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Changes of saliva microbiota in nasopharyngeal carcinoma patients under chemoradiation therapy

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ABSTRACT

Objective: A growing body of evidence has implicated human oral microbiota in the aetiology of oral and systemic diseases. Nasopharyngeal carcinoma (NPC), an epithelial-originated malignancy, has a complex aetiology not yet fully understood. Chemoradiation therapy of NPC can affect oral microbiota and is usually accompanied by plaque accumulation. Thus, the study aimed to understand the diversity, divergence and development of the oral microbiota in NPC patients and their associated treatment, which might provide useful insights into disease aetiology and treatment side effects.

Design: A longitudinal study was designed that included three Chinese adults with NPC. Saliva samples were collected at three time points: prior to the chemoradiation treatment (carcinoma baseline, or CB), 7 months post-treatment (carcinoma-after-therapy phase 1 or CA1) and 12 months post-treatment (carcinoma-after-therapy phase 2 or CA2). Pyrosequencing of the bacterial 16S ribosomal DNA (rDNA) V1–V3 hypervariable region was employed to characterise the microbiota. Saliva samples of three healthy subjects from our former study were employed as healthy controls. Principal coordinates analysis (PCoA), Metastats and random forest prediction models were used to reveal the key microbial members associated with NPC and its treatment programme.

Results: (1) In total, 412 bacterial species from at least 107 genera and 13 phyla were found in the saliva samples of the NPC patients. (2) PCoA revealed that not only were the microbiota from NPC patients distinct from those of healthy controls ($p < 0.001$) but also that separation was found on the saliva microbiota between pre- and post-therapy ($p < 0.001$) in the NPC samples. (3) At the genus level and the operational taxonomic unit (OTU) level, *Streptococcus* was found with lower abundance in NPC samples. (4) Chemoradiation therapy did not incur similar changes in microbiota structure among the three NPC patients; the microbiota in one of them stayed largely steady, while those in the other two showed significant alteration.

Conclusions: This is the first study employing culture-independent techniques to interrogate the phylogenetic diversity, divergence and temporal development of oral microbiota in NPC

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patients. Our results indicated that certain bacterial taxa might be associated with NPC and that oral microbiota of NPC patients might respond to the chemoradiation therapy in a host-specific manner. Further investigation with larger sample size should help to validate the links between oral microbiota and NPC.

1. Background

Nasopharyngeal carcinoma (NPC) is an epithelial-tissue-originated malignancy with an annual incidence rate of less than 1 per 100,000 in most populations. However, the southern Chinese population of Guangdong Province has been an exception, where the annual incidence of more than 20 cases per 100,000 is reported; yet, the underlying mechanism has remained elusive.[1] Multiple factors including environmental, genetic factors and chronic infection of Epstein–Barr virus (EBV) might all play a role in the aetiology of NPC.[2] Due to the radio- and chemo-sensitivity of NPC, radiotherapy and chemotherapy have become the standard treatment option. However, chemoradiation-based treatment of the NPC was usually found associated with several unwelcome side effects such as severe oral mucositis, gingivitis, oral candidiasis, cellulitis and radiation caries,[3–10] suggesting the perturbation to the oral ecosystem.

The oral ecosystem is home to a plethora of human symbionts, most of which are bacteria.[11,12] There is increasing evidence that the oral microbiota play a major role in health and in diseases.[13–19] Although the role of oral microbiota has remained elusive, NPC and its associated treatment might cause structural and functional changes of oral microbiota. In fact, chemoradiation therapy was found usually accompanied by plaque accumulation (likely due to the decreased salivary secretion rate), potentially resulting in an increase of opportunistic infections.[3,4] This suggested that thorough understanding of the diversity, divergence and development of the oral microbiota in NPC might provide useful insights into disease aetiology as well as the side effects of NPC.

However, two major challenges have hindered the investigation into the role of human microbiota in NPC. Culture-dependent approaches for microbial survey in NPC revealed a shift in plaque bacterial memberships between pre- and post-chemoradiation-based treatments, which included a relative increase of *Lactobacillus* and *Candida* species after treatment.[20,21] However, the majority of oral bacterial species remained unculturable,[22] which has posed a key challenge to culture-dependent microbial surveys. However, one practical challenge in pinpointing the role of oral microbiota in NPC has been the difficulty in sampling tissues and the associated microbiota, as the location of the carcinoma in the nasopharynx is usually deep inside the head and, thus, not easily examined or sampled.

Pyrosequencing of 16S ribosomal RNA (rRNA), which is independent of microbial cultivation, has allowed us and others to characterise human oral microbiota and test their links to a series of oral and systemic diseases such as caries,[14,23] gingivitis,[24] periodontitis,[25] pancreatic cancer[19] and gastrointestinal cancer[18] without the sampling biases inherent in culture-dependent approaches.[26,27] Moreover, our recent works and other reports have shown that saliva microbiota are sensitive to a number of oral and systemic disease states of the hosts,[14,18,19,24] suggesting saliva might be a non-invasive, readily accessible, yet meaningful venue to interrogate the link between oral microbiota and NPC.

Here, employing pyrosequencing of 16S genes, we have sampled saliva microbiota in a longitudinal study in three NPC patients, each at three time points: prior to the chemoradiotherapy treatment, 7 months after the treatment and 12 months after the treatment. The diversity and structure of the saliva microbiota pre- and post-treatment were reconstructed and compared.

2. Methods

2.1. *Subject selection and sampling protocol*

Between 24 November 2010 and 3 July 2012, NPC patients enrolled at Sun Yat-sen University Cancer Center (SYSUCC) in Guangzhou (Guangdong, China) were screened for the longitudinal study of the oral microbiome with the approval of the Research Ethics Board of Sun Yat-sen University Cancer Center (Table 1). Written informed consent was obtained. The inclusion criteria included: first-visit patients with diagnosis of NPC, unrelated individuals of both sexes and an age between 34 and 45 years. In addition, the following exclusion criteria were applied and patients who met any of the criteria were excluded: smoking; ongoing participation in another clinical study; use of antibiotic, anti-inflammatory or

Table 1 – Metadata for the three subjects sampled in this study.

Subject ID	Gender	Age	Therapeutic strategies	Radiological dose	Times of therapy	DMFT	Chemical therapy
T1	M	36	IMRT	68 Gy	30	3	2 stages
T3	M	45	2DRT	70 Gy	35	6	5 stages
T4	M	34	IMRT	70 Gy	30	0	NO
pg1.H1	F	22	NO	NO	NO	2	NO
pg1.H6	F	23	NO	NO	NO	4	NO
pg1.H11	F	21	NO	NO	NO	3	NO