CHAPTER

Oral metagenomics changes the game in carcinogenesis

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9.1 Introduction

Microbial metagenomics has provided advances in the recognition of either known or unknown microbiota. Also, the advantages of metagenomic analysis have been applied in various fields, such as the composition of normal and disease microbiomes. Oral microbiota is associated with many aspects of human health, systemic implications, and pathologies. The oral cavity is exposed to microbiota that includes diverse species of bacteria, viruses, archaea, fungi, and protozoa that preserve the oral ecosystem against pathogens (Girija and Ganesh, 2022). Oral healthy microbiota has balance in their unavoidable interactions. Homeostasis of microbiota in their microenvironment in the oral cavity depends on complex signaling, intrinsic host factors, and external factors (Cornejo Ulloa et al., 2019). These sensitive ecosystem changes impact microbiota niches and threaten oral and systemic health (Marsh, 2018). The oral microbiota directly affects pathogenicity in oral and other organ diseases, either with translocation or overload of microorganisms and their byproducts or indirectly releasing inflammatory mediators that weaken the immune system. Also, out-of-balance in oral microbes is demonstrated to result in systemic diseases, such as cancer, cardiovascular, neurological, and metabolic diseases. Dysbiosis in the oral microbiota composition typically causes systemic diseases (Sudhakara et al., 2018).

Oral bacteria are associated with various pathological processes (Fig. 9.1). The analysis of oral cavity microbiota demonstrated that many bacterial families are more engaged in oral squamous cell carcinoma (OSCC) (Zhang et al., 2019; Banakar et al., 2022). Oral microbiotas' role in tumorigenesis has been confirmed

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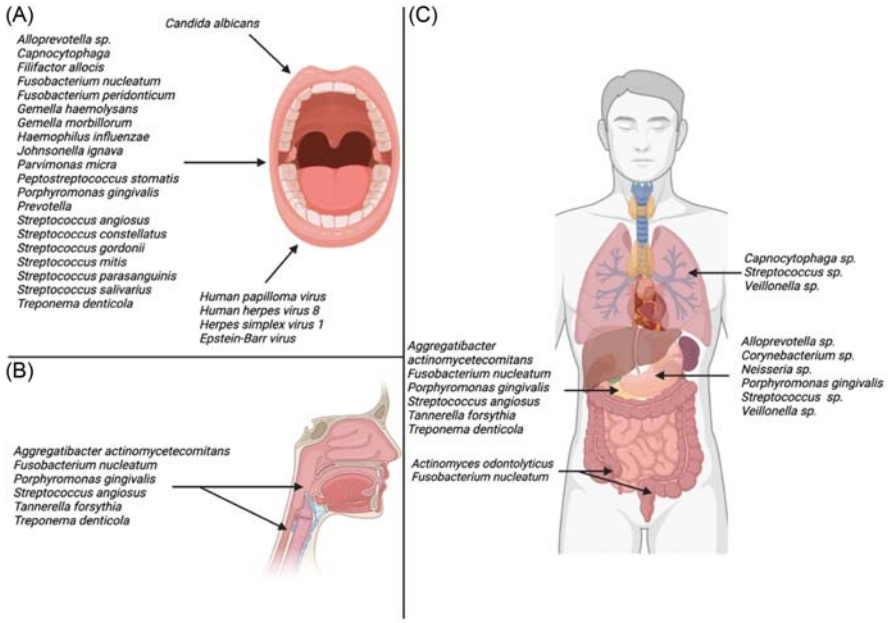

FIGURE 9.1

The oral microbiota has been linked to the following cancers: (A) oral; (B) esophageal; (C) lung, gastric, pancreatic, and colorectal (Stasiewicz and Karpiński, 2022).

in throat cancer (Wang et al., 2019) and as the promoting factor for colorectal cancer (CRC). Also, they can be used as biomarkers for diagnosing pancreatic cancer (Stasiewicz et al., 2021) and gastrointestinal carcinomas (Chen et al., 2019). Also, oral microbiomes, such as intratumor microbiomes, improve cancer progression (Liu and Zhang, 2022) and lymph node metastasis (Eun et al., 2021). Moreover, microorganisms play a role in resisting tumor progression (Bhatt et al., 2017), and it has been approved that modulating the microbiome could improve the therapeutic response (McQuade et al., 2019). Different mechanisms and factors affect the microbiome complexity that can lead to cancer. Three mechanisms including chronic inflammation, inhibition of cellular apoptosis by activation of NF-B, and bacteria products that act in a carcinogenic manner are engaged in carcinogenesis (Zhang et al., 2018). Also, genetic and epigenetic factors can alter the complexity of the microbiome. New methods such as metagenomic next-generation sequencing (mNGS) have introduced a novel gene profiling method and an accurate analysis method for microbiota. This method could be more useful for oral metagenomics because the oral cavity microbiota is available in dentistry sewage and saliva (Wang et al., 2022). The findings showed that the oral microbiome gene profiles could be exploited as diagnostic indicators and therapeutic targets. Also, the studies showed that the genetic polymorphisms and

frequencies of bitter-taste receptors control the risk and resistance of cancers (Yamaki et al., 2017). On the other hand, this NGS demonstrates that the genetic variants in taste-related genes are interrelated to the composition of bacteria or fungi in oral plaques (de Jesus et al., 2022).

Many recent studies have focused on oral metagenomics to study the microbiota associated with oral and systemic diseases, including different cancers. So, understanding the compositions of oral microbiota communities that mediate carcinogenesis could help cancer prediction, diagnosis, and treatment. In this chapter, we reviewed the importance of oral metagenomics to present the new approach to predicting, diagnosing, and targeting cancer treatment. Moreover, we emphasize using dentistry sewage and saliva as waste per person along with the high-resolution NGS method. The megabank library improves diagnosis accuracy, efficient treatment, and personalized health care. Finally, we introduce the advantages and challenges of big data analysis.

9.2 Oral microbiota and cancer

Microbiota present throughout the human body constitutes various microhabitats in diverse regions such as the oral cavity, gastrointestinal system, and rectal. Microbial infection is one of the most important causes of cancer, and commensal microbiota also has a large impact on the initiation and progression of cancer.

The oral microbiota of normal and tumoral tissues could determine various physiological conditions. The analysis of microbial genera in healthy individuals' oral microbiota showed a high diversity between oral microhabitats. The method of 16S Rrna NGS supports the accurate core oral microbiota that can be provided as a reference for eubiosis definition (Caselli et al., 2020). Oral microbiome dysbiosis is implicated in tumorigenesis in the oral cavity and other tissues. In the oral cavity, various oral microbiota in the salivary microbiome and bacterial biofilm profiles have been correlated with carcinogenesis and cancer progression by interacting with the host immune system through several mechanisms (Fig. 9.2). Accordingly, the salivary microbiota profile could be a source of cancer biomarkers (Yang et al., 2018).

The oral microbiota in the human body is found in many distinct sites. It has been linked to numerous cancers, such as oral cancer (Su Mun et al., 2021), esophageal cancer (Kawasaki et al., 2021), lung cancer (Rajpoot et al., 2018), liver cancer (Li et al., 2020), CRC (Nosho et al., 2016), gastric cancer (Huang et al., 2021), pancreatic cancer (Nagata et al., 2022), head and neck cancer (Hayes et al., 2018), and breast cancer (Wu et al., 2022). Some oral anaerobic and aerobic bacteria are associated with periodontitis, and appendicitis is the most common pathobionts in carcinomas under certain conditions. In this way, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* are oral microbiomes that correlated with increased biomarkers of colorectal (Huh and Roh, 2020) and

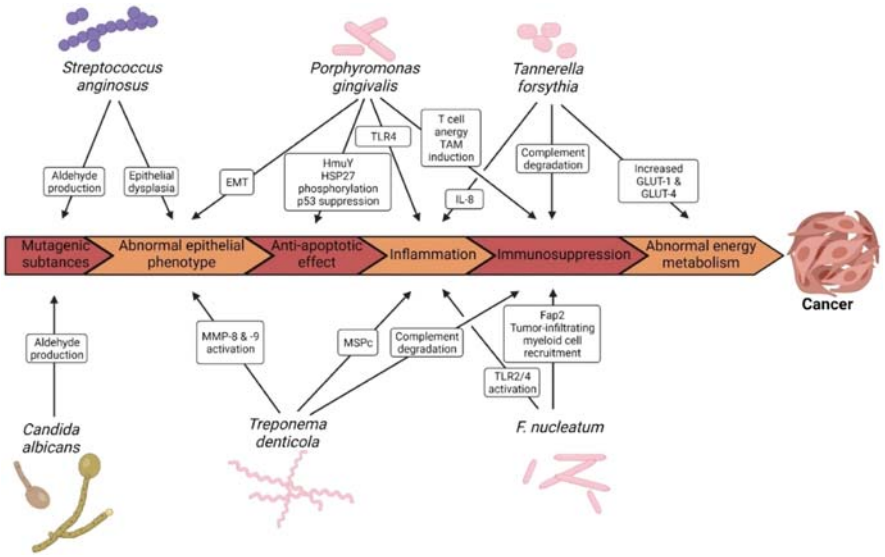

FIGURE 9.2

Carcinogenic mechanisms are employed by select members of the oral microbiota (Stasiewicz and Karpiński, 2022).

pancreatic cancer (Fan et al., 2018), and the microbiota such as *Porphyromonas* spp., *Fusobacterium* spp., *Neisseria* spp., and *Corynebacterium* spp. affect OSCC (behavior) by regulating mRNA and cytokines and altering the gene expression level related to inflammation, migration, invasion, and cell cycle (Hu et al., 2021). Also, the role of HPV in the oropharyngeal is well known, but more studies are needed for oral fungi and parasites concerning carcinogenesis.

The microbiota has been identified as using different mechanisms in cancer progression. The first mechanism involves the immune system response and inflammation against microbiota. Preclinical studies on IL-10 knockout mice support the relationship between inflammation and cancer. An immune-suppressive cytokine, IL-10, prevents improper immune reactions targeting commensal gut bacteria. This study demonstrated a correlation between the tumor burden and the inflammatory phenotype of IL-10 knockout mice. Toll-like receptors (TLRs) detect bacterial antigens and innate immune response mediated by the MyD88 adapter and NF-κB transcription factors and its intracellular signaling cascades that link microbiota-induced inflammation (Cooper et al., 2014) and CRC (Uronis et al., 2009). Proinflammatory TH_{17} cells depend on microbiota, such as segmented filamentous bacteria (SFB) in GI (Ivanov et al., 2009).

Additionally, several toxins have been shown to induce tumors by activating STAT3 and promoting TH_{17}-mediated inflammation in the colon (Wu et al., 2009). Microbial-derived butyrate can induce the differentiation of T_{Reg}-cell fate

(Furusawa et al., 2013). Furthermore, dysbiotic pathogens increase inflammatory dendritic cells, promoting T_{reg} and Th_{17} responses and leading to chronic inflammatory diseases such as periodontitis (Meghil and Cutler, 2020). Both Th-$_{17}$ and T_{Reg} cells have ambiguous roles in cancer. The second mechanism is related to microbial products that damage DNA and the DNA repair process, which causes carcinogenesis. Microbiota genotoxins can directly break the host-cell DNA (Nougayrède et al., 2006) or indirectly have the potential to produce reactive oxygen species (ROS) and free radicals that damage host DNA strands and result in tumorigenesis (Goodwin et al., 2011). Moreover, the microbiome can promote systemic diseases by modifying metabolites (Barbour et al., 2022). Additionally, a change in diet results in dysbiosis that modifies microbiota, including depletion of butyrate producers and an increase in mucus-degrading bacteria, which influence barrier functions and could be a risk factor for CRC (Desai et al., 2016). On the other hand, several probiotics, such as *Lactobacillus* and *Bifidobacterium*, are used as useful microbiota to improve barrier functions (Banakar, 2023; Kelly, 2015). Moreover, the activation of oncogenic and inflammatory genes, inactivation of tumor suppressor genes through B-catenin activation, and hypermethylation of promotors' CpG islands are other mechanisms that promote tumorigenesis concerning microbiota (Shen et al., 2022).

9.3 Oral metagenomics leads to oral megabank

The microbiota DNA is the metagenome and is far bigger than the human cell's whole genome. The old methods were based on culture and microbiota staining, which had limitations for nonculturable microbial communities. The development in molecular methods such as NGS and metagenomics which need fewer culture organisms has revolutionized the study of the microbiome. Metagenomic sequencing has revealed the interplay between the human microbiome and carcinogenesis. NGS produces large volumes of new information, and bioinformatic tools make it possible to assess species diversity and microbial composition dynamically. Metagenomics analysis applied DNA sequencing to determine the microbial community's composition in health and disease conditions (Willis and Gabaldón, 2020). Shotgun metagenomic sequencing is used to sequence microbiomes for species identification and detection of rare and low-abundance microbes.

Furthermore, RNA sequencing is used in metatranscriptome analysis to investigate microbial species and their change in the microbial community. So, microbiome changes at the strain level can be discovered using whole-genome shotgun sequencing and bioinformatics techniques. Furthermore, metagenomic sequencing has some drawbacks, such as the inability to determine if a particular microbiota alteration is a cause or an effect of cancer and the inability to understand the spatial distribution of microorganisms when analyzing a biofilm.

The rich and highly diverse microbiota content of the oral cavity makes it an important place to evaluate the interaction between metagenomic with infection and tumorigenesis (Burczynska et al., 2017). The saliva, biofilm, and gingival crevicular fluid are the available resources for an individual's oral microbiota. Methods for analyzing genes and sequencing metagenomes from the mouth have been developed, allowing researchers to compare the microbiome of people with cancer to that of healthy individuals. Many studies have applied saliva and other dentistry sewage analyses for multiple types of cancer and observed microbiome changes. Dysbiosis in cancer conditions leads to decreased oral microbial diversity and community stability. However, the combined effects, such as the aggregation of *H. pylori* and oncogenic viruses, are thought to be more intense.

Most research institutions now have the means to look for novel human tumor infections that cause human cancer, thanks to advancements in sequencing technology (NGS). The databases of whole-genome and transcriptome sequencing are the sources of metagenomics. Establishing numerous biobanks and integrating them to expand these databases is critical. So, we need an integrated megabank that includes various communities of oral metagenomics linked to health, pathology, and disease states. Many research groups have used this approach, focusing on metagenomic sequencing in population-based studies or in vitro organoid models.

Reference genome databases constitute megabanks through projects such as the Human Microbiome Project (HMP) and European Metagenomics of the Human Intestinal Tract (MetaHIT), which use NGS-based data established for the human microbiome. Furthermore, many projects run to create oral megabanks of different populations for characterized human oral metagenomic and maturing its database. Tohoku Medical Megabank (TMM) Organization used 16S ribosomal RNA gene sequencing to study the differences in oral microbial composition and community in saliva and plaque of a Japanese population (Saito et al., 2020). Another study in Taiwan applied 16S ribosomal RNA gene sequencing to investigate OSCC-associated metagenomics (Su et al., 2020). Researchers also use organoid studies to investigate host-cell genetic and epigenetic alterations, cocultures with microbes, and host−microbiota interactions in disease conditions such as cancer (Puschhof et al., 2021). Also, researchers have focused on various bacterial and fungal microbial profiles and bacterial−fungal interactions in healthy oral microbiomes in the Chinese population by sequencing the bacterial 16S rRNA gene and fungal internal transcribed space (Cheung et al., 2022). As mentioned, NGS technology introduces a high-throughput and cost-effective method for metagenomic detection. Furthermore, bioinformatic approaches can simplify the analysis and interpretation of the metadata. Therefore access to this massive data and machine-learning data mining methods for screening require cutting-edge technologies (Pasolli et al., 2016). Also, the sequencing data could be applicable when the data of metagenomic studies are merged and made accessible in a public database. The MG-RAST portal is the earliest metagenomic database that includes amplicon sequencing of the marker genes, shotgun DNA sequencing, and all RNA as metatranscriptomic (Oliveira et al., 2021).

9.4 Oral metagenomics applications

Some significant infectious agents are contemporary with a type of cancer, and the importance of identifying infections in human cancer can lead to the prediction, control, and treatment of cancer. Since individual microbial species and their ecosystems have been implicated in cancer progression, they have significant potential to be used as cancer indicators. Furthermore, the composition of commensal, symbiotic, and pathobiont microbiota in the oral microbiome and their interactions influence the host immune response. Then, the understanding of immune activities against the microbiome could be helpful in the development of therapeutic strategies such as modifying the microbial composition.

Comprehensive genetic analyses of oral metagenomic profiles and host whole-exome sequencing, by considering host−microbiome interaction, give a deeper understanding of an individual's pathogen related to diseases, which improves treatment and detection of disease biomarkers. Thus metagenomic information in dentistry sewage and saliva mixed with water waste in the dental unit can help to develop screening methods for detecting personalized oral bacteria. Moreover, the intraindividual oral microbiota composition and the evaluation of the microbial proteins and peptide biomarkers could help predict the risk of diseases (Fernandez-Gutierrez et al., 2020; Belibasakis et al., 2019; Paqué et al., 2022). Recent advances in oral metagenomic analysis and noninvasive sampling have revolutionized cancer diagnosis, prognosis, and treatment approaches, leading to personalized therapy correlated with advanced genetic methods.

9.4.1 Oral metagenomics in cancer screening

The noninvasiveness and simplicity of sampling make oral microbiota a good choice to use as a biomarker in health and disease conditions. The microbiome compositions are very diverse in health. They are capable of resisting changes during different physiological stresses (Nearing et al., 2020). Consequently, in disease conditions, a decrease in microbial diversity leads to limiting beneficial microbes and inflammation. Many oral infectious diseases may be associated with cancer and chronic immune-inflammatory diseases. Since changes in the microbiome can cause cancer, and the microbiome is associated with the initiation and progression of tumors, the microbiome could be used as an early cancer detection biomarker. So, assessing the complexity and abundance of the oral metagenome could be used as an indirect approach to detecting the cancer-associated microbiome. Oral metagenomic monitoring uncovers mechanisms of how an altering microbial diversity leads to cancer and predicts microbial invasion in cancer patients (Shelburne et al., 2015). A noninvasive diagnostic approach through oral metagenomics is based on the strong supply of biomarkers for detecting cancer patients and their prognoses (Zhou et al., 2021). Therefore numerous studies have investigated oral metagenomic signatures that reliably predict malignancies and

distant pathologies (Zaura, 2022). The research on oral and intestinal metagenomic profiles reveals microbial biomarkers that accurately predict pancreatic ductal cancer (PDAC) (Nagata et al., 2022). Also, saliva metagenomic and metaproteomic analysis showed that compositional modulation of the oral microbiome is correlated with cancer stage and tumor properties in OSCC patients. Oral metagenomics has the potential to diagnose and oral prognosis cancer, as well as predict overall patient survival, by analyzing the composition of the oral microbiome (Granato et al., 2021). Moreover, the expression of inflammatory cytokines exaggerated by oral metagenomics can be used as a noninvasive biomarker in more efficient screening and early detection of OSCC patients (Rai et al., 2021). The megabank of oral microbiota and the metadata processing of oral metagenomic provide the potential for prediction and biomarker discovery of cancer markers, making population-wide cancer screening possible.

9.4.2 Oral metagenomics in cancer therapeutics

Cancer patients' responses to checkpoint inhibitors appear to depend on the patient's gut microbiome, which interacts with the immune system. As a result, it is not surprising that the clinical responses to interactions between individual microbiota and immune checkpoint inhibitors were different. However, immunotherapy studies have shown that the mechanisms of different microbiota actions when using different checkpoint blockades were similar in dendritic cell activation and effector T-cell function. Patients' microbial ecosystems change as a result of chemotherapy. Furthermore, it is significant that the unique composition of microbiota can influence the anticancer response to conventional chemotherapeutics that result in tumor retardation effects. Therefore antibiotics in a conditioning regimen affect host gene expression and anticancer responses. Consequently, the proinflammatory potential linked to the recruitment of immune cells for tumor regression was decreased by antibiotic treatment. Conversely, oral microbiota such as *F. nucleatum* promotes drug resistance and contributes to malignancies (Da et al., 2021).

Therefore new strategies for cancer prevention, enhancing anticancer responses and overcoming tumor resistance, are concentrated on manipulating the microbiome composition. So, modifying oral metagenomic has been used to address oral dysbiosis via antibiotics, probiotics, and microbial transplants by interrupting tumorigenesis and improving therapeutic efficacy. Human microbiome modulation can be done by microbial supplements or microbial suppression strategies with antibiotics (Xia et al., 2021). Also, a recent study has confirmed the potential of probiotic strains as anticancer therapeutics in the liver and oral cancers (Singh et al., 2021). Probiotics such as *Neisseria sicca* and *Corynebacterium matruchotii* affect genome stability and inhibit OSCC (Shen et al., 2022). Moreover, recent research on targeting microbial cancer drugs has focused on their potential to decrease the side effects of microbiota dysbiosis that lead to cancer (Xia et al., 2021).

9.4.3 Oral metagenomics in cancer precision medicine

Biofilms are the composite of microbiota colonies, and multispecies biofilms form dental plaque on the surfaces of the oral cavity. Saliva plays a crucial role in preserving microbiota homeostasis and composition in the oral cavity. Salivary flow and metagenomic profile are diverse interindividuals (Zarco et al., 2012). Interpersonal microbiome diversity is one of the biggest challenges in metagenomic studies in health and disease conditions. Different microbial compositions, metabolites, and functions influence the efficiency of specific therapeutic interventions in patients. Precision medicine has significant potential to optimize treatment for individual diversity, genetic heterogeneity, and different lifestyles. So, the presence or absence of specific bacterial content and their metabolite can alter specific cancers' prevalence and severity, making them appropriate as prognostic biomarkers. In recent research, the pharmacogenomics study merges with the microbiome and its metabolites to predict precise dosing and improve anticancer therapeutic responses. Furthermore, the design of a suitable microbiome makeup plays an effective role in personalized cancer treatment. In a new area of cancer therapy research, the microbiome is being targeted for therapeutic intentions through personalized medicines. Some personalized bacterial treatments for cancer involve using specific bacteria and their metabolites. For example, *Barnesiella intestinihominis* bacteria or its metabolite β-glucuronidase could be used to treat cancer patients who are receiving immunotherapy and the chemotherapy drug irinotecan. Metagenomics has improved precision medicine through whole-genome and transcriptome sequencing methods. The application of oral metagenomics in precision medicine is about targeting specific oral microbiome compositions to remedy oral microbial dysbiosis that correlates with specific diseases (Khor et al., 2021). Selective manipulation of oral metagenomic composition can provide an accurate concentration of a specific peptide that can conjugate with immunoglobulin G (IgG) antibodies that exhibit antimicrobial activity against *P. gingivalis* and *F. nucleatum* (Franzman et al., 2009). Furthermore, oral microbiota modification enabled a precision medicine approach in pancreatic cancer patients (Herremans et al., 2022). Recently, mNGS technology revealed that oral periodontitis pathogens such as *Prevotella denticola* are associated with brain abscesses (Ma et al., 2021). Therefore precision medicine improves antibiotic therapy and targeted treatment through genomic analysis.

9.4.4 Oral metagenomics in advanced research

The treatment techniques based on mesenchymal stem cells (MSCs) have shown extraordinary efficacies. Oral tissue−derived MSCs have considerable potential in tissue engineering and regenerative medicine due to their robust self-renewal and multilineage differentiation capabilities, as well as their numerous sources and the ease of accessibility. MSCs modulate inflammatory reactions that cause

immunosuppressive functions such as suppressing T-cell proliferation, promoting T_{Reg}-cell differentiation, downregulation of proinflammatory factors, and upregulation of antiinflammatory cytokines (Shang et al., 2021). Oral MSC-based disease treatment and tissue regeneration focused on immune tolerance by cell−cell interaction and paracrine molecules (Hong et al., 2017). The immunomodulatory activities of different kinds of dental MSCs, such as dental pulp (DP-MSCs) and dental follicle (DF-MSCs), are functionally varied (Tomic et al., 2011).

MSCs have antimicrobial activity through direct cell-to-cell contact and indirect paracrine mechanisms. Dental MSCs produce antimicrobial peptides (AMPs) that are regulated by bacterial products, inflammatory cytokines, and vitamin D3. Actually, AMPs have immunomodulatory abilities that indirectly improve therapeutic approaches of MSCs in dentistry through TLR signaling in dental MSCs physiology, which is essential in regeneration (Andrukhov et al., 2021). Furthermore, MSCs have the ability to modulate the activity of immune cells and control periodontal disease progression (Iliopoulos et al., 2022).

Periodontal disease is caused by an abnormal inflammatory response, which can harm oral tissues or lead to the development of systemic disorders like cancer due to an imbalance of the oral microbiota and genetic susceptibility. Periodontal disease is linked to specific bacteria and their possible functions, as shown by metagenomic sequencing (Fig. 9.3). Standard therapies for periodontitis today focus on eliminating the microbial infections that cause the disease and regenerating the gums and teeth. Stem cells have been applied in regenerative medicine by cell-based therapy and cell-free treatment (Junxian et al., n.d.). Cell-based therapy tends to transplant cells for repairing and regenerating tissue. A recent study demonstrated that stem cell−loaded hydrogel microcapsules (SC-HM) could optimize the gut microbiome (Kim et al., 2022). Immunomodulation by dental MSCs is a promising therapeutic approach that eliminates the microbial pathogen and leads to periodontal regeneration. So, periodontal tissue engineering that includes MSCs could be an alternative therapeutic approach in periodontal disease and immunomodulation (Amato et al., 2022). Also, the effects of interaction between oral MSCs and microbiota in stem-cell therapy are confirmed in oral regenerative medicine (Tu et al., 2022). Also, MSC−derived exosomes (MSC-Exos) were introduced as a cell-free treatment (Keshtkar et al., 2021). MSC therapy has therapeutic potential for microbial diseases (Wu et al., 2021) and oral infectious disease therapy by regulating immunoinflammatory responses (Jafari et al., 2021). MSC-Exo promotes tissue regeneration and is often used with scaffolds in tissue engineering.

Moreover, many studies in salivary gland tissue engineering have applied different approaches for tissue regeneration, such as gene-based therapy by the administration of AQP-1 cDNA, stem-cell therapy by cryopreserved salivary gland integrin-$\alpha 6\beta 1$ cells, and tissue engineering approaches based on hydrogel scaffolds (Khan et al., 2020). These cutting-edge studies demonstrate that metagenomics has great potential to improve oral tissue regeneration.

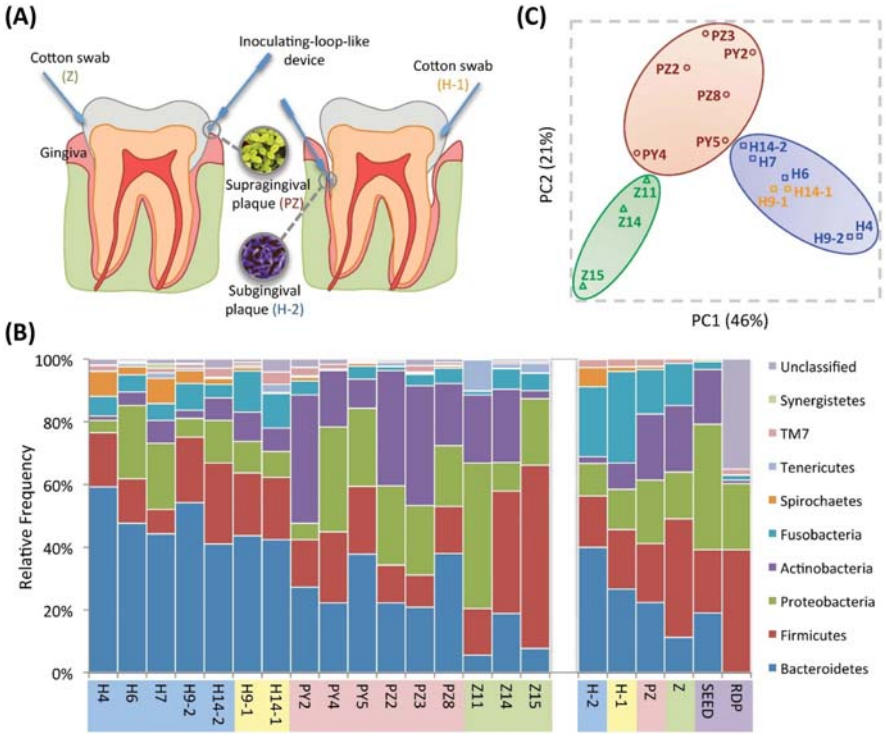

FIGURE 9.3

Analysis of bacterial communities in periodontal health and disease, focusing on sample collection, composition, and clustering relationships. (A) A diagram illustrating the process of sample collection. Dental surfaces of healthy individuals were swabbed, and supragingival plaques were collected from the gingival margin. Dental surfaces in chronic periodontal patients were swabbed for the H-1 group, while subgingival plaques were collected from the bottom of the periodontal pocket for the H-2 group. (B) The distribution of major phyla in the bacterial communities of periodontal health and disease will be analyzed. The bacterial phyla were identified in columns H4-Z15 using NR-BLAST. Columns Z, PZ, H-1, and H-2 displayed the bacterial components of each group according to 16S rRNA gene sequences. The columns SEED and RDP display the bacterial distributions of two reference samples (MG-RAST IDs: 4446622.3 and 4444448.3) obtained from periodontally healthy volunteers. These two reference data sets used the SEED and RDP classification systems for bacterial classifications. (C) Principal component analysis was conducted on 16 periodontal bacterial communities at the genus level using metagenomic data. PC1 and PC2 account for 67% of the variance in the data. Three different periodontal states are represented by different colors: Z, PZ, and H-1 and HP-2 (Wang et al., 2013).

9.4.5 Dental wastewater and cancer

Dental wastewater (DWW) contains heavy metals, antibiotic-resistant bacteria, and antibiotic-resistant genes (ARGs) (Jiao et al., 2023). Dental effluents, which are the waste products generated during dental procedures, can play a role in preventing, diagnosing, and treating oral cancers through oral metagenomics. Dental effluents have shown promise as noninvasive diagnostic tools for oral cancer. Salivary diagnostics, in particular, is a rapidly evolving field, with saliva being an excellent diagnostic fluid due to its accessibility and ease of collection. Oral metagenomic studies have identified several microbial signatures associated with oral cancer. For example, higher levels of *Capnocytophaga gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis* have been found in the saliva of oral cancer patients compared to healthy individuals. Such microbial signatures, identified through NGS of dental effluents, could serve as novel diagnostic markers for oral cancer, aiding early detection and improving patient outcomes (Banakar et al., 2022; Hema Shree et al., 2019; Rodríguez-Molinero et al., 2022).

Analyzing dental effluents such as saliva and dental plaque, rich in oral microbiota, allows researchers to identify potential dysbiosis early, potentially preventing the onset or progression of oral cancer. For example, certain bacterial species, such as *P. gingivalis* and *F. nucleatum*, have been associated with oral cancer development (Binder Gallimidi et al., 2015). Moreover, the oral microbiome's metabolic products, such as short-chain fatty acids and nitrosamines, can have carcinogenic potential (Zvanych et al., 2015). By analyzing these metabolites in dental effluents, it might be possible to modulate the diet or use prebiotics or probiotics to maintain a healthy oral microbiome and prevent oral cancer (Banakar et al., 2022; Zvanych et al., 2015).

Understanding the oral microbiome's role in oral cancer can also contribute to treatment strategies. There is increasing evidence that the oral microbiome can influence the response to cancer therapy, including immunotherapy (Preissner et al., 2023; Routy et al., 2018). Microbial modulation, such as antibiotics, probiotics, or fecal microbiota transplantation, could potentially enhance treatment response. For instance, altering the oral microbiome to favor bacterial species that boost the immune response might make immunotherapy more effective (Routy et al., 2018). As we unravel the complex interactions between the oral microbiome and oral cancer, this is expected to open new avenues for improving oral cancer management. Moreover, understanding the microbial interactions in dental waste can help develop strategies for managing dental waste and mitigating the spread of antibiotic resistance in the environment (Sung, 2021). For instance, metagenomic analysis of treated and untreated DWW can provide valuable information on the effectiveness of current treatment methods and identify areas for improvement (Jiao et al., 2023; Guo et al., 2021).

9.5 Conclusion

The balance of the oral microbiome is an important factor in assessing health and microbiota-related diseases. The studies demonstrate that changes in the oral

microbiome composition and community may lead to inflammation and tumorigenesis through different mechanisms. Since most studies about oral metagenomics focus on bacteria, there is a gap in knowledge about other oral microbiota like viruses and protozoa. Oral metagenomic analysis and standardization in sampling and advanced sequencing tools provide great potential in personalized healthcare. Dental effluents can significantly prevent, diagnose, and treat oral cancers through oral metagenomics. By studying the oral microbiome, researchers can identify potential risk factors, develop diagnostic markers, and optimize treatment regimens for oral cancer patients. Further research and clinical trials are needed to fully understand the potential of dental effluents and oral metagenomics in managing oral cancers. Oral metagenomics and their megabanks are essential for customized cancer diagnosis and treatment.

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