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REVIEW

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The human microbiome: redefining cancer pathogenesis and therapy

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Abstract

The human microbiome has always been an important determinant of health and recently, its role has also been described in cancer. The altered microbiome could aid cancer progression, modulate chemoresistance and significantly alter drug efficacy. The broad implications of microbes in cancer have prompted researchers to investigate the microbe-cancer axis and identify whether modifying the microbiome could sensitize cancer cells for therapy and improve the survival outcome of cancer patients. The preclinical data has shown that enhancing the number of specific microbial species could restore the patients' response to cancer drugs and the microbial biomarkers may play a vital role in cancer diagnostics. The elucidation of detailed interactions of the human microbiota with cancer would not only help identify the novel drug targets but would also enhance the efficacy of existing drugs. The field exploring the emerging roles of microbiome in cancer is at a nascent stage and an in-depth scientific perspective on this topic would make it more accessible to a wider audience. In this review, we discuss the scientific evidence connecting the human microbiome to the origin and progression of cancer. We also discuss the potential mechanisms by which microbiota affects initiation of cancer, metastasis and chemoresistance. We highlight the significance of the microbiome in therapeutic outcome and evaluate the potential of microbe-based cancer therapy.

Keywords Gut microbiota, Cancer, Chemotherapy, Immunotherapy, Chemoresistance

Introduction

Human body harbours an extraordinary number of microbes including bacteria, fungi, yeast, archaea, protozoa and viruses, collectively called as commensal microbiota. The term microbiota includes bacteria, fungi, virus and other microbes, which influence gut and systemic homeostasis [1]. They affect metabolism, inflammation, nerve transmission, immune function, and haematopoiesis. Bacteria are the most common among these microorganisms. It is astonishing that a human body of 70 kg harbours around 39 trillion bacterial cells, compared to just 30 trillion human cells [2]. Both commensal microbiota and human body lives in harmony as they display symbiotic relationship [3]. The microbiota niche includes gut, skin, oral cavity, respiratory tract, urinary tract and reproductive tract [2, 4, 5]. The microbiota contributes significantly to human health and any alterations have been known to cause several pathological conditions. Interestingly, topographical location and dietary intake influence an individual's microbial population. For instance, people living in different locations harbour different set of gut microbiota, which induce distinct microbial signalling pathways in them [6].

Cancer is a highly lethal and devastating disease with over 19 million newly diagnosed cancer cases and almost 10 million cancer deaths worldwide, reported in 2022 (https://gco.iarc.fr/today/fact-sheets-cancers). In

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addition to the patient suffering, cancer poses tremendous economic burden with remarkable healthcare costs [7]. While cancer is a complex, multi-step, chronic disease, that is caused by the accumulation of spontaneous mutations acquired during DNA replication in actively dividing cells, environment also impacts cancer risk considerably. Several risk factors associated with cancer include lifestyle factors, microbial infection, tobacco use, physical activity, diet and energy balance [8]. In addition, dysregulation of the gut microbiota has gained widespread attention as a potential risk factor for cancer.

Recently, microbiota has been added to the list of hallmarks of cancer, due to their capability to affect cancer phenotypes. The microbes are known to generate several metabolites such as short chain fatty acid (SCFA) and Tryptophan (Trp), which confer genetic and epigenetic changes in cells, disposing them to become cancerous [9]. An amplitude of literature suggests a crosstalk between gut microbiota and the intestinal mucosal surface of the host which plays a principal role in tumorigenesis [10–12]. Microbes may increase the risk of tumorigenesis by inducing DNA damage via oxidative stress in the gastric mucosa, enhancing epithelial inflammation and disrupting the mucosal barrier [13, 14]. Dysbiosis, which signifies an imbalance in a person's microbiota, has been observed in the gut of numerous cancer patients. It has been proposed that dysbiosis enables the microbes to spread to distant organ locations via systemic circulation and leads to the development of chronic inflammation and immunosuppressive microenvironment. This, in turn, increases the risk of cancer development [15].

In addition to gut microbiota, emerging evidence highlights the importance of intratumoral microbiota. The intratumoral microbes are considered an essential component of the tumor microenvironment (TME) [16] and their composition, richness, and spatial distribution vary among different tumors [17]. Four potential sources for intratumoral microbiota have been proposed: compromised mucosal barriers, spread from adjacent normal tissues, dissemination via blood vessels, and lymphatics. Understanding how these microbes colonize and inhabit tumor tissues remains a major area of research. The potential of intratumoral microbiota has been highlighted in great depth in recently published reviews [10, 18, 19].

Microbiota, aging and cancer

Aging refers to progressive, time dependent deterioration of cellular and physiological processes. This functional decline is considered as the major risk factor for key diseases, including cancer. The fundamental hallmarks of aging include genomic instability, epigenetic alterations, oxidative damage, telomere attrition, mitochondrial dysregulation, cellular senescence and disturbance of proteostasis [20–22]. Studies on mice aged in the same environment has shown that microbiota diversity and abundance decreases in natural aging. Older mice show a boost in creatine degradation, leading to muscle wasting, and a decline in the biotin, cobalamin biosynthesis, and underrepresented SOS genes associated with DNA repair [23]. Studies on human aging cohort indicate that a group of genera including *Akkermansia*, *Anaerotruncus*, *Eggerthella*, and *Bilophila* prominently increases with aging, while, abundance of *Faecalibacterium*, *Prevotella*, and *Bacteroides* was comparatively compromised in aged adults. An enrichment in pathobionts, which are opportunistic pro-inflammatory bacteria, has been observed in aged adults, that can nurture the physiological inflammation in these people [24, 25]. It has been proposed that make up of gut microbiota changes gradually during aging in humans also, despite the substantial inter-individual heterogeneity and impact of external factors such as life style, diet and treatment [26, 27].

Several reports suggest that dysbiosis increases during aging and leads to abundance of pro-inflammatory commensals as compared to beneficial microbes. SCFAs, including butyrate, can help slow down cellular aging. They achieve this by suppressing histone deacetylases, which modulates metabolic processes, boosts insulin secretion, and regulates immune responses [28–30]. In contrast, pathogenic bacterial byproducts such as LPS enhance cellular senescence, a hallmark of aging, by accelerating inflammation and increasing oxidative stress [31]. In addition, the toxins produced by *Pseudomonas aeruginosa* and *Helicobacter pylori* cause DNA damage and elevate oxidative stress in the host. As a result, this exacerbates the DNA damage response, genomic instability, and cellular senescence [32, 33].

Dysbiosis and bacterial toxins facilitate the accumulation of senescent cells, DNA damage, and proinflammatory microenvironment in aged people. This leads to metabolic disturbance and creates a TME that promotes the survival and propagation of neoplastic cells, ultimately resulting in cancer (Fig. 1) [26, 34, 35].

Cancer initiation and progression

Cancer is a complex, dynamic, and multi-step disease composed of heterogenous cell populations and associated TME [36]. Due to its non-linear nature and dependency on multiple variables, carcinogenesis is controlled by numerous risk factors, indicating that each tumour is unique. However, a common outline of the process has been established by identifying the molecular events involved in the initiation and progression of cancer [37].

Cancer often starts with an alteration in genetic or epigenetic pathway in the somatic cells making them

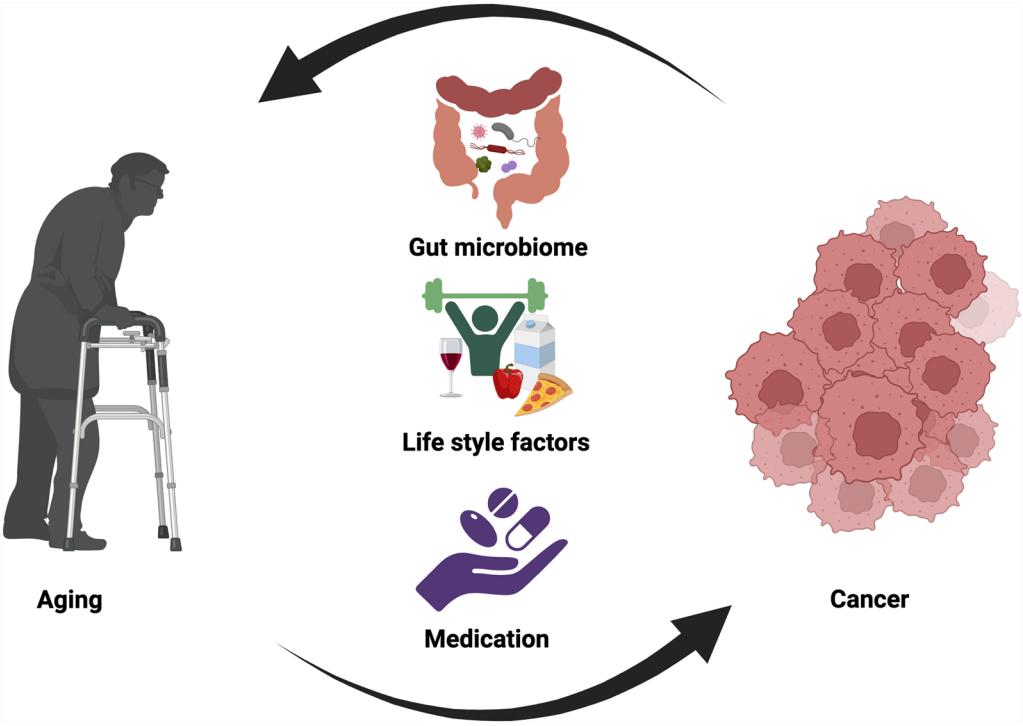

Fig. 1 Aging can culminate into cancer and cancer treatment specifically leads to premature aging. Risk factors of aging and cancer include gut microbiome, life style factors and medication

tumorous, due to the presence of a carcinogen. This is followed by a qualitative change in the genetic traits of the tumor cells. This change typically leads to malignancy, even though there may not be any observable physical changes [38]. Genetic mutation in tumor suppressor gene, oncogene, DNA repair gene or cell cycle gene may confer growth advantages, resistance to death signals and thus lead to abnormal proliferation and initiation of tumors [39]. Various hallmarks have been found to be associated with the neoplastic growth stages, which a normal cell undergoes while becoming malignant [1]. Metabolic reprogramming is one such key hallmark. Cancer progression occurs through the different metabolic phenotypes that cancer cells acquire, which facilitate their rapid division, dissemination, and invasion into different parts of the body. One such metabolic characteristic is the accumulation of oncometabolite, D-2-hydroxyglutarate, in tumors by the successive mutations of isocitrate dehydrogenase-1/2. This is shown to directly encourage cancer initiation and further progression by suppressing differentiation [40]. Other metabolic activities that have often been found to be associated with tumours in cultured cancer cells include aerobic glycolysis, macromolecular synthesis, redox homeostasis and glutamine catabolism – all assisting rapid cellular proliferation and growth. However, tumour heterogeneity has been widely discovered in various studies–leading to differences in metabolic properties in separate regions of the tumor [41].

TME is another component that plays a vital role in cancer initiation and progression. It is an orchestrated vascularized ecosystem, which harbours cancer cells along with several non-cancerous cells such as immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, adipocytes, neurons and the extracellular matrix (ECM) [42, 43]. Cancer cells further promote tumor-supportive niche by rewiring surrounding non-cancerous cells and by facilitating angiogenesis and modulating ECM. For instance, tumor-associated macrophages (TAMs) secrete growth factors and cytokines to stimulate angiogenesis and invasion. Tumor cells are known to reprogram CAFs to secrete ECM proteins and vascular factors including VEGF-A, contributing to tumor supportive niche [42]. The ECM serves as a reservoir for secreted molecules, thereby enhancing intercellular communication, cell adhesion, and migration [44]. Therefore, different components of TME contribute to cancer initiation, progression and migration by affecting various cellular processes.

Better understanding of molecular mechanisms that regulate cancer progression and plasticity can aid in

developing precise and targeted therapies for cancer treatment and prevent recurrence.

Evidence of association between microbiota and cancer

The most well studied direct connection between microbiota and cancer is the role of oncogenic viruses in inducing cancer such as *Helicobacter pylori* and Human Papilloma Virus acting as a causal factor for gastric adenocarcinoma and cervical cancer, respectively. The studies on animal models using antibiotics have confirmed that microbes can promote the initiation and progression of several cancers including breast cancer [45], gastric cancer [46], hepatocellular carcinoma (HCC) [47], colorectal cancer [48] and pancreatic ductal adenocarcinoma [49]. Association studies have identified critical microbes, which can impact cancer initiation, progression, therapeutic response, and chemoresistance in a positive or negative manner (Fig. 2) [1]. The high throughput DNA sequencing experiments have demonstrated the alteration of bacterial, viral and fungal communities of a human being as a trigger for carcinogenesis [50].

Analysis of gastric cancer specimens revealed greater microbial abundance, diversity and increased complexity in cancerous tissues as compared to non-cancerous tissues [51]. For instance, development of oral squamous cell carcinoma (OSCC) is often associated with the elevated enrichment of *Fusobacterium* spp [52]. Concurrently, OSCC patients were shown to display abundance of *Fusobacterium* in specific tumor sites, and reduced levels in saliva and oral rinse samples [53]. Notably, oral microbiome can also influence gut microbiota as gastric cancers harboured increased enrichment of microbial taxa associated with the oral microbiota [51].

Metagenomic sequencing helped identify the presence of specific microbial communities in different cancers (Table 1) [8]. The enrichment of *Streptococcus sanguinis*, *Anaerococcus mediterraneensis*, *Fusobacterium nucleatum*, and *Fusobacterium Hwasookii* was observed in primary colorectal tumour samples [54]. The gut microbial signatures were also found to be different among breast cancer patients responsive to neoadjuvant chemotherapy (NAC) when compared with patients resistant to NAC [55]. In addition, HER2 + breast cancer patients exhibited lower abundance of *Firmicutes* and higher abundance of *Bacteroidetes* in comparison to HER2- breast cancer patients in a study of 37 patients [56]. In HCC, microbial translocation to liver has been proposed due to aberrant gut epithelial barrier. Several dominant phyla such as *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* and *Ruminococcus gnavus* have been identified in tumor samples resected from patients of viral HCC [47]. Different

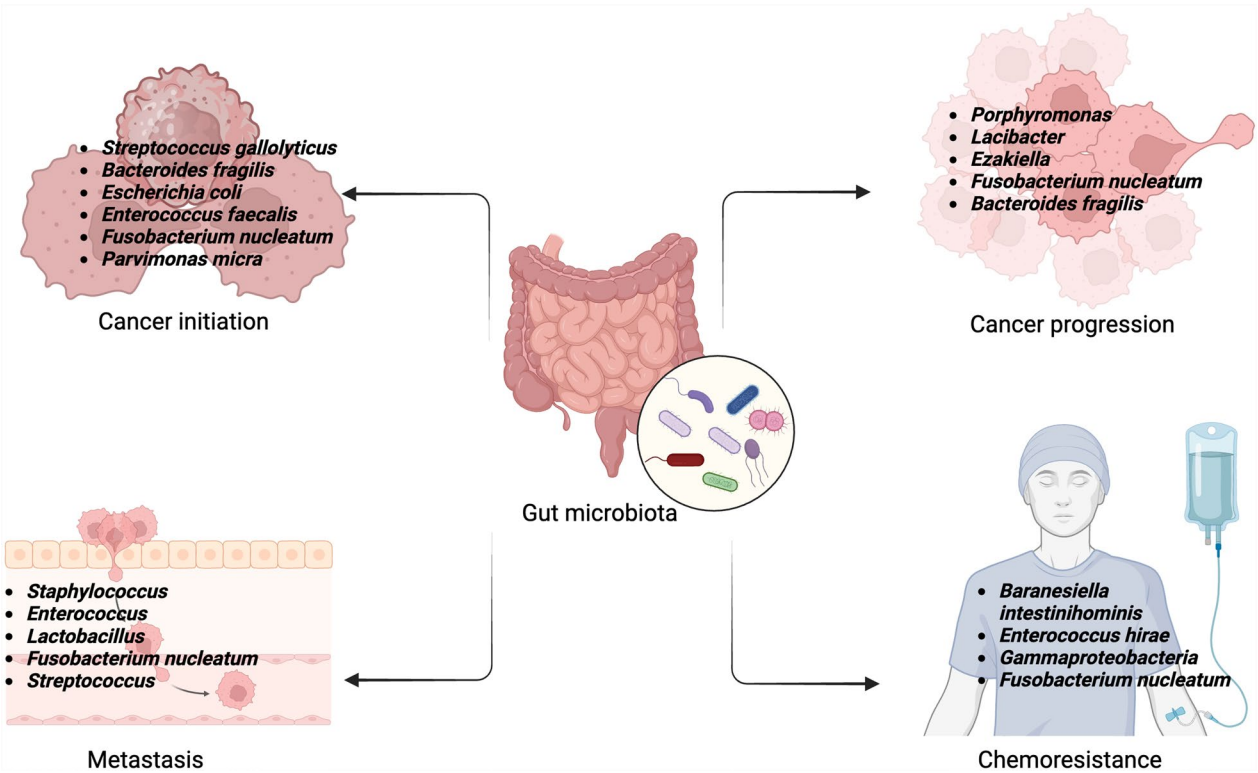

Fig. 2 Specific microbes within the gut microbiota impacts cancer initiation, progression, metastasis and chemoresistance

Table 1 Microbial species associated with the respective cancer

Cancer type	Microbes associated with increased risk	Microbes associated with decreased risk	References
Colorectal cancer	*Streptococcus gallolyticus*, *Bacteroides fragilis*, *Solobacterium moorei*, *Fusobacterium nucleatum*		[202–204]
Pancreatic cancer	*Porphyromonas*, *A. Actinomycetemcomitans Ascomycota*, *Basidiomycota*, *Malassezia*		[205–207]
Brain cancer /glioma	*Enrerobacteriaceae*, *Fusobacterium*, *Akkermansia*		[72, 208]
Gastric cancer	*Helicobacter pylori*		[209]
Ovarian cancer	*Neisseria gonorrhoeae* or *Chlamydia trachomatis*, *Bacteriodete*, *Firmicutes*		[210, 211]
Lung cancer	*Actinomyces*, *Haemophilus*, *Streptococcus*		[212, 213]
Breast cancer	*Lactobacilli*, *Bacteroide fragilis*, *Clostridia*, *Fusobacterium nucleatum*, *Escherichia coli*	*Lactobacillus helvecticus R389*, *Lactobacillus casei*, *Lactobacillus acidophilus*	[127]
Cervical	*Fusobacterium* spp, *Mycoplasma genitalium*, *Chlamydia trachomatis*		[214–216]
Glioblastoma	*Peptostreptococcaceae*, *Eubacterium brachy*	*Ruminococcaceae*, *Anaerostipes*, *Faecalibacterium*, *LachnospiraceaeUCG004*, *Phascolarctobacterium*, *Prevotella7*, *Streptococcus*	[217, 218]
Larynx	*Fusobacterium nucleatum*, *Streptococcus* spp, *Prevotella* spp, *Helicobactor pylori*	*Streptococcus* spp	[219, 220]
Prostate	*Cutibacterium acnes*, *Shewanella*, *Microbacterium* sp, *Escherichia coli*, *Streptococcus anginosusi*, *Propionibacterium acnesi*	*Botulinum toxin A*, *Staphylococcus aureus*	[220–224]
Oral squamous cell carcinoma	*Capnocytophaga gingivalis*, *Fusobacterium nucleatum*, *Carnobacterium* spp, *Tannerella* spp, *Parvimonas* spp, *Filifactor* spp, *Candida*	*Lactobacillus plantarum*	[225–229]
Bladder	*Prevotella* spp, *Alistipes* spp, *Barnesiella* spp, *Parabacteroides* spp, *Lachnospiracea_incertae_sedis*, *Staphylococcus* spp, *Parvimonas* spp, *Proteniphilum* spp, *Saccharofermentans* spp, *Klebsiella* spp	*Actinobacteria*	[230–232]
Head and neck	*Stenotrophomonas*, *Comamonadacea*, *Fusobacterium*, *Peptostreptococcus*		[233, 238]

mechanisms have been proposed to be responsible for microbe-associated tumorigenesis including epigenetic modulations, DNA damage, altered DNA damage response, dysregulated signaling pathways and modulated immune response [10].

Role of microbiota in development and progression of cancer

Many studies have reported dual role of microbes as protumorigenic and anti-tumorigenic in cancer initiation and progression depending on the stage of tumor progression and functional and spatio-temporal properties of microbiota [57–60].

Several microbes including *Fusobacterium nucleatum* and *Streptococcus gallolyticus* subsp *Gallolyticus* are involved in carcinogenesis. For instance, colorectal cancer (CRC) has been found to be closely associated with *Streptococcus gallolyticus*, *Bacteroides fragilis*, *Escherichia coli*, *Enterococcus faecalis*, *Fusobacterium nucleatum* and *Parvimonas micra* [61, 62]. These pathogens are considered to play a role in CRC tumor formation and development as they are abundantly expressed in the gut of CRC patients [63, 64]. These pathogens affect CRC development and metastasis through epigenetic mechanisms such as DNA methylation and histone modifications via producing metabolites such as short chain fatty acids [65, 66].

The abundance and diversity of various microorganisms including *Porphyromonas*, *Neisseria*, *Actinomycetes*, *Streptococcus*, *Bifidobacteria*, *Bacteroides* and *Fusobacterium* species have been associated with the origin and progression of pancreatic cancer. These microbes cause inflammation and immune suppression and thus affect tumor growth and development [67, 68]. The oral cavity microbiota and *Helicobacter pylori* act as risk factor for pancreatic cancer by inducing inflammation [69]. In addition, microbiota can also modulate the risk of breast cancer. They can modulate the function of immune system by producing metabolites, such as antibiotics, and by regulating bioenergetics and steroid hormone levels. Analysis of 16S rRNA gene sequence of human breast cancer tissues revealed a higher abundance of *Porphyromonas*, *Lacibacter*, *Ezakiella*, and *Fusobacterium* in advanced-stage breast tumors compared to early-stage

ones [70]. *Fusobacterium nucleatum* and *Bacteroides fragilis* have been shown to aggravate breast cancer growth and metastasis by inhabiting breast tumors and promoting self-renewal of cancer cells [45, 71].

Brain cancer patients harbour *Enterobacteriaceae*, *Fusobacterium* and *Akkermansia* as the major gut bacterial colonies but are deficient in SCFA-producing probiotics [72]. These microbes are believed to affect gliomas through immune system suppression, activation of inflammation, limiting cell death, and promoting angiogenesis and invasiveness [73]. The microbes are unable to cross blood–brain barrier, however they release certain extracellular vesicles, which have the ability to cross the barrier and make their way into the brain [74]. Several studies have designed models to link these microorganisms with brain cancer and predict treatment outcomes [73, 74].

Further instances of microbiota involvement in origin of other cancers include *Helicobacter pylori* in Gastric cancer [75], *Fusobacterium nucleatum* in colorectal carcinoma [76, 77], *Proteobacteria* in ovarian cancer [78], Human Papillomavirus in cervical cancer [79] and *Actinomyces* and *Peptostreptococcus* in lung cancer [80]. These species were found to be in abundance in respective cancers and were believed to elevate tumor susceptibility by modifying metabolism and immune response, enhancing inflammation and toxicity and altering signaling pathways [58, 81].

Beside these pro-tumorigenic roles of microbes, a few of them such as *Lactobacillus*, *Bifidobacterium*, *Faecalibaculum rodentium*, *Streptococcus thermophiles* have been observed to have anti-tumorigenic properties [82–85]. Generally, the mechanisms through which they inhibit tumor growth include modulation of the immune system, metabolic effects, and the mutualistic and competitive relationships among commensal and pathogenic bacteria [10, 86].

A consortium of commensal gut bacteria such as *Paraprevotella xylaniphila*, *Bacteroides dorei*, and *Parabacteroides distasonis* can induce IFNγ production by CD8 T cells, resist against *Listeria monocytogenes* infection and enhance efficacy of immune checkpoint inhibitors in mice [87]. Other bacteria, such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Clostridium difficile*, have demonstrated anti-tumor properties in melanoma, pancreatic, and breast cancer, respectively. They achieve this by producing toxins that inhibit proliferation, arrest cells in the G1-S phase, and induce apoptosis, thereby promoting anti-cancer activity [88–90]. Notably, *Streptococcus thermophilus* also suppresses cell proliferation, elicits cell cycle arrest, enhances apoptosis of colorectal cells in vitro and reduces the growth of CRC xenograft [85]. Additionally, gut microbiota restricts progression of colorectal cancer in mice by suppressing colonic lncRNA Snhg9 and upregulating p53 expression [91].

Microbiota and their products, like Coley toxins, have also been shown to confer anti-tumor immunity to the host [92]. *Clostridium perfringens* enterotoxin (CPE) also cause cell death in cancer cells by binding to highly expressed tight junction proteins claudin-3 and − 4 in breast, prostate and colon cancer [93–95]. A recent report shows enhanced effectiveness of anti-programmed cell death protein 1 (PD-1) therapy in colon cancer by targeting bacterial S100A11, which remarkably abolished tumour growth and infiltration of myeloid-derived suppressor cells (MDSC) in the tumor site [96]. *Akkermansia muciniphila* also mitigates liver steatosis and efficiently abrogates the tumor growth by reducing the number of monocytic MDSCs and M2 macrophages in orthotopic Metabolic Dysfunction-Associated Fatty Liver Disease-Hepatocellular Carcinoma (MAFLD-HCC) mouse models. This bacterium also improves the efficiency of PD1 treatment in several MAFLD-HCC mouse models [97].

Not only microbial toxins but metabolites produced by microbiota also contribute to anti-tumorigenic roles of the microbes. Short-chain fatty acid (SCFA) is one such metabolite produced by commensal bacteria in the gut. Sodium butyrate, a well-known SCFA, abolishes tumor cell growth, induces cell cycle arrest, promotes apoptosis, and alter immune responses in non-small cell lung cancer [98]. Further, isobutyric acid augments the efficiency of anti-PD-1 immunotherapy in colon cancer mice model by decreasing tumour volume [99].

Tryptophan is another metabolite produced by microbes that can impact the progression of cancer. It is an essential amino acid that plays a critical role in various physiological processes, including protein synthesis and neurotransmitter production. The metabolism of tryptophan occurs through three primary pathways: the serotonin pathway, the kynurenine pathway, and the indole pathway. The gut microbiota like *Bacteroides*, *Clostridium sporogenes*, *Eubacterium* and *Ruminococcus gnavus* significantly influences tryptophan metabolism, producing various indole metabolites that have been shown to impact tumor development and progression [100].

The indole pathway, unique to gut microbiota, produces metabolites such as indole-3-lactic acid (ILA), indole-3-propionic acid (IPA), and indole-3-acetic acid (IAA). These metabolites modulate the TME by affecting immune responses, promoting tumor growth, and influencing cancer cell metabolism. Dysbiosis can disrupt tryptophan metabolism, contributing to cancer progression and immune evasion [101].

Recent research has highlighted the potential therapeutic implications of targeting tryptophan metabolism and indole metabolites in cancer treatment. Studies have

shown that indole metabolites can enhance the effectiveness of cancer immunotherapy and chemotherapy by modulating the immune response and creating an unfavorable environment for tumor growth. However, the complex interactions between gut microbiota, tryptophan metabolism, and tumor biology necessitate further research to develop standardized protocols and therapeutic strategies.

Presence of several microbes in TME indicates interaction between TME and microbiota that plays a paramount role in progression of cancer [102]. Fap2 protein of *Fusobacterium nucleatum* binds to TIGIT, an inhibitory receptor present on all human NK cells and T cells, due to which it suppresses the action of NK cells, $CD4^+$ and $CD8^+$ T cells in eradicating tumors [103]. *H. pylori* interacts with macrophages in the TME and induces their polarization to M2-like macrophages. This causes a decline in the antigen presentation abilities and alter macrophage secretions, collectively promoting the progression of gastric cancer [104].

Intratumoral microbiota also plays a role in oncogenesis and tumor progression [17, 105, 106]. In a recent report, six bacterial genera including *Fusobacterium*, *Longibaculum*, *Intestinimonas*, *Pasteurella*, *Limosilactobacillus*, and *Arthrobacter* were found to be enriched in glioma tissues. Out of these, *Fusobacterium nucleatum* was found to increase glioma proliferation and secrete pro-inflammatory cytokines such as CCL2, CXCL1, and CXCL2 [107]. An enrichment of various *Bacillus* species has been observed in metastatic breast tumors. Culture of breast tumor cells with *Bacillus thermoamylovorans* enhances the metastatic capabilities of these cells by almost three times as compared to control cells [108]. However, a few intratumoral microbes have also been reported to enhance anti-tumor immune response and thus inhibit tumor progression [109–111].

Microbiota and cancer metastasis

Metastasis is a key hallmark of cancer cells which require significant ingenuity on part of cancer cells. The later stages of most cancer types are often characterized by the onset of metastasis. It is undoubtedly the leading cause of mortality among cancer patients [112]. Metastasis involves translocation of cancer cells from the site of primary tumor to secondary sites in other organs through blood circulation. It involves epithelial-mesenchymal transition (EMT), migration, invasion, extravasation and colonisation at the secondary site. Metastasis is however a challenging process, both physically and chemically, for the cancer cells as they have to encounter the stiff extracellular matrix, fluid shear stress and immunosurveillance before colonizing a distant site that may have a very distinct physiology from the primary site of tumor formation. Strikingly, in all the aforementioned cellular processes for metastasis, intratumoral microbiota may play a remarkable role (Table 2).

In a recent study by Fu et al., it was reported that while microbiota was responsible for the tumor growth, it was indeed the intratumoral bacteria which played a key role in metastasis [113]. The treatment of tumor cells with antibiotics that can penetrate the cell membrane revealed that majority of the bacterial population in the tumor tissue was intracellular. The intracellular microbes in the tumor belonged to the genus *Staphylococcus*, *Lactobacillus*, *Enterococcus*, and *Streptococcus*. Since the microbes were intracellular, the metastasis of breast tumor to lung carried these microbes to the lung tissue. This study also showed that once metastasis has occurred, the microbial abundance would be dependent on the target organ microenvironment, for instance, lung metastasis would aid the growth of aerobic bacteria and impede the facultative anaerobes. The intracellular microbes in tumor cells including *Staphylococcus xylosus*, *Lactobacillus animalis*, and *S. cuniculi* have been shown to protect the metastasizing cells against apoptosis induced by fluid stress during circulation by inhibiting RhoA and ROCK proteins (Fig. 3).

In a retrospective study on pancreatic cancer patients, it was observed that antibiotic usage for more than 48 h had a positive impact on the overall survival and progression free survival in metastatic patients [114]. There was, however, no impact of antibiotic usage on the resectable tumor patients. The effect on both overall survival and progression free survival was observed when antibiotic usage was combined with gemcitabine as primary chemotherapeutic while only progression free survival showed a positive impact when 5-Fluorouracil was used as a primary chemotherapeutic. This could most likely be attributed to the targeting of microbes by the antibiotic which could be offering resistance to the chemotherapy. Although, other possible reasons for better outcome with antibiotics could be targeting of systemic infections or the regulation of immunomodulatory genes like CD47 or STAT3.

Fusobacterium nucleatum (Fn) has been implicated to induce metastasis in breast, colorectal and laryngeal cancer [71, 115–117]. It is a common gram-negative bacteria present in the oral cavity and often associated with periodontal diseases and halitosis [118]. Two independent studies revealed the metastatic role of Fn in colorectal cancer. Kong et al. showed that Fn activates TLR4/Keap1/NRF2 axis which induces the expression of CYP2J2 and 12,13-EpOME. Rubinstein et al., showed that Fn through Fad protein is capable of binding to E-cadherin on CRC cells, which leads to the activation of Wnt signalling pathway. Fn colonises CRC cells through Fap2 protein which

Table 2 Role of intratumoral microbiome in regulating cancer metastasis

Cancer	Microbe	Effect on metastasis	Mode of action	Model organism/cell line	References
Breast	*Staphylococcus*, *Lactobacillus*, *Enterococcus*, and *Streptococcus*	Pro-metastatic	Modulation of host cell actin network and protects against fluid stress during circulation	Mice	[113]
Larynx	*Fusobacterium nucleatum*	Pro-metastatic	enhanced the expression of miR-155-5p and miR-205-5p, leading to activation of ADH1B and TGFBR2 PI3K/Akt	LSCC patient samples, LSCC cell line	[115]
Prostate	*Trichomonas vaginalis*	Pro-metastatic	Production of cytokines such as IL-6, CCL2 and CXCL8, which lead to polarization of M2 macrophages	PC3, DU145 and LNCaP cells	[234]
Oral squamous cell carcinoma	*Porphyromonas gingivalis*	Pro-metastatic	Increase in cancer stem cell marker genes, CD44 and CD133, increased MMP1 and MMP10 and increased EMT regulators, Slug, Snail, and Zeb1. Increased vimentin and decrease E-cadherin	Ca9-22 cells	[235, 236]
Colon	*Lactobacillus plantarum* YYC-3	Anti-metastatic	Inhibition of MMP2, MMP9, and VEGFA	Caco-2 and HT-29 cells	[121]
Bladder	*Oscillatoria*	Anti-metastatic	Reduced EMT by regulating E-cadherin, Vimentin, Snail, Slug and Twist1	Patient sample data from TCGA	[122]
Bladder	*E. coli*	Pro-metastatic	Induces EMT	Patient sample data from TCGA	[122]
Lung	*Legionella*	Pro-metastatic	Not described	Patient samples	[237]

binds Gal-GalNAc signal expressed by CRC cells [119]. Notably, Fn uses the same mechanism to colonize breast tumor too [71]. Fn was also found to be enriched in the laryngeal squamous cell carcinoma (LSCC) patient with a prior history of alcohol consumption. The enrichment of Fn was shown to enhance proliferation and metastasis of the cancer cells [115]. In the presence of alcohol, Fn enhanced the expression of miR-155-5p and miR-205-5p, which in turn inhibited alcohol dehydrogenase 1B (ADH1B) and transforming growth factor b receptor 2 (TGFBR2) expression. This caused the activation of PI3K/Akt pathway ultimately leading to epithelial-mesenchymal transition (EMT). The amount of Fn was also negatively correlated with disease free survival, thereby implying the potential of this bacterium in the prognosis and clinical management of LSCC. It can, therefore, be concluded that targeting Fn could be an effective treatment strategy to suppress the metastasis in multiple cancer types (Fig. 3).

The microbes do not always promote metastasis, rather many microbial species are known to inhibit metastasis too, thereby providing a natural way to prevent cancer progression. In colorectal cancer, *Lactobacillus* and *Lactococcus* species and their metabolites have been shown to have anti-metastatic effect. Nisin, a metabolite secreted by *Lactococcus lactis* inhibits metastasis by suppressing the expression of MMP2F and CEA, marker genes for colorectal cancer metastasis [120]. The metabolites from *Lactobacillus plantarum YYC-3* were shown to mediate their anti-metastatic effect by regulating VEGF-MMP2/9 pathway [121].

In the muscle invasive bladder cancer, the intratumoral microbial species were found to show strong correlation with the expression of EMT and ECM genes [122]. For instance, *Oscillatoria* spp showed negative correlation with mesenchymal markers (Vimentin, Snail, Slug and Twist1) but positive correlation with epithelial marker (E-cadherin) while *Saccharomonospora viridis*, *E. coli* and butyrate secreting bacteria showed opposite correlation with these EMT marker genes.

Recently, Ma et al. reported that the intratumoral microbiome consisted of both pro- and anti-cancer microbes by analysing the transcriptomic data from TCGA. The presence of microbes including *Listeria monocytogenes*, *Methylobacterium radiotolerans*, *Xanthomonas albilineans*, and *Bradyrhizobium japonicum* showed negative correlation with prostate cancer biomarkers [123]. *Methylobacterium radiotolerans JCM 2831*, in particular, showed negative correlation with tumor node metastasis. The presence of many bacteria including *Staphylococcus aureus*, *Paraburkholderia phymatum*, *Pseudomonas putida* and *Haemophilus*

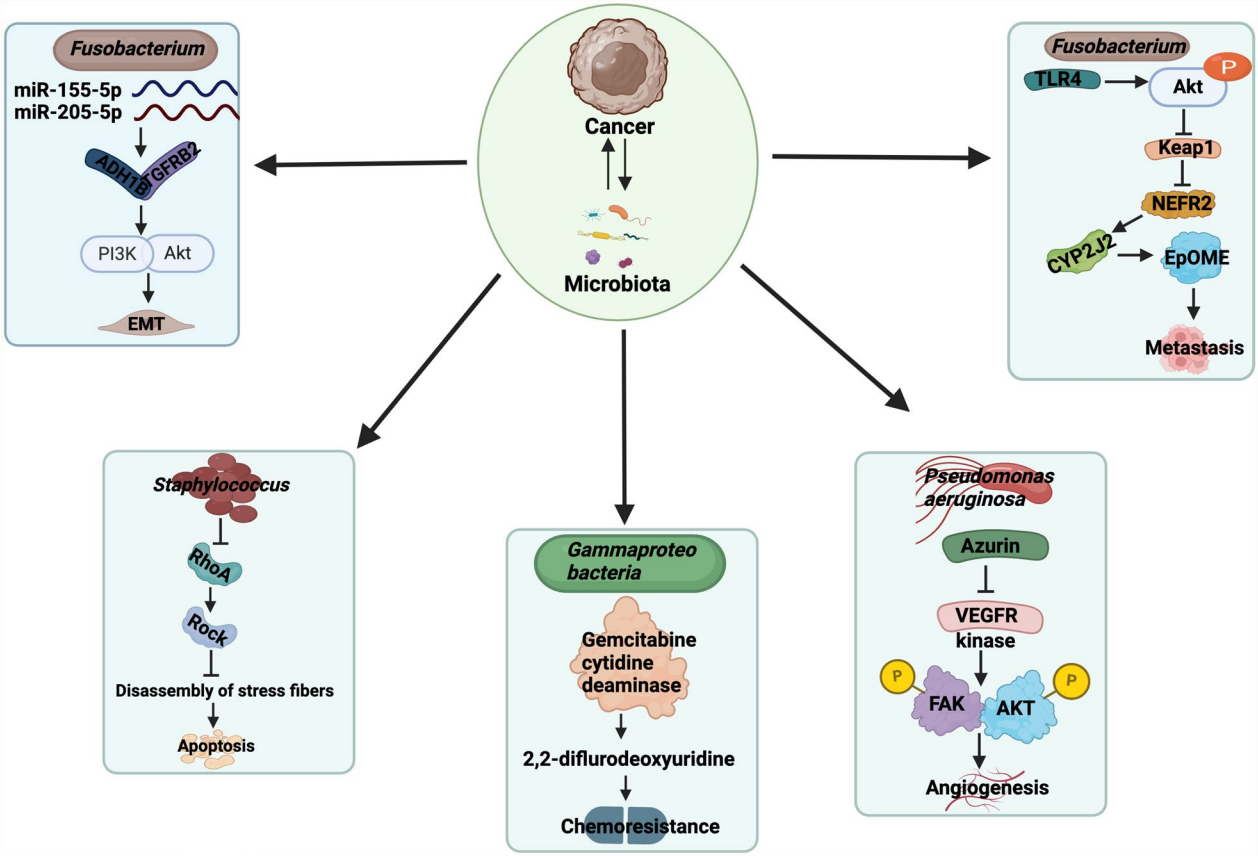

Fig. 3 Mechanism of action of microbiota involved in cancer initiation, progression, angiogenesis, metastasis and chemoresistance: microbiota is a critical parameter for origin and progression of cancer. The cancer cells too can promote the growth of specific microbes while preventing the growth of others. The figure highlights a few examples of microbes regulating different hallmarks of cancer. *Fusobacterium* has been shown to regulate EMT and metastasis through miR/PI3K/Akt pathway in laryngeal squamous cell carcinoma and TLR4/Keap1/NRF2 axis in colorectal cancer, respectively. *Staphylococcus* prevents apoptosis in metastasizing lung cancer cells by inhibiting the expression of RhoA and ROCK proteins. Gammaproteobacteria promotes chemoresistance towards gemcitabine by converting it into an inactive form through the action of its enzyme, cytidine demainase in colon cancer. *Pseudomonas aeruginosa* produces a protein, Azurin which can prevent angiogenesis by inhibiting the phosphorylation of FAK and Akt protein in melanoma xenograft mice models

parainfluenzae correlated with the expression of many stem cell genes. Since the cancer stem cells are often associated with metastatic properties, these bacteria may promote metastasis [123].

The importance of intratumoral microbial species in metastasis have been demonstrated in other cancers too (Table 2). All these studies indicate that the specific targeting of microbes in cancer could inhibit metastasis and thus serve as an important part of anti-cancer combination therapies.

Microbiota and chemoresistance

Besides the fundamental role of microbiota in different tumors, certain microbes have been observed to exhibit resistance to anti-cancer therapies. Bacterial and fungal microbiota has been known to contribute to chemoresistance, specifically by affecting the drug metabolism and transport, by altering drug efficacy and cytotoxicity [124]. Cyclophosphamide (CTX), an anti-cancer drug for haematological malignancies and solid tumors, was shown to modulate gut microbial composition and promote the transfer of specific gram-positive bacteria from the small intestine into secondary lymphoid organs. This causes the activation of pathogenic T-helper cells, thereby, promoting the anti-cancer activity of CTX [125, 126]. Abolishing the gut microbiota, specifically, *Barnesiella intestinihominis* and *Enterococcus hirae* in either germ free mice or by antibiotic administration causes drug resistance to cyclophosphamide, highlighting the role of gut microbiota in chemoresistance [125]. However, antibiotics have also been linked with increased resistance in the bacteria leading to counterintuitive results [59, 127].

Studies further indicate that microbiota modulation helps in increasing the efficiency of cancer therapies and

promote a better prognosis via altering metabolism and immune response. García-González et al. demonstrated that administration of *E.coli* impacts the efficiency of 5-fluoro-2′-deoxyuridine (FUDR) chemotherapy in *C. elegans* by modulating metabolism [128]. Similarly, *Faecalibacterium* in melanoma patients caused massive immune response by increasing immune cells and antigen presentation, higher infiltration of the tumor bed by cytotoxic CD8 + T cells during anti-PD-1 therapy [129]. It was proposed that microbiota may also be used as a biomarker to predict the therapeutic response and efficacy of chemo and immunotherapy in patients [64]. Ni et al. have further revealed the diagnostic potential of human microbiota by calculating the dysbiosis index (Ddys), which reflects microbial disturbance in the fecal samples of HCC patients. The dysbiosis index was calculated based on the relative abundance of probiotic bacteria in comparison to harmful bacteria in fecal samples of HCC patients [130]. Further, using pre-clinical models, it has been observed that microbiota modulation by employing probiotics or microbial products can boost therapeutic response and decrease tumor growth and invasion. Not only that, live engineered or attenuated bacteria and their purified products such as proteins or peptides have also been developed as anti-cancer agents. For instance, Azurin, a small protein produced by *Pseudomonas aeruginosa*, acts as a potent anti-cancer agent with higher affinity by inducing cancer cell toxicity and preventing angiogenesis (Fig. 3) [131, 132]. In addition, bacteria can be engineered to express tumor-specific antigens, checkpoint blocking antibodies and a linker polydopamine on its surface, and display specific targeted immune response to tumor [133]. The tumor-targeting *Salmonella typhimurium* strain, VPN20009 was transformed to produce Violacein, which shows cytotoxic effects in vitro and in vivo [134]. Such genetically engineered bacteria or their products suppress cancer growth by affecting metabolism and defence mechanism [57].

Thus, the microbiota has great potential in developing more efficient cancer treatments. The microbes can also be engineered for specific treatment purposes. However, further research studies are warranted on the composition and diversity of the microbiota and their correlation with respect to the specific cancer.

Chemotherapy induced alterations in microbiota

Chemotherapy has been known to induce major changes in the gut microbiota too. The gut microbiota, in response, modulates the efficacy and toxicity of chemotherapy through metabolic flux and immunomodulation. In the intestine, chemotherapy can damage the mucus layer, thus enabling some of the intestinal microbiota to penetrate the lamina propria and induce immunogenic responses. Chemotherapy also modulates richness and diversity in the intestinal microbiota of colorectal cancer patients which can in turn influence chemotherapeutic outcomes [135]. Cong et al. studied the microbial ecological networks in fecal matter to understand the changes in intestinal microbiota following chemotherapy of colorectal cancer patients. They showed that in patients undergoing chemotherapy, the connectivity among the microbial networks increased by over 50% while the modularity decreased by over 40% in comparison to healthy individuals. This highlights that the interspecies interaction among the microbial species in patients undergoing chemotherapy is tightly linked (connectivity) but the links with similar modules have reduced (modularity). The inconsistency in connectivity and modularity further implies that there is significant imbalance in the microbial networks in colorectal cancer patients following chemotherapy. Strikingly, interactions among the species were negative in nature, which signifies the competition or predation among microbial species in colorectal cancer patients undergoing chemotherapy. The negative links decreased significantly after five rounds of chemotherapy. They also found that the species, *Fusobacterium*, *Bacteroides*, and *Faecalibacterium* showed correlation with the tumour markers, CEA, CA724, and CA242, respectively, but their abundance was not affected by different chemotherapy stages [135]. It is, therefore, quite obvious to imagine that the chemotherapy associated side effects could be circumvented by favouring the growth of specific gut microbiota. Blaustein et al. reported that specific microbial species which show resistance against chemotherapeutic drugs through biotransformation and not by efflux mechanisms, can alter the toxic effects of drugs [136]. They used a reductionist approach to identify the microbes which could overcome the negative effects of doxorubicin. *Clostridium innocuum*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Lactobacillus rhamnosus* were grown in anaerobic conditions and treated with doxorubicin. It was found that *Escherichia coli* and *Klebsiella pneumoniae* were able to resist the drug at all concentrations and at higher concentrations, they could transform the bioactive drug. *K. pneumonia* was more effective drug transformer than *E. coli*. Notably, the bacterial transformation of doxorubicin had a positive effect on the other microbial communities including *Clostridium innocuum* and *Enterococcus faecium*. In future, such probiotics could be harnessed, which promotes the protective microbiota in the gut and thus prevent the gastrointestinal side effects of chemotherapeutic drugs. The microbiota can also abrogate the drug efficacy by metabolizing the drug into its inactive form. For instance, in colon cancer mice model, gemcitabine was shown to be metabolized into its

inactive state, 2′,2′-difluorodeoxyuridine by gammaproteobacterial enzyme, cytidine deaminase (Fig. 3) [137]. Gemcitabine is also used for pancreatic cancer and the study further showed that 76% of the pancreatic cancer patients exhibited the presence of gamma proteobacteria and culturing these bacteria with colon cancer cell lines was sufficient to make them resistant to gemcitabine. The enzyme, cytidine deaminase, is also expressed by *Mycoplasma hyorhinis* and its infection in tumor cell cultures abrogates the effect of gemcitabine. The addition of deaminase inhibitor restored the anti-cancer activity of gemcitabine [138].

5-fluorouracil (5FU) is among the most widely used anti-cancer drugs. It is used in the treatment of breast cancer, colorectal cancer, gastric cancer, pancreatic cancer and stomach cancer. Its mode of action involves DNA damage leading to apoptosis or inhibition of RNA synthesis [139]. 5FU induces intestinal mucositis through NFkB/MAPK pathway and is often accompanied by alterations in the gut microbiota and the cytokine/chemokine profile [140]. 5FU reduced the abundance of firmicutes, proteobacteria and cyanobacteria in faeces. The fecal microbiota transplant from healthy mice was shown to reduce the severity of 5FU induced mucositis. In contrast, another study showed that the gut microbiota enhances the efficacy of 5FU. The treatment of colorectal mice model with antibiotics significantly reduced the anti-cancer effect of 5FU. This was because the administration of antibiotic resulted in decrease of *Lactobacillus*, *Alistipes* and *Rikenella* and increase of pathogenic bacteria such as *Escherichia shigella* and *Enterobacter* [141].

Oxaliplatin forms the first line of chemotherapy for patients with advanced colorectal cancer. It was recently shown that butyrate, a metabolite produced by gut microbiota, could promote the anti-tumor effect of oxaliplatin. Butyrate was shown to activate CD8 + T-cells in an ID2-dependent manner. In the colorectal cancer patients, the responders exhibited higher levels of serum butyrate in contrast to the non-responders. This observation implies that butyrate produced by the gut microbes could be the deciding factor for patient response to oxaliplatin [142].

Radiotherapy and microbiota

It has been shown in multiple studies that radiotherapy and microbiota have an intertwining relationship. While radiation therapy could kill beneficial microbiota, certain microbial species could also enhance the sensitivity of radiotherapy by influencing immune system of the patient. *Fusobacterium nucleatum* present in the buccal cavity was shown to relocate to the colorectal tumor and negatively impact the therapeutic efficacy of radiotherapy. The treatment with metronidazole, and antibiotic against *Fusobacterium*, was shown to act as a radiosensitizer in colorectal mice models [143].

The synthesis of butyrate by *Lachnospiraceae* is also associated with radiotherapy resistance. The butyrate was shown to suppress STING-activated expression of IFN 1 in dendritic cells by blocking the phosphorylation of TBK1 and IRF3. This suppression disrupted the function of cytotoxic T-lymphocytes induced by radiation, thereby protecting the tumour cells. The treatment with vancomycin eliminated *Lachnospiraceae* resulting in better response to radiotherapy in cancer [144].

In a comparative study on the effect of bacteria and fungi on radiotherapy in induced melanoma and breast cancer in mice models revealed interesting insights. It was observed that while removing fungi improved the effectiveness of radiotherapy, eliminating bacteria diminished the response to radiotherapy. The knockdown of Dectin-1, a receptor present in immune cells which serve as a sensor for fungal infection, also enhanced the response to radiotherapy. The high expression of Dectin-1 is also known to be correlated with poor survival in breast cancer patients [124].

Impact of intratumoral microbiota on therapeutic response

Emerging evidence indicate the role of intratumoral microbiota in modulating the efficacy of and resistance to anti-cancer therapy. Intratumoral administration of *E. coli* in a mouse model of colorectal carcinoma reduced the anti-tumor activity of Gemcitabine and enhanced chemoresistance by biotransforming the drug [145]. Incubation of cervical cancer cells with intratumoral *Lactobacillus iners* causes resistance to chemotherapy and radiation by enhancing tumor metabolism and modulating lactate signalling pathway [146]. Notably, administration of *Peptostreptococcus anaerobius* in a mouse model of CRC abrogated the effectiveness of anti-PD1 therapy by triggering the immunosuppressive activities of intratumoral MDSCs [147]. However, administration of *Fusobacterium nucleatum* in mouse allografts and humanized mice model of microsatellite-stable colorectal cancers sensitizes tumor cells to anti-PD-1 therapy through the secretion of butyric acid [148]. Thus, the effectiveness of anti-cancer therapy can be either enhanced or diminished by intratumoral microbiota, depending on the presence of specific microbial species.

Microbiota modulation for cancer therapy

Conventional cancer therapies often pose numerous limitations such as the collateral damage to normal cells, the possibility of therapy resistance and their inability to fully penetrate the tumor. Thus, there is an urgent need to develop new and better approaches for amelioration of

cancer [149–151]. Literature suggests that intervention of the intestinal microbiota in cancer patients can potentiate the current anti-cancer drug regimens such as chemo and immunotherapy (Table 3) [73]. It has sparked the interest of many researchers in bacteria-mediated cancer therapy. This approach utilizes bacterial components such as proteins, peptides and immunotoxins to target and colonize the specific tumor. These components possess cancer specific cytotoxic activity that led to the elimination of tumor cells. This method may be combined with other approaches for an integrated and efficient treatment of cancer patients [152].

This approach was first brought to light in 1868 by Dr. William B. Coley, who observed cancer regression in a patient after a bacterial infection [153]. Later, Dr. William Coley administered "Coley toxins"—a cocktail of various bacterial strains, including *Streptococcus* in patients for tumor reduction [154, 155]. Since then, advancements in rDNA technology and a better understanding of the TME have led to identification of various obligate or facultative anaerobes as potential candidates for bacteria mediated anti-cancer therapies [151]. These bacteria, belonging to *Clostridium*, *Listeria*, *E. coli*, *Salmonella* and *Bifidobacterium species*, possess cellular constituents such as cell membranes, vesicles and others that exhibit tumor targeting and tumor killing properties [156]. For instance, successful treatment of bladder cancer involved the use of Bacillus Calmetter-Guerin (BCG). It is a live, attenuated strain of *Mycobacterium bovis*, which was administered directly into the bladder for treating transitional cell carcinoma [151].

Recently, *Salmonella* has garnered a lot of attention for its efficient colonization of solid and semi-solid tumors [157]. *Salmonella* promotes apoptosis after migrating towards tumor core from tumor edge within 48 h and colonizes the entire tumor mass within 72 h post injection [158]. Different strains of *Salmonella* also destroy tumor by inducing apoptosis and autophagy through various mechanisms including triggering immune cells and immune reaction of the host [149]. Among other salmonella strains, *S. Typhimurium* is widely studied for its tumor destroying properties [157]. In another independent study, researchers engineered obligate anaerobic *S. typhimurium* strain YB1 and observed suppression of tumor growth by injecting this modified bacteria in the tumor core in neuroblastoma murine model [159]. Some other bacterial strains displaying inherent cancer cytotoxic properties include *Streptomyces fradiae* against colorectal cancer, *Pseudomonas aeruginosa* against prostate carcinoma, *Clostridium novyi* against colon carcinomas, *Enterobacter cloacae* against leukemia and *Brevibacillus* spp. against breast cancer [160–163]. Notably, supplementation of αPD-1 immunotherapy with *Lactobacillus johnsonii* increases the efficiency of immunotherapy via production of IPA, which alters the stemness of $CD8^+$ T cells in breast, melanoma and colorectal tumors [164].

The bacterial strains exhibit varied mechanisms to target cancer cells. They may target and colonize the specific tumor sites and thereby inhibit tumor progression or they may modulate the TME and trigger the immune response. Their specificity and precision, further, prevents damage to the neighbouring normal tissues surrounding the tumor. Moreover, they possess extensive motility, which helps in deeper penetration of the tumor and hence can even act as delivery vectors, enhancing the effect of chemotherapeutic drugs [149–152]. For instance, *Bifidobacterium longum* has been engineered to display WT1 protein and upon administration in mice, the bacterium potentiates tumor infiltration of CD4 + T and CD8 + T cells, cytokine production, and cytotoxic activity in a WT1-specific manner without any side effects [165]. Recombinant attenuated *Salmonella* strain SL7207 was used as a vehicle for delivery of engineered tumor vaccine in melanoma mice model [166]. Live bacteria can also be conjugated with nanoparticles to create an efficient drug delivery system [150].

However, bacteria mediated anti-cancer therapeutics present several challenges including short half-life, DNA instability and intrinsic pathogenic potential of the microbe. These barriers can be overcome by using genetic engineering approaches. Genetic engineering has helped in deletion of some of the virulence genes of pathogenic strains, and thus their anti-tumor activity, specificity and colonization can be controlled [167]. Currently, several clinical trials are determining the effect of functionalized *Salmonella Typhimurium* strains. These strains have either been engineered through various genetic techniques or have undergone surface modifications by nanoparticles or other agents to exhibit desired tumor targeting and colonization [155].

Other recent strategies for microbial intervention in cancer therapy includes fecal microbiota transplantation, supplementation with probiotics and prebiotics.

Fecal microbiota transplantation (FMT) in cancer therapy

FMT is an emerging therapeutic approach that involves transferring gut microbiota from healthy donors to patients. Recent studies have shown promising results in using FMT to reshape microbial dysbiosis and potentially inhibit cancer progression, particularly colorectal cancer. CRC is usually accompanied by intestinal microbial dysbiosis. The administration of fecal samples from CRC patients to both germ-free and conventional mice made them develop more intestinal tumors compared to those

Table 3 Microbiota that increases the efficiency of immunotherapy in various tumors

Cancer	Microbe	Effect on immunotherapy	Mode of action	Model organism/cell line	References
Melanoma	*Bifidobacterium* spp	Enhance the efficacy of PD-L1 inhibitors	Activation of dendritic cells and enhancement of tumor-specific CD8 + T cells	C57BL/6 mice with B16.SIY melanoma	[196]
Colorectal cancer	*Lactobacillus rhamnosus*	Synergized the efficacy of PD-L1 inhibitors and increased tumor inhibition	Increased the relative abundance of beneficial bacteria	Specific pathogen-free (SPF) BALB/c mice	[239]
Non-small cell lung cancer (NSCLC)	Probiotics	Enhance the efficacy of PD-L1 inhibitors	Enhanced progression-free survival and favorable clinical outcomes	Patients	[240]
Epithelial tumors (Sarcoma, Melanoma, NSCLC, Renal cell carcinoma)	*Akkermansia muciniphila*	Restored the efficacy of PD-1 blockade	Promote the recruitment of CCR9 + CXCR3 + CD4 + T lymphocytes	C57BL/6 and BALB/c Mice model, Patients	[241]
Melanoma, Sarcoma, NSCLC	*Bacteroides fragilis*	Reestablished the reduced antitumor effects of CTLA-4 blockade	Modulates IL-12–dependent TH1 immune response	Patients and mice model	[242]
Renal cell carcinoma	*Clostridium butyricum*(CBM 588)	Enhance the clinical outcome of nivolumab-ipilimumab treatment	Enhance the abundance of Bifidobacterium, Lactobacillus, and Lactococcus spp, increase the levels of IL-1β, G-CSF, IL-10, IL-12, GM-CSF, CCL4, TNF-α, and increase the number of IL-17A-producing cells (including γδT cells and CD4 cells)	Patients and mice model	[243, 244]
Subcutaneous tumour	Consortium of 7 Bacteroidales and 4 non-Bacteroidales species	Enhances therapeutic efficacy of anti-PD-1 antibody	Increases interferon-γ-producing CD8 + T cells and expression of MHC class I	germ-free mice	[87]
Melanoma	*Bifidobacterium longum*	Increased efficacy of anti-PD-L1 therapy	Increased frequency of dendritic cells, activated T cell's response, induced number of SIY-specific CD8 + T cells,	Patients and mice model	[245]
Melanoma	*Clostridiales/Ruminococcaceae*, *Faecalibacterium* spp	Enhances the efficacy of anti-PD-L1 therapy and augment progression free survival	Enhanced systemic and antitumor immunity by inducingamino acid biosynthesis	Patients and germ-free recipient mice	[129]

receiving fecal samples from healthy individuals. This finding underscores the significant role of gut microbiota in CRC [168].

In a study involving a CRC mouse model, researchers found that FMT from healthy mice significantly reversed gut dysbiosis in CRC mice. This led to a suppression of cancer progression, increased survival rates, and enhanced anti-cancer immune responses. The transplantation resulted in a higher infiltration of immune cells like CD8 + T cells and NK cells, which can directly attack cancer cells, and reduced the number of immunosuppressive cells like Foxp3 + Treg cells. The modulation of inflammatory cytokines was also observed, with a decrease in pro-inflammatory cytokines (IL1a, IL6, IL12a, IL12b, IL17a) and an increase in anti-inflammatory cytokine IL10. This suggests that FMT can help in creating a more favourable immune environment for fighting cancer [169].

While these findings are promising, more research is needed to fully understand the mechanisms and to translate these results into clinical practice.

Clinical trials with FMT

The potential of FMT as a cancer therapy is also being explored in the clinical trials. In phase-II trial (NCT04951583) on advanced cutaneous melanoma (FMT-LUMINate Trial), the safety and efficacy of FMT in combination with immunotherapy (ipilimumab and nivolumab) was evaluated in patients. It showed objective response rate (ORR) of 70%, with 2 complete responses (CR) and 12 partial responses (PR). However, immune-related adverse events (irAEs) were common, with diarrhoea or colitis being the most frequent grade 3 irAE. The metagenomic profile showed an enrichment of *Prevotella copri*, *Ruminoccocaceae* and *Eubacterium* post-FMT in responders. Further, the faeces of responders one-month post-FMT were demonstrated to inhibit the tumor growth in murine models when compared with feces of non-responders [170].

The same trial (NCT04951583) also evaluated FMT treatment with pembrolizumab (immune checkpoint inhibitor) in patients with advanced or unresectable non-small cell lung cancer. There were less irAEs recorded with NSCLC patients than in melanoma patients; indicating that microbiota can show differential irAEs which may be attributed to the specific donor used in these trials [171]. The ORR and CR of patients with NSCLC is yet to be published.

FMT with Nivolumab as therapy for advanced solid cancers was also evaluated in clinical trial (NCT04264975). The patients enrolled for this study had advanced, unresectable, or metastatic solid cancer which has progressed during anti-PD-(L)1 therapy. The FMT from the anti-PD1 responder in 13 patients with anti-PD-1-refractory advanced solid cancers resulted in significant microbiota changes and clinical benefits in 6 patients with ORR of 7.7%. The FMT responders showed the presence of *Prevotella merdae* Immunoactis which could suppress tumor growth by enhancing the cytotoxic T-lymphocyte infiltration to the tumor site [172].

FMT has also been tested in melanoma patients who have not responded to PD-1 inhibitors such as pembrolizumab or nivolumab (NCT03341143). The patients received FMT from anti-PD1 responders which was then followed by further cycles of pembrolizumab. The combination resulted in stable transformation of microbiota and offered clinical benefits to 6 out of 15 patients. It resulted in noticeable changes in the microbiota composition, increased CD8 + T cell activation, and reduced frequency of interleukin-8-expressing myeloid cells. CD8 + T cells are responsible for targeted killing of tumor cells and IL-8 expressing myeloid cells are involved in immunosuppression, which aids the tumor progression [173].

Many such clinical trials involving FMT are currently underway, and a brief summary of the trials, where some preliminary results are available, is presented in Table 4.

Probiotics

Considering the significance of microbiota in cancer, probiotics are being explored for promoting the growth of healthy gut microbiota and as a component of cancer therapy regimen. Their mechanism of action includes alteration of immunity, reduction of growth of pathogens, and enhance the intestinal barrier function [86]. A few clinical trials showed a promising outcome of patients after probiotics supplementation, while others did not observe any major impact produced by probiotics [174, 175]. In a recent cohort study, low and moderate intake of probiotics has been found to be significantly associated with decreased risk of cancer mortality [176]. *Lactobacillus rhamnosus* GG (LGG), is a probiotic, which protects mouse epithelium from radiation by releasing lipoteichoic acid (LTA), which in turn activates pericryptal macrophages by binding to TLR2 to secrete chemokine CXCL12. This chemokine then causes the migration of mesenchymal stem cells by binding to CXCR4 receptor, thus protecting epithelial stem cells from radiation-induced apoptosis [177]. Another probiotic, Prohep, which is composed of *Lactobacillus rhamnosus* GG (LGG), viable *Escherichia coli* Nissle 1917 (EcN), and heat-inactivated VSL3, was used to reduce the tumor growth by diminishing Th17 cells and IL-17 cytokine in a mouse model of HCC [178]. In addition to inhibition of cancer progression, probiotic *Akkermansia muciniphila* has been used in orchestrating liposome for improving pharmacokinetic profile and targeted delivery

Table 4 Clinical trials involving fecal microbiota transplantation

Trial name	Cancer type	Intervention type	Key findings	Phase	Clinical trial number	References
TACITO trial	Metastatic renal cell carcinoma	FMT + Immunotherapy + Chemotherapy (anti PD-1/PD-L1 + VEGFR-TKIs)	Preliminary results suggest FMT may improve response to pembrolizumab plus axitinib. 1-year PFS rate was 66.7% with FMT vs. 35.0% with placebo. ORR was 52% with FMT vs 28% with placebo	2	NCT04758507	[246]
FMT-LUMINATE	Non-small cell lung cancer	FMT + Immunotherapy (pembrolizumab)	irAE occurred in 79% patients. Out of 20 patients, ORR was 77% in 14 patients. Final ORR not yet available as the study is ongoing	2	NCT04951583	[171]
FMT-LUMINATE	Melanoma	FMT + Immunotherapy (ipilimumab and nivolumab)	irAE occurred in 80% patients and ORR was 70%	2	NCT04951583	[170]
MITRIC	Solid tumors (melanoma, head and neck cancer, renal clear cell carcinoma, non-small cell lung cancer)	FMT + Immunotherapy	The study is ongoing. Preliminary data shows that irAE occurred in 3 of 9 patients. One patient showed clinical benefit	2	NCT05286294	[247]
CBM588 study	Metastatic renal cell carcinoma	Live bacterial product (CBM588) + Immunotherapy + Chemotherapy (nivolumab + cabozantinib)	ORR was 63% in patients receiving CBM588 + nivolumab + cabozantinib. ORR was 33% if treated with only nivolumab + cabozantinib	1	NCT05122546	[248]
FMT in melanoma patients	Melanoma	FMT + Immunotherapy (Pembrolizumab)	Clinical benefit in 6 out 15 patients. Minimal adverse events and improved ORR	2	NCT03341143	[173]
Utilization of microbiome as biomarkers and therapeutics in immuno-oncology	Solid cancers (gastrointestinal cancers, including GC, ESCC, and HCC)	FMT + Immunotherapy	Clinical benefit in 6 out of 13 patients. Responders showed increased abundance of beneficial microbial taxa, enhanced CD8 + T cell activation, and reduced interleukin-8-expressing myeloid cells	1	NCT04264975	[64]

ICI immune checkpoint inhibitor, *ORR* overall response rate, *irAE* immune related adverse effect

of 5FU, which is a first-line chemotherapy for CRC [179]. *E. coli* as a probiotic has been engineered to express a collagenase and an immunotoxin to degrade the collagen present in TME and reduced tumor growth in breast cancer. The anti-tumor effects of this engineered probiotic have been further enhanced in combination with a chemotherapeutic drug, doxorubicin [180]. Another example of engineered probiotic includes *Saccharomyces cerevisiae* var. boulardii (Sb), a yeast with anti-cancer activity. The yeast was engineered to secrete an antibody that can bind to programmed death ligand 1 (Sb_haPD-1), which decreased the tumor growth and improved the immune cell profile and microbial composition in an ICI-refractory CRC mouse model [181]. While probiotics can offer benefits, they can also have adverse effects during cancer treatment. The therapeutic response of anti–PD-1-based therapy in melanoma mice models got impaired following the supplementation of probiotics [182]. Therefore, due to the limited research in this field, the use of probiotics should be approached cautiously to prevent potential adverse effects, especially in immunocompromised patients.

Prebiotics

Prebiotics refer to selectively fermented ingredients including dietary fibers and carbohydrate polymers that specifically facilitate the growth and/or metabolic activity of targeted gut microbiota, alter their composition and confers health benefits to host [183]. Inulin, a prebiotic fiber not only inhibits tumor growth in colon cancer and NRAS mutant melanoma syngeneic mouse models by increasing tumor-infiltrating lymphocytes (TILs) and altering gut microbiota, but also augments the efficacy of MEK inhibitor in melanoma and overcome the drug resistance [184]. Ginseng polysaccharides, another prebiotic, boosts the antitumour response to PD-1 antibody in syngeneic mouse models by reducing L-kynurenine and kynurenine/tryptophan ratio, and by enhancing the valeric acid, which in turn increases the population of T effector cells [185]. Recently, a prebiotic gum odina—sodium alginate conjugate was used to produce biopolymeric microspheres with capecitabine, which is a first line chemotherapy drug for colon cancer. These microspheres reduce drug elimination, promote capecitabine concentration within tumor and thus, reduce the tumor growth in colon cancer mouse model [186]. Additionally, following irradiation, psyllium plus inulin supplementation was shown to reduce the tumor size and markedly hinder the tumor growth by increasing $CD8^+$ cells and increasing the levels of acetate, butyrate and propionate in a mouse model of bladder cancer [187].

Therefore, supplementing with prebiotics and probiotics may provide significant benefits when combined with other anti-cancer therapies, enhancing both safety and efficacy.

Metabolism of anti-cancer drugs

The gut microbiota is also involved in biotransformation and metabolism of anti-cancer drugs leading to differential absorption and bioavailability of these drugs [188]. During the biotransformation of drugs, the microbiota employs various mechanisms such as deamination, hydrolysis, demethylation, glucuronidation and other reactive reactions [189]. The bacterial metabolism was shown to be responsible for conversion of ulcerative colitis prodrug sulfasalazine into its active ingredient 5-aminosalicylic acid (5-ASA) azo and toxic by-product sulfapyridine [190]. Sometimes, bacterial biotransformation of drug worsens the toxic side effects of the drug as seen in case of irinotecan (CPT11), which is used as a primary anti-cancer drug for colorectal cancer patients. It gets metabolized in liver into its active ingredient SN-38, which targets dividing cells and therefore, is detrimental not only to cancer cells but also to the healthy intestinal epithelium. In liver, SN-38 is converted to SN-38 glucuronide (SN-38G) by UDP-glucuronosyltransferases (UGTs). SN-38G is harmless to the intestinal epithelium, however, microbial β-glucuronidases, present in gut, can alter SN-38G into SN-38 by removing glucuronide group, thus, resulting in severe diarrhoea as a side effect of the drug [190, 191]. Proteobacteria and firmicutes transform 5FU to its inactive metabolite dihydrofluorouracil (DHFU) and exhibit metabolic mechanism of drug inactivation [192]. Recently, Wu et al. have shown that chitooligosaccharides exhibit protective potential against colorectal carcinomas by reducing the density of *Enterococcus*, *Escherichia-Shigella* and *Turicibacter* and enhancing the growth of butyrate producing bacteria [193]. Advances have been made in restoring healthy or beneficial microbiota using targeted interventions; however, the field is still in its infancy and additional studies are warranted to uncover its true potential.

Conclusion, challenges and future perspective

The global cancer statistics clearly demonstrate that the overall cure and survival rates of cancer patients remain relatively low despite the amazing advancements in the development of anti-cancer therapeutics. This accentuates the need for further investigation into complementary treatments, in order to extend the clinical benefits of anti-cancer therapy to a larger cohort of cancer patients. The increasing significance of the gut microbiota in cancer initiation, progression, metastasis, and chemoresistance has received a lot of attention lately in the hunt for alternative cancer therapeutics [194]. For instance, an edible fungus, *Auricularia delicate* exhibits protective

effects in colitis-associated colorectal cancer mice model by modulating intestinal microbiota, inhibiting NF-kB pathway and reducing inflammation [195]. The patient's microbiota is also reported to interact with immune checkpoint inhibitors used in cancer treatment, which ultimately influences the outcome of cancer immunotherapy (Table 5) [8]. Fecal microbiota transplant from JAX mice treated with anti-PD-L1 immunotherapy into Taconic mice enhanced the immunotherapy outcome. This was attributed to the role of *Bifidobacterium*. Probiotic colonization of *Bifidobacterium* enhanced the efficacy of anti-PD-L1 immunotherapy by modulating the activation of CD8 + T cells [196]. Further, the efficacy and outcome of some anti-cancer chemotherapeutic drugs such as oxaliplatin and cyclophosphamide depend on the presence of specific microbiota including gram-positive bacteria, which modify TME or increase the CD8/TReg ratio [125, 197]. However, some chemotherapeutics such as irinotecan and doxorubicin cause severe adverse effects in intestine. Therefore, the usage of such drugs requires clearance of GI tract microbiota by concurrent treatment of specific antibiotics to lessen the drug toxicity [198].

Investigators across the globe have documented modifications in microbiota during cancer initiation, progression and therapy, but there exists a vast array of methodological heterogeneity in their experimentation including differences in methods of sample selection and collection, sample size, techniques used, quality and analysis of data. This makes it challenging to comprehend the role of microbiota in cancer and anti-cancer drugs with complete fidelity. Collection of different samples from same individual, contamination of samples and next gen sequencing techniques used for different samples have further added biasness and increased heterogeneity in the results [10, 199]. To circumvent these challenges, unified methodologies and stringent standard operating procedures (SOPs) should be developed and employed across globe.

The biological and ecological variations of a patient may also cause hindrance to the wider application of microbe-based anti-cancer therapy. The therapeutic regimens for cancer are already quite complex and drug resistance, drug toxicity and recurrence further adds to the roadblocks in cancer therapy [200]. Adding another complex layer of microbes to anti-cancer treatments will introduce multifaceted challenges to cancer therapy.

To achieve homogeneity and explore specific microbe-associated mechanisms, future studies should focus on distinct microbiota stratification, creating individual microbe profiles for the unique microbes present in different individuals. In addition, specialized cell culture preclinical models like patient-derived organotypic tumor spheroids or stem cell derived organoids in 3D cultures may be employed to capture the molecular mechanistic insight into host-microbe interactions [10].

Preclinical studies suggest that personalized dietary habits combined with the intake of specific microbiota can support good gut health. This approach may enhance patients' responses to anti-cancer therapy and improve their overall quality of life [201]. In recent years, gut microbiota-based therapeutic approaches

Table 5 Abundance of microbiota in patients responding to immunotherapy

Cancer	Type of immunotherapy	Abundant microbes in responders	References
Melanoma	Anti-PD-1	*Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*	[245]
	Anti-PD-1	*Actinobacteria* phylumand the*Lachnospiraceae/Ruminococcaceae*families of Firmicutes	[249]
	Anti-PD-1	*Ruminococcaceae family*	[129]
Hepatocellular carcinoma	Anti-PD-1 antibody	Akkermansiamuciniphila and Ruminococcaceae spp.	[250]
Epithelial tumors	Anti-PD-1	*Akkermansiamuciniphila*	[241]
Colorectal cancer	Regorafenib and Toripalimab	*Fusobacteriota* and *decreased Proteobacteria* phylum in non-responders	[251]
Gastrointestinal cancer	Anti-PD-1/PD-L1	*Prevotella*, *Ruminococcaceae*, and *Lachnospiraceae*	[252]
Lung cancer	Anti-PD-1 PD-L1 ICIs	*Escherichia*, *Akkermansia*, *Shigella*, *Olsenella*, *Veillonelladispar*, *Neisseria*, *Faecalibacterium*	[253–255]
	Anti-PD-1 antibodies	*Desulfovibrio*, *Actinomycetales*, *Bifidobacterium*, *Odoribacteraceae*, *Anaerostipes*, *Rikenellaceae*, *Faecalibacterium*, and *Alistipes*	[256]
	Anti-PD-1 antibodies	*Alistipes putredinis*, *Bifidobacterium longum*, *Prevotella copri*	[257]
	PD-1 inhibitor	*Enterococcal prophage*	[258]
Breast cancer	PD-1, PD-L1, ICIs	*Bifidobacterium longum*, *Collinsela aerofacience*	[241, 259]
Gastric cancer	PD-1, PD-L1	*Prevotella*, *Ruminococcacea*, *Lachnospiracea*	[252]
Glioblastoma	Viroimmunotherapy	*Bifidobacterium* and *Akkermansia*in treated mice	[260]

have shown a lot of promise in improving the efficacy and reducing the adverse effects of anti-cancer therapy in cancer patients, thus encouraging the usage of microbiota-based precision medicine.

Despite the evidence of microbiota modulation in cancer patients, clinical interventions targeting microbes have not yet reached cancer patients due to individual variability in genetics, age, diet, sex, geography and microbial sensitivity. Therefore, more preclinical and clinical trial-oriented research is warranted for the development of a comprehensive approach that can integrate microbiota modulation strategy with the existing anti-cancer therapies. Such an approach will foster the development of precise and effective cancer therapies.

Abbreviations

SCFA	Short chain fatty acid
TME	Tumor microenvironment
CSCs	Cancer stem cells
OCT4	Octamer-binding transcription factor 4
SOX2	SRY-related HMG box 2
OSCC	Oral squamous cell carcinoma
NAC	Neoadjuvant chemotherapy
CRC	Colorectal cancer cells
Fn	*Fusobacterium nucleatum*
LSCC	Laryngeal squamous cell carcinoma
ADH1B	Alcohol dehydrogenase 1B
TGFBR2	Transforming growth factor b receptor 2
EMT	Epithelial-mesenchymal transition
CTX	Cyclophosphamide
FUDR	5-Fluoro-2′-deoxyuridine
HCC	Hepatocellular carcinoma

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Author contributions
YKA conceived the idea. YKA and RC designed the concept of the manuscript. YKA and RC wrote the initial draft of the manuscript. YKA and RC finalized, revised, and presented the final concept of the manuscript. YKA and RC prepared the figures and the tables of the manuscript. All authors have read and approved the final manuscript.

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Declarations

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