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Review Article

Gut Microbiome in Cancer: the Next big Opportunity for Better Patient Outcomes?

Key words

gut microbiome;
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Abstract: The gut microbiome, a diverse community of microorganisms in the human body, plays an important role in maintaining health and influences various processes such as digestion, immunity, and protection against pathogens. A person's unique gut microbiome, shaped by factors such as birth method, diet, antibiotics, and lifestyle, contributes to bodily functions such as nutrient metabolism, drug processing, and immune regulation. Changes in the gut microbiome are associated with a predisposition to cancer and can influence the effectiveness of cancer treatments. Dysbiosis in the gut microbiome can lead to inflammation, tumor development, and metastasis, highlighting its importance in cancer research and prevention. The gut microbiota significantly influences cancer development and treatment outcomes. Certain bacteria enhance the effects of therapies such as cyclophosphamide and contribute to the body's immune response against tumors. Microbes produce anti-cancer molecules and probiotic compounds, making them potential tools in cancer prevention and treatment. Future research aims to develop targeted antibiotics and explore fecal microbiota transfer to selectively manipulate the microbiota for improved cancer treatment. Due to genetic and physiological similarities, mouse models are invaluable in biomedical research. However, because the gut microbiome of humans and mice and the composition of the tumor microenvironment differ, direct comparison between these two models can be challenging in research. Bridging these gaps is crucial for comparative medicine, especially in cancer research where the microbiome plays an important role in treatment outcomes. One important area where the gut microbiome could offer potential new treatment options is in primary brain tumors such as gliomas. To date, there are no long-lasting effective treatments for this type of cancer, but research in mouse models shows a link between tumor progression and response to treatment with changes in the gut microbiome. Overall, the gut microbiome and its modulation represent an opportunity for more efficient future cancer treatment.

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Introduction

The gut microbiome is known to play a crucial role in maintaining normal homeostasis in humans. The microbiome is a collection of all microorganisms, such as bacteria, fungi, viruses, and their genes, that naturally reside on and in our bodies. Often referred to as the "forgotten organ", the microbiome contains a metagenome that is 100 times larger than our own genome and performs key functions that are vital to human health (1). For instance, the human body comprises approximately 40-100 trillion microbial cells, which is ten times more than the number of human somatic cells. A healthy individual's gut contains around 300-500 different species of bacteria (2), although some sources mention up to 3500 bacterial species (3).

Over time, the microbiome and the host have evolved into a complex "superorganism" with their symbiosis benefiting

the host in numerous ways, such as food metabolism, protection against pathogens, and assistance in the development of the immune system. The majority (99%) of microbial mass resides in the gastrointestinal tract (GI) and functions both locally and over long distances. As a result, the gastrointestinal microbiome not only has the most substantial impact on overall health and metabolic status among all microbiomes but is also the most extensively studied microbiome, serving as a model for understanding interactions between the host, microorganisms, and diseases (4, 5).

The role of a normal gut microbiome

A hypothesis suggests that the GI's microbiome plays a significant role in maintaining an individual's gut health and is crucial for overall human health (6, 7). Everyone has a unique gut microbiome profile, which serves specific functions in host nutrient metabolism, xenobiotic metabolism, maintenance of the structural integrity of the intestinal mucosal barrier, immunomodulation, and protection against pathogens.

Under normal circumstances, the host's immune system recognizes markers that are specific to pathogenic microorganisms, making it easier to eliminate them. The fact is that the majority of the host's microbiome is non-pathogenic and lives in symbiosis with the host's immune system. Intestinal bacteria play an important role in this, as they inhibit the growth and spread of pathogens, provide essential nutrients, and assist in nutrient and drug metabolism. In the meantime, the host's immune system must prevent the invasion of both pathogenic and non-pathogenic microbes. Immune cells, including macrophages, phagocytes, and dendritic cells, closely interact with the intestinal microbiome and its metabolites thus maintaining intestinal homeostasis and recognizing bacteria that might be pathogenic (8).

The gut microbiome of each individual is formed early in life, and several factors play a role in its development. These factors include in what way a baby is born (vaginal or cesarean section), childhood diet (breast milk or formula), adult diet (vegan or meat-based), as well as the use of antibiotics or antibiotic-like molecules derived from the environment or the gut's commensal community (7). The gut microbiome of an adult host is relatively stable. Still, it varies from person to person, mainly due to differences in lifestyle, including frequency of physical activity and cultural practices, as well as enterotype, body mass index (BMI), and dietary habits. Alpha and beta diversity are two metrics used to look at microbiome diversity. Alpha diversity measures how many types of species live in a given area in a person or a single sample and beta diversity measures the differences in the microbiome composition between body sites or people (9).

As a result, there is no unique optimal composition of the gut microbiome, as it differs for everyone. However, despite this diverse composition, statistics show that dominant types of gut microbes include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* comprising 90% of gut microbes (10, 11).

The gut microbiome and cancer

Microbiota-induced tumors are estimated to represent about 20% of all tumors worldwide (12). In the past decade alone, the number of cancer patients in the USA has increased from 13.8 to 18.1 million, and it is projected to continue rising in the upcoming years. Additionally, a diet high in fat and heavily processed foods is believed to influence the diversity of gut microbiomes. A recent study showed that higher fat consumption in healthy young adults is associated with unfavorable changes in gut microbiomes, which could impact the overall health of the host (13). Numerous studies point out that changes in the gut microbiome can lead to a predisposition to various types of cancer. Moreover, bacteria and their metabolites have been found not only to contribute to cancer development, such as colorectal cancer but also to alter the pharmacodynamics of cancer drugs (14, 15). When the balance in the gut microbiome is disrupted, bacteria can penetrate the intestinal mucosa and surrounding tissues, causing inflammation. As the inflammatory process promotes tumor development and progression, it accelerates the invasion of tumor cells and may eventually lead to metastasis. Increased levels of inflammatory cytokines can directly damage the DNA of epithelial cells, triggering inflammation-associated cancer (16, 17).

For instance, the presence of the bacterium *Helicobacter pylori* can promote an immune response and chronic inflammation, which can cause stomach cancer. Many products from this bacterium disrupt the regulation of normal cell homeostasis, resulting in a build-up of cytokines and other signaling molecules that cause stomach and esophageal cancer. In healthy individuals, the esophagus is densely populated with *Firmicutes* and *Streptococcus* (mostly Gram-positive bacteria). In cases of dysbiosis, Gram-negative bacteria (anaerobic and microaerophilic) replace Gram-positive bacteria, which can later lead to esophagitis or inflammation of the esophagus. Among other gut microbes associated with esophageal cancer are *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (18).

One of the more significant health problems across the world is colorectal cancer. Many studies have proven that several bacterial species play a role in colorectal carcinogenesis, including *Escherichia coli*, *Bacteroides fragilis*, *Fusobacterium spp.*, *Enterococcus faecalis*, *Streptococcus bovis*, *H. pylori* and *Clostridium septicum*. The colon contains ten times more bacteria than the small intestine,

which means that the likelihood of developing colorectal cancer is about 12 times higher in the colon than in the small intestine, emphasizing the role of commensal bacteria (19, 20). Other research has indicated that obesity may be associated with the gut microbiome composition and increased liver cancer. In obese individuals, the epithelium junctions are damaged, allowing gut bacteria to enter the bloodstream and cause systemic infections. Additionally, the gut microbiota––driven COX2 pathway secretes secondary metabolites and other small molecules such as lipoteichoic acid (LTA), lipopolysaccharides (LPS), and bile acids, which cause inactivation of the immune system in the liver, possibly leading to liver cancer (21–24).

The role of the gut microbiome in tumorigenesis

The symbiotic microbiome is recognized for its vital function in upholding human well-being and fortifying the host's immune defenses. Nevertheless, a group of bacteria remains associated, either directly or indirectly, with the advancement and progression of cancer (25). In an imbalanced gut ecosystem, harmful microbes can inflict various damages upon the host's organism in several ways (Figure 1). Research has proven that the intestinal flora can infiltrate deep within bodily tissues, instigating tumor formation in mouse models deficient in IL-10, a critical cytokine pivotal in the host's anti-cancer immunity (26–29).

When there is a microbial imbalance in the gut microbiota, pathogenic bacteria can generate and discharge an array of toxins. These toxins can make the genome unstable by instigating breaks in the host's DNA, triggering the development of tumor in predisposed tissues. One example of such a toxin is cytotoxin-associated gene A (CagA), synthesized by *H. pylori*. Through this cytokine, CagA can degrade the tumor suppressor protein p53 in gastric cells, disrupting the host's serine/threonine kinase pathway which leads to inhibition of cell apoptosis, survival of damaged cells and stomach cancer development (Figure 2). Similarly, E. coli produces colibactin, and CDT+ strains produce a cytotoxic toxin (CDT) with DNase activity that leads to host cell apoptosis. When these toxins are released near the intestinal epithelium, they create double-strand breaks in the host's DNA, culminating in genetic mutations and the development of tumors. By producing enzymes like Virulence A (VirA) and inositol phosphate phosphatases D (IpgD), *Shigella flexneri* can induce degradation of the p53 protein in host cells and disrupt DNA damage and repair pathways (30, 31).

Pathogenic bacteria are capable of indirectly influencing tumorigenesis in host cells through various mechanisms. For instance, they can produce molecules such as different bile acid metabolites that inhibit the host's immune response and enhance inflammation and with this help cancer cells evade the immune system (32). Bacteria can also generate oxidative stress, which subsequently drives genetic mutations in host cells (33, 34). For example, flavoenzyme (spermine oxidase) can be activated in host cells by *Helicobacter pylori* and *Bacteroides fragilis*, creating hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS), which adds to DNA damage accumulation. Bacteria *Enterococcus faecalis* can infiltrate host cells by producing reactive oxygen species, accelerating host DNA damage (35–37). *Fusobacterium nucleatum* can block the cytotoxic

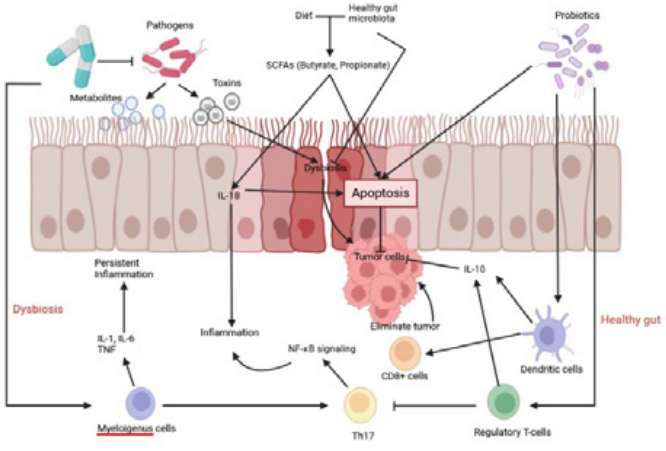

Figure 1: The interaction between the gut microbiota and the immune system. In an imbalanced gut ecosystem, harmful microbes can inflict various damages upon the host's organism and affect local and systemic immune responses. Adapted from (30). The Scheme was created using Biorender.com

Abbreviations: interleukin (IL), short-chain fatty acids (SCFAs), tumor necrosis factor (TNF), nuclear factor kappa B (NF-κB), T helper 17 cell (Th17)

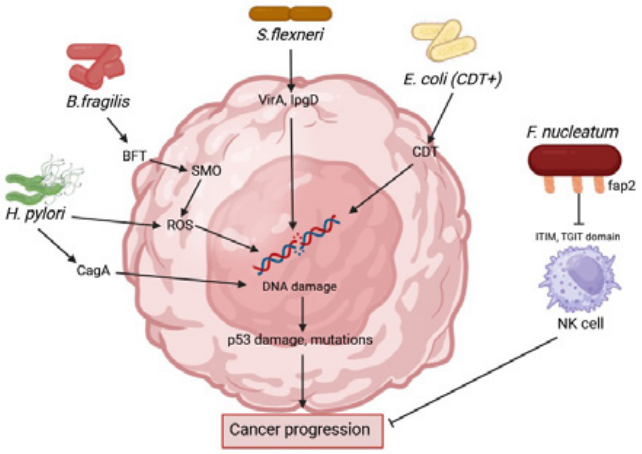

Figure 2: The role of different bacteria in cancer progression. An illustration of how numerous bacteria can contribute to or even outright cause cancer progression and their mechanism of action. The Scheme was created using Biorender.com

Abbreviations: B. fragilis toxin (BFT), cytotoxin-associated gene A (CagA), Cytolethal distending toxins (CDT), fibroblast activation protein-2 (Fap2), inositol phosphate phosphatases D (IpgD), natural killer (NK), reactive oxygen species (ROS), spermine oxidase (SMO), virulence A (VirA)

activity of natural killer cells (NK) by producing a virulence factor (Fap2), which can bind to the NK inhibitory receptor TGIT and ITIM domain, preventing NK cells from attacking cancer cells (25, 38).

Anti-cancer properties of intestinal microbiota

Microbiota plays a crucial role in tumor formation and the outcomes of anticancer therapies (39, 40). The interaction between the host's immune system and gut microbiota enables immune cells to recognize and eliminate opportunistic bacteria before they can invade the host's body. Apart from that, the interaction also impacts food digestion and the elimination of metabolites against gastrointestinal antigens (41). Moreover, the microbiota influences both innate and acquired immune systems systemically. In studies with germ-free mouse models lacking gut microbiota, scientists have observed a lack of the mucus layer, altered immunoglobulin A (IgA) levels, and mesenteric lymphadenitis (41, 42). Additionally, microbiota deficiency has been shown to negatively affect the efficacy of therapeutic interventions (43).

Cyclophosphamide (CTX) is a popular chemotherapeutic drug that stimulates the host's T-cell immune response. It is used as a treatment for different types of cancers, and it works by crossing the small intestine and breaching the epithelial membrane. In this way commensal gut microbiota is transferred to the spleen and mesenteric lymph nodes, thus stimulating helper T cells (T_H17 cells) and further inducing anti-cancer effects against tumor development (25, 44). Studies on mouse models have shown that several bacterial species such as *Branesiella intestinihominis*, *Enterococcus hirae*, and *Lactobacillus johnsonii* enhance the anti-cancer activity of CTX (44, 45). On the other hand, antibiotic-treated and germ-free mouse models have shown reduced immune responses due to the lack of Gram-positive bacteria in their intestines. Based on these studies, it has been found that commensal bacteria can alter the efficacy of immunotherapy and chemotherapy drugs (44).

Bacteria *Bifidobacterium longum* and *B. breve* have been shown to enhance the dendritic cell function. Active dendritic cells can then trigger the recruitment of cytotoxic T cells in the tumor microenvironment. Cytotoxic T cells and NK cells are the main components of the immune system responsible for eliminating cancer cells (25). Numerous studies analyzing 1000 patients with sarcoma have found out that heat-killed bacteria *Serratia* and *Streptococcus pyogenes* can increase the survival rate of patients by approximately 80% over five years. Additionally, heat-killed microorganisms may activate a sustained immune response and potentially exhibit anti-cancer effects against sarcomas. It is presumed that CD8+ cells can effectively infiltrate infected cells or tissues in solid tumors because of the gut microbiota (46). For example, it was shown that CTLA-4 inhibitors have anti-cancer effects and depend on the gut microbiota, especially Gram-negative obligate anaerobic bacteria. The CTLA-4 inhibitor had no anti-cancer effects in germ-free mice, but when Gram-negative obligate anaerobic bacteria were introduced into sterile mice, the inhibitor's anti-cancer efficiency was restored (47).

Molecules or components derived from microorganisms have potent anti-cancer properties. Short-chain fatty acids (SCFA), produced by the gut microbiota, play a crucial role in suppressing tumors/cancer (48–50). Common SCFAs produced by commensal gut bacteria like butyrate and propionate have effective anti-cancer effects (51). They inhibit the histone deacetylases of cancer cells and induce the programmed cell death known as apoptosis. In patients with colorectal cancer there were lower levels of bacteria producing butyrate found. Butyrate activates the GPR109A receptor, which then induces IL-18 production in epithelial cells of the intestinal mucosa, which may trigger the repair mechanisms of the mucous layer (52).

Metabolites obtained from probiotics can initiate an indirect immune response against tumor formation by modifying the host's immune system. For instance, lipopolysaccharides (LPS) can activate Toll-like receptor 4 (TLR 4), which further enhances the T cell immune response against tumor cells (53). Likewise, monophosphoryl lipid A from the bacterium *Salmonella enterica* which has a high efficacy against cervical cancer is used as an adjuvant in vaccine development (54). Some gut bacteria can produce probiotic molecules with anti-tumor effects. For instance, *Lactobacillus casei* produces ferrichrome, which activates the c-Jun N-terminal kinase signaling pathway and ultimately induces programmed cell death in cancer cells (55). *Lactobacilli* are also believed to play a role in an anti-tumor response by stimulating the host's immune system such as dendritic cells, NK cells, and T_H1 cells (38).

Bacteria and viruses in the intestines influence the effects of chemotherapy, immunotherapy, and the immune response. Studies have shown that mice with tumors that do not typically respond to immunotherapy drugs can start responding if they receive specific gut bacteria from mice that have positively responded to the drugs (56). The same phenomenon has been observed in humans, where altering the composition of bacteria in the gut microbiota using fecal transplants can improve the condition of some patients with tumors who did not respond to immunotherapy or drugs. The most well-known donor of his feces is Zion Levy, who was diagnosed with melanoma but showed very good responsiveness to immune treatment with nivolumab. Doctors concluded that with his feces, he could also help other patients who do not respond to immunotherapy as well. This led to the first such research studies at Sheba Medical Center in Israeli study and a study led by scientists at Memorial Sloan Kettering Cancer Center in New York City and scientists at the University of Minnesota Medical School in the USA in which the transfer of microbes from

feces (FMT) improved the response to immunotherapy in patients. The results of these two studies, published in the journal Science (56, 57), were modest - out of 26 people who did not previously respond to immunotherapy, about one in three responded after FMT. However, they attracted a lot of attention. In the Israeli study, ten individuals with advanced melanoma were included, whose cancer had progressed despite treatment with checkpoint inhibitors. Only three out of ten overcame treatment resistance, and only two of them partially. Interestingly, all three responders received FMT from donor Levy. None of the five participants who received material from another donor who also survived cancer responded. Due to encouraging results, there are now at least 30 clinical studies of fecal microbiota transplantation being conducted (58).

The role of probiotics in cancer prevention

Probiotics are generally considered safe live microorganisms and can play a key role in preventing various diseases, including different types of tumors (59). Besides live probiotics, it has also been proven that dead probiotics, such as bacterial components (cell wall), have numerous benefits in managing various diseases, including cancer (60). Although probiotics are generally considered safe, they should be carefully considered before giving them to cancer patients since they are immunocompromised. There may be a risk of antibiotic resistance transfer and the development of opportunistic infections (61). Nonetheless, probiotics have many positive effects in cancer patients, as they can prevent diarrhea and other gastrointestinal issues and increase the populations of beneficial bacteria in the gut, thereby enabling the establishment of a healthy gut microbiota (62). For example, in a study where a patient received the probiotic strains *Lactobacillus johnsonii* and *Bifidobacterium longum*, the strain successfully adhered to the intestinal mucosa and eliminated pathogenic bacteria by triggering a local immune response (63). A similar study showed that *Bifidobacterium longum* and *Lactobacillus acidophilus* significantly reduced severe diarrhea during pelvic radiotherapy indicating their probiotic efficacy (64). Likewise, a blend of ten probiotic strains not only alleviated diarrhea but also demonstrated decreased chemotherapy-induced cytotoxicity in the treatment of certain patients battling metastatic colorectal cancer (65). Probiotic bacteria can indirectly prevent colorectal cancer by attenuating the activity of intestinal enzymes responsible for converting amines and complex aromatic hydrocarbons into active carcinogens (66). Furthermore, research has established that *Bifidobacterium animalis* subspecies *Lactis* and other probiotic strains modulate the host's immune response by activating phagocytic cells, which subsequently target and eliminate cancer cells in their early developmental stages (67–69). The direct consumption of probiotics by colorectal cancer patients has been associated with proapoptotic effects on cancer cells. For example, *Lactobacillus delbrueckii* significantly upregulates the expression of caspase 3, thereby triggering programmed cell death in human colorectal cancer cells. Consequently, probiotic bacteria stand as promising biotherapeutic agents capable of preventing intestinal dysbiosis, enhancing the host's immune response, and eliminating various curable and difficult-to-cure diseases, including different types of cancers (70).

Microbiota of the gut interacts not only with the immune system but also engages gut epithelial cells via inflammasome activation. These inflammasomes, expressed by both intestinal and immune cells, enable the distinction between toxic and non-toxic molecules produced by pathogenic and non-pathogenic microorganisms based on the nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) (71). When homeostasis in the body is disrupted for any reason, inflammasomes become active and mediate a strong immune response. They activate caspase 1, which in turn triggers the secretion of pro-inflammatory cytokines (IL-1β), IL-18, and ultimately leads to apoptosis (72). Dysregulation of inflammasomes is implicated in various diseases, including autoimmune conditions, neurodegenerative and metabolic disorders, and cancer, with the gut microbiome emerging as a pivotal factor in their activation (73).

Comparative studies of microbiome between human and animal models

Scientists have been using animals to study human diseases for over a hundred years. In this regard, mice have been particularly useful, as they share many biological characteristics with humans. Moreover, they share over 80% of their genome with humans. Due to their phylogenetic similarity, physiological resemblance to humans, ease of maintenance and breeding in laboratories, and the availability of numerous inbred strains, domestic mice (*Mus musculus*) have long served as models for human biology and diseases, including cancer (74).

However, despite the anatomical, histological, and physiological similarities between mouse and human intestines, there are significant differences in size, metabolism rate, and dietary habits. The use of mice as model organisms for studying human biology is based on genetic and physiological similarities between them. It is important to be aware that despite their phylogenetic closeness, mice, and humans have evolved and adapted to different environments, leading to significant differences in their characteristics. This is also why mice often respond to experimental interventions in ways that differ considerably from humans. Mice models in the laboratory are 'specific-pathogen-free' (SPF) mice. These differences are also reflected in the development of the gut microbiome compared to humans. Instead of SPF mice, microbially exposed, or ‘dirty’ mice model that better mimics the diverse infectious history that is typical of most humans, can be used (75). For example,

a study done by Sjaastad et al found the potential limitation of exclusive use of SPF mice when testing vaccine efficacy compared with "dirty" mice, which may also be one of the reasons that some new therapies work in experimental mice and then the efficacy is lost when transferred to humans (76).

The anatomy of the gastrointestinal tract plays a crucial role in these differences, with significant variations between the two species. The ratio of the length of the small and large intestines is greater in mice than in humans, mice have a distinct cecum, and they lack an appendix. An important site for microbial fermentation of undigested food in mice is the cecum. Thus, both species provide different environments that support the growth of different gastrointestinal microbiota (77).

An example where the impact of anatomy can be observed is in mice with "cecal lymphoid patches, " which can be synonymous with the human appendix. Here, the flora of these two compartments differs, with *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* being predominant in the human appendix in terms of abundance, whereas the mouse cecal lymphoid patches consist of Bacteroidetes, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* (78, 79).

Previous studies have shown that human and mouse gut flora share 90% and 89% similarity in species and genera, respectively. However, more recent research has shown that the differences are much larger than previously thought (80). A study from 2021 revealed that more than half of the species in both human and mouse microbiomes belonged to the *Firmicutes_A* species. *Firmicutes_A* and *Bacteroidota* (*Bacteroidetes*) were the most common species in both human and mouse microbiomes. *Firmicutes_B* was more common in mice than in humans, while *Firmicutes_C* was less represented. In general, 16 species were common to both human and mouse microbiomes, with 5 species found only in humans and not in mice. In contrast, species such as *Deferribacterota*, *Thermoto gota* and two species *Chlamydia muridarum* and *Chlamydophila psittaci* were specific to mice. No archaea were reconstructed from the mouse gut metagenome, whereas 0.4% of genomes in the human gut were attributed to this domain. At the family level, humans and mice shared 88 out of 109 taxa, and their average abundances in human and mouse microbiota were strongly correlated. Two families *Lachnospiraceae* and *Oscillospiraceae* which dominate *Firmicutes_A* were highly present in both humans and mice. In mice, the *Muribacula ceae* family was more than 30 times more abundant than in humans, while the *Bacteroidaceae* family was 14 times smaller. While 255 out of 412 taxa were shared at the genus level, the abundance of genera showed a moderate correlation (r = 0.44), consistent with the results of 16S rDNA sequencing (81). Interestingly, the genus *Collinsella* (phylum *Actinobacteria*), associated with atherosclerosis and rheumatoid arthritis, was represented by 579 species in humans but was not found in the mouse metagenome. Surprisingly, out of 1573 CMMG (comprehensive mouse microbiota genome) species, only 170 (10.8%) were identified in the human gut microbiota. Common species, on average, represented 13% of the composition of the mouse gut microbiome. Mapping mouse metagenome samples to the human reference database and vice versa achieved only a 30% mapping rate (82).

While these numbers may initially suggest a high degree of similarity in the gut microbiota, a closer look reveals key deviations, especially in terms of microbial composition and abundance. This demonstrates that mice and the human microbiome are significantly different. These results challenge our analogy between the human and mouse microbiota. Such changes in the microbiome composition can have a significant impact on experimental plans and research approaches to studying the human gut microbiome using mice as intermediaries.

Considering the significant influence that the microbiome can have on the efficacy of various drugs, the differences between the human and mouse gut microbiomes present a considerable challenge. Establishing a humanized gnotobiotic mouse model by transplanting human fecal microbiota into mice without their own microbiota represents an innovative and powerful tool for mimicking the human microbial system in mice. However, creating such a model requires careful consideration of various factors, from aspects related to human donors to the genetic background of the mice, all of which can influence the final research outcomes (83). It is also important to question how much of the human microbiome mice can retain, given the anatomical differences in the gastrointestinal tract between humans and mice.

While mice models are the most prevalent models to study the gut microbiome (84), there are also other animal candidates for these studies. For example, non-human primates have the most similar microbiome to human primates than to any other animal (85), therefore various studies have been conducted using them to explore the influence of different diets, for example, Western and Mediterranean diets on the gut microbiome (86, 87). We need to note here that of course there are differences in abundance of certain bacterial taxa between species. It was found that in non-human primates and in rats there is a higher abundance of *Prevotella* compared to humans and mice (88). As many diseases, disorders, and cancer progression have been linked to an abnormal gut microbiome in humans, research has now expanded to other animal models such as horses (89, 90) and dogs (91) to name a few. Researchers are also trying to create pig models that resemble the human gut microbiome to make future experiments easier and more reliable (92, 93).

For now, using mouse models to study the gut microbiome seems to be the optimal option, and the potential of optimized humanized gnotobiotic mouse models is something to look forward to in the future.

Studying the microbiome using alternative *in vitro* and *ex vivo* models

Despite great progress being made in deciphering the role of the gut microbiota in connection with various diseases using different animal models, especially mouse models, there are still several limitations with those models. They are time-consuming, under ethical considerations, and often fail to copy real human conditions because of inter-species differences and the complexity of the gut microbiome in general (94). Advances in three-dimensional cell biology and bioengineering enabled researchers to come up with alternative *in vitro* and *ex vivo* cellular and tissue models to study the microbiome. These models could decrease the number of animal experiments in the future.

Organoids are gaining more and more attraction since they have been proven to be valuable *in vitro* systems for modeling different human diseases (95). They are 3D self-assembled tissue constructs that contain highly polarized cells that mimic the *in vivo* organization and architecture of the tissue of origin (96). Organoids can be derived either from pluripotent stem cells (PCSs) or adult stem cells (ASCs). In the intestines, ACSs are located at the bottom of the intestinal crypts which can then be grown in extracellular matrix Matrigel to make organoid models which contain fully mature goblets cells, enteroendocrine cells, enterocytes and Paneth cells (97). When using PCSs to form organoids, cells are often either derived from embryonic stem cells or from induced pluripotent stem cells which are then treated with specific growth factors that direct the tissue-specific development of the cells (98). The PCS-derived organoids can contain also mesenchymal cells in comparison to intestinal epithelial cell types seen in ASC-derived organoids (99).

Gastrointestinal organoids either from ASC or PCS-derived cells have the basal membrane displayed outwards and the lumen in the center of the construct. The most popular method to deliver the bacteria or their metabolites to form a relevant microbiome organoid model is through microinjection into the lumen. This method mimics bacteria that normally also infects the host from the lumen but has also downsides since it is a difficult method to perform, and organoid damage often happens during the whole process. There is also a method where organoids in suspensions are mixed with microbes and then cultivated to reform 3D organoids but again this method does not appropriately capture the mechanisms by which microbes infect the cells (100, 101). One very important limitation researchers should be aware of includes the lack of cellular components of the microenvironment particularly in ASC-derived organoids (102). Among cellular components, organoids lack mesenchymal cell heterogeneity and architecture, vasculature, neuronal connections, and interaction with immune cells and the intestinal microbial flora (103).

Because of the emerging connection of microbiome influence on tumor development and progression, scientists also try to use different cancer organoid models to study this correlation. Traditional 2D cell cultures fail to mimic the complex tumor microenvironment and the interaction of tumor and non-tumor cells as well as the interaction with the extracellular matrix. The results so far show that tumor cells in organoids react differently to chemotherapy than 2D cell cultures or just tumor cells embedded in 3D gels, showing a promising future for the use of organoids (104).

Another very promising *in vitro* model is organs-on-chips, which can mimic the physiology, structure, function, and pathology of human organs. Scientists have invented organ chip models of the human intestine which are novel cell culture devices that offer greater control over important biological parameters such as oxygen availability and pH levels using different micro-fluidic channels (105). The chips complexity over the last years has increased and can now even mimic intestinal peristaltic-like motions and flow, using different mechanical forces (106). The advancements also include a variety of channels that are surrounded by commensal microbes, pathogens, immune cells, and human microvascular endothelium and can also enable villus-crypt formation and added mucus layer (105). For example, researchers have made an intestine chip that contains epithelial cells from human intestinal biopsies that successfully mimics real human physiological conditions (107). Apart from primary cells, other types of cells can be grown on such chips for example ASC or PCS-derived organoids (108, 109) or immortalized cell lines (110). In the end, these models could serve as a great option in addition to animal models to study the influence of the gut microbiome as well as being a valuable tool to study different kinds of tissues.

Alternative cell and tissue models in the laboratory will allow us to study the interactions between tissue, tumor, and microbiome by mimicking microbiome-host-cancer interactions as a function of species. This will allow us to understand the molecular mechanisms and develop alternative treatment approaches for various diseases, including cancer.

Gut microbiota in patients with aggressive primary brain tumors

We know that gut microbiota influences tumor growth and progression. Since gliomas are the most aggressive primary brain tumors in adults and are challenging to treat there is ongoing research about the connection to the gut microbiome trying to find new ways to improve current treatment outcomes (111). Researchers have found out that glioma tumor growth leads to dysbiosis in the gut microbiome in mice before weight loss occurs. They found a significant decrease in the *Firmicutes* to *Bacteroides* (F/B) ratio following tumor growth. They observed a decrease in *Firmicutes* and an increase in *Verrucomicrobia phyla*. Interestingly,

after treating the mice with the oral chemotherapy drug temozolomide (TMZ) there was no glioma-induced dysbiosis seen since there was no significant difference in the F/B ratio. They later showed that TMZ administration in healthy mice causes dysbiosis but with no significant change in *Verrucomicrobia*. When they analyzed fecal samples from human glioma patients and healthy controls they found a correlation with their mice results, with similar microbiome changes observed in both species (112).

We mentioned in previous sections the effects of short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate have anti-cancer properties. In a study made by A. Dono, *et al.* they show that the short fatty acids were all decreased in mouse models after glioma growth after an analysis of fecal metabolites. Apart from that, they saw a decrease in important neurotransmitters such as norepinephrine and 5-hydroxyindoleacetic acid (5-HIAA) after tumor development and an increase in serotonin, 3-methyl valerate, caproate, and acetylcholine. This study also found the same increases in the *Verrucomicrobia* phylum and a decrease in *Bacteroidetes* which coincides with the findings of the study mentioned in the previous paragraph since *Bacteroides* belong to the *Bacteroidetes* phylum of Gram-negative bacteria. After treating the mice with TMZ there was no significant change in SCFAs which is consistent with the findings that glioma growth impacts the gut microbiome. Similar data was also obtained from human samples which means that we can draw parallels between the mouse model and actual human samples (113).

Another study found conflicting results with the previously mentioned studies. After the growth of glioma in mouse models they observed a decrease in the abundance of *Bacteroidia* (new name for *Bacteroidetes*) and an increase in the abundance of *Firmicutes*. However, the results still suggest that glioma growth contributed to dysbiosis in the gut. They also found that after administering antibiotics to mice, the glioma progression is worse than in non-antibiotic treated mice. These results confirm the hypothesis that gut dysbiosis can worsen glioma progression. After detecting expression levels of CD8 and Foxp3 in mouse brain tissues in antibiotic and non-antibiotic-treated mice, they found out that CD8 expression levels were not significantly different between the two groups. On the other hand, there was a decrease in Foxp3 expression in antibiotic-treated mice compared to the control group suggesting that gut microbiome dysbiosis downregulated Foxp3 expression in glioma tissue and Foxp3 may act as a tumor suppressor protein (114).

The importance of gut microbiota in glioma development was highlighted in another study where they also found that glioma growth increases in mice treated with antibiotics. They also observed changes in microglia phenotype and a reduction in $CD27^+/CD11b^+$ NK cells that are involved in tumor cell lysis which could explain the tumor size increase in antibiotic-treated mice (115). The *Bifidobacterium* genus was shown to have antitumor effects (116) and in a study where mixtures of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* were administered into an orthotopic mouse model of glioma via gavage treatment, the tumor volume was reduced, and the lifespan of the mice was prolonged (117). This experiment gives us promising new therapeutic options for future glioma treatments.

Glioblastoma is the most aggressive type of glioma with a median survival of 12-15 months (118). The most effective treatment so far is the combination of surgical removal of the tumor, radiotherapy, and chemotherapy with TMZ. Immunotherapy is successful only in preclinical mouse models however, it is not effective in humans. The reason could be in the difference between the gut microbiome in humans and mouse models since studies report that 85% of bacterial genera found in the mouse gut microbiota are not present in human (77). A study using humanized mouse models, where they transplanted the human gut microbiome into mice, found that mice with different human gut donors responded differently to immunotherapy using the checkpoint inhibitor anti-PD-1 drug. Out of five tested human mouse models only 2 responded to the treatment and displayed a significant increase in survival compared to the control groups. The two responsive mouse models had an abundance of *Bacteroides cellulosilyticus*, while the non-responsive models had an abundance of *Bacteroides intestinalis* and *Bacteroides uniformis*. They also found an increase in cytotoxic $CD8^+$ and $CD4^+$ T-cells producing IFN-γ after anti-PD-1 treatment in a humanized mouse model that was responsive to immunotherapy. The same results were not observed in the humanized mouse model that did not respond to immunotherapy (119). A recent study comparing healthy individuals with glioblastoma brain tumor (GBM) patients has shown that the GBM patients had a higher gut microbial diversity compared to the healthy individuals. The GBM group had a decrease in *Firmicutes* and an increase in the Proteobacteria phylum (120).

The gut microbiota also plays a role in the metabolism of various amino acids, which has different effects on the progression of gliomas. For example, the gut microbiome plays an important role in tryptophan metabolism, the product of which are AHR agonists. AHR stands for the aryl hydrocarbon receptor, a transcription factor that is activated by various ligands and is involved in cell proliferation, differentiation, cell death, and cell adhesion (121). The receptor is expressed in gliomas with the highest expression seen in glioblastomas (122). The AHR agonists produced by gut bacteria can activate the AHR receptor which then increases $FoxP3^+$ regulatory T cells through different mechanisms. It also regulates other T cell function as well as the differentiation and function of dendritic cells (123). In the case of arginine metabolism, the gut microbiota can turn dietary arginine into polyamines and nitric oxide which arrive to the brain through the blood-brain barrier (124, 125). Polyamine can affect tumor growth by up-regulating the expression of

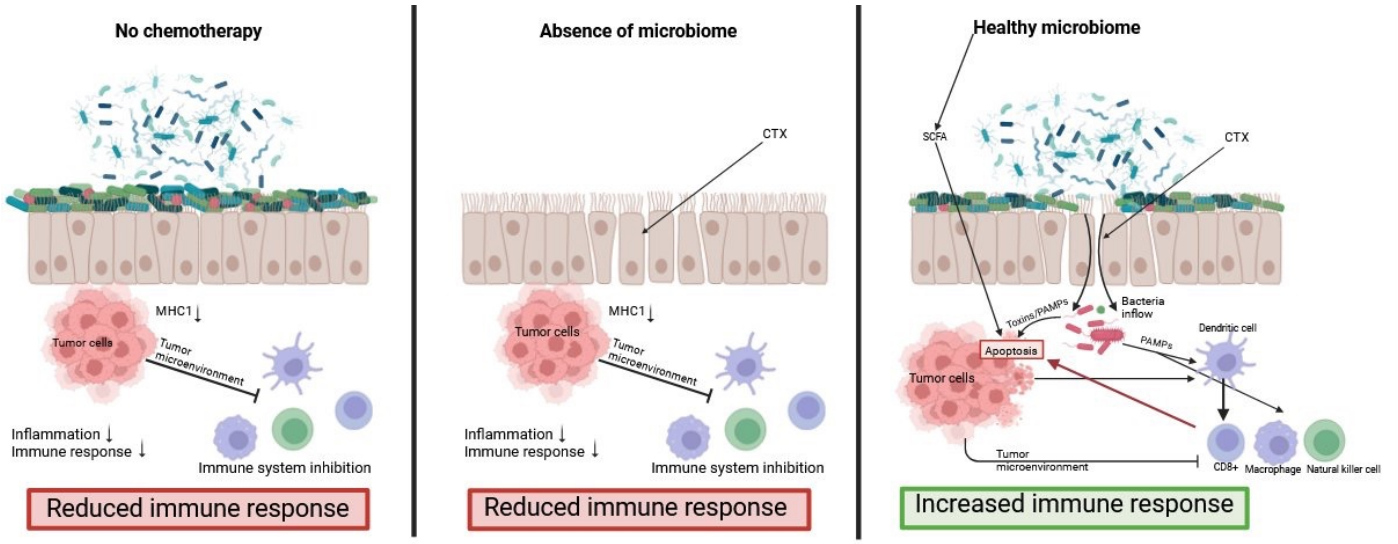

Figure 3: The influence of a healthy gut microbiome on chemotherapy treatment. On the left we can see that without chemotherapy the tumor microenvironment can suppress the immune system and hence hindering adequate immune response to eliminate the tumor. In the middle we can see that the absence of a gut microbiome, in gnotobiotic mice for instance, after administration of a chemotherapeutic drug CTX we can see no improvement in immune response. On the right where we have a healthy gut microbiome, the administration of CTX increases immune response and therefore impacts the natural immune system and increases the anti-tumor immune response. The Scheme was created using Biorender.com

Abbreviations: major histocompatibility complex 1 (MHC1), short-chain fatty acids (SCFAs), cyclophosphamide (CTX), pathogen-associated molecular pattern molecules (PAMPs).

ornithine decarboxylase, spermidine, spermine acetyltransferase, and Akt1 which can induce tumor cell proliferation and metastasis (126). The effects of nitric oxide on glioma are still not fully known but since nitric oxide can interfere with T cell function by promoting T cell apoptosis it can be speculated that it could promote glioma development (127, 128).

These studies suggest that the gut microbiome is also crucial for distant tumors, such as primary brain tumors, and that the microbiome is also found in the microenvironment of brain tumors (129), where its role remains to be explored. In this regard, the microbiome should be exploited to increase the efficacy of current therapies and/or to develop new, more efficient treatments.

Future outlook and conclusions

The gut microbiome has been shown to influence the success of various cancer therapies (Figure 3). Some bacteria have a tumor-inhibiting effect and help the individual fight cancer, while others have the opposite effect and worsen the state of health. Ideally, it would be desirable to eliminate the harmful bacteria while promoting the proliferation of the beneficial ones. However, current antibiotics have a broad spectrum of action that affects both types of bacteria. For this reason, research is being carried out into the synthesis of specific antibiotics that only eliminate the bad bacteria and leave the good bacteria untouched. Another possible solution would be the use of bacteriophages, which only infect and destroy certain bacteria, thus creating a microbiome that benefits the patient. Research on fecal microbiome transfer seems promising, but scientists have yet to determine why some donors' feces are more successful in increasing the effectiveness of immunotherapy than others. Nonetheless, this area of research is gaining increasing attention and could prove to be a crucial approach to cancer treatment in the future.

The gut microbiome is a dynamic and influential component of human health. While it offers many benefits, disturbances in its balance can contribute to the development and progression of cancer. It plays an important role in the anti-tumor response, as demonstrated by successful cancer remission after fecal transplantation in mice and humans. Understanding the intricate relationship between the gut microbiome and cancer is crucial for the development of new strategies to prevent and treat this complex disease. Further research is needed to explore the full extent of these interactions and their potential therapeutic implications so that we can improve treatment outcomes for difficult malignancies such as gliomas in the future. Because mouse models have genetic and physiological similarities to humans, they are invaluable for biomedical research, including research into human disease. However, it is important to recognize the microbiome-related differences between these two species, as this is the only way we can achieve more relevant results. New in vitro models such as organoids on chip organs offer a promising alternative to animal models to study the gut microbiome in a more controlled and species-specific way. This emerging field of research could help us fight cancer and other diseases in a novel way that could significantly improve our overall well-being.

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References

1. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013; 13(11): 800–12. doi: 10.1038/nrc3610
2. Gomaa EZ. Human gut microbiota/microbiome in health and diseases: a review. Antonie Van Leeuwenhoek 2020; 113(12): 2019–40. doi: 10.1007/s10482-020-01474-7
3. Frank DN, St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 2007; 104(34): 13780–5. doi: 10.1073/pnas.0706625104
4. Bielanski A, Haber J. Structure, function and diversity of the healthy human microbiome. Nature 2012;486(7402): 207–14. doi: 10.1038/nature11234
5. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol 2011 ;9(4): 244–53. doi: 10.1038/nrmicro2537
6. Chen D, Jin D, Huang S, Wu J, Xu M, Liu T, et al. Clostridium butyricum, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. Cancer Lett 2020; 469: 456–67. doi: 10.1016/j.canlet.2019.11.019
7. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World J Gastroenterol 2015; 21(29): 8787–803. doi: 10.3748/wjg.v21.i29.8787
8. Jiao Y, Wu L, Huntington ND, Zhang X. Crosstalk Between Gut Microbiota and Innate Immunity and Its Implication in Autoimmune Diseases. Front Immunol 2020; 11: 282. doi: 10.3389/fimmu.2020.00282
9. Rosas-Plaza S, Hernández-Terán A, Navarro-Díaz M, Escalante AE, Morales-Espinosa R, Cerritos R. Human gut microbiome across different lifestyles: from hunter-gatherers to urban populations. Front Microbiol 2022; 13: 843170. doi: 10.3389/fmicb.2022.843170
10. Shu YZ, Arcuri M, Kozlowski M, et al. Haloemodins, a new class of endothelin-1 type B (ETB) receptor binding inhibitors. J Antibiot 1994;47(11):1328–32.
11. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. Immunol Rev 2017; 279(1): 90–105. doi: 10.1111/imr.12563
12. De Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13(6): 607–15. doi: 10.1016/S1470-2045(12)70137-7
13. Wan Y, Wang F, Yuan J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut Microbiota 2019; 68(8): 1417–29. doi:10.1136/gutjnl-2018-317609
14. Lam W, Bussom S, Guan F, et al. The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity. Sci Transl Med 2010; 2(45): 45ra59. doi: 10.1126/scitranslmed.3001270
15. Wallace BD, Wang H, Lane KT, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010; 330(6005): 831–5. doi: 10.1126/science.1191175
16. Hattori N, Ushijima T. Epigenetic impact of infection on carcinogenesis: mechanisms and applications. Genome Med 2016; 8(1): 10. doi: 10.1186/s13073-016-0267-2
17. Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. Genomics Proteomics Bioinformatics 2018; 16(1): 33–49. doi: 10.1016/j.gpb.2017.06.002
18. Nasrollahzadeh D, Malekzadeh R, Ploner A, et al. Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. Sci Rep 2015; 5: 8820. doi: 10.1038/srep08820
19. Proctor LM. The human microbiome project in 2011 and beyond. Cell Host Microbe 2011; 10(4): 287–91. doi: 10.1016/j.chom.2011.10.001
20. Gagnière J, Raisch J, Veziant J, et al. Gut microbiota imbalance and colorectal cancer. World J Gastroenterol 2016; 22(2): 501–18. doi: 10.3748/wjg.v22.i2.501
21. Edwards PT, Kashyap PC, Preidis GA. Microbiota on biotics: Probiotics, prebiotics, and synbiotics to optimize growth and metabolism. Am J Physiol Gastrointest Liver Physiol. 2020;319(3):G382–90.
22. Yoshimoto S, Loo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013; 499(7456): 97–101. doi: 10.1038/nature12347
23. Loo TM, Kamachi F, Watanabe Y, et al. Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. Cancer Discov 2017; 7(5): 522–38. doi: 10.1158/2159-8290.CD-16-0932
24. Ma C, Han M, Heinrich B, et al. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. Science 2018; 360(6391): eaan5931. doi: 10.1126/science.aan5931
25. Cheng WY, Wu CY, Yu J. The role of gut microbiota in cancer treatment: friend or foe? Gut 2020; 69(10): 1867–76. doi: 10.1136/gutjnl-2020-321153
26. Nougayrède JP, Homburg S, Taieb F, et al. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science 2006; 313(5788): 848–51. doi: 10.1126/science.1127059
27. Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012; 338(6103): 120–3. doi: 10.1126/science.1224820
28. Bultman SJ. Emerging roles of the microbiome in cancer. Carcinogenesis 2014; 35(2): 249–55. doi: 10.1093/carcin/bgt392
29. Dennis KL, Blatner NR, Gounari F, Khazaie K. Current status of interleukin-10 and regulatory T-cells in cancer. Curr Opin Oncol 2013; 25(6): 637–45. doi: 10.1097/CCO.0000000000000006
30. Akbar N, Khan NA, Muhammad JS, Siddiqui R. The role of gut microbiome in cancer genesis and cancer prevention. Health Sci Rev 2022; 2(3): 100010. 10.1016/j.hsr.2021.100010
31. Bergounioux J, Elisee R, Prunier AL, et al. Calpain activation by the Shigella flexneri effector VirA regulates key steps in the formation and life of the bacterium's epithelial niche. Cell Host Microbe 2012; 11(3): 240–52. doi: 10.1016/j.chom.2012.01.013
32. Fomby P, Cherlin AJ. Role of microbiota in immunity and inflammation. National Institute of Health 2011;72(2): 181–204.
33. Wada Y, Takemura K, Tummala P, et al. Helicobacter pylori induces somatic mutations in TP53 via overexpression of CHAC1 in infected gastric epithelial cells. FEBS Open Bio 2018; 8(4): 671–9. doi: 10.1002/2211-5463.12402

34. Ding SZ, Minohara Y, Xue JF, et al. Helicobacter pylori infection induces oxidative stress and programmed cell death in human gastric epithelial cells. Infect Immun 2007; 75(8): 4030–9. doi: 10.1128/IAI.00172-07

35. Huycke MM, Moore D, Joyce W, et al. Extracellular superoxide production by Enterococcus faecalis requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases. Mol Microbiol 2001; 42(3): 729–40. doi: 10.1046/j.1365-2958.2001.02638.x

36. Chaturvedi R, Asim M, Romero-Gallo J, et al. Spermine oxidase mediates the gastric cancer risk associated with Helicobacter pylori CagA. Gastroenterology 2011; 141(5): 1696-1708.e1–2. doi: 10.1053/j.gastro.2011.07.045

37. Goodwin AC, Destefano Shields CE, Wu, et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc Natl Acad Sci U S A 2011; 108(37): 15354–9. doi: 10.1073/pnas.1010203108

38. Vivarelli S, Salemi R, Candido S, et al. Gut Microbiota and cancer: from pathogenesis to therapy. Cancers (Basel) 2019; 11(1): 38. doi: 10.3390/cancers11010038

39. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016; 14(8): e1002533. doi: 10.1371/journal.pbio.1002533

40. Morgan XC, Huttenhower C. Chapter 12: Human microbiome analysis. PLoS Comput Biol 2012; 8(12): e1002808. doi: 10.1371/journal.pcbi.1002808

41. Johansson MEV, Jakobsson HE, Holmén-Larsson J, et al. Normalization of host intestinal mucus layers requires long-term microbial colonization. Cell Host Microbe 2015; 18(5): 582–92. doi: 10.1016/j.chom.2015.10.007

42. Spiljar M, Merkler D, Trajkovski M. The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 2017; 8: 1353. doi: 10.3389/fimmu.2017.01353

43. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013; 14(7): 685–90. doi: 10.1038/ni.2608

44. Viaud S, Saccheri F, Mignot G, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342(6161): 971–6. doi: 10.1126/science.1240537

45. Daillère R, Vétizou M, Waldschmitt N, et al. Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced therapeutic immunomodulatory effects. Immunity 2016; 45(4): 931–43. doi: 10.1016/j.immuni.2016.09.009

46. Lin C, Cai X, Zhang J, et al. Role of gut microbiota in the development and treatment of colorectal cancer. Digestion 2019; 100(1): 72–8. doi: 10.1159/000494052

47. Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350(6264): 1079–84. doi: 10.1126/science.aad1329

48. Jan G, Belzacq AS, Haouzi D, et al. Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ 2002; 9(2): 179–88. doi: 10.1038/sj.cdd.4400935

49. Wei W, Sun W, Yu S, Yang Y, Ai L. Butyrate production from high-fiber diet protects against lymphoma tumor. Leuk Lymphoma 2016; 57(10): 2401–8. doi: 103109/1042819420161144879.

50. Zhang J, Xia Y, Sun J. Breast and gut microbiome in health and cancer. Genes Dis 2020; 8(5): 581–9. doi: 10.1016/j.gendis.2020.08.002

51. Sánchez-Alcoholado L, Ramos-Molina B, Otero A, et al. The role of the gut microbiome in colorectal cancer development and therapy response. Cancers (Basel) 2020; 12(6): 1406. doi: 10.3390/cancers12061406

52. Salcedo R, Worschech A, Cardone M, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med 2010; 207(8): 1625–36. doi: 10.1084/jem.20100199

53. Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. J Clin Invest 2007; 117(8): 2197–204. doi: 10.1172/JCI32205

54. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009; 374(9686): 301–14. doi: 10.1016/S0140-6736(09)61248-4

55. Konishi H, Fujiya M, Tanaka H, Ueno N, Moriichi K, Sasajima J, et al. Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. Nature Communications 2016; 7: 12365. doi: 10.1038/ncomms12365

56. Davar D, Dzutsev AK, McCulloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 2021; 371(6529): 595–602. doi: 10.1126/science.abf3363

57. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021; 371(6529): 602–9. doi: 10.1126/science.abb5920

58. Erdmann J. How gut bacteria could boost cancer treatments. Nature 2022; 607(7919): 436–9. doi: 10.1038/d41586-022-01959-7

59. Legesse Bedada T, Feto TK, Awoke KS, Garedew AD, Yifat FT, Birri DJ. Probiotics for cancer alternative prevention and treatment. Biomed Pharmacother 2020; 129: 110409. doi: 10.1016/j.biopha.2020.110409

60. Rasouli BS, Ghadimi-Darsajini A, Nekouian R, Iragian GR. In vitro activity of probiotic Lactobacillus reuteri against gastric cancer progression by downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression. J Cancer Res Ther 2017; 13(2): 246–51. doi: 10.4103/0973-1482.204897

61. Redman MG, Ward EJ, Phillips RS. The efficacy and safety of probiotics in people with cancer: a systematic review. Ann Oncol 2014; 25(10): 1919–29. doi: 10.1093/annonc/mdu106

62. Mego M, Holec V, Drgona L, Hainova K, Ciernikova S, Zajac V. Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. Complement Ther Med 2013; 21(6): 712–23. doi: 10.1016/j.ctim.2013.08.018

63. Gianotti L, Morelli L, Galbiati F, et al. A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. World J Gastroenterol 2010; 16(2): 167–75. doi: 10.3748/wjg.v16.i2.167

64. Demers M, Dagnault A, Desjardins J. A randomized double-blind controlled trial: impact of probiotics on diarrhea in patients treated with pelvic radiation. Clin Nutr 2014; 33(5): 761–7. doi: 10.1016/j.clnu.2013.10.015

65. Mego M, Chovanec J, Vochyanova-Andrezalova I, et al. Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. Complement Ther Med 2015; 23(3): 356–62. doi: 10.1016/j.ctim.2015.03.008

66. Hatakka K, Saxelin M, Mutanen M, et al. Lactobacillus rhamnosus LC705 Together with Propionibacterium freudenreichii ssp shermanii JS administered in capsules is ineffective in lowering serum lipids. 2013; 27(4): 441–7. doi: 101080/07315724200810719723

67. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018; 359(6371): 97–103. doi: 10.1126/science.aan4236

68. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018; 359(6371): 104–8. doi: 10.1126/science.aao3290

69. Chen Q, Wang C, Chen G, Hu Q, Gu Z. Delivery Strategies for Immune Checkpoint Blockade. Adv Healthc Mater 2018; 7(20): e1800424. doi: 10.1002/adhm.201800424

70. Chaput N, Lepage P, Coutzac C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. Ann Oncol 2017; 28(6): 1368–79. doi: 10.1093/annonc/mdx108

71. Levy M, Thaiss CA, Katz MN, Suez J, Elinav E. Inflammasomes and the microbiota—partners in the preservation of mucosal homeostasis. Semin Immunopathol 2015; 37(1): 39–46. doi: 10.1007/s00281-014-0451-7

72. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nature Immunol 2009; 10(3): 241–7. doi: 10.1038/ni.1703

73. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. Nature Immunol 2012; 13(4): 343–51. doi: 10.1038/ni.2224

74. Morse HC. Building a better mouse: one hundred years of genetics and biology. In: Fox JG, eds. The mouse in biomedical research: history, wild mice, and genetics. 2nd ed. Amsterdam: Elsevier, 2006.

75. Li Y, Baldridge MT. Modelling human immune responses using microbial exposures in rodents. Nat Microbiol 2023; 8(3): 363–6. doi: 10.1038/s41564-023-01334-w

76. Sjaastad FV, Huggins MA, Lucas ED, et al. Reduced T cell priming in microbially experienced "dirty" mice results from limited il-27 production by xcr1+ dendritic cells. J Immunol 2022; 209(11): 2149–59.

77. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech. 2015 Jan 1;8(1):1–16.

78. Guinane CM, Tadrous A, Fouhy F, et al. Microbial composition of human appendices from patients following appendectomy. mBio 2013; 4(1): e00366–12. doi: 10.1128/mBio.00366-12

79. Alkadhi S, Kunde D, Cheluvappa R, Randall-Demllo S, Eri R. The murine appendiceal microbiome is altered in spontaneous colitis and its pathological progression. Gut Pathog 2014; 6: 25. doi: 10.1186/1757-4749-6-25

80. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. PLoS One 2013; 8(5): e62578. doi: 10.1371/journal.pone.0062578

81. Almeida A, Nayfach S, Boland M, et al. A unified catalog of 204, 938 reference genomes from the human gut microbiome. Nat Biotechnol 2021; 39(1): 105–14. doi: 10.1038/s41587-020-0603-3

82. Kieser S, Zdobnov EM, Trajkovski M. Comprehensive mouse microbiota genome catalog reveals major difference to its human counterpart. PLoS Comput Biol 2022; 18(3): e1009947. doi: 10.1371/journal.pcbi.1009947

83. Park JC, Im SH. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. Exp Mol Med 2020; 52(9): 1383–96. doi: 10.1038/s12276-020-0473-2

84. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. Front Physiol 2018; 9: 1534. doi: 10.3389/fphys.2018.01534

85. Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. Science 2008; 320(5883): 1647–51. doi: 10.1126/science.1155725

86. Amato KR, Yeoman CJ, Cerda G, et al. Variable responses of human and non-human primate gut microbiomes to a Western diet. Microbiome 2015; 3(1): 53. doi: 10.1186/s40168-015-0120-7

87. Nagpal R, Shively CA, Appt SA, et al. Gut microbiome composition in non-human primates consuming a western or mediterranean diet. Front Nutr 2018; 5: 28. doi: 10.3389/fnut.2018.00028

88. Nagpal R, Wang S, Solberg Woods LC, et al. Comparative microbiome signatures and short-chain fatty acids in mouse, rat, non-human primate, and human feces. Front Microbiol 2018; 9: 2897. doi: 10.3389/fmicb.2018.02897

89. Garrett LA, Brown R, Poxton IR. A comparative study of the intestinal microbiota of healthy horses and those suffering from equine grass sickness. Vet Microbiol 2002; 87(1): 81–8. doi: 10.1016/s0378-1135(02)00018-4

90. Costa MC, Arroyo LG, Allen-Vercoe E, et al. Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the v3-v5 region of the 16s rrna gene. PLoS One 2012; 7(7): e41484. doi: 10.1371/journal.pone.0041484

91. Rostaher A, Morsy Y, Favrot C, Unterer S, Schnyder M, Scharl M, et al. Comparison of the gut microbiome between atopic and healthy dogs—preliminary data. Animals (Basel) 2022; 12(18): 2377. doi: 10.3390/ani12182377

92. Heinritz SN, Mosenthin R, Weiss E. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr Res Rev 2013; 26(2): 191–209. doi: 10.1017/S0954422413000152

93. Zhang Q, Widmer G, Tzipori S. A pig model of the human gastrointestinal tract. Gut Microbes 2013; 4(3): 193–200. doi: 10.4161/gmic.23867

94. Paul W, Marta C, Tom V de W. Resolving host–microbe interactions in the gut: the promise of in vitro models to complement in vivo research. Curr Opin Microbiol 2018; 44: 28–33. doi: 10.1016/j.mib.2018.07.001

95. Schweiger PJ, Jensen KB. Modeling human disease using organotypic cultures. Curr Opin Cell Biol 2016; 43: 22–9. doi: 10.1016/j.ceb.2016.07.003

96. Hill DR, Spence JR. Gastrointestinal organoids: understanding the molecular basis of the host–microbe interface. Cell Mol Gastroenterol Hepatol 2017; 3(2): 138–49. doi: 10.1016/j.jcmgh.2016.11.007

97. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009; 459(7244): 262–5. doi: 10.1038/nature07935

98. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011; 470(7332): 105–9. doi: 10.1038/nature09691

99. Forbester JL, Goulding D, Vallier L, et al. Interaction of Salmonella enterica serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells. Infect Immun 2015; 83(7): 2926–34. doi: 10.1128/IAI.00161-15

100. Williamson IA, Arnold JW, Samsa LA, et al. A high-throughput organoid microinjection platform to study gastrointestinal microbiota and luminal physiology. Cell Mol Gastroenterol Hepatol 2018; 6(3): 301–19. doi: 10.1016/j.jcmgh.2018.05.004

101. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-derived 3d organoid systems. Trends Mol Med 2017; 23(5): 393–410. doi: 10.1016/j.molmed.2017.02.007

102. Günther C, Winner B, Neurath MF, Stappenbeck TS. Organoids in gastrointestinal diseases: from experimental models to clinical translation. Gut 2022; 71(9): 1892–908. doi: 10.1136/gutjnl-2021-326560

103. Taelman J, Diaz M, Guiu J. Human intestinal organoids: promise and challenge. Front Cell Dev Biol 2022; 10: 854740. doi: 10.3389/fcell.2022.854740

104. Shelkey E, Oommen D, Stirling ER, et al. Immuno-reactive cancer organoid model to assess effects of the microbiome on cancer immunotherapy. Sci Rep 2022; 12(1): 9983. doi: 10.1038/s41598-022-13930-7

105. Bein A, Shin W, Jalili-Firoozinezhad S, et al. Microfluidic organ-on-a-chip models of human intestine. Cell Mol Gastroenterol Hepatol 2018; 5(4): 659–68. doi: 10.1016/j.jcmgh.2017.12.010

106. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 2012; 12(12): 2165–74. doi: 10.1039/c2lc40074j

107. Kasendra M, Tovaglieri A, Sontheimer-Phelps A, et al. Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. Sci Rep 2018; 8(1): 2871. doi: 10.1038/s41598-018-21201-7

108. Nikolaev M, Mitrofanova O, Broguiere N, et al. Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature 2020; 585(7826): 574–8. doi: 10.1038/s41586-020-2724-8

109. Naumovska E, Aalderink G, Valencia CW, et al. Direct On-Chip Differentiation of Intestinal Tubules from Induced Pluripotent Stem Cells. Int J Mol Sci 2020; 21(14): 4964. doi: 10.3390/ijms21144964

110. Beaurivage C, Naumovska E, Chang YX, et al. Development of a gut-on-a-chip model for high throughput disease modeling and drug discovery. Int J Mol Sci. 2019; 20(22): 5661. doi: 10.3390/ijms20225661

111. Bush NAO, Chang SM, Berger MS. Current and future strategies for treatment of glioma. Neurosurg Rev 2017; 40(1): 1–14. doi: 10.1007/s10143-016-0709-8

112. Patrizz A, Dono A, Zorofchian S, et al. Glioma and temozolomide induced alterations in gut microbiome. Sci Rep 2020; 10(1): 21002. doi: 10.1038/s41598-020-77919-w

113. Dono A, Patrizz A, McCormack RM, et al. Glioma induced alterations in fecal short-chain fatty acids and neurotransmitters. CNS Oncol 2020; 9(2): CNS57. doi: 10.2217/cns-2020-0007

114. Fan Y, Su Q, Chen J, Wang Y, He S. Gut Microbiome alterations affect glioma development and foxp3 expression in tumor microenvironment in mice. Front Oncol 2022; 12: 836953. doi: 10.3389/fonc.2022.836953

115. D'Alessandro G, Antonangeli F, Marrocco F, et al. Gut microbiota alterations affect glioma growth and innate immune cells involved in tumor immunosurveillance in mice. Eur J Immunol 2020; 50(5): 705–11. doi: 10.1002/eji.201948354

116. Wei H, Chen L, Lian G, et al. Antitumor mechanisms of bifidobacteria . Oncol Lett 2018; 16(1): 3–8. doi: 10.3892/ol.2018.8692

117. Fan H, Wang Y, Han M, et al. Multi-omics-based investigation of Bifidobacterium's inhibitory effect on glioma: regulation of tumor and gut microbiota, and MEK/ERK cascade. Front Microbiol 2024; 15: 1344284. doi: 10.3389/fmicb.2024.1344284

118. Dunn GP, Rinne ML, Wykosky, et al. Emerging insights into the molecular and cellular basis of glioblastoma. Gen Dev 2012; 26(8): 756–84. doi: 10.1101/gad.187922.112

119. Dees KJ, Koo H, Humphreys JF, et al. Human gut microbial communities dictate efficacy of anti-PD-1 therapy in a humanized microbiome mouse model of glioma. Neurooncol Adv 2021; 3(1): vdab023. doi: 10.1093/noajnl/vdab023

120. Ishaq HM, Yasin R, Mohammad IS, et al. The gut-brain-axis: a positive relationship between gut microbial dysbiosis and glioblastoma brain tumour. Heliyon 2024; 10(9): e30494. doi: 10.1016/j.heliyon.2024.e30494

121. Zaragoza-Ojeda M, Apatiga-Vega E, Arenas-Huertero F. Role of aryl hydrocarbon receptor in central nervous system tumors: biological and therapeutic implications . Oncol Lett 2021; 21(6): 460. doi: 10.3892/ol.2021.12721

122. Jin UH, Michelhaugh SK, Polin LA, Shrestha R, Mittal S, Safe S. Omeprazole inhibits glioblastoma cell invasion and tumor growth. Cancers 2020; 12(8): 2097. doi: 10.3390/cancers12082097

123. Gutiérrez-Vázquez C, Quintana FJ. Regulation of the immune response by the aryl hydrocarbon receptor. Immunity 2018; 48(1): 19–33. doi: 10.1016/j.immuni.2017.12.012

124. Dai Z, Wu Z, Hang S, Zhu W, Wu G. Amino acid metabolism in intestinal bacteria and its potential implications for mammalian reproduction. Mol Hum Reprod 2015; 21(5): 389–409. doi: 10.1093/molehr/gav003

125. Kao CC, Cope JL, Hsu JW, et al. The microbiome, intestinal function, and arginine metabolism of healthy indian women are different from those of american and jamaican women. J Nutr 2015; 146(4): 706–13. doi: 10.3945/jn.115.227579

126. Dai F, Yu W, Song J, Li Q, Wang C, Xie S. Extracellular polyamines-induced proliferation and migration of cancer cells by ODC, SSAT, and Akt1-mediated pathway. Anticancer Drugs 2017; 28(4): 457–64. doi: 10.1097/CAD.0000000000000465

127. Rivoltini L, Carrabba M, Huber V, et al. Immunity to cancer: attack and escape in T lymphocyte–tumor cell interaction. Immunol Rev 2002; 188(1): 97–113. doi: 10.1034/j.1600-065x.2002.18809.x

128. Harari O, Liao JK. Inhibition of MHC II Gene Transcription by Nitric Oxide and Antioxidants. Curr Pharm Des 2004; 10(8): 893–8. doi: 10.2174/1381612043452893

129. Nejman D, Livyatan I, Fuks G, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. Science 2020; 368(6494): 973–80. doi: 10.1126/science.aay9189

Črevesni mikrobiom pri raku: naslednja velika priložnost za boljši izid zdravljenja?

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Izvleček: Črevesni mikrobiom, raznolika skupnost mikroorganizmov v človeškem telesu, igra pomembno vlogo pri ohranjanju zdravja in vpliva na različne telesne procese. Edinstven črevesni mikrobiom posameznika, ki ga oblikujejo dejavniki, kot so način rojstva, prehrana, vnos antibiotikov in življenjski slog, prispeva k različnim telesnim funkcijam. Te funkcije so presnova hranil, metabolizem zdravil in uravnavanje imunskega sistema. Spremembe v črevesnem mikrobiomu so povezane s predispozicijo za nastanek raka in lahko vplivajo na učinkovitost njegovega zdravljenja. Porušeno črevesno ravnovesje oziroma disbioza v črevesnem mikrobiomu lahko vodi do vnetja, razvoja tumorjev in metastaz, kar poudarja njegov pomen v raziskavah raka. Črevesna mikrobiota pomembno vpliva na razvoj raka in rezultate zdravljenja. Nekatere bakterije povečajo učinke terapij, kot je ciklofosfamid, in prispevajo k boljšemu imunskemu odzivu proti raku. Mikroorganizmi proizvajajo protirakave molekule in probiotične spojine, ki so pomembno orodje pri preprečevanju in zdravljenju raka. Z nadaljnjimi raziskavami si znanstveniki želijo razviti ciljne antibiotike in raziskati prenos fekalne mikrobiote za selektivno manipulacijo mikrobiote. Zaradi genetskih in fizioloških podobnosti so mišji modeli neprecenljivi v biomedicinskih raziskavah, vendar pa zaradi razlik v črevesnem mikrobiomu ljudi in miši ter sestavi tumorskega mikrookolja neposredna primerjava med tema dvema modeloma lahko predstavlja izziv. Premostitev teh vrzeli je ključna za primerjalno medicino zlasti pri raziskavah raka, kjer mikrobiom igra pomembno vlogo pri izidih zdravljenja. Pri možganskih tumorjih gliomih lahko črevesni mikrobiom izkoristimo za potencialne nove možnosti zdravljenja. Dolgoročnega učinkovitega zdravljenja za to vrsto raka še ni, vendar raziskave na mišjih modelih kažejo povezavo med napredovanjem tumorja in odzivom na zdravljenje ter spremembami v črevesnem mikrobiomu. Črevesni mikrobiom in njegova modulacija predstavljata priložnost za učinkovitejše zdravljenje raka v prihodnosti.

Ključne besede: črevesni mikrobiom; rak; izid zdravljenja; tumorski modeli; gliom