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Abstract

Microbes, which live in the human body, affect a large set of pathophysiological processes. Changes in the composition and proportion of the microbiome are associated with metabolic diseases (Fulbright et al., PLoS Pathog 13:e1006480, 2017; Maruvada et al., Cell Host Microbe 22:589–599, 2017), psychiatric disorders (Macfabe, Glob Adv Health Med 2:52–66, 2013; Kundu et al., Cell 171:1481–1493, 2017), and neoplastic diseases (Plottel and Blaser, Cell Host Microbe 10:324–335, 2011; Schwabe and Jobin, Nat Rev Cancer 13:800–812, 2013; Zitvogel et al., Cell 165:276–287, 2016). However, the number of directly tumorigenic bacteria is extremely low. Microbial dysbiosis is connected to cancers of the urinary tract (Yu, Arch Med Sci 11:385–394, 2015), cervix (Chase, Gynecol Oncol 138:190–200, 2015), skin (Yu et al., J Drugs Dermatol 14:461–465, 2015), airways (Gui et al., Genet Mol Res 14:5642–5651, 2015), colon (Garrett, Science 348:80–86, 2015), lymphomas (Yamamoto and Schiestl, Int J Environ Res Public Health 11:9038–9049, 2014; Yamamoto and Schiestl, Cancer J 20:190–194, 2014), prostate (Yu, Arch Med Sci 11:385–394, 2015), and breast (Flores et al., J Transl Med 10:253, 2012; Fuhrman et al., J Clin Endocrinol Metab 99:4632–4640, 2014; Xuan et al., PLoS One 9:e83744, 2014; Goedert et al., J Natl Cancer Inst 107:djv147, 2015; Chan et al., Sci Rep 6:28061, 2016; Hieken et al., Sci Rep 6:30751, 2016; Urbaniak et al., Appl Environ Microbiol 82:5039–5048, 2016; Goedert et al., Br J Cancer 118:471–479, 2018). Microbial dysbiosis can influence organs in direct contact with the microbiome and organs that are located at distant sites of the body. The altered microbiota can lead to a disruption of the mucosal barrier (Plottel and Blaser, Cell Host Microbe 10:324–335, 2011) or promote or inhibit tumorigenesis through the modification of immune responses (Kawai and Akira, Int Immunol 21:317–337, 2009; Dapito et al., Cancer Cell 21:504–516, 2012) and microbiome-derived metabolites, such as estrogens (Flores et al., J Transl Med 10:253, 2012; Fuhrman et al., J Clin Endocrinol Metab 99:4632–4640, 2014), secondary bile acids (Rowland, Role of the gut flora in toxicity and cancer, Academic Press, London, p x, 517 p., 1988; Yoshimoto et al., Nature 499:97–101, 2013; Xie et al., Int J Cancer 139:1764–1775, 2016; Shellman et al., Clin Otolaryngol 42:969–973, 2017; Luu et al., Cell Oncol (Dordr) 41:13–24, 2018; Miko et al., Biochim Biophys Acta Bioenerg 1859:958–974, 2018), short-chain fatty acids (Bindels et al., Br J Cancer 107:1337–1344, 2012), lipopolysaccharide (Dapito et al., Cancer Cell 21:504–516, 2012), and genotoxins (Fulbright et al., PLoS Pathog 13:e1006480, 2017). Thus, altered gut microbiota may change the efficacy of chemotherapy and radiation therapy (McCarron et al., Br J Biomed Sci 69:14–17, 2012; Viaud et al., Science 342:971–976, 2013; Montassier et al., Aliment Pharmacol Ther 42:515–528, 2015; Buchta Rosean et al., Adv Cancer Res 143:255–294, 2019). Taken together, microbial dysbiosis has intricate connections with neoplastic diseases; hereby, we aim to highlight the major contact routes.

Keywords (separated by " - ")

Microbiome - Breast cancer - Tumor microenvironment - Bacterial metabolite - Bacterial metabolism - Antitumor immunity - Tumor metabolism - Epithelial-mesenchymal transition - Tumorigenesis - Metastasis - Chemotherapy

The Microbiome as a Component of the Tumor Microenvironment

10

Tünde Kovács, Edit Mikó, Gyula Ujlaki, Zsanett Sári, and Péter Bai

Abstract

Microbes, which live in the human body, affect a large set of pathophysiological processes. Changes in the composition and proportion of the microbiome are associated with metabolic diseases (Fulbright et al., PLoS Pathog 13:e1006480, 2017; Maruvada et al., Cell Host Microbe 22:589–599, 2017), psychiatric disorders (Macfabe, Glob Adv Health Med 2:52–66, 2013; Kundu et al., Cell 171:1481–1493, 2017), and neoplastic diseases (Plottel and Blaser, Cell Host Microbe 10:324–335, 2011; Schwabe and Jobin, Nat Rev Cancer 13:800–812, 2013; Zitvogel et al., Cell 165:276–287, 2016). However, the number of directly tumorigenic bacteria is extremely low. Microbial dysbiosis is connected to cancers of the urinary tract (Yu, Arch Med Sci 11:385–394, 2015), cervix (Chase, Gynecol Oncol 138:190–200, 2015), skin (Yu et al., J Drugs Dermatol 14:461–465, 2015), airways (Gui et al., Genet Mol Res 14:5642–5651, 2015), colon (Garrett, Science 348:80–86, 2015), lymphomas (Yamamoto and Schiestl, Int J Environ Res Public Health 11:9038–9049, 2014; Yamamoto and Schiestl, Cancer J 20:190–194, 2014), prostate (Yu, Arch Med Sci 11:385–394, 2015), and breast (Flores et al., J Transl Med 10:253, 2012; Fuhrman et al., J Clin Endocrinol Metab 99:4632–4640, 2014; Xuan et al., PLoS One 9:e83744, 2014; Goedert et al., J Natl Cancer Inst 107:djv147, 2015; Chan et al., Sci Rep 6:28061, 2016; Hieken et al., Sci Rep 6:30751, 2016; Urbaniak et al., Appl Environ Microbiol 82:5039–5048, 2016; Goedert et al., Br J Cancer 118:471–479, 2018). Microbial dysbiosis can influence organs in direct contact with the microbiome and organs that are located at distant sites of the body. The altered microbiota can lead to a disruption of the mucosal barrier (Plottel and Blaser, Cell Host Microbe 10:324–335, 2011) or promote or inhibit tumorigenesis through the modification of immune responses (Kawai and Akira, Int Immunol 21:317–337, 2009; Dapito et al., Cancer Cell 21:504–516, 2012) and

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microbiome-derived metabolites, such as estrogens (Flores et al., J Transl Med 10:253, 2012; Fuhrman et al., J Clin Endocrinol Metab 99:4632–4640, 2014), secondary bile acids (Rowland, Role of the gut flora in toxicity and cancer, Academic Press, London, p x, 517 p., 1988; Yoshimoto et al., Nature 499:97–101, 2013; Xie et al., Int J Cancer 139:1764–1775, 2016; Shellman et al., Clin Otolaryngol 42:969–973, 2017; Luu et al., Cell Oncol (Dordr) 41:13–24, 2018; Miko et al., Biochim Biophys Acta Bioenerg 1859:958–974, 2018), short-chain fatty acids (Bindels et al., Br J Cancer 107:1337–1344, 2012), lipopolysaccharide (Dapito et al., Cancer Cell 21:504–516, 2012), and genotoxins (Fulbright et al., PLoS Pathog 13:e1006480, 2017). Thus, altered gut microbiota may change the efficacy of chemotherapy and radiation therapy (McCarron et al., Br J Biomed Sci 69:14–17, 2012; Viaud et al., Science 342:971–976, 2013; Montassier et al., Aliment Pharmacol Ther 42:515–528, 2015; Buchta Rosean et al., Adv Cancer Res 143:255–294, 2019). Taken together, microbial dysbiosis has intricate connections with neoplastic diseases; hereby, we aim to highlight the major contact routes.

Keywords

Microbiome · Breast cancer · Tumor microenvironment · Bacterial metabolite · Bacterial metabolism · Antitumor immunity · Tumor metabolism · Epithelial-mesenchymal transition · Tumorigenesis · Metastasis · Chemotherapy

10.1 The Human Microbiome

The human body harbors different kinds of symbiotic, commensal, and pathogenic bacteria that live on the surface and the cavities of the body. Microbiota is a collective term that refers to the group of microbes colonizing the human body, and the collection of genes they encode is known as our microbiome [36]. The number of colonizing microbial cells (>10^{14}) is 10 times more than the total sum of human somatic and germ cells. Therefore, their collective genome—called the metagenome—contains a large number of genes that exceed the human genome by 150 times. This metagenome performs key functions relevant to human health [37].

Each anatomical niche possesses a unique mixture of microbial populations (gut, skin, vagina, mouth, nose, and conjunctiva) that have important and functionally relevant individual variability (at the levels of genus, species, and strain) [5]. The great majority of microorganisms live in the gastrointestinal (GI) lumen. These microbes compete and collaborate with other organisms in this niche, resulting in a functionally and genetically plastic metagenome [5]. The GI microbiota plays a crucial role in digestion, maturation, immune response, protection against pathogen overgrowth, maintenance of intestinal barrier function, regulation of intestinal endocrine functions, neurologic signaling, bone density, biosynthesis of vitamins, neurotransmission, metabolism of bile salts, reaction or modification of drugs, elimination of exogenous toxins, and maintenance of the energy homeostasis of the host [38].

10.2 Bidirectional Microbiome-Host Connection

There is increasing evidence for complex and dynamic microbial interactions with hosts. The microbe-human symbiotic connection is a result of millions of years of coevolution, coadaptation, and codependence. Bacterial colonization begins at birth and progresses through childhood to adulthood. The adaptation process is nonrandom [39] and depends on the body habitat, lifestyle, physiological conditions, genotype of the host, and presence of other microbes in the niche [40]. The function and composition of the microbiome are determined by the diet of the host, probiotic or antibiotic consumption, stress, and short- or long-term travel. Besides these external factors, the host can affect the dynamics of the microbiome through its genetics, immune system, and personal hygiene [38]. Given the diverse func-

tional repertoire of the microbiome, it is not surprising that dysbiosis is associated with a broad range of diseases from neurological disorders to metabolic diseases and cancer [12]. Numerous studies highlight the relationship between changes in the function, composition, and proportion of microbes—also called microbial dysbiosis—and the progression of certain diseases. Koch's concept that one microbe is responsible for the formation of one disease ("one microbe-one disease hypothesis") was shown to be an oversimplification. Recent advances have shown that the loss of balance in microbial communities and the global change in our microbiome are directly or indirectly connected to carcinogenesis, rather than the presence of a single causative microbe [41]. Nevertheless, there are directly tumorigenic bacteria, although their number is extremely low, including about 10 species (e.g., *Helicobacter pylori* promote the development of gastric cancer). Dysbiosis is associated with cancers of the urinary tract, cervix, skin, airways, colon, lymphomas, prostate, and breast [42]. However, it is still unclear whether cancer is the product of alterations of the microbiota or modifications in the "normal" microbiome are the consequences of cancer progression.

10.3 The Tumor Microenvironment

Cancers are not just masses of homogenous malignant cells. Tumors have been recognized as complex organs, whose complexity may exceed that of normal healthy tissues. Interactions between malignant and recruited non-transformed cells create the tumor microenvironment (TME). Nonmalignant cells include immune cells, cells of the vasculature and lymphatic system, cancer-associated fibroblasts, pericytes, and adipocytes [43]. The role of nonmalignant cells in the TME is to support cancer growth. Nonmalignant cells have a dynamic tumor-promoting function at all stages of carcinogenesis. The communication between cell types is driven by an extremely complex network of cytokines, chemokines, growth factors, other inflammatory mediators, and matrix remodeling enzymes [44]. Cancer cell metabolism is strictly regulated by the tumor microenvironment. The microbiome is a new component of the tumor microenvironment that impairs tumor cell metabolism by maintaining a healthy barrier, inducing inflammation, and producing genotoxins and bacterial metabolites with different features. Below, we review the modalities of how dysbiosis interferes with carcinogenesis (Fig. 10.1).

10.4 Bacteria-Driven Carcinogenesis Through Physical Interaction

The most relevant pathomechanism for microbiome-derived carcinogenesis is *barrier failure*. In healthy humans, numerous commensal bacteria are found in the intestinal lumen, where some bacteria are in direct association with the epithelium. The microbiota is vital in preserving the functional luminal barrier, by maintaining epithelial cell turnover, facilitating mucin production, and competing for resources and, thereby, suppressing the growth of pathogens [45]. The physical and chemical barrier of gut epithelial cells prevents microbial translocation to the underlying connective tissue. Defects in protein-coding genes (e.g., laminin) that are essential for the maintenance of a normal barrier, infections, inflammation, carcinogenesis, or microbial dysbiosis may induce barrier failure. Inflammation and carcinogenesis may trigger barrier failure, but barrier failure also promotes inflammation and carcinogenesis, suggesting a forward-amplifying loop [6]. Breakdown of the intestinal barrier leads to translocation of bacteria and the development of a systemic inflammatory response [46].

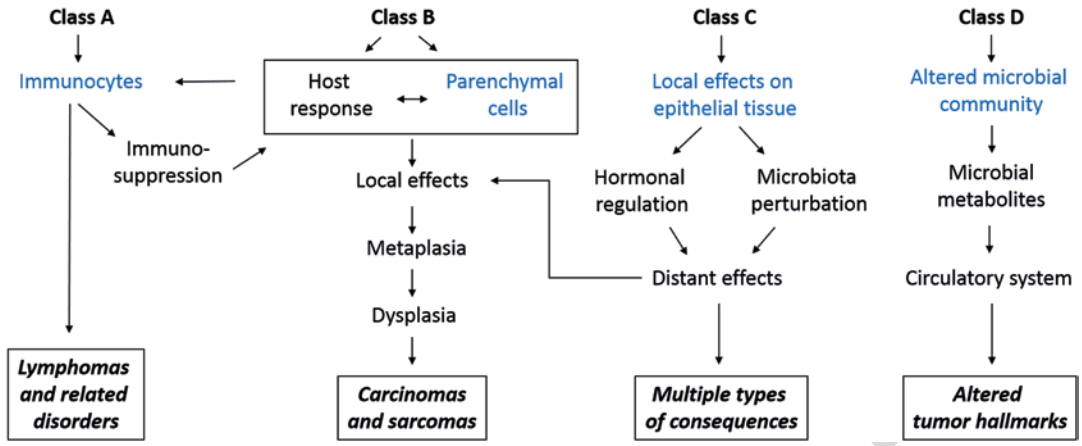

Fig. 10.1 Schematic picture of the classification of microbiota-associated human malignancies. Class A is defined by the involvement of the immune response, Class B requires direct microbial interactions with parenchymal cells, Class C covers distant effects from local interactions, and Class D shows the consequences of altered microbiome composition. (Modified figure from [5])

10.5 Microbiome-Immune System Interactions in Tumorigenesis

Microbiome-immune system interactions play multifaceted roles in tumorigenesis. The microbiome may promote tumorigenesis by inducing chronic inflammation, disrupting the balance between cell proliferation and cell death, and triggering immune responses. The physical loss of the natural gut epithelial barrier—barrier failure—or the loss of the antibacterial defense system enables the movement of cellular components and microbes across the barrier, where they cause an innate inflammatory response. The mammalian immune system detects the presence of microbial infection through *pattern recognition receptors (PRRs). Toll-like receptors (TLRs)* and *NOD-like receptors (NLR)* belong to the PRR family and recognize different but overlapping microbial components. They are expressed in different cellular compartments (cell surface, cytoplasm, lysosome, and endosome) and activate specific signaling pathways that promote inflammation, tumor proliferation, or resistance to cell death [23].

TLRs are one of the most powerful pro-inflammatory stimuli. These structures recognize microbe-associated molecular patterns, such as lipopolysaccharides (LPS), peptidoglycan, flagella, or microbial DNA/RNA. TLR2 recognizes peptidoglycan and lipoteichoic acid (bacterial cell wall components) and promotes gastric cancer, while TLR4 detects LPS (Gram-negative cell wall component) and contributes to skin, pancreas, liver, and colon cancer development [6]. Carcinogenesis is promoted through TLRs of epithelial cells, macrophages, and fibroblasts. TLR induction leads to the production of pro-inflammatory cytokines, such as interleukins and TNFα. Downstream effectors of TLR signaling induce cell survival and suppress apoptosis through NF-κB (nuclear factor-κB) and STAT3 signaling, which is in line with the role of MYD88 mutations that induce NF-κB and STAT3 in many human lymphomas [24]. Tumor formation is reduced by pharmacologic inhibition of interleukins (IL-17 and IL-23), antibiotic treatment, or MYD88 inactivation [6].

Although a direct link between endogenous bacteria and tumor-associated angiogenesis has not been shown, the microbiome is required for normal development of the vasculature. LPS, produced by the microbiome, may promote angiogenesis through TLRs. IL-17 is produced by T-helper-17 (Th17), suggesting that bacteria also impact the tumor microenvironment by stimulating Th17 lymphocytes. A connection between

breast cancer and immunoglobulins has been established. Secretory immunoglobulin A (IgA) helps to maintain the integrity of the mucosal barrier, attenuates the host immune response, and regulates the composition of the gut microbial community.

Several bacterial species induce immunity in tumor development. *Lactococcus* species help maintain the cytotoxic activity of natural killer (NK) cells, while *Sphingomonas yanoikuyae* have an important role in maintaining breast tissue health. Cytotoxic immune cells (cytotoxic T lymphocytes) are essential for identifying and destroying precancerous and cancerous cells; *Fusobacterium nucleatum* destroy this protective mechanism and enable tumor progression, while others stimulate anticancer immunity. *Bifidobacterium*, *Bacteroides thetaiotaomicron*, and *Bacteroides fragilis* enhance dendritic cell function and antitumor cytotoxic T cell immunity [1]. TLRs may also promote cancer cell proliferation through different growth factor receptor ligands (amphiregulin, epiregulin, and hepatocyte growth factors), which exert both local and long-distance effects.

In carcinogenesis, the microbiota induce activation of NOD-like receptors (NLRs) as well. Many studies focus on NOD2, because loss of NOD2 activity is connected with Crohn's disease. NOD2 has a key role in the activation of NF-κB signaling and the formation of a bacterial community. Thus, NOD2 loss-of -function mutations may lead to intestinal dysbiosis and an enhanced risk of developing colorectal carcinoma (CRC). Genetically induced CRC is also evoked by NOD1 deficiency, which plays an important role in intestinal defense against bacteria. NLRP6, another NLR, is important in microbiota-tumorigenesis interactions. NRRP6 is a component and key activator of inflammasomes (multiprotein oligomers responsible for the activation of inflammatory responses), which are downregulated in dysbiosis-driven carcinogenesis, together with decreased IL-18 production [6].

Immunotherapy is used to eliminate residual cancer cells after chemotherapy or radiation therapy. In therapy, monoclonal antibodies target molecules, such as anti-T-lymphocyte-associated antigen 4 (CTLA-4) and anti-programmed death 1 (PD-1) or its ligand anti-PD-L1. The advantage of immunotherapy is that it stimulates and supports the immune system of the host to fight cancer cells. The gut microbiome can stimulate the T cell response and improve inflammatory signaling through PRRs that potentiate the immune system to directly eliminate cancer cells. Antibodies against immune checkpoints improve T cell function and proliferation and, thereby, improve the anticancer immune response, providing an effective therapeutic approach in patients with various types of cancers, such as in advanced melanoma [47], renal cell carcinoma [48], or non-small cell lung cancer [49]. Alterations in commensal gut bacteria influence therapeutic responses to inhibition of CTLA-4 and PD-1. Following CTLA-4 therapy, the microbial composition shifts; *Bacteroidales* and *Burkholderiales* abundance decreases and *Bacteroides* and *Clostridiales* are enriched [50]. *Bacteroides fragilis* is capable of promoting T-helper 1 (Th1) responses and activating antigen-presenting cells (dendritic cells) through the induction of IL-12. Thus, an improvement in anti-CTLA-4 effectiveness may be partially due to the enrichment of *Bacteroides fragilis.* Improved effectiveness of anti-CTLA-4 therapy was observed in melanoma patients with increased abundance of *Bacteroides*, *Bacteroides thetaiotaomicron*, and *Bacteroides fragilis* [50]. The main bacterial component driving these processes was found to be the LPS of *Bacteroides* species. Thus, inhibition of CTLA-4 can alter the composition of the gut microbiome that in turn influences responsiveness to immunotherapy. Studies on anti-PD-1 or anti-PD-L1 therapy showed similar bacteria-driven differences in tumor outgrowth. In a mouse model of melanoma, increased effectiveness of anti-PD-L1 therapy was associated with enhanced *Bifidobacterium* (*Bifidobacterium longum* and *B. breve*) abundance in the gut and a consequent activation of dendritic cells [51]. In metastatic melanoma patients receiving anti-PD-1 and anti-PD-L1 treatment, patients with greater alpha diversity with an enrichment of *Clostridiales*,

Faecalibacterium, and *Ruminococcaceae* species and decrement in *Bacteroidales* had longer survival. These beneficial effects were partly due to an enhanced T cell response (connected mainly to $CD8^+$ T lymphocytes) and the upregulation of antigen-presenting pathways [52]. Increased $CD8^+$ T cell activation was shown in another study in advanced melanoma patients. Patients that responded to anti-PD-L1 therapy had elevated levels of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*. Moreover, all patients that responded to treatment carried *Akkermansia muciniphila* [53]. Better survival was shown in urothelial carcinoma, renal cell carcinoma, or non-small cell lung carcinoma patients undergoing anti-PD-1 treatment who did not receive antibiotics during or after treatment and carried elevated levels of *Akkermansia* and *Alistipes* species. These findings were mainly connected to $CD4^+$ T cell activation [54] and demonstrated that antibiotic-induced dysbiosis could negatively influence responses to immunotherapy.

However, the mechanisms that contribute to dysbiosis and changes in the microbial community are not well understood. Host-driven immune and inflammatory responses are important driving factors that shape the bacterial community composition. The composition of the microbiome, innate immunity, and inflammation determine the outgrowth of different types of specific bacteria by changing the production of metabolites, such as nitrate. Nitrate may provide a unique energy source for facultative anaerobic bacteria (e.g., *Enterobacteriaceae*). Inflammation may promote bacterial fitness and adaptation by inducing the expression of stress-response genes in bacteria (e.g., *Escherichia coli*) [6].

10.6 Genotoxins and Microbiota-Driven Genomic Instability

Inflammation enhances tumorigenesis by inducing DNA damage and altering the mechanism of DNA repair. Macrophage release of reactive oxygen species (ROS) in response to inflammatory cytokines directly induces DNA breakage and mutations, and their downstream pathways stimulate transcription factors (NRF2, NF-κB) that impair cellular growth to produce cancer [36]. *Enterococcus faecalis* can generate large amounts of superoxide, while *Fusobacteria* species and *Deltaproteobacteria* produce hydrogen sulfide; both *Fusobacteria* species and *Deltaproteobacteria* are associated with CRC.

Hydrogen sulfide is a product of sulfate reduction from dietary taurine and sulfur-containing amino acids and has a wide effect on the host. Hydrogen sulfide is highly inflammatory and toxic to colonocytes. Furthermore, hydrogen sulfide can enhance colonocyte proliferation through the ERK1/2 pathway [55], inhibit mucus synthesis and butyrate oxidation while impairing the activity of cytochrome oxidase, and generate free radicals that lead to genotoxicity.

Although the ability of microorganisms to produce ROS [56] contributes to tumorigenesis, bacteria can also release specific *toxins* that induce DNA damage responses, which also contribute to tumorigenesis (Fig. 10.2). Damaged barrier function may also allow the bacteria to transfer or deliver toxins, including cytolethal distending toxin (CDT), colibactin, cytotoxic necrotizing factor 1 (CNF1), and *Bacteroides fragilis* toxin. CDT and colibactin are true genotoxins, which directly damage the DNA and activate the ataxia signaling pathway and histone phosphorylation, which lead to G2/M cell cycle arrest [6]. CDT is created by Gram-negative bacteria (*E. coli, Helicobacter species,* and *Salmonella typhi*) and is relevant to colorectal, gastric, and gallbladder cancer. Colibactin is produced by *E. coli*, *Enterobacteriaceae*, *Proteus mirabilis*, and *Klebsiella pneumoniae* and is important in the development of CRC. Colibactin produced by *E. coli* induces DNA double-strand brakes, cell cycle arrest, and improper cell division [1]. *Bacteroides fragilis* toxin activates the Wnt/β--catenin signaling pathway, which promotes epithelial proliferation, by promoting the cleavage of the adhesion molecule, E-cadherin. The cleavage of E-cadherin leads to β-catenin translocation to the nucleus and enables the transcription of proto-oncogene c-myc, leading to colonic epithelial hyperplasia [1].

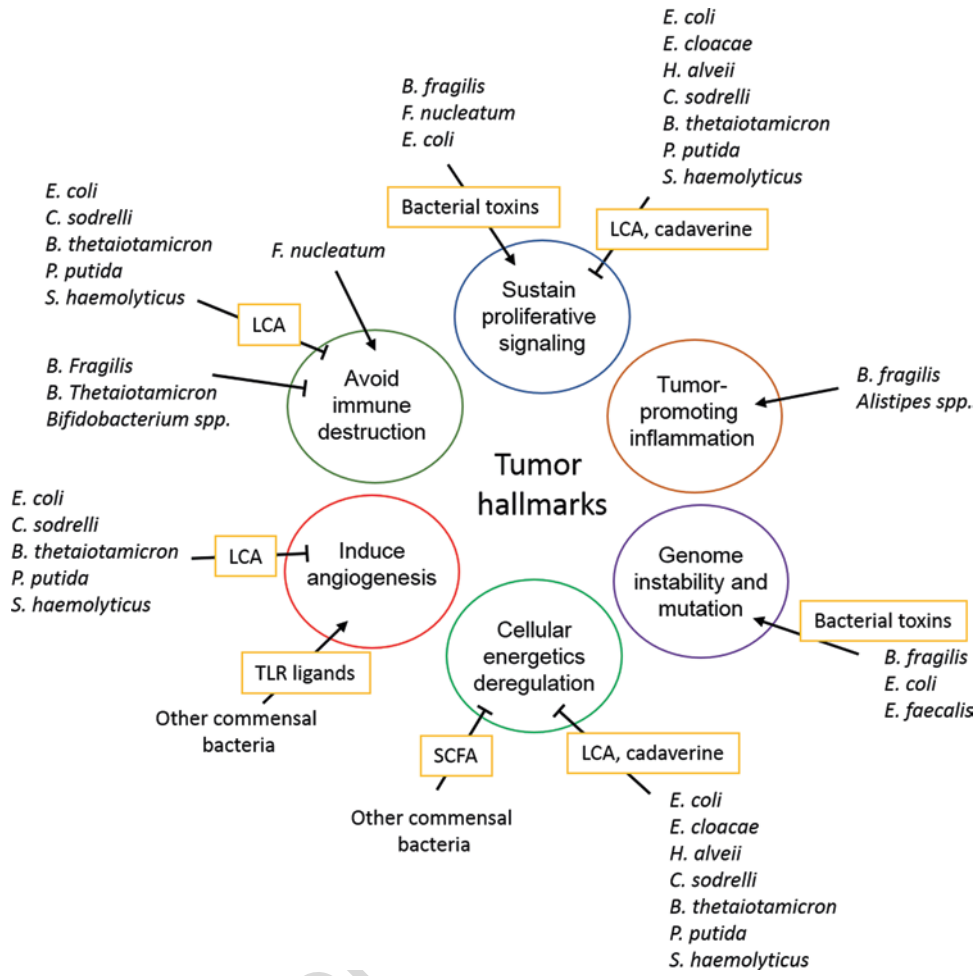

Fig. 10.2 The intestinal microbiota can modulate several hallmarks of cancer through different mechanisms

10.7 Bacterial Metabolites in Carcinogenesis

A major pathway in microbiome-host signaling is the production of bacterial metabolites. These metabolites, which are synthesized by the microbiome, enter the circulation at the site of production and travel to distant organs, where they exert their biological effects [57]. Bacterial metabolites behave like human hormones in the sense that they are synthesized by an "organ" (the microbiome) and are then transferred to the site of action by the circulation [57].

Microbiota have the potential to metabolize hormones, such as *estrogen*. The gut microbiome is a key determinant of estrogen levels in the body. β-Glucuronidases are the enzymes responsible for estrogen deconjugation. Deconjugation of excreted estrogen is important in estrogen reuptake and, thus, modulation of systemic estrogen availability and the regulation of estrogen-associated pathways. Numerous bacterial species can express β-glucuronidases, including *Firmicutes and Bacteroidetes*: *Alistipes, Bacteroides, Bifidobacterium, Citrobacter, Clostridium, Collinsella, Dermabacter, Edwardsiella, Escherichia, Faecalibacterium, Lactobacillus, Marvinbryantia, Propionibacterium, Roseburia*, and *Tannerella.* Thus, these bacterial species affect circulating and excreted estrogen levels. Reactivated estrogen increases the serum estrogen levels and act

through estrogen receptors (ERα and ERβ) to modulate the expression of several genes, including mitochondrial genes. Elevated oxidative phosphorylation was shown to support metastasis [58], contribute to therapy failure [59], and, thereby, render the tumors more aggressive. Taken together, bacterial estrogen deconjugation promotes breast cancer progression and changes the risk for development and progression of estrogen-dependent cancers [6, 57].

The fermentation of nondigestible carbohydrates is beneficial for the host due to the generation of *short-chain fatty acids (SCFAs)*, such as acetate, butyrate, formate, lactate, and propionate. SCFAs are novel potential targets for the management of obesity, metabolic disorders, and lipomas, due to their ability to influence adipocyte differentiation [60]. SCFAs have known anti-inflammatory, antiproliferative, and antineoplastic effects. In addition, SCFAs can regulate autophagy. Thus, SCFAs have a protective effect on the colonic mucosa and play a significant role in the protection against colon and liver cancer [6]. In the gut, acetate, butyrate, and propionate production are associated with a large group of bacteria. Acetate production is widespread, while the production of butyrate is connected to *Faecalibacterium prausnitzii, Eubacterium hallii, Eubacterium rectale, Roseburia faecalis, Odoribacter*, and *Anaerotruncus* species. The majority of propionate production is associated with *Bacteroidetes, Lachnospiraceae*, and *Negativicutes* species, as well as to *Roseburia inulinivorans* and *Ruminococcus obeum.* In line with this, the abundance of *Akkermansia muciniphila*, a propionate-producing bacterium, is associated with the richness of the gut microbiome [61]. SCFAs have both positive and negative effects on breast cancer. Stroma and cancer cells have free fatty acid receptors, through which SCFAs modulate several hallmarks of cancer: cell proliferation, invasion, apoptosis, metabolism, and the expression level of certain genes. Lactate can be used as a direct energy substrate; thus, the inhibition of lactate metabolism reduces cancer cell viability. Butyrate enhances mitochondrial ROS level, induces apoptosis, and inhibits histone deacetylases, which lead to elevated anticancer activity [57].

The intestinal microbiota regulate *bile acid* metabolism and are involved in producing the secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), through the deconjugation, oxidation, and dehydroxylation of primary bile acids. The enzyme responsible for the conversion of primary bile acids to secondary bile acids is 7α/β hydroxysteroid dehydrogenase (HSDH). Conversion to secondary bile acids increases the hydrophobicity of bile salts allowing recovery through the colonic epithelium. Secondary bile acids have both pro- and anticancer activity. The consumption of a high-fat diet changes the gut microbiome and enhances the level of DCA via 7/α-dehydroxylase, which is produced by bacteria, mainly clostridia. DCA is a promoter of carcinogenesis in certain cancers. DCA-elicited cell signaling is connected to protein kinase C and ERK1/2 signaling through epidermal growth receptors, resulting in enhanced cell proliferation. DCA is known to increase CRC development and promote colon and esophageal cancers [6]. Moreover, bile acids disrupt cell membranes through their amphipathic properties and the generation of ROS and reactive nitrogen species. Bile acids also exert antimicrobial activity that changes the composition of the intestinal community. LCA is synthesized through 7α-dehydroxylation of chenodeoxycholic acid (CDCA) or 7β-dehydroxylation of ursodeoxycholic acid (UDCA). The enzyme responsible for LCA synthesis is encoded by the bile acid-inducible (baiH) operon and expressed by aerobic and anaerobic bacteria, including *Bacteroides fragilis, Bacteroides intestinalis, Clostridium scindens, Clostridium sordellii, Clostridium hylemonae*, and *E. coli*. These bacteria belong to the phyla *Bacteroides, Firmicutes*, and *Proteobacteria.* LCA inhibits the epithelial-to-mesenchymal transition, vascular endothelial growth factor (VEGF) production, and metastasis formation of breast cancer cells, changes the metabolic features of the cells, and enhances antitumor immunity of the host [30]. In line with these observations, human serum levels of LCA and the ability of the microbiome to produce LCA are

largely reduced in breast cancer; this is most pronounced in in situ and early stage carcinoma (stages 0 and 1) [30]. LCA can potentially exert its effects through the farnesoid X receptor (FXR), liver X receptor (LXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR), and G-protein-coupled bile acid receptor 1 (TGR5). In breast cancer, the main receptor is TGR5. Activation of TGR5 signaling was shown to induce OXPHOS, mitochondrial biogenesis through NRF1, AMPK, and PGC-1β signaling. The expression of mitochondrial proteins (cytochrome c, atp5g1, and ndufb5) consequently increases mitochondrial activity and exerts anti-Warburg effects in breast cancer models [30]. In supraphysiological concentrations (>1 μM), LCA was shown to inhibit fatty acid production and induce cell death and the expression of multidrug-resistant proteins [62].

When undigested dietary compounds reach the large intestine, they are fermented through anaerobic respiration. High protein consumption is associated with elevated colonic fermentation. *Bioactive products*, similar to bile salts, can produce or inhibit carcinogenesis. Cadaverine, a *biogenic amine*, is synthesized from L-lysine by bacterial lysine decarboxylase enzymes (LdcC and CadA). Cadaverine also has a human origin, but it seems that bacterial production is more important as it highly exceeds human biosynthesis. The main cadaverine-producing bacteria include *Aeromonas veronii, Clostridium perfringens, E. coli, Enterobacteriaceae bacteria, Edwardsiella tarda, Hafnia alvei, Raoultella ornithinolytica, Staphylococcus*, and *Streptomyces* species. These species belong to the *Acinetobacteria, Bacteroides, Firmicutes, Fusobacteria,* and *Proteobacteria* phyla. Trace amine-associated receptors (TAARs) were shown to be responsible for mediating cadaverine-elicited effects. Through TAARs, cadaverine inhibits epithelial-to-mesenchymal transition, proliferation, movement, and invasion of breast cancer cells. Moreover, cadaverine treatment inhibits primary tumor infiltration to the surrounding tissue and reduces the proportion of cancer stem cells [42].

Many bacteria in the GI tract have alcohol dehydrogenase activity, which enables the bacteria to metabolize ethanol and produce reactive and toxic *acetaldehyde*. The most important gastric pathogen, *H. pylori,* and some skin bacteria have high alcohol dehydrogenase activity. The colonic mucosa has a low aldehyde dehydrogenase activity, resulting in acetaldehyde accumulation in the colon. High acetaldehyde levels contribute to the pathogenesis of alcohol-induced diarrhea and the increased risk of colon polyps and colon cancer [63] (Fig. 10.3).

10.8 The Interference of the Microbiome with Chemotherapy

Bacteria of the intestinal microbiome can interfere with therapeutic agents during cancer treatment and management. The microbiome can modulate the efficacy of both chemotherapy and radiotherapy. Bacteria can inactivate or activate chemotherapeutic drugs, alter immune responses, or interfere with the side effects of the therapy. The relationship is reciprocal, as tumor therapy can influence the composition and function of the microbiome [57].

Chemotherapeutic compounds, such as cisplatin or oxaliplatin, exert their cytotoxic effects through DNA damage, the upregulation of apoptotic pathways, or the promotion of antitumor immune responses (through a TLR4-dependent mechanism). The antitumor effects of *platinum compounds* significantly decrease upon broad-spectrum antibiotic treatment or in microbiota-deficient mice. In addition, tumor-infiltrating cells show reduced production of ROS after antibiotic treatment [35]. In this scenario, commensal microbes prime tumor-infiltrating cells for ROS production through the connection to PRRs, with the involvement of MYD88 signaling (described previously) [6, 56]. *Lactobacillus acidophilus* supplementation can restore the antitumor effects of cisplatin in mice [11]. *Cyclophosphamides* have been used for anticancer therapy for almost 60 years. In high doses, cyclophosphamides are immunosuppressive,

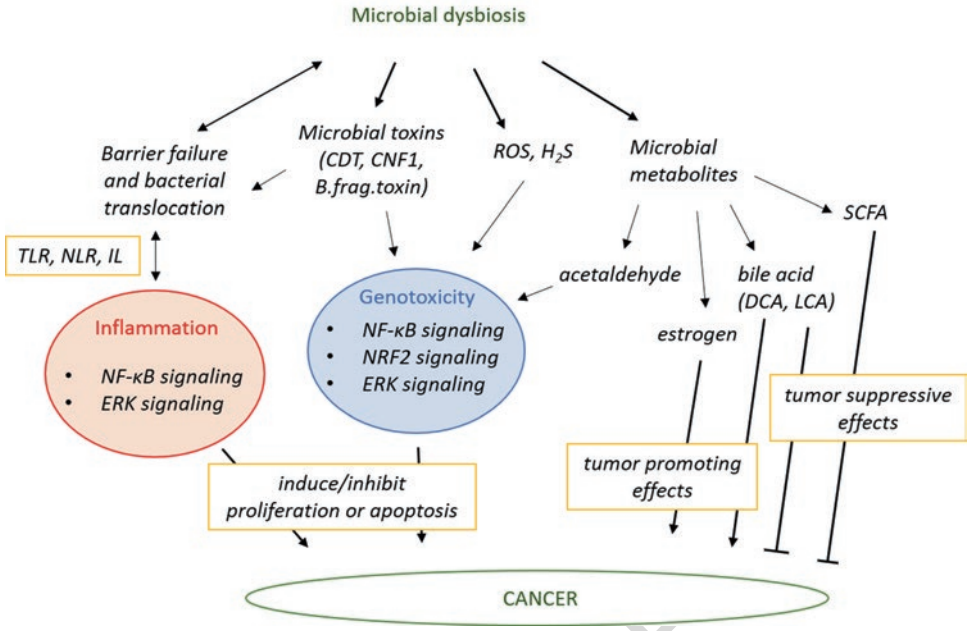

Fig. 10.3 Mechanisms by which microbial dysbiosis modulates carcinogenesis

while in low doses, cyclophosphamides promote the antitumor immune response through activation of cytotoxic T cells and induction of immunogenic cell death [33]. Cyclophosphamides are used in the therapy of breast cancer; however, cyclophosphamides cause damage to the gut mucosa, making the gut leaky and allowing gut bacteria to enter the circulation. A rich microbiome and elevated levels of *Lactobacillus plantarum* are protective against cyclophosphamide-induced mucosal injury [57]. Cyclophosphamide treatment causes the overrepresentation of Gram-negative species, such as *Barnesiella intestinihominis* that enhance effector T cells (cytotoxic $CD8^+$ T cell), and *Enterococcus hirae*, Gram-positive bacteria that enhance MYD88-dependent $CD8^+$ T cell activation in a tumor-specific manner. Both bacteria are regulated by intestinal NOD2 receptors that promote a pro-inflammatory tumor environment and drive antitumor immune responses [35]. T cell-mediated immune responses against *B. intestinihominis* and *E. hirae* have clinical relevance in chemotherapy-treated patients with lung and ovarian cancers.

In addition to cyclophosphamides, anthracyclines, selective estrogen receptor modulators (SERMs), taxanes, and antimetabolites have key roles in breast cancer therapy. *Anthracyclines* are produced by *Streptomyces* species. Anthracyclines act mainly by intercalating into DNA and interfering with DNA metabolism and RNA production, or by generating excessive ROS. Anthracyclines can be bacteriostatic; they decrease the abundance of *Acinetobacter* species [32]. No bacterial drug metabolism was associated with SERMs (tamoxifen, raloxifene). Tamoxifen can modulate the composition of the microbiome, while tamoxifen resistance can also be modulated by the microbiome. SERMs are toxic to different species in the GI tract, including *Acinetobacter baumannii, Bacillus stearothermophilus, Enterococcus faecium, Klebsiella pneumoniae, Porphyromonas gingivalis, Pseudomonas aeruginosa,* and *Streptococcus mutans* [57]. *Taxanes* (paclitaxel, docetaxel) are widely used as chemotherapy agents. Taxanes disrupt microtubule formation and, hence, block cell division and proliferation. Taxanes may change the composition of the microbial community or interfere with bacterial LPS, while activat-

ing the immune system. PARP inhibitors are drugs used in the treatment of ovarian cancer with a potential to be used for other neoplasias (e.g., breast cancer, prostate cancer). PARP inhibitors were shown to induce the diversity of the gut microbiome [64].

Drugs are often used in combinations to enhance treatment efficacy. *Irinotecan* is used to treat colon cancer and small cell lung carcinoma. For treating colon cancer, irinotecan is generally used in combination with 5-fluorouracil (5FU), whereas for the treatment of small cell lung cancer, irinotecan is combined with cisplatin. Bacterial reactivation of irinotecan by bacterial β-glucuronidase leads to severe side effects, such as diarrhea, vomiting, bone marrow suppression, hair loss, shortness of breath, and fever. Antibiotic treatment or β-glucuronidase inhibition prevents most of these side effects [6]. When *5FU* is used in combination with irinotecan, dysbiosis-induced mucositis leads to bacterial translocation from the GI tract. Both 5FU and *gemcitabine* undergo bacterial activation and bacterial deactivation. In human pancreatic ductal adenocarcinoma, *Gammaproteobacteria* was found to be the most important player in deactivating gemcitabine. In tumors, levels of *Gammaproteobacteria* were elevated in tumor patients as compared to healthy individuals, underlining its role in the regulation of gemcitabine availability. Both 5FU and gemcitabine have bactericidal properties; therefore, they can alter the composition of the GI microbial community [57].

Chemotherapy is often not specific for one or two bacterial species, but change the proportion and diversity of the microbiome. After chemotherapy, both the alpha diversity, which represents species richness (the number of different species in a sample), and beta diversity, which refers to the diversity in the microbial community between different environments, are altered as compared to samples without chemotherapy. These changes are independent of covariates (age, sex, previous antibiotic consumption, and previous chemotherapeutic treatment) and show increases in *Citrobacter, Enterococcus, Klebsiella, Megasphaera,* and *Parabacteroides* species, while showing decrements in the abundance of *Adlercreutzia, Anaerostipes, Bifidobacterium, Blautia, Clostridium, Collinsella, Coprococcus, Dorea, Lachnospira, Roseburia*, and *Ruminococcus* species. Some bacteria showed resistance to chemotherapy; thus their abundance did not change upon treatment, including *Actinomyces, Erysipelotrichaceae, Mobiluncus, Mitsuokella, Oxalobacter, Prevotella, Scardovia*, and *Slackia* [34].

Besides inducing taxonomic dysbiosis, chemotherapy can disrupt microbial function. Several metabolic pathways can be suppressed by chemotherapy, including amino acid, carbohydrate, and nucleotide metabolism, as well as the metabolism of vitamins and cofactors. Other pathways are enhanced by chemotherapy, including signal transduction, xenobiotic degradation, and glycan metabolism. Glycan metabolism, together with disrupted carbohydrate and amino acid metabolism, contributes to enhanced intestinal inflammation [65] and upregulation of nitrogen, sulfate, and riboflavin pathways, which is associated with inflammatory diseases, increased ROS production, and bacterial translocation [66]. Moreover, chemotherapy increases bacterial motility proteins and flagella assembly (essential for bacterial pathogenesis, motility, adhesion, and invasion).

Dysregulated microbiota plays a significant role in the development of GI mucositis. Mucositis is a painful inflammation of the mucous membranes of the digestive system, usually as an unpleasant side effect of chemotherapy and radiotherapy for cancer. In the first step of this process, the microbiome enhances the activation of NF-κB and TNFα signaling, leading to long-lasting inflammation. Several bacteria are reduced after chemotherapy, including *Bifidobacterium, Coprococcus, Clostridium, Dorea, Faecalibacterium, Lachnospira, Roseburia*, and *Ruminococcus*, which inhibit inflammation through blocking NF-κB and produce mucosa-protecting metabolites (SCFAs), whereas *Citrobacter* and other species, which participate in LPS biosynthesis and enhance intestinal inflammation, are increased during chemotherapy [34]. Subsequently, GI mucositis

barrier dysfunction develops, leading to increased intestinal permeability, which coincides with a decrease in the amount of the previously mentioned protective bacteria. The microbiome may modulate the composition of the mucus layer, as the terminal step of mucositis induction. *Citrobacter*, which increases after chemotherapy, may participate in the degradation of the mucosal barrier through the expression of mucus-degrading enzymes (mucinase, glycosidase), and *Enterobacteriaceae* can disrupt the mucus layer. Butyrate-producing bacteria protect the mucin layer, as butyrate increase mucin synthesis. A decrement in cysteine, proline, and methionine metabolism, which occurs during chemotherapy, can also be responsible for altered mucin composition and the development of GI mucositis after chemotherapy [34].

Radiation therapy is used as a primary treatment in cancers that are localized to one area of the body to prevent tumor recurrence after surgery or applied together with chemotherapeutic agents. Radiation itself is genotoxic, resulting in cancer cell death. However, radiation can also abolish nontarget cells due to the activation of the immune system by radiation-induced inflammation. The microbiota is known to be involved in these off-target effects due to intestinal mucosa damage and toxicity. Radiotherapy decreases both the diversity and the total amount of gut bacteria, particularly *Bacteroidetes, Enterobacteriaceae, Firmicutes*, and *Lactobacillus* species, while enriching *Fusobacterium* and *Proteobacteria*, which are connected with increased production of pro-inflammatory cytokines [35].

10.9 Modulation of the Microbiome to Enhance the Efficacy of Chemotherapy

Probiotics and prebiotics are widely used to shift the composition of the microbiome, and these interventions are potentially useful in restoring the microbiome after chemotherapy. Probiotics contain live bacteria that can be administered orally, while prebiotics (dietary prebiotics) are compounds in food, which provide substrates that stimulate the growth or activity of advantageous bacteria colonizing the gut. Prebiotics and probiotics prevent infection and moderate the side effects of cancer treatment. Administration of various strains of *Lactobacillus*, such as *Lactobacillus acidophilus,* is associated with enhanced cisplatin sensitivity and longer survival in lung cancer [35]. *Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus casei*, and *Lactobacillus rhamnosus* decrease the toxicity associated with 5FU chemotherapy and, consequently, reduce abdominal discomfort and diarrhea. In addition, *Bifidobacterium* and *Lactobacillus* species in combination were able to moderate the side effects after radiation treatment. Current clinical trials are focused on the efficacy of probiotic treatment for colorectal, kidney, breast, gynecologic, and lung cancer [35].

Fecal microbiota transplantation (FMT), also known as stool transplantation, is the process of transplanting fecal bacteria from a healthy individual into a diseased subject. FMT is an effective therapy to shift the composition of the microbiome. FMT is effective in the treatment of *Clostridium difficile,* where FMT is curative through enhancement of the diversity of the microbiome [67]. FMT could be potentially effective after chemotherapy or radiotherapy in cancer patients by avoiding gut toxicity or preventing infections. However, FMT has numerous side effects (fever, diarrhea, vomiting), including serious side effects, such as GI bleeding or perforation, that limit its applicability in cancer patients [35].

As a developing future therapy, bacterial engineering offers the opportunity to treat cancer without reconfiguring the gut microbiome. Biologically engineered bacteria could be applied effectively to target cancer cells or to deliver therapeutic agents, thereby avoiding serious side effect-eliciting anticancer therapies. Bacterial cells can be easily and rapidly transfected with vectors encoding interfering RNAs, cytokines, toxins, antiangiogenic factors, or antibodies. *Listeria* and *Shigella* species could invade hypoxic tumor tissues, and, given their quick rep-

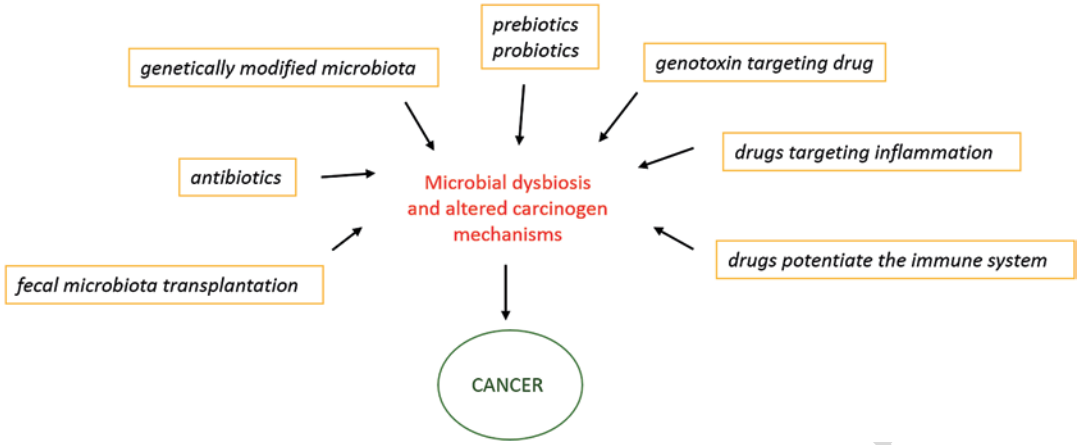

Fig. 10.4 Targeting the microbiome for modulation of carcinogenesis

lication rate, these bacteria could amplify their transgene(s) within the tumor microenvironment. Upon the application of bacteria, finding a good balance is necessary; one must seed a sufficient number of bacteria to elicit therapeutic effect but should avoid suppressing the immune system at the same time [35] (Fig. 10.4).

10.10 Type of Cancers Related to Microbial Dysbiosis

Besides the GI tract, other organs are colonized by a unique microbial community, such as the skin, oral cavity, and germinal tracts. Growing evidence confirms a significant relevance of bacterial microbiota in the carcinogenesis of the colon, liver, breast, lung, oral cavity, and pancreas.

The liver receives 70% of its blood supply from the intestinal vein. This close functional relationship between the liver and GI tract results in constant exposure to nutrients, toxins, microbial metabolites, and microbes. Various types of immune cells (NK cells, macrophages, lymphocytes) defend this organ against harmful agents derived from the intestine. An altered microbiome may contribute to the development of *hepatocellular carcinoma* (HCC), which is preceded by chronic liver disease, fibrosis, and cirrhosis [68]. The disrupted microbiome may drive this process through the loss of intestinal barrier function, the activation of the NF-κB pathway, the production of pro-inflammatory cytokines, and increased anti-apoptotic signals.

Pancreatic cancer is an aggressive cancer type with low therapeutic success and survival rate. Periodontal disease, low oral hygiene, obesity, smoking, and alcohol consumption are well-known risk factors for pancreatic cancer, because they facilitate the translocation of bacteria through disrupted barrier layers. Bacteria can reach the pancreas through the circulation. Furthermore, although the pancreas does not have a microbiome, carcinogenesis of this organ is enhanced by distant dysbiotic microbiota [6], through the involvement of inflammatory responses, LPS expression, and TLR4 activation [69].

About 90% of all lung cancer cases are attributed to smoking, while only 15% of smokers develop *lung cancer*, suggesting other mechanisms and influences. The interface of the lung is continuously connected to the outside environment, and the microbiota of the lung reflect the microaspiration of oral microbiota. The lung has a unique microbiome with different species of *Proteobacteria*. The connection between lung cancer and chronic pulmonary disease is assigned to toxic pro-inflammatory and neoplasia-causing compounds. Different bacteria species, such as *Moraxella catarrhalis, Haemophilus influenza*, and *Streptococcus pneumoniae*, are associated with 50% of chronic pulmonary disease, and

their presence can elicit chronic inflammatory responses [70].

The oral cavity harbors diverse individual microbiota. Moreover, the composition of the microbiota differs between microenvironments within the oral cavity; the lateral and dorsal tongue and tooth surface all have unique microbial communities. The normal oral microbiome includes *Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Haemophilus, Neisseria, Prevotella, Proteobacteria, Streptococcus*, and *Veillonella* species. *Capnocytophaga gingivalis, Prevotella melaninogenica,* and *Streptococcus mitis* are found in *oral squamous cell carcinoma (OSCC)* and are considered biomarkers of this disease. Risk factors for OSCC, which are connected to anaerobic, Gram-negative bacteria that liberate inflammatory markers, include smoking, heavy alcohol consumption, poor oral hygiene, and periodontal disease [71].

Genetic factors, infection, inflammation, and diet are well-known risk factors for *colorectal carcinoma* (CRC). CRC is associated with other diseases, such as inflammatory bowel disease, autoimmune, allergic reactions, obesity, and diabetes. Despite the great diversity of bacterial species of the GI tract, CRC is closely related to changes in the diversity and activity of microbes. Microbes produce metabolically active molecules that alter homeostasis or carcinogenesis [72]. The microbiota may contribute to CRC through different mechanisms that result in an imbalance between cellular proliferation and apoptosis pathways, such as PRR signaling and inflammation, metabolites that induce DNA damage and chromosome instability, or the loss of protective metabolites (due to microbial dysbiosis), such as SCFAs, secondary bile acids, or bioactive amines [73].

Recent research showed a strong correlation between gut microbiome dysbiosis and *breast cancer*. In addition to the gut microbiome, the breast has a unique microbiome that shows drastic changes in breast cancer. The microenvironment of breast cancer cells is modulated by bacterial metabolites (SCFAs, secondary bile acids, amino acid degradation products, and estrogen derivatives) that are produced in the intestine and reach cancer cells of the breast via the circulatory system. In breast cancer, various pathways are disrupted or altered in addition to the general changes in glycolysis and mitochondrial function, including glutamine, fatty acid, cholesterol metabolism, protein translation, and glutamine-serine pathways in cancer cells. These changes are the consequence of the rearrangement of a complex homeostatic system and energy sensors and lead to changes in cell proliferation and angiogenesis. Microbial dysbiosis occurs in both the fecal flora and the breast microbiome in breast cancer [20]. Fecal samples of breast cancer patients contain increased levels of *Clostridiaceae, Faecalibacterium*, and *Ruminococcaceae* and decreased levels of *Dorea* and *Lachnospiraceae* species [18]. Moreover, the microbiota composition differs not only between cancerous persons and healthy volunteers but also between breast cancer stages and grades and according to different tumor subtypes (triple-negative breast cancer associated with unique microbiome) [74]. For example, patients with grade III cancer have an increased number of *Blautia* species, compared with grade I patients, and samples from stage II/III showed elevated absolute numbers of *Bacteroidetes, Clostridium*, and *Blautia* species [75].

10.11 Future Prospects

The recent emergence of studies on the microbiome in various diseases highlights the importance of bacterial dysbiosis in different cancers. Despite the increasing literature on colorectal cancer, the data and observations on those cancers that are not in direct contact with the (gut) microbiome are limited and the available studies are often restricted to observational studies. Hence, mechanistic studies are largely missing. Minor microbiome compartments are understudied, in terms of the number of bacteria (e.g., lower airways). These caveats will need to be filled in the future.

The currently available data suggest that prebiotics and probiotics may have beneficial effects in restoring/preventing the microbiome dysbiosis, but these findings will have to be assessed in well-controlled clinical studies. Along those same lines, the use of antibiotics in cancer patients will need to be assessed in detail. Finally, the microbiome-drug interactions, a key element in cancer-related personalized medicine, will need to be precisely mapped.

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