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INVITED REVIEW

The microbiome(s) and cancer: know thy neighbor(s)

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Abstract

The human microbiome is essential for the correct functioning of many host physiological processes, including metabolic regulation and immune responses. Increasing evidence indicates that the microbiome may also influence cancer development, progression, and the response to therapy. Although most studies have focused on the effect of the gut microbiome, many other organs such as the skin, vagina, and lungs harbor their own microbiomes that are different from the gut. Tumor development has been associated with dysbiosis not only in the gut but also in the tissue from which the tumor originated. Furthermore, the intratumoral microbiota has a distinct signature in each tumor type. Here, we review the mechanisms by which the organ-specific microbiome can contribute to carcinogenesis: release of toxins that cause DNA damage and barrier failure; alteration of immune responses to create a local inflammatory or immunosuppressive environment; and regulation of nutrient levels in the tumor microenvironment through metabolite production and consumption. Solving the puzzle of how the microbiome influences the carcinogenesis process and treatment response requires an understanding of the two ways the microbiome can interact with cancer cells and the tumor microenvironment: through systemic effects exerted by the gut microbiota and local effects of the intratumoral microbiota.

Keywords: microbiome; local microbiota; intratumoral microbiota; dysbiosis; cancer; tumor microenvironment; DNA damage; inflammation; microbial toxins; microbial metabolites

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Cancer and the microbiome

Cancer is a major health threat and follows only cardiovascular disease as a leading cause of death worldwide [1,2]. Cancer is a multifactorial disease; although much focus has been placed on genetic causes, epidemiological studies have shown that a high percentage are linked to environmental factors [3]. For example, human papilloma virus infection and tobacco smoking are responsible for up to 90% of cervical squamous cell carcinomas and lung cancers, respectively [4]. Among the extensive list of environmental risk factors, the commensal microbiota is emerging as a major modulator of carcinogenesis, immune response, and treatment efficacy [5–7].

The human microbiota is defined as the community of all microorganisms living in association with the human body. It contains members of different kingdoms, including bacteria, archaea, fungi, small eukaryotes, and viruses. This collection of microbes, their genomes, the metabolites they produce, and the proteins they express are known as the microbiome. The number of microbial genes alone is estimated to be 100 times greater than the human genome [8], so it is no surprise that the microbiome carries out key functions in the human body [9,10]. The complex microbiome–host ecosystem is the result of millions of years of coevolution, establishing what has been described as the 'super-organism' [11]. Microbial colonization of the human body begins at birth and develops throughout childhood until reaching its adult composition [12,13], a process that depends on both external (lifestyle) and internal factors (genetics or immune system) [14]. The microbiome is beneficial to humans in many ways, including its fundamental contribution to the development and education of the immune system [15]. However, failure to maintain microbiome–host homeostasis is directly and closely related to many diseases [13,16–19].

It is estimated that individual microbial pathogens contribute to cancer development in approximately 15–20% of total cases [20]. However, recent studies have suggested that cancer initiation and progression are impacted not only by single pathogens, but also by global changes to the microbiome (referred to as dysbiosis) [21,22]. The interactions between the microbiome and host during cancer are complex. Animal studies using germ-free mice or antibiotics to deplete the intestinal microbiota have illustrated the role of the microbiome in promoting different types of cancer [23–25]. On the other hand, microbial antitumoral effects have also been reported [21,26], with several bacterial toxins

and some pathogen-associated molecular patterns that prevent tumor growth by activating the immune system [26]. All these findings highlight the need for new types of microbiome intervention that encourage the expansion of beneficial over pathogenic microorganisms.

Several lifestyle factors associated with cancer risk have been shown to act via microbiota-related mechanisms. The Western diet, and specifically the consumption of processed meats, is associated with different types of cancer [27], and the microbiota has been shown to be essential in mediating some of the carcinogenic effects. Heme iron, the pigment of red meat, induces epithelial damage in the colon and leads to hyperplasia only in the presence of gut microbiota [28]. Sulfur compounds used to preserve processed meat are metabolized by sulfur-reducing bacteria present in the colon to hydrogen sulfide, a metabolite implicated in carcinogenesis through numerous mechanisms [29]. Apart from diet, epidemiological studies suggest that obesity is a risk factor for certain types of cancer [30]. Recently, the obesity-associated gut microbiota has been proposed to underlie some aspects of this relationship. Evidence indicates that changes in gut microbiota induced by obesity cause a senescence-associated secretory phenotype in hepatic stellate cells, which secrete inflammatory and tumor-promoting factors in the liver [31]. These alterations in the gut microbiota have also been linked to histone methylation and acetylation associated with signaling pathways central to the development of colon cancer [32]. Finally, aspirin and other non-steroidal anti-inflammatory drugs have been suggested to contribute to cancer risk reduction [33], in part due to alteration of the microbial communities [34]. Findings from a clinical trial indicated a possible beneficial effect of aspirin on the gut microbiota, reducing several bacteria linked to inflammation and colorectal cancer (CRC) [35]. Similar results have been reported in an animal study [36], which also revealed that aspirin's chemopreventative effects depend on the gut microbial composition. Therefore, the interplay between the microbiome and cancer is not simple, and it is affected not only by the genetic landscape, but also by many epidemiological factors, including diet, lifestyle, and aging, among others. Moreover, all these factors influence the microbiome in a continuous manner, and one limitation of microbiota studies in cancer is the use of dichotomy models, i.e. antibiotics versus control. Given this intricate relationship, it has been proposed that interdisciplinary approaches, such as molecular pathological epidemiology [37], are necessary to finally establish the role of the microbiome in cancer [38,39].

The vast majority of the human microbiota resides in the gastrointestinal tract, particularly in the colon, and it can interact both locally and systemically with cancer cells. This may be why the gut microbiome is the most studied, and the model system to understand the microbiome–host relationship [22]. The identification of microorganisms in other parts of the body has been challenging due to their low biomass. Indeed, other organs, such as the lungs or breast tissue, were considered to be sterile until a few years ago [40,41]. Together, this has led to a disregard of the role of the local (organ-specific) microbiome in carcinogenesis. In recent years, the scientific community has made a big effort to detect and characterize the microorganisms present in healthy organs and tissues. Specific microbial populations have been described for many organs [40–45], revealing a different microbiome signature for each [46–48]. Thus, the specific local microbiome probably plays a key role in the development of cancer in organs distant from the gut.

Intratumoral microbiota, a new component of the tumor microenvironment (TME)

Even though they show extensive diversity, it has been proposed that all tumors share some key alterations to cell physiology, such as sustained proliferative signaling and resistance to cell death, which ultimately lead to the accumulation of mutations and carcinogenesis [49]. Another common feature of tumors is that they modify their immediate environment through paracrine signaling, creating a particular niche that is required for the proliferation of cancer cells [50–53]. The TME is made up of all the non-tumor cells and soluble molecules surrounding the tumor. The classical vision of the TME includes immune cells, vascular and lymphatic endothelial cells, fibroblasts, adipocytes, pericytes, and factors secreted by both tumor and non-tumor cells [54,55]. The microbiome is a newly recognized component of the TME [56]. In order to really understand its influence on cancer, it is important to distinguish between the two ways the microbiome can shape the TME and interact with cancer cells: systemically or locally (Figure 1).

From a distance, the microbiota living in the gastrointestinal tract can modulate the fate of tumors arising in other organs. Metabolites and immune signals produced by gut microbiota enter the circulation and reach tumors distal to the gut [57,58], becoming a part of their TME. These microbial metabolites may interact directly with cancer cells or may regulate carcinogenesis by interacting with other components of the TME, participating in immune responses or angiogenesis [59–63]. Locally, a direct effect of the lung microbiome on lung cancer has been established [24]. In pancreatic cancer, the bacterial diversity in tumor samples from pancreatic ductal adenocarcinoma patients correlates with survival [64,65]; long-term pancreatic ductal adenocarcinoma survivors had higher intratumoral bacterial diversity and the microbiome signature was significantly different from that of short-term survivors. Interestingly, three enriched genera were identified in long-term survivors (*Saccharopolyspora*, *Pseudoxanthomonas*, and *Streptomyces*), which had a positive correlation with the number of $CD8^+$ T cells, suggesting their role in the antitumoral immune response [65]. Moreover, a broad study analyzing seven human tumor types revealed a distinct microbiome composition in each, and that most bacteria were localized intracellularly within cancer and immune cells of the TME [66]. These findings indicate a strong physical relationship between microorganisms and

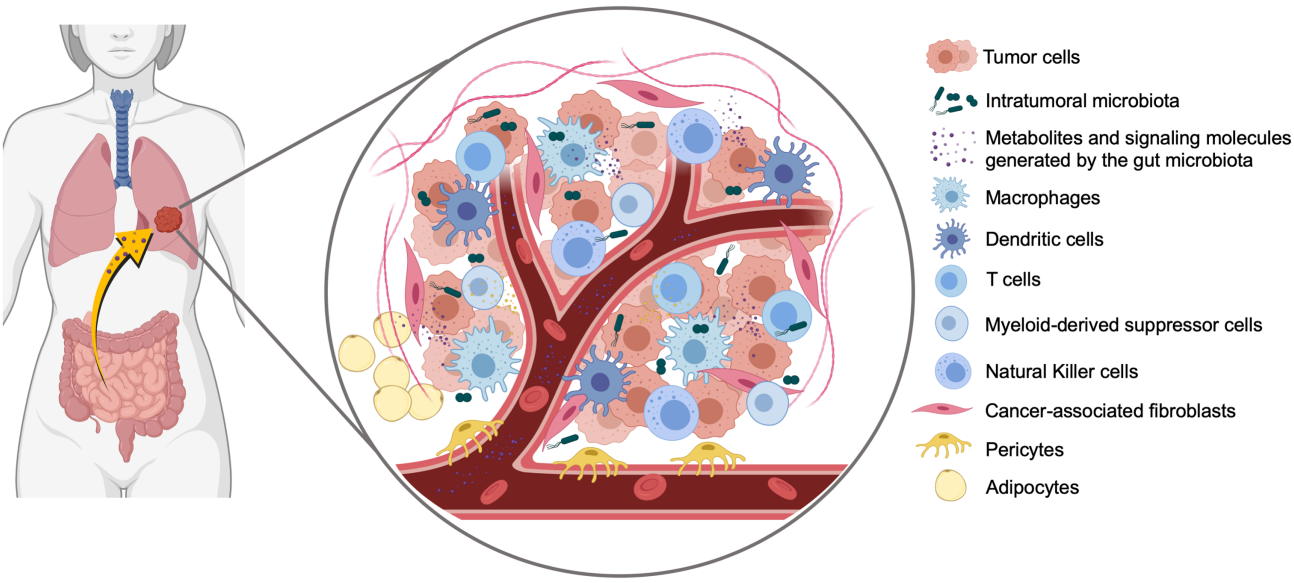

Figure 1. The microbiome in the TME. The microbiota can shape the TME systemically and locally. Metabolites and signaling molecules synthesized by the gut microbiota (purple dots) enter the circulation and travel to distant organs (yellow arrow), where they can feed or interact with cancer cells and other cells in the TME. Moreover, the microbiota is also localized within tumor and immune cells, forming part of the TME together with macrophages, dendritic cells, T cells, myeloid-derived suppressor cells, natural killer cells, cancer-associated fibroblasts, pericytes, adipocytes, and blood vessels. The influence of the microbiome in carcinogenesis is a combination of both effects: the systemic effects exerted by the gut microbiome and the local effects from the intratumoral microbiome.

cancer cells at a local level. In agreement with this, human CRC shares its microbiome with its metastatic lesions in the liver, suggesting that the microbiome travels with the primary tumor cells to distant sites. Importantly, the microbiome was linked to the malignancy of the metastatic lesions [67]. In breast cancer, the intratumoral microbiome differs based on type [68] and stage of cancer [69]. Beyond bacteria, pancreatic colonization by the fungal genus *Malassezia* promotes tumorigenesis via activation of mannose-binding lectin in mice and humans [70]. Additionally, viral infections can influence the susceptibility to develop certain cancers by integration of oncogenes into the human genome [71–73].

Understanding the role of the microbiome within a specific type of tumor requires study of the combined local and long-distant effects. The systemic effects of metabolites and small molecules produced by the gut microbiota on cancer have been the topic of many excellent reviews [74–76]. Instead, we will explore the local effects of the organ-specific microbiome on carcinogenesis, with a focus on the bacterial populations. The role of fungal and viral infections has been widely reviewed elsewhere [77–79].

Contribution of the intratumoral microbiota to carcinogenesis

Barrier failure

A cooperative relationship between the microbiota and host is possible due to mechanisms that tightly regulate their intercellular interactions. One mechanism is the use of barriers to physically separate microbial and host cells, preventing uncontrolled systemic spread of potentially dangerous pathogens [80]. Such barriers are found in the skin [81] and the gastrointestinal [82], respiratory [83], and urinogenital tracts [84], and are composed of epithelial linings. With the exception of the skin, they also contain a mucosal layer, which serves as the primary point of interaction between microorganisms and human cells [85]. Within these barriers, organ-specific cells (e.g. Paneth cells in the gut) secrete antimicrobial peptides to control the microbial population [86]. Moreover, the microbiome also participates in this defensive role [87]. In the vaginal mucosal surface, *Lactobacillus* spp. improves barrier function by acidification of the local microenvironment, and by producing metabolites that increase antimicrobial cytokine levels [88]. The skin microbiome plays a role in control of local immune responses through the modulation of resident lymphocytes and T cells [89]. The commensal microbiome also protects the host against local pathogen infections through the release of antimicrobial peptides [90].

Failure to maintain proper barrier functions has been linked to a variety of diseases, including cancer (Figure 2) [91]. Several events may lead to a breach in epithelial barriers, including genetic mutations affecting the structure and function of the barrier [92], infection by pathogens, dysbiosis [93], inflammation, or carcinogenesis [94]. Barrier disruption often results in translocation of microorganisms to sterile compartments, promoting dysbiosis and the initiation of the host immune response [95]. At the same time, inflammation-induced barrier damage in the gut may also disturb microbial–host homeostasis, triggering dysbiosis [96,97]. Determination of the causal factor is challenging due to the interdependence between these two events [94,98].

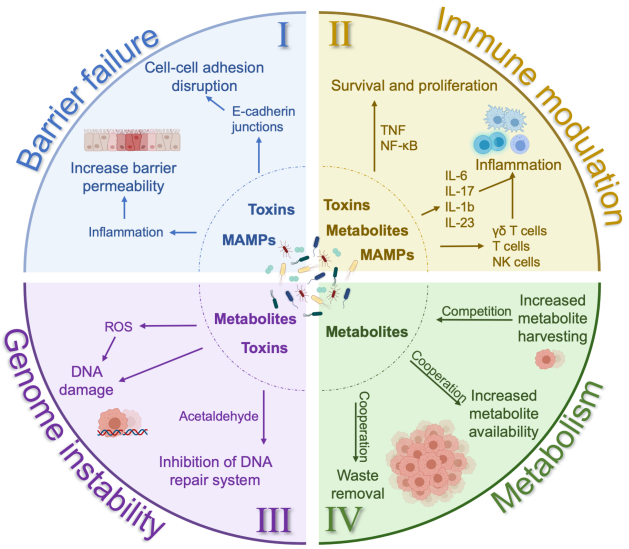

Figure 2. Contribution of the intratumoral microbiota to carcinogenesis. Through the release of toxins, microorganism-associated molecular patterns, and metabolites, the intratumoral microbiota can influence cancer development and progression in several ways: (I) disrupting cell–cell adhesion and increasing barrier permeability, which leads to barrier failure; (II) modulating immune responses by increasing the levels of signaling molecules, IL, and activating some immune populations to create an inflammatory or immunosuppressive environment; (III) damaging DNA and creating genome instability; and (IV) regulating the metabolism of cancer cells (and other cells in the TME) by competing for nutrients or cooperating through waste removal and increasing the levels of some metabolites used by cancer cells.

Invasion by pathogens and dysbiosis can both promote epithelial barrier disruption, creating a proinflammatory milieu that favors carcinogenesis [99]. This has been extensively studied in the context of CRC [100], where bacteria use different mechanisms to break through epithelial barriers and invade the host [93]. Pathogenic bacteria can adhere to the epithelium using pili and surface adhesive molecules, promoting an immune reaction and disruption to the barrier [99]. *Fusobacterium nucleatum* promotes CRC by generating an inflammatory environment via NF-κB activation [101] and direct modulation of Wnt-β-catenin signaling [102]. This signaling pathway is also modulated by other bacteria, such as *Bacteroides fragilis* and *Helicobacter pylori*, associated with CRC and gastric cancer, respectively [103]. The cell–cell adhesion molecule E-cadherin is a common target of *F. nucleatum* and the *B. fragilis* toxin. Through different mechanisms, both disrupt the E-cadherin junctions in epithelial cells, increasing endothelial permeability and allowing bacteria to cross the intestinal barrier [104,105]. Dysbiosis in the vaginal microbiome can also damage the vaginal epithelial barrier and modify the local immune response, which has been linked to an increased susceptibility to sexually transmitted infections [106,107]. The role of barrier failure in vaginal cancer has been proposed, but mechanistic studies are lacking [108]. Finally, a relationship between skin barrier failure and skin cancer has been established, but in this case, the skin microbial contribution is completely unknown [109,110].

Microbial toxins

Failure of the barriers mentioned above allows direct interaction between bacterial toxins and epithelial and immune cells, which can compromise the stability of the host's DNA (Figure 2). Released toxins can interact directly with host DNA and increase the occurrence of oncogenic mutations. Colibactin is a genotoxin that has the ability to induce double-strand breaks in host DNA [111]. It is produced by the B2 phylogenetic group of *Escherichia coli*, which possess the 54-kb *pks* genomic island. The *pks* island encodes a polyketide–peptide hybrid that is finally responsible for colibactin synthesis [112]. The expression level of pks^+ *E. coli* has been correlated with the development of CRC [113], and its presence was significantly higher in CRC patients compared with healthy controls [114,115]. A study using human intestinal organoids recently showed a distinct mutational signature after exposure to pks^+ *E. coli* and, more importantly, the same mutational signature was detected in human cancer genomes, predominantly in CRC [116]. Moreover, a second study corroborated these findings using human colorectal cells infected with pks^- or pks^+ *E. coli* [117]. These two studies provided the first evidence of an etiological role of a bacterial genotoxin in human cancer. Other bacteria, such as *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Citrobacter koseri*, also harbor *pks* islands in their genomes [118]. Although *K. pneumoniae* has been linked to colitis in a mouse model [119], a promoting role in cancer has not been determined. *Escherichia coli* was also found to be one of the most prevalent bacterial species in breast tumors [120]. In that study, *E. coli* isolated from breast tumor tissue was shown to induce double-strand DNA breaks in HeLa cells. Furthermore, *Staphylococcus epidermis* isolated from the same samples induced similar DNA breaks [120]. Cytolethal distending toxin (CDT) is another well-known genotoxin that is produced by a broad group of Gram-negative bacteria that colonize

the gut [121]. The CDT protein is made up of three subunits: CdtA and CdtC are responsible for the target cell internalization of CdtB, the subunit that reaches the nucleus and generates single- and double-strand breaks [122–124]. Although the role of CDT in the etiology of human cancer is not clear, CDT-producing bacteria have been shown to induce tumorigenesis in different CRC mouse models. In A/JCr mice, the CDT produced by *Helicobacter hepaticus* played a critical role in the induction of hepatocarcinogenesis [125]. A study using germ-free $Apc^{Min/+}$ mice colonized with a human clinical isolate of *Campylobacter jejuni* indicated that CDT production by this strain was involved in CRC development [126]. Another CDT-producing bacterium associated with human CRC is *E. coli*, which is overrepresented in human CRC samples [127], although further investigation is needed to determine the mechanism. Additionally, some strains of *E. coli* release cytotoxic necrotizing factor 1, which can induce several alterations, including protection of epithelial cells from apoptosis and promotion of cellular mobility [128]. The stomach cancer risk caused by different strains of *H. pylori* in the gastric epithelium has been linked (in part) to several toxins (cytotoxin-associated gene A protein; vacuolating cytotoxin; urease and several others) that promote chronic inflammation, oxidative stress, and host DNA damage [129]. Microcystin is a toxin secreted by the phylum *Cyanobacteria* and has been detected in non-small cell lung cancer patients [130]. In an *in silico* analysis, the presence of microcystin was related to decreased CD36 and increased PARP1 levels, suggesting a role of this toxin in inflammatory processes in lung carcinogenesis [130].

Apart from bacterial toxins that interact directly with host DNA, various bacteria also have the ability to generate reactive oxygen species (ROS), which are known to cause oxidative DNA lesions and carcinogenesis [131]. Many species of the *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* genera that colonize the oral cavity can generate hydrogen peroxide, which increases the risk of DNA damage [132]. Other oncogenic molecules produced by the oral microbiota include hydroxy ethyl and hydroxyl radicals, subproducts of ethanol metabolism carried out by some species of the *Streptococcus* genus [133]. Oral microbial dysbiosis with increased representation of all these genera has been associated with oral cancer [134]. The production of extracellular superoxide by *Enterococcus faecalis* in the gut has been described as a critical step to promote CRC in the $IL\text{-}10^{-/-}$ mouse model. In this model, an increase in superoxide levels induced 4-hydroxy-2-nonenal production by macrophages, a molecule that led to genome instability [135].

More experiments are needed to clarify the direct role of DNA damage by bacterial toxins in the induction of cancer. It has been suggested that the primary role of these toxins is not the generation of oncogenic mutations in host DNA, but rather the consequent activation of the immune system that establishes a vulnerable environment [136].

Microbial metabolites

The microbiota interacts directly with host cells through the exchange of metabolites and signaling molecules. In this bidirectional relationship, metabolites produced by host cells affect the dynamics of microbial communities and, at the same time, microbial metabolites are essential for the correct functioning of metabolic pathways in host cells. Regarding cancer, many microbial metabolites have been classified as carcinogenic, with three main mechanisms by which they affect tumor development: (1) promotion of DNA damage; (2) immune system modulation; or (3) alteration of metabolite availability (Figure 2). In addition to circulating metabolites from the gut microbiota [137], microbes that reside within tumors of other organs are able to synthesize a multitude of metabolites. However, the vast majority of studied mechanisms have been in the context of the gut microbiome and CRC development.

DNA damage

Many microbial metabolites are known to induce host DNA damage, either by direct interaction with DNA or by increasing ROS generation. Sulfur-reducing bacteria present in the colon have the ability to produce hydrogen sulfide through the metabolism of different molecules. Hydrogen sulfide has been shown to induce direct radical-associated DNA damage [138] and oxidative DNA damage through an increase in ROS [139]. Hydrogen sulfide can be generated by cysteine degradation by *F. nucleatum* and *E. coli*, taurine breakdown by *Bilophila wadsworthia* and general sulfonate degradation by *Desulfovibrio desulfuricans* [139,140]. Increased relative abundance of these bacteria has been linked to CRC [139]. In the oral cavity, the increase in these bacteria and others from the *Bacteroidetes* and *Firmicutes* phyla has been associated with oral squamous cell carcinoma through the same mechanism [132]. Deoxycholic acid (DCA), a secondary bile acid synthesized by the gut microbiota, has been shown to induce DNA damage [141] and promote carcinogenesis in CRC mouse models [142], inducing mitochondrial oxidative stress and increasing ROS levels [143]. Alcohol consumption is one of the main risk factors of oral cancer, and the production of its metabolite acetaldehyde plays a role in this association [144]. Many species of bacteria in the oral cavity and gut are involved in alcohol metabolism, and those expressing the enzyme alcohol dehydrogenase are the main source of acetaldehyde in the oral cavity [145]. The concentration of this metabolite is especially elevated in the oral cavity due to the limited number of bacteria that can convert acetaldehyde to acetate [146]. Two of the main reasons acetaldehyde is considered to contribute to oral cancer are its capacity to damage mitochondria (and thereby increase ROS) and its inhibition of the DNA repair system [146]. Several oral *Streptococcus* spp., including *S. gordonii*, *S. mitis*, *S. oralis*, *S. salivarius*, and *S. sanguinis*, are involved in this metabolism and are increased in oral cancer [132].

An *in silico* metabolic analysis of the microbiota over-represented in breast cancer tissue predicted an increase in levels of the enzyme β-glucuronidase [147]. An increase in this enzyme activity has also been reported in patients with CRC, thought to be responsible for the production of reactive intermediates from 2-amino-3-methylimidazo [4,5-f] quinoline that induce DNA damage in colon cells [148].

Modulation of immune response

The immune system can be modulated by metabolites produced by microorganisms, eliciting proinflammatory or immunosuppressive responses. It is important to mention that although all the metabolites and toxins released by bacteria that damage DNA also induce an immune response, there are also metabolites that can directly impact immune cells. Short-chain fatty acids produced from gut microbe metabolism, such as acetate, butyrate, and propionate, are known to exert an anti-inflammatory response. Animal studies have shown that in the colon, butyrate interacts with epithelial cells to increase IL-18 expression [149]. Butyrate also interacts with intestinal macrophages and dendritic cells, inducing the anti-inflammatory molecules IL-10 and Aldh1a, which trigger differentiation of naïve T cells into T_{reg} cells and suppression of Th17 cells [149]. Moreover, butyrate may stimulate T_{reg} induction by enhanced histone acetylation of colonic $CD4^+$ T cells and epigenetic regulation mechanisms [150]. Overall, this creates an environment that prevents inflammation and protects the colon against carcinogenesis. Many butyrate-producing bacteria belong to the phylum *Firmicutes* [151], and analysis of the colon microbiota revealed a decrease in these bacteria in patients with colon cancer [152]. Similar mechanisms have been associated with propionate and its immunosuppressive and antitumoral effect on colon cancer [153]. Synthesis of short-chain fatty acids by the skin microbiome also affects the local immune system. The production of propionate and valerate by the skin microbe *Propionibacterium acnes* inhibited histone deacetylase activity and induced cytokine expression in response to Toll-like receptor (TLR) ligands [154,155]. However, there is no clear relationship between these metabolites produced by the skin microbiome in skin cancer. Secondary bile acids have the opposite effect on intestinal inflammation. Although low-concentration secondary bile acids may decrease proinflammatory cytokine levels [156], an increased level of DCA and lithocholic acid is considered a risk factor for intestinal inflammation and colon cancer [157]. The metabolites DCA and lithocholic acid provoke inflammation as a consequence of DNA damage and also by increasing levels of IL-6 and TNF [158]. A recent bioinformatic study aiming to understand the interplay between vaginal dysbiosis and inflammation in cervical cancer found several metabolites modulated by the local microbiota that were associated with inflammation [159]. Among them, an increase in glycochenodeoxycholate and carnitine metabolism have been predicted to be associated with genital inflammation in cervical cancer patients. Indeed, the abundance of these metabolites correlate negatively with *Lactobacillus*, a genus that colonizes the healthy vaginal microbiome but it is drastically reduced in cancer patients. Moreover, high levels of adenosine and cytosine correlated negatively with inflammation and positively with *Lactobacillus* [159]. The protective role of adenosine against inflammation has been shown previously [160].

Metabolism of cancer cells and other cells in the TME

Local nutrient availability is an environmental pressure that modulates the metabolic reprogramming of cancer cells [161]. Thus, metabolite production and consumption by the intratumoral microbiota is probably an important factor shaping the metabolic phenotype of cancer cells as well as other cells in the TME.

Comparing the breast microbiota in patients with breast cancer and those with benign breast disease showed that not only did the bacterial communities differ, but also the metabolism of the bacteria. Bioinformatics predicted an increase in cysteine and methionine metabolism, glycosyltransferases, and fatty acid biosynthesis in the microbiota present in benign breast tissue. In contrast, bacteria colonizing breast tumors (enriched in *Fusobacterium*, *Atopobium*, *Hydrogenophaga*, *Gluconacetobacter*, and *Lactobacillus*) showed a decrease in inositol phosphate metabolism [48]. Levels of the hormone estrogen are strongly associated with the development of breast cancers expressing the estrogen receptor, and the breast microbiome is thought to increase the availability of estrogen in breast tissue [147]. In the lungs, differences in the microbial population have been associated with an increase in specific metabolites. A study comparing the lung microbiome between HIV-infected and non-infected individuals found that bacteria from *Caulobacteraceae*, *Staphylococcaceae*, *Nocardioidaceae* families, and the *Streptococcus* genus were linked to alterations in the glycerophospholipid and lineolate metabolic pathways in the lung [162]. Enrichment of the *Prevotella* and *Veillonella* genera in the lung microbiota correlated with high levels of palmitoleic acid, arachidonic acid, 4-hydroxybenzoate, and glycerol, whereas enrichment of *Pseudomonas*, *Sphingomonas*, *Chryseobacterium*, *Burkholderia*, and *Janthinobacterium* associated with glyceric acid, isothreonic acid, erythritol, threitol, cholesterol, and fucose-rhamnose [163]. A pathogenic metabolic relationship has been established between *Pseudomonas aeruginosa* and macrophages in the lungs. *Pseudomonas aeruginosa* exploits macrophages to produce itaconate, a metabolite that promotes biofilm formation and resistance to antibiotic treatments [164]. Thus, the lung dysbiosis known to accompany lung tumors is expected to play an essential role in the metabolic reprogramming of cancer and non-cancer cells in the TME. The vaginal microbiome composition has also been found to impact the cervicovaginal metabolome. Bioinformatic analysis predicted that bacterial dysbiosis found in cervical cancer patients, shifting from

Lactobacillus dominance to an enrichment in *Gardnerella*, *Prevotella*, *Streptococcus*, and *Atopobium* spp., perturbed amino acid and nucleotide metabolism [159].

Microbial metabolites have also been proposed to influence pH in the TME [165]. In the oral cavity, several bacteria from the *Lactococcus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, and *Pediococcus* genera are known to release lactic acid. This and other acids may decrease the pH in the TME, creating a favorable environment for tumor progression and metastasis [165]. Interestingly, most of the bacteria isolated from oral squamous cell carcinoma patients belong to aciduric species [166]. Furthermore, the metabolic activity of bacteria can interfere with therapeutic treatments by affecting drug availability and toxicity. For example, the β-glucuronidase enzymes of gut bacteria metabolize irinotecan, a chemotherapeutic agent commonly used in CRC treatment, thereby decreasing its effectiveness and leading to severe side-effects [167]. The addition of β-glucuronidase inhibitors to irinotecan treatment has been shown to alleviate many side-effects and improve its antitumoral efficacy [7].

At the local level, correlation of tumor development with metabolic communication between microbes and cancer cells is weak, and more experimental evidence is needed in order to determine its relevance. Interestingly, examples in the literature have reported opposite functions for the same bacterial genus or even the same metabolite in different tumor types. This highlights the need to study not only the bacterial communities within the tumor, but also bacterial metabolism and its impact on cancer cells [120].

Microbial-associated molecular pattern and immune response

Apart from metabolic interactions, the host senses the microbiota via pattern recognition receptors, which control the immune response to microorganism-associated molecular patterns (Figure 2). Microbial recognition by TLRs, a family of pattern recognition receptors, is critical to maintain epithelial barriers and has been shown to participate in carcinogenesis. Activation of NF-κB is a common protumoral event downstream of TLR recognition of microorganism-associated molecular patterns [168]. Signaling by the intestinal bacterium *F. nucleatum* within the TME promotes CRC by TLR interaction and NF-κB induction, creating a proinflammatory environment that promotes resistance to cell death [80]. In the lungs, TLR4 activation by the Gram-negative bacterial cell wall component lipopolysaccharide was reported to increase IL-6 and stimulate alveolar macrophages in the presence of specific lung microbiota enriched in the *Prevotella* and *Veillonella* genera [163]. Local lung antibiotic treatment decreased implantation of tumor cells in a mouse model of lung metastasis. The antitumoral effect of antibiotics was associated with a reduction in regulatory T cells and enhanced activation of T and NK cells [169]. Moreover, another study using antibiotics has also reported a direct relationship between the intratumoral microbiota and lung tumor development in the $Kras^{LSL\text{-}G12D}$; $p53^{flox/flox}$ mouse model. In that study, the lung microbiome stimulated IL-1b and IL-23 production from myeloid cells, inducing γδ T cells that produced IL-17 and other molecules to promote inflammation and tumor cell proliferation [24]. Antibiotic treatment also suppressed tumor growth in a pancreatic cancer mouse model. Depletion of the microbiota with antibiotics correlated with a decrease in myeloid-derived suppressor cells and an increase in M1 macrophage differentiation, inducing a rise in the number of $CD4^+$ T helper-1 and cytotoxic $CD8^+$ T cells in the TME [170]. However, a recent study showed that some intratumoral bacteria were associated with an antitumoral immune response in pancreatic cancer patients. Specifically, a pancreatic microbiota signature enriched in *Pseudoxanthomonas*, *Saccharopolyspora*, and *Streptomyces* spp. was associated with an increased infiltration of tumors with $CD8^+$ T cells and long-term survival [65].

Microbial dysbiosis in cancer: cause or consequence

Throughout this review we have highlighted how the microbiota and its products impact host health. Infection or changes in the microbial composition in different tissues can lead to a dysregulation of physiological functions that can eventually promote cancer. However, the opposite idea may also be true, wherein the rise of cancer or a proinflammatory process in a specific tissue can modify the environment, favoring the growth of some bacterial species over others. This dysbiosis may trigger the overgrowth of bacteria that promote tumor progression, creating a forward-amplifying loop. Many studies have focused on the mechanisms by which the increase in certain species of bacteria alters tumor progression, but few have focused on how the growth of cancer cells may trigger dysbiosis. It is also unclear whether dysbiosis (due to host genetics or diet) necessarily precedes inflammation or, on the contrary, an inflammatory process triggers dysbiosis. This question has been discussed in the framework of different diseases, including cancer [22,171,172].

Besides the role of inflammation *per se*, cancer cells and non-cancer cells in the TME can alter the microenvironment's metabolome via nutrient depletion and metabolite production. These changes impose specific metabolic conditions that in turn support the growth of certain microorganisms. Generation of nitrate in the gut after an inflammatory response promoted growth of the commensal bacterium *E. coli*, which is able to use nitrates as the terminal electron acceptor for anaerobic respiration and energy production [173]. This ability gave *E. coli* a competitive growth advantage against other intestinal bacteria (*Bacteroidetes* and *Firmicutes* phyla) who, as obligate anaerobes, lack the ability to utilize nitrate and thus had to depend on fermentation. The

nitrates were generated by the host, as mice deficient in inducible nitric oxide synthase did not support the growth of *E. coli* [173]. In breast tissue, higher levels of *Proteobacteria* and *Firmicutes* were proposed to be a consequence of local host fatty acid production [41]. Another study reported an association between an increased abundance of *Proteobacteria* in the gut and specific changes to the expression of metabolic pathway genes [174].

That tumors create a niche for bacteria to colonize has been exploited for therapeutic targeting. Interestingly, although systemically administrated bacteria reach both tumor and healthy tissues, only those bacteria in the tumor persist and proliferate, supporting the idea that the immunosuppressive and unique metabolic characteristics of the TME can shape the local microbiome [175]. Collectively, these studies show the importance of understanding both directions in this complex metabolic relationship: not only how microorganisms regulate host metabolism, but also how changes in host metabolism (a hallmark of cancer) modulate the dynamics of bacterial communities throughout the body.

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Author contributions statement

PGS and GMD were responsible for conceptualization. PGS prepared the original draft. PGS and GMD were responsible for reviewing, writing and editing the manuscript.

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