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REVIEW

Intra-tumoral bacteria in head and neck cancer: holistic integrative insight

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ABSTRACT Intra-tumoral bacteria are pivotal in the initiation and progression of head and neck squamous cell carcinoma (HNSCC), exerting a significant influence on tumor cell biology, immune responses, and the tumor microenvironment (TME). Different types and distribution of bacteria threaten the balance of metabolism and the immune environment of tumor cells. Taking advantage of this disrupted homeostasis, intra-tumoral bacteria stimulate the secretion of metabolites or influence specific immune cell types to produce inflammatory or chemokines, thereby influencing the anti-tumor immune response while regulating the level of inflammation and immunosuppression within the TME. Some intra-tumoral bacteria are used as diagnostic and prognostic markers in clinical practice. Based on the unique characteristics of bacteria, the use of engineered bacteria and outer membrane vesicles for drug delivery and biological intervention is a promising new therapeutic strategy. The presence of intra-tumoral bacteria also makes chemoradiotherapy tolerable, resulting in a poor treatment effect. However, due to the immune-related complexity of intra-tumoral bacteria, there may be unexpected effects in immunotherapy. In this review the patterns of intra-tumoral bacteria involvement in HNSCC are discussed, elucidating the dual roles, while exploring the relevance to anti-tumor immune responses in the clinical context and the prospects and limitations of the use of bacteria in targeted therapy.

KEYWORDS Head and neck squamous cell carcinoma; intra-tumoral bacteria; tumor progression; immunity; therapy

Introduction

Intra-tumoral bacteria affect the occurrence and development of cancer and the immune response *via* structural characteristics and biological functions and subsequently have unique effects on antitumor therapy[1]. Indeed, of head and neck cancer (HNC) pathogenic factors are inseparable from microorganisms[2]. Head and neck squamous cell carcinoma (HNSCC) is the predominant subtype of HNC, accounting for approximately 90% of all HNC cases[3]. HNSCC primarily originates from the mucosal epithelium of the oral cavity, pharynx, and larynx[3]. As the gateway between the respiratory and digestive tracts, these anatomic sites have extensive bacterial accumulation. Some studies have shown that there are microbial differences between healthy and abnormal oral cavity tissues, which further confirms the suspected involvement of bacteria in tumor development[4]. While intestinal flora research has dominated cancer-related bacterial studies, investigations into intra-tumoral bacteria are emerging[5,6].

Bacteria and their metabolites often induce microenvironmental changes that are closely associated with inflammation and tumor progression[7]. *Fusobacterium nucleatum* is more active and abundant in HNSCC tissues than non-cancerous tissues and *Porphyromonas gingivalis* is closely associated with the development of HNSCC[8-12]. Both *F. nucleatum* and *P. gingivalis* establish chronic infections, persist intracellularly in epithelial cells, spread systemically, cause extraoral infections, and possess distinct immunomodulatory properties[13]. Different types of bacteria pose a threat to the development of tumor cells and the microenvironment through metabolites, destroy

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the original order of the tumor microenvironment (TME), and promote the invasion and migration of tumor cells by promoting the secretion of interleukin (IL)-6, IL-8, matrix metalloproteinases (MMPs), and other inflammatory factors and markers such as plasminogen activator inhibitor-1 (PAI-1), chitinase 3-like-1 (CHI3L1), epidermal growth factor (EGF), C-X-C motif chemokine ligand 5 (CXCL5), CXCL1, C-C motif chemokine ligand 2 (CCL2), and CCL3[12,14]. Bacteria interact with a variety of immune cells, including T cells, macrophages, and antigen collecting cells, to further affect the expression and binding of programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1), resulting in an abnormal tumor immune response and immune evasion[15]. However, the specific mechanisms and biological behaviors of intra-tumoral bacteria in tumors have not been fully elucidated.

The therapeutic effect of anti-tumor therapies is often affected by many underlying factors. Because there are a variety of intra-tumoral bacteria found in tumor tissues, it is undeniable that bacteria have an impact on the efficacy of anti-tumor therapy, including radiotherapy and chemotherapy[16,17]. In addition, intra-tumoral bacteria are involved in multiple immune responses to tumors, so intra-tumoral bacteria have synergistic or inhibitory effects on immunotherapy. *P. gingivalis* may inhibit immunotherapy by upregulating PD-L1 expression in tumor or immune cells[18,19]. Combining novel materials that allow the advantageous growth of *Peptostreptococcus* and immune checkpoint inhibitors may improve the efficacy of immunotherapy[20]. It is also possible to improve the efficacy of immune checkpoint-targeted therapy by ablating the microbiota[21] or enhancing the sensitivity of immunotherapy by activating immune cells to improve the tumor immune microenvironment[22]. The microbiota can also be used as the target of antibiotics to further activate and enhance the immune response by renewing the antigen[23]. In contrast, engineering bacteria to kill tumor cells or serve as drug delivery systems by taking advantage of the targeting properties of bacteria and the characteristics of the secreted vesicles is an intriguing possibility[24,25].

This review explores the role and potential mechanisms of intra-tumoral bacteria in promoting and suppressing HNSCC with a focus on the mode of involvement and impact on tumor-associated immunity. The impact of intra-tumoral bacteria on anti-tumor efficacy is demonstrated with a special focus on anti-tumor therapeutic strategies that utilize bacteria and the prognostic and diagnostic implications. Additionally, the potential and limitations of bacteria-based personalized cancer therapies are discussed. This study provides a theoretical basis for scholars to study the mechanism and biological behavior of bacteria in HNSCC tumors and provides a basis for the combination of theory and clinical practice.

Characteristics of intra-tumoral bacteria in HNC

The behavior of bacteria in tumors is diverse using bacterial and metabolite properties to have a variety of participation modes. The differences in bacterial flora among different tumor subtypes also suggest the preference of bacteria for the occurrence and development of tumors at different sites. In addition to the regulation of the surrounding environment, *in vitro* factors also have potential effects on intra-tumoral bacteria.

Patterns of involvement of intra-tumoral bacteria

Most studies have shown that intra-tumoral bacteria mainly exist in tumor and immune cells in the TME and participate in immune regulation *via* innate components[26,27]. Intracellular bacteria enter host cells by conventional modes of invasion. Intra-tumoral bacteria can directly stimulate related receptors to affect the genetic material of host cells and activate signaling pathways to affect tumor cells. Intra-tumoral bacteria can also indirectly participate in tumor-related activities and behaviors by using bacterial membrane vesicles and metabolites.

Bacteria invade host cells directly

Intracellular bacteria invade non-phagocytic host cells by conventional trigger or zipper mechanisms. That is, the surface invasion and bacterial membrane adhesins interact with receptors on the host membrane. This interaction triggers a signaling cascade that leads to host cytoskeletal reorganization through actin polymerization, which leads to the formation of membrane protrusions that surround and internalize the bacteria. Triggers involve bacteria that insert effector molecules into host cells, resulting in actin rearrangement and membrane protrusion, which allows bacteria to enter the host and function[28,29].

Induction of autophagy based on the pathogen-associated molecular patterns (PAMPs)-pattern recognition receptors (PRRs) model

Combining the relevant mechanisms underlying PAMPs, the connection between intracellular bacteria PAMPs and cancer

development has also been gradually validated[30]. Most of the bacterial products carrying PAMPs, such as lipopolysaccharide (LPS), can be recognized by the PRRs of the innate immune system and induce the production of inflammatory factors[31]. Bacteria initially trigger and continuously stimulate an inflammatory response, which subsequently intensifies, leading to the attraction of more pro-inflammatory cells to the infected site and an increase in the release of cytokines. This process also involves participation of the immune system. These chain reactions may cause an imbalance in oxidative stress, genetic mutations in carcinogenic genes, and cellular malignant transformation, ultimately leading to the development and spread of tumors[30,32]. The PAMPs-PRRs interactions within intracellular bacteria have a crucial role in stimulating anti- or pro-tumor innate immune responses in the TME[33]. Bacteria further induce autophagy through the contact of PAMP-PRRs with tumor cells[34]. Due to the specificity of tumors and the TME, autophagy has both positive and negative consequences. Some scholars have argued that during the early nutrient-rich stage of a tumor, autophagy ensures the function of immune surveillance by clearing away carcinogenic factors. However, during the late nutrient-poor stage of a tumor, autophagy is the concentration of nutrients to tumor cells, thus maintaining tumor survival[30].

Bacterial membrane vesicle (BMV) communication

BMVs are important mediators that regulate the interactions between host cells and bacteria. BMVs include inner membrane vesicles, outer membrane vesicles (OMVs) and double-membrane vesicles. OMVs are the most studied and used BMVs. OMVs secreted by Gram-negative bacteria are one type of BMV. OMVs contain bacterial payloads, including LPS, peptidoglycan, proteins, nucleic acids, and bacterial toxins[35]. OMVs participate in long-distance signal exchange through the host circulatory system[36,37], regulate gene expression by delivering a payload[38], and participate in biological behaviors, such as reprogramming immune responses. Bacterial payloads, such as LPS and flagellin, also transmit a series of signaling molecules through toll-like receptors (TLRs), thereby activating the inflammatory microenvironment[39].

Bacterial metabolite communication

Bacterial metabolites are also a means by which bacteria participate in tumor progression. Butyrate is a short-chain fatty acid (SCFA) that promotes the response to immune checkpoint inhibitors by inhibiting histone deacetylase activity and inducing DNA-binding 2 inhibitor expression in $CD8^+$ T cells, thereby increasing T cell activation and reducing T cell exhaustion[40]. In clinical studies related to bacterial metabolites, some researchers have proposed that the excellent photo-thermal stability and conversion efficiency of synthetic materials may further enhance the anti-cancer effects of phototherapy by reducing microbial metabolic pathways and metabolites[41]. Specific products produced by specific bacteria often serve as mediators of bacterial involvement in tumor behavior. Gingipains secreted by *P. gingivalis* are the key virulence factors on which *P. gingivalis* promotes carcinogenesis. Gingipains and the *P. gingivalis*-mediated AKT-STAT3 signaling pathway have a critical role in upregulation of PD-L1 on dendritic cells (DCs) and the subsequent suppression of killer T cell activity[18]. Another example involves *F. nucleatum*-produced *Fusobacterium* adhesin A (FadA), which causes DNA damage and leads to an inflammatory tumor microenvironment by regulating E-cadherin/WNT/β-catenin signaling[42-44]. Lactate-producing *Lactobacillus iners* has been shown to initiate the growth and proliferation of cancer cells using lactate instead of glucose as fuel, thereby altering the oxidative stress signaling pathway after radiotherapy and thus resistance to treatment[45]. In addition, bacterial metabolites may reverse gene function. As a tumor suppressor gene, *p53* usually has a protective role. However, when inflammation or immune surveillance function is dysregulated, as in the case of microbial production of gallic acid, *p53* mutation occurs and has a role in promoting cancer[46,47].

In summary, intra-tumoral bacteria participate in tumor progression mainly through specific recognition mechanisms based on traditional contacts that trigger inflammatory and immune responses, extracellular vesicles, and the influence of bacterial metabolites. These findings shed light on the working mode of bacteria within tumor cells.

Distribution and source of intra-tumoral bacteria

With the development of sequencing technology and updated databases, increasing evidence has shown that the bacterial flora in head and neck tumors significantly differs from normal para-cancerous tissue[48]. The major bacterial composition in HNSCC based on existing studies is summarized in **Figure 1** and **Table 1**.

Studies using 16S rRNA sequencing and alpha/beta analyses have shown an increased relative abundance of *Fusobacterium* and decreased abundance of *Streptococcus* and *Actinomyces* in tumor tissues compared to normal tissues[50,61]. *Streptococcus* acts as an early colonizer in oral squamous cell carcinoma (OSCC),

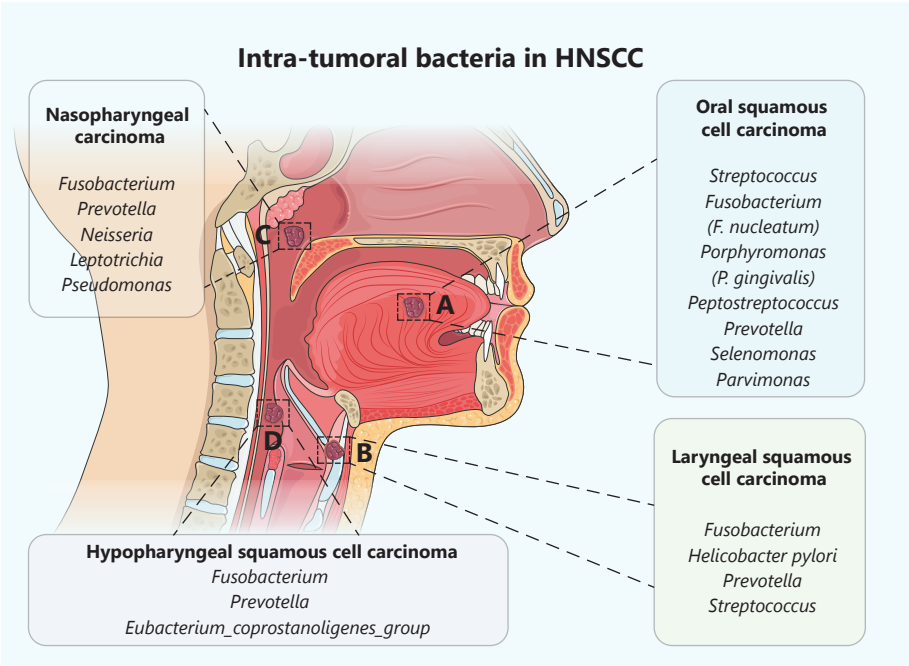


Figure 1 Common intra-tumoral bacteria in each major subtype of HNSCC. (A) **OSCC**: The common bacterial flora in OSCC is shown in the figure. *P. gingivalis* and *F. nucleatum* are the most common and most studied intra-tumoral bacteria in *Porphyromonas* and *Fusobacterium*, respectively, while *Streptococcus* has been observed to have reduced abundance in most studies. (B) **LSCC**: *Fusobacterium* is also the most common species in LSCC. *Helicobacter pylori* is a specific bacterial species found in LSCC. (C) **NPC**: *Fusobacterium* and *Prevotella* are common in NPC and intra-tumoral bacteria in NPC are often associated with prognosis. (D) **HPSCC**: The intra-tumoral bacteria in HPSCC are like other subtypes. Specifically, the *Eubacterium coprostanoligenes* group comprises specific bacteria that inhibit the tumor. HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma; NPC, nasopharyngeal carcinoma; HPSCC, hypopharyngeal squamous cell carcinoma.

while *F. nucleatum* serves as a transitional bacterium between early and late colonizers and has the ability to coaggregate[62]. Moreover, *Streptococcus* has been shown to attenuate *F. nucleatum*-induced pro-inflammatory responses in oral epithelial cells[63]. Some researchers suggest that Firmicutes and Actinobacteria are sensitive to cancer-related environments and a significant decrease in the proportion of Firmicutes and Actinobacteria, along with an increase in *F. nucleatum*, may indicate a pre-cancerous state[51].

The origin of intra-tumoral bacteria is of great significance for research. The intra-tumoral bacterial sources found in > 10 head and neck tumors can be broadly divided into 3 categories. Some oral *Streptococcus* species are found in the tumors of patients with oral cancer and are derived from the patients' oral microbiome[64]. In addition, bacteria can also enter the tumor tissue through the damaged mucosal barrier or through normal adjacent tissues[65]. The bacterial composition of hypopharyngeal squamous cell carcinoma (HPSCC) is dominated by *Fusobacterium* and *Prevotella*. *Fusobacterium*, *Micromonas*, and *Dialister* tend to be abundant in advanced T classification and TNM staging[66]. Bacteria spread through the blood or lymphatic circulation in metastatic tumor tissue and migrate into the tumor. Intestinal flora, such as *Klebsiella*, *Escherichia*, and *Enterococcus*, have been detected in cervical lymph nodes adjacent to oral cancer tumor tissue[64].

These findings suggest the possible existence of oncogenic mechanisms associated with alterations in oral bacteria and provide new perspectives on the role of bacterial communities in head and neck tumors. Moreover, this evidence suggests that finding specific bacteria and identifying changes in the number and distribution of bacteria in tumors[67] are key links to clarify the pathogenesis of this disease.

Predominant intra-tumoral bacteria in HNSCC

Based on the available studies, the intra-tumoral bacteria associated with HNSCC have been shown to be mainly related to

Table 1 Major features of the intra-tumoral bacteria in HNSCC

Tumor type	Bacterial composition	Effect	Mechanism	References
OSCC	*Streptococcus*	Promotes tumor metastasis and progression	Initiates the EMT program	49
	Fusobacterium (F. nucleatum)	Promotes tumor progression	Activates carcinogenic pathways	50-52
		Inhibits anti-tumor immunity		
	Porphyromonas	Promotes tumor progression	Activates carcinogenic pathways	51
	(P. gingivalis)	Inhibits tumor initiation and progression	Induces cell cycle arrest	12
		Enhances anti-tumor immune response	Promotes inflammatory factor secretion	
	Peptostreptococcus	Promotes tumor metastasis		53
	Prevotella	Promotes tumor progression and metastasis	Promotes chronic inflammation	52,53
	Selenomonas			52
	Parvimonas			52
LSCC	*Fusobacterium*	Promotes tumor progression, metastasis, and recurrence	Induces ethanol metabolism reprogramming and initiates the EMT program	50,54
	Helicobacter pylori	Promotes tumor progression	Interacts with viruses	55
	Prevotella	Promotes tumor progression	Stimulates angiogenesis	8
	Streptococcus	Antagonistic *Fusobacterium*		8
NPC	*Fusobacterium*	Promotes tumor progression	Remodels the tumor microenvironment and promotes oxidative stress	56,57
	Prevotella	Promotes tumor progression	Remodels the tumor microenvironment	56
	Neisseria	Promotes tumor progression	Produces carcinogens	58
	Leptotrichia	Promotes tumor initiation	Promotes chronic inflammation and interacts with viruses	56
	Pseudomonas			58
HPSCC	*Fusobacterium*	Promotes tumor progression	Promotes oxidative stress	59
	Prevotella	Promotes tumor metastasis and recurrence		60
	Eubacterium coprostanoligenes group	Inhibits tumor metastasis and recurrence	Involved in cholesterol metabolism	

HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma; NPC, nasopharyngeal carcinoma; HPSCC, hypopharyngeal squamous cell carcinoma; EMT, epithelial-mesenchymal transition.

Fusobacterium and *Streptococcus*. *Porphyromonas* is particularly prevalent in oral-associated tumors. In addition, the role of bacteria, such as *Lactobacillus* and *Actinomycetes*, warrants further study.

Fusobacterium, which is characterized as a Gram-negative anaerobic bacteria, is frequently observed to be enriched across diverse subsets of HNSCC and these bacteria are implicated in a spectrum of oral maladies as well as anaerobic

infections[68,69]. The relative abundance of *Fusobacterium* and *Streptococcus* is increased in primary and metastatic tumor tissues compared to normal healthy tissues of HNSCC patients[70,71]. However, other studies have reported a reduced abundance of *Streptococcus* in head and neck tumor samples[50,67]. The inconsistent findings may potentially arise from patient-specific variables, lifestyle factors, medication usage, and variations in sample collection methods. The impact of lifestyle on microbiota abundance in HNSCC is apparent. The log abundance of Firmicutes and *Peptostreptococcus* is significantly decreased in smokers, while the log abundance of *Fusobacterium* and *Actinomycetes* is significantly increased[72,73]. The ethanol-associated increase in the abundance of *Neisseria* that produce acetaldehyde and the decrease in the abundance of Lactobacillales that are involved in acetaldehyde degradation have been demonstrated in smokers and alcohol consumers[74]. Oral *Corynebacterium* and *Kingella* are involved in xenobiotic degradation and are associated with various metabolic pathways, including the capacity to process several toxicants present in cigarette smoke[75]. Thus, a reduced risk of HNSCC is associated with an increased abundance of *Corynebacterium* and *Kingella* species[76,77]. In particular, laryngeal squamous cell carcinoma (LSCC) tends to have the strongest association with smoking and alcohol consumption, mainly because of alterations that alter the laryngeal ecologic balance and thereby affects the development of LSCC[11].

Impact of tobacco and alcohol on intra-tumoral bacteria

The results of tobacco and alcohol use often act synergistically with the cancer-promoting effects of intra-tumoral bacteria. The mechanisms underlying these changes may be the effects of smoking on DNA damage and repair capacity[78], as well as the effect on inflammatory responses[79]. HNSCC is highly inflammatory in nature and expresses a variety of cytokines and growth factors involved in inflammation[80]. Pre-treatment serum IL-6 levels predict recurrence and poor survival in HNSCC[81]. Smoking is strongly associated with high IL-6 levels[82], which laterally assists intra-tumoral bacteria in promoting progression of TME inflammation[83]. *F. nucleatum* inhibits ethanol metabolism by downregulating the tumor suppressor enzyme, alcohol dehydrogenase 1B (ADH1B), which eventually leads to the accumulation of ethanol and causes metabolic stress in LSCC. Local ethanol accumulation also promotes the proliferation of *F. nucleatum*. Such a mutual promotion pattern explains the increase of *F. nucleatum* in LSCC patients and the promotion of tumor development[54]. In addition, several commensal bacteria metabolize ethanol to carcinogenic acetaldehyde, further exerting a role in alcohol-related carcinogenesis[84]. Acetaldehyde dehydrogenase generated by *Streptococcus* catalyzes the formation of mutagenic levels of acetaldehyde under aerobic or microaerophilic conditions, which leads to elevated acetaldehyde concentrations and the induction of tumors by hydroxyethyl and hydroxyl radicals[85]. In addition, acidogenic *Streptococcus mutans* synthesizes extracellular polymeric substances in the presence of sucrose to form polysaccharide reserves, thereby providing energy for tumor progression[86].

Studies have shown that *P. gingivalis*, together with other oral bacteria, such as *Fusobacterium*, affect the heterogeneity of cancer cells and change the transcriptional program, thereby affecting specific cellular behaviors[6,87,88]. This evidence suggests that personalized studies should be conducted involving specific bacterial flora based on the identification of specific tumor types.

Role of intra-tumoral bacteria in the development of HNCs

Intra-tumoral bacteria have a dual role in HNSCC, either promoting or inhibiting tumor progression. Intra-tumoral bacteria are intricately involved in various biological processes of cancer cells. Variations in bacterial species and disruptions to the microbial balance can significantly influence the trajectory of cancer development.

Intra-tumoral bacteria promote the occurrence and development of tumors

Intra-tumoral bacteria primarily promote cancer progression through mechanisms, such as DNA damage repair disruption, chronic inflammation, regulation of oxidative stress, and interactions with viruses. Bacterial infections induce DNA damage in host cells, increasing the susceptibility to malignant transformation. Additionally, bacterial infections activate various inflammatory signaling pathways, promoting inflammation in surrounding tissues. Disruption of reactive oxygen species (ROS) homeostasis also contributes significantly to tumor

progression. Furthermore, the interplay between bacteria and viruses within the TME acts synergistically to enhance tumor initiation and progression.

Promotion of DNA damage

F. nucleatum is closely related to DNA mismatch repair deficiency (MMR) and microsatellite instability (MSI). The bacteria upregulate miR-205-5p through the TLR4/MyD88-dependent innate immune signaling pathway and inhibit the expression of MMR-related genes, such as *MLH1*, *MSH2*, and *MSH6*, leading to DNA damage and cell proliferation[89]. In addition, intracellular bacteria can initiate gene signatures that are consistent with the invasive and metastatic behaviors of cancer cells, as well as DNA damage repair mechanisms by activating transcription factors of the JUN and FOS families and forming heterodimers as an integral part of the activator protein 1 (AP-1) complex. The continuous activation of JUN and FOS has a regulatory role in enhancing DNA repair pathways, thereby equipping tumor cells with improved adaptation to adverse environments and facilitating their progression[6,90,91]. Another study showed that *F. nucleatum* directly induces double-stranded DNA breaks to cause damage and inhibits the Ku70/p53 pathway to improve cancer cell proliferation, leading to the occurrence of oral cancer[92].

Promotion of chronic inflammation

F. nucleatum and *P. gingivalis* trigger TLR signaling to promote the production of IL-6 and activate STAT3, which further induces the proliferation and invasion of OSCC[93]. Intra-tumoral bacteria can use LPS or other metabolites to activate TLRs on immune cells in the TME through surface PAMPs for IL-6 production and secretion[51]. In addition, intra-tumoral bacteria can activate NF-κB and ERK/MYC signaling pathways in tumor or immune cells that regulate the expression of a variety of pro-inflammatory genes. Thus, IL-6 secretion is induced directly or indirectly[26,94]. These mechanisms promote the production of IL-6, which leads to the excessive expression of IL-6 in HNSCC, then promote inflammation, immune escape, and malignant progression of the tumor.

In addition, human beta-defense (HBD), an antimicrobial peptide that is expressed in oral epithelial cells, including cancer cells, antagonizes Gram-negative bacteria, affects bacteria survival in tumors, and affects the development of tumors through passive changes in expression[95,96]. HBD2 expression is downregulated in some OSCCs, which renders cancer tissue more susceptible to bacterial infection and exacerbates bacteria-induced inflammation, promoting a microenvironment conducive to tumor growth and progression[95]. HBD2-induced tumor necrosis factor-alpha (TNF-α) in OSCC cells may further affect bacterial survival[97]. Inhibition of TNF-α may be a contributing factor to bacterial survival in the context of HNSCC[98]. HBD1 and HBD2 have been identified as factors that inhibit tumor progression. HBD3 is considered a carcinogenic factor that may promote the proliferation and invasion of OSCC cells by regulating the NF-κB pathway[99]. Interestingly, there are studies that have reported a different phenomenon. HBD2 and HBD3 promote the progression of OSCC and HBD2 has a stronger effect than HBD3. Chronic inflammation caused by intra-tumoral bacteria may promote the expression of HBD2 and HBD3 and inhibit the tumor-suppressive effect and expression of HBD1 through inflammatory factors, including transforming growth factor beta1 (TGF-β1) and IL-1β[100,101]. Cross-regulation of defensins reveals the dynamic balance between tumor bacteria and defensins during tumor progression.

Regulation of oxidative stress

F. nucleatum infection in HPSCC downregulates miR-361-3p, relieving suppression of nudix hydrolase 1 (NUDT1) and leading to NUDT1 upregulation. NUDT1 has a key role in oxidative stress homeostasis and inflammation regulation. NUDT1 upregulation activates DNA damage response (DDR) signaling and promotes autophagy. Notably, autophagy and DDR interact in a feedback loop. Specifically, autophagy mitigates oxidative stress and damaged cellular components and indirectly activates DDR, while DDR further enhances autophagy as a cytoprotective mechanism to resolve damage, maintains homeostasis, and drives tumor progression[59]. *F. nucleatum* inhibits purine degradation and lowers uric acid levels in HNSCC, thereby disrupting ROS homeostasis, promoting tumor progression, and worsening patient prognosis[102]. In addition, *F. nucleatum* infection stimulates ROS generation by activating the NF-κB and AKT signaling pathways and drives nasopharyngeal carcinomas (NPC) tumorigenesis by activating the AKT/mTOR pathway and inhibiting *p53* signaling through ROS[57,103,104].

Synergy with viruses

Bacterial involvement also impacts intra-tumoral viruses. A significant positive correlation was demonstrated between the abundance of oral-translocation microbes and local Epstein-Barr virus (EBV) load in NPC and an altered virus-associated

microenvironment in intra-tumoral meta-transcriptome data was found[56]. Earlier studies collectively showed that the *F. nucleatum* culture medium effectively induces EBV-associated antigens *in vitro*[105]. The researchers hypothesized that the translocated oral microbiota might act synergistically with EBV in the pathogenesis of NPC, together creating favorable conditions for latent infection[56]. Patients with periodontitis have a higher incidence of human papillomavirus (HPV)-positive HNSCC. We speculate that *P. gingivalis* and *F. nucleatum* in the HNSCC may synergistically interact with HPV[106]. In addition, *Streptococcus* in the oral cavity contributes to the development of HPV-related HNSCC by stimulating the host inflammatory response and producing carcinogenic metabolites, such as acetaldehyde[107]. However, these studies are still preliminary and further prospective studies with larger sample sizes are needed to confirm these findings **(Figure 2)**.

Intra-tumoral bacteria inhibit tumor progression

Studies suggesting a suppressive role of intra-tumoral bacteria in HNSCC are limited with intra-tumoral bacteria, often aiding in cancer progression. However, *P. gingivalis* has a different role, exhibiting suppressive effects in HNSCC.

Inhibition of tumor cell proliferation

P. gingivalis inhibits mucin and chemokines, allowing *P. gingivalis* to further invade tumor tissue, inducing cell cycle arrest in the G2/M phase and accelerating OSCC cell death, indicating the anti-tumor properties[12]. Periodontitis-negative bacteria, such as *Neisseria sicca* and *Corynebacterium matruchotii*, may activate ATR-CHK1 signaling and NLRP3/GSDMD-mediated pyroptosis, potentially promoting genomic stability in OSCC[108].

Promotion of tumor cell apoptosis

In addition, taste receptor family 2 (T2R) is a G protein-coupled receptor (GPCR) and the expression of GPCRs is elevated in solid tumors compared to normal tissues[109]. Intracellular T2R activation by bacterial metabolites with bitter agonist-related ligands may inhibit HNSCC proliferation and induce apoptosis, establishing a novel mechanistic link between Ca^{2+}_{nuc} influx and apoptosis[110]. Generally, these intra-tumoral bacteria impede cancer progression by disrupting cancer cell replication and promoting cell death.

Relationships between intra-tumoral bacteria and tumor metastasis

Intra-tumoral bacteria mainly initiate or promote tumor metastasis by altering mechanical stress and promoting initiation of the epithelial-mesenchymal transition (EMT) program. Intra-tumoral bacteria initiate a fluid shear stress response upon invasion of host cancer cells and this response is associated with the ability of bacterial species to promote metastasis. Cancer cells invaded by bacteria can carry the bacteria, move to distant organs, and promote cancer cell survival[27]. The bacteria can regulate the cytoskeletal reorganization pathway, allowing cancer cells to become more resistant to mechanical stress.

In addition to physically inducing cancer cell migration, intra-tumoral bacteria also interfere with signaling pathways. *F. nucleatum* triggers TLR signaling to cause IL-6 production and activates STAT3, thereby promoting the production of cyclin D1 and MMP and subsequently activating EMT to promote the invasion and migration of OSCC cells[51]. Moreover, OMVs activate the autophagy pathway in tumor cells, promote the EMT of tumor cells, and promote the formation of lung metastasis[111]. *P. gingivalis* increases the invasion of OSCC cells through IL-8-dependent upregulation of MMPs, enhances the expression of mesenchymal intermediate filament protein and MMP, decreases the expression of E-cadherin and β-catenin, regulates EMT, and promotes the migration of infected cells[112]. Metastasis to cervical lymph nodes is more common in cancer cells harboring bacterial infections. Mechanistically, the compromised oral mucosa in oral cancer facilitates colonization of the oral surface by new bacterial species, which can subsequently migrate to local lymph nodes. Notably, *Streptococcus oralis* is the most frequently isolated bacterial species from cervical lymph nodes in these cases[64].

Interestingly, the *F. nucleatum* culture supernatant, which contains LPS, induces IL-8 and MMP expression, suggesting that direct bacterial contact may not be necessary for carcinogenesis[113]. Another study demonstrated that LPS from *F. nucleatum* increases miR-155-5p and miR-205-5p expression through the TLR4/MyD88 pathway, which targets ADH1B and transforming growth factor-β (TGFBR2), respectively, to promote LSCC EMP, leading to tumor development and invasion[54]. *S. mutans* induces inflammation *via* IL-6, accelerates tumor progression, upregulates MMP9, promotes basement membrane degradation and invasiveness, and establishes an immune-inhibitory TME[49].

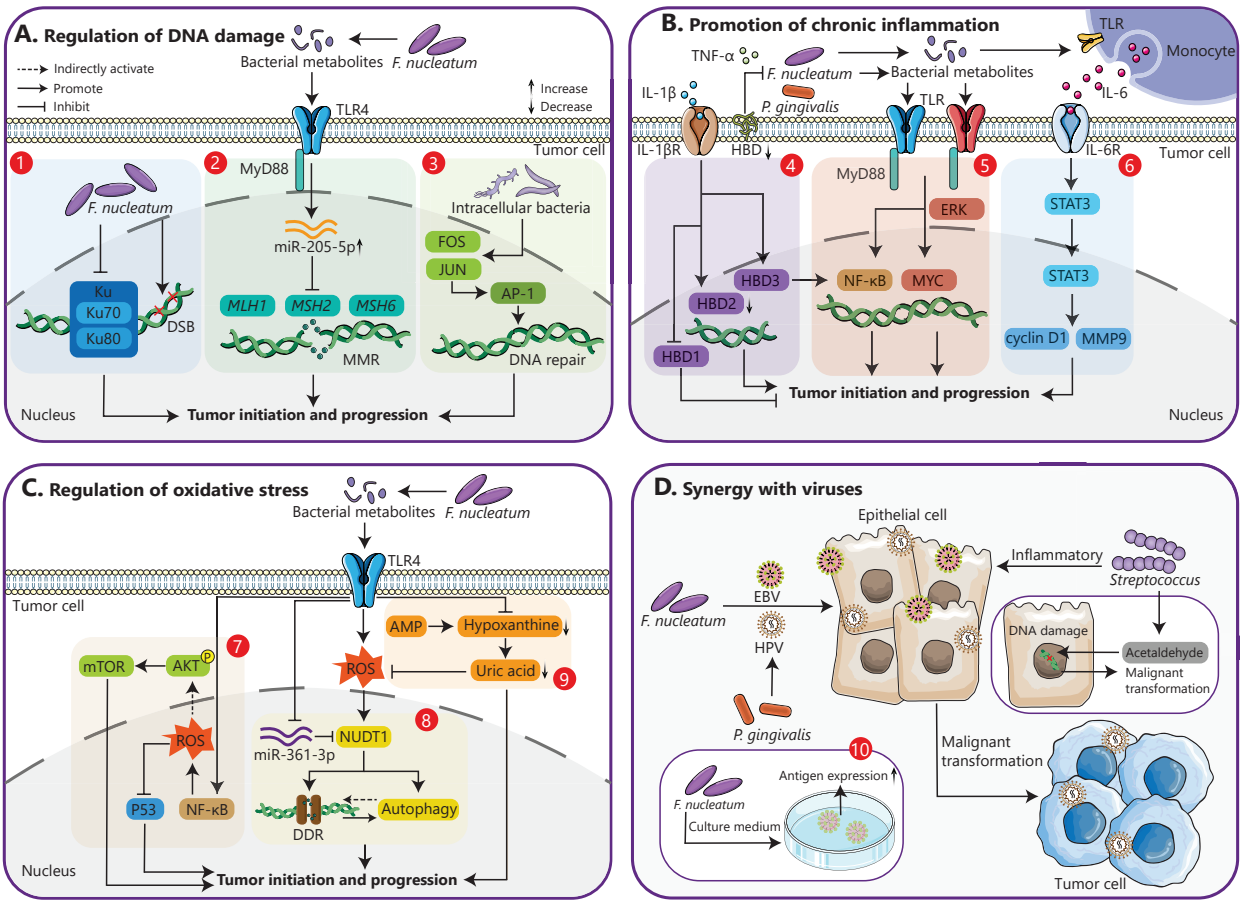


Figure 2 Intra-tumoral bacteria promote tumor initiation and progression. (A) **Regulation of DNA damage**: ① *F. nucleatum* may directly cause DSB and disrupt Ku 70/80 complex in inhibiting DNA repair. ② Metabolites of *F. nucleatum* activate TLR4/MyD88 followed by miR-205-5p upregulation, which inhibits MMR gene expression, including *MLH1*, *MSH2*, and *MSH6*. ③ Intracellular bacteria may promote the formation of JUN and FOS heterodimers and the AP-1 complex for transcriptional activation of DNA damage repair genes, such as *ERCC-1* and *XPC*. (B) **Promotion of chronic inflammation**: ④ Downregulation of antibacterial HBD1 coupled with IL-1β–mediated induction of oncogenic HBD2 and HBD3 facilitates bacterial persistence and contributes to tumor progression. ⑤ *F. nucleatum* and *P. gingivalis* activate the NF-κB and ERK/MYC pathways through the TLR/MyD88 axis, driving tumor progression. ⑥ Intra-tumoral bacteria induce monocyte secretion of IL-6 *via* metabolites and chemokines to activate IL-6/STAT3 signaling and promote effectors, such as cyclin D1 and MMP9. (C) **Regulation of oxidative stress**: ⑦ *F. nucleatum* promotes tumor growth by driving NF-κB activation, which increases ROS production, indirectly activates the AKT/mTOR pathway, and suppresses the p53 pathway. ⑧ *F. nucleatum* regulates oxidative stress by activating the miR-361-3p/NUDT1 axis *via* TLR4, relieving miR-361-3p inhibition of NUDT1 to promote autophagy and DDR. Autophagy indirectly activates DDR, while DDR alleviates DNA damage stress through autophagy, forming a feedback loop that reinforces both processes and drives tumor progression. ⑨ *F. nucleatum* inhibits ROS production by suppressing purine degradation and uric acid production, a key antioxidant. (D) **Synergy with viruses**: ⑩ Co-culture of *F. nucleatum* with EBV enhances EBV antigen expression. Periodontal pathogens activate and exacerbate the oncogenic potential of EBV and HPV through their metabolites, synergistically promoting malignant transformation. *Streptococcus* species contribute to carcinogenesis by inducing inflammation and generating carcinogens, such as acetaldehyde. AMP, adenosine monophosphate; AP-1, activator protein 1; DDR, DNA-damage response; DSB, double-strand break; EBV, Epstein–Barr virus; HBD, human beta-defense; HPV, human papillomavirus; MMP9, matrix metalloprotein 9; MMR, mismatch repair; MyD88, myeloid differentiation primary response gene 88; NUDT1, nudix hydrolase 1; ROS, reactive oxygen species; TLR, Toll-like receptor.

Intra-tumoral bacteria and anti-tumor immunity

Bacterial products and activities within tumors influence tumor immunity by modulating tumor cell immune evasion and reshaping the TME. The association between intra-tumoral bacteria and the PD-1/PD-L1 axis highlights the complex interplay with immune cells in tumor-related immune regulation.

Intra-tumoral bacteria enhance anti-tumor immune responses

Intra-tumoral bacteria enhance the immune response in HNSCC primarily by recruiting immune cells through chemokines, downregulating PD-L1 expression, and boosting the anti-tumor activity of immune cells. Immunosuppressive cells and processes are simultaneously diminished or inhibited, further strengthening anti-tumor immunity.

Recruitment of immune cells

Intra-tumoral bacteria recruit immune cells by regulating chemokines to promote anti-tumor immunity. Higher $CD4^+$ T and $CD8^+$ T levels are associated with improved overall survival (OS) and relapse-free survival[114]. The CXCL17 in the HNSCC TME suppresses anti-tumor immunity by reducing the infiltration of $CD4^+$ and $CD8^+$ T cells and promoting myeloid-derived suppressor cells (MDSCs). However, *P. gingivalis* counteracts this effect by downregulating CXCL17 expression *via* the suppression of pleomorphic epithelial mucin 1 (MUC1), a membrane-bound glycoprotein expressed on cancer cells that contributes to immunosuppression. *P. gingivalis* reduces the transcription of PD-L1 through downregulation of MUC1, thereby inhibiting immune evasion mechanisms. This process enhances the recruitment and infiltration of $CD4^+$ and $CD8^+$ T cells into tumor tissues, reshaping the TME, reversing immunosuppression, and ultimately strengthening anti-tumor immune responses[12,14].

Regulation of inflammation

Lachnoclostridium, *Flammeovirga*, and *Luteibacter* are significantly enriched in TMEs. *Lachnoclostridium* enhances anti-tumor immunity by promoting $CD8^+$ T cell infiltration and activation, while butyric acid metabolites exert anti-inflammatory effects[115,116]. Additionally, *Flammeovirga* and *Luteibacter* are positively associated with the presence of infiltrating $CD8^+$ T cells and the expression of chemokines, such as CXCL9, CXCL10, and CCL5[117]. Notably, the co-existence of these three bacterial genera has been linked to poor prognosis, potentially inducing chronic inflammation. Inflammation exhibits a dual role in the context of cancer; specifically, chronic inflammation fosters tumor initiation and progression, whereas acute inflammation enhances therapeutic efficacy[118]. This duality underscores the complex and context-dependent impact of intra-tumoral bacteria on tumorigenesis and clinical outcomes.

Interestingly, *F. nucleatum* is more prevalent in elderly OSCC patients who abstain from alcohol consumption and is associated with reduced lymph node invasion, lower distant recurrence rates, and improved survival outcomes. *F. nucleatum* is negatively correlated with macrophage M2 polarization and TLR4 expression, suggesting a potential role in mitigating immunosuppressive mechanisms[119]. Similarly, *F. nucleatum* has been implicated in anti-tumor activity in patients with colon cancer with autoinducer-2 (AI-2), a quorum-sensing signal molecule, activating the TNFSF9/IL-1β signaling pathways in macrophages to exert immunomodulatory effects[120,121].

These findings are in contrast with the predominant evidence suggesting that intra-tumoral bacteria promotes tumor progression and is linked to a poor prognosis. This finding highlights the complex and context-dependent roles of intra-tumoral bacteria in cancer immune responses, underscoring the need for multidimensional investigations into the diverse mechanisms of action (**Figure 3**).

Intra-tumoral bacteria inhibit anti-tumor immune responses

Intra-tumoral bacteria mainly rely on activation of PD-1/PD-L1 to affect tumor immune escape. In addition, intra-tumoral bacteria promote the secretion of inflammatory factors by inhibiting relevant immune cells as well as conventional signaling pathways, thereby inducing chronic inflammation, which affects TME and inhibits anti-tumor immune responses.

Immunosuppressive potential

P. gingivalis OMVs activate the mTOR signaling pathway through a TLR4-dependent pathway, leading to a selective reduction in the TNF response of monocytes to live bacteria and this tolerance is mediated by IL-10. Notably, the normal reactivity of monocytes to *P. gingivalis* is restored by blocking TLR4[122]. Specific bacterial genera show significant correlations

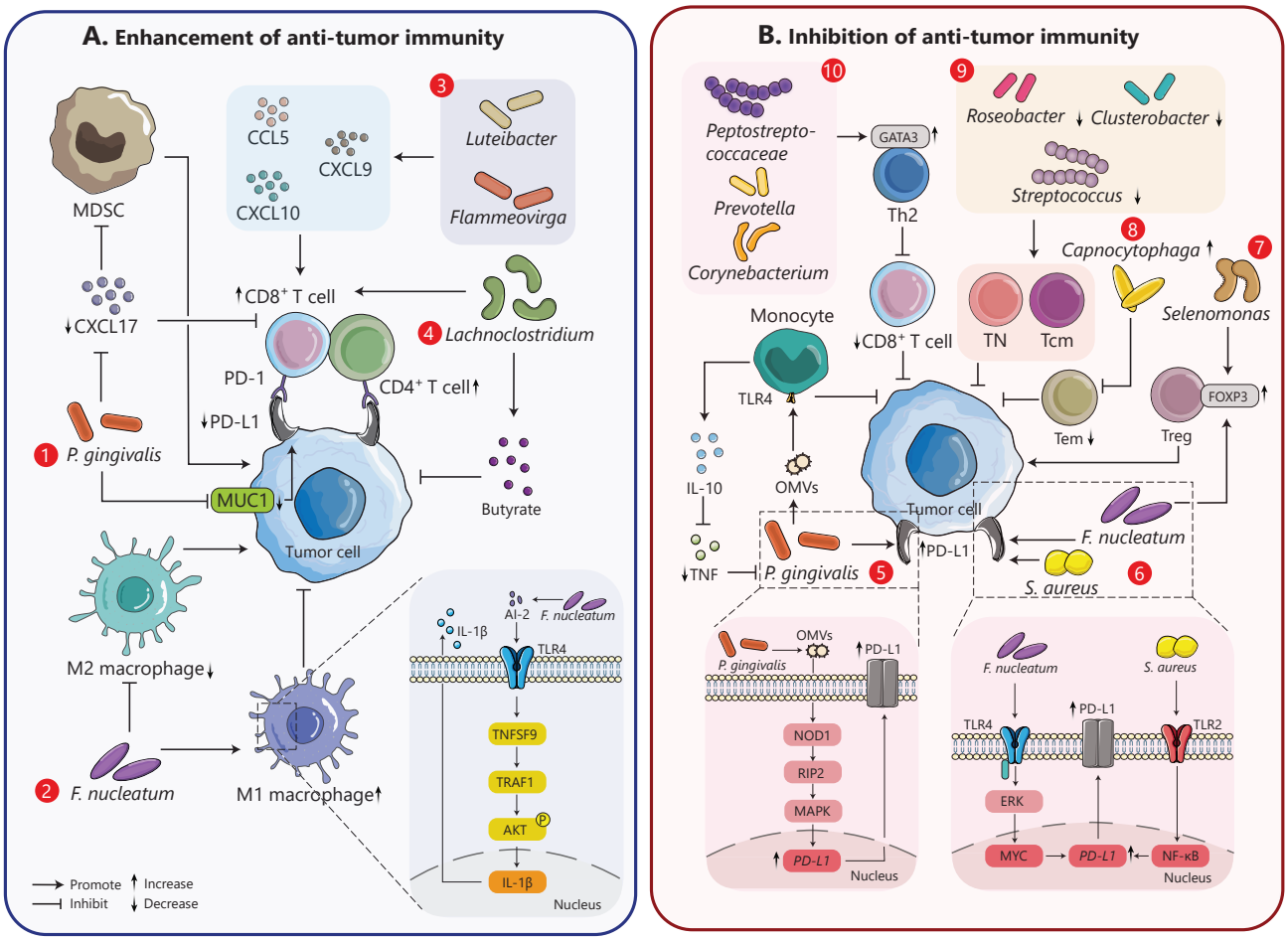


Figure 3 Dual role of intra-tumoral bacteria in regulating the anti-tumor immune response. (A) **Enhancement of anti-tumor immunity:** ① *P. gingivalis* downregulates MUC1 to reduce PD-L1 and inhibit MDSC, thereby enhancing anti-tumor immunity. ② *F. nucleatum* has a negative correlation with M2 macrophages and a positive correlation with M1 macrophages, suggesting involvement in tumor suppression, potentially through activation of the TNFSF9/IL-1β axis. ③ *Flammeovirga* and *Luteibacter* are positively associated with chemokines, such as CXCL9, CXCL10, and CCL5, facilitating the recruitment and activation of $CD8^+$ T cell. ④ *Lachnoclostridium* further supports anti-tumor immunity by promoting $CD8^+$ T cell activity and producing the anti-inflammatory metabolite, butyrate, which exerts tumor-suppressive effects. (B) **Inhibition of anti-tumor immunity:** ⑤ *P. gingivalis* activates NOD1 in tumor cells *via* teichoic acid carried by OMVs, upregulating PD-L1. OMVs also stimulate monocytes to secrete IL-10 and inhibit TNF production, enhancing *P. gingivalis* survival and promoting immune evasion. ⑥ *F. nucleatum* and *S. aureus* utilize TLR signaling to activate ERK/MYC and NF-κB pathways, driving PD-L1 and suppressing T cell activation to facilitate tumor progression. ⑦ *F. nucleatum* and *Selenomonas* are positively correlated with FOXP3, a marker of Treg, suggesting an association with Treg. ⑧ High abundance of *Capnocytophaga* showed a negative correlation with Tem, further linking them to immunosuppressive effects. ⑨ *Roseobacter*, *Streptococcus*, and *Clusterobacter* are associated with the abundance of TN and Tcm, yet significantly reductions of TN and Tcm in tumors may impair the efficacy of these T cell subsets. ⑩ *Corynebacterium*, *Prevotella*, and members of the *Peptostreptococcaceae* family show positive correlations with the Th2 marker GATA3, suggesting roles as potential immunosuppressive agents in the tumor microenvironment. AI-2, autoinducer-2; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; MDSC, myeloid-derived suppressor cell; MUC1, pleomorphic epithelial mucin 1; NOD1, nucleotide-binding and oligomerization domain 1; OMVs, outer membrane vesicles; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; RIP2, receptor-interacting protein kinase 2; Tcm, central memory T cell; Tem, effector memory T cells; Th2, T helper 2 cell; TLR, toll-like receptor; TN, naive T cell; TNF, tumor necrosis factor; TNFSF9, tumor necrosis factor ligand superfamily member 9; TRAF1, tumor necrosis factor receptor-associated factor 1; Treg, regulatory T cell.

with the abundance of T cell subsets in the TME. *Roseobacter*, *Streptococcus*, and *Clusterobacter* are positively correlated with the abundance of naive T cells (TNs) and central memory T cells (Tcms), but the abundance is significantly reduced in the TME, suggesting that the loss may promote tumor growth by inhibiting T cell activity and weaken anti-tumor adaptive immune response[123]. The high abundance of *Capnocytophaga* is inversely correlated with the abundance of effector memory T cells (Tems) with antitumor activity, especially in the TME, which further implies that these bacteria may have a role in tumor immune escape[123-125].

Regulatory T cells (Tregs) are another subset of T cells with pro-tumor activity characterized by transcription factor forkhead box P3 (FOXP3) expression[126]. The abundance of *Fusobacterium* and *Selenomonas* in the TME is positively correlated with FOXP3 expression, suggesting that these bacteria may have an immunosuppressive role in the TME[123]. T helper 2 (Th2) cells are recognized as pro-tumorigenic due to an ability to suppress $CD8^+$ T cell-mediated anti-tumor immune responses through the secretion of the anti-inflammatory cytokine, IL-10[127]. Furthermore, a positive correlation has been observed between bacterial taxa, including *Corynebacterium*, *Prevotella*, and *Peptostreptococcaceae*, and the expression of Th2 cell markers [GATA binding protein 3 (GATA3) and IL-10]. These findings suggest that these bacterial species may function as immunosuppressive agents within the TME, contributing to immune evasion and tumor progression[123].

Regulation of immune checkpoints

Intra-tumoral bacteria regulate the tumor immune response by increasing PD-L1 expression on the cell surface. *P. gingivalis* OMVs contain cell wall components, particularly peptidoglycan, which are internalized by host cells and activate cytoplasmic nucleotide-binding and oligomerization domain 1 (NOD1). NOD1 subsequently interacts with receptor-interacting protein kinase 2 (RIP2) to form a filamentous structure of RIP2, leading to downstream activation of the MAPK signaling pathway. This interaction results in phosphorylation and activation of transcription factors, ultimately upregulating PD-L1 expression. As a result, T cell function is inhibited and immune evasion of tumor cells is promoted[15,128]. Another study reported that *F. nucleatum* infection may induce PD-L1 expression in the TME through ERK/MYC signaling[94].

The binding of *Staphylococcus aureus* to TLRs on tumor cells in the TME further promote immune escape. When microbial ligands bind to TLR2 on the surface of tumor cells, a signal transduction cascade will be triggered, which eventually leads to activation of the NF-κB pathway. Then, PD-L1 expression is upregulated and T cell cytotoxicity is further inhibited, thus promoting tumor cell immune escape[129].

At present, the relevant research results have not been fully integrated and there are scientific problems that need to be solved and improved urgently. In the future, more comprehensive and in-depth research is needed to turn it into a complete story.

Intra-tumoral bacteria and anti-tumor therapy

Intra-tumoral bacteria significantly impact the efficacy of traditional anti-tumor therapies with the effects varying based on unique characteristics. Leveraging these properties, numerous innovative therapeutic strategies are being developed to harness the potential of intra-tumoral bacteria in enhancing treatment outcomes (**Figure 4**).

Intra-tumoral bacteria and radiotherapy

A recent clinical study revealed that radiotherapy positively influences *Streptococcus* and *Lactobacillus* abundance, which provides direct evidence of oral microbiota modulation by radiotherapy[130]. Oral tumors exhibit higher hypoxia levels than other head and neck tumor sites, potentially contributing to radiation resistance. Subsite-specific microbial populations are overrepresented in hypoxic tumors[16].

Follow-up evaluations post-radiotherapy often reveals mucositis, xerostomia, colitis, radiation enteritis, and inflammatory bowel disease due to microbial dysbiosis. Research has increasingly focused on the interaction of radiotherapy with the intestinal and oral microbiota, including the use of probiotics to mitigate radiation reactions and alter the intestinal flora composition to enhance radiation sensitivity[131]. Anaerobe levels increase during radiation, suggesting the prevalence on tumor surfaces and potential involvement in radiation support. Scholars advocate the use of tailored antibacterial regimens in OSCC diagnostics to mitigate radiation mucositis deterioration caused by Gram-negative bacill[132].

While current research primarily concentrates on the intestinal flora, it is plausible that tumor bacteria impact radiotherapy efficacy and complications *via* hypoxia-induced pathways affecting tumor and para-carcinoma tissue.

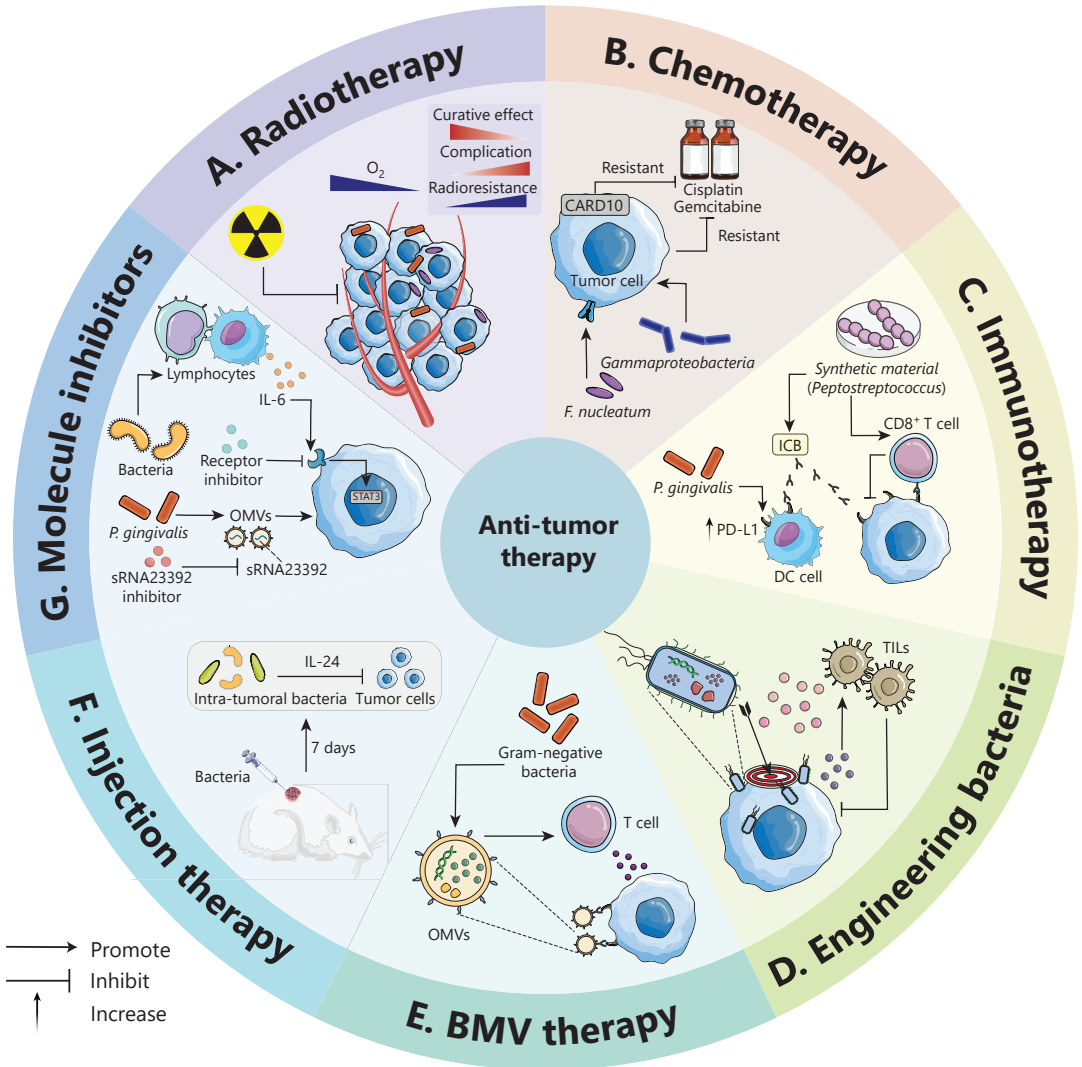


Figure 4 Anti-tumor therapy associated with intra-tumoral bacteria. (A) **Radiotherapy**: Anaerobes associated with vascular distribution can induce radiation resistance of tumor cells in hypoxic areas. (B) **Chemotherapy:** In traditional chemoradiotherapy, intra-tumoral bacteria induce resistance to cisplatin and gemcitabine through intrinsic components of tumor cells. (C) **Immunotherapy**: *P. gingivalis* induces the upregulation of PD-L1 expression in DCs and attenuates ICB efficacy, while the synthetic material containing *Peptostreptococcus* has a synergistic effect with ICB. (D) **Engineering bacteria**: The design of engineering bacteria is an effective means of targeted drug delivery in TME, improving the response rate to anti-PD-1 drugs and reversing immunosuppression. (E) **BMV therapy**: The membrane vesicles of intra-tumoral bacteria are often designed to have anti-tumor effects and positive implications in adjuvant therapy. (F) **Injection therapy**: Direct injection of bacteria or supernatant into tumor cells tended to inhibit tumor growth. (G) **Molecular inhibitors**: Bacteria promote the occurrence and progression of tumors through a variety of signaling molecules and the use of corresponding molecular inhibitors is a potential treatment method. BMVs, bacterial membrane vesicles; CARD10, caspase recruitment domain family member 10; DC, dendritic cell; ICB, immune checkpoint blockade; OMVs, outer membrane vesicles; TILs, tumor infiltrating lymphocyte.

Intra-tumoral bacteria and chemotherapy

Research on the effect of intra-tumoral bacteria on chemotherapy is warranted in the future. *F. nucleatum*-induced miR-146a-5p expression *via* TLR2 activation inhibits downstream caspase recruitment domain family member 10 (CARD10), rendering OSCC cells resistant to cisplatin-induced chemotherapy[51]. Additionally, intra-tumoral bacteria may influence the cancer therapy response through direct interactions with the tumor immune microenvironment. For example,

Gammaproteobacteria expressing cytidine deaminase within tumors mediate gemcitabine resistance[17]. These findings underscore the critical need to further elucidate the mechanisms by which intra-tumoral bacteria influence chemotherapeutic outcomes.

Intra-tumoral bacteria and immunotherapy

Intra-tumoral bacteria are widely involved in the immune response of tumors and have an impact on the survival and function of immune cells. Therefore, immunotherapies for tumors exhibit synergistic or inhibitory effects depending on the immune response effects.

P. gingivalis infection increases PD-L1 expression on the surface of DCs and significantly inhibits the cytotoxic activity of $CD8^+$ T cells[18]. PD-L1 expression on the surface of DCs is a key factor for tumor immunotherapy. PD-L1 inhibitors achieve therapeutic effects by inhibiting PD-L1 expression on DCs, increasing the number of infiltrating T cells or increasing the activity of depleted T cells[19]. This finding suggests that *P. gingivalis* infection may lead to reduced immunotherapy efficacy.

However, intra-tumoral bacteria also have a synergistic effect on immunotherapy. In our previous work we reported that patients with OSCC had a higher abundance of *Peptostreptococcus* in tumors and a higher likelihood of a good prognosis. Synthetic material containing bacteria alone or in combination with a PD-1 blocker inhibited tumor growth and increased the number of $CD8^+$ T cells in draining lymph nodes. The levels of *Peptostreptococcus* could be increased in tumors with low *Peptostreptococcus* content by the addition of exogenous *Peptostreptococcus anaerobius*, leading to an enhanced immune response and anti-tumor effect. The direct cytotoxic effect of *Peptostreptococcus* on tumor cells is limited and the anti-tumor effect is mainly achieved by regulating the host immune response, such as promoting maturation of DCs[20].

Intra-tumoral bacterial engineering

Engineering bacteria to treat cancer is an emerging therapeutic strategy. Engineered bacteria are used to localize a payload inside or outside tumor cells and release the payload through mechanisms, such as secretion, diffusion, or lysis[24]. Based on this concept, some scholars have proposed use of the quorum sensing (QS) genetic circuits principle to enable engineered bacteria to achieve targeted drug delivery through the accumulation of the QS molecule, acyl homoserine lactone[133]. Upon entry of engineered bacteria into the tumor, neoantigens are released into the TME through the function of antigen-presenting cells, where neoantigens recruit and activate tumor infiltrating lymphocytes (TILs) at specific sites and promote the production of pro-inflammatory cytokines and chemokines[24].

It is also possible to combine engineered bacteria with aggregation ultrasound to visualize the localization of bacteria within the tumor[134]. The combination of engineered bacteria with magnetic guidance and interactions with 3D materials also appears to be a new therapeutic strategy[135]. Moreover, engineered bacteria can act as synthetic antigens between chimeric antigen receptor T-cell and tumors to delay tumor progression[136].

Intra-tumoral bacteria BMVs

BMV engineering is an important treatment method, among which OMVs are more widely studied and applied. OMVs are more capable of triggering an inflammatory response than bacterial metabolites, such as LPS alone to stimulate the production of pro-inflammatory cytokines and chemokines by different cells in the body[137]. Some studies have shown that OMVs are safe and effective delivery vectors for tumor drugs because OMVs can deliver encapsulated drugs to tumor tissues, improve drug pharmacokinetic properties, and reduce drug toxicity[138]. In addition to tumor targeting, OMV-based drug delivery platforms can also delay drug release[25] and can be used to regulate the pharmacokinetic parameters of OMV delivery platforms.

According to the characteristics of OMVs, scholars have constructed *Escherichia coli* strains expressing PD-1 to produce PD-1-rich OMVs. OMV-PD-1 accumulates at tumor sites, effectively binds to PD-L1 on tumor cells, inhibits the interaction between tumor PD-L1 and PD-1 on $CD8^+$ T cells, and blocks the PD-1/PD-L1 immune checkpoint axis. These findings protect the function of $CD8^+$ T cells and allow $CD8^+$ T cells to exert normal anti-tumor activity[139].

Intra-tumoral bacterial injection therapy

Intra-tumoral bacteria demonstrate potential for tumor ablation. For example, direct injection of lactic acid bacteria into human squamous cell carcinomas in nude mice inhibited tumor growth, although with associated risks. These

findings suggest that utilizing *Lactobacillus casei* cell supernatants may offer a safer and effective approach for tumor treatment[140]. Furthermore, a novel recombinant *Listeria monocytogenes* tumor vaccine, Lm-ActA-E7, induced E7-specific cytotoxic T lymphocytes in HPV-positive HNSCC patients, resulting in tumor regression without harming normal tissue[141].

The expression of target genes *in vitro* showed that *Bacillus brevis* inhibits the occurrence and development of tumors by increasing IL-24 expression at the mRNA level. The experiment proved that the bacteria were only detected in the tumor tissues 7 days after injection of bacteria. The specific ability of *Bifidobacterium* strains to target and colonize tumor tissues *in vivo* was demonstrated. This new anti-cancer gene, *IL-24*, can be used for *in vivo* tumor treatment to provide a safe and acceptable clinical treatment[142].

Signaling molecule inhibitor therapy

Intra-tumoral bacteria often act through a variety of signaling pathways and key cytokines, so related inhibitor studies may be potential treatment methods and strategies. IL-6 is an important therapeutic target in HNSCC. Several IL-6 receptor antagonists, such as tocilizumab and sarilumab, are currently in clinical trials. These drugs inhibit IL-6-mediated signaling by blocking IL-6 binding to its receptor. Tocilizumab has been shown to have some anti-tumor activity in an HNSCC study[143]. In addition, direct targeting of IL-6 protein using neutralizing antibodies reduces IL-6 levels and slows tumor progression. Several neutralizing antibodies to IL-6 have entered clinical studies, and preliminary results have shown a promising safety profile and modest therapeutic efficacy[144]. In addition to directly targeting IL-6 or its receptor, researchers are also exploring key molecules in the IL-6 signaling pathway, such as JAK and STAT3. JAK inhibitors, such as ruxolitinib, and STAT3 inhibitors, such as stattic, have shown modest anti-tumor efficacy in laboratory studies and early-phase clinical trials[145]. Because IL-6 has a complex role in the TME, IL-6-targeted therapy alone may have limited efficacy. Therefore, researchers are exploring strategies to combine IL-6-targeted therapy with other therapeutic methods, such as chemotherapy and immunotherapy, to enhance the therapeutic effects[146].

In addition, use of inhibitors to inhibit the signaling molecules that are caused by bacteria is a strategy that cannot be ignored. *P. gingivalis* promotes the migration and invasion of HNSCC cells by targeting desmocollin-2 (DSC2), a small RNA released by OMVs (sRNA23392) to reduce the expression of DSC2, thereby affecting cell adhesion and invasion. Inhibition using sRNA23392 attenuates *P. gingivalis*-derived OMV-induced adhesion, migration, and invasion and restores DSC2 expression in HNSCC cells, further suggesting a critical role of sRNA23392 in bacteria-induced tumor progression. These findings suggest that inhibitors targeting sRNA23392 and its target (DSC2) may become a new strategy for the prevention and treatment of *P. gingivalis*-associated HNSCC, providing new ideas for the prevention and treatment of such cancers[38].

Most of the treatment strategies discussed remain at the theoretical and pre-clinical research stage. However, several scholars have already been translated into clinical applications by researchers. We identified an ongoing clinical trial combining three strategies (NCT03435952). Researchers genetically modified *Clostridium novyi*-NT to remove toxic substances and simultaneously injected *Clostridium novyi*-NT into the tumor with combined use of pembrolizumab to solve the problem of a poor chemotherapy effect due to vascular distribution in the tumor area. This finding also suggests that we should focus on multi-strategy combination therapy when considering treatment strategies.

Diagnostic and prognostic roles of intra-tumoral bacteria in HNCs

Intra-tumoral bacteria populations have the potential to be used as diagnostic and prognostic tools due to differences in the composition and abundance of bacteria in head and neck tumors (**Table 2**).

Diagnostic role of intra-tumoral bacteria

Intra-tumoral bacteria in head and neck tumors regulate the TME through various mechanisms, which influence tumor progression. Furthermore, significant differences in the abundance of these bacteria before and after tumorigenesis suggest the potential as diagnostic markers[51,123,150].

Due to the dynamic and heterogeneous nature of predictive markers, such as PD-1, researchers are exploring bacterial populations and metabolites as potential predictive biomarkers for head and neck tumors. Bacteria produce volatile organic metabolites (VOMs) with unique biological characteristics

Table 2 Intra-tumoral bacteria as a diagnostic/prognostic marker in HNC patients

Diagnostic/ prognostic	Bacteria	Clinical role	References
Diagnosis	*Fusobacterium*	Distinguish tumor from normal tissue	48
	Lautropia, Asteroleplasma, Parvimonas, Peptostreptococcus, Roseburia, and *Propionibacterium*	Diagnose metastatic tumor	53
Prognosis	*Leptotrichia*	Improves patient survival	147
	P. gingivalis, Bacteroides fragilis	Promotes tumor spread	148
	Fusobacterium	High levels indicate tumor recurrence or metastasis	54,148
	Corynebacterium, Staphylococcus	Bacterial load correlated with tumor	149
	Prevotella	High levels indicate tumor recurrence or metastasis	60
	Eubacterium coprostanoligenes group	Low levels indicate tumor recurrence or metastasis	

HNC, head and neck cancer.

and it has been shown that a systematic analytical model of VOMs in urine can be used as a diagnostic tool for tumors, especially HNSCC[150-152].

Changes in intra-tumoral bacterial populations can enhance the accuracy of diagnosing head and neck tumors[150]. A study involving Fanconi anemia patients with tongue squamous cell carcinoma utilizing 16S RNA sequencing revealed distinct flora between tumor and healthy sites, with *Mycobacterium salivarius* predominating in tumors and potentially serving as a predictive biomarker for tumorigenesis[153]. Some researchers have identified diverse oral tumor microbiota with genera, such as *Lautropia*, *Asteroleplasma*, *Parvimonas*, *Peptostreptococcus*, *Pyramidobacter*, *Roseburia*, and *Propionibacterium*, showing high diagnostic efficacy for OSCC metastasis[53]. Analysis of the bacterial community data in cancer lesions and controls showed that Bacteroidetes, Proteobacteria, Firmicutes, Fusobacteria, and Actinobacteria accounted for 98.62% of all sequences[154]. Bacteroidetes accounted for 37.6% of the sequences and could serve as an indicator of OSCC occurrence and development[154]. In a prospective observational study by Torralba et al. the data showed a greater number of bacteria belonging to the phyla Fusobacteria, Bacteroidetes, and Firmicutes associated with tumor tissue compared to all other sample types[155]. These data suggest that determining the number of specific bacteria within a tumor will greatly improve the diagnosis of OSCC[53].

It is worth noting that the use of biomarkers for diagnosing head and neck tumors faces challenges. Similarities between pathogens in these tumors and neck abscesses may cause misdiagnosis, necessitating excisional biopsies for histologic examination for confirmation[156].

Additionally, systemic hematoporphyrin derivative fluorescence may not distinguish between normal tissue autofluorescence and endogenous porphyrin fluorescence from bacteria within tumor tissues, potentially leading to false-positive cancer identification[157]. In summary, while changes in bacterial abundance and metabolites hold diagnostic promise, the challenge of identifying and validating predictive biomarkers remains for future research.

Prognostic role of intra-tumoral bacteria

The distribution patterns of intra-tumoral bacterial communities allow such communities to serve as new prognostic markers due to interactions with tumors[46]. Specifically, the nasopharynx tumor tissue microbiome shows a specific structure and contains a higher bacterial biomass compared with normal nasopharyngeal tissues. Patients with NPC have lower microbial diversity in tumor tissues compared to patients with chronic nasopharyngitis. The intra-tumoral bacterial burden is closely related to the prognosis of patients with NPC. A high bacterial burden is associated with worse disease-free survival and OS. In addition, an inverse

correlation has been reported between bacterial biomass and T cell infiltration, suggesting that the tumor microbiome may influence the tumor immune microenvironment, which in turn affects patient outcomes[158,159]. In a clinical study in which samples from > 802 NPC patients were analyzed, intra-tumoral bacterial burden was identified as a reliable prognostic tool that distinguishes the risk of malignant progression[149]. Differences in bacterial composition were noted across tumor stages, with *Fusobacterium* linked to early cancer stages[123]. High *Fusobacterium* abundance correlates with poor survival in HNSCC patients and its cut-off value in HNSCC specimens shows promising sensitivity and specificity in prognostic models, suggesting its potential as a meaningful microbial marker for predicting poor outcomes[89]. Several studies have shown that the number of *P. gingivalis* bacteria within the tumor has a large impact on the prognosis of HNSCC. Higher *P. gingivalis* abundance is associated with increased mortality from oral cancer[160]. If OSCC is chronically exposed to *P. gingivalis*, the probability of tumor migration is greatly increased (approximately 3 times that of uninfected controls)[161].

There is also a correlation between intra-tumoral bacteria and tumor recurrence. The higher abundance of *Prevotella* and the lower abundance of the *Eubacterium coprostanoligenes* group in HPSCC are indicators of higher recurrence and metastasis rates. Specifically, the *Eubacterium coprostanoligenes* group from the primary tumor affected cholesterol metabolism by converting local cholesterol into fecal steroids, which could not be directly utilized by tumor cells, and further inhibited tumor recurrence and metastasis[60]. Previous studies have indicated that antimicrobial therapy impacts HNSCC prognosis with increased survival observed with increased proportions of *Leptotrichia*, suggesting the need to avoid ciliate-targeted therapy[147]. Radiation induces specific microbial changes in head and neck tumors, serving as biomarkers for treatment response and aiding in personalized radiation therapy planning[131].

In summary, intra-tumoral bacterial load and composition differences hold promise as prognostic indicators for head and neck tumor patients, guiding therapeutic decisions based on risk levels for malignant progression.

Conclusions and perspectives

The presence of intra-tumoral bacteria influences the occurrence and progression of HNSCC to varying degrees. Different bacterial species exert distinct effects on tumor cell biology and immune responses with the same bacterial species potentially having dual roles in tumor development. Consequently, the complex interactions between intra-tumoral bacteria and tumor cells require further in-depth investigation in basic research and clinical practice.

Characteristics and diversity

The origin and distribution of bacteria within head and neck tumors are not random but rather follow a certain logic[6]. However, it is not clear if a direct relationship exists with the intra-tumoral bacterial species. It is well-known that the bacterial Gram classification is closely related to bacterial structure, but it is unknown whether the vesicles or metabolites produced by the bacterial cell wall or outer membrane structure are related to the bacterial parasitic preference for host tumor cells. At present, there is a lack of macroscopic studies at the bacterial species level in HNSCC-related fields. Moreover, there is a lack of diversity in the existing studies. OSCC and other common bacteria, such as *F. nucleatum* and *P. gingivalis*, have also been the focus of research. However, for bacterial research, the interaction between bacteria and bacteria, bacteria and tumors, and the interaction between the former two is an important cornerstone in both basic research and clinical applications.

Molecular mechanism

Given that intra-tumoral microflora can promote the occurrence and development of tumors through immunosuppression, inflammation promotion, and EMT signaling pathways but the underlying mechanism is still unclear. It is therefore necessary to elucidate the composition of the main cytokines induced by intra-tumoral microflora and the related mechanisms[6,54,162,163]. *F. nucleatum* can change the status of the TME by promoting the secretion of pro-inflammatory factors in tumors, thereby inhibiting the proliferation of anti-tumor immune cells, promoting the immune escape of tumor cells and promoting the occurrence and development of tumors. In addition, macro-autophagy promoted by oral cancer cells is an adaptive mechanism for *P. gingivalis* to invade tumors and a survival mechanism to limit bacterial toxicity[112]. As mentioned in the previous section, intra-tumoral bacteria have dual roles in tumorigenesis and tumor development and intra-tumoral bacteria do not have a single role in the tumor immune

response and need to be studied from multiple perspectives. In addition, from the bacterial species perspective, *F. nucleatum* and *P. gingivalis* are both Gram-negative anaerobic bacteria, so we hypothesized that the link between HNSCC and intra-tumoral bacteria might be more focused on Gram-negative anaerobic bacteria. However, few studies have addressed the bacterial species associated with HNSCC. It is necessary to closely follow the latest progress in this field and elucidate the underlying mechanisms involved. Moreover, the existing evidence strongly suggests that intra-tumoral bacteria are closely related to PD-1/PD-L1[43,164], suggesting that researchers should not ignore the role of intra-tumoral bacteria when seeking to improve the PD-1 response rate and their role may be crucial.

Many studies on the mechanism by which bacteria affect anti-tumor immunity have made progress but the current research results are not uniform, which hinders the clinical application of bacteria-related therapeutic strategies. More attention should be given to the correlation between the specific mechanisms of various tumor-promoting and -suppressing pathways and intra-tumoral bacteria in the future.

Strategies for intra-tumoral bacterial therapy

Due to the hypoxic environment of the tumor and the metabolism of chemotherapeutic drugs, intra-tumoral bacteria can lead to drug resistance and radiation resistance in tumor cells, thus affecting the therapeutic efficacy in HNSCC patients[16]. Intra-tumoral bacteria influence the efficacy of radiotherapy and chemotherapy, while these treatment modalities also alter the balance of the oral microbiota in patients[131]. However, the mechanisms underlying how these changes impact HNSCC progression or suppression remain unclear and warrant further investigation. Immunotherapy targeting intra-tumoral bacteria will be a promising potential treatment option. According to the different flora and working mechanism, intra-tumoral bacteria have different effects on immunotherapy, which also suggests that there is still a huge space for exploration of intra-tumoral bacteria[18-20]. Targeting intra-tumoral bacteria allows intra-tumoral bacteria to be engineered to deliver drugs. Therefore, imaging the location of bacteria within the tumor cannot be ignored. Some scholars have proposed that the special prodrug, NR-NO_2, can be used to detect the unique fluorescence signal effect of Gram-negative bacteria[165]. Moreover, encapsulation of drugs in bacteria-produced OMVs appears to be more biocompatible. However, the difficulty lies in the production accuracy and efficiency, which is one of the difficulties in the translation of basic research results. Notably, intra-tumoral bacteria have tumor ablative potential. Some studies have reported that direct injection of lactic acid bacteria into human squamous cell carcinomas in nude mice retards tumor growth[140]. However, the risk to other healthy tissues remains a significant concern. Cytokines and chemokines caused by bacteria promotes tumor progression using signal molecules inhibitors to inhibit tumor progression and treat cancer patients. This is also a part of the field of future HNSCC treatment strategy research that should not be neglected. However, whether the normal tissue related signaling pathways will be inhibited and lead to undesirable results needs to be considered.

Intra-tumoral bacteria represent an emerging and promising area of research in HNSCC. Current findings hold significant implications for future investigations and offer valuable insights into improving cancer prognosis with broad potential for clinical applications. The origin, distribution, and abundance of intra-tumoral bacteria in HNSCC patients are closely linked to tumor etiology. These bacteria actively influence tumor-associated immunity and modulate the biological behavior of tumor cells. Furthermore, intra-tumoral bacteria have been explored for potential clinical applications, including use in diagnosis, prognosis, and therapeutic strategies. The results of recent studies underscore the critical role of intra-tumoral bacteria in cancer research and highlight the expansive prospects for advancing patient care.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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References

1. Blake SJ, Wolf Y, Boursi B, Lynn DJ. Role of the microbiota in response to and recovery from cancer therapy. Nat Rev Immunol. 2024; 24: 308-25.
2. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020; 6: 92.
3. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018; 18: 269-82.
4. Li K, Bihan M, Yooseph S, Methé BA. Analyses of the microbial diversity across the human microbiome. PLoS One. 2012; 7: e32118.
5. Sfanos KS. Intratumoral bacteria as mediators of cancer immunotherapy response. Cancer Res. 2023; 83: 2985-6.
6. Galeano Niño JL, Wu H, LaCourse KD, Kempchinsky AG, Baryiames A, Barber B, et al. Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. Nature. 2022; 611: 810-7.
7. Nwabo Kamdje AH, Tagne Simo R, Fogang Dongmo HP, Bidias AR, Masumbe Netongo P. Role of signaling pathways in the interaction between microbial, inflammation and cancer. Holist Integr Oncol. 2023; 2: 42.
8. Gong HL, Shi Y, Zhou L, Wu CP, Cao PY, Tao L, et al. The composition of microbiome in larynx and the throat biodiversity between laryngeal squamous cell carcinoma patients and control population. PLoS One. 2013; 8: e66476.
9. Gong H, Shi Y, Zhou X, Wu C, Cao P, Xu C, et al. Microbiota in the throat and risk factors for laryngeal carcinoma. Appl Environ Microbiol. 2014; 80: 7356-63.
10. Gong H, Shi Y, Xiao X, Cao P, Wu C, Tao L, et al. Alterations of microbiota structure in the larynx relevant to laryngeal carcinoma. Sci Rep. 2017; 7: 5507.
11. Hsueh CY, Gong H, Cong N, Sun J, Lau HC, Guo Y, et al. Throat microbial community structure and functional changes in postsurgery laryngeal carcinoma patients. Appl Environ Microbiol. 2020; 86: e01849-20.
12. Lan Z, Zou KL, Cui H, Zhao YY, Yu GT. Porphyromonas gingivalis suppresses oral squamous cell carcinoma progression by inhibiting MUC1 expression and remodeling the tumor microenvironment. Mol Oncol. 2024; 18: 1174-88.
13. Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res. 2013; 92: 485-91.
14. Beatson R, Tajadura-Ortega V, Achkova D, Picco G, Tsourouktsoglou TD, Klausing S, et al. The mucin MUC1 modulates the tumor immunological microenvironment through engagement of the lectin Siglec-9. Nat Immunol. 2016; 17: 1273-81.
15. Groeger S, Jarzina F, Mamat U, Meyle J. Induction of B7-H1 receptor by bacterial cells fractions of Porphyromonas gingivalis on human oral epithelial cells: B7-H1 induction by Porphyromonas gingivalis fractions. Immunobiology. 2017; 222: 137-47.
16. Dhakal A, Upadhyay R, Wheeler C, Hoyd R, Karivedu V, Gamez ME, et al. Association between tumor microbiome and hypoxia across anatomic subsites of head and neck cancers. Int J Mol Sci. 2022; 23: 15531.
17. Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science. 2017; 357: 1156-60.
18. Ren J, Han X, Lohner H, Hoyle RG, Li J, Liang S, et al. P. gingivalis infection upregulates PD-L1 expression on dendritic cells, suppresses CD8+ T-cell responses, and aggravates oral cancer. Cancer Immunol Res. 2023; 11: 290-305.
19. Oh SA, Wu DC, Cheung J, Navarro A, Xiong H, Cubas R, et al. PD-L1 expression by dendritic cells is a key regulator of T-cell immunity in cancer. Nat Cancer. 2020; 1: 681-91.
20. Zheng DW, Deng WW, Song WF, Wu CC, Liu J, Hong S, et al. Biomaterial-mediated modulation of oral microbiota synergizes with PD-1 blockade in mice with oral squamous cell carcinoma. Nat Biomed Eng. 2022; 6: 32-43.
21. Pushalkar S, Hundeyin M, Daley D, Zambirinis CP, Kurz E, Mishra A, et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov. 2018; 8: 403-16.
22. Riquelme E, Maitra A, McAllister F. Immunotherapy for pancreatic cancer: more than just a gut feeling. Cancer Discov. 2018; 8: 386-8.
23. Wang M, Rousseau B, Qiu K, Huang G, Zhang Y, Su H, et al. Killing tumor-associated bacteria with a liposomal antibiotic generates neoantigens that induce anti-tumor immune responses. Nat Biotechnol. 2024; 42: 1263-74.
24. Gurbatri CR, Arpaia N, Danino T. Engineering bacteria as interactive cancer therapies. Science. 2022; 378: 858-64.
25. Huang J, Wu Z, Xu J. Effects of biofilm nano-composite drugs OMVS-MSN-5-FU on cervical lymph node metastases from oral squamous cell carcinoma. Front Oncol. 2022; 12: 881910.
26. Yang L, Li A, Wang Y, Zhang Y. Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy. Signal Transduct Target Ther. 2023; 8: 35.
27. Fu A, Yao B, Dong T, Chen Y, Yao J, Liu Y, et al. Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. Cell. 2022; 185: 1356-72.e26.
28. Cossart P, Sansonetti PJ. Bacterial invasion: the paradigms of enteroinvasive pathogens. Science. 2004; 304: 242-8.

29. Schorr L, Mathies M, Elinav E, Puschhof J. Intracellular bacteria in cancer-prospects and debates. NPJ Biofilms Microbiomes. 2023; 9: 76.
30. Fu Y, Li J, Cai W, Huang Y, Liu X, Ma Z, et al. The emerging tumor microbe microenvironment: from delineation to multidisciplinary approach-based interventions. Acta Pharm Sin B. 2024; 14: 1560-91.
31. Akira S, Hemmi H. Recognition of pathogen-associated molecular patterns by TLR family. Immunol Lett. 2003; 85: 85-95.
32. Wei MY, Shi S, Liang C, Meng QC, Hua J, Zhang YY, et al. The microbiota and microbiome in pancreatic cancer: more influential than expected. Mol Cancer. 2019; 18: 97.
33. Demento SL, Siefert AL, Bandyopadhyay A, Sharp FA, Fahmy TM. Pathogen-associated molecular patterns on biomaterials: a paradigm for engineering new vaccines. Trends Biotechnol. 2011; 29: 294-306.
34. Deretic V, Delgado M, Vergne I, Master S, De Haro S, Ponpuak M, et al. Autophagy in immunity against mycobacterium tuberculosis: a model system to dissect immunological roles of autophagy. Curr Top Microbiol Immunol. 2009; 335: 169-88.
35. Guerrero-Mandujano A, Hernández-Cortez C, Ibarra JA, Castro-Escarpulli G. The outer membrane vesicles: secretion system type zero. Traffic. 2017; 18: 425-32.
36. Amatya SB, Salmi S, Kainulainen V, Karihtala P, Reunanen J. Bacterial extracellular vesicles in gastrointestinal tract cancer: an unexplored territory. Cancers (Basel). 2021; 13: 5450.
37. Jang SC, Kim SR, Yoon YJ, Park KS, Kim JH, Lee J, et al. In vivo kinetic biodistribution of nano-sized outer membrane vesicles derived from bacteria. Small. 2015; 11: 456-61.
38. Liu D, Liu S, Liu J, Miao L, Zhang S, Pan Y. sRNA23392 packaged by Porphyromonas gingivalis outer membrane vesicles promotes oral squamous cell carcinomas migration and invasion by targeting desmocollin-2. Mol Oral Microbiol. 2021; 36: 182-91.
39. Li D, Zhu L, Wang Y, Zhou X, Li Y. Bacterial outer membrane vesicles in cancer: biogenesis, pathogenesis, and clinical application. Biomed Pharmacother. 2023; 165: 115120.
40. He Y, Fu L, Li Y, Wang W, Gong M, Zhang J, et al. Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic CD8$^+$ T cell immunity. Cell Metab. 2021; 33: 988-1000.e7.
41. Kong F, Fang C, Zhang Y, Duan L, Du D, Xu G, et al. Abundance and metabolism disruptions of intratumoral microbiota by chemical and physical actions unfreeze tumor treatment resistance. Adv Sci (Weinh). 2022; 9: e2105523.
42. Guo P, Tian Z, Kong X, Yang L, Shan X, Dong B, et al. FadA promotes DNA damage and progression of Fusobacterium nucleatum-induced colorectal cancer through up-regulation of chk2. J Exp Clin Cancer Res. 2020; 39: 202.
43. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe. 2013; 14: 195-206.
44. Chang CM, Pekkle Lam HY. Intratumoral microbiota: unraveling their oncogenic impact on cancer progression with focus on breast cancer therapeutic outcomes. Anticancer Res. 2024; 44: 2271-85.
45. Colbert LE, El Alam MB, Wang R, Karpinets T, Lo D, Lynn EJ, et al. Tumor-resident Lactobacillus iners confer chemoradiation resistance through lactate-induced metabolic rewiring. Cancer Cell. 2023; 41: 1945-62.e11.
46. Kadosh E, Snir-Alkalay I, Venkatachalam A, May S, Lasry A, Elyada E, et al. The gut microbiome switches mutant p53 from tumour-suppressive to oncogenic. Nature. 2020; 586: 133-8.
47. Chen L, Huang M. Oncometabolites in cancer: from cancer cells to the tumor microenvironment. Holist Integr Oncol. 2024; 3: 26.
48. Wang Y, Wang Y, Wang J. A comprehensive analysis of intratumor microbiome in head and neck squamous cell carcinoma. Eur Arch Otorhinolaryngol. 2022; 279: 4127-36.
49. Tsai MS, Chen YY, Chen WC, Chen MF. *Streptococcus mutans* promotes tumor progression in oral squamous cell carcinoma. J Cancer. 2022; 13: 3358-67.
50. Shin JM, Luo T, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Microbial communities associated with primary and metastatic head and neck squamous cell carcinoma - a high fusobacterial and low streptococcal signature. Sci Rep. 2017; 7: 9934.
51. Li Q, Hu Y, Zhou X, Liu S, Han Q, Cheng L. Role of oral bacteria in the development of oral squamous cell carcinoma. Cancers (Basel). 2020; 12: 2797.
52. Zeng B, Tan J, Guo G, Li Z, Yang L, Lao X, et al. The oral cancer microbiome contains tumor space-specific and clinicopathology-specific bacteria. Front Cell Infect Microbiol. 2022; 12: 942328.
53. Nie F, Wang L, Huang Y, Yang P, Gong P, Feng Q, et al. Characteristics of microbial distribution in different oral niches of oral squamous cell carcinoma. Front Cell Infect Microbiol. 2022; 12: 905653.
54. Hsueh CY, Huang Q, Gong H, Shen Y, Sun J, Lau HC, et al. A positive feed-forward loop between *Fusobacterium nucleatum* and ethanol metabolism reprogramming drives laryngeal cancer progression and metastasis. iScience. 2022; 25: 103829.
55. Gong H, Shi Y, Zhou L, Tao L, Shi Y, Cao W, et al. Helicobacter pylori infection of the larynx may be an emerging risk factor for laryngeal squamous cell carcinoma. Clin Transl Oncol. 2012; 14: 905-10.
56. Liao Y, Wu YX, Tang M, Chen YW, Xie JR, Du Y, et al. Microbes translocation from oral cavity to nasopharyngeal carcinoma in patients. Nat Commun. 2024; 15: 1645.
57. Ashique S, Houshyari M, Islam A, Pal R, Ghazanfar S, Taghizadeh-Hesary F. The role of microbiota in nasopharyngeal cancer: where do we stand? Oral Oncol. 2024; 158: 106982.
58. Liu QY, Liao Y, Wu YX, Diao H, Du Y, Chen YW, et al. The oral microbiome as mediator between oral hygiene and its impact on nasopharyngeal carcinoma. Microorganisms. 2023; 11: 719.
59. Lau HC, Yuan X, Huang H, Zhang M, Hsueh CY, Gong H. Fusobacterium nucleatum facilitates proliferation and autophagy by activating miR-361-3p/NUDT1 axis through oxidative stress in hypopharyngeal squamous cell carcinoma. BMC Cancer. 2023; 23: 990.
60. Yuan X, Lau HC, Shen Y, Huang Q, Huang H, Zhang M, et al. Tumour microbiota structure predicts hypopharyngeal carcinoma recurrence and metastasis. J Oral Microbiol. 2023; 15: 2146378.

61. Wang H, Funchain P, Bebek G, Altemus J, Zhang H, Niazi F, et al. Microbiomic differences in tumor and paired-normal tissue in head and neck squamous cell carcinomas. Genome Med. 2017; 9: 14.
62. Nobbs AH, Jenkinson HF, Jakubovics NS. Stick to your gums: mechanisms of oral microbial adherence. J Dent Res. 2011; 90: 1271-8.
63. Zhang G, Chen R, Rudney JD. Streptococcus cristatus modulates the Fusobacterium nucleatum-induced epithelial interleukin-8 response through the nuclear factor-kappa B pathway. J Periodontal Res. 2011; 46: 558-67.
64. Sakamoto H, Naito H, Ohta Y, Tanakna R, Maeda N, Sasaki J, et al. Isolation of bacteria from cervical lymph nodes in patients with oral cancer. Arch Oral Biol. 1999; 44: 789-93.
65. El Tekle G, Garrett WS. Bacteria in cancer initiation, promotion and progression. Nat Rev Cancer. 2023; 23: 600-18.
66. Lau HC, Shen Y, Huang H, Yuan X, Ji M, Gong H, et al. Cross-comparison of microbiota in the oropharynx, hypopharyngeal squamous cell carcinoma and their adjacent tissues through quantitative microbiome profiling. J Oral Microbiol. 2022; 14: 2073860.
67. Lang S, Brandau S, Marchesi JR, Jablonska J, Thurnher D, Mattheis S, et al. The microbiome in head and neck tumors-initial findings and outlook. HNO 2020; 68: 905-10.
68. Stokowa-Sołtys K, Wojtkowiak K, Jagiełło K. Fusobacterium nucleatum – friend or foe? J Inorg Biochem. 2021; 224: 111586.
69. Gopinath D, Menon RK, Banerjee M, Su Yuxiong R, Botelho MG, Johnson NW. Culture-independent studies on bacterial dysbiosis in oral and oropharyngeal squamous cell carcinoma: a systematic review. Crit Rev Oncol Hematol. 2019; 139: 31-40.
70. Mukherjee PK, Wang H, Retuerto M, Zhang H, Burkey B, Ghannoum MA, et al. Bacteriome and mycobiome associations in oral tongue cancer. Oncotarget. 2017; 8: 97273-89.
71. Bronzato JD, Bomfim RA, Edwards DH, Crouch D, Hector MP, Gomes BPFA. Detection of Fusobacterium in oral and head and neck cancer samples: a systematic review and meta-analysis. Arch Oral Biol. 2020; 112: 104669.
72. He X, Ma X, Meng Z, Han Z, Chen W. Localization of Fusobacterium nucleatum in oral squamous cell carcinoma and its possible directly interacting protein molecules: a case series. Histol Histopathol. 2023; 38: 929-39.
73. Thomas AM, Gleber-Netto FO, Fernandes GR, Amorim M, Barbosa LF, Francisco AL, et al. Alcohol and tobacco consumption affects bacterial richness in oral cavity mucosa biofilms. BMC Microbiol. 2014; 14: 250.
74. Fan X, Peters BA, Jacobs EJ, Gapstur SM, Purdue MP, Freedman ND, et al. Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. Microbiome. 2018; 6: 59.
75. Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, et al. Cigarette smoking and the oral microbiome in a large study of American adults. ISME J. 2016; 10: 2435-46.
76. Marron M, Boffetta P, Zhang ZF, Zaridze D, Wünsch-Filho V, Winn DM, et al. Cessation of alcohol drinking, tobacco smoking and the reversal of head and neck cancer risk. Int J Epidemiol. 2010; 39: 182-96.
77. Hayes RB, Ahn J, Fan X, Peters BA, Ma Y, Yang L, et al. Association of oral microbiome with risk for incident head and neck squamous cell cancer. JAMA Oncol. 2018; 4: 358-65.
78. Fracasso ME, Doria D, Franceschetti P, Perbellini L, Romeo L. DNA damage and repair capacity by comet assay in lymphocytes of white-collar active smokers and passive smokers (non- and ex-smokers) at workplace. Toxicol Lett. 2006; 167: 131-41.
79. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest. 2007; 131: 1557-66.
80. Wang F, Arun P, Friedman J, Chen Z, Van Waes C. Current and potential inflammation targeted therapies in head and neck cancer. Curr Opin Pharmacol. 2009; 9: 389-95.
81. Duffy SA, Taylor JM, Terrell JE, Islam M, Li Y, Fowler KE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. Cancer. 2008; 113: 750-7.
82. Duffy SA, Teknos T, Taylor JM, Fowler KE, Islam M, Wolf GT, et al. Health behaviors predict higher interleukin-6 levels among patients newly diagnosed with head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2013; 22: 374-81.
83. Sharp L, McDevitt J, Carsin AE, Brown C, Comber H. Smoking at diagnosis is an independent prognostic factor for cancer-specific survival in head and neck cancer: findings from a large, population-based study. Cancer Epidemiol Biomarkers Prev. 2014; 23: 2579-90.
84. Moritani K, Takeshita T, Shibata Y, Ninomiya T, Kiyohara Y, Yamashita Y. Acetaldehyde production by major oral microbes. Oral Dis. 2015; 21: 748-54.
85. Perera M, Al-Hebshi NN, Speicher DJ, Perera I, Johnson NW. Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio-pathogenic bacteria. J Oral Microbiol. 2016; 8: 32762.
86. Forssten SD, Björklund M, Ouwehand AC. Streptococcus mutans, caries and simulation models. Nutrients. 2010; 2: 290-8.
87. Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. Technol Cancer Res Treat. 2019; 18: 1533033819867354.
88. Wang Y, Du J, Wu X, Abdelrehem A, Ren Y, Liu C, et al. Crosstalk between autophagy and microbiota in cancer progression. Mol Cancer. 2021; 20: 163.
89. Hsueh CY, Lau HC, Huang Q, Gong H, Sun J, Cao P, et al. Fusobacterium nucleatum impairs DNA mismatch repair and stability in patients with squamous cell carcinoma of the head and neck. Cancer. 2022; 128: 3170-84.
90. Abate C, Baker SJ, Lees-Miller SP, Anderson CW, Marshak DR, Curran T. Dimerization and DNA binding alter phosphorylation of Fos and Jun. Proc Natl Acad Sci U S A. 1993; 90: 6766-70.
91. Yao H, Wu Y, Zhong Y, Huang C, Guo Z, Jin Y, et al. Role of c-Fos in DNA damage repair. J Cell Physiol. 2024; 239: e31216.
92. Geng F, Zhang Y, Lu Z, Zhang S, Pan Y. *Fusobacterium nucleatum* caused DNA damage and promoted cell proliferation by the *Ku70/p53* pathway in oral cancer cells. DNA Cell Biol. 2020; 39: 144-51.

93. Binder Gallimidi A, Fischman S, Revach B, Bulvik R, Maliutina A, Rubinstein AM, et al. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. Oncotarget. 2015; 6: 22613-23.
94. Michikawa C, Gopalakrishnan V, Harrandah AM, Karpinets TV, Garg RR, Chu RA, et al. Fusobacterium is enriched in oral cancer and promotes induction of programmed death-ligand 1 (PD-L1). Neoplasia. 2022; 31: 100813.
95. Abiko Y, Mitamura J, Nishimura M, Muramatsu T, Inoue T, Shimono M, et al. Pattern of expression of beta-defensins in oral squamous cell carcinoma. Cancer Lett. 1999; 143: 37-43.
96. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999; 341: 738-46.
97. Joly S, Maze C, McCray PB Jr, Guthmiller JM. Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. J Clin Microbiol. 2004; 42: 1024-9.
98. Lam-Ubol A, Hopkin D, Letuchy EM, Kurago ZB. Squamous carcinoma cells influence monocyte phenotype and suppress lipopolysaccharide-induced TNF-alpha in monocytes. Inflammation. 2010; 33: 207-23.
99. Chen M, Hu Z, Shi J, Xie Z. Human β-defensins and their synthetic analogs: natural defenders and prospective new drugs of oral health. Life Sci. 2024; 346: 122591.
100. Kawsar HI, Ghosh SK, Hirsch SA, Koon HB, Weinberg A, Jin G. Expression of human beta-defensin-2 in intratumoral vascular endothelium and in endothelial cells induced by transforming growth factor beta. Peptides. 2010; 31: 195-201.
101. Winter J, Pantelis A, Reich R, Martini M, Kraus D, Jepsen S, et al. Human beta-defensin-1, -2, and -3 exhibit opposite effects on oral squamous cell carcinoma cell proliferation. Cancer Invest. 2011; 29: 196-201.
102. Li F, Huang H, Xu J, Tao L, Zhou L, Hsueh C, et al. Fusobacterium nucleatum-triggered purine metabolic reprogramming drives tumorigenesis in head and neck carcinoma. Discov Oncol. 2023; 14: 120.
103. Kang W, Jia Z, Tang D, Zhang Z, Gao H, He K, et al. *Fusobacterium nucleatum* facilitates apoptosis, ros generation, and inflammatory cytokine production by activating AKT/MAPK and NF-κB signaling pathways in human gingival fibroblasts. Oxid Med Cell Longev. 2019; 2019: 1681972.
104. Ran H, Zhang J, Zeng X, Wang Z, Liu P, Kang C, et al. TOM40 regulates the progression of nasopharyngeal carcinoma through ROS-mediated AKT/mTOR and p53 signaling. Discov Oncol. 2023; 14: 109.
105. Ito Y, Kishishita M, Yanase S. Induction of Epstein-Barr virus antigens in human lymphoblastoid p3HR-1 cells with culture fluid of Fusobacterium nucleatum. Cancer Res. 1980; 40: 4329-30.
106. Doolittle JM, Webster-Cyriaque J. Polymicrobial infection and bacterium-mediated epigenetic modification of DNA tumor viruses contribute to pathogenesis. mBio. 2014; 5: e01015-14.
107. Schwartz J. Poly-microbial interaction with human papilloma virus leading to increased risk for head and neck squamous cell carcinoma and oral squamous cell carcinomas. In: Radosevich JA, editor. HPV and cancer. Dordrecht: Springer Netherlands; 2012. p. 75-106.
108. Shen X, Zhang B, Hu X, Li J, Wu M, Yan C, et al. *Neisseria sicca* and *Corynebacterium matruchotii* inhibited oral squamous cell carcinomas by regulating genome stability. Bioengineered. 2022; 13: 14094-106.
109. Sriram K, Moyung K, Corriden R, Carter H, Insel PA. GPCRS show widespread differential mRNA expression and frequent mutation and copy number variation in solid tumors. PLoS Biol. 2019; 17: e3000434.
110. Carey RM, McMahon DB, Miller ZA, Kim T, Rajasekaran K, Gopallawa I, et al. T2R bitter taste receptors regulate apoptosis and may be associated with survival in head and neck squamous cell carcinoma. Mol Oncol. 2022; 16: 1474-92.
111. Chen G, Gao C, Jiang S, Cai Q, Li R, Sun Q, et al. Fusobacterium nucleatum outer membrane vesicles activate autophagy to promote oral cancer metastasis. J Adv Res. 2024; 56: 167-79.
112. Olsen I, Yilmaz Ö. Possible role of *Porphyromonas gingivalis* in orodigestive cancers. J Oral Microbiol. 2019; 11: 1563410.
113. Harrandah AM, Chukkapalli SS, Bhattacharyya I, Progulske-Fox A, Chan EKL. Fusobacteria modulate oral carcinogenesis and promote cancer progression. J Oral Microbiol. 2020; 13: 1849493.
114. Wang G, Zhang M, Cheng M, Wang X, Li K, Chen J, et al. Tumor microenvironment in head and neck squamous cell carcinoma: functions and regulatory mechanisms. Cancer Lett. 2021; 507: 55-69.
115. Yu AI, Zhao L, Eaton KA, Ho S, Chen J, Poe S, et al. Gut microbiota modulate CD8 T cell responses to influence colitis-associated tumorigenesis. Cell Rep. 2020; 31: 107471.
116. Hu M, Coleman S, Fadlullah MZH, Spakowicz D, Chung CH, Tan AC. Deciphering the tumor-immune-microbe interactions in HPV-negative head and neck cancer. Genes (Basel). 2023; 14: 1599.
117. Zhu G, Su H, Johnson CH, Khan SA, Kluger H, Lu L. Intratumour microbiome associated with the infiltration of cytotoxic CD8+ T cells and patient survival in cutaneous melanoma. Eur J Cancer. 2021; 151: 25-34.
118. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010; 140: 883-99.
119. Neuzillet C, Marchais M, Vacher S, Hilmi M, Schnitzler A, Meseure D, et al. Prognostic value of intratumoral Fusobacterium nucleatum and association with immune-related gene expression in oral squamous cell carcinoma patients. Sci Rep. 2021; 11: 7870.
120. Thompson JA, Oliveira RA, Djukovic A, Ubeda C, Xavier KB. Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. Cell Rep. 2015; 10: 1861-71.
121. Wu J, Li K, Peng W, Li H, Li Q, Wang X, et al. Autoinducer-2 of Fusobacterium nucleatum promotes macrophage M1 polarization via TNFSF9/IL-1β signaling. Int Immunopharmacol. 2019; 74: 105724.
122. Waller T, Kesper L, Hirschfeld J, Dommisch H, Kölpin J, Oldenburg J, et al. Porphyromonas gingivalis outer membrane vesicles induce selective tumor necrosis factor tolerance in a toll-like receptor

4- and mTOR-dependent manner. Infect Immun. 2016; 84: 1194-204.
123. Pratap Singh R, Kumari N, Gupta S, Jaiswal R, Mehrotra D, Singh S, et al. Intratumoral microbiota changes with tumor stage and influences the immune signature of oral squamous cell carcinoma. Microbiol Spectr. 2023; 11: e0459622.
124. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000; 100: 655-69.
125. Liu Q, Sun Z, Chen L. Memory T cells: strategies for optimizing tumor immunotherapy. Protein Cell. 2020; 11: 549-64.
126. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. Nat Rev Clin Oncol. 2019; 16: 356-71.
127. Ellyard JI, Simson L, Parish CR. Th2-mediated anti-tumour immunity: friend or foe? Tissue Antigens. 2007; 70: 1-11.
128. Groeger S, Denter F, Lochnit G, Schmitz ML, Meyle J. Porphyromonas gingivalis cell wall components induce programmed death ligand 1 (PD-L1) expression on human oral carcinoma cells by a receptor-interacting protein kinase 2 (RIP2)-dependent mechanism. Infect Immun. 2020; 88: e00051-20.
129. Mann JE, Ludwig ML, Kulkarni A, Scheftz EB, Murray IR, Zhai JY, et al. Microbe-mediated activation of toll-like receptor 2 drives PDL1 expression in HNSCC. Cancers (Basel). 2021; 13: 4782.
130. Arrifin A, Heidari E, Burke M, Fenlon MR, Banerjee A. The effect of radiotherapy for treatment of head and neck cancer on oral flora and saliva. Oral Health Prev Dent. 2018; 16: 425-9.
131. Joseph NT, Shankar SR, Narasimhamurthy RK, Rao SBS, Mumbrekar KD. Bi-directional interactions between microbiota and ionizing radiation in head and neck and pelvic radiotherapy-clinical relevance. Int J Radiat Biol. 2020; 96: 961-71.
132. Sonalika WG, Amsavardani Tayaar S, Bhat KG, Patil BR, Muddapur MV. Oral microbial carriage in oral squamous cell carcinoma patients at the time of diagnosis and during radiotherapy - a comparative study. Oral Oncol. 2012; 48: 881-6.
133. Swofford CA, Van Dessel N, Forbes NS. Quorum-sensing salmonella selectively trigger protein expression within tumors. Proc Natl Acad Sci U S A. 2015; 112: 3457-62.
134. Bourdeau RW, Lee-Gosselin A, Lakshmanan A, Farhadi A, Kumar SR, Nety SP, et al. Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. Nature. 2018; 553: 86-90.
135. Gwisai T, Mirkhani N, Christiansen MG, Nguyen TT, Ling V, Schuerle S. Magnetic torque-driven living microrobots for increased tumor infiltration. Sci Robot. 2022; 7: eabo0665.
136. Vincent RL, Gurbatri CR, Li F, Vardoshvili A, Coker C, Im J, et al. Probiotic-guided CAR-T cells for solid tumor targeting. Science. 2023; 382: 211-8.
137. Pfalzgraff A, Correa W, Heinbockel L, Schromm AB, Lübow C, Gisch N, et al. LPS-neutralizing peptides reduce outer membrane vesicle-induced inflammatory responses. Biochim Biophys Acta Mol Cell Biol Lipids. 2019; 1864: 1503-13.
138. Kuerban K, Gao X, Zhang H, Liu J, Dong M, Wu L, et al. Doxorubicin-loaded bacterial outer-membrane vesicles exert enhanced anti-tumor efficacy in non-small-cell lung cancer. Acta Pharm Sin B. 2020; 10: 1534-48.
139. Li Y, Zhao R, Cheng K, Zhang K, Wang Y, Zhang Y, et al. Bacterial outer membrane vesicles presenting programmed death 1 for improved cancer immunotherapy *via* immune activation and checkpoint inhibition. ACS Nano. 2020; 14: 16698-711.
140. Miyaguchi J, Shiga K, Ogawa K, Suzuki F, Katagiri K, Saito D, et al. Treatment with lactobacillus retards the tumor growth of head and neck squamous cell carcinoma cells inoculated in mice. Tohoku J Exp Med. 2018; 245: 269-75.
141. Sewell DA, Douven D, Pan ZK, Rodriguez A, Paterson Y. Regression of HPV-positive tumors treated with a new Listeria monocytogenes vaccine. Arch Otolaryngol Head Neck Surg. 2004; 130: 92-7.
142. Wang L, Vuletic I, Deng D, Crielaard W, Xie Z, Zhou K, et al. Bifidobacterium breve as a delivery vector of IL-24 gene therapy for head and neck squamous cell carcinoma in vivo. Gene Ther. 2017; 24: 699-705.
143. Xia T, Li J, Ren X, Liu C, Sun C. Research progress of phenolic compounds regulating IL-6 to exert antitumor effects. Phytother Res. 2021; 35: 6720-34.
144. Peppler WT, Townsend LK, Wright DC. Recent advances in the role of interleukin-6 in health and disease. Curr Opin Pharmacol. 2020; 52: 47-51.
145. Wang Y, Liu C, Yang L, Xu H, Sun N. 1304p interleukin-6 (IL-6) as a potential predictive marker and a desensitizer for immunotherapy response in non-small cell lung cancer. Ann Oncol. 2021; 32: S1007-8.
146. Rose-John S, Jenkins BJ, Garbers C, Moll JM, Scheller J. Targeting IL-6 trans-signalling: past, present and future prospects. Nat Rev Immunol. 2023; 23: 666-81.
147. Hamada M, Inaba H, Nishiyama K, Yoshida S, Yura Y, Matsumoto-Nakano M, et al. Potential role of the intratumoral microbiota in prognosis of head and neck cancer. Int J Mol Sci. 2023; 24: 15456.
148. Wang J, Gao B. Mechanisms and potential clinical implications of oral microbiome in oral squamous cell carcinoma. Curr Oncol. 2023; 31: 168-82.
149. Qiao H, Tan XR, Li H, Li JY, Chen XZ, Li YQ, et al. Association of intratumoral microbiota with prognosis in patients with nasopharyngeal carcinoma from 2 hospitals in China. JAMA Oncol. 2022; 8: 1301-9.
150. Inchingolo AM, Malcangi G, Piras F, Palmieri G, Settanni V, Riccaldo L, et al. Precision medicine on the effects of microbiota on head-neck diseases and biomarkers diagnosis. J Pers Med. 2023; 13: 933.
151. Elbehi AM, Anu RI, Ekine-Afolabi B, Cash E. Emerging role of immune checkpoint inhibitors and predictive biomarkers in head and neck cancers. Oral Oncol. 2020; 109: 104977.
152. Opitz P, Herbarth O. The volatilome – investigation of volatile organic metabolites (VOM) as potential tumor markers in patients with head and neck squamous cell carcinoma (HNSCC). J Otolaryngol Head Neck Surg. 2018; 47: 42.
153. Henrich B, Rumming M, Sczyrba A, Velleuer E, Dietrich R, Gerlach W, et al. Mycoplasma salivarium as a dominant coloniser of Fanconi anaemia associated oral carcinoma. PLoS One. 2014; 9: e92297.

154. Zhao H, Chu M, Huang Z, Yang X, Ran S, Hu B, et al. Variations in oral microbiota associated with oral cancer. Sci Rep. 2017; 7: 11773.
155. Torralba MG, Aleti G, Li W, Moncera KJ, Lin YH, Yu Y, et al. Oral microbial species and virulence factors associated with oral squamous cell carcinoma. Microb Ecol. 2021; 82: 1030-46.
156. Ridder GJ, Eglinger CF, Sander A, Technau-Ihling K. Deep neck abscess as first manifestation of head and neck carcinoma – implications for diagnostic management. Laryngorhinootologie. 2000; 79: 604-8.
157. Harris DM, Werkhaven J. Endogenous porphyrin fluorescence in tumors. Lasers Surg Med. 1987; 7: 467-72.
158. Zhong G, Wei W, Liao W, Wang R, Peng Y, Zhou Y, et al. Tumor microbiome in nasopharyngeal carcinoma and its association with prognosis. Front Oncol. 2022; 12: 859721.
159. Wang X. Highlights the recent important findings in cancer heterogeneity. Holist Integr Oncol. 2023; 2: 15.
160. Ahn J, Segers S, Hayes RB. Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. Carcinogenesis. 2012; 33: 1055-8.
161. Ha NH, Woo BH, Kim DJ, Ha ES, Choi JI, Kim SJ, et al. Prolonged and repetitive exposure to Porphyromonas gingivalis increases aggressiveness of oral cancer cells by promoting acquisition of cancer stem cell properties. Tumour Biol. 2015; 36: 9947-60.
162. Harrandah AM. The role of Fusobacteria in oral cancer and immune evasion. Curr Opin Oncol. 2023; 35: 125-31.
163. Xue Y, Xiao H, Guo S, Xu B, Liao Y, Wu Y, et al. Indoleamine 2,3-dioxygenase expression regulates the survival and proliferation of Fusobacterium nucleatum in THP-1-derived macrophages. Cell Death Dis. 2018; 9: 355.
164. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013; 14: 207-15.
165. Karan S, Cho MY, Lee H, Kim HM, Park HS, Han EH, et al. Hypoxia-directed and self-immolative theranostic agent: imaging and treatment of cancer and bacterial infections. J Med Chem. 2023; 66: 14175-87.

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