DOI:10.31557/APJCC.2026.11.6.929

REVIEW

The Dual Function of Bacteria in Cancer: Causes and Treatments

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Abstract

Introduction: Cancer continues to be a primary cause of death worldwide, with growing evidence linking the microbiome to its development and therapeutic response. The development involves the gradual accumulation of genetic and epigenetic alterations, shaped by the tumor microenvironment and host factors. This study investigates the dual role of bacteria in cancer growth and therapy, emphasizing pathogenic mechanisms, diagnostic tools, and the rising promise of bacterium-based cancer therapies. **Results:** The human microbiome, which comprises bacteria, viruses, fungi, and protozoa, plays a crucial role in regulating health and disease, including carcinogenesis, according to mounting data. Dysbiosis has been closely linked to cancer and other illnesses, even though the microbiome normally maintains a symbiotic relationship with the host. Through processes such as immune evasion, DNA damage, and chronic inflammation, some bacteria contribute to cancer development. Interestingly, *Helicobacter pylori* has been conclusively connected to stomach cancer, which is a significant advancement in our knowledge of infection-related cancers. On the other hand, some bacteria can produce anti-cancer chemicals or specifically colonize tumors, demonstrating protective or therapeutic potential. **Conclusion:** In conclusion, the bacteria and cancer as egg and chicken relation one of them leading to another, the bacteria not always leading to cancer also can be use in treatment.

Keywords: Cancer, Bacteria, , Tumor

Asian Pac J Cancer Care, **11 (6)**, 929-940 Submission Date: 07/12/2026 Acceptance Date: 09/03/2026

Introduction

Cancer is a deadly illness that has claimed many lives worldwide, with estimates indicating that by 2030, there will be about 20 million cancer-related fatalities [1]. The development of several genetic and epigenetic alterations in host cells over many years promotes oncogenic characteristics and carcinogenesis, ultimately leading to cancer. It is also critical to remember that the environment in which tumors develop provides distinct signals. These signals influence the development, survival, migration, and metastasis of cancer cells [2]. Protozoa, eukaryotic fungi, viruses, and bacteria make up the human microbiome [3]. Under normal circumstances, the human microbiome and host exist in a symbiotic relationship. The human host can create a healthy microenvironment that supports the microbiome, which aids digestion and metabolism [4]. Numerous pieces of evidence demonstrate a strong association between human disease, including cancer, infectious diseases, metabolic disorders, and digestive diseases, and the microbiome [3]. Human bodies host 3 trillion microorganisms that can regulate the intricate interactions between physiological functions and illness susceptibility [5]. The vast genetic diversity of bacteria encodes diverse metabolic and mechanistic abilities that regulate host tissue-specific and immune cell functions, as well as contributing to their own microbial niche [6]. Additionally, bacteria may contribute to health by generating compounds that combat cancer [7].

Before the idea was fully developed, they also observed early that bacteria in the intestines might convert nitrates into nitrites or potentially carcinogenic N-nitroso compounds. One significant area of ongoing research is the connection between chronic inflammation, a disordered microbiota, and oncogenesis [8]. In 1979, the potential direct association between a bacterium and

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stomach cancer was proposed when Australian pathologist John Robin Warren became intrigued by his discovery of germs on the surface of a single gastric biopsy specimen. Over the next two years, he found a link between these microbes and chronic gastritis. He had trouble locating normal control gastric biopsies from the antral region of the stomach. Ultimately, he collected twenty of these specimens, none of which contained the germs he had discovered in samples exhibiting ulceration or inflammation of the stomach. In 1983, Warren discovered that biopsies from patients with active gastritis showed Campylobacter-like bacteria in the antral mucosa of the stomach [8]. Howard William Steer and Duncan G. Colin-Jones found gram-negative bacteria in the gastric mucosa of 80% of 50 gastric ulcer patients in 1975. The bacteria persisted following therapy. Later studies found that histamine-2 receptor antagonists, such as cimetidine, repaired ulcer craters but did not improve antral gastritis, resulting in a high rate of ulcer recurrence [9]. The spiral gastric bacterium initially named "Campylobacter pylori" was physically and biochemically incompatible with the "Campylobacter" genus. Due to these differences, the bacterium was renamed "*Helicobacter pylori*" in 1989, establishing the new genus "Helicobacter". Papers from 1989 to 1992 showed that "*Helicobacter pylori*" was linked to two-thirds of stomach cancer cases globally [10]. In 2002, 75% of stomach cancers were attributable to "*H. pylori*", implying that this bacterium caused 5.5% of all cancers globally, the highest percentage attributable to any infectious agent. In 2002, nearly 20% of malignancies worldwide were caused by viruses [11].

Intestinal bacteria metabolize butyrate, which provides colonocytes with energy and inhibits inflammation and carcinogenesis by influencing immunity, gene expression, and epigenetic alterations [7]. Certain bacteria have shown a strong capacity to invade and colonize tumor sites, leading to tumor clearance and a slowdown in tumor growth. These bacteria include Lactococcus, Clostridium, Shigella, Bifidobacteria, Listeria, Vibrio, Salmonella, and Escherichia. Furthermore, certain bacteria, such as Bifidobacterium longum and Clostridium strains, can survive and proliferate in the hypoxic tumor environment, thereby eradicating it [12]. More focus has recently been placed on the part bacteria play in the development of cancer (Table 1).

The review focused on the mechanisms by which bacteria, such as *Helicobacter pylori* and *Fusobacterium nucleatum*, induce DNA damage, chronic inflammation, and immune evasion to promote carcinogenesis, and summarized methods for identifying bacteria that cause cancer. Based on the principle that bacteria are a cause of cancer and a key element in its development. This article will discuss the possibility of using bacteria as a safe therapeutic tool to reduce the spread of cancer or even treat it.

Bacteria as Carcinogens

It has been shown that long-term bacterial colonization leads to a persistent inflammatory state, which is essential for cancer development as Figure 1 [13, 14]. In addition to causing direct tissue damage, the persistent presence of bacteria creates an immunosuppressive environment that increases tumor survival and growth. [15]. Pro-inflammatory cytokines such as *TNF-α, IL-6, IL-8, IL-1β, IL-17F, IL-21, IL-22*, and *IL-23* are released over time in response to persistent infections. Additionally, they encourage the release of reactive oxygen species (ROS) and reactive nitrogen species (ROS), which damage DNA continuously and impair DNA repair mechanisms, resulting in the accumulation of mutations that induce malignant transformation, such as in colorectal mucosa [16, 17]. By causing dysregulated immune responses through the ongoing recruitment of regulatory immune cells (such as cytotoxic T cells and NK cells) and the production of immunomodulatory molecules, chronic bacterial infections with prolonged antigen exposure further contribute to immune evasion by preventing effective antitumor immunity [18]. Additionally, bacteria attract additional suppressive cell types (such as regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages) and reduce the cytotoxic function of CD8+ T cells by releasing immunomodulatory molecules and altering the local cytokine balance. This creates a tumor-permissive niche that promotes the progression of cancer [15, 16]. The ability of bacteria to physically connect with host cells and alter signaling pathways that support tumorigenesis, invasion, and metastasis is known as direct tumor interactions [13, 19]. Some bacteria release virulence factors that trigger oncogenic signaling cascades and interfere with normal cell-cell adhesion [13]. For example, secreted poisons and bacterial adhesins attach to cellular receptors. One such instance is *H. pylori*, which delivers the *E-cadherin*-interacting *CagA* protein to epithelial cells, activating intracellular signaling cascades such as the Wnt/beta-catenin pathway and upregulating oncogenes like cyclin D1 and c-Myc, which, in turn, promote cell survival and proliferation [13, 16, 17]. Comparably, *Fusobacterium*

Table 1. The more Frequent Bacterial Species to Each Cancer Type [92]

Cancer type	Bacteria species according to cancer
Colorectal cancer	Fusobacterium, Selenomonas, and Leptotrichia species are highly prevalent in cancerous tissues.
Esophageal cancer	high levels of Veillonella, Prevotella, and Streptococcus species in cancerous tissues.
Pancreatic cancer	Enterobacteriaceae, Pseudomonadaceae, Moraxellaceae, and Enterococcaceae are abundant in cancer tissues.
Head and neck cancer	Gemella, Prevotella, and Fusobacterium species are highly prevalent in cancerous tissues. Lower quantities of Rothia and Streptococcus species were discovered in cancerous tissues.
Breast cancer	Prevotella, Veillonella, and Streptococcus species are highly prevalent in cancerous tissues.
Prostatic cancer	Propionibacterium acnes is highly prevalent in cancerous tissues.

nucleatum produces adhesins that bind epithelial receptors (*E-cadherin*) and trigger intracellular signaling pathways that promote colorectal Cancer (CRC) cellular invasion and proliferation [20]. Through these direct interactions, bacteria cause normal cells to become malignant and create a tumor microenvironment that promotes cancer spread. Through a complex series of events that combine direct DNA damage with epigenetic instability, bacterial infections contribute to oncogenesis [19, 21]. Specifically, it has been shown that some bacteria induce both oxidative stress, which directly damages DNA, and epigenetic changes that interfere with normal gene regulation, such as changes in DNA methylation and histone modifications as Table 2 [21].

The mechanism of bacterial carcinogenesis

Bacteria induce cancer via several mechanisms, including 1. Chronic inflammation

Helicobacter pylori and stomach cancer are a well-established example of a bacterial association with human cancer [3], as Figure 1. *H. pylori* produces a cytotoxin-associated gene A (*CagA*), which is among the first bacterial proteins known to cause cancer in humans. In addition to interfering with cell cycle and cell death and raising the risk of gastric cancer, *CagA* may activate carcinogenic signal transduction pathways [22]. *H. pylori* is one of just 11 organisms that have been officially identified as possible causes of human malignancies [23]. The International Agency for Research on Cancer (IARC) has identified only one bacterium. The IARC's list of protumorigenic microbes hasn't been updated in over ten years, despite current developments

Table 2. Human Metagenomic Research that Links the Microbiota to Cancerous Tissues [29].

Type of tissue	Differences between species according to the infected tissue.	The mechanism of bacterial action
Breast Cancer	*Methylobacterium, Sphingomonas*	The mechanism differs among bacterial species.
Esophageal Cancer	There were more Streptococcus, Prevotella, and Veillonella species in cancerous tissues.	The oral cavity is a normal niche for bacteria. However, it can contribute to inflammation and alter tissue nature.
Colorectal Cancer	In cancer tissues, there was an increase in *Fusobacterium, Selenomonas*, and *Leptotrichia* species.	Responsible for increasing the number of tumor cells.
Head and Neck Cancer	In cancer tissues, there was an increase in *Fusobacterium, Prevotella*, and *Gemella species. Rothia* and *Streptococcus* species declined in cancerous tissues.	Causing the overexpression of PD-L1, an
Prostate cancer	*Propionibacterium* increased in cancer tissues.	Causing chronic inflammation and change hormonal environment.
Pancreatic Cancer	In cancer tissues, there was an increase in Enterobacteriaceae, Pseudomonadaceae, Moraxellaceae, and Enterococcaceae.	Causing cancer progression by the foundation LPS that activates the inflammation factors and suppresses immune responses

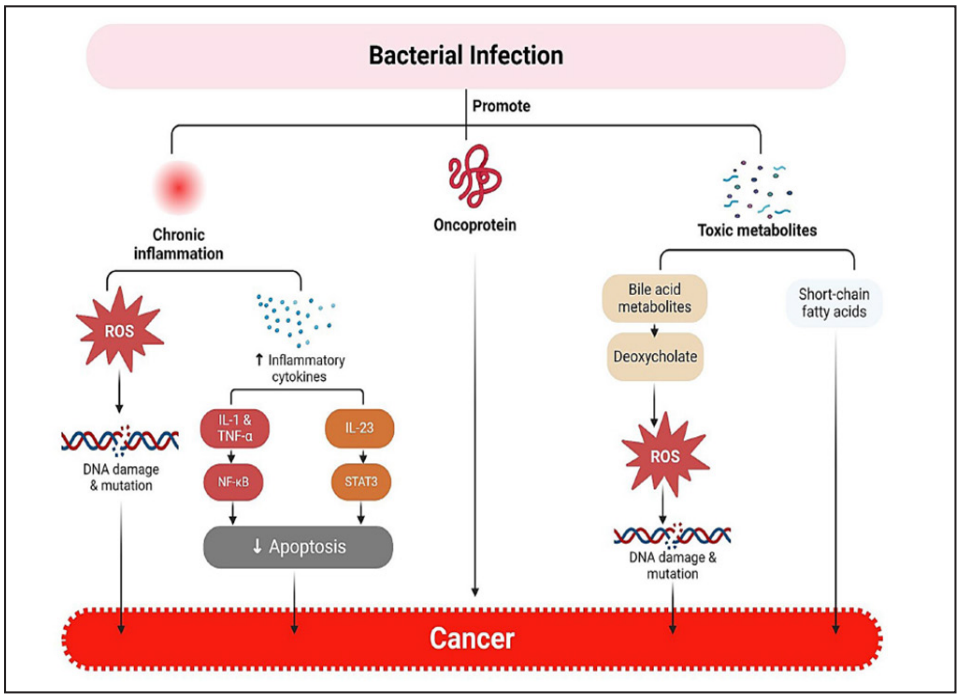


Figure 1. Bacterial Infection Triggers Many Molecular Pathways that Lead to the Development of Cancer. According to recent research, bacteria can directly influence the physiology of host cells by a number of processes, including invasion and immune evasion, inflammation, altered cell signaling, DNA damage initiation, and the production of oncoproteins that cause mutagenesis or mitogenesis [93].

in microbiological and microbiome research. According to recent research, hundreds of other bacteria besides *H. pylori* may influence or be involved in malignancies [24]. Bacteria can mechanically induce malignant tumors by negatively altering the host's physiological functions, such as persistent inflammation [25], antigen-induced lymphocyte proliferation [26], hormone-induced epithelial cell proliferation [27], and direct alterations in cell metamorphosis to influence oncogenesis [28]. It interferes with the regulation of cell development by disrupting the cellular signal through the generation of hazardous chemicals or carcinogenic metabolites [7]. About a century ago, the first known bacteria were found in human malignancies [29].

Why does H. pylori consider a carcinogen?

H. pylori, which is linked to gastric epithelial cancer and lymphoma, seems to cause cancer by several concurrent mechanisms: Helicobacter, which causes stomach MALT lymphoma and gastric adenocarcinoma, is the most well-known and extensively researched example of how microorganisms contribute to carcinogenesis [30]. It has been determined that *H. pylori* is a class 1 carcinogen. Thorough investigations of *H. pylori* have demonstrated its capacity to colonize hosts, influence the immune system, and confer important cancer characteristics, such as inflammation that promotes tumor growth, downregulation of antitumor immune responses, and increased proliferative signals [31].

Initiation stage for making H. pylori carcinogen?

Because of its many persistence mechanisms, *H. pylori* can gradually drive tumorigenesis. In particular, the presence of many adhesins, such as SabA and BabA, enables *H. pylori* to adhere to gastric epithelial cells and to penetrate the thick gastric mucus layer via its flagella [32]. *H. pylori* uses its type IV secretion system to deposit *CagA* and other virulence factors on human cells when it has formed a close relationship with the epithelium. Uncontrolled cell growth and motility result from *CagA*'s dysregulation of the host oncoprotein SHP2 once it is inside the cell. Additional virulence factors, such as VacA, create holes in host membranes, which results in increased cell turnover and cell death [33].

Inflammation and the effect of H. pylori on host immunity

In addition to directly affecting the epithelium through its toxigenic substances, *H. pylori* also manipulates the inflammatory milieu, triggering innate immune responses and creating a cauldron of chronic inflammation. *Th1* immunological activity in the gut is ultimately driven by the generation of inflammatory cytokines, such as *IL-1β*, tumor necrosis *factor-α*, and *IL-8*, as well as by reactive oxygen species (ROS) produced by neutrophils and macrophages in response to *H. pylori* infection. Furthermore, patients with infections exhibit higher NF-κB levels, which fuel the inflammatory processes that drive tumor development and spread. *H. pylori*'s catalase and superoxide dismutase proteins shield it from these ROS, as is the case with other infections, and bystander effects cause harm to host tissues as Figure 2 [34].

In vitro, infection with *H. pylori* increased DNA damage in host cells. Increased mutational burdens result from DNA damage over time [20]. Furthermore, it has been demonstrated that *H. pylori* induces programmed death-ligand 1 (PD-L1) expression on gastric epithelial cells. This, in turn, controls programmed cell death 1 (*PD-1*) on T cells, leading to a loss of immune surveillance. This is an essential tumor escape mechanism that has been a key therapeutic target, as it is linked to checkpoint-mediated tumor growth [34].

An oral cavity opportunistic commensal anaerobe, *Fusobacterium nucleatum*, is linked to several periodontal disorders. It is among the most common extraoral infections. *F. nucleatum* was connected to colorectal cancer development. (CRC) and was strongly expressed

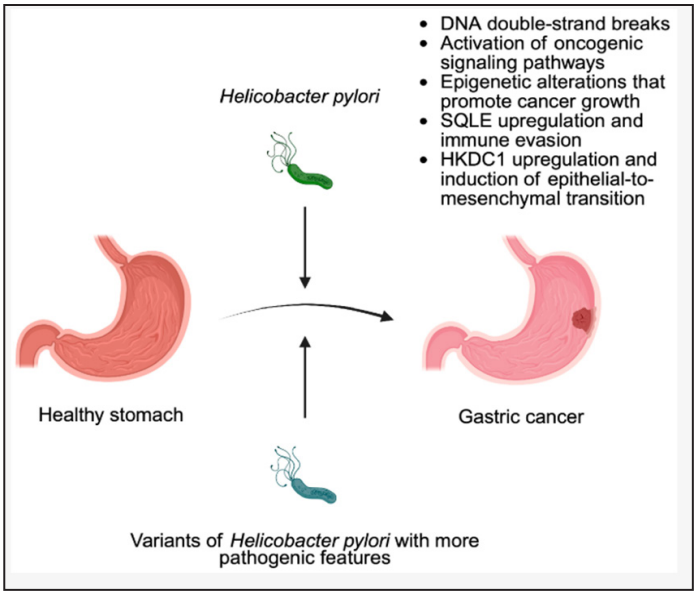


Figure 2. An Overview of the Processes that *Helicobacter pylori* Causes that Propel the Development of Stomach Cancer [94].

in CRC, suggesting that an oral pathogen linked to OSCC may also be linked to distant malignancies [3]. Further indicating its involvement in the formation of Cancer, *F. nucleatum* was also linked to liver metastases. It uses FadA to attach to and infiltrate endothelial and epithelial cells, enabling it to spread throughout the body [35]. This may be one way that *F. nucleatum* infiltrates OSCC cells since it causes pathogens to internalize and mediates the pro-inflammatory cascade by inducing nuclear factor (NF)-kB (NFkB) and interleukin-6. [3]. As demonstrated in HEK293 cells, FadA, which is generated on the bacterial surface and highly conserved in *F. nucleatum*, may specifically attach to the EC5 region of *E-cadherin* on CRC cells to penetrate them. *F. nucleatum* is also common in ESCC tissues compared to neighboring non-tumor tissues [36].

2. The role of Bacterial Genotoxic and Non-genotoxic to cause cancer

It has been demonstrated that certain microorganisms produce genotoxic compounds and other toxins that cause cancer. For instance, the polyketide calobactin, produced by enterotoxic Escherichia coli strains that carry the pks gene cluster, causes double-strand breaks in the DNA of mammalian cells [37].

When *E. coli* harboring the gene is fed to IL-10 knockout mice susceptible to colorectal cancer (CRC), tumor numbers increase. Similar inflammation scores were produced by other inflammatory microbe species, such as Enterococcus faecalis or *E. coli* without pks; however, they did not worsen carcinogenesis [38]. Consequently, this model distinguishes between the effects of a specific *E. coli*-borne genotoxin and the consequences of inflammation. There is a high correlation between microbial gene sequences and colorectal cancer (CRC), as demonstrated by the finding that only 5 of 24 healthy control individuals and 14 of 21 CRC patients harbor the toxigenic pks gene cluster in the metagenome of mucosa-associated colon tissue specimens [38]. On the other hand, B. fragilis strains that produced enterotoxins induced tumorigenesis in APCmin/+ mice via an inflammation-dependent mechanism. In particular, Stat3 activation was required for Th17 differentiation. Therefore, bacteria may either produce specific genotoxins to accelerate carcinogenesis or induce inflammation to increase the overall risk of carcinogenesis [29].

One of the key factors influencing cancer development is bacterial inflammation. Cancer is caused by increased inflammation, which increases cell division [39]. Bacterial infections are carcinogenic and tumor-stimulating, which accelerates the development of cancer. Furthermore, bacterial infections disrupt regulatory signals governing cell growth and proliferation by producing toxins and inducing genomic instability [20].

Some bacterial strains can cause cancer, but not all do. Examples include gallbladder carcinoma caused by S. typhi and gastric cancer caused by *Helicobacter pylori* [20]. Other bacterial strains have also been linked to cancer. According to [40], Citrobacter rodentium is linked to human colorectal cancer, Mycobacterium tuberculosis to lung cancer, and Campylobacter jejuni to small intestine lymphomas [41].

3. Immune Modulation

Research indicates that bacterial infections may promote tumor development and spread through several mechanisms. Bacteria, for example, can trigger a prolonged inflammatory response, keeping the immune system active. By altering the tumor microenvironment, this may also improve metastasis in addition to encouraging tumor cell proliferation [42]. For example, bacterial infections recruit immune cells into the tumor microenvironment, particularly neutrophils and macrophages, which have dual roles in tumor progression: boosting metastasis and preventing tumor growth [43].

Chronic lung infections caused by Pseudomonas aeruginosa may attract neutrophils with high levels of MHC class II, altering the pulmonary milieu and favoring BC lung metastases [44]. Endotoxins from bacterial infections may activate host inflammatory signaling pathways and enhance tumor cell migration and invasion [45, 46]. Breast duct colonization by enterotoxigenic Bacteroides fragilis leads to tumor cell proliferation and metastasis via Notch1 and β-catenin pathways [47]. Some bacteria produce immunosuppressive compounds that boost regulatory T cells and reduce antitumor immunity [48]. Campylobacter is more frequent in metastatic lesions of primary colorectal cancer in rat models of hepatic or pulmonary metastasis, and its cytolethal distending toxin promotes CRC propagation via the JAK2-STAT3-MMP9 pathway [49]. Gut microbiota affects immune responses, and Akkermansia muciniphila and Bifidobacterium longum promote antitumor immunity [50, 51]. Colon cancer is linked to dysregulated intestinal microbiota, which may lead to tumorigenesis and metastases by activating pro-inflammatory pathways such as NF-κB [52].

Additionally, gut bacteria's metabolites can be used by some tumor cells, such as melanoma, to alter their immune evasion strategies and increase their chances of survival [53]. Furthermore, bacteria can affect immune cell function by producing extracellular vesicles that carry tumor antigens or immunosuppressive substances, thereby facilitating immune evasion [54, 55]. By releasing toll-like receptor 4 (TLR4), which interacts with BC cells via extracellular vesicles, *Fusobacterium nucleatum* has been shown to promote cancer growth and metastasis [56]. On the other hand, Lactobacillus casei and Lactobacillus reuteri have been shown to reduce TLR4 expression, thereby inhibiting the growth and migration of pancreatic cancer cells. Additionally, these bacteria encourage the differentiation of M1 macrophages and prevent pancreatic cancer cells from inducing M2 macrophages [57].

Intratumoral microbiome and metastasis

The primary reason for treatment failure and cancer patient mortality is tumor metastasis. Both the intrinsic characteristics of tumor cells and external variables, such as microbes, affect the complex metastatic process. Interestingly, certain microbiota, collectively referred to

as intratumoral microbiota, have been found to infiltrate tumor tissues. Through a variety of mechanisms, such as immune response regulation, genomic instability and gene mutation induction, metabolic pathway modification, epigenetic pathway control, and disruption of cancer-related signaling pathways, intratumoral microbiota can affect tumor growth. Additionally, it has been demonstrated that intratumoral microbiota directly affects tumor metastasis by controlling mechanical stresses, cell adhesion, stem cell plasticity and stemness, and the epithelial-mesenchymal transition. By altering the tumor microenvironment and host immune system, they may indirectly impact tumor metastasis[58].

Subsequent studies have confirmed the existence of intratumoral microbiota using advanced techniques. Numerous of these microbes have been linked to tumor metastasis. Eun et al. investigated the microbial profile in individuals with lymph node (LN) metastases from oral squamous cell carcinoma (OSCC) [59]. Fusobacterium, Tannerella, Prevotella, Stomatobaculum, Bifidobacterium, Peptostreptococcaceae, Shuttleworthia, and Finegoldia were among the bacteria they identified as showing altered abundance in LN metastatic patients [59]. The most notable differences between patients with and without metastases were observed in Prevotella, Stomatobaculum, Bifidobacterium, and Fusobacterium [59]. *F. nucleatum* levels were clearly elevated in individuals with esophageal squamous cell carcinoma (ESCC), suggesting that it may be involved in metastasis [60]. The well-researched Gram-negative anaerobic bacterium *F. nucleatum* is an enriched oncogenic bacterium that promotes tumor development across a variety of cancer types [61,62]. Numerous studies have examined its function in the development and spread of colorectal cancer (CRC) [62,63]. The significance of *F. nucleatum* in CRC metastasis was demonstrated by Zhang et al. (48). Additionally, Guo et al. found that *F. nucleatum*-secreted exosomes can infect CRC cells, accelerating metastasis [64]. Additionally, *F. nucleatum* is associated with the spread of breast cancer [65]. Different bacterial communities, including Staphylococcus, Enterococcus, Streptococcus, and Lactobacillus, have been found as markers to differentiate normal tissues from lung or breast tumor tissues in some mouse tumor models [66]. This discovery suggests complex relationships between these microbial communities and tumor biology, indicating the significant influence of microbiota from one body part on tumor behavior in another[58].

Together, these investigations demonstrate the established links between tumor metastasis and the intratumoral microbiome. It is expected that the discovery of additional intratumoral microbiota will aid the development of innovative treatment strategies targeting specific microbes or microbiota-related pathways [58].

Properties of the Tumor Microenvironment (TME)

Hypoxia and Necrosis: When solid tumors, such as those in the liver and breast, grow faster than the blood supply, they frequently develop necrotic cores. For facultative and obligatory anaerobes, such as Salmonella, *E. coli*, and Bifidobacterium, this habitat serves as a particular niche [67].

1. Immune Privilege: Because tumors frequently cause immunosuppression, germs can avoid detection.

2. Leaky Vasculature: A process known as “homing” enables germs to become stuck inside tumors due to chaotic tumor blood vessels (angiogenesis) [67].

3. Tissue-Specific Tropism: Metabolic Availability: Certain nutrients, such as hydroxyproline in bone tumors or unique metabolic pathways in lung or liver malignancies, are provided by different cancers and promote the proliferation of specific bacterial taxa.

4. Anatomical Location: Mucosal organs (colon, esophagus, lung, cervix) and breast tissue exhibit unique, enduring microbiomes, indicating that the anatomical site strongly influences the microbiome composition.

5. Mechanisms of Selection and Survival: Adhesion and Biofilms: Certain bacteria, such as FadA, use adhesins to attach to cancer cells, such as *E-cadherin* in colorectal cancer.

6. Metabolic Adaptation: Bacteria that can adjust to the changed metabolism of cancer cells include *Fusobacterium nucleatum* and *E. coli*.

7. Immune Evasion: To promote tumor growth, pathogenic bacteria such as Enterotoxigenic Bacteroides fragilis (ETBF) can alter the TME [68].

The Identification Method for Intratumoral Microbiome

1- The sequencing of the 16S rRNA gene

Popular amplicon sequencing methods include 16S rRNA gene sequencing. 16S rRNA is preserved and hypervariable the nine hypervariable regions (V1–V9) of a 16S rRNA fragment alternate with conserved regions. Bacterial diversity analysis and annotation are best performed in the V4 region due to its specificity and the amount of data available [69]. Interestingly, Nejman et al. amplified and sequenced five sections of the 16S rRNA gene using multiplex PCR [70].

Compared with conventional V4 or V3–V4 amplification, this technique demonstrated better coverage and resolution in bacterial species detection. PCR, purified, and sequenced typically amplify 16S rRNA. The sequences are then grouped into operational taxonomic units (OTUs) according to similarity, and each OTU’s representative sequences are used to compare them with database entries already in existence[71].

2- WMS

WMS uses non-targeted sequencing to extract the DNA of all microorganisms in ambient samples and is the core of metagenomics research [72]. WMS does not require microbial isolation, culture, or purification, and metagenomics research measures the environment’s DNA content rather than that of individual microorganisms or cells [73]. WMS yields more comprehensive findings and is more accurate, costly, and sophisticated than 16S rRNA gene sequencing.

3- IHC

IHC relies on antigen-antibody binding. This procedure usually requires fixing the sample and coincubating it with a blocking solution. After color development and primary and secondary antibody coincubation, the sample is microscoped. IHC provides location-specific quantitative and qualitative data. Researchers use antibodies against bacterial lipopolysaccharide (LPS) and lipoteichoic acid to detect Gram-positive and Gram-negative bacteria in samples [74].

4- FISH

Using fluorescently labeled probes targeting the bacterial 16S rRNA gene, FISH can detect bacterial DNA or RNA in tissues. The complementary base pairing principle of nucleic acids is exploited by FISH. The target DNA or RNA is hybridized to a fluorescein-labeled nucleic acid probe. Fusobacterium enrichment in colorectal Cancer (CRC) was investigated by Kostic et al. using FISH [75].

5- D-alanine-based techniques

Researchers often mark living bacteria with fluorescent D-alanine. Alanine arginase helps most bacteria produce D-alanine and peptidoglycan, essential cell wall components [76]. Fresh tumor tissue may be cultivated in vitro, and fluorescently tagged D-alanine may be added to demonstrate that tumors contain metabolically active bacteria.

How do microorganisms affect cancer cells?

Rubinstein et al. [72] showed that FadA may facilitate *F. nucleatum* adhesion and cell invasion by binding to CRC cell *E-cadherin*. FadA promotes CRC cell proliferation by activating β-catenin signaling, leading to increased transcription of transcription factors, oncogenes, Wnt genes, and inflammatory genes [72]. FadA directly regulates Wnt and oncogene activation in CRC cells, although clathrin-mediated FadA internalization also activates inflammatory genes [20]. In mice, *F. nucleatum*-infected CRC cells proliferate, invade, and form xenograft tumors [77].

Another study found that *F. nucleatum* may activate NFkB by signaling through this enhanced miR21 synthesis and lowered RAS GTPase RASA1 expression. *F. nucleatum* enhanced cancer cell proliferation, migration, and invasion and decreased ESCC survival. It may infect ESCC cells and stimulate the tumor-growing NFkB pathway via NOD1/RIPK2. Even after eliminating cancer cell bacteria, lung metastasis was reduced in a mouse model of spontaneous breast cancers, but tumor weight remained the same. This suggests that intracellular bacteria affect breast cancer metastasis rather than growth [68]. The RhoA-ROCK signaling pathway may be activated when bacteria penetrate breast cancer cells, thereby protecting them against blood pressure and metastasis. Consequently, bacteria-carrying breast cancer cells are better able to spread to distant regions [68].

The Treatment of Cancer by Bacteria

It has been investigated how well eukaryotic and prokaryotic expression systems can deliver therapeutic payloads, such as immunomodulators, prodrug invertase, cytotoxic drugs, tumor matrix-targeting compounds, and siRNA as Figure 3 [3]. The most used techniques are the bacterial expression systems. They rely on bacterial prokaryotic plasmids to encode specific genes [78]. Transduction of host cells, such as immune or tumor cells, with eukaryotic plasmids encoding cDNAs of target genes is a component of eukaryotic expression systems [79].

1-Bacteria as therapeutic target (antibiotics, antimicrobial peptides)

Recent advances in bioengineering and microbiology have rekindled interest in developing cancer treatments based on bacteria. By addressing several concerns, including systemic toxicity, limited penetration into solid tumor tissues, and the emergence of drug resistance, these

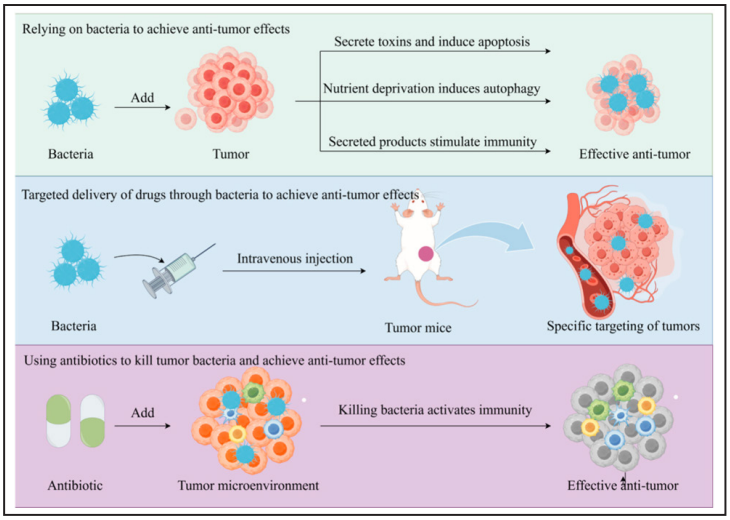


Figure 3. Three Modes of Action of Tumor Bacteria in Tumor Control are Depicted in the Introductory Diagram (mode 1 is the anti-tumor effect achieved by relying on the bacteria's own components or products; mode 2 is the tumor-targeting effect of some specific bacteria; and mode 3 is the anti-tumor effect achieved by relying on the activation of autoimmunity after the tumor bacteria are destroyed by antibiotics) [95].

medicines offer promising alternatives to conventional cancer treatments. Naturally, because they can multiply preferentially in tumor microenvironments (TMEs), which are often hypoxic and nutrient-rich, tumor-targeting bacteria are the subject of much research. This selective proliferation can inhibit tumor growth, thereby enhancing treatment effectiveness. Their promise has been further extended by advances in bacterial bioengineering, which have enabled the creation of strains with improved safety profiles and therapeutic potential. The creation of anti-cancer proteins, the targeted delivery of therapeutic chemicals, and the control of immune responses have all been enabled by genetic alterations, opening new possibilities for customizing bacterial therapeutics to target specific tumor types. One important tactic that has increased the promise of modified bacterial strains as potent cancer therapy tools is the regulated production of therapeutic proteins. Furthermore, bacteria are being investigated as natural drug-delivery vehicles, offering the benefit of localized therapy that reduces systemic adverse effects [80].

In CRC tissues, *F. nucleatum* is highly expressed. In *F. nucleatum*-positive colorectal cancer, metronidazole reduces *F. nucleatum*, cancer cell proliferation, and cancer growth. Therefore, microorganisms may be targets for cancer treatment. BCG was first used in bladder perfusion treatment in the 1970s to prevent and cure non-muscle-invasive bladder cancer (NMIBC). BCG administered intravenously can dramatically lower the risk of cancer recurrence in NMIBC patients [81].

2-Modulating microbiome to enhance therapy

A. Chemotherapy

Bacteria can control the effects of chemotherapy even though they are therapeutic targets in cancer treatment. Escherichia coli may alter the effects of chemotherapy with gemcitabine and CB1954 by triggering cytotoxicity and drug resistance, respectively [82]. Gammaproteobacteria, a cell taxon found in pancreatic cancers, can express a cytidine deaminase isoform that renders gemcitabine inactive, reducing its tumor concentration and causing treatment resistance [82]. When comparing CRC patients who relapsed after chemotherapy with those who did not, *F. nucleatum* levels were higher in the relapsed group [83]. *F. nucleatum* can biologically regulate chemotherapy resistance in colorectal cancer by coordinating the autophagy networks of TLR4-MyD88, miR18a, and miR4802, and ULK1/ATG7 [83]. This aids in formulating a crucial clinical question: are individuals with colorectal Cancer (CRC) who express high levels of *F. nucleatum* suitable candidates for traditional chemotherapy regimens that include capecitabine and oxaliplatin? We propose that anti-*F. nucleatum* medication or autophagy inhibitors should be included in chemotherapy regimens for patients with high *F. nucleatum* expression. Irinotecan's significant intestinal toxicity is a major side effect of cancer treatment. Hepatic UDP-glucuronyltransferase then converts SN38, the active metabolite of irinotecan, to SN38-G. Irinotecan's inactive metabolite, SN38-G, is eliminated from the body after passing through the small intestine. The intestinal mucosa can be directly damaged by β-glucuronidases produced by intestinal bacteria, which convert SN38-G to SN38. The composition of the intestinal microbiome may be affected by chemotherapy and the resulting mucosal damage. Additionally, by boosting Gram-negative anaerobic bacteria, 5-fluorouracil (5-FU) can control the oral and fecal microbiota of lab animals [84].

B) Radiotherapy

Radiotherapy was linked to gut flora ecological imbalance. An increase in Bacteroides and Enterococcus and a reduction in symbiotic bifidobacteria, fecal bacilli, and Clostridium characterize the radiation therapy-induced imbalance in the gut microbiota [85]. In vitro studies show that oral vancomycin reduces Gram-positive bacteria in the colon and improves the efficacy of radiation therapy for cervical, lung, and melanoma cancers. This effect may be due to mechanisms that are dependent on interferon-g and CD8 T cells [86]. Conversely, antibiotics caused intestinal yeast to grow and Clostridium to decrease in the mouse model of radiation therapy for breast cancer, which encouraged a tumor response mediated by macrophages [87]. Additionally, intestinal bacteria may cross the compromised intestinal barrier to reduce radiation toxicity, thereby exacerbating tissue damage and an uncontrollable intestinal immune response [88]. Bacteria have a particular advantage as carriers for cancer therapy compared to most conventional drug-delivery methods. They target and aggregate in cancer tissues despite physical barriers, prompting an immunological response [89]. Recently identified bacteria in cancer cells may support the idea that bacterial transporters might precisely deliver medications [72]. Genetically and chemically changing microorganisms to generate and transport anti-cancer drugs to cancer tissues may improve cancer treatment safety and reduce healthy cell cytotoxicity [3].

3- Bacteria as delivery systems (safety, specificity, regulation)

Additionally, microorganisms are used in gene therapy as vectors to achieve therapeutic potential. When a vector reaches its target, it acts as a "drug". It initiates the production of a new protein that corrects a malfunctioning cellular function. Because the vectors contain antineoplastic drugs, such as chemotherapeutics or enzymes that aid in killing cancer cells, they do not have any negative consequences. As a result, they are employed to deliver a chemotherapeutic medication straight to the tumor and to use their byproducts, including toxins [90]. Drug loading within bacteria can be achieved by either directly loading pharmaceuticals into bacterial cells or by using gene-editing technologies to load drugs as nucleic acids. Drugs or nanoparticles can enter bacteria via passive diffusion and active transport mechanisms for the uptake of foreign molecules.

The bacteria produce the medication via specific reactions when they reach the tumor, killing cancer cells. Because of their tumor-targeting, oncolytic, and immunomodulatory properties, bacteria represent a unique

delivery technology with significant promise in cancer therapy [91].

In conclusions, the complex relationships between bacteria and cancer present both major obstacles and fascinating possibilities. A clearer understanding of these connections may enable the development of innovative methods for cancer diagnosis, therapy, and prevention. We may identify novel treatment targets and pathways by integrating microbiome research into cancer biology, thereby improving patient outcomes. To fully harness bacteria's potential in the fight against cancer and lay the foundation for future advances in oncology, more research in this area is essential.

Limitation

However, further research is still needed to determine how other bacteria penetrate cancer cells and how illnesses impact the growth of tumors. Numerous questions remain to be investigated. How did microorganisms get involved in cancer? Does the development of cancer alter the makeup of the bacteria? Are microorganisms able to "travel" to metastatic sites with cancer cells? Are any microorganisms found at metastatic locations that are more pertinent to the new site? To improve cancer treatment, we should focus on investigating and resolving the issues.

Conflict of Interest

The authors conducted the study without any financial or commercial ties that could potentially create a conflict of interest.

Author Contributions

All authors have the same role in the review's reading and writing.

Originality Declaration for Figures

All figures included in this manuscript are original and have been created by the authors specifically for the purposes of this study. No previously published or copyrighted images have been used. The authors confirm that all graphical elements, illustrations, and visual materials were generated from the data obtained in the course of this research or designed uniquely for this manuscript.

Acknowledgments

The authors would like to express their sincere appreciation to Biology department staff at science college of Basrah University.

Declaration on generative AI and AI-assisted technologies in the writing process:

No declaration on generative AI.

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