An Orchestrated Impact of the Microbiome on Oral Cancer

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Introduction

Head and neck cancers are a group of malignancies affecting the mouth, nose, pharynx, larynx, sinuses, and salivary glands (Chow, 2020), with an annual incidence of comprising approximately 600,000 new cases all over the world (Johnson et al., 2020). As the predominant histopathological subtype of epithelial-cell-derived malignant tumors occurred in head and neck, oral squamous cell carcinomas (OSCCs) account for around 90% of all head-neck malignancies (Vigneswaran and Williams, 2014; Jou and Hess, 2017). The major anatomical sites of OSCC embrace anterior tongue, buccal mucosa, lower and upper gum, floor of the mouth, hard palate, retromolar trigone as well as lips (Huang and O’Sullivan, 2017; Almangush et al., 2020) (Figure 1). Due to its high morbidity and mortality (Sung et al., 2021) (377,713 new cases and 177,757 deaths annually) estimated by GLOBOCAN (http://globocan.iarc.fr/), settling the tumorigenesis of OSCC has important clinical value whereby it will help for improving the poor prognosis.

Tumor progression is governed by various pro-tumorigenic cytokines and growth factors in the tumor microenvironment (TME) (Hanahan and Weinberg, 2011). For OSCC, TME that mainly functions in its aggravation is created not only through some kinds of stromal cells, but also through malignant epithelial cells. It produces a marked effect in tumor growth and invasiveness coupled with early metastasis, increased recurrences and acquired therapeutic resistance in oral cancer (Naik et al., 2017). In recent decades great headways have made in the field of OSCC research, however, there seems to be a void regarding the role that the oral microbiome may play in OSCC by contrast with the traditional risk factors (e.g. tobacco smoking, alcohol consumption, human papillomavirus (HPV) infection, betel quid intake (Kumar et al., 2016; Alsahafi et al., 2019)). Bad habits of oral hygiene are established to be another risk that acts synergistically to enhance the oral cancer (Hashim et al., 2016; Perera et al., 2018; Chang et al., 2019). It is noteworthy that poor oral hygiene can also significantly determine the microbial habitat and its ecology in oral cavity (Kilian, 2018). Hence, the inner-relationship...
between oral microbiota and TME could hypothetically trigger the occurrence of OSCC.

Part 1 – Oral microbial distribution in human mouth

Human oral microbiome is defined as all microorganisms along with their genomes in human beings’ oral cavity (Dewhirst et al., 2010). According to the expanded Human Oral Microbiome Database (eHOMD, http://www.homd.org/), more than 770 identified bacterial strains are hosted in the mouth, but herein only 70% species are cultivable in the laboratory, and the remains of 30% can be detected with the existence of whole genome sequences of over 480 taxa (Verma et al., 2018). Oro-maxillofacial region is composed of many spaces, such as frontal sinus, sphenoid sinus, ethmoidal sinus, maxillary sinus, oral cavity, nasal cavity and so on. These structures and constitutes make an ideal niche, wherein the stable habitat of constant temperature (37 Celsius degree) or saliva pH value (6.5–7.5) provided, for the growth whether they are aerobic bacteria or anaerobes. And both aerobes and anaerobic bacteria can prevent themselves from external-environment through the oral biofilms shaping, as well as supply nutrients and keep hydrated by saliva (Takahashi, 2005; Arweiler and Netuschil, 2016). With regard to microflora, the most densely populated habitats can be arisen from the environmental diversity of oral cavity boosting the establishment of distinct microbial communities of physiological/pathological areas as follows, surfaces of teeth, gingival sulcus, supragingival and subgingival plaque, buccal mucosa, hard and soft palates, tonsils, and tongue coating (Aas et al., 2005). In addition, most of them

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Figure 1. There is Marked Diversity of Tumors’ Primary Location Arising in the Oral Cavity

Figure 2. Schematic Picture of the Classification of Microbiota-Associated Human Malignancies. Class A is defined by the involvement of the immune response; Class B requires direct microbial interactions with parenchymal cells; Class C covers distant effects from local interactions; and Class D shows the consequences of altered microbiome composition.
may be found in the saliva attaching to exfoliated human epithelial cells (Dawes, 2003). Host-derived nutrition (e.g. proteins or glycoproteins from gingival crevicular fluid or saliva), moist and warm conditions are responsible for the maintenance of ecological equilibrium within microbial flora (van ‘t Hof et al., 2014).

Oral microorganisms are classified into five categories in line with their oxygen requirements (Rosan and Lamont, 2000; Avila et al., 2009): a. obligate aerobes are isolated only under pathogenic circumstances considering as pathogens, such as progressive periodontitis (Mitchell, 1984); b. microaerophiles (e.g. Actinomyces) which grow best at low O2 concentrations (2%~5%, >10% growth inhibition); c. facultative anaerobes (e.g. Streptococci); d. obligate anaerobe (e.g. Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola) can be detected abnormal increase in patients with dentoalveolar abscesses (Sklavounos et al., 1986), stomatitis (Koopmans et al., 1988), advanced periodontitis (Kobayashi et al., 1990), pericoronitis (Sixou et al., 2003), etc; e. capnophiles (e.g. Aggregatibacter actinomycetemcomitans) which grow best at high concentrations of CO2 (5%~10%), can be detected in juvenile/localized aggressive periodontitis acting as the aetiology of this oral disease (Gholizadeh et al., 2017).

The distribution of bacterial species depends on their specific responses toward different biological zones in the oral cavity (Zarco et al., 2012). The colonization of bacterial species is ensured by a so-called “lock and key” or “ligand-receptor” mechanism holding assorted adhesins (adhesion molecules) (Piatti et al., 1997). Predominant microbial communities in oral cavity and oropharyngeal region are summarized in Tab. 1 (Lim et al., 2017).

### Part 2 – An overview of the oral microbiome in OSCC

As the most frequent malignancies in the mouth, OSCCs represent 4.3% of all cancers globally (see the web-based platform of Global Cancer Observatory (GCO)). The radical resection in oral and maxillofacial region is very challenging in front of the subunits of complex anatomical structure and vital physiological functions. Correspondingly, the requirements of both aesthetic recovery and functional reconstruction make a heavy financial burden for the treatment (Huber and Tantiwangkosi, 2014). Additionally, an essential factor in OSCC deaths is the high level of recurrence after treatment (Huber and Vermorken, 2020). Several studies, which contain practically 1,500 cases, have shown with approximately 30% as the overall recurrence rate (Mücke et al., 2009; Rogers et al., 2009; Wang et al., 2013). It would be of paramount importance that, the recurrence rate could be as high as ranging from 70% to 92% in the first 36 months (Boysen et al., 1992; Schwartz et al., 2000; Braakhuis et al., 2002; Mücke et al., 2009). Besides, the moment in which OSCC recurrence happens usually decides the subsequent 5-year survival status. Concretely, the survival rate drops to between 20.5% and 27.55% if relapse happens within the 18 months after treatment, whereas it might relatively improve to 38.1% to 42.3% if relapse happens afterwards (Liu et al., 2007; Mücke et al., 2009). All in all, given the overall survival rate associated with OSCC has still no substantial changes in the last forty years, despite multitudinous research advances in our knowledge of its causes and risk factors, a brand-new perspective is urgently needed to offer for early diagnostic

| Colonia location* | Bacterial species** |
|-------------------|--------------------|
| Tooth surface     | Streptococcus mutans, Actinomyces, Eubacterium, Peptostreptococcus |
| Gingival crevice   | Fusobacterium, Prevotella, Porphyromonas |
| Dental plaque     | Actinomyces, Rothia, Kocuria, Arsenicoccus, Microbacterium, Propionibacterium, Mycobacterium, Dietzia, Turicella, Corynebacterium, Bifidobacterium, Scardovia, Parascardovia |
| Tongue            | Veillonella atypica, Porphyromonas gingivalis, Selenomonas species, Actinobacillus actinomycetemcomitans Prevotella intermedia, Capnocytophaga species, Streptococcus faecalis, Eikenella corrodens |
| Tonsil            | Streptococcus viridans, Neisseria species, Haemophilus influenzae, coagulase-negative Staphylococci |
| Oropharynx        | Streptococcus salivarius, Streptococcus mutans, Streptococcus anginosus, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae |

*Bacteria bind with complementary receptors of the host, colonizing on non-shedding surfaces of the teeth and continually shedding surfaces of the mucosal epithelia; **Streptococci are commonly found genera in human oral cavity, but this group is highly genetically heterogeneous (Itzek et al., 2010).
Table 2. Oral Microbiota in Craniomaxillofacial-Cervical Squamous Cell Carcinomas

| Cancer category | Microorganisms | Sample source | Bibliography |
|-----------------|----------------|---------------|--------------|
| HNSCC           | phyla Fusobacteria | Meta-analysis | (Bronzato et al., 2020) |
| HNSCC           | Streptococcus anginosus | Tumor tissue | (Tateda et al., 2000) |
| HNSCC           | Firmicutes, Proteobacteria, Bacteroidetes (Streptococcus, Prevotella, Haemophilus, Lactobacillus, Veillonella) | Saliva | (Guerrero-Preston et al., 2016) |
| OSCC            | Streptococcus anginosus | Tumor tissue | (Sasaki et al., 2005) |
| OSCC            | Capnocytophaga gingivalis, Prevotella melaninogenica, Streptococcus mitis | Saliva | (Mager et al., 2005) |
| OSCC            | Micrococcus luteus, Prevotella melaninogenica, Exiguobacterium oxidotolerans, Fusobacterium naviforme, Staphylococcus aureus, Veillonella parvula, Prevotella sp. (oral clone BE073 phylotype), Rothia mucilaginosa, Streptococcus salivarius, Actinomyces odontolyticus, Moraxella osloensis, Prevotella veroralis, Propionibacterium acnes, Ato pobi um par vulum, Streptococcus par asanguinis, Veillonella dispar, Streptococcus mitis/oralis | Tumor tissue | (Hooper et al., 2006) |
| OSCC            | Fusobacterium periodonticum, Parvimonas micra, Streptococcus constellatus, Haemophilus influenza, Filifactor alocis | Oral rinse | (Yang et al., 2018) |
| OSCC            | Streptococcus sp. (oral taxon 058), Streptococcus salivarius, Streptococcus gordoni, Streptococcus par asanguinis, Peptostreptococcus stomatis, Gemella haemoly ans, Gemella morbillorum, Johnsonella ignava | Tumor tissue | (Pushkarkar et al., 2012) |
| HNSCC           | Actinomyces depleted in HNSCC, Parvimonas increased in HNSCC | Tumor tissue | (Wang et al., 2017) |
| OSCC            | Fusobacterium, Dialister, Peptostreptococcus, Filifactor, Peptococcus, Catonella, Parvimonas | Swabs of oral lesions | (Zhao et al., 2017) |
| Primary OECs    | Porphyromonas gingivalis promoted cell migration which was slightly enhanced by co-infection with Fusobacterium nucleatum | Primary OECs | (Lee et al., 2017) |
| OSCC            | Acinetobacter, Fusobacterium, Streptococcus, Prevotella | Tumor tissue, saliva, mouthwash | (Zhang et al., 2019) |
| Gingival squamous cell carcinoma | Porphyromonas gingivalis | Paraffin embedded cancer samples | (Katz et al., 2011) |
| Oral mucosal cancer | Oral streptococi (Streptococcus intermedius, Strep. constellatus, Strep. oralis, Strep. mitis, Strep. sanguis, Strep. salivarius), aerobic enteric bacteria (Enterococcus, Escherichia, Klebsiella etc.), anaerobic bacteria, Peptostreptococcus spp. | The regional lymph nodes in the neck | (Sakamoto et al., 1999) |
| OSCC            | Veillonella, Fusobacterium, Prevotella, Porphyromonas, Actinomyces and Clostridium (anaerobes), and Haemophilus, Enterobacteriaceae and Streptococcus spp. (aerobes), Candida albicans | Biofilm from the central surface of the lesions | (Nagy et al., 1998) |
| Malignant oral leukoplakia | Fusobacterium, Leptotrichia, Campylobacter species, Rothia mucilaginosa, Leptotrichia spp. Campylobacter concisus | Swabs | (Amer et al., 2017) |
| OSCC            | Porphyromonas gingivalis | Paraffin embedded cancer samples | (Guo et al., 2021) |

and therapeutic work. Viruses have long been related with the risk of developing oral carcinomas. First and foremost to be mentioned is human papillomavirus (HPV), with an almost thirty years’ history being widely researched, in which is treated as the aetiological factor in OSCCs (Wittekindt et al., 2018). The oncogenic potential of HPV is well demonstrated in the context of OSCC via several comprehensive meta-analyses founded by about 150 studies addressing HPV and OSCC in human cases, and that has indicated that by the increment of HPV infection the risk of oral cancer adds up to three-fold (Liyanage et al., 2013; Hardefeldt et al., 2014). The prevalence of HPV infection among OSCC patients, estimating via HPV DNA detection in OSCC tumors, is close to 25% (Syrjänen K and Syrjänen S, 2013; Hardefeldt et al., 2014; Petrick et al., 2014). HPV-negative OSCCs occupy the vast majority of oral carcinoma cases nevertheless. Accordingly, the next goal gradually comes into our view.

In recent years, there has been increasing focus on the oral microbiome with the respect to its contribution to tumor development. There are evidences that bacteria have a direct causal association with oncogenesis in some types of cancers, including Salmonella typhi in gallbladder cancer (Di Domenico et al., 2017), Helicobacter pylori in gastric cancer (Amieva and Peek, 2016), though, no exactly distinguishable linkage between the oral microbiome and OSCC has been yet tapped. Tab. 2 presents a couple of studies concerning the effect of bacteria’s presence to cancers of the oral cavity and pharynx (Nagy et al., 1998; Sakamoto et al., 1999; Tateda et al., 2000; Mager et al., 2005).
Figure 3. Actions of Microorganism Contribute to the Pathogenesis of Oral Inflammation and Increase the Risk of OSCC.

2005; Sasaki et al., 2005; Hooper et al., 2006; Katz et al., 2011; Pushalkar et al., 2012; Guerrero-Preston et al., 2016; Amer et al., 2017; Lee et al., 2017; Wang et al., 2017; Zhao et al., 2017; Yang et al., 2018; Zhang et al., 2019; Bronzato et al., 2020; Guo et al., 2021).

Part 3 – The main immune-related cells of tumor microenvironment (TME)

In pace with a growing recognition that tumor could be a ‘unique organ’ whose complex property approaches to or might even exceed what normal healthy tissues are like, a concept of TME is proposed for the first time (Hanahan and Weinberg, 2011). From this path-breaking perspective, the tumors’ biological phenomena and outbreak regularity can only be understood by studying the individual specialized cell types within TME as well as the behaviors that they manifest during the course of multistep tumorigenesis. Further, since Stephen Paget firstly proposed the hypothesis of ‘seeds (pro-metastatic tumor cells) and soil (the supportive microenvironment in specific organ sites)’ that is essential prerequisite for tumor development and progression (Paget, 1889), this theory of biological importance of TME in fostering cancer recommitted has come to be widely accepted. TME is highly heterogeneous characterized with a principal compartment of host stromal cells as well as its inner extracellular matrix (ECM) which is a collection of fibrous proteins, fibronectins and collagens for example, that is

Table 3. CAFs’ Morphological, Phenotypical, and Functional Variability

| Characteristics of CAFs | Descriptions | Bibliography |
|-------------------------|--------------|--------------|
| **Cellular origins of CAFs** | CAFs can originate from various cell types such as MSCs, fibrocytes, adipocytes, endothelial cells via EndMT, smooth muscle cells, stellate cells, epithelial cells via EMT, pericytes as well as normal fibroblasts. | (Radisky et al., 2007; Potenta et al., 2008; Spaeth et al., 2009; Wikström et al., 2009; Bochet et al., 2013; Shiga et al., 2015; Kalluri, 2016; Bartoschek et al., 2018; Helms et al., 2021) |
| **Markers of activated CAFs** | Several molecules, such as α-SMA, FAP, FSP-1, PDGF α/β, vimentin, TGF-β1, FGF, SDF-1, and EGF are considered some of the markers of activated CAFs. | (Zeisberg et al., 2007; Council and Hameed, 2009; Kalluri and Weinberg, 2009; Toullec et al., 2010; Jia et al., 2013; Kim et al., 2015; Öhlund et al., 2017) |
| **Heterogeneity of CAFs** | CAFs as well as activated fibroblasts are known to be very heterogeneous, displaying different expression patterns. Resting fibroblast cells are morphologically spindle shaped in contrast to activated fibroblast cells which are stellate shaped. Activated fibroblast cells express the above molecular markers (mainly α-SMA, FAP and PDGFβ). Resting fibroblast cells express FSP-1 (rarely), and α1β1 integrin. | (Chang et al., 2002; Kalluri, 2016) |

α-SMA, alpha-smooth muscle actin; CAFs, cancer-associated fibroblasts; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; EndMT, endothelial to mesenchymal transition; FAP, fibroblast activation protein; FGF, fibroblast growth factor; FSP-1, fibroblast-specific protein-1; MSCs, mesenchymal stem cells; PDGF α/β, platelet-derived growth factor receptor α/β; SDF-1, stromal derived factor 1; TGF-β1, transforming growth factor-beta1
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Invasion in oral cancer cells YD10B and Proposed mechanism

Proliferation, migration in oral cancer cells Increases MMP1 in both cancer cells and CAFs. (Li et al., 2017)
Proliferation, migration in oral cancer cells Migration, invasion in oral cancer cells SCC9 and CAL27; in vivo tumor growth using SCC9 Induces ROS and subsequent activation of PI3k/ Akt-mTOR in cancer cells and CAFs. In cancer, CCL2 further increases Cyclin D/E and CDK4. (Li et al., 2014)

IL-8* Proliferation, migration in oral cancer cells SCC9 and CAL27 Over-expression of miR-124 in CAFs-OSCCs co-culture abrogated CAFs-promoted OSCCs cell growth and migration, and this inhibitory effect can be rescued by addition of CCL2. (Li et al., 2017)

IL-6** Proliferation, migration, invasion in oral cancer cells SCC25 and CAL27 Regulates osteopontin expression in a STAT3 dependent-way. Osteopontin binds to an integrin receptor and activates NF-κB in cancer. (Qin et al., 2018)

BDNF*** Migration, invasion in oral and pharyngeal cancer cells; in vivo tumor growth using OSC19 Interacts with TrkB and upregulates MMP9 in cancer cells. (Jiffar et al., 2017)

HGF** Glycolysis in head and neck cancer cells HN5 and SCC1 Induces the expression of key glycolytic enzymes and lactate efflux. Regulates bFGF expression in cancer cells. (Kumar et al., 2018)
Proliferation in head and neck cancer cells HN5 and UM-SCC-1; migration and invasion in HN5, UM-SCC-1 and OSC-19 Interacts with c-Met and phosphorylates STAT3 via a JAK dependent route in cancer cells. (Kumar et al., 2015)

HGF*** Migration in oropharyngeal cancer cells SCC072 and SCC089 A synergistic relationship between HGF and IL-6 in the support of migration that relates JAK activation to HGF responsiveness in HPV-negative lines. In vitro evidence to support the clinical application of c-Met inhibitors in the control of early HPV-negative oropharyngeal carcinomas. (Bolt et al., 2018)

MMPs** Cetuximab resistance in laryngeal cancer cell line UT-SCC9 (40%) and tongue cancer cell line UT-SCC-24A (60%) CAF-dependent modulation of cetuximab sensitivity and suggest that inhibiting MMPs may improve the effects of EGFR-targeted therapy. (Johansson et al., 2012)

MMP-2* Invasion in oral cancer cell H357 Senescent CAFs from genetically unstable OSCC promote a more aggressive oral cancer phenotype by production of active MMP-2, disruption of epithelial adhesion and induction of keratinocyte invasion. (Hassona et al., 2014)

Periostin** Stemness in head and neck cancer cells SCC-25 and HN6 Activates PTK7 and Wnt/β-Catenin in cancer cells. (Yu et al., 2018)
Proliferation, migration, invasion in head and neck cancer cells HN13 and rat oral cancer cells Rca-T Binds integrin receptors. (Qin et al., 2016)

MFAP5* Proliferation, migration in tongue cancer cell SCC25 Increases phosphorylation of PDK1, Akt and decreases cRAF, PTEN. (Principe et al., 2018)

CXCL12* EMT, invasion in oral cancer cells SCC9 and CAL27 EMT and angiogenesis formation in tongue cancer through myofibroblast differentiation via SDF1 secretion. (Zhou et al., 2015)

CXCL1* Invasion in oral cancer cells YD10B and YD38 CXCL1 can transform NFs into senescent CAFs via an autocrine mechanism. (Kim et al., 2018)

CXCL11* Invasion in oral cancer cell SAS Increases MMP1 in both cancer cells and CAFs. (Wei et al., 2019)

*Normal fibroblasts (NFs) activated in co-culture used as a surrogate for CAFs; *Derived from a primary culture of oral cancer; **Derived from a primary culture of head and neck cancer, without specifying the subsite; ***Derived from a primary culture of the oral cavity and oropharyngeal area. Abbreviations: Akt, protein kinase B; BDNF, brain derived neurotrophic factor; bFGF, basic fibroblast growth factor; CCL2, chemokine (C-C motif) ligand-2; c-Met, c-mesenchymal-epithelial transition factor; CXCL, C-X-C motif chemokine ligand; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; HGF, hepatocyte growth factor; IL, interleukin; JAK, Janus tyrosine kinase; MFAP5, microfibrillar-associated protein 5; MMP9, matrix metalloproteinase-9; mTOR, mammalian target of the rapamycin; NF-κB, nuclear factor-kappaB; PDK1, pyruvate dehydrogenase kinase isoenzyme-1; PI3K, phosphatidylinositol-3-kinase; PTEN, gene of phosphate and tension homology deleted on chromosome 10; PTK7, protein tyrosine kinase 7; ROS, reactive oxygen species; SDF1, stromal cell-derived factor-1; STAT3, signal transducer and activator of transcription-3; TrkB, tyrosine kinase receptor B; Wnt (Wingless / Integrated).

Table 4. CAF-Derived Proteins, Enzymes, Cytokines and Factors that have a Functional Impact on HPV-Negative HNSCC, OSCC.
| Phenotypes | Stimuli | Markers | Potential signal axis | Functions | Bibliography |
|------------|---------|---------|-----------------------|-----------|--------------|
| M1         | IFN-α, TNF-α, LPS, GM-CSF | IL-12 high/IL-10 low, IL-6, TNF-α, CCL19, CCL20, CD80, CD86, iNOS | Expression of M1-associated transcripts was increased in TH-1 cells transfected with mimics of miR-29b, miR-125a-5p, or miR-155. The apparent inflammatory property of miR-29b and miR-125a-5p can be at least partially explained by repression of TNFAIP3, a negative regulator of NF-κB signaling. | Pro-inflammation, microbial effect, tumor resistance | (Martinez et al., 2008; Mosser and Edwards, 2008; Graff et al., 2012; Colin et al., 2014; Eigsti et al., 2014; Gensel and Zhang, 2015; Ito et al., 2016; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2a        | IL-4, IL-13 | "CCL17, IL-1R, CD206, Dectin-1, IL-10, DC-SIGN (CD209)* | miRNAs that are uniquely regulated in human macrophages polarized toward M2a (miR-193b) phenotype. | Anti-inflammatory, wound healing | (Martinez et al., 2008; Mosser and Edwards, 2008; Graff et al., 2012; Eigsti et al., 2014; Gensel and Zhang, 2015; Nakamura et al., 2015; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2b        | LPS+IC, IL-10, IL-12 | IL-10 high/IL-12 low, CD86, TNF-α, CCL1, IL-6 | miRNAs that are uniquely regulated in human macrophages polarized toward M2b (miR-27a*, miR-29b-1*, miR-132*, and miR-222*) phenotypes. | Immune-regulation, promoting infection, tumor progression | (Sironi et al., 2006; Martinez et al., 2008; Mosser and Edwards, 2008; Kobayashi et al., 2011; Graff et al., 2012; Orme and Mohan, 2012; Colin et al., 2014; Ohlsson et al., 2014; Gensel and Zhang, 2015; Chen et al., 2016; Fujiwara et al., 2016; Ito et al., 2016; Schulert et al., 2016; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2c        | IL-10, glucocorticoids | "CD206, CD163, IL-10, TGF-β, CCL13, MerTK* | Increased expression of MerTK, which binds indirectly to phosphatidyl serine through the bridging molecules GAS-6 and protein S, promoting the uptake of apoptotic cells, in active macroscopic polyangiitis and in the M2c subtype of macrophages. | Immunosuppression, phagocytosis, tissue remodeling | (Martinez et al., 2008; Graff et al., 2012; Gensel and Zhang, 2015; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2d        | LPS+A2R, ligands, IL-6 | VEGF, IL-10, TGF-β | Junonji domain containing-3 IRF4 pathway has been implicated in M2d activation. | Tumor progression, angiogenesis | (Duluc et al., 2007; Martinez et al., 2008; Ferrante and Leibovich, 2012; Shapouri-Moghaddam et al., 2018) |
| M1         | IFN-α, TNF-α, LPS, GM-CSF | IL-12 high/IL-10 low, IL-6, TNF-α, CCL19, CD80, CD86, iNOS | Not applicable | Pro-inflammation, microbial effect, tumor resistance | (Martinez et al., 2008; Mosser and Edwards, 2008; Colin et al., 2014; Gensel and Zhang, 2015; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2a        | IL-4, IL-13 | CCL17, IL-1R, Dectin-1, IL-10, Arg-1, Chil3, FIZZ1 | Anti-inflammatory, wound healing | (Martinez et al., 2008; Mosser and Edwards, 2008; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2c        | IL-10, glucocorticoids | "CD206, CD163, IL-10, TGF-β, CCL13, MerTK, Arg-1" | Immunosuppression, phagocytosis, tissue remodeling | (Martinez et al., 2008; Gensel and Zhang, 2015; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |
| M2d        | LPS+A2R, ligands, IL-6 | VEGF, IL-10, TGF-β, iNOS | Tumor progression, angiogenesis | (Martinez et al., 2008; Wang et al., 2010; Ferrante and Leibovich, 2012; De Paoli et al., 2014; Gensel and Zhang, 2015; Liberale et al., 2017; Shapouri-Moghaddam et al., 2018) |

Abbreviations: Arg-1, arginase 1; CCL, C-C motif chemokine ligand; CD, cluster of differentiation; CCL, C-X-C motif chemokine ligand; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-, interferon gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; LPS, lipopolysaccharide; miRNA, micro ribonucleic acid; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.
secreted by mesenchymal cells and provides structural and biochemical support to the surrounding cells.

**Cancer-associated fibroblasts (CAFs)**

CAFs, a type of perpetually activated fibroblasts, are recognized as a prime source of collagen-producing cells. They can directly communicate with the cancer cells and other types of stromal cells (e.g., inflammatory cells and endotheliocytes) (Ishii et al., 2016). Extensive evidences have been implicated to support the premise of the biological importance of CAFs in cancerous progression (Zhang et al., 2017; Li et al., 2018; Qin et al., 2018; Qin et al., 2019; Matos et al., 2020). Nevertheless, CAF different subtypes with heterogeneous biological properties make distinct functional contributions (Tab. 3) (Chang et al., 2002; Orimo and Weinberg, 2007; Radisky et al., 2007; Zeisberg et al., 2007; Potenta et al., 2008; Council and Hameed, 2009; Kalluri and Weinberg, 2009; Spaeth et al., 2009; Wikström et al., 2009; Toullec et al., 2010; Bochet et al., 2013; Jia et al., 2013; Kim et al., 2015; Shiga et al., 2015; Kalluri, 2016; Ohms et al., 2020). M2 macrophages are induced by diverse stimuli such as interleukin (IL)-4, IL-13, prostaglandin E2 (PGE2), transforming growth factor-beta (TGF-β) and they show anti-inflammatory effects. They can promote the occurrence and metastasis of tumor cells, enhance tumor cell invasion, motility and intravasation, stimulate tumor angiogenesis, inhibit T and natural killer (NK) cell-mediated anti-tumor immune response, and lead to tumor progression and prevent tumor cells from chemo- or immuno-therapy (Funes et al., 2018; Pan et al., 2020). M2 macrophages may be further divided into subpopulations such as M2a, M2b, M2c and M2d (Ji et al., 2016; Wang et al., 2019; Pan et al., 2020). The role of TAMs in tumor progress, as well as inducible factors for M1/M2 polarization, and M1/M2 macrophages’ secretory proteins associating with the biological function, are displayed in Tab. 5.

**Tumor-associated neutrophils (TANs)**

Neutrophils (i.e. also known like polymorphonuclear leukocytes, PMNs) are the most preponderant leukocyte population existing in blood circulation, and are crucial effector cells originated from the innate immune system (Welch et al., 1989). Beside TAMs, another pivotal constitution as infiltrating immune cells engaged into the TME through cytokines and chemokines, is TANs, which can be distinguished in accordance with their activation status and influences on tumor cells growing in N1 (antitumor) or N2 (protumor) TANs (Masucci et al., 2019; Ohms et al., 2020). N2 subtype is characterized by increased expression of angiogenesis enhancing and invasion promoting factors CXCR4, VEGF and MMP-9 with absent IFN-β (Jablonska et al., 2010) and is acquired by neutrophils following the TGF-β treatment (Fridlender et al., 2009). However, neutrophils can revert back to the

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Table 6. Tumor-Associated Neutrophils (TANs) can Either Promote or Inhibit Tumor Growth Depending on Their Polarization States

| Phenotype | Identificationmarkers/axis | Function                  | Bibliography                                    |
|-----------|-----------------------------|---------------------------|-------------------------------------------------|
| N 1       | Arginase-1, ROS, STAT3, STAT/IRF-8, LOX-1, mPR3, MPO/hydrogen peroxide | Protumor T/NK cell suppression | (el-Hag and Clark, 1987; Cemerski et al., 2002; Rotondo et al., 2009; Chalmin et al., 2010; Wight et al., 2013; Condamine et al., 2010; Waight et al., 2016; Yang et al., 2018) |
|           | VEGF, MMP-9, TNF-α, IL-8, NAMPT/STAT3, Oncostatin M, NETs, NE | Angiogenesis/metastasis | (McCourt et al., 1999; Kerfoot et al., 2001; Huang et al., 2002; Queen et al., 2005; Ardi et al., 2007; Houghton et al., 2010; Bald et al., 2014; Park et al., 2016; Chen et al., 2018; Pylaeva et al., 2019) |
|           | CCL2, CCL17 | Immune cell recruitment | (Mishalian et al., 2014; Zhou et al., 2016) |

| N 2       | ROS, Granzyme B, MET signaling, Trogoptosis,TNF-α | Antitumor Tumor cytotoxicity | (Finisguerra et al., 2015; Comen et al., 2016; Gershkovitz et al., 2018; Martin et al., 2018; Matlung et al., 2018) |
| Mechanism unknown,NETs | T-cell activation/priming | (Beauvillain et al., 2007; Tillack et al., 2012; Eruslanov et al., 2014) |

Abbreviations: CCL, chemokine (C-C motif) ligand; LOX-1, low-density lipoprotein receptor-1; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; mPR3, membrane-associated proteinase 3; NAMPT, nicotinamide phosphoribosyltransferase; NE, neutrophil elastase; NET, neutrophil extracellular trap; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.
The extracellular matrix (ECM) is a non-cellular network of macromolecules, including glycoproteins, proteoglycans, fibrous structural proteins, and growth factors that form a frame providing other surrounding cells with biochemical and physical support (Ziober et al., 2017). The progression of cancer has been found directly linked to the dysregulation, disorganization and degradation of the ECM (Walker et al., 2018) with recent studies showing that matrix metalloproteinases (MMPs) have been reported to activate growth factors or release them from the matrix, along with ECM degradation, eventually leading to the formation of primary tumours which can further metastasize (Johnson et al., 2014). Fascinatingly, the ECM components as a potential target for therapeutics will be a great ordeal as they are linked to various signalling pathways and may pave way for blocking the life-threatening metastasis of oral cancer.

**Part 4 – Pathogenic impact of the microbiome on TME**

Recent work has indicated that a certain association between oral microbial dysbiosis and cancer (Su et al., 2021); more to the point, tumor microenvironment (TME) could be decisive therein. Both pro-tumorigenic and anti-tumorigenic roles functioned through TME have been associated with the microbiome. Within TME, the communication between homogenous cell types is driven by an extremely complex network of chemokines, cytokines, growth factors, other inflammatory mediators, and matrix remodeling enzymes (Balkwill et al., 2012). Now, the microbiome can be served as a potent modulator among diverse non-malignant cells of the TME to impact the host immune responses. Considering that the metabolism of cancerous cells is strictly regulated by the TME, the microbiome may be a new component in the TME that impairs tumor cell metabolism maintaining a healthy barrier, inducing inflammation, and producing genotoxins and bacterial metabolites with different features. Below, the possible modalities of how dysbiosis interferes with carcinogenesis, and the potential mechanisms by which microbial dysbiosis modulates carcinogenesis in OSCC are reviewed in Fig. 2 and 3. Rationale of how microbiome and cancer microenvironment change each other, is forging a metagenomics cross-talk that the regulatory interplay between immunogenomics and the microbiome. The human microbiome encompasses up to 100-fold more genes than the host genome, is frequently referred to as the ‘second genome’. Importantly, the microbial genome is flexible and amendable to alter during the host’s lifetime, in contrast to human genome. The bi-directional interaction between microbial and host genome has been revealed in some diseases’ development, such as obesity, inflammatory bowel disease (e.g. Crohn’s disease, and ulcerative colitis) (Levy et al., 2015). Although the identification of key host genes and microbe-derived signals involved in TME immunity is still in its infancy, it could be hypothesized that, the influence of the microbiota on genetic and epigenetic regulation of gene expression in the immunocytes of TME is especially apparent via the host immune system.

**Part 5 – Effect of the microbiome in the outcomes of oral cancer therapy**

The effect of human microorganism on the treatment of oral malignancy is merely starting to be investigated, in spite of its clinical importance. There is now proof indicating that the oral microbiome can impact patient reactions to the cancer treatment. Specifically, embracing chemotherapy and radiotherapy, it has been implicated in modulating the efficacy and cytotoxicity during cancer therapy (Dunnack et al., 2021). Moreover, preclinical data suggest that the microbiome modulation could become a novel strategy for improving the efficacy of immune-based therapies for cancer (Fessler et al., 2019). The oral microbiota has the potential to affect the capacity of cancer therapy. The microbiota, when affected by dysbiosis, may profoundly influence both cancer pathogenesis and its therapeutic outcome. In particular, the regulation of such a therapeutic outcome is related to the oral microbiota’s competence to process anti-cancer compounds and modify the host’s immune response. These two effects combined could clarify the substantive participation of the patient’s microbiome composition in affecting the efficiency of anti-cancer therapy (Irfan et al., 2020). We anticipate that the human microbiome will bit by bit play an undeniably conspicuous role in oral cancer treatment. As of now, the system of the microbiome’s impacts in cancer treatment
is not surely known; be that as it may, some studies in respect of clinical pilot should be designed to assist with uncovering the ability of the microbiome in malignant tumor-relevant treatment. We reckon that the advancement of these clinical preliminaries will expel impediments for utilizing the microbiome to improve and help treatment utilizing immune check point inhibitors (Irfan et al., 2020).

Future prospects
The field of microbiome-immunity-cancer has made huge strides over the past several years, nevertheless, few aim at microbiome-mediated regulation in the TME system of OSCC. Additional research is required to expand on correlative or functional observations toward mechanistic understanding and translation of these findings to the clinics.

Author Contribution Statement
LC, GZ and LH conceptualized, and designed the outline of the study; contributed to paper interpretation. LC and LH reviewed all the relevant articles. LC was in charge of findings’ acquisition, original draft writing, tables and figures preparing; and manuscript revision. GZ supervised the study. GZ and LH critically revised the main text, and obtained the financial body. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. The requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work.

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Ethical approval will not be required because this article is a kind of secondary research that retrieved and synthesized data from already published studies.

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Not applicable.

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The authors declare that they have no competing interests.

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