The Oral Microbiome and Cancer

A. Ross Kerr DDS, MSD

Introduction

The human microbiome is defined as the collective genomes of the microbes (composed of bacteria, bacteriophages, fungi, protozoa and viruses) that live inside and on the human body, and there are approximately 10 microbes and 100 microbial genes for each human cell and gene respectively. Collectively the human genome and microbiome is known as the metagenome. The oral microflora comprises a number of specific ecological surface niches (biofilms) that evolve from birth through to death: initially as populations adherent to mucosal surfaces passed on from maternal flora, to tooth-adherent populations following eruption of the dentitions, and with changes in both supra- and subgingival niches (ie dental plaque/biofilm). In diseases states, there is a shift in the equilibrium away from the dynamic synergistic interplay of these healthy oral microbial populations towards a narrower diversity of healthy populations in antagonistic interplay with pathologic populations, and coupled with variable inflammatory host immune responses. The structure and function of the oral microflora (and associated microbiome) has been investigated in numerous oral diseases caused by bacteria, fungi and viruses (eg. periodontal diseases) and the systemic diseases linked to chronic infections (eg. diabetes mellitus, cardiovascular disease, and cancer). The purpose of this lecture is to provide an updated understanding about the oral microbiome in health and disease, with a particular emphasis on the relationship with cancer, not only oral and pharyngeal cancers, but also other cancer sites.

Our understanding of the microbiome has been limited by our inability to detect important microbial populations using culture-based methods. Advances in high-throughput genome sequencing led the National Institutes of Health (NIH) to launch the Human Microbiome Project (HMP) as an extension of the Human Genome Project (see http://commonfund.nih.gov/hmp), catalyzing multiple studies to explore the diversity of the microbiome across different body habitats in both health and disease states. An initial landmark study has explored the human bacteriome in health by sampling multiple habitats (ie: oral, gut, urogenital and skin sites) over two time points in a cohort of more than 240 "healthy" adults.[1,2] An analysis of bacterial diversity was performed using complex methodology including 16S ribosomal RNA gene profiling and shotgun metagenomic sequencing.[3] In general, the results showed that there is considerable intra- and interpersonal variation in the composition of the microbiome, yet despite such complexity, sophisticated data analysis incorporating demographics (eg. gender, education levels), life-style factors (eg. diet) and environmental exposures (eg. breast feeding), has allowed a distillation into distinct groups or communities within habitats that share similar signatures. Further investigation is needed to establish if these communities predict risk of disease.[4]

In general, the oral microbiome is diverse, and oral wash samples (surrogates for the oral flora) from 20 healthy subjects analyzed using high-throughput methods revealed the presence of 5 major phyla (Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria) and that Streptococcus, Veillonella, Leptotrichia, Prevotella, and Haemophilus genera were the most abundant.[5] In an effort to discern differences across the different oral niches, a landmark HMP study has explored the microbiome of samples collected from 9 distinct oral/pharyngeal sites: saliva, supragingival plaque, subgingival plaque, keratinized gingiva, buccal mucosa, tongue dorsum, hard palate, palatine tonsils, and posterior pharyngeal wall. Similar phyla were represented in these samples, and statistical analysis allowed a distillation into three distinct community groups:

- Group 1 (buccal mucosa, keratinized gingiva, and hard palate) demonstrated a predominance of organisms from the phylum Firmicutes (with a very high proportion [approximately 50%] from the genus Strepto-

coccus) followed in relative abundance by the phyla Proteobacteria, Bacteroidetes and either Actinobacteria or Fusobacteria
- Group 2 (saliva, tongue, tonsils, and posterior pharyngeal wall) demonstrated a decreased relative abundance of Firmicutes compared to Group 1 replaced by increased levels of four phyla: Bacteroidetes, Fusobacteria, Actinobacteria and the candidate phylum TM7, and with a predominance of Streptococcus (approximately 20%), followed by approximately equal abundance of the genera Veillonella, Prevotella, Neisseria, Fusobacterium, Actinomyces and Leptotrichia
- Group 3, (the sub- and supra-gingival plaque biofilm) showed the greatest bacterial diversity and had a further decrease in Firmicutes compared to Groups 1 and 2, with a marked increase in the relative abundance of Actinobacteria and with a similar profile of genera as Group 2 plus Corynebacterium, Capnocytophaga, Rothia and Porphyromonas[6]

Further analysis of these groups revealed a low but non-zero abundance of known bacterial pathogens in the oral cavity habitat were also consistently detected in these healthy subjects, namely Treponema, Aggregatibacter, Porphyromonas, and Tannerella species. Also, comparison of the supra-gingival and sub-gingival sub-sites epitomized niche specialization and confirmed the physiological distinctions known between these two sites: with facultative anaerobic and obligate anaerobic genera populating the supra-gingival and subgingival sites respectively.

Despite the focus on the oral bacteriome, the diversity of both the oral mycobiome and virome, and their interplay with bacterial communities has been explored. In a study of 20 healthy individuals sampled by an oral rinse at baseline, 85 genera and 101 fungal species were detected. Candida species were the most frequently obtained genera, isolated from 75% of all study participants, followed by Cladosporium (65%), Aureobasidium, Saccharomycetales (50% for both), Aspergillus (35%), Fusarium (30%), and Cryptococcus (20%), suggesting that fungi play an important role, not only in disease states but also in the healthy microbiome.[7] The oral virome is mainly comprised of "commensal" bacteriophages mirroring the diversity of the oral bacteriome rather than pathogenic eukaryotic viruses.[8] Bacteriophages are involved in the exchange of genetic material and hence provide another intricate layer of complexity to the microbiome. Human papillomavirus communities across various habitats in healthy patients have also recently been described.[9]

In terms of the functional attributes of the oral microbiome in health, little is currently understood and more studies are needed to identify the significance of the communities (ie. the metaproteome or metametabalome). Techniques such as shotgun metagenomic sequencing data provides some insight into the metabolic pathways, and as an example, bacterial small sugar transporters were shown to be of particular abundance in the oral cavity sites.

There is a large literature exploring the oral microbiome in various disease states and a discussion of this literature is beyond the scope of this lecture. In terms of cancer however, it was the discovery of the association of Helicobacter pylori infection with gastric adenocarcinoma that spawned an exploration for other cancer-infectious disease associations. Epidemiologic studies have long reported an alleged association of periodontal diseases and tooth loss with cancer, and there is data to support an association with oral, esophageal, gastric, and pancreatic cancer, even after controlling for confounding factors such as tobacco use.[10,11] More recently, the principal periodontal pathogen Porphyromonas gingivalis has been identified as a biomarker for orodigestive tract cancer death (colorectal and possibly pancreatic cancer).[12] Recent microbiome studies lend support for the association of upper digestive tract flora with gastric and esophageal cancers.[13] There is also some evidence to support associations between both oral fungal and viral organisms and cancer. As an example, human papillomavirus 16 (HPV-16) infection is an established cause for the majority of oropharyngeal squamous cell carcinomas.[14]

The mechanisms by which oral bacterial flora might cause carcinogenesis are hypothetical, particularly for sites distant to the oral cavity, and may include local activation of carcinogens by oral microbes (eg. conversion of ethanol to acetaldehyde),[15] or release of pro-inflammatory mediators that can dysregulate cellular cycling, disrupt signaling mechanisms, and act as tumor promoters.[16]

Early studies using culture-dependent assays concluded that oral squamous cell carcinomas (compared to normal tissues with the same patient) have a significantly increased abundance of both aerobic and anaerobic bacteria with in-

creases in Veillonella, Fusobacterium, Prevotella, Porphyromonas, Actinomyces and Clostridium (anaerobes), and Haemophilus, Enterobacteriaceae and Streptococcus species (aerobes). In addition, approximately 30% of cancers were shown to harbor Candida albicans, but not at control sites.[17] The oral microbiome in oral squamous cell carcinomas has been recently studied using culture-independent assays. In one pilot study, the microbiome in a series of 10 oral tongue/floor of mouth cancers was compared to that of normal tissue in the same patients using a 16s rRNA assay coupled with denaturing gradient gel electrophoresis (DGGE). Streptococcus intermedius was present in 70% of both cancer and normal tissues. Streptococcus sp. oral taxon 058, Peptostreptococcus stomatis, Streptococcus salivarius, Streptococcus gordonii, Gemella haemolysans, Gemella morbillorum, Johnsonella ignava and Streptococcus parasanguinis were highly associated with the cancers and Granulicatella adiacens was prevalent the normal tissue.[18] Recently, a cohort of oral cancers and premalignant oral lesions matched with normal contralateral tissue sites from the same patient were profiled by sequencing 16S rDNA hypervariable region amplicons. In cancer samples, the abundance of the phyla Firmicutes (especially Streptococcus) and Actinobacteria (especially Rothia) were significantly decreased relative to contralateral normal samples. Significant decreases in abundance of these phyla were observed for pre-cancers, but not when comparing samples from contralateral sites (tongue and floor of mouth) from healthy individuals.[19]

In summary, technological advances have provided insights about the structure of the oral microbiome in health and, to a lesser extent, in disease. Further research is needed to explore the functional implications of the oral microbiome in terms of diagnosis and risk assessment of disease (ie. cancer), or possibly therapeutic strategies to restore the health of the oral ecosystem.

References

1. Huttenhower C, Gevers D, et al (The Human Microbiome Project Consortium). Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486(7402):207-14.

2. Methe BA, Nelson KE et al (The Human Microbiome Project Consortium). A framework for human microbiome research. *Nature* 2012;486(7402):215-21.

3. Li K, Bihan M, Yooseph S, Methe BA. Analyses of the microbial diversity across the human microbiome. *PLoS One* 2012;7(6):e32118.

4. Ding T, Schloss PD. Dynamics and associations of microbial community types across the human body. *Nature* 2014;509(7500):357-60.

5. Ahn J, Yang L, Paster BJ, et al. Oral microbiome profiles: 16S rRNA pyrosequencing and microarray assay comparison. *PLoS One* 2011;6(7):e22788.

6. Segata N, Haake SK, Mannon P, et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol* 2012;13(6):R42.

7. Ghannoum MA, Jurevic RJ, Mukherjee PK, et al. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathog* 2010;6(1):e1000713.

8. Abeles SR, Pride DT. Molecular Bases and Role of Viruses in the Human Microbiome. *J Mol Biol* 2014.

9. Ma Y, Madupu R, Karaoz U, et al. Human papillomavirus community in healthy persons, defined by metagenomics analysis of human microbiome project shotgun sequencing data sets. *J Virol* 2014;88(9):4786-97.

10. Meyer MS, Joshipura K, Giovannucci E, Michaud DS. A review of the relationship between tooth loss, periodontal disease, and cancer. *Cancer Causes Control* 2008;19(9):895-907.

11. Fitzpatrick SG, Katz J. The association between periodontal disease and cancer: a review of the literature. *J Dent* 2010;38(2):83-95.

12. Ahn J, Segers S, Hayes RB. Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. *Carcinogenesis* 2012;33(5):1055-8.

13. Yu G, Gail MH, Shi J, et al. Association between upper digestive tract microbiota and cancer-predisposing states in the esophagus and stomach. *Cancer Epidemiol Biomarkers Prev* 2014;23(5):735-41.

14. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;356(19):1944-56.

15. Meurman JH, Uittamo J. Oral micro-organisms in the etiology of cancer. *Acta Odontol Scand* 2008;66(6):321-6.

16. Meurman JH. Oral microbiota and cancer. *J Oral Microbiol* 2010;2.

17. Nagy KN, Sonkodi I, Szoke I, Nagy E, Newman HN. The microflora associated with human oral carcinomas. *Oral Oncol* 1998;34(4):304-8.

18. Pushalkar S, Ji X, Li Y, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. *BMC Microbiol* 2012;12:144.

19. Schmidt BL, Kuczynski J, Bhattacharya A, et al. Changes in abundance of oral microbiota associated with oral cancer. *PLoS One* 2014;9(6):e98741.