The oral microbiome and lung cancer risk

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The oral cavity is instrumental to the human body in several ways. It is the entry point for both ingested (to the GI tract) and inhaled (to the respirable tract) substances. The oral microbiome resides within biofilms throughout the oral cavity and forms an ecosystem that helps to maintain health. The breadth and depth of the oral microbiome is impressive: 1 mL of saliva contains 10^8 microbial cells and 700 distinct prokaryotic taxa. There are rich communities such as bacteria, fungi, viruses, archaea and protists, among which about 54% are cultivatable and identified, 14% are cultivatable, but not identified, and 32% are not even cultivatable.[1] Caselli *et al* defined the oral microbiome in 20 healthy individuals from Europe by whole genome sequencing (WGS) and reported that α-diversity differed significantly among the different microsites in the mouth of each participant, but not among the participants, supporting the notion of a recognisable healthy oral microbiome.[1]

With its rich microenvironment, the oral cavity remains less understood than the gut as to its health effects. Metagenomic studies have associated the oral microbiome with oral cancer and at least one with oesophageal cancer, and another with head and neck cancer.[2]

The use of recent 16 s rRNA gene next-generation sequencing methods have provided an important look into the complexity of the bacterial inhabitants of the oral microbiome and have helped to distinguish the differences between healthy microbiome and disease states of the oral cavity.

Shotgun metagenomic sequencing is a sophisticated technique that is based on unrestricted DNA sequencing of all genetic material within a sample to allow for a deeper taxonomical analysis of the microbiome. The assembly of short sequencing reads into larger fragments can increase the discriminatory power between microbes, enhancing taxonomic resolution up to the species or strain level. This technique provides more detailed insight into the functional capacity of commensals within a community and a unique understanding about the intestinal tract.[3] The intrasubject versus intersubject variability in the oral (and lung) microbiome is still not well understood. We are still learning whether there is a predictable healthy state oral microbiome signal in this variability. A large population-based study of healthy adults characterised the oral microbiome by 16 s sequencing and described the influence of tobacco smoking on oral ecology and functional consequences.[4] Overall oral microbiome composition differed significantly between current and non-current (former and never) smokers. Current smokers had lower relative abundance of the phylum Proteobacteria (4.6%) compared with never smokers (11.7%), with no difference between former and never smokers. The depletion of Proteobacteria in current smokers was also observed at class, genus and Operational Taxonomic Unit (OTU) levels. Taxa not belonging to Proteobacteria were also associated with smoking. The genera *Capnocytophaga*, *Peptostreptococcus* and *Leptotrichia* were depleted, while *Atopobium* and *Streptococcus* were enriched, in current compared with never smokers. Functional analysis from inferred metagenomes showed that bacterial genera depleted by smoking were related to carbohydrate and energy metabolism, as well as to xenobiotic metabolism. These findings demonstrate that smoking alters significantly the oral microbiome, potentially leading to shifts in functional pathways with implications for smoking-related diseases.

There are several postulated mechanisms of carcinogenic action of oral microbes. One is bacterial stimulation of chronic inflammation, which can cause cell proliferation, mutagenesis, oncogene activation and angiogenesis. Another mechanism is that bacteria can affect cell proliferation, cytoskeletal rearrangements, activation of nuclear factor kappa-beta (NF-kB) and inhibition of apoptosis.[5]

In this issue of *Thorax*, Hosgood *et al*[6] present an interesting study of the association between the oral microbiome and lung cancer in prediagnostic samples collected from a nested case-control study in two large cohorts of lifelong non-smokers from Shanghai, China, enrolled between 1996 and 2006. Metagenomic shotgun sequencing measured the community structure and abundance of the oral microbiome in prediagnostic oral rinse samples of each case and control, and analyses focused on lung cancer risk with α-diversity metrics and relative abundance of taxa. The Microbiome Regression-Based Kernel Association Test evaluated the association between risk and microbiome β-diversity. Multivariate analyses were adjusted for matching factors and adjustments made for multiple comparisons. The authors analysed data excluding participants who used antibiotics in the week before saliva collection, further strengthening their observations. They found that lower α-diversity was associated with greater risk of lung cancer. Moreover, certain specific taxa abundance was associated with altered risk: increased relative abundance within the Bacteroidetes and Spirochaetes phyla was associated with reduced risk of lung cancer, whereas increased abundance within the Firmicutes phyla was associated with increased risk of lung cancer.

The study does have some limitations that should be considered in interpreting the results and conclusions. The final reported analysis is not matched, that the participant numbers are relatively small, especially for men. Also, there are no data on the association of the oral microbiome measurements with markers of immune status of the participants (eg, serum or oral cytokines, cell markers, etc).

Notwithstanding these limitations, in light of careful adjustments for covariates collected in these populations, and the fact that these findings replicate those of smaller studies done earlier in another part of China in non-smokers suggest that the results are robust and the observed loss of α-diversity, combined with specific taxa abundance may well play a mechanistic role in the genesis of lung cancer in non-smokers. There are a number of questions that are provoked by this study. First, how stable is the human oral microbiome over time? Second, if the human oral microbiome varies over time, what determines that variability? Third, how does the ambient environment such as exposure to air pollutants, affect the oral (and lung) microbiome? A recent study by Wu *et al*[7] described the environmental microbiome in metalworking fluid and human microbiome in lung to answer questions of pathogenesis in an occupational lung disease characterised by bronchiolitis, alveolar ductitis and emphysema with B cell primary lymphoid follicles (BADE) among patients exposed to metalworking fluids. They provide the first evidence in humans that the microbiome of one's environment can seed and shape the lung microbiome. Finally, in terms of questions being provoked by the study, it remains

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unclear whether the oral microbiome as measured in this (and other) epidemiological study represents a causative agent or only a marker of disease or immune activity. If it is the former, then it will be important to understand whether the oral microbiome actually seeds the lung microbiome and thus acts locally.

It will be interesting to see more studies of the oral, nasal and lower respiratory tracts in relation to environmental exposures and various lung disorders. The oral cavity and nasopharynx are the foyers to the lung and further understanding of the functional aspects of the microbiome in these compartments will surely help shed light on disease pathogenesis and disease modification.

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