Review Article
The effect of oral bacterial infection on DNA damage response in host cells

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Abstract: Maintaining and transferring intact genomes from one generation to another plays a pivotal role in all living organisms. DNA damage caused by numerous endogenous and exogenous factors must be adequately repaired, as unrepaired and accumulated DNA mutations can cause severe deleterious effects, such as cell death and cancer. To prevent adverse consequences, cells have established DNA damage response mechanisms that address different forms of DNA damage, including DNA double-strand breaks, mismatches, nucleotide excision, and base excision. Among several sources of exogenous DNA damage, bacterial infections cause inflammation in the host, generating reactive oxygen species (ROS) and causing oxidative DNA damage. Recent studies have revealed the importance of the oral microbiome in inflammation and several systemic host diseases. Dysbiosis of oral bacteria can induce chronic inflammation, which enhances ROS-induced DNA damage, and improperly repaired damage can lead to carcinogenesis. This review describes the various DNA repair pathways that are affected by chronic inflammation and the discovery of the DNA damage response induced by oral bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.

Keywords: Oral bacteria, bacterial infection, DNA damage, DNA damage repair, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*

Introduction

Intact genome transfer to daughter cells is an important task in living organisms. Various exogenous and endogenous factors constantly affect genomic integrity, and the resulting genomic damage can have deleterious effects on cells, such as cancer [1]. In addition, accumulated mutations in oncogenes and tumor suppressor genes caused by DNA-damaging agents are associated with cancer development [2, 3]. Exogenous factors include ultraviolet (UV) radiation, ionizing radiation, toxic chemicals, bacterial or viral infections, and chronic inflammation, which can cause various types of DNA damage [4-6]. As an endogenous damaging factor, DNA can be damaged during replication. In addition, reactive oxygen species (ROS) and oxidative chemicals produced by the mitochondria cause DNA damage [7]. To preserve the intact genome from various factors that cause DNA damage, cells develop several DNA repair mechanisms, including DNA double-strand break repair (DSBR), mismatch repair (MMR), nucleotide excision repair (NER), and base excision repair (BER), depending on the type of DNA damage [8, 9].

Bacterial infections induce various immune responses, including inflammation, in host cells [7, 10]. Chronic infections cause the release of various inflammatory cytokines that induce chronic inflammatory responses in host cells. In addition to inflammation, bacterial infections generate ROS and reactive nitrogen species (RNS) in response to the bacteria or their metabolic products, which can lead to the accumulation of genetic mutations in host DNA [11, 12]. Furthermore, DNA damage increases the risk of cancer (**Figure 1**) [13].
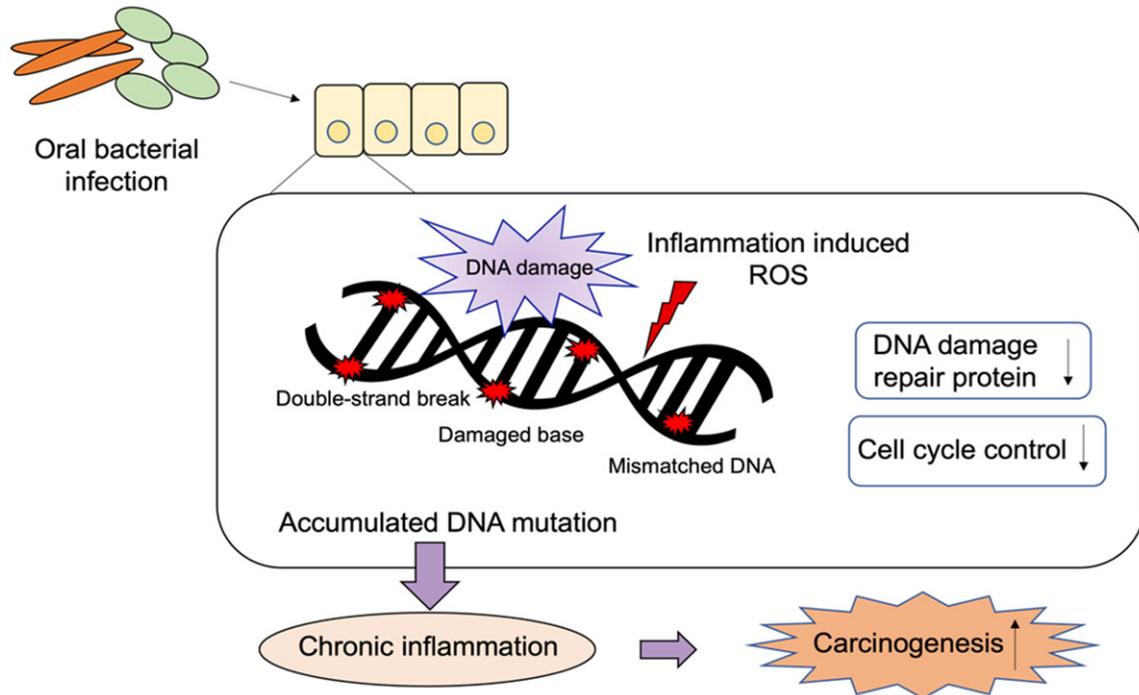
Figure 1. Proposed model of how oral bacterial infection affects the DNA damage response.

Emerging evidence has suggested that bacterial infections are risk factors for various types of cancer [3]. *Helicobacter pylori (H. pylori)* infection induces chronic inflammation and increases the risk of gastric cancer [14, 15]. Several studies have reported the detection of *Fusobacterium nucleatum (F. nucleatum)* in colorectal cancer tissues, thus explaining bacterial infection-mediated carcinogenesis [7, 16, 17]. The oral cavity contains the second-largest microbiota after the gut [18]. Approximately 6 million bacteria from >700 species reside in the oral cavity, and it is well known that bacterial dysbiosis is related to various types of cancer, diabetes, and several systemic diseases, including periodontal disease [12, 19]. Among the major periodontal bacteria, the roles of *F. nucleatum* and *Porphyromonas gingivalis (P. gingivalis)* in different types of cancers have been thoroughly studied. Oral bacterial infection is known to induce DNA damage and regulate the expression of DNA damage response genes [12, 20, 21]. **Table 1** lists the DNA damage response triggered by bacterial infections in various cell types.

This review discusses DNA damage repair pathways, including DSBR, MMR, BER, and NER,

which are affected by chronic inflammation, recent findings on the effect of the oral microbiome on the DNA damage response, and their possible link to cancer development.

DNA damage repair

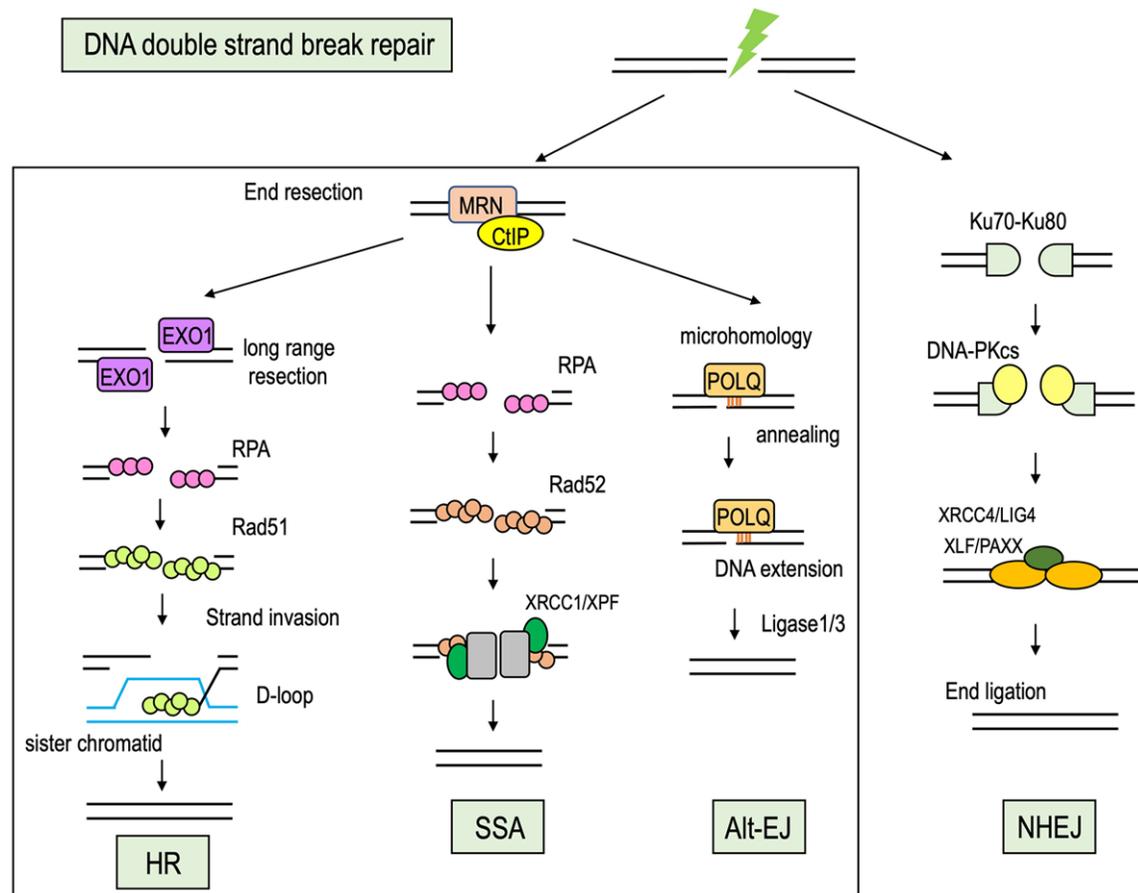*DNA double-strand break repair (DSBR)*

DSBs are considered the most dangerous type of DNA damage because both strands of DNA are affected, and unrepaired DNA commonly causes mutations, such as chromosome translocation and large insertion and deletion mutations. DSBs are induced by various stimuli, including ionizing radiation, X-rays, toxic chemicals, and ROS [8, 22]. As ROS generate DSBs, bacterial infection, and infection-mediated inflammation, which are the major sources of ROS, are highly related to DSBR. DSBs are repaired through four pathways: homologous recombination (HR), nonhomologous end joining (NHEJ), single-strand annealing (SSA), and alternative end joining (alt-EJ) (**Figure 2**) [8, 23, 24].

Among the four DSBR pathways, HR and NHEJ are well studied. The choice of repair mechanism among the four DSBRs depends on the

Table 1. Bacterial infection-mediated DNA damage response

Cell type	Bacterial infection	DNA damage response	Reference
Oral squamous cell carcinoma	*Fusobacterium nucleatum*	NHEJ (Ku70)	[40]
Head and neck squamous cell carcinoma	*Fusobacterium nucleatum*	MMR (MSH2, MSH6, MLH1)	[21]
Human trophoblast	*Porphyromonas gingivalis*	ATR, p-ATR (Ser428)	[88]
Gastric epithelial cells	*Helicobacter pylori*	MMR (MSH2, MSH6)	[61]
Gastric epithelial cells	*Helicobacter pylori*	BER (APE1)	[78, 79]
Colorectal cancer cell	*Enteropathogenic Escherichia coli*	MMR (MSH2, MLH1)	[97]

Figure 2. DNA double-strand break repair pathway.

length of the 3' single-stranded DNA (ssDNA) generated through DNA end resection, which is an early step in the repair process, and the phase of the cell cycle [8]. HR primarily occurs in proliferating cells and during the S and G2 cell cycle phases because of the use of sister chromatids as templates to replace damaged DNA, distinguishing it from other repair mechanisms [8]. Ataxia-telangiectasia mutated (ATM), and ATM- and Rad3-Related (ATR) recognize DSBs and activate BRCA1. Subsequently, the MRE11-RAD50-NBS1 (MRN) complex initiates the resection of the broken DNA ends, which generates 3' overhangs in ssDNA [25, 26]. Once short-range DNA resection is initiated by the MRN complex together with CtIP, the Ku protein, which belongs to the initial NHEJ process, cannot bind to the resected DNA; therefore, the downstream repair process is restricted to HR, alt-EJ, and SSA [8]. Long-range resection then occurs through EXO1 or Bloom syndrome protein (BLM)/Werner syndrome helicases (WRN) together with DNA replication helicase/nuclease 2 (DNA2), and the resulting

long ssDNA is coated with replication protein A (RPA) [27-29]. Next, the RPA subunits are replaced with RAD51 recombinase through BRCA2 and PALB2, and the RAD51 filament searches for a homologous sequence in the sister chromatid [29]. After the homologous sequences in the sister chromatid are recognized by RAD51, strand invasion occurs, generating a displacement loop (D-loop). Polymerase δ then synthesizes a new sequence using sister chromatid information, creating a cross-shaped structure called the Holliday junction. Several enzymes are involved in the dissolution and resolution of the Holliday junction, including the BLM helicase-topoisomerase IIIa-RMI1-RMI2 complex, GEN1 endonuclease, and MUS81-EME1 [29-32]. Because HR uses sister chromatid information as a template, the repaired DNA is less mutagenic than that repaired using Alt-EJ, SSA, or NHEJ.

The SSA anneals the end resection that occurs across tandem repeat sequences. In the SSA, ssDNA coated with RPA is replaced with RAD52, and the remaining nonhomologous 3' ssDNA tails are removed by the ERCC1/XPF complex [33]. DNA ligase I then seal the nicks. During this process, some deletions may occur in tandem repeats [34].

After short-range resection of the MRN complex, ssDNA with microhomology (3-6 homologous sequences) is repaired by alt-EJ, also referred to as DNA polymerase θ (POLθ)-mediated end-joining (TMEJ) [35]. Because POLθ does not have a 3' to 5' proofreading function, it exhibits error-prone polymerase characteristics [36]. The TMEJ process involves the base pairing of microhomologous sequences, flap trimming, gap filling, and ligation. During this process, mutations such as deletions, insertions, and base substitutions may occur. POLθ synthesizes DNA using primers containing microhomologous sequences, and the gap is ligated by ligases I and III and XRCC1 [37].

NHEJ repairs DSBs with blunt ends and short ssDNA structures at the damage site, irrespective of the cell cycle phase [8]. In NHEJ, the Ku heterodimer (Ku70/Ku80) binds to DSBs and recruits downstream proteins to process broken ends [38]. NHEJ repairs diverse types of broken DNA ends, including 5' overhangs, 3' overhangs, blunt ends, and damaged bases. Consequently, various NHEJ proteins are re-

cruited for processing these ends. The Ku heterodimer, together with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), forms a complex called DNA-dependent protein kinase (DNA-PK) [39]. *F. nucleatum* infection reduces the expression of Ku70 in oral squamous cell carcinoma (OSCC) [40]. This indicates that *F. nucleatum* infection may inhibit the initial steps of NHEJ repair. To remove damaged DNA, the recruited DNA-PKcs and the endonuclease Artemis are activated to initiate end processing. X-family DNA polymerases, such as DNA polβ, polμ, and polλ, participate in NHEJ. Polμ and polλ bind to Ku through the N-terminal BRCA1 C terminus (BRCT) domain. These polymerases fill structural gaps [41]. Then several downstrem ligase complexes are recruited, such as X-ray repair cross-complementing protein 4 (XRCC4), XRCC4-like factor (XLF), DNA ligase IV, and paralogs of XRCC4 and XLF (PAXX), with the help of aprataxin and PNK-like factor (APLE) [42-46].

DSBRs are frequently disrupted in numerous types of cancers. Mutations in either the germline or somatic cells of the *BRCA1* and *BRCA2* genes, which are important for HR, increase the susceptibility to ovarian, breast, prostate, and pancreatic cancers [47-49]. Furthermore, mutations in *RAD51* and *PALB2* have been observed in breast and ovarian cancers [50-52]. In addition to HR-related genes, NHEJ factors such as Ku70/Ku80, *DNA-PKcs*, *XRCC4*, and *LIG4* are associated with the development of various types of cancer [53].

Mismatch repair (MMR)

MMR proteins recognize DNA mismatches and insertion/deletion loops (IDLs) at DNA damage sites [54]. Germline mutations in several MMR genes accelerate the onset of various cancers, such as nonpolyposis colorectal cancer (HNPCC) [55, 56]. MMR protein expression can be altered by mutations in MMR genes and chronic inflammation, which increases the levels of inflammatory cytokines and ROS [57]. Mismatched DNA is recognized by two heterodimeric protein complexes: *Escherichia coli* MutSα (eukaryotes MSH2 and MSH6) and MutSβ (eukaryotes MSH2 and MSH3). MutSα recognizes one or two mismatches, whereas MutSβ recognizes larger mismatches and IDLs [58]. After the MutS heterodimer binds to mismatched DNA, MutL complexes (eukaryotic
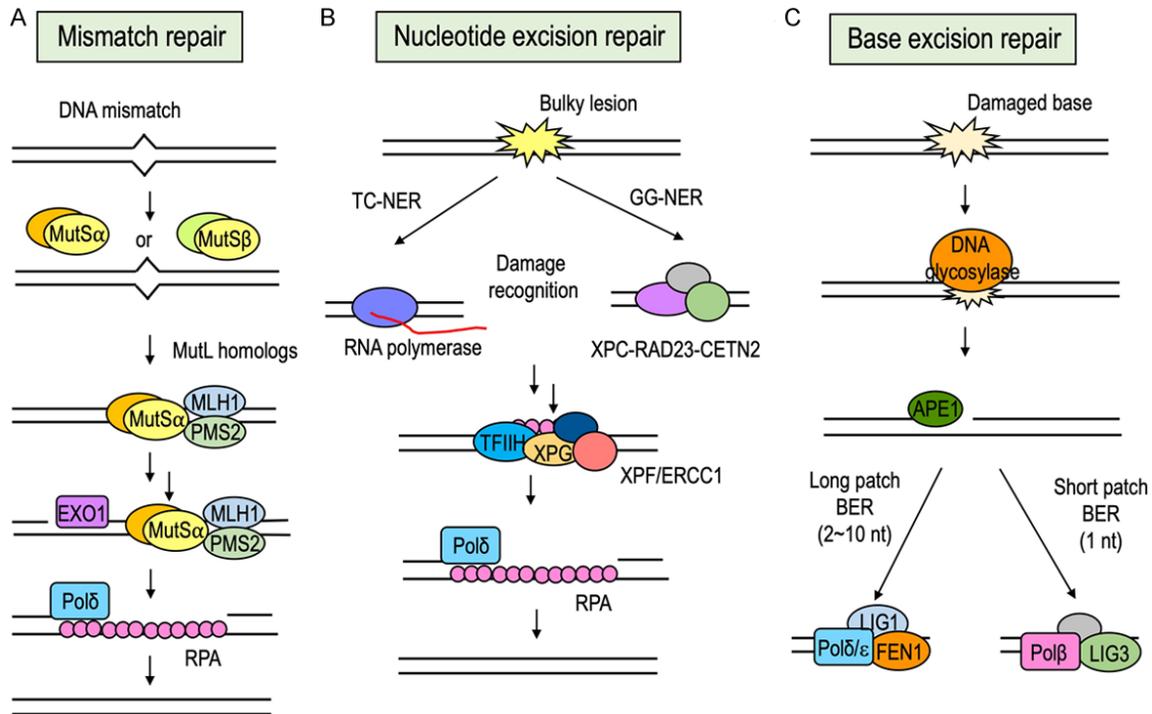
Figure 3. Model of DNA damage repair pathway. A. Mismatch repair (MMR). B. Nucleotide excision repair (NER). C. Base excision repair (BER).

MLH1-PMS1, MLH1-PMS2, or MLH1-MSH3) are recruited, and the downstream repair process is activated. To distinguish mismatched DNA from the existing normal DNA strand, *E. coli* uses DNA methylation at the A residue in the 5'-GATC-3' sequence. The existing DNA strand is methylated at A, whereas the newly synthesized DNA is not. Therefore, the MutL complex can distinguish and repair error-containing strands using the methylation code. Various methods are used to recognize DNA sequences containing mismatches in eukaryotes. In the lagging strand, short Okazaki fragments with gaps are identified as newly synthesized DNA. In the leading strand, proliferating cell nuclear antigen (PCNA) binding may indicate newly synthesized DNA. The MLH1-PMS2 interaction with the back face of PCNA in the direction of DNA synthesis can be used to discriminate newly synthesized DNA [59]. MutL creates a single-stranded nick near the lesion, EXO1 removes the nucleotides of the mismatched strand, and the polymerase δ synthesizes a new sequence (**Figure 3A**) [60]. *H. pylori* infection reduces the expression of MMR proteins such as MSH2 and MSH6 in gastric epithelial cells [61]. This suggests that *H. pylori* infection

enhances the accumulation of DNA mutations and increases the risk of carcinogenesis. In addition, *F. nucleatum* infection is associated with the MMR pathway. In head and neck squamous cell carcinoma (HNSCC), *F. nucleatum* infection reduces the expression of MSH2, MSH6, and MLH1 [21]. These findings indicate that both *H. pylori* and *F. nucleatum* infections accelerate cancer cell development by inhibiting the MMR pathway.

Nucleotide excision repair (NER)

NER recognizes and eliminates various bulky adducts and helix-distorting DNA lesions, such as pyrimidine dimers, 6-4 photoproducts, and cyclobutene pyrimidine dimers (CPD), which are generated by UV irradiation and genotoxic chemicals [62]. Defects in the NER genes have been implicated in various autosomal recessive disorders, including Cockayne syndrome, xeroderma pigmentosum, and trichothiodystrophy [63]. There are two pathways of eukaryotic NER: global genomic NER (GG-NER) and transcription-coupled NER (TC-NER), which are distinguished by the methods used to recognize DNA lesions (**Figure 3B**) [63]. In GG-NER, dam-

age-sensing proteins, including XPC-RAD23-CETN2, recognize helix distortions in both transcribed and untranscribed DNA [64, 65]. TC-NER repairs actively transcribed DNA lesions. Stalled RNA polymerase in DNA lesions serves as a recognition signal in the TC-NER. In addition to the initial recognition step, GG-NER and TC-NER use the same incision, repair, and ligation processes. After damage recognition, a dual incision is created to remove the lesion by various proteins, including transcription factor II H, XPG, and the XPF/ERCC1 complex [66]. Then, DNA polymerases δ, ε, and κ fill the gap and ligaseIII-XRCC1 seals the nick [67, 68].

Base excision repair (BER)

BER recognizes and removes various small and non-helical distorting base lesions generated by deamination, methylation, alkylation, and oxidation [69]. This damage is caused by several factors, such as spontaneous DNA decay, ROS, radiation, and toxic chemicals [70]. Because BER repairs a variety of DNA damage induced by ROS, it is considered a critical defense repair mechanism against ROS-induced mutations. DNA lesions repaired by BER typically involve small base adducts that are not sufficiently destructive to induce apoptosis. However, the accumulation of mutations in oncogenes and tumor suppressor genes can ultimately lead to carcinogenesis [22]. Abnormalities in BER genes, such as *UNG*, *TDG*, and *SMUG*, have been associated with various cancers, including colorectal, gastric, and breast cancer [71-74].

BER is initiated by DNA glycosylases, such as UNG, SMUG, MBD4, TDG, and MPG, which recognize and remove damaged bases and form apurine/apyrimidine (AP) sites (**Figure 3C**). These AP sites are cleaved by an AP endonuclease (APE1), followed by DNA synthesis and ligation, to complete the BER process [75, 76]. The cleaved AP sites are processed through two pathways: short-patch BER and long-patch BER. Short-patch BER removes and replaces only a single damaged base, whereas long-patch BER removes 2-10 nucleotides near the damaged base and synthesizes them [69]. Short-patch BER occurs in both proliferating and non-proliferating cells through APE1, DNA polymerase β, and DNA ligase I or III (LIG1/3) [77]. Long-patch BER mainly occurs in prolifer-

ating cells, and DNA polymerase δ or ε recognizes the AP sites cleaved by APE1 and synthesizes 2-10 DNA nucleotides. The synthesized 5' flap sequence is cleaved by FEN1, flap endonuclease, and DNA ligase I (LIG1) to seal the gap [77]. Several studies have shown that microbiome dysbiosis affects BER repair. *H. pylori* infection increases APE1 expression in human gastric epithelial cells [78]. Another study demonstrated that APE1 induction decreases p53-mediated apoptosis in *H. pylori*-infected gastric epithelial cells [79]. In a mouse model of defective MPG, *H. pylori* infection resulted in more severe gastric lesions [80].

Oral bacteria with DNA-damaging effects

Fusobacterium nucleatum

F. nucleatum is a gram-negative bacterium and a major oral microbe associated with the development of colon cancer and OSCC. The significant role of *F. nucleatum* in OSCC and colorectal cancer has been recognized in several studies [12, 81-83]. *F. nucleatum* causes chronic inflammation and downregulates multiple DNA repair pathways, suggesting that *F. nucleatum* is an important contributor to carcinogenesis.

Geng et al. showed that *F. nucleatum* infection increases the expression of the phosphorylated form of Histone H2A (γH2AX), a hallmark of DNA damage [40]. Together with the increased number of DSBs in the infected cells, the DNA damage repair proteins Ku70 and p53 were decreased. In addition, they showed that cell proliferation was increased by reducing p27 expression, a cell cycle regulator, following *F. nucleatum* infection [40]. These data suggest that *F. nucleatum* infection increases the proliferation of tongue squamous cell carcinoma by inducing DNA damage through the Ku70/p53 pathway.

FadA, a pathogen of *F. nucleatum*, is known to induce DNA damage and increase cell proliferation in colorectal cancer in Apc(Min/+) mice through the upregulation of chk2, which is activated through the E-cadherin/β-catenin pathway [84]. In contrast, infection of *F. nucleatum* with a FadA knockout strain did not have the same effect, indicating the importance of FadA of *F. nucleatum* in the DNA damage response. A cohort study showed that *F. nucleatum* induc-

es a DNA damage response in colorectal cancer. Okita et al. reported that colorectal cancer tissues with high microsatellite instability (MSI) features contain more *F. nucleatum* DNA than those with MSI-low features [85]. It was also shown that *F. nucleatum* infection induced γH2AX expression in several colon cancer cell lines. These data suggest that *F. nucleatum* infection leads to increased DNA damage in human colorectal cancer. Hsueh et al. reported that *F. nucleatum* impairs DNA MMR and MSI in HNSCC [21]. The presence of *F. nucleatum* was negatively correlated with DNA MMR proteins, including MSH2, MSH6, and MLH1, in HNSCC tissues. The expression of MMR genes is suppressed by the upregulation of miR-205-5p, which is activated by TLR4- and MYD88-dependent signaling pathways in *F. nucleatum*-infected cancer cell [21].

Porphyromonas gingivalis

P. gingivalis, a gram-negative anaerobe, is a well-known periodontal bacterium that is strongly associated with the pathogenesis and inflammation of periodontal disease [86]. *P. gingivalis* has been found in several types of cancers of the digestive system, including OSCC, esophageal squamous carcinoma, and pancreatic cancer [87]. Chronic infection with *P. gingivalis* and *F. nucleatum* promotes OSCC by activating IL-6-STAT3 signaling in a mouse model [83]. Gallimidi et al. also showed that *P. gingivalis* and *F. nucleatum* infections increase IL-6 expression through Toll-like receptors in human OSCC cells. In addition, infection with both pathogens induced the expression of genes related to cancer cell survival, proliferation, and aggressiveness, such as TNF-α, cyclin D1, MMP-9, and heparinase. Therefore, the proliferation of human OSCC cells is stimulated by infection [83]. Inaba et al. reported that the DNA damage response was stimulated by *P. gingivalis* infection through FAS and p53 accumulation [88]. They showed that total ATR and phospho-ATR (Ser428) levels increased after *P. gingivalis* infection and activated downstream Chk2 and p53 phosphorylation. These processes increased G1 cell cycle arrest and apoptosis in *P. gingivalis*-infected human trophoblast HTR-8 cells [88]. In addition, it has been reported that *P. gingivalis* infection activates the p38 and JNK pathways, together with the activation of downstream HSP27 and p21, which cause G1 cell cycle arrest and apoptosis

in HTR-8 cells [89]. Aquino-Martinez et al. reported a connection between *P. gingivalis* infection and osteocyte senescence, which is important for *P. gingivalis*-induced inflammation-related periodontal diseases. *P. gingivalis*-derived lipopolysaccharides (LPS) treatment increased γH2AX expression and senescence-associated secretory phenotype factors, such as *Icam1, Il6, Mmp12*, and *Mmp13*, in primary osteocyte-like bone cells [20]. The mRNA expression of p16, p21, and p53 and the percentage of SA-b-gal-positive cells also increased in *P. gingivalis*-derived LPS-treated cells. These data suggest that *P. gingivalis* LPS induces DNA damage and senescence in osteocytes.

Conclusion

Approximately 20% of all human cancers are linked to bacterial and viral infections, such as those caused by *H. pylori*, human papillomavirus (HPV), and hepatitis B and C virus [90-92]. Emerging evidence has shown that infection and infection-mediated chronic inflammation have numerous effects on cancer development. Furthermore, DNA mutations and disruption of DNA damage repair are the leading causes of cancer development due to infections.

Extensive research has been conducted on the effect of gut microbiota dysbiosis on inflammation and the development of colorectal cancers. For instance, *pks*-positive *E. coli* has been found to promote the formation of colorectal cancer [93]. Additionally, infection by *Streptococcus bovis* contributes to cancer development by enhancing the inflammatory process [94]. *Enterococcus faecalis* produces hydroxyl radicals and H_2O_2 that can cause DNA damage in the colonic epithelial cells of the host [95].

The oral microbiome is particularly well known for its association with periodontal diseases, which are closely related to the development of various cancers, including oral, pancreatic, digestive tract, prostate, breast, lung, and lymphatic cancers [12, 96]. Several studies on the DNA damage response of the oral microbiome have focused on *F. nucleatum* and *P. gingivalis*. As the importance of the effects of oral microbiomes on human health increases, further studies must focus on the effects of these oral microbiomes on cells, especially on the DNA damage response.

Disclosure of conflict of interest

None.

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