Exp Oncol 2020
42, 2, 82–93

REVIEW

Experimental ONCOLOGY

HUMAN MICROBIOTA AND EFFECTIVENESS OF CANCER CHEMOTHERAPY

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This review presents up-to-date information on the effects of microbiota on the individual chemotherapy sensitivity in cancer treatment. Recent studies have shown that a fine balance between the intestinal microbiota and the immune system is crucial for maintaining an efficacy of cancer chemotherapy. A number of antitumor drugs have complex mechanisms of action involving not only direct effects but also the activity of the intestinal microbiota and the immune system. A unique combination of these factors contributes to the individual chemotherapy sensitivity.

***Key Words*: chemotherapy, microbiota, cancer, tumor-microbiota relationships.**

DOI: 10.32471/exp-oncology.2312-8852.vol-42-no-2.14611

The outbreak of Covid-19 infection makes us think again about the fact that there are individual features of the infectious process. Similarly, the individual characteristics of the macroorganism determine the predisposition to the development of malignant neoplasms, the effectiveness of chemotherapy, radiotherapy and immunotherapy. Recently, more and more attention is paid to the microbiota of the human body as a key factor that determines the individual features of metabolism and functions of vital body systems: digestive, immune, hormonal, nervous.

Individual sensitivity of patients to therapeutic influences is a key problem in the treatment of any human pathology, including cancer, and is caused mostly by the genetic and epigenetic characteristics of a particular individual. However, apart from these, there are other important factors. Today it is known that the human body is a "superorganism" or "holobiont", i.e. is a whole ecosystem inhabited by trillions of bacteria, protozoa, viruses, which generally form the microbiota of each individual macroorganism [1–4].

The microbiota of bacterial origin is one of the most studied and influential. In modern research projects Human Microbiome Projects 1 and 2, the species diversity of bacteria that inhabit the human body has been studied, and the impact of microorganisms on human health and pathology has been determined [5, 6]. According to recent estimates, the number of bacterial cells in the human body slightly exceeds the number of own cells of the macroorganism (the ratio of bacterial cells to eukaryotic cells of the human body is 1.3/1) [7]. The microbiota of the human body accounts up to ~ 2000 species of bacteria, ~ 800 of which are inhabitants of the intestine. The intestinal microbiota is the most numerous and has a tremendous effect on metabolic processes in the human body. All representatives of the intestinal microbiota belong to ~ 30 phyla, but the largest number of bacteria belongs to only 5 phyla: Firmicutes (*Clostridia*, *Bacilli*, *Lachnospiraceae*, *Ruminicoccaceae*, *Veillonellaceae*), Bacteroidetes (*Bacteroidaceae*), Actinobacteria (*Bifidobacteria*), Proteobacteria (*Enterobacteriaceae*), Verrucomicrobia (*Akkermansia muciniphila*) [8, 9] (Fig. 1). The most abundant are two phyla — Firmicutes and Bacteroidetes. The composition of the microbiota changes during human life and depends on many factors [10, 11] (see Fig. 1).

It was estimated that in the total number of genes of the holobiont, the genes of the human body make up only 1%, while 99% account for the set of genes of the human microbiota, which is called the microbiome. The microbiome encodes a wide variety of enzymes that carry out the biodegradation of various substances, foods and drugs that enter the human body [12–14]. Sometimes the human microbiota is called a "metabolic organ" and some scientists even use the term "forgotten organ" because the value of the microbiota in human health and pathological conditions has long been underestimated [15].

To date, it has been investigated that bacterial cells may be present in various organs and systems that were previously considered sterile. Bacteria are the components of the microenvironment of many types of tumors: tumors of the gastrointestinal tract, lungs, reproductive system, breast. The activity of these bacteria may be one of the causes of tumor development, as well as partially determine the resistance of the tumor to chemotherapy [16, 17]. It is also necessary to recognize that the effects of microbiota on cancer chemotherapy are mostly realized through various physiological systems of the host, especially, the immune system.

Submitted: May 10, 2020.

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Abbreviations used: 5-FU – 5-fluorouracil; ALL – acute lymphoblastic leukemia; CDD_L – cytidine deaminase long form; GF – germ free; ICI – immune checkpoint inhibitor; ROS – reactive oxygen species.

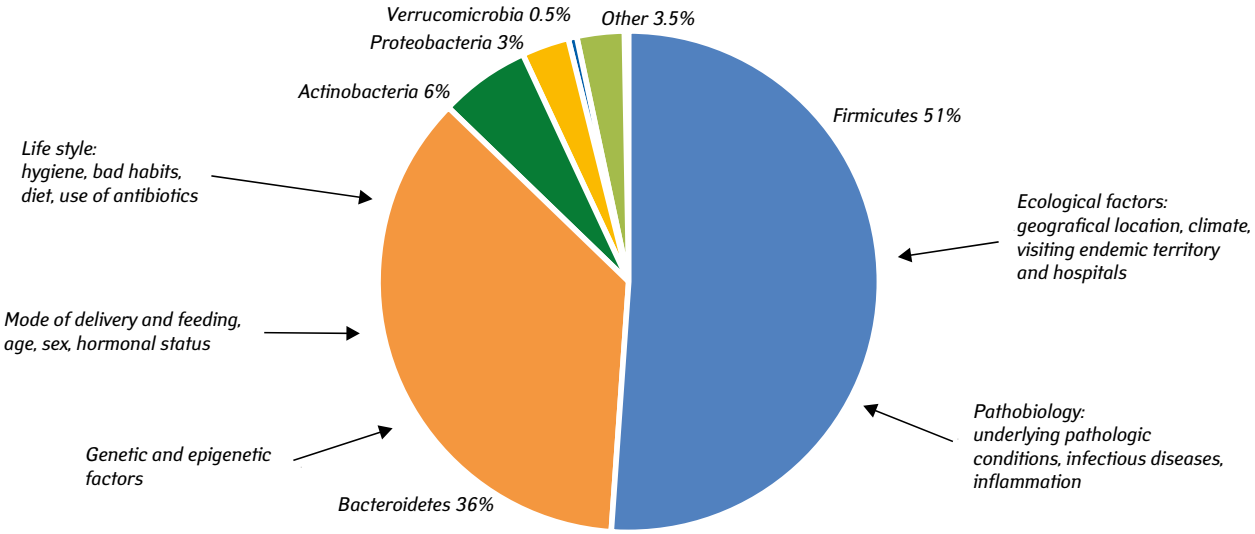

Fig. 1. Taxonomic distribution of human intestinal microbiota by phyla and the key factors influencing composition of microbiota

To study the effect of microbiota on the effectiveness of cancer chemotherapy, the animals that are born and live in germ-free (GF) conditions or the animals, in which the microbiota is eliminated by the use of broad-spectrum antibiotics, are being used. Both systems have their advantages and disadvantages. For example, in GF animals, all organs and systems develop in the complete absence of microorganisms. Therefore, the immune system of such animals is significantly underdeveloped, both structurally and functionally. Since the effectiveness of chemotherapy depends largely on the activity of the immune system, the GF model of animals, in this sense, is not adequate. When using animals in which the microbiota is eliminated by antibiotics, it should be understood that antibiotics, like any chemotherapy, have a number of toxic side effects on those organs and systems whose normal activity is important for the effectiveness of antitumor chemotherapeutics. Studies using GF animals have shown that the use of antitumor drugs in such animals with grafted tumors is not effective. The effectiveness was restored when these animals were contaminated by some microbiota representatives [3].

In the case of studying the effect of microbiota on the effectiveness of cancer chemotherapy in humans, special attention is paid to the study of those cases where chemotherapy occurs simultaneously with antimicrobial therapy.

When considering the interaction of microbiota and cancer chemotherapy, three logical areas of research could be identified: the effect of chemotherapy on the microbiota, the effect of microbiota on the effectiveness of cancer chemotherapy and the effect of microbiota on toxic side effects of chemotherapy. Also extremely attractive is the idea of changing the composition of the microbiota for increasing the effectiveness of chemotherapy and decreasing its toxicity. What are the natural ways to correct the microbiota? Isn't dietary therapy the safest and most environmentally friendly approach to such task?

THE EFFECT OF CHEMOTHERAPY ON THE SPECIES COMPOSITION OF THE MICROBIOTA

Chemotherapeutic anticancer agents are divided into certain categories depending on their structure and mechanisms of action. Thus, the key groups are: alkylating drugs, antimetabolites, cytotoxic antibiotics, protein kinase inhibitors, topoisomerase inhibitors, cell division spindle blockers, antihormonal drugs. The effect of some drugs on the composition and translocation of the intestinal microbiota has been studied (Table).

Cancer chemotherapeutics are usually administered parenterally and orally. In the case of parenteral administration, from the first minutes > 90% of the chemotherapeutic agent is in circulation and acts directly on the cells of various organs and systems, including tumor tissue [3]. When administered parenterally, xenobiotics undergo the first stages of metabolism in the liver, then enter the small intestine through the bile ducts, where they are subjected to secondary metabolism with the participation of microbiota and then reabsorbed in the intestine. When administered orally, the drugs are first metabolized by the enzymatic systems of the intestinal microbiota, and then, after absorption in the intestine and transport through the portal vein, undergo metabolic transformations in the liver. It is believed that only ~ 10% of the active form of the drug enters the circulation and acts on the tumor and other tissues of the body when administered orally.

The results of clinical observations of patients undergoing chemotherapy indicate that most patients develop dysbacteriosis of the intestine, oral cavity, and vagina [18]. A number of antitumor drugs have a proven adverse effects on the intestinal microbiota [19–21]. Moreover, chemotherapy induces the changes not only in intestinal microbiota but also in microbiota of tumor tissue.

It is known that representatives of the human intestinal microbiota belonging to different species possess different sensitivity to the action of antitumor drugs [21]. Mitomycin C has been shown to be effec-

Table. Effect of anticancer agents on microbiota in experiment

Chemotherapeutic drug	Effect on the microbiota and the immune system
Cyclophosphamide	Induction of dysbacteriosis, reduction of intestinal microbiota diversity, bacterial translocation; Firmicutes ↑[1], Bacteroides ↓[1]; Decrease in the number of Treg, increase in the number of CD8+ T cells, Th1 та Th17 *in vivo*
Cisplatin	Induction of dysbacteriosis, bacterial translocation; Firmicutes ↓[1], *Lactobacillus* ↓[1], *Ruminococcusgnavus* ↓[1], *Bacteroidaceae* ↑[1],*Erysipelotrichaceae* ↑[1], *Helicobacter* ↑[1], *Lactobacillus* ↓[2],*Coprococcus* ↓[2], *Escherichia* ↑[2], *Bacteroides* ↑[2], *Clostridium* ↑[2]
5-Fluorouracil	Induction of dysbacteriosis; *Clostridium* ↓[2], *Lactobacillus* ↓[2], *Streptococcus* ↓[2], *Enterococcus* ↓[2], *Staphylococcus* ↑[2]
Methotrexate	Induction of dysbacteriosis; *Lachnospiraceae* ↑[1], *Ruminococcaceae* ↓[1],*Bacteroidales* ↓[1],
Doxorubicin	Induction of dysbacteriosis of oral cavity and intestines in humans, Firmicutes/Bacteroidetes ↓[1], *Lachnospiraceae* ↓[1], *Clostridium*_IV↓[1], *Roseburia* ↓[1], *Clostridium*_XIVa↓[1], *Oscillibacter* ↓[1], *Butyricicoccus* ↓[1], *Clostridiales* ↓[1], *Akkermansia* ↑[1]
Mitomycin C	Induction of dysbacteriosis; *Pseudomonas aeruginosa* ↓[3], *Escherichia coli* ↓[3], *Klebsiella pneumoniae* ↓[3], *Enterobacter cloacae* ↓[3]
Irinotecan	Induction of dysbacteriosis; *Escherichia* spp. ↑[2], *Clostridium* spp. ↑[2], *Enterococcus* spp. ↑[2], *Serratia* spp. ↑[2], *Staphylococcus* spp. ↑[2], *Proteus* spp. ↑[2], *Clostridium* spp. ↑[2], *Peptostreptococcus* ↑[2]; *Bacillus* spp. ↓[2], *Bifidobacterium* spp. ↓[2]

Notes: [1]data confirmed in experiments using mice; [2]data confirmed in experiments using rats; [3]results of *in vitro* studies.

tive against opportunistic gram-negative bacteria: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter cloacae* [20]. In *in vitro* studies, the sensitivity of bacteria in the human intestinal microbiota belonging to 34 species to 12 most commonly used antitumor chemotherapeutics have been examined [21]. The sensitivity of bacteria belonging to the genera *Lactococcus, Lactobacillus, Bifidobacterium, Bacteroides, Blautia, Slackia* and representatives of the species *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Faecalibacterium prausnitzice* and *Serratiamarcescens* was studied [21]. The studied bacteria were shown to be resistant to taxoids, docytaxel and paclitaxel, the drugs that disrupt the eukaryotic cell division spindle. Drugs such as erlotinib, gefitinib, and afatinib, which are inhibitors of EGF-R-dependent tyrosine kinase pathways, exerted different effects on the studied bacteria. It is believed that in this case, cross-reactivity of chemotherapeutics is possible due to the similarity of target proteins between pro- and eukaryotic cells. Most of the bacteria studied were resistant to so-called prodrugs such as irinotecan and capecitabine. These drugs are converted into active therapeutic forms only if they are metabolized by enzymes in the human body. Lactobacilli were resistant to cyclophosphamide. However, it was shown that lactobacilli and bifidobacteria were sensitive to 5-fluorouracil (5-FU), doxorubicin, gemcitabine and pemetrexed.

It was shown that the use of cisplatin in C57BL/6 mice with grafted ovarian carcinoma led to the development of dysbacteriosis [22]. In the feces of mice, there was observed an increased content of bacteria from the families *Bacteroidaceae* (eg, *Bacteroides uniformis*) *and Erysipelotrichaceae* and significantly decreased content of bacteria of *Ruminococcus gnavus*. This study also showed that the administration of cisplatin to animals led to the destruction of the epithelial cells of the ileum and colon with the subsequent development of mucositis. In another study, when C57BL/6/J mice were injected with cisplatin, in the intestinal microbiota a significant decrease of Firmicutes phylum bacteria (by 27%), including members of *Lactobacillus* genus, and an increased *Helicobacter* counts were observed [23]. The use of cyclophosphamide in mice reduced the diversity of intestinal microbiota, decreased the content of Bacteroidetes phylum and increased the content of Firmicutes phylum [24].

Disturbed intestinal epithelial integrity along with the development of tissue inflammation was also observed in BALB/c mice after methotrexate administration [25]. The use of methotrexate led to the development of mucositis and intestinal dysbacteriosis in mice — decreased number of members of the family *Ruminococcaceae*, and increased number of bacteria of the family *Lachnospiraceae*. There was also observed a significant decrease of the counts of bacteria from genus *Bacteroidales*, especially bacteria of the key to the human intestine and mice species *Bacteroides fragilis*. It is known that the bacterium *B. fragilis* has tolerogenic effect on local immune response. In this study, the authors also showed that *B. fragilis* promoted the reduction of the polarization of macrophages in the direction of the proinflammatory M1 phenotype. The use of metronidazole, an antibiotic to which *B. fragilis* is sensitive, disrupted the tolerogenic effect of these bacteria and exacerbated the development of inflammation of the intestinal mucosa in response to methotrexate [25]. Therefore, the introduction of the cytostatics, in addition to a direct toxic effect on the cells of the intestinal epithelium, also exerted the effect mediated by the microbiota, which leads to increased inflammation.

In a study performed on rats, administration of cisplatin at therapeutic concentrations to these animals resulted in a reduction in the number of bacteria belonging to the genera *Lactobacillus* and *Coprococcus* and an increase in the number of bacteria belonging to the genera *Escherichia*, *Oscillospira*, *Paraprevotella*, *Bacteroides*, *Clostridium*, *Desulfovibrio* and *Mucispirillum* [26].

The use of 5-FU led to the development of intestinal dysbacteriosis in rats [27]. It has been shown that after chemotherapy with the use of 5-FU in the small intestine of animals the number of members of the genera *Clostridium, Lactobacillus* and *Streptococcus* decreased and the number of members of the genus *Escherichia*

increased. In the large intestine of rats, 5-FU caused a decrease in the number of members of the genera *Enterococcus*, *Lactobacillus* and *Streptococcus*. In these animals the development of mucositis was also observed, which was accompanied by disruption of mucosal cells and mucus secretion [27].

Administration of doxorubicin to rats resulted in a decrease in the Firmicutes/Bacteroidetes ratio in their intestines. Also, there was observed a decrease in the number of representatives of *Lachnospiraceae*, *Clostridium IV*, *Roseburia*, *Clostridium XlVa*, *Oscillibacter*, *Butyricicoccus,* and an increase in the number of members of the genus *Akkermansia* [28].

Neoadjuvant polychemotherapy using doxorubicin, cyclophosphamide and taxotere led to an increase the number of bacteria of the genera *Pseudomonas* and *Streptococcus* and a decrease in the genus *Prevotella* in the tissue of breast tumors in female patients [29]. It was shown that in healthy breast tissue the content of *P. aeruginosa yields* up to ~ 5% of the microbiota, in breast tumors — 20%, while neoadjuvant chemotherapy caused such a redistribution of microbiota that the content of *P. aeruginosa* was 85% [29]. In an *in vitro* study, doxorubicin was shown to promote the formation of *P. aeruginosa* biofilms [30]. It is known that the formation of biofilms is among adaptations of bacteria to survive in adverse conditions.

The study of the intestinal microbiota in pediatric patients with acute lymphoblastic leukemia (ALL) showed that the microbiota of ALL patients differs from that of healthy children from the control group [31]. Thus, in children with ALL, Bacteroidetes accounted for 64% of the intestinal microbiota and Firmicutes — 31%, while in healthy children, the content of Bacteroidetes was twice lower (30%), and Firmicutes — 54%. After chemotherapy course, the ratio of bacteria of the key phyla changed toward normalization (close to the indexes of healthy children) — 42% for Bacteroidetes phyla representatives and 48% for Firmicutes [31].

The use of a number of chemotherapeutics, such as doxorubicin and 5-FU caused the development of mucositis in the oral cavity of patients. It has been shown that oral mucositis is also associated with dysbacteriosis. A decrease in the number of members of the genera *Streptococcus, Actinomyces, Gemella, Granulicatella* and *Veillonella*, which are associated with a healthy state of the oral microbiocenosis, and an increase in the number of *Fusobacterium nucleatum*, a bacterium associated with the development of pathological conditions of the oral cavity and intestine, has been shown [18].

There is another important aspect of the effect of anticancer chemotherapeutics on the organism as a whole. After all, drugs that directly or indirectly disrupt bacterial DNA activate the SOS signaling response in bacteria [32]. "Low-precision" DNA polymerases are involved in this reparative response, leading to a significant increase in mutations in the bacterial genome. Such processes underlie the development of drug resistance in bacteria. Therefore, during repeated courses of chemotherapy, microbiota often develops resistance to antibiotics, especially rifampicin and fluoroquinolones. In addition, the activation of the SOS response in bacteria activates the processes of horizontal gene transfer involving mobile genetic elements. Many mobile genetic elements encode pathogenic factors (e.g., toxins). Therefore, the action of cytotoxic anticancer agents leads to an increase in the pathogenic potential of the microbiota. These events are extremely undesirable in an organism with an immune system weakened by chemotherapy [33]. The combined use of antitumor chemotherapy and antibiotic therapy may increase the risk of infection caused by multidrug-resistant bacterial strains.

It is known that representatives of key species of intestinal microbiota exert colonization resistance, i.e. prevent the colonization of mucosa by bacteria of opportunistic and pathogenic species. In addition, many commensal bacteria are active in restoring the mucin layer and mucosal epithelial cells, as well as suppressing inflammatory processes in the intestine. For example, representatives of the species *Faecalibacterium prausnitzii* and *Clostridium* cluster XlVa, produce butyrates, which help to restore the epithelial cells of the intestine and maintain the integrity of the mucosa. Representatives of the species *Bacteroides thetaiotaomicron* and *Bifidobacterium infantis* exert anti-inflammatory activity suppressing signaling pathways that lead to the activation of NF-kB [33].

Thus, the mucous membrane of the human body and representatives of the microbiota exist in a state of certain mutually beneficial interaction and balance. Violation of one of the components of this balance by certain factors leads to a violation of this complex system as a whole. Thus, changes in the microbiota caused by chemotherapy can be mediated by biochemical changes in the microbiotope where bacteria live, namely: destruction of the epithelium, the development of inflammation in the epithelial tissue and changes in the activity of local immune factors [18].

The microbiota of the mouse, rat and human body changes its composition in response to chemotherapy. As can be seen from the above data, the number of bacteria with positive properties for the macroorganism usually decreases and the number of opportunistic pathogens increases. However, whether the effects of chemotherapy are direct or indirect remains an open question.

THE EFFECT OF MICROBIOTA ON THE EFFECTIVENESS OF CANCER CHEMOTHERAPY

Scientists have proposed a number of mechanisms by which the microbiota exerts its influence on the effectiveness of cancer chemotherapy. The authors of one study referred to these mechanisms as "TIMER" (Translocation, Immunomodulation, Metabolism, Enzymatic degradation, Reduced diversity) [34]. To these mechanisms, one could can add the effect of the microbiota on the signal transduction pathways,