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Review Article

Intratumoral microbiota: understanding microbial environment of tumors

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ABSTRACT

Microorganisms within the human body are integral to numerous health and disease mechanisms. Studies demonstrate that several bacterial species are associated with multiple cancer forms. In addition to its role in cancer start and progression, the microbiome shows potential as a biomarker for cancer diagnosis, risk assessment, and prognosis determination. Intratumoral microorganisms significantly influence tumour biology by governing tumour initiation and progression, as well as altering responses to chemotherapy, radiation, and immunotherapy. A comprehensive understanding of the intratumoral microbiome's function in cancer necessitates additional research into its impacts and underlying mechanisms. This study examines the importance of intratumoral bacteria in cancer start, development, and metastasis, their influence on treatment results, and the methodologies utilized for profiling the intratumoral microbiome.

Introduction

The human body comprises between 21 × 10^{13} eukaryotic cells in women and 30 × 10^{13} in men, in addition to various microbes involved in symbiosis, commensalism, and parasitism. These interactions influence coevolution and fluctuate with external stimuli and host conditions [1]. The microbiota comprises bacteria found in the skin, oral cavity, respiratory, gastrointestinal, urinary, and reproductive systems, whereas the microbiome denotes its aggregate genetic material. Recent studies have discovered bacteria in organs previously considered sterile, including the kidney, prostate, lung, liver, pancreas, and breast.

The gut contains around 100 trillion bacterial cells, with microbiota-associated cells constituting approximately 90% of the human body [2]. These bacteria are essential to host physiology, especially in the immunological, metabolic, structural, and neurological systems. Furthermore, dysbiosis of microbiota is associated with numerous diseases, including neurological disorders (Parkinson's, Alzheimer's), cardiovascular conditions (hypertension, atherosclerosis), immune-related issues (allergies, autoimmune), metabolic disorders (obesity, diabetes), and cancer. The association between the microbiota and cancer has a lengthy history. In 1886, Doyen isolated Micrococcus neoformans from several tumours and confirmed its tumorigenicity in animal models [3]. Subsequently, in 1911, Rouse showed that avian sarcoma leucosis could be conveyed via a filter of tumor-free cell extracts, leading to the development of cancer. Consequently, he was the inaugural individual to identify viruses as a contributing factor to cancer. Marshall and Warren's 1983 study presented the initial

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evidence of the involvement of bacteria in cancerogenesis. By isolating H. pylori from biopsy samples of the intact antral mucosa and observing its presence in nearly all patients with active chronic gastritis, duodenal ulcers, or gastric ulcers, they demonstrated the bacterium's involvement in these diseases and gastric cancer [4]. Research indicates that many bacterial species are implicated in multiple cancer types, including esophageal, breast, head and neck, prostate, and pancreatic cancers. Microorganisms are expected to account for 15%–20% of cancer cases, the second biggest cause of death globally [5]. The microbiome's influence on cancer progression beyond direct infections, profoundly affecting the tumour microenvironment (TME). The tumour microenvironment (TME) is a multifaceted network consisting of fibroblasts, immune cells, vascular structures, adipocytes, pericytes, and extracellular matrix components that collectively affect tumour behaviour via biochemical and mechanical interactions. Increasing research highlights the microbiota as a significant external component influencing tumour growth through its metabolic byproducts, immunological interactions, and signalling effects inside the tumour microenvironment (TME). Microbial metabolites serve as bioactive chemicals that modulate critical processes like inflammation, angiogenesis, immunological response, and epithelial-mesenchymal transition (EMT). Secondary bile acids, such as deoxycholic acid (DCA), induce a pro-tumorigenic transformation in cancer-associated fibroblasts (CAFs) by activating various metabolic and signalling pathways. Lithocholic acid (LCA) similarly affects immune regulation by altering the differentiation of T-helper 17 (Th17) and regulatory T cells (Treg), hence facilitating tumour immune evasion. Bacterial lipopolysaccharides (LPS) can directly influence epithelial cells, facilitating epithelial-mesenchymal transition (EMT) and activating vascular endothelial growth factor (VEGF) signalling, hence enhancing angiogenesis and increasing metastatic potential [6]. In addition to metabolic impacts, certain bacterial species facilitate carcinogenesis by disrupting host cell signalling pathways. Bacteria secrete toxins that disturb cellular homeostasis, resulting in genetic instability and inflammation that promotes tumour proliferation. The complex interplay between microbial components and host mechanisms contributes to tumour genesis and progression. Conversely, microorganisms have been explored as potential therapies for cancer. More than a century ago, Dr. William B. Coley noted spontaneous tumour reduction in patients with streptococcal infections and developed "Coley's toxins," a preparation of heat-killed bacteria that had potential in cancer treatment. Based on these findings, Bacillus Calmette-Guerin (BCG) is the sole FDA-approved bacterial agent for the treatment of superficial, non-muscle invasive bladder cancer (NMIBC) [7]. Bacteriophages have lately attracted interest for their capacity to affect tumour growth, underscoring the microbiome's dual function in cancer progression and therapy. The microbiome influences systemic immune responses from remote body locations, hence affecting tumour behaviour. The capacity of microbial metabolites to induce either tumor-promoting or tumor-suppressing effects—contingent upon their context and concentration—further exemplifies the complex dynamics of microbiome-tumor microenvironment interactions. In addition to its involvement in carcinogenesis and cancer progression, the microbiome has surfaced as a potential biomarker for cancer diagnosis, risk evaluation, and prognosis [8]. Considering its significance in the diagnosis, progression, and treatment of several malignancies, more investigation into the features of intratumoral microbiota and their impact on tumour growth is essential. Furthermore, enhancing methodologies for examining tumor-associated microorganisms will yield greater understanding of their therapeutic potential. Acquiring a comprehensive understanding of these systemic impacts offers critical insights into prospective therapeutic options that utilise microbiome manipulation to affect tumour microenvironment dynamics and eventually regulate tumour growth [9].

The genesis of Intratumoral microbiota

Notwithstanding the significant relevance of intratumoral microorganisms, their origin remains unidentified. Recent research indicates three potential origins for the intratumoral microbiota. The initial method is via the mucosal barrier. Mucosa-colonizing microorganisms may infiltrate the tumour via compromised mucosa. Consequently, they evolve into intratumoral microbiota capable of executing intricate activities. Intratumoral microbiota is commonly seen in malignancies originating from mucosal tissues, such as colorectal, pancreatic, cervical, and lung cancers [10]. While human mucosal organs include diverse microbiomes, the dominant belief that intratumoral microbiota solely derives from the mucosal site across the mucosal barrier does not account for the complete spectrum of intratumoral microbial communities. Certain identified intratumoral bacteria are rarely seen in the mucosal organs linked to their corresponding tumours, while others are commonly observed in non-mucosal tumours. This suggests the potential for alternative sources of intratumoral microorganisms [11]. The alternative pathway involves the surrounding normal tissue as a 2020 study indicates that the bacterial composition of normal adjacent tissues closely resembles that of tumour tissues [12]. Related

investigations proliferated, revealing the presence of germs in tissues previously considered sterile. The bacteria present in normal adjacent tissues (NATs) may have originated from tumour microenvironments (TMEs), perhaps elucidating this resemblance. Consequently, it remains uncertain if NATs are a source of intratumor bacteria, necessitating further investigation.

The circulatory system, comprising blood, lymphatic fluid, and the internal channels of the alimentary tract, constitutes the final and third source of intratumoral bacteria. This method allows bacteria from the oral cavity, intestines, and other non-sterile locations to be transferred to the tumour site through the circulatory system and infiltrate the tumour via compromised blood arteries. Fusobacterium nucleatum is a significant constituent of the human oral microbiome; these bacteria utilise a haematogenous pathway to access colon cancer [13]. Microbial organisms in the circulatory system may directly infiltrate tumour tissues. Microbes entering the bloodstream from diverse sites may be conveyed to the tumour microenvironment via debris produced by necrotic cells in tumours or through the chemotactic gradient. Moreover, erythrocytes have been proposed as potential carriers of bacteria to tumours [14]. Intratumoral bacteria generally arise from several sources and are strongly associated with the oral and intestinal microbiota. Moreover, studies indicate that bacteria infiltrate tumours via many mechanisms.

Variability and Distinctions of Intratumoral Microbiota

Given the potential diversity of microbial origins in tumours, the microbial composition differs among various cancer types. Investigations on the microbiomes of seven cancer types—lung, breast, pancreatic, ovarian, brain, bone, and melanoma—have revealed that each tumour exhibits a unique microbiome makeup [12]. Recent studies identified DNA and fungal cells in various prevalent human cancers. The compositions of microbiome communities differed according to the kind of cancer. Bacteria predominated in the microbiological communities of the tumour, but fungi were minimal. Moreover, same community compositions were identified in adjacent normal tissues [12]. Specific bacteria have been identified in numerous malignancies. The frequency, however, fluctuates based on the cancer kind. Cancerous tissue exhibits reduced microbial diversity compared to normal tissue, creating a distinct environment that promotes certain bacterial species. The majority of these bacteria are commensal species that predominantly inhabit intracellular compartments. The existence of diverse bacterial communities in cancer tissues indicates possible multifunctional interactions with cancer cells, affecting tumour growth and microenvironment dynamics [15]. The heterogeneity of the intratumoral microbiota presents challenges that can substantially hinder research endeavours. Tumour dynamics are affected by various factors, including cellular proliferation, genetics, microbial interactions, and metagenomics. The intratumoral microbiota influences the tumour microenvironment by altering immune responses, inflammation, and metabolic processes. Furthermore, microbial makeup differs throughout various cancer stages, hence complicating the investigation of tumor-resident microbiota [16]. For example, in oral squamous cell carcinoma (OSCC) and colorectal cancer (CRC), these microbial alterations significantly affect tumour aggressiveness and immune responses. In OSCC, Capnocytophaga, Fusobacterium, and Treponema proliferate in advanced stages, whereas Streptococcus and Rothia are more prevalent in precancerous stages. Advanced-stage cancer (T4) is characterised by diminished bacterial diversity, marked by a decline in Streptococcus, the absence of Rothia, and the predominance of Capnocytophaga [17]. These alterations influence immunological activation, promoting microorganisms that inhibit immune responses. In colorectal cancer (CRC), microbial composition changes with disease advancement, with Fusobacterium nucleatum being markedly enriched in advanced stages (III/IV), facilitating immune evasion and tumour progression. Early-stage colorectal cancer (CRC) demonstrates increased microbial diversity, characterised by a higher prevalence of Bacteroides and Prevotella, while late-stage CRC exhibits diminished variety. Bifidobacteria is significantly linked to signet ring cell carcinoma, a more aggressive subtype of colorectal cancer, whereas virulence-associated bacterial genes increase in prevalence in advanced colorectal cancer, perhaps facilitating metastasis. The microbial alterations in OSCC and CRC highlight the significant influence of intratumoral bacteria on cancer advancement and immunological regulation [18]. These data highlight the intrinsic variability of intratumoral microbiota among patients and cancer stages, complicating attempts to establish standardised microbial markers for disease progression. In light of these challenges, additional research utilising tumour tissue biopsy specimens is essential to accurately identify tumor-invading bacteria and clarify their interactions with the intratumoral immune system. A thorough comprehension of the dynamic alterations in microbial communities across various cancer types and stages is crucial for formulating tailored treatment and diagnostic approaches.

The intratumoral microbiota present in the tumour microenvironment displays unique composition and

distribution characteristics resulting from selective forces and microbial adaptation. The distribution of intratumoral microbiota differs across various tumour areas, as noted in CRC and adenoma [19]. Specific bacterial clusters are associated with particular tumour cell characteristics, including reduced p53 expression, underscoring the diversity of micro-niches inside the tumour microenvironment (TME). Tumor-associated microbial communities frequently exhibit substantial differences from those in neighbouring healthy tissue at the phylum, order, or genus level, indicating that tumours impose selective forces that alter microbial composition distinctively from normal tissue. Intratumoral microbiota may derive from the local microbiome of tumor-bearing tissues or migrate from distant locations, such as the gut or oral cavity, through compromised mucosal barriers or the circulatory system. The wider tumour microenvironment is affected by these translocating microorganisms, which may subsequently colonise the tumour. Intratumoral bacteria directly engage with immune cells, stromal cells, and the extracellular matrix (ECM), affecting microbial composition and tumour advancement. These interactions foster a dynamic tumour environment.

Furthermore, tumours exhibit distinct circumstances like hypoxia, acidity, nutrition competition, and immunological activity, which preferentially promote the survival of particular microbial species. Anaerobic bacteria flourish in hypoxic areas, but acidophilic organisms such as Lactobacillus acclimatise to the acidic tumour milieu [19]. Comprehending these variances is crucial for elucidating the function of microbial communities in tumour advancement and treatment response.

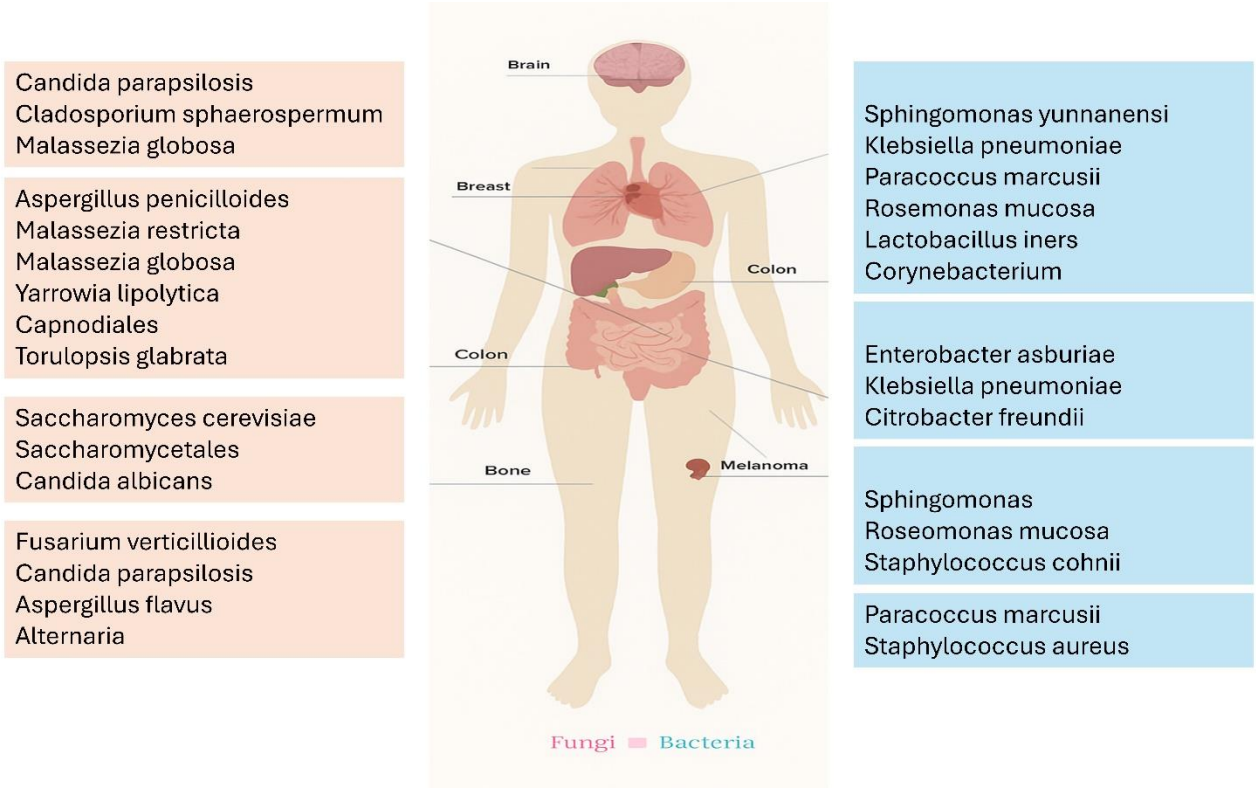

Figure 1: The diversity of intratumoral microbiota.

The effects of intratumoral microbiome on cancer progression

The microbiome's potential role in cancer genesis and progression remains uncertain, however it may influence essential tumor-promoting processes in both malignant and non-malignant cells. Understanding these mechanisms can improve the efficacy of cancer diagnosis and treatment. The following section will outline the essential roles of intratumoral bacteria in promoting carcinogenesis and tumour formation.

Stimulate DNA damage

Some bacteria possess mechanisms that can damage DNA, potentially resulting in mutations and ultimately leading to cancer. Excessive DNA damage that exceeds the repair capabilities of the host cell may lead to apoptosis, cellular demise, or neoplastic changes. Consequently, DNA damage is a critical element in carcinogenesis [20]. Carcinogenic bacteria have evolved many ways to inflict damage on the host's DNA, including DNA-damaging chemicals, proteins, and metabolites. Such products possess the capacity to

directly or indirectly engage with the host's DNA, resulting in alterations. The metabolites comprise cytolethal distending toxin (CDT), colibactin, and Bacteroides fragilis toxin (BFT), which cause DNA damage and provoke mutations. Gram-negative bacteria from the gamma and epsilon classes of the phylum Proteobacteria produce CDT. CDT is an exotoxin distinguished by unusual characteristics that allow it to be categorised as both a cyclomodulin and a genotoxin. CDT is a heteromultimeric protein composed of three subunits: CdtA, CdtB, and CdtC. Each subunit fulfils a specific purpose within the overall operation of CDT. CdtB has sequence homology, structural similarity, and functional parallels with DNase I, resulting in DNA damage [21]. CdtB demonstrates its function in a dose-dependent fashion. The effect of CdtB activity is contingent upon the concentration or dosage of this protein. Consequently, as the dosage escalates, the impact transitions from causing single-stranded DNA breaks to double-stranded breaks. Aberrant reactions to DNA damage can lead to genomic instability and precipitate cancer. Some E. coli strains possess genomic islands referred to as "pks islands," which are biosynthetic gene clusters. This gene cluster encodes a combination of non-ribosomal peptide synthase (NRPS), polyketide synthase (PKS), and colibactin. Colibactin can cause DNA double-strand breaks (DSBs), hence elevating genomic instability and mutation frequencies. Colibactin demonstrates genotoxic effects on the DNA of both the infected host cells and the bacteria that produce it. Bacteria have developed multiple ways to safeguard DNA from colibactin's effects, including efflux mechanisms and the ClbS hydrolase enzyme. BFT has been associated with diarrhoea, inflammatory bowel disease, and colon cancer in multiple studies. In a murine model of colon cancer, pks + E. coli was found to exert a synergistic impact with enterotoxigenic Bacteroides fragilis (ETBF), resulting in DNA damage to colon epithelial cells and elevating the risk of cancer development [22].

Bacterial metabolites may exert an indirect genotoxic effect by generating free radicals and reactive oxygen species (ROS). For instance, Enterococcus faecalis, a commensal bacterium in the human gastrointestinal tract, can produce significant quantities of extracellular superoxide (O_2) and reactive oxygen species, including H_2O_2 and hydroxyl radicals, via the autoxidation of membrane-bound dimethyl menaquinone. These oxidants may induce chromosomal instability (CIN) and facilitate the development of colorectal cancer and adenomatous polyps.

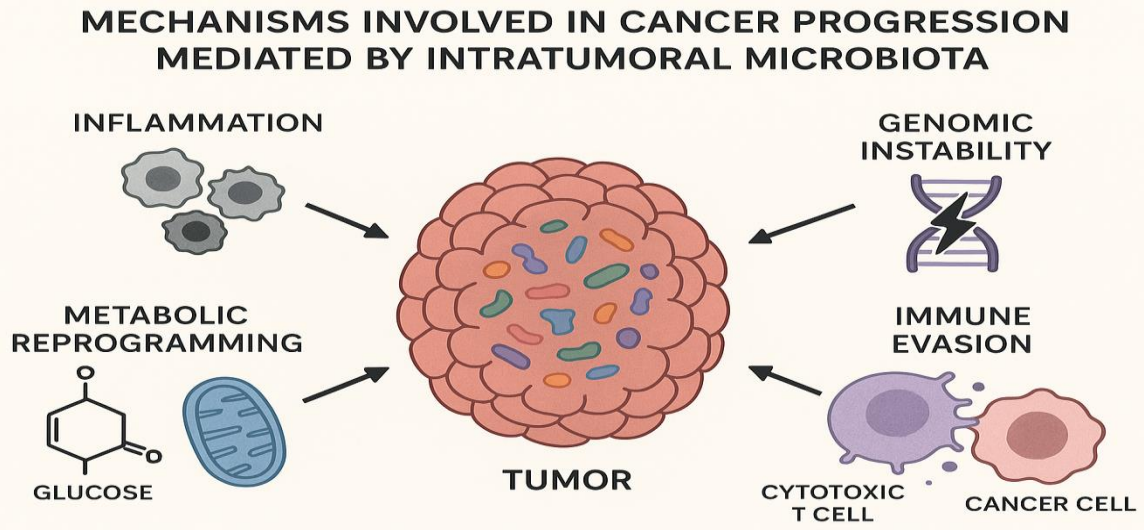

Figure 1: Mechanisms involved in cancer progression mediated by intratumoral microbiota.

Epigenetic Alteration

In mammals, epigenetic mechanisms are essential for the development and preservation of tissue-specific gene expression patterns. Chromatin consists of repeated nucleosome units, and epigenetic mechanisms can modify chromatin structure. Mammalian cells can adjust their transcriptional program in response to environmental stimuli via epigenetic modifications, allowing changes in gene expression without affecting the genetic coding [23]. One drawback is that epigenetic mechanisms might significantly contribute to oncogenesis by improperly suppressing tumour suppressor genes (TSG) and activating oncogenes. Numerous bacteria can endure, multiply, and elude the host's immune response by altering the host's epigenome. Epigenetic modifications, such as DNA methylation, histone abnormalities, miRNA-mediated regulation, and chromatin remodelling, are frequently detected in several malignancies, including colorectal cancer. Furthermore, infection with H. pylori can lead to abnormal DNA methylation, increasing the risk of

gastric cancer (GC). Histone proteins may experience numerous post-translational changes, such as methylation, acetylation, phosphorylation, and ubiquitination. Histone acetylation has received considerable focus in microbiological research concerning several malignancies, especially breast cancer. Microorganisms participate in the manufacture and metabolism of several substances that act as epigenetic substrates and cofactors or influence the activity of epigenetic enzymes. These interactions indirectly affect host epigenetic changes. DNA and histone methylation predominantly depend on substrates like folate and other B vitamins. Folate is a crucial component of beneficial gut microbes, including the probiotic species Bifidobacterium and Lactobacillus. It is involved in one-carbon metabolism, producing S-adenosylmethionine (SAM), an essential substrate for DNA and histone methylation. A significant group of epigenetically associated chemicals is short-chain fatty acids (SCFAs), generated by commensal microbes via the fermentation of indigestible complex carbohydrates and fibre [23]. Short-chain fatty acids influence genomic epigenetic modifications via impacting the activities of histone acetyltransferases and deacetylases. A recent analysis revealed a correlation between microbiome alteration and miRNA expression in various cancer types. Additionally, miR-515-5p and miR-1226-5p can promote the proliferation of Fusobacterium nucleatum and E. coli, alongside their targeting of nucleic acid sequences [24].

Comprehensive studies have demonstrated that intratumoral microorganisms can influence host epigenetic modifications, including alterations in DNA, histones, RNA, and non-coding RNA, either directly or indirectly. However, the specific molecular pathways governing these epigenetic alterations caused by intratumoral microorganisms necessitate additional research.

Inflammation

Inflammation is intricately linked to all phases of cancer development and malignant progression, as well as the effectiveness of anticancer treatment. Acute inflammation induces cancer cell apoptosis through the activation of an antitumor immune response, but persistent inflammation fosters treatment resistance and cancer progression. Chronic inflammation may induce immunosuppression, creating a conducive milieu for carcinogenesis, tumour advancement, and metastasis. Intratumoral bacteria can activate inflammatory pathways by engaging pattern recognition receptors in the tumour microenvironment, including Toll-like receptors (TLRs). For instance, TLR4 in non-small-cell lung carcinoma cells can be stimulated by gram-negative bacteria, facilitating tumour proliferation and metastasis [25]. Toll-like receptors (TLRs) are crucial for linking innate and adaptive immunity through the regulation of antigen-presenting cell activation and key cytokines. F. nucleatum engages with TLRs within the tumour microenvironment, hence activating the TLR4/MYD88/NF-κB signalling cascade. Activating this pathway fosters a pro-inflammatory milieu conducive to the survival of colorectal cancer cells while inhibiting apoptosis. This generates a positive feedback loop that initiates pro-inflammatory responses and hastens the progression of CRC. Alongside F. nucleatum, some strains of B. fragilis and E. coli can elicit pro-inflammatory responses. These reactions promote the mobilisation of immune cells, including neutrophils and myeloid-derived suppressor cells (MDSCs), to the tumour location. These cells are paradoxical, as their interactions with microorganisms and the host can either facilitate or obstruct tumour development [26].

Inflammation arises when the immune system reacts to detrimental stimuli, including infections, injured cells, toxic agents, or radiation exposure. It eradicates these detrimental stimuli and commences the healing process, serving as an essential defence mechanism for health preservation. Nevertheless, unchecked acute inflammation may advance to a chronic state, leading to numerous chronic inflammatory diseases. The persistent inflammatory microenvironment in cancer can evolve into an immunosuppressive milieu, facilitating tumour progression and suppressing the antitumor immune response. Furthermore, inflammatory cells can produce reactive oxygen species (ROS), which serve as a mediator of DNA damage induction [27].

Modulation of Oncogenic Pathways

Microorganisms can promote tumorigenesis by modulating oncogenes and signalling pathways, such as Wnt/β-catenin and β-Catenin is a multifaceted protein that is crucial for physiological equilibrium. Excessive expression of β-Catenin leads to various diseases, including cancer. It functions as a transcriptional co-regulator and an intracellular adhesion adaptor protein. Wnt is the principal regulator of β-catenin, a group of 19 glycoproteins that govern both the β-catenin-dependent (canonical Wnt) and catenin-independent (non-canonical Wnt) signalling pathways [28]. Fusobacterium nucleatum activates the β-catenin signalling pathway via Toll-like receptor 4. Activation of the β-catenin pathway can stimulate downstream oncogenes, such as cyclin D-1 and c-Myc, hence facilitating cancer proliferation. H. pylori synthesises CagA, which enhances β-catenin signalling and contributes to stomach cancer. Specific S. typhi strains

produce AvrA, which stimulates β-catenin and is associated with hepatobiliary cancer (Lu et al., 2014) [29].

In addition to the Wnt/β-catenin pathway, microbes may also activate other cancer-associated signalling pathways. B. fragilis triggered the Notch1 and β-catenin pathways, resulting in breast tissue carcinogenesis and progression. The JAK-STAT pathway plays a crucial role in colorectal cancer and other malignancies, frequently exhibiting aberrant activation. ETBF can induce STAT3 activation in colorectal tumours by phosphorylation and subsequent nuclear translocation [30].

Tumor metastasis

While the precise processes by which intratumoral bacteria affect tumour metastasis remain unclear, emerging data indicates that these microorganisms may contribute to the initiation of tumour spread. Microorganisms in different tumour types might facilitate tumour genesis, development, and metastasis by affecting several signalling pathways [31]. Exosomes secreted by infected cancer cells may constitute an additional mechanism. Exosomes, also referred to as extracellular vesicles (EVs), are generally membrane structures measuring 40–100 nm. These substances are secreted into fluids by diverse human body cells and comprise proteins, mRNA, miRNA, and signalling molecules. Exosomes are essential for the transfer of proteins and RNA between cells and, from an immunological standpoint, they possess the ability to deliver antigens. Tumor-derived exosomes (TEXs) have emerged as crucial elements originating from tumours that participate in the metastatic process. Evidence suggests that TEXs can interact with host immune, epithelial, and tumour cells. TEXs can alter and reprogram host cells through these interactions, hence advancing tumour growth and enabling cancer metastasis. Research findings demonstrate that tumour cells infected with bacteria release an increased quantity of exosomes [32].

Moreover, intracellular bacteria within tumours markedly improve the survival of tumour cells under mechanical stress during blood circulation. Cancer cells entering the bloodstream often experience apoptosis during metastasis due to fluid shear stress. Tumour cells containing bacteria demonstrate enhanced viability compared to those devoid of bacteria, likely because intracellular bacteria influence the cellular stress response [33].

Effects of intratumoral microorganisms on cancer treatment

The core anticancer treatments are radiotherapy, chemotherapy, and immunotherapy, each utilising unique techniques to inhibit tumour growth and advancement. Radiotherapy utilises ionising radiation to inflict damage on cancer cell DNA, whereas chemotherapy employs cytotoxic chemicals to impede cell division. Immunotherapy utilises the body's immune system to recognise and eliminate cancerous cells, providing a focused therapeutic approach.

Chemotherapy and the Microbiome

The application of chemotherapy is accomplished by the use of genotoxic chemicals, which cause damage to the DNA of the tumour cells that are currently present and prevent the formation of new DNA during the process of cell division [34]. The microbiome possesses a wide range of enzymatic capabilities, which influence the response to chemotherapy as well as its toxicity. According to Lehouritis et al. (2015), the inherent enzymes of intratumoral bacteria have the ability to change the efficacy of chemotherapeutic drugs through a process that is referred to as its biotransformation. The gut microbiota has been shown to have an effect on chemotherapy for cancer, particularly chemotherapy regimens that use cyclophosphamide (CTX) and oxaliplatin, this has been proved by research [35]. It is primarily owing to the stimulation of antitumor immune responses via several immunological pathways that CTX is able to exert its anticancer effects. These immune responses help Th1 and Th17 cells in their efforts to regulate the proliferation of cancer cells. It has been indicated in previous research that the administration of cyclophosphamide can result in changes to the composition of the microbiota in the gut, which can then lead to the migration of certain gram-positive bacteria to secondary lymphoid organs. According to Viaud et al. (2013), this causes the production of pathogenic T helper 17 (pTh17) cells and enhances the response of the host immune system, which is driven by memory T helper 1 (Th1) cells during the immunological response [36]. Studies have shown that the oral administration of Enterococcus hirae can restore the anticancer effects that are mediated by CTX. As a result, Enterococcus hirae is recognised as a valuable oncomicrobiotic [37].

Oxaliplatin is an anticancer drug that is based on platinum that is used. It has applications in the treatment of a variety of illnesses, including neuroendocrine tumours, malignancies of the stomach and oesophagus, and advanced pancreatic cancer. According to Chambers and Illingworth (2023), the mechanism of action of this chemotherapy treatment involves DNA damage, which ultimately results in the death of cancer cells [38].

In colorectal cancer patients, the bacterium F. nucleatum, which is found in the gut, has been shown to be capable of promoting resistance to cytotoxic chemotherapy medicines when combined with oxaliplatin and capecitabine [39]. Gemcitabine, which is a nucleoside analogue, is widely utilised in the treatment of malignancies of the pancreas, lungs, breast, stomach, and bladder. One of the most abundant species in pancreatic ductal adenocarcinoma (PDAC) tissues is Gammaproteobacteria, which is responsible for the predominant expression of the long isoform of the bacterial enzyme cytidine deaminase (CDDL). This is mostly due to Gammaproteobacteria. It was observed that intratumoral bacteria that express CDDL are able to metabolise gemcitabine in a passive manner, which results in the development of resistance in tumours to this chemotherapeutic medication. The administration of ciprofloxacin has been shown to be effective in preventing the development of chemoresistance to gemcitabine in mice models of colon cancer [40]. Consequently, the term "pharmacomicrobiomics" is becoming increasingly well-known as a new subject within the realm of chemotherapeutic research.

Radiotherapy and the microbiome

At least two-thirds of cancer treatment protocols in Western nations use radiotherapy, which is a substantial curative treatment approach for uncomplicated loco-regional tumours. Radiotherapy is also incorporated into an increasing number of cancer treatment protocols. Radiotherapy is driven by two main ideas, which are as follows: In the first place, it eliminates cancer cells by directly destroying the DNA of cancer cells by the use of ionising radiation. Second, it targets cancer cells in a roundabout way by causing DNA damage through the use of reactive oxygen species. By targeting cancer cells, radiotherapy (RT) can have a negative impact on healthy tissues as well as the commensal bacteria that are found in the body, particularly those that are found in the gut. Both radiotherapy and the microbiota in the gut have an effect on one another that is mutually beneficial. Dysbiosis of the gut microbiota is a common adverse effect that can occur as a result of radiation. Typically, this condition is characterised by a decrease in the number of beneficial microbes, such as Bifidobacterium, and an increase in the number of harmful microorganisms, such as Fusobacteria and Proteobacteria. Radiation-related problems, such as radiation enteropathy, are made worse by these alterations in the composition of the microbiota in the gut. In spite of this, certain commensal bacteria play a significant part in boosting the efficacy of radiation and minimising the adverse events that are connected with it. Intestinal fungus has been shown to influence antitumor immune responses following radiation therapy in mice models of breast cancer and melanoma, according to recent research. Bacteria, on the other hand, play the opposite role and are responsible for increasing the response rate. According to the findings of another study, probiotics such as Lachnospiraceae and Enterococcaceae have the ability to alleviate the negative effects of radiation therapy, which include exhaustion, nausea, vomiting, and diarrhoea. Through the modulation of the gut microbiome, these probiotics may be able to assist in the reduction of damage caused by radiation [41].

Despite the fact that there is still a lack of direct evidence regarding the microbiome's influence on the effectiveness of radiation treatment, the connection between the side effects of radiation therapy and the gut microbiome suggests that there is the possibility of modifying the composition of the gut microbiome in order to reduce the toxicity that is associated with radiation. Modulation of this kind has the potential to improve the prognosis for individuals who are undergoing radiation therapy. There is a possibility that future study will uncover the precise mechanisms that link the microbiota of the host to the responsiveness and negative effects of radiation therapy. As a result, the interaction between bacteria in the gut, tumours, and radiotherapy is complex, which presents a wide area for investigation.

Immunotherapy and microbiome

For the therapeutic management of cancer, immunotherapy has introduced fresh approaches alongside established treatments such as chemotherapy and radiotherapy. In recent years, immunotherapy has shown promising outcomes, offering novel approaches. Research conducted by Iwai et al. (2017) shown that antitumor immunotherapies enhance the capacity of the host immune system to recognise and kill malignant cells [42]. It is possible to highlight two key methodologies that are utilised within the field of immunotherapy. To begin, immune checkpoint blockade is a targeting strategy that explicitly targets molecules like CTLA-4 and PD-1. The second example of adoptive T-cell therapy is the CAR-T therapy, which was published by [43].

Even though immunotherapy is quite effective, there are still a substantial proportion of patients who do not show any signs of responding to the treatment. According to Bai et al.'s research from 2020, it is even more concerning that some patients who initially show promising responses to immunotherapy eventually develop resistance to the treatment. Importantly, there is growing data that suggests that bacteria that are present in the intratumoral space can have an effect on the efficacy of immunotherapy. As a consequence of

this, various research are investigating the possibility of medicinal benefits from modifying the microbiome. Some of the methods that have been proposed include the use of probiotics, the utilisation of foetal microbiota transplantation (FMT), and the direct application of antibiotics. One study, for example, discovered that Clostridium was more prevalent in the melanomas of patients who reacted to immune checkpoint inhibition, but Gardnerella vaginalis was more prevalent in those who did not respond to the treatment. An additional study was conducted in which researchers found that increasing the levels of Bacteroides fragilis, Burkholderia cepacia, and Faecalibacterium in the gastrointestinal tract of patients who were receiving CTLA-4-based immunotherapy improved the therapeutic effect and decreased the number of adverse side effects, such as colitis [44].

The use of FMT in immunotherapy is definitely promising, especially when one considers how feasible it is to acquire stool samples from human donors. It has been established through clinical trials that FMT can have a beneficial effect on patients suffering from melanoma, which may give prospective benefits. According to the findings of these investigations, FMT led to an increase in the proliferation of bacterial species that had been previously associated with favourable responses to anti-PD-1 therapy, an increase in the activation of CD8+ T cells, and a decrease in the presence of myeloid cells that expressed interleukin-8. Despite the fact that it has the potential to be therapeutic, FMT is not risk-free. Most short-term adverse effects, such as temporary diarrhoea, stomach discomfort, bloating, and constipation, are moderate and self-limiting, despite the fact that they are widely regarded to be safely associated with the medication. The transmission of live microbes, on the other hand, poses a more significant risk, particularly for persons who are immunocompromised. Despite the fact that studies have shown that FMT is well tolerated even in high-risk groups, there have been reports of consequences that are extremely uncommon yet severe. According to DeFilipp et al. (2019), cases of bloodstream infections that have been linked to extended-spectrum beta-lactamase (ESBL)-producing E. coli and enteropathogenic E. coli (EPEC) underline the crucial need of rigorous donor screening and the continuous revision of safety standards. Furthermore, the concerns extend beyond the hazards associated with infectious diseases, since there is a possibility that FMT could potentially influence ailments that are not infectious, such as metabolic disorders, neuropsychiatric issues, and even cancer. Despite the fact that long-term data have not revealed any substantial safety problems, continued surveillance, which includes programs like as the FMT National Registry, is still necessary in order to have a complete understanding of the hazards that are connected with this developing medication [45]. Furthermore, merging immunotherapy with probiotic nutrition is a viable route for research. This strategy takes a strategic approach to combining one or more beneficial microorganisms into a single solution. Probiotic supplements combined with OncoTherad was found to have a number of impacts, according to the findings of a study conducted by experts. The process of controlling weight loss, activating the canonical TLR2/TLR4 signalling pathway (which is dependent on MyD88), diminishing the non-canonical signalling pathway (which is dependent on TRF), suppressing the proliferative pathway driven by Ki-67 and the KRAS oncogene, and significantly increasing the production of IL-10 and TGF-β cytokines were all observed by Reis et al. in their study published in 2022. In addition, research has shown that the use of probiotics that are available for purchase in the market without any specific purpose may not improve the efficacy of immunotherapy and may even result in autoimmune reactions that are related to immunotherapy. According to the findings of Tlaskalová-Hogenová et al. (2011), improper use of probiotics has been linked to a wide variety of diseases. These diseases include inflammatory bowel diseases (IBD), coeliac disease, type 1 diabetes (which is dependent on insulin), neurological and mental disorders, rheumatic conditions, obesity, cardiovascular issues, atherosclerosis, allergies, and cancer [46]. It is therefore essential to carefully select patients, conduct strain-specific assessments, and administer probiotics in a controlled manner in order to minimise the chances of harmful effects, despite the fact that probiotics may have potential benefits in the treatment of cancer. It is necessary to do additional research in order to uncover the mechanisms that lie beneath these microbial interventions and to develop personalised probiotics that are tailored to patients who have a variety of living situations and eating habits. There is a dearth of research on the influence of tumour bacteria on the efficacy of immunotherapy, despite the fact that the majority of the studies that are now being conducted focus on gut microbes. There is still a lack of clarity on the existence of communication between the microbes in the gut and those in the intratumoral space, as well as the possible influence of changing gut microorganisms on the host immunological milieu and the makeup of the intratumoral microbes. Additional research should be conducted in these areas.

Conclusion

When it comes to the microecology of tumours, intratumoral bacteria play a crucial role, as they have the ability to influence the progression of cancer, the

response to medicines, and the potential results of treatment. The investigation of microbiota that are connected with tumours is a relatively new topic that is gradually expanding our knowledge of the role that microbes play in the processes that cause cancer. The promotion of DNA damage, epigenetic alterations, inflammation, the regulation of carcinogenic pathways, and the facilitation of metastasis are all examples of these aspects of the contribution. Furthermore, intratumoral microbiota presents a prospective frontier for the development of novel therapeutic methods, such as diagnostics based on biomarkers and complementary medications. In the future, research should concentrate on turning these discoveries into practical applications, such as personalised microbiota-based medicines that are tailored to the specific tumour profiles and immunological microenvironments of particular patients. This may involve the use of microbiome editing technologies, such as precision probiotics or modified bacterial strains, with the goal of improving medicinal efficacy while simultaneously reducing the extent of any side effects. Furthermore, the use of machine learning into the investigation of the microbiome possesses a tremendous potential to simplify the interpretation of intricate microbial interactions and to forecast the results of therapeutic interventions. Diagnostics could be improved with the help of advanced computational methods, which could also be used to uncover microbiological signatures of treatment response and direct the development of personalised therapies. By focussing on these promising areas, the discipline is poised to revolutionise our approach to cancer detection and treatment, thereby paving the way for oncology care that is both more effective and more individualised.

Conflict of Interest

The authors declare no conflict of interest.

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