[37] Plus, in addition to tobacco and alcohol use, poor oral hygiene may itself be an independent risk factor for oral cancer. Clinicians have long noticed an association between poor oral hygiene, poor dental status, and oral cancer. Unfortunately, hard evidence of a correlation is difficult to come by as issues of socioeconomic background, tobacco use, alcohol consumption, nutrition, and other associates of cancer risk usually confound these factors.[15] Nevertheless, a few epidemiological studies have suggested that the number of teeth lost was an indicator of increased oral cancer risk.[38,39] Furthermore, a lower risk was associated with increased teeth brushing and greater frequency of dental check-ups.[39–41] Additionally, at least 1 preliminary study has indicated an association between periodontal disease and the presence of precancerous and neoplastic oral lesions. In an analysis of 13,798 subjects aged 20 years and older, clinical attachment loss was measured as a representation of the severity of periodontal disease and compared against 3 separate variables: the presence or absence of a tumor, a precancerous lesion, or another soft-tissue lesion in the oral cavity. Descriptive statistics suggested associations between periodontal disease and the risk for precancerous lesions and tumors.[42]

The observation that current smokers were about 4 times more likely to have periodontitis than nonsmokers[43] has prompted the theory that tobacco use may alter the levels of certain pathogenic bacteria in the microflora. However, different studies into the composition of the subgingival microflora in smoking and nonsmoking patients with periodontitis report conflicting findings. For instance, in some groups of patients statistically higher risks of infection with such pathogenic species as *Treponema denticola*, *Tannerella forsythensis* (formerly *Bacteroides forsythus*), *Prevotella intermedia, Porphyromonas gingivalis, Peptostreptococcus micros, Fusobacterium nucleatum, Eikenella corrodens, Campylobacter rectus,* and *Aggregatibacter actinomycetemcomitans* were found in smokers than nonsmokers.[44,45] Yet, in other, molecular-based comparisons of the prevalence of these species plus *Prevotella nigrescens, Selenomonas noxia,* and *Streptococcus intermedius*, no significant differences were found between smoking and nonsmoking cohorts.[46–48] Conversely, some evidence suggests that, rather than the presence or absence of specific microorganisms, it may be the host response to bacterial challenge that determines susceptibility to periodontitis.[49]

Nevertheless, there is some evidence that cigarette smoking can lead to the selective growth of certain strains, if not species, of bacteria. For example, in 1 study, all but a few oral strains of *Staphylococcus aureus* did not grow in the presence of cigarette-smoke condensates. At least 1 of these tar-resistant isolates was found to be able to induce inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), in vitro.[50] The increased production of TNF-α is an important part of inflammation and has been heavily implicated in oral carcinogenesis,[51] suggesting that the tar-resistant *S. aureus* may be carcinogenic. The reasons behind any possible correlation between carcinogenic potential and resistance to cigarette smoke in certain bacterial strains are unclear.

Consumption of alcohol may also alter the composition of the oral microflora. A recent study has reported that salivary levels of *Streptococcus anginosus* are significantly higher in alcoholics compared with nonalcoholic patients.[52] At the time of writing, no other more comprehensive studies were carried out to determine if this trend is true with other microbial species.

Increased growth of the microflora and greater numbers of bacteria and *Candida* are

associated with poor oral hygiene, periodontal disease, and known clinical risk factors for oral cancer. There is strong evidence that the microflora may be a common denominator that links microorganisms with oral cancer.

FUNGAL INFECTIONS ASSOCIATED WITH ORAL CARCINOMA

Species of *Candida* are members of the oral microflora and are generally regarded as being commensals. However, they are capable of causing a range of opportunistic infections, referred to as candidoses, which are especially important in elderly, debilitated, or immunocompromised patients.[53] *Candida albicans* has also been found to be present at higher levels in biofilms on the surface of human OSCCs in contrast to healthy control sites.[54] Infection with *Candida* has been associated with malignant development in the oral cavity ever since it was found to cause candidal oral leukoplakias[55,56] and correlate with oral epithelial dysplasia.[57] Candidal leukoplakia was observed in rats when their tongues were artificially inoculated with *Candida*. Long-term infection of the rat tongue resulted in hyperplasia and dysplasia of the epithelium.[56] That epithelial dysplasia can improve following elimination of *Candida* from infected tissue also supports the idea of a causal link.[53] *Candida*-infected leukoplakias appear to have a higher rate of malignant transformation than other types.[58] Chronic hyperplastic candidosis, a form of candidosis characterized by hyphal invasion of the oral epithelium, is estimated to develop into a neoplasm in up to 10% of cases.[59]

Further epidemiological evidence of a link between *Candida* infections and oral cancer comes from cases of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a rare autosomal recessive disease caused by mutations of the autoimmune regulator gene. Most patients with the disorder have chronic oral candidosis since early childhood and also exhibit a highly increased risk for developing oral carcinoma at a young age.[60]

BACTERIA ASSOCIATED WITH ORAL CARCINOMA

Several reports have observed that significant numbers of patients with intraoral cancer have had "abnormal" bacterial flora, containing noticeable numbers of potential pathogens, both before and after treatment.[61–63] Furthermore, at least 1 study has observed that patients with oral cancer who maintain a normal flora have a better prognosis than those who do not.[64] Since these original observations, only a few investigations into the relationship between patients with oral cancer, or patients with a high risk of developing oral cancer, and their intraoral microflora have been reported. One such study found, using standard clinical microbiological culture analyses, that biofilms from the surface of human OSCC harbor increased number of bacteria compared with healthy control sites. The bacteria detected were a range of aerobes and anaerobes, including *Veillonella, Fusobacterium, Prevotella, Porphyromonas, Actinomyces, Clostridium, Haemophilus, Enterobacteriaceae,* and *Streptococcus* species.[54]

We have recently detected a rich diversity of apparently viable bacteria present within human oral carcinoma using both standard clinical culture and molecular-based approaches.[65,66] Interestingly, these studies correlated with others that have used culture-independent methods to look for and detect the bacteria *S. anginosus, Streptococcus mitis,* and *Treponema denticola* in various upper digestive tract carcinomas.[67–71] The majority of these intratumor bacteria are of species known to reside in the human mouth and could very well have originated from the oral cavity. For instance, it has been suggested that dental plaque could act as a reservoir of the *S. anginosus* that seems to frequently infect oral and esophageal carcinoma.[72] The suggestion that oral bacteria may pass through mucosal tissue to within a tumor mass is not surprising; bacteria originating from the mouth have previously been shown to infect systemically and may translocate to within lymph nodes.[73,74]

There is evidence that the presence of oral carcinoma tissue may affect the microflora throughout the mouth, as well as support bacteria within and on their surface. It has been reported that patients with OSCC tend to possess notably raised concentrations of certain bacterial species in their saliva compared with OSCC-free individuals. Using checkerboard DNA-DNA hybridization, counts of *Capnocytophaga gingivalis, Prevotella melaninogenica,* and *S. mitis* were all found to be elevated in the subjects with OSCC.[75] As the authors of this study discuss, this apparent alteration of the salivary microflora in the presence of OSCC lesions is of particular interest because of its potential application as a

noninvasive diagnostic tool for predicting oral cancer.

POSSIBLE MECHANISMS BY WHICH MICROORGANISMS MAY PROMOTE CANCER

Of course, it is always of interest to know the microflora that inhabits lesions and malignancies, only because increased numbers in the regional microflora may increase the risk of local and systemic polymicrobial infections, which may complicate the morbidity of the immunocompromised cancer patient.[54,76,77] However, the presence of these species does not, by itself, automatically mean there is any connection to the cancers' etiology. However, evidence that bacteria and yeasts can promote carcinogenesis, either directly or indirectly, does exist.

The development of OSCC comprises a multistep process requiring genetic damage, presumably caused by exogenous carcinogens, and influenced by environmental factors and chronic inflammation. Damage to the DNA can lead to mutation, which can bring about a loss of function or aberrant expression of oncogenes and tumor-suppressor genes. An accumulation of dysregulated tumor-suppressor genes may release a stem cell from the normal cell cycle, allowing it to proliferate and prevent it from being killed by programmed cell death.[78–80] It can be seen, therefore, that there are many stages at which the development of cancer can be promoted or inhibited. Considerable evidence exists to suggest that there are a variety of microbial species capable of encouraging the initiation, promotion, or progression of human malignancies. Much of this evidence is the subject of previous review articles, but some examples of how bacteria and *Candida* may be associated with carcinogenesis in humans and how this may relate to cancer of the oral cavity are listed in Table 1 and summarized in the text.

Candidal Production of Nitrosamine. Despite the strong association described above, the exact role of *Candida* in malignant change is uncertain. However, nitrosamine compounds produced by *Candida* species may directly, or in concert with other carcinogens, activate specific proto-oncogenes and thus initiate the development of a malignant lesion. *N*-nitroso-benzylmethylamine (NBMA) is a compound able to induce carcinoma of the esophagus and the oral cavity in the rat.[81] When strains of *Candida* were isolated from leukoplakia lesions and normal mucosa and then assessed for nitrosation potential, those with a relatively high potential for producing NBMA from salivary precursor molecules were comparatively more frequently isolated from lesions with more advanced precancerous changes.[82]

Activation of Procarcinogens. Perhaps the most significant hypothesis as to how bacteria may be associated to oral cancer development relates to the activation of procarcinogenic chemicals. The majority of known chemical carcinogens need to be metabolized before they are able to interact with cellular macromolecules and initiate carcinogenesis in humans. This process of activation can potentially be performed by the hosts' own cells, via the action of xenobiotic-metabolising enzymes,[83] or by local bacterial species.

An example of this mechanism of carcinogenesis can be found in the human intestinal tract

Table 1. A summary of the proposed mechanisms by which microorganisms may be involved in the etiology of oral cancer.

Potential carcinogenic mechanism	Associated oral microorganisms
Production of carcinogens	
Nitrosamine production	*Candida* spp.
Metabolism of procarcinogens	
Conversion of ethanol to acetaldehyde	*Candida* spp. *Neisseria* spp. *Streptococcus* spp. Other Gram-positive species
Induction of chronic inflammation	
Stimulation of procarcinogenic inflammatory mediators	Periodontopathogenic bacteria *Streptococcus* spp.
Direct influence of bacteria on human cell cycle/signaling	
Promotion of cellular proliferation	Periodontopathogenic bacteria
Inhibition of cellular apoptosis	*Mycoplasma* spp.

Note: At the time of writing, the strongest body of evidence indicates the production or metabolism of carcinogenic substances as a possible etiological link, although other mechanisms have been hypothesized.

where an association between colon cancer and the colonic microflora has been indicated by both laboratory and epidemiological studies.[84] Heterocyclic aromatic amines (HAs) and other procarcinogenic chemicals, which are found in common foodstuffs such as cooked meats and fish, are frequently ingested. Much work on the effects of different dietary mixtures on the health of laboratory animals has implicated HAs as initiators of carcinogenesis and shown them to be capable of producing tumors at multiple organ sites.[85,86] Furthermore, studies have indicated that intestinal bacteria have a strong impact on the genotoxicity of at least 1 of these carcinogenic HAs: DNA damage caused by the HA chemical 2-amino-3-methylimidazo[4,5,f]quinoline (IQ) was 3- to 5-fold greater in rats containing a natural microflora as opposed to germfree specimens.[87] It should be noted, however, that this effect is dependent on the species comprising the microflora of the individual. For example, whereas *Bacteroides fragilis* can cause a distinct increase in mutagenicity in the presence of HAs, lactobacilli species result in a decrease.[86]

Similarly, despite alcohol being considered an important risk factor for cancer of the upper-aerodigestive tract, pure ethanol has been found to have no carcinogenic effects.[17,88] However, acetaldehyde, the first metabolite of ethanol, is carcinogenic in both animal models and in vitro. Acetaldehyde has been shown to produce mutagenic effects, such as DNA adducts, DNA cross-linking, aneuploidy, or chromosomal aberrations.[88,89] This reaction can not only be catalyzed in the drinker's mouth by alcohol dehydrogenase (ADH) enzymes from the epithelium,[88–91] but also by the oral microflora. An increasing number of studies provide evidence for the importance of this hypothesis and, to date, streptococci, other gram-positive aerobic bacteria, and yeasts have been associated with acetaldehyde production.[92–95] Additionally, *Neisseria* species, traditionally regarded as nonpathogenic residents of the oral cavity, have been reported to exhibit extremely high levels of ADH activity and produce significant amounts of acetaldehyde in the presence of ethanol.[96] Moreover, salivary analysis shows that increases in microbial acetaldehyde production correlates with smoking and heavy alcohol consumption.[92] As mentioned previously, the combined use of tobacco and alcohol has a multiplicative effect on the risk of oral cancer, and this effect appears true for acetaldehyde levels as well, with smokers exposed to ethanol demonstrating up to 7 times higher concentrations of salivary acetaldehyde compared with nonsmokers.[97,98] This explanation for some of the epidemiological trends seen in the clinic certainly seems to indicate that acetaldehyde is a key factor in the etiology of oral cancer. And so, as the microflora plays a key role in the production of acetaldehyde, an important link between microorganisms and the pathogenesis of alcohol and tobacco-related carcinogenesis seems evident.

Infection, Inflammation, and Carcinogenesis. A critical discovery from recent molecular investigations into carcinogenesis has been the importance of inflammation to the entire process. The inflammatory microenvironment surrounding the tumor is now known to actively participate in the induction, selection, and expansion of malignant cells.[99] These findings have led to a better understanding of the observed links between several chronic inflammatory disorders and the development of cancer. At the clinical level, several such associations have been known for a long time. For example, inflammation appears to increase the risk of cancer at many different body sites, including the pancreas,[100] stomach,[101] colon,[102] liver,[103] bladder,[104] prostate,[105] and ovaries.[106] Epithelial inflammation is also a factor in oral carcinogenesis, and it is thought that inflammation is the mechanism that links the use of smokeless tobacco products and areca nut extract to an increased risk of cancer.[51,107]

Inflammation can have several effects on cancer. Acute inflammation has been said to counteract cancer, whereas, on the other hand, chronic inflammation has been seen to promote cancer development.[108] Chronic inflammation is often as such from the outset, but may also develop from an acute inflammatory response if the causal agent persists. Any persistent stimulus of the immune system, including chemical irritants such as asbestos and silica or an infection by a viral or bacterial pathogen, is capable of causing chronic inflammation that can often last for years.[109,110] How inflammation can increase the likelihood of cancer development has been well-described in detail elsewhere, but in summary inflammatory mediators including enzymes, complement proteins, coagulation factors, growth factors, cytokines, reactive metabolites of oxygen and nitric oxide, whereas offering protection by destroying invading pathogens can inhibit apoptosis and enhance cell proliferation,

both of which can promote mutation and carcinogenesis.[109–113]

The most recent research indicates that it is the mechanisms of chronic inflammation that are responsible for the observed link between *H. pylori* and carcinogenesis. Infections with *H. pylori*, especially with strains positive for the cagA virulence factor, predictably cause an inflammatory response, which includes the induction of cyclooxygenase (COX)-2 expression[114] and the invasion of the local tissue by neutrophils and phagocytes, accompanied by the production of proinflammatory cytokines.[22,24] Peptides from *H. pylori* have also been shown to induce the activation of NADPH oxidase and produce oxygen radicals.[115] And inflammation in the stomach is not caused solely by *H. pylori*; other species of bacteria can infect the stomach and hence may also play a role in gastric carcinogenesis. *Acinetobacter lwoffi*, for example, can also result in chronic gastritis independently of *H. pylori*.[116]

Several other associations between certain bacteria and cancers are based on inflammatory mechanisms. For instance, it has been suggested that *Propionibacterium acnes* infection may possibly be linked with the development of prostate cancer. *Propionibacterium acnes* was positively associated with a higher degree of prostatic inflammation, a condition which has in turn been implicated with carcinogenesis.[117] *Propionibacterium acnes* is known to stimulate the production of inflammatory mediators.[118,119] Similarly, the induction of inflammation may also explain the observed links between infections with *Chlamydophila pneumoniae* and *S. bovis* and an increased risk of lung and colon cancer, respectively. *C. pneumoniae* can infect human lung epithelial cells and induce the expression of proinflammatory cytokines, including interleukin (IL)-8, interferon-γ, and TNF-α.[120] Likewise, *S. bovis* releases proteins that are able to stimulate intestinal cells to produce inflammatory mediators such as IL-8 and prostaglandin E2 (PGE2). This has also been seen to promote the progression of preneoplastic lesions in the colonic mucosa of rats.[28,121]

The multitudes of bacteria that reside in the human oral cavity do so without necessarily causing inflammation. However, as our knowledge of periodontal disease shows, given the correct circumstances some species of oral bacteria can initiate inflammation in their host.[122,123] For example, *Porphyromonas gingivalis* can induce COX-2 expression[124] and bring about an increased production of proinflammatory mediators such as TNF-α and cytokines including IL-6, IL-8, and IL-1β.[125] Likewise, the periodontopathic species *Eikenella corrodens* is able to stimulate human oral epithelial cells to produce various mediators including IL-6 and IL-8, and PGE2, seemingly via the secretion of soluble proteins.[126]

Oral species of *Streptococcus* isolated from carcinoma tissues (see above) have been found to be capable of promoting an inflammatory response. *S. anginosus* and *S. mitis* were observed to induce the production of inflammatory cytokines in human esophageal epithelial cell lines.[68] Similarly, supernatants from cultures of *S. anginosus* strain NCTC 10713 contained an antigen which was found to induce nitric oxide synthesis as well as produce inflammatory cytokines in murine peritoneal exudate cells.[127] Patients with periodontitis whose saliva tested positive for *S. anginosus* have also been found to exhibit significantly higher levels of 8-hydroxy-deoxyguanosine (8-OHdG), a commonly used marker for evaluating inflammatory cell infiltration and oxidative DNA damage, than patients tested negative for the bacterium. Increases in 8-OHdG levels have previously been associated with human premalignant lesions and cancerous tissues. Although the salivary levels of *S. anginosus* were relatively low in these patients, there was a correlation between the level of *S. anginosus* and 8-OHdG.[128] It has been hypothesized that *S. anginosus* in particular may play a significant role in many cases of esophageal cancer by causing inflammation and promoting the carcinogenic process. Eradication of these streptococci may decrease the risk of recurrence of esophageal cancer.[68]

Direct Influence of Bacteria on Human Cell Signaling (Cellular Microbiology). The relatively new discipline of cellular microbiology focuses on the myriad of interactions between bacteria and the cells of their host. It is these interactions, or disruptions of the normal 2-way communications, that can lead to infection rather than simple colonization with bacteria.[129] Several species of bacteria have been discovered to directly interfere with eukaryotic cellular signaling in a way that is characteristic of tumor promoters.[130] Examples of these are discussed in more detail later in the text.

Cellular proliferation has a pivotal role in carcinogenesis. Mutations in DNA regularly arise from exposure to exogenous or endogenous mutagens. Hyperproliferation simultaneously reduces the time available to repair any of these mutations and also increases the risk of spontaneous mutation due to errors in DNA replication. The likelihood that mutations get transmitted into the next generation is thereby significantly increased.[131]

Several, phylogenetically unrelated species of bacteria are known to increase the proliferation of eukaryotic cells, directly via the secretion of virulence factors. For instance, *Pasteurella multocida*, a commensal of the digestive and respiratory tracts of many warm-blooded animals, releases a protein referred to as *Pasteurella multocida* toxin (PMT) that can induce the proliferation of quiescent cells at picomolar concentrations. Human growth factors, such as platelet derived growth factor (PDGF), require more than 300 times the concentration of PMT to achieve the same level of effect making PMT the most potent eukaryotic mitogen ever reported.[132–134]

Potentially more significantly for oral carcinogenesis, it appears that common members of the oral cavity microflora can also promote the proliferation of host cells. For instance, *Porphyromonas gingivalis*, a notorious pathogen associated with periodontal disease, contains proteins and lipopolysaccharides on its outer surface that have been shown to stimulate human fibroblasts to proliferate in vitro.[135–137]

Apoptosis, or programmed cell death, is the mechanism by which multicellular organisms dispose off damaged and atypical cells in response to physiological and pathologic stresses. When a cell is transformed and becomes such that homeostasis and the intricate system of cell signaling is disrupted, apoptosis can be triggered, destroying the cell and preventing it from developing into a malignant tumor. Thus, any agent that can impede apoptosis promotes the atypical build-up of cancerous cells.[138,139] There have been several examples of bacteria suppressing apoptosis and potentially promoting carcinogenesis.

For instance, *E. coli* releases a range of virulence factors including cytotoxic necrotizing factor type 1 (CNF1), which prevents apoptosis in epithelial cells, ostensibly by activating a cell signaling cascade and promoting the expression of antiapoptotic members of the Bcl-2 gene family.[140,141] Also, *C. pneumoniae*-infected epithelial cells are resistant to apoptosis induced by chemicals or death receptors, seemingly, in part, because of the bacterium's ability to induce the expression of IL-10, which can down-regulate the expression of major histocompatibility complex class I molecules.[120]

Species of *Mycoplasma* such as *M. fermentans* and *M. penetrans* have also been shown to prevent apoptosis in vitro. Cultures of the mouse myeloid cell line 32D ordinarily undergo apoptosis on withdrawal of IL-3 from the culture medium. However, this phenomenon does not occur when 32D is cultured in the presence of either live or heat-killed *Mycoplasma* cells. Furthermore, when infected with live *Mycoplasma* for periods of 4 to 5 weeks, 32D cells underwent malignant transformation, after which they required neither IL-3 supplements nor *Mycoplasma* to survive.[142] *M. fermentans* has also been shown to inhibit apoptosis in a human cell line, the myelomonocytic U937 cell line, by affecting the TNF-α signaling pathway.[143] A number of mycoplasmal species, including *M. fermentans*, have been detected as members of the normal oral microflora in saliva, on the mucosal surfaces, and in plaque.[144–146] However, at the time of writing, there is no evidence to link *Mycoplasma*-related suppression of apoptosis with carcinogenesis of the oral cavity.

CONCLUSION

Interest in the possible relationships between bacteria and the different stages of cancer development has been increasing since the classification of *H. pylori* by the World Health Organization as a definite (class 1) carcinogen. Subsequently, links between infections and the onset of cancer in various body sites were discovered. Numerous mechanisms by which different species of bacteria may initiate or promote carcinogenesis have also since been proposed and are currently under investigation. These include the induction of chronic inflammation by interference, either directly or indirectly, with eukaryotic cell cycle and signaling pathways, or via the metabolism of potentially carcinogenic substances.

Oral cancer is a lethal disease with an increasing incidence that cannot be wholly explained by the traditional risk factors, but it is only relatively recently that investigations have

begun into the associations between microorganisms and cancer of the oral cavity. Yet, as summarized in this review, there is evidence to suggest that epidemiological and etiological links between microbial infection in the oral cavity and oral cancer could exist. Perhaps most compelling are the indications that the activation of procarcinogenic substances by the oral microflora, specifically the conversion of ethanol to acetaldehyde, may be an important etiological factor. In particular there is also strong evidence of link with *Candida* infections, which are already often considered as clinical risk factors for OSCC, and streptococci, which are not but have been reported to be present within carcinoma of the upper aerodigestive tract in several studies to date.

Whether or not there is a direct causal link between microbes and cancer, there is also a possibility that changes in the commensal microflora occur in conjunction with cancer development, which could have the potential of being used as a diagnostic indicator. The microflora associated with oral malignancy and how microorganisms interact with the oral mucosa on a cellular level are concepts that warrant further investigation.

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