Review Article

Oral microbiome and oral cancer – The probable nexus

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Abstract

Oral squamous cell carcinoma is one of the most common malignancies and is the leading cause of morbidity and mortality. The known risk factors for oral cancer are tobacco, alcohol consumption and betel quid chewing. Nutritional deficiencies and certain microorganisms are also associated with oral cancer. Oral cavity is a host to numerous microorganisms, majority of which are bacterial communities along with fungi and viruses. A possibility of the dysregulation of the oral microbiome cannot be ignored. Oral microbiome is defined as the collective genome of microorganisms that reside in the oral cavity. With the development of culture-independent techniques, the detection and identification of the bacteria which cannot be cultured has become possible. Revolution in technology has led to increased research in this area in an attempt to find the role of microbiome in health and disease. Before identifying the exact role the microbiome plays in the development of oral cancer, it is essential to profile the microbiome in healthy individuals and patients with oral cancer. It is essential to note that oral cancer may sometimes occur without any habit too!! This article is an attempt to review the role of oral microbiome in oral cancer with a focus on the bacteriome, its related studies and in brief about the omics technologies in understanding the microbiome.

Keywords: Omics, oral cancer, oral microbiome

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INTRODUCTION

The microbiome in humans is known to be associated with many different types of cancers in the body. Since the last two decades, bacteria have been implicated in the etiology of some cancers such as *Helicobacter pylori* in gastric cancer and mucosa-associated lymphoid tissue lymphomas, *Chlamydia trachomatis* in cervical cancer, *Salmonella typhi* in gallbladder cancer and *Bacteroides fragilis* and *Fusobacterium nucleatum* in colon cancer.[1,2]

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This has encouraged and triggered a considerable amount of research to identify the possible role of bacteria in oral carcinogenesis.[1]

Oral cancer is a multifactorial disease. Host genetics and environmental factors play a role in the causation of this disease. Tobacco, alcohol consumption, betel quid chewing and human papillomavirus (HPV) infections are well-known risk factors. A possible etiology could be attributed to the microbes in around 15% of oral cancer patients without known risk factors.[3]

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The squamous epithelium of the oral cavity is continuously exposed to a variety of microbial challenges, at both cellular and molecular levels.[4]

There is a wide inter-individual diversity in microbial colonization in the oral cavity. This micro-environmental variability connected to the oral microbial flora is likely to modify tumor cell genotype and phenotype. This leads to heterogeneity in the tumor cells, a characteristic that might be quite exceptional for oral squamous cell carcinoma (OSCC) and not hold true for other cancers in the body.[5]

It is, therefore, justifiable to discuss the changes in the composition of the normal oral microbiome, which comprises of more than 600 different bacterial species. The changes in the composition of the oral microbiome are certainly associated with periodontal disease which is known to be a polymicrobial disease. Variations of oral bacterial and fungal microbiome have been noted in association with oral precancers and cancers. Chronic bacterial infections could promote or cause oral cancer.[6]

Theories which indicate a link between cancer and the micro-organisms have been studied in the last few decades.[7]

Specific bacteria have been identified that strongly correlate with OSCCs, such as *Streptococcus*, *Peptostreptococcus*, *Prevotella*, *Porphyromonas gingivalis* and *Capnocytophaga gingivalis*.[8]

Most research so far have been focused on the bacterial component of the oral microbiome – the oral bacteriome, as bacteria are the predominant microorganisms in the oral cavity. However, there is a growing interest in the less abundant microbial communities such as fungi and viruses.[9]

However, there is no consensus among the reports regarding changes in the oral microbiome in association with oral cancer; this is because previous studies were confined to the investigation of a relatively small number of known bacteria and bacteria which can be cultured. Culture-independent techniques, particularly next-generation sequencing (NGS) of the hypervariable region of the 16S ribosomal subunit, provides a means for more comprehensive and accurate profiling of the microbiome in health and disease.[6]

BACTERIA AND ORAL CARCINOGENESIS

Cancer has been described as a molecular disease of cell membrane glycoconjugates. Certain glycoconjugates are receptors for distinct bacteria. Recent studies support the understanding that shifts in the colonization of different cancer cells are linked with observed changes in cell surface receptors. It is now identified that bacteria bind to and colonize the mucosal surfaces in a very particular manner via a "lock and key" mechanism. There is a specific binding between adhesins on bacteria to complementary receptors on the mucosal surfaces of the host.[10]

Bacteria may induce carcinogenesis by the following mechanisms:[9]

1. Stimulation of chronic inflammation
2. Cell proliferation
3. Inhibition of cellular apoptosis
4. Promotion of cellular invasion
5. Production of carcinogenic substances.[9]

Stimulation of chronic inflammation

Chronic or dysregulated inflammation plays a role in tumor development partly through the modulation of tumor microenvironment.[11]

Inflammation is triggered by infections or environmental exposures and plays a crucial role in all stages of carcinogenesis including induction, progression, invasion and metastasis.

Microorganisms and their products activate inflammatory cells, fibroblasts and epithelial cells. Reactive species such as hydrogen peroxide and oxygen radicals, reactive nitrogen species such as nitric oxides, reactive lipids and metabolites (malondialdehyde and hydorxy-2-nonenal) and matrix metalloproteases are generated by the activation of these cells. These compounds can directly affect tumor growth. They induce DNA damage in epithelial cells by activating tumor cell toll-like receptors that ultimately leads to nuclear translocation of the transcription factor nuclear *factor*-kappa β (NF-kβ) and cytokine production. These cytokines are formed in a dysregulated manner and play a role in cell growth, invasion and interruption of tumor suppression, immune status and even survival.

It is not clear whether these mediators are critical for the growth of tumors and/or whether they constitute a conducive habitat for the spread of malignancies. Increased levels of certain pro-inflammatory, pro-angiogenic NF-kβ-dependent cytokines such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor and vascular endothelial growth factor were observed in serum, saliva and tissue specimens of patients with oral cancer.[12]

P. gingivalis and *F. nucleatum* persist intracellularly within the epithelial cells and can cause chronic infections and initiate inflammatory responses. They can also spread systemically and cause infections beyond the oral cavity where they cause major disruptions of the host immune response mechanisms.[13]

Cell proliferation

Bacteria can cause chronic infections or produce toxins that induce cellular proliferations and DNA replication by the activation of mitogen-activated kinase pathways and cyclin D1. This leads to disturbance in the cell cycle and alteration in cell growth. This increases tumor development through an increase in the genetic mutations.[14]

The microbial endotoxins may directly induce mutations in tumor suppressor genes and proto-oncogenes. They may also alter signaling pathways that affect multiplication of and/or survival of epithelial cells.[12]

Inhibition of apoptosis

P. gingivalis is recognized to halt the apoptosis of gingival epithelial cells by different mechanisms, hence allowing itself to sustain.[15]

The response of the epithelial cells to *P. gingivalis* infection is changes to apoptosis and cell division. *P. gingivalis* is greatly antiapoptotic and can surely suppress chemically induced apoptosis in primary cultures of gingival epithelial cells.[16] It stimulates JAK1/STAT3 and PI3K/Akt signaling. This signaling regulates intrinsic mitochondrial apoptosis pathways.[1]

Promotion of cellular invasion

P. gingivalis infection activates the ERK 1/2-Ets 1, p38/HSP27 and PAR2/NF-KB pathways. This induces the expression of promatrix metalloproteinase MMP-9. P. gingivalis also produces Gingipains, cysteine proteinases, which play a dual role in this process.

They both engage the PAR2 receptor and cleave the MMP-9 proenzyme into the mature active form. MMP-9 causes degradation of the basement membrane and extracellular matrix. This promotes the migration and invasion of the cancer cells and thus allows carcinoma cells to enter the lymphatic system and blood vessels for spread and metastatic growth at distant sites. In this way, P. gingivalis may contribute to the metastasis of OSCC.[16]

Production of carcinogenic substances

The microflora which exists in the tumor microenvironment may aid in tumorigenesis by conversion of ethanol into acetaldehyde, its carcinogenic derivative to levels which are able to induce damage to the DNA, mutagenesis and secondary hyperproliferation of the epithelium.[14] The International Agency for Research on Cancer has also classified acetaldehyde associated with alcohol consumption as a Group I carcinogen to humans.[1]

Oral bacteria are found much more commonly on the surface of primary oral cancer tissue and in the metastatic lymph nodes as compared to normal oral mucosa and nonmetastatic lymph nodes. Bacteria in the oral cavity may invade through areas of tissue surface destruction due to oral cancer and further flow into the cervical lymph nodes. This suggests a contribution of oral bacteria to progression of oral cancer.[15]

VIRUSES AND ORAL CANCER

Viral infections of latent or chronic nature cause malignant transformation by interfering with the cell cycle machinery of the host. Cell growth and proliferation is affected by these viral genes and gene products. Certain viral genes are proto-oncogenes. These proto-oncogenes become oncogenes when inserted into the host's DNA and ultimately result in malignant transformation.[17]

Oncogenic viruses have the capacity to disrupt checkpoints in the cell cycle induced by genotoxic stress. P53 and connecting cellular proteins are involved in downstream activities. They are inactivated by viral antigens either by releasing cells from cell cycle checkpoints or protecting cells from the p53-dependent apoptotic pathway.[18]

DNA viruses have the capacity of transforming cells to a malignant phenotype and are found in many different cancers. HPV has been proposed as a risk factor in the development of OSCC and the most important subtype is HPV 16. Other oncogenic virus species that have been proposed to be involved in oral carcinogenesis are the Epstein–Barr virus and Herpes simplex virus type 1.[19]

FUNGI AND ORAL CANCER

It is a known fact that *Candida* species are normal commensals of the oral cavity in up to 50% of healthy population.[18]

Candida might induce OSCC by the production of carcinogenic compounds such as nitrosamines. The tubular hyphal structure of *Candida albicans* might allow the ingress of precursors from saliva and release of the nitrosamine products to keratinocytes, thus initiating OSCC.[20]

Candida species present in the oral cavity contain the enzyme alcohol dehydrogenase. This enzyme catalyzes the

production of mutagenic amounts of acetaldehyde under aerobic or microaerophilic conditions.[1]

STUDIES ON ORAL MICROBIOME AND ORAL CANCER

Nagy *et al.* in 1998 investigated the microbial contents of the biofilms by aerobic and anerobic culture methods on the surfaces of OSCCs. They concluded that human oral carcinoma surface biofilms show significantly more number of aerobes and anaerobes when compared with the healthy mucosa of the same patient.[21]

Sakamoto *et al.* in 1999 isolated bacteria in oral cancer patients. Viable bacteria were isolated from cervical lymph nodes from 25 patients. The most commonly isolated bacteria were oral Streptococci.[22]

Lax and Thomas in 2002 attempted to explain the role of bacteria in the causation of cancer.[23]

Morita *et al.* in 2003 used PCR to determine whether *Streptococcus anginosus* is associated with oral cancer tissues. Their results suggested that *S. anginosus* is not closely related with oral cancer but is associated with esophageal cancer.[24]

Muthu *et al.* in 2004 studied the changes in the flora in the oro-pharyngeal region in head-and-neck malignancy post radiotherapy and found a significant decrease in alpha hemolytic Streptococci and *Neisseria* species post radiotherapy.[25]

Chambers *et al.* in 2005 conducted a pilot study to examine elevated *Mutans Streptococci* in xerostomic cancer patients after pilocarpine therapy.[26]

Mager *et al.* in 2005 investigated to determine if salivary counts of oral bacteria differed in patients with OSCC and cancer-free controls and concluded that high counts of *C. gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis* may be diagnostic indicators of OSCC.[27]

Hooper *et al.* in 2006 isolated nighty species mostly bacterial from within the tissue of twenty OSCC and provided some evidence for tumor specificity of bacteria.[28]

Hooper *et al.* in 2007 characterized bacterial microbiota in oral cancerous lesions and noted apparent differences between the composition of tumorous and nontumorous tissues; tumorous tissue showed the presence of saccharolytic and aciduric species.[29]

Lee *et al.* 2010, explored the relevance of HPV infection to carcinogenesis of oral tongue cancer.[30]

Katz *et al.* in 2011 investigated *P. gingivalis* in squamous cell carcinoma specimens by immuno-histochemical staining and suggested a potential association between the bacteria and gingival squamous cell carcinoma.[31]

Pushalkar *et al.* in 2011 evaluated the diversity and relative abundance of bacteria in the progression of OSCC using saliva and found that majority of classified sequences belonged to phyla *Firmicutes* (45%) and *Bacteroidetes* (25%).[32]

Schmidt *et al.* in 2014 investigated the changes in the microbiome associated with oral cancers by profiling cancers and anatomically matched contralateral normal tissue from the same patient by sequencing 16S rDNA hypervariable region amplicons. They found a significant decrease in the abundance of *Firmicutes* (especially *Streptococcus*) and *Actinobacteria* (especially *Rothia*) relative to contralateral normal sample from the same patient.[6]

Lee *et al.* in 2017 investigated the differences in microbiota between normal, epithelial precursor patients and cancer patients with different lifestyles using NGS and the overall microbiome composition of five genera and revealed significant differences between epithelial precursor lesion and cancer patients.[33]

Zhao *et al.* in 2017 studied the variations in oral microbiota associated with oral cancer. They profiled the bacteria within OSCC lesion surface samples at the species level using NGS and comprehensively investigated bacterial community composition and functional genes in these samples and observed significantly greater bacterial diversity in cancer samples than in normal samples.[34]

Banerjee *et al.* in 2017 studied the microbial signature associated with oropharyngeal and OSCCs by using a pan-pathogen array technology (Pathochip) coupled with NGS. They found different microbiome signatures in OSCC tissue than those in adjacent clinically normal controls or oral tissue from otherwise healthy controls.[35]

Furquim *et al.* in 2017 studied salivary microbiome and oral cancer risk in Fanconi anemia patients by 16S rRNA sequencing of the V3–V4 hypervariable region and the most abundant phyla found were *Firmicutes* and *Bacteroidetes*.[36]

Mok *et al.* in 2017 studied the community variations in oral microbiome associated with normal, potentially malignant disorders and malignant lesions of the oral cavity using 16S rDNA sequencing. They identified a core microbiome in the form of common bacterial phyla and genera in normal,

OPMD and oral cancer patients. The predominant phylum and genus were Firmicutes and *Streptococcus,* respectively.[37]

Yang *et al.* in 2018 studied oral microbiota community dynamics associated with OSCC staging. The oral microbiota communities from Stage 4 patients showed significantly higher complexity than those from healthy controls. Their results revealed changes during the progression of oral cancer. A marker panel of upregulated *F. periodonticum* and downregulated *S. mitis* and *P. pasteri* bacteria can discriminate OSCC Stage 4 patients from healthy controls.[38]

Hayes *et al.* in March 2018 examined the association between the oral microbiome and incident head-and-neck squamous cell cancer prospectively and concluded that greater oral abundance of commensal *Corynebacterium* and *Kingella* is associated with decreased risk of HNSCC.[39]

Kengo *et al.* in 2018 studied the changes in the oral microbiome profiles in OSCC and revealed some shifts in the oral microbial communities and concluded that salivary microbiome changes may have potential application as a useful diagnostic tool for early detection of OSCC and malignant transformation of precancerous regions. They observed that the frequency of *Bacteroidetes* was more abundant in OSCC patients compared with leukoplakia and normal controls. The genera *Streptococcus* and *Rothia* were significantly lower in OSCC patients compared to leukoplakia and normal controls.[40]

ORAL MICROBIOME IN CANCER AT EXTRA-ORAL SITES

Oral microbiome is known to be involved in the initiation and progression of disease not only in the oral cavity, but also at distant sites.[41]

There are data suggesting that bacteria in the oral cavity play a role in tumors of the gastrointestinal tract.[8]

Bacteria and fungi present in the oral cavity and gut are also associated with the pathogenesis of colorectal cancer (CRC).[42]

P. gingivalis has been related with the promotion of CRC. Metagenomics studies using fecal samples, identify *Parvimonas micra* and *Pasteurella stomatis* bacteria from the oral cavity as markers of CRC. These bacteria have been reported in an oral microbe-induced colorectal tumorigenesis model. Hence, it is important to consider the role of oral microbial communities in the development of CRC.[43]

A study by Fan *et al.* provides supportive documentation that oral microbiota may play a role in the etiology of pancreatic cancer.[44]

OMICS TECHNOLOGIES FOR ORAL CANCER MICROBIOME RESEARCH

The word "omics" refers to a field of study in biological sciences that ends with –omics, such as genomics, transcriptomics, proteomics or metabolomics. The ending –ome is used to describe the object of study of such fields, such as genome, transcriptome, proteome or metabolome.[45]

The microbial community can be characterized by its composition (Metagenome), by the analysis of transcripts (Metatranscriptome), by the analysis of proteins (Metaproteome) or by the analysis of the final products resulting from the collective action of the community (Metabolomics).[46]

Metagenomics

Metagenomics (meta-beyond) is the study of the genetic material of entire communities of organisms. The main purpose of metagenomics is to infer taxonomic profile of a microbial community. It is the first step to study the microbiome.[47]

It gives insight of the bacterial profiles, detects specific metabolic pathways and the associated genes.[48]

Metataxonomic sequencing is a technique in which bacterial composition of a sample is determined by PCR amplification and sequencing one or more variable regions of the 16S rRNA gene present in the bacterial genomes from the extracted DNA sample.[49]

The 16S rRNA gene is present in all prokaryotes and is a highly conserved genetic marker.[50]

16S rRNA sequencing only determines the presence or abundance of bacterial species and allows researchers to draw observational conclusions.[51]

16S rRNA gene surveys are generally quoted as metagenomics studies but they are not. In 16S rRNA gene surveys, the study is focused on a single gene used as a taxonomic marker while structural metagenomics intends to investigate the genomes of microbial community members.[52]

Metatranscriptomics

The transcriptome is the total mRNA in a cell or organism. It is the template for protein synthesis in a process called translation.[47]

It reflects the genes that are actively expressed at any given moment.[53]

Metatranscriptomics is emerging as a powerful approach for functional characterization of complex microbial communities.[54]

Metaproteomics

The proteome is defined as the set of all expressed proteins in a cell, tissue or organism. Proteomics aims to characterize information flow within the cell and organism via protein pathways and network. The eventual aim of proteomics is understanding the functional relevance of proteins.[47]

Metaproteomics allows us to study the presence and abundances of proteins in any microbial community.[55]

Metabolomics

Metabolomics can generally be defined as the study of global metabolite profiles in a system (cell, tissue or organism). It is the final downstream product of gene transcription and therefore changes in the metabolome are amplified relative to changes in the transcriptome and proteome.[47] Oral metabolomic analysis has been used for the exploration of biomarkers in different types of cancers, including oral cancer.[46]

CONCLUSION

Human body is a host to numerous microorganisms. These microorganisms along with their genetic material form a significant part of the human body which is called as the microbiome. Alterations in the composition of the normal microbiome are referred to as dysbiosis which leads to diseased states. Microbes have been associated with oral diseases such as dental caries, periodontal diseases and many others including oral cancer. Bacteria and viruses have been implicated in the etiology of oral cancer. Microbiome research has become the current buzzword. Omics technologies have revolutionized our understanding of the microbiome. We are at an embryonic stage of microbiome research. Studies have shown alterations of the oral microbiome in oral cancer. The first step is understanding the changes in the composition of the oral microbiome from normal to precancer to cancer. The next level of research should be focusing on the functional microbiome, i.e., metagenomics, meta-transcriptomics, meta-proteomics and metabolomics. This will concentrate on the metabolic pathways, host–microbiome interactions and how the microbiome contributes to carcinogenesis, which may help in knowing why a therapy works for specific patients only. This may assist in targeted therapies, precision and personalized medicine and better treatment planning and thus contribute to health care.

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Conflicts of interest

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