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REVIEW

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HUMAN MICROBIOTA AND BREAST CANCER

*Yu.V. Shvets[1, *], O.O. Lykhova[2], V.F. Chekhun[2]*

[1]NSC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Kyiv 01033, Ukraine

[2]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv 03022, Ukraine

Breast cancer is the leading malignancy in women worldwide. To date, much is known about the molecular subtypes of these malignant neoplasms and the mechanisms of drug resistance. Significant success has been achieved in approaches to early diagnosis, which allows identifying the tumor process in the early stages of development. Recently, the study of the influence of the human body microbiota on cancer development and the effectiveness of treatment has become an actively developing field of research. This review presents an analysis of the literature data on this issue.

***Key Words*: breast cancer, microbiota.**

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Modern research projects Metagenomics of the Human Intestinal Tract Consortium (MetaHIT) and Human Microbiome Project (HMP) using genetic research methods have significantly deepened our knowledge about the diversity, number and importance of bacteria inhabiting the human body [1–3]. Further development of these studies has shown that microorganisms significantly affect multiple metabolic processes that occur in the human body [4, 5]. There is no doubt that bacteria affect the development and functioning of many physiological systems of the macroorganism, such as immune, nervous, respiratory, digestive, hormonal, and metabolic [6, 7]. It has been shown that certain compositions of microbiota can be associated both with the healthy state of the human organism and with certain pathological processes, such as metabolic syndromes, cardiovascular pathologies and malignant neoplasms [8, 9]. It is known that approximately 20% of tumors that occur in humans are caused by the activity of microorganisms [10, 11]. It has long been known that certain microorganisms have a direct carcinogenic activity or are associated with the development of human malignancies. For example, *Helicobacter pylori* is associated with carcinomas and lymphomas of the stomach [12], *Fusobacterium nucleatum* — with tumors of the colon [13], and highly oncogenic types of human papillomavirus cause malignancies of the cervix, vagina, and anus [14, 15]. It has recently been found that a microscopic fungus, a representative of the skin microbiota, *Malassezia globosa*, is associated with malignant neoplasms of the pancreatic ducts [16]. In 2020, the journal Science published the results of a large-scale study of 1,563 samples of tumors of various histogenesis (melanoma, tumors of the lung, breast, pancreas, bone, brain) and healthy adjacent tissues for the presence of bacteria. Bacterial cells were found in samples of all studied tumors in much greater numbers than in compatible healthy tissues [17]. Interestingly, many of the bacteria found were present in tumors as intracellular L-forms. Of all the tumors studied, breast tumors were characterized by the highest content and diversity of bacteria. Reviews [18, 19] based on the analysis of literature data showed that certain representatives of the microbiota are involved in the induction of tumor growth, affect the growth and metastasis of tumors, the formation of immune defense mechanisms against tumor cells, and determine the sensitivity of tumors to drug therapy.

In the epidemiology of malignant neoplasms of women, breast cancer (BC) occupies the first place [20, 21]. It is now known that the genetic predisposition to BC accounts for only 10–20% of the total number of various influencing factors [22, 23]. Therefore, there is considerable interest in how the origin and development of these tumors can be associated with the microbiota. This review analyzes current literature data on the relationship and influence of microbiota on the development of breast malignancies in women. In the study of this problem we will consider the following questions: 1) the difference between composition and activity of the microbiota in healthy and malignantly transformed breast tissue; 2) how the composition of intestinal microbiota can be associated with the development of breast tumors; 3) how the microbiota affects the hormonal status of women; 4) whether there is a link between diet, microbiota and BC in women.

COMPOSITION OF THE MICROBIOTA IN NORMAL AND MALIGNANTLY TRANSFORMED BREAST TISSUE

The breast is an organ that has a certain structure and function in mammals. The main function of the breast is the production of milk for breastfeeding. The main types of cells present in the breast are epithelial cells, connective tissue and immune system cells. The ducts of the breast are lined with epithelium that has a secretory function.

Currently, there is no doubt that the breast has its own microbiota, the composition of which is unique and

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*Correspondence: E-mail: julia73_shvets@ukr.net

Abbreviations used: BC – breast cancer; BMI – body mass index; DCA – deoxycholic acid; F/B – Firmicutes/Bacteroidetes; HDAC – histone deacetylase; MeD – Mediterranean diet; NK – natural killer cells; SCFA – short-chain fatty acids; TNF-α – tumor necrosis factor-α; WeD – Western type diet.

different from the microbiota of other biotopes of the human body [24, 25]. According to current research on the breast tissue, the most common are representatives of the phylum Proteobacteria, then Firmicutes, as well as Bacteroides and Actinobacteria [26, 27]. Currently, there are two models of breast microbiota formation: 1) constant flow model; 2) model of mucosal interaction. The constant flow model assumes that the breast does not have a resident microbiota, and those microorganisms that appear in the breast and breast milk are a kind of "tourists". This explains the relatively small number of microorganisms in the breast. It is known that the epithelium of the breast does not specialize in the production of mucins. Also, there is still no experimental evidence that bacteria are able to attach to the epithelium of the ducts of the breast and multiply in the ducts of the organ. The "mucosal interface" model assumes that the ducts of the breast contain their self-renewing composition of the microbiota. Interestingly, the microbiota of two breasts in one woman is also somewhat different [28].

Throughout a woman's life, the breast is subject to development and is involved in performing such an important physiological function as lactation. It is known that normal human breast milk contains a total of up to 800 species of bacteria in the amount of approximately 1000 bacterial cells per 1 ml [29]. It has been estimated that a breastfed infant consumes 800,000 bacteria daily with breast milk. The most frequent representatives in breast milk are: *Staphylococcus spp., Streptococcus spp., Cutibacterium acnes, Corynebacterium spp., Bifidobacterium spp., Veillonella spp., Bacteroides spp., Parabacteroides spp., Clostridium spp., Collinsella spp., Faecalibacterium spp., Coprococcus spp.* and *Blautia spp.* [30].

The presence of skin microbiota in the milk is easy to understand (representatives of the genera *Staphylococcus*, *Streptococcus*). For a long time, it remained a mystery how intestinal bacteria got into milk. Microbial associations of breast milk are unique and do not correspond to that of woman's intestines. Moreover, the content of the representatives of certain genera (*Bacteroides*, *Enterococcus*, *Faecalibacterium*) in the milk is higher than the representatives of other genera, which are rarely found in breast milk (for example, *Escherichia*, *Eubacterium*). This suggests the existence of a special selective mechanism of vertical transport, which provides the representation in the microbiota of breast milk, the types of bacteria from the mother's intestine that are most "useful" for the developing baby [31]. In addition, milk contains so-called bifidogenic factors (human milk oligosaccharides, HMOs), which promote colonization of the child's intestines by members of the genus *Bifidobacterium* and *Lactobacillus* [32], as well as prevent the attachment of pathogenic microorganisms in the biotope. By the way, breastfeeding is associated with a reduced risk of breast tumors [33, 34], possibly because milk supports the development of beneficial bacteria. Transport of bacteria from the intestines to the breast occurs in non-pregnant and non-lactating primates. This confirms the fact that bacteria are present in the breast before pregnancy and lactation [35].

Thus, three main physiological mechanisms of microorganisms entering the breast are currently being considered: colonization of bacteria from the microenvironment of the skin [36], from the oral cavity of the infant during lactation [35] and by transporting bacteria to the breast from woman's intestines [37]. The idea of transferring bacteria from the intestine is also confirmed by the results of studies of breast milk in women after oral probiotics. The probiotic strains of bacteria were later detected in breast milk [38].

The data on the breast microbiota in health and in the tumor process are often contradictory. This is due to the difference in approaches to the use of histological material for research. For example, in some studies, the microbiota of tumor tissue and adjacent healthy tissue is investigated in the same woman. The result of such studies, for the most part, indicates a minimal difference or no difference in the composition of the microbiota of tumor tissue and adjacent healthy breast tissue [27]. This approach raises some doubts because the tumor develops in a certain microenvironment, which is significantly different from healthy tissue. Therefore, the tumor tissue is surrounded by healthy tissues with altered microbiota, which contributed to the development of the tumor [39]. In other studies, in which the microbiota of breast tumor tissue is compared with the microbiota found in histological samples from women without BC, differences in the composition of microbiota are more common [28, 40].

It is now established that the microbiota of the breast of women of different countries, ethnic groups, races is different. For example, one study has shown that the composition of the breast microbiota of women from Canada and Ireland differed [41]. In particular, Canadian breast samples were dominated by bacteria of the genera *Bacillus* (11.4%), *Acinetobacter* (10.0%), *Enterobacteriaceae* (8.3%), *Pseudomonas* (6.5%), *Staphylococcus* (6.5%), *Propionibacterium* (5.8%), *Comamonadaceae* (5.7%), *Prevotella* (5.0%) and *Gammaproteobacteria* (5.0%). Bacteria of the *Enterobacteriaceae* family (30.8%) and representatives of genera *Staphylococcus* (12.7%), *Propionibacterium* (10.1%), *Pseudomonas* (5.3%), and *Listeria welshimeri* (12.1%) predominated in breast tissue from Irish population. Another study showed different composition of the breast microbiota in women of different races with the same histological subtypes of the BC [42].

The results of studies generally indicate that the microbiota of breast tumors is dominated by bacteria belonging to the phyla of Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria (Table).

One study evaluated the microbiota in the tissue adjacent to the tumor and the microbiota of healthy breast tissue. Bacteria of the genera *Lactococcus*, *Streptococcus*, *Prevotella*, *Corynebacterium*, and *Micrococcus* have been shown to predominate in healthy breast tissue. In contrast, women with BC had an increased content of *Staphylococcus*, *Bacillus*, and *Enterobacteriaceae*, *Comamonadaceae*, and *Bacteroidetes* [43]. It was further investigated that isolates of *Escherichia coli* and *Staphylococcus epidermidis* derived from healthy breast tissue adjacent to tumor had the ability to induce double-stranded DNA breaks in HeLa cells *in vitro*. The accumulation of such

disorders in cells usually leads to genomic instability and malignant transformation of cells (Fig. 1). The elevated levels of *Bacillus cereus* were also found in breast tissue involved in tumor process. *B. cereus* is not a representative of the microbiota of the human body, but can come from the environment: soil, plant foods.

Another study showed the increased content of bacteria of the families *Pseudomonadaceae*, *Dietziaceae*, *Genellaceae*, *Neisseriaceae* [28] in breast tumor tissue. According to the authors, it may be associated with tumor development. Bacteria of the family *Genellaceae* are representatives of the intestinal microbiota of newborns and are found in amniotic fluid and therefore, probably, get into the breast from infants.

An increase in the number of members of the genera *Fusobacterium, Lactobacillus*, *Atopobium*, *Gluconacetobacter*, *Hydrogenophaga* [40] and *Ralstonia* [27] has been documented in BC tissue by other authors. From this list of bacteria, *Fusobacterium nucleatum* has significant carcinogenic potential and is associated with tumors of the colon.

One study [48] analyzed the microbiota of the breast, mouth and urine lavage of women with BC and healthy women. No significant differences were found between the oral microbiota in two study groups. Increased numbers of gram-positive bacteria, members of the genera *Staphylococcus*, *Corynebacterium* and *Actinomyces*, were found in the urinary microbiota in women with BC. In the breast tissue of women with tumors, a decreased number of bacteria of the genus *Methylobacterium* was found compared with healthy women. In another study, the authors found the presence of *Methylobacterium radiotolerans* bacteria in 100% of breast tumor tissue samples and revealed no significant differences in the content of these bacteria between healthy breast tissue samples and breast tumors [49]. Note that in this case, healthy breast tissue was examined in the same patients in whom breast tumors were detected.

In metagenomic studies, Banerjee *et al*. [46, 47] analyzed the breast tissue of patients with different histological subtypes for microbiota content. Differences between the composition of the microbiota in different histological subtypes of BC were determined (Table). In addition, in these studies, genetic material from viruses, microscopic fungi, and protozoa was found in women's breast tissue.

In one large-scale study involving 61 researchers, 9 medical centers from 4 countries analyzed the histological material of 355 samples of breast tumors [17]. It was determined that the microbiota of breast tumors is much more numerous and diverse in species composition compared to breast tissue of healthy women. Of particular interest is the fact that many bacteria have been found in the form of intracellular L-forms. The frequency of detection of certain types of bacteria in samples of breast tumors was: 30–50% for *Streptococcus infantis*, *Lactobacillus inners*; 25–30% *Corynebacterium* US_1715 (US, unknown species); 20–25% *Fusobacterium nucleatum*; 15–20% *Paracoccus marcusii*, *Staphylococcus cohnii*; 10–15% *Staphylococcus aureus*, *Acinetobacter* US_424, *Enterobacter cloacae* [17]. In this study, it was also determined that histological subtypes of breast tumors (ER+, HER2+ and triple negative) are also characterized by a slightly different microbiota. In addition, certain features of metabolic activity of BC tissue microbiota were revealed. It was determined that the microbiota of ER+ breast tumors was characterized by such metabolic activity as detoxification of arsenic and mycothiol synthesis. The chemical element arsenic is a group I carcinogen. Its effect on a woman's body increases the risk of developing breast tumors. It is also known that arsenic induces the expression of estrogen receptors on breast cells [50]. Mycothiol is a substance used by bacteria to neutralize reactive oxygen species. It was studied that the microenvironment of breast tumors with the ER+ phenotype is more oxidative than other histological subtypes of BC [51], which explains the production of mycothiol by bacterial cells. The increased expression of anaerobic bacterial enzymes was observed in samples of all histological subtypes of breast tumors. The microbiota of breast tumors of different histological subtypes was also characterized by increased activity of anaerobic respiratory enzymes.

Thus, the microbiota of the breast has its own specific composition, which is different in women of different races and ethnic groups. The species composition of the microbiota changes with the development of the tumor process [52]. Some representatives of the microbiota of the breast have a certain carcinogenic activity. This activity could be realized by direct DNA damage and/or induction of regional inflammation (Fig. 1). In addition, there is an associative relationship between the composition of the microbiota and histological subtypes of malignant neoplasms of the breast. The reasons for such changes and associations still need more detailed study.

INTESTINAL MICROBIOTA AND BREAST TUMORS

The microbiota of the human intestine plays an extremely important role in the life of the human body. Bacteria carry out colonization resistance, break down a number of nutrients inaccessible to human digestive enzymes, produce vitamins (B and K), short-chain fatty

Table. Microbiota of human breast tissue in health and BC [43–47]

Histological type	Bacteria type
Breast tissue of healthy women	*Lactococcus, Streptococcus, Prevotella, Corynebacterium, Micrococcus [43];*
Adjacent to tumor healthy tissue	*Buttiauxella, Nitrosomonas, Sphingobium, Sphingomonas, Bacillus, Staphylococcus*
The endocrine receptor positive BC tissue	*Arcanobacterium, Bifidobacterium, Cardiobacterium, Citrobacter, Escherichia [47];*
The human epidermal growth factor receptor 2 positive BC tissue	*Streptococcus [47];*
The triple positive BC tissue	*Bordetella, Campylobacter, Chlamydia, Chlamydophila, Legionella, Pasteurella [47];*
The triple negative BC tissue	*Aerococcus, Arcobacter, Geobacillus, Orientia, Rothia [47]; Arcanobacterium, Brevundimonas, Sphingobacteria, Providencia, Prevotella, Brucella, Eschherichia, Actinomyces, Mobiluncus, Propionibacteria, Geobacillus, Rothia, Peptinophilus, Capnocytophaga [46]*

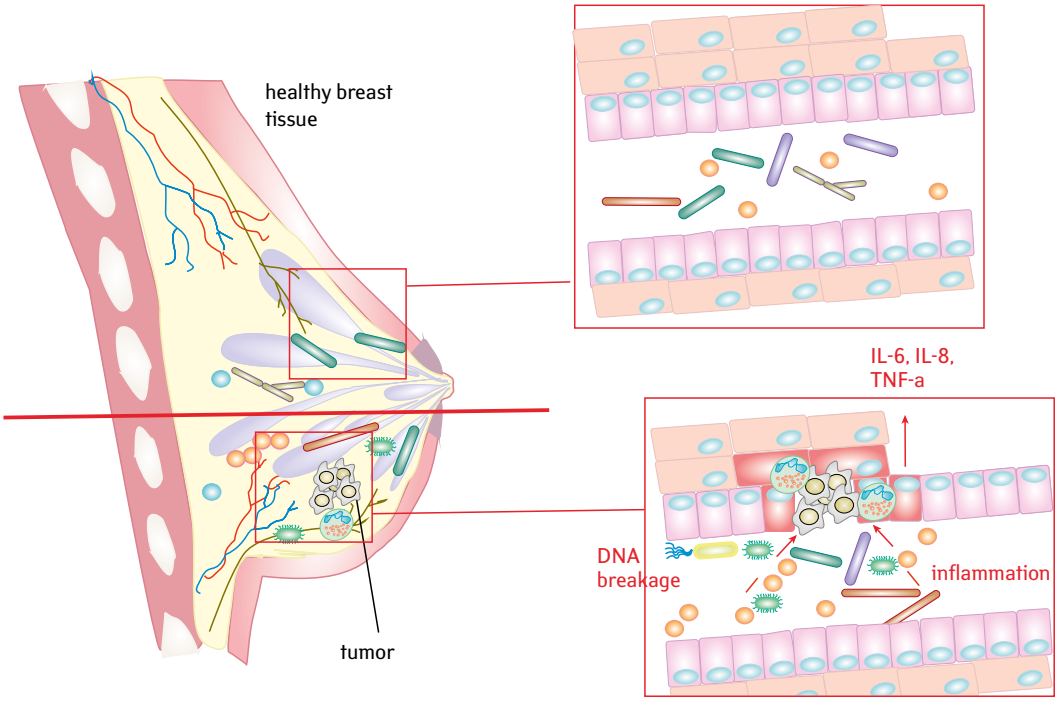

Fig. 1. Influence of microbiota on the development of breast tumors in women

acids, affect the metabolic transformation of some biologically active substances of the human body (bile acids, hormones), and promote the development of such physiological systems of the body as digestive and immune. The composition of intestinal microbiota is influenced by such factors as genetic characteristics, age, sex, lifestyle, hygiene, diet. The intestinal microbiota, which is the most numerous in comparison with other biotopes of the human body, has a significant impact on the metabolism of the human body, and is also the cause of systemic inflammatory processes. Currently, the phenomenon of "leaky gut", which is associated with many pathological conditions of the human body, is being actively studied. The development of leaky gut can be induced by even minor stressful situations and changes in the composition of intestinal microbiota (dysbiosis). Due to the impaired intestinal permeability, intestinal microbiota, bacterial cell components and metabolites can penetrate the altered intestinal tight junctions and enter the bloodstream that finally may result in systemic inflammation [53].

Human intestinal microbiota comprises many phyla, the key of which are Firmicutes (*Clostridium*, *Faecalibacterium*, *Eubacterium*, *Ruminococcus*, *Peptostreptococcus*, *Lactobacillus*, *Streptococcus*) and Bacteroidetes (*Bacteroides*). Representatives of these phyla make up about 80–90% of the intestinal microbiota. The ratio of the number of bacteria of these species Firmicutes/Bacteroidetes (F/B ratio) is a fairly stable indicator in a healthy ecological system of the macroorganism [49, 54]. For example, it has been studied that the F/B index is increased in obese patients. The remaining 10–20% of microbiota are Actinobacteria (*Streptomyces*, *Bifidobacterium*), *Proteobacteria*, *Fusobacteria*, Verrucomicrobia (*Akkermansia*), Tenericutes, Lentisphaerae. Intestinal microbiota significantly affects the development of tumors in the intestine and in distant parts of the human body.

The study of the composition of the intestinal microbiota of women with BC and women in the relevant control group must be considered in the context of age and body mass index (BMI), as these indicators significantly affect the results. It is generally known that the development of BC in postmenopausal women is often associated with metabolic disorders accompanied by weight gain.

It has been shown that postmenopausal overweight women have a higher risk of developing BC compared to women of the same age group with normal weight. Some studies have shown that the F/B ratio is higher in overweight patients [55]. Another study showed an increased content of bacteria of the genera *Bacteroides*, *Clostridia* and *Lactobacillus* in the intestinal microbiota of premenopausal BC patients [56].

It has been studied that in postmenopausal women with BC, the composition of each woman's fecal microbiota (α-diversity) is less diverse than in healthy women [57, 58]. Another study showed that the diversity of bacterial species within the group of women studied (β-diversity) was more pronounced in the group of women with breast tumors than in the control group [59].

In women with postmenopausal BC, the intestinal microbiota had an increased content of *Clostridiaceae*, *Faecalibacterium*, *Ruminococcaceae*, and a decrease in *Dorea* and *Lachnospiraceae* levels. It has also been shown that BC is often associated with conditions such as obesity, insulin resistance, dyslipidemia, leukocytosis and the elevated levels of C-reactive protein in peripheral blood [56].

In postmenopausal women with BC, Jhu *et al*. [59] demonstrated an increase in the intestinal microbiota of 38 bacterial species was observed including *Escherich-*

ia coli, *Citrobacter koseri*, *Acinetobacter radioresistens*, *Enterococcus gallinarum*, *Shewanella putrefaciens*, *Erwinia amylovora*, *Actinomyces* spp. HPA0247, *Salmonella enterica*, *Fusobacterium nucleatum*, *Klebsiella* sp_1_1_55 and *Prevotella amnii*. In contrast, a decrease in the number of bacteria of 7 species, such as *Porphyromonas uenonis*, *Eubacterium eligens*, *Eubacterium eligens*, *Roseburia inulinivorans* and *Lactobacillus vaginalis*, was observed. At the same time, no significant difference in the composition of intestinal microbiota in premenopausal women with BC and healthy premenopausal women was found [59]. This study also showed the association of intestinal microbiota enterotypes with a BC diagnosis. The concept of enterotypes was first proposed by Arumugam *et al*. in 2011 p. [60]. Three key enterotypes with a predominance of bacteria of the genera *Bacteroides*, *Prevotella* and *Ruminococcus*, belonging to the 1st, 2nd and 3rd enterotypes, respectively, were identified. No associations with intestinal enterotypes were found in women with BC [59].

In a study by Luu *et al*. [61], an increase in the number of *Blautia* spp. cells was observed in patients with stage III BC in the intestinal microbiota compared with patients with stage I [61]. It was also shown that the number of representatives of Bacteroidetes, *Clostridium coccoides* cluster, *C. leptum* cluster, *Faecalibacterium prausnitzii* was significantly higher in clinical groups of patients with BC stage II and III compared to patients with stage 0 and I.

What mechanisms of microbiota influence on the development of BC are studied today? Breast tumors are associated with estrogen-dependent and estrogen-independent functions of the intestinal microbiota. Consider estrogen-independent functions of microbiota, which can be both tumor stimulating and antitumor (Fig. 2).

The intestinal microbiota is known to affect the development of systemic inflammation in the human body. Such systemic inflammation is a favorable background for the development of breast tumors. For example, increased circulating neutrophils and increased neutrophil/lymphocyte ratios, as well as circulating cytokines interleukin (IL)-6, IL-8, tumor necrosis factor-α (TNF-α), and C-reactive protein, have been shown to be associated with BC [56].

Certain representatives of the intestinal microbiota are able to break down dietary fiber to form short-chain fatty acids (SCFA), which are mostly represented by butyrates, propionates and acetates. Butyrate is a group of short-chain fatty acids that are a source of energy for the epithelial cells of the large intestine. The producers of butyrates are the bacteria *Roseburia inulinivorans*. Butyrates are known to have anti-inflammatory and immunomodulatory activity, which helps maintain the integrity of the intestinal mucosa [62]. One of the known mechanisms of anti-inflammatory activity of butyrates is an inhibition of proinflammatory transcription factor NF-κB in the intestinal epithelial cells.

The studies of the intestinal microbiota of women with BC have shown a decrease in the number of bacteria *Roseburia inulinivorans*, which, in turn, leads to the development of local and systemic inflammatory processes [59]. In particular, in the microbiota of postmenopausal women with BC, there was a decrease in the expression of butyrate metabolism genes and an increase in the expression of lipopolysaccharide biosynthesis genes, iron and vitamin B_{12} transport systems, phosphotransferase systems and bacterial secretion systems. This profile of bacterial gene expression indicates the formation of such bacterial substances that contribute to the development of systemic inflammation.

Butyrates and propionates also inhibit the activity of histone deacetylase enzymes (histone deacetylases — HDACs) [63, 64]. The disruption of the intestinal microbiota by antibiotics in mice leads to an increase in HDAC2 levels in tissues, which is further associated with carcinogenesis. In malignant neoplasms of the breast, elevated HDAC2 levels correlate with increased metastasis, increased expression of Ki67 cell proliferation antigen, and resistance to chemotherapy [65]. Today, HDAC2 is considered as one of the markers of tumor progression in BC. Thus, the intestinal microbiota through the production of SCFA, namely, butyrates and propionates, has an epigenetic regulatory effect on the expression of genes in the human body. Therefore, changes in the composition of the intestinal microbiota are related to the sensitivity/resistance to chemotherapy.

Representatives of the microbiota are involved in the metabolic transformations of certain biologically active substances produced in the human body, such as bile acids [66]. Primary bile acids are synthesized in the liver from cholesterol and are mostly represented by cholic acid and chenodeoxycholic acid, which are further subject to conjugation with glycine or taurine. Most of the primary bile acids that come from the bile into the intestine are actively reabsorbed in the terminal parts of the ileum and returned to the liver through the portal vein. However, approximately 5% of bile acids enter the large intestine, where microbiota biotransformation/deconjugation of primary bile acids with their conversion into secondary bile acids such as deoxycholic acid (DCA), lithocholic acid, ursodeoxycholic acid. Secondary bile acids are also partially absorbed in the large intestine into the portal vein of the liver. After entering the liver, secondary bile acids are re-conjugated with glycine or taurine and, together with primary bile acids, enter the bile and are reused.

Representatives of the ileal microbiota of the genera *Clostridium*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Bacteroides*, as well as *Methanobrevibacter smithii*, *Methanosphera stadmanae* contain enzymes — hydrolases of bile acids (bile salt hydrolase), which deconjugate bile acids with the creation of secondary bile acids like DCA [67].

Primary and secondary bile acids, in addition to performing functions in the digestive processes, perform certain signaling functions, which scientists are still finding out. As signaling molecules, bile acids interact with certain bile acid receptors: farnesoid X receptor and G-protein bile acid receptor 1 on the surface of muscle cells, neurons and intestinal endothelium. These receptors are also widely expressed by immune system cells: monocytes, macrophages, dendritic cells, natural killer cells (NK) and NKT cells [68]. Signals involving bile acid

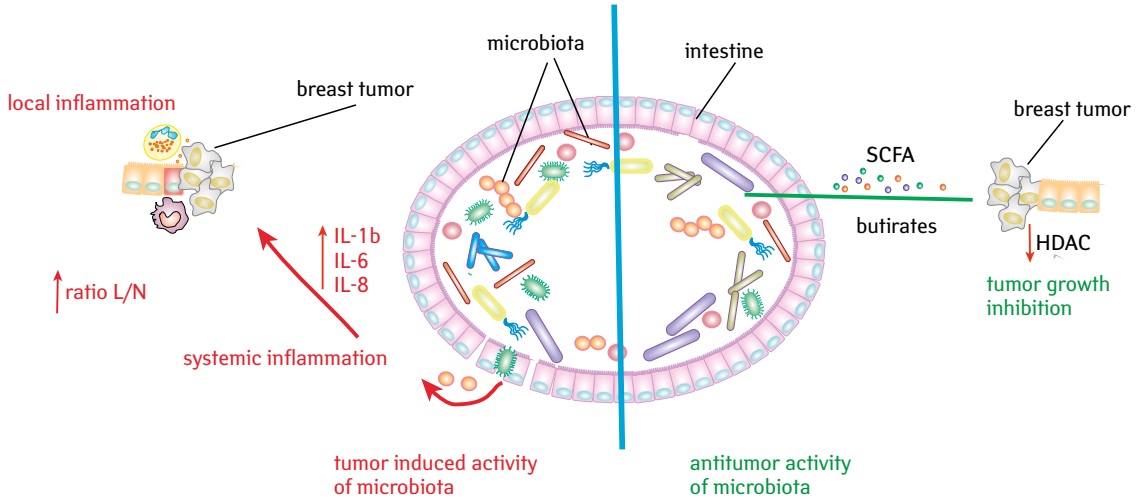

Fig. 2. Influence of human intestinal microbiota on the development of malignant neoplasms of the breast

receptor on dendritic cells and NKT macrophages lead to the development of such reactions, which are inhibitory by their nature [69]. Thus, due to the involvement of bile acid receptor receptors, a tolerogenic signal is transmitted to the cells of the natural part of the immune system of the liver and intestines.

Currently, information on the effect of bile acid metabolites on the tumor process is somewhat contradictory. It has been investigated that elevated levels of secondary bile acid DCA were elevated in the blood plasma of BC patients compared to healthy women [70]. In mice, DCA was shown to act on Ito liver cells by stimulating them to produce pro-inflammatory cytokines. The effect of chemical carcinogens on the macroorganism against the background of chronic inflammation further leads to the development of hepatocellular carcinoma in mice [71]. Blocking the formation of DCA by intestinal bacteria led to inhibition of hepatocellular carcinoma in these animals [71]. An *in vitro* study using BC cell lines: T47D, ZR-75-30, MDA-MB-175-VII showed that cell proliferation was significantly reduced by exposure to DCA. Genes whose products are involved in DNA replication, DNA repair, and cell cycle have been shown to be repressed by DCA [71]. Another *in vitro* study showed that treatment of BC cell lines MCF-7, MDA-MB-231, MDA-MB-468 with chenodeoxycholic acid resulted in tumor cell death by apoptosis [72]. A retrospective study of the metabolism of breast tissue samples showed that the accumulation of primary bile acids and certain metabolites in breast tissue is associated with low tumor cell proliferation and less aggressive type of tumor and increased survival [71]. For example, it was investigated that the content of primary bile acid DCA conjugated with glycine (glycochenodeoxycholate) increased in certain types of breast tumors and is associated with better survival of patients [71]. The level of bile acid accumulation in tumor tissue was higher than in adjacent healthy tissue of the same patients. It was shown that in luminal A histological subtype the level of glycochenodeoxycholate accumulation was higher. Thus, primary bile acids and microbiota-derived secondary bile acids accumulate in breast tumor tissue and inhibit tumor growth and improve patient survival [71].

Indirect evidence of the involvement of intestinal microbiota in tumor processes is the increased risk of tumors, as well as recurrence of tumors after frequent and prolonged use of antibiotics that disrupt the normal composition of microbiota and induce dysbiosis [73, 74].

Of great interest are studies using laboratory animals. After all, animals can be used to model and investigate situations that cannot be created in humans. For example, in female C57BL/6 ApcMinRag2- mice, an increase in the incidence of breast tumors was observed in *Helicobacter hepaticus* infection. In mice, changes in the composition of the intestinal microbiota were also observed with the development of breast tumors [75].

In one study in C57BL/6 mice, intestinal dysbacteriosis was induced by the use of antibiotics. Then, against the background of dysbacteriosis, the animals were transplanted with breast tumors. Under conditions of dysbacteriosis, such tumors metastasized faster and were infiltrated by myeloid cells [76]. It was shown that most tumor macrophages belonged to the M2 phenotype. M2 macrophages promote angiogenesis, suppress effective antitumor immune response, promote metastasis of tumor cells. The dysbiotic microbiota of the mice was then transferred to other mice sterilized by antibiotics, which were also similarly inoculated with breast tumors. Increased levels of tumor metastasis and tumor infiltration by myeloid cells were also observed in these mice. and local inflammation in the tumor tissue of the breast, which contributes to the progression of the tumor process. Thus, intestinal dysbiosis induces the development of systemic inflammation and local inflammation in the breast tumor tissue, which contribute to the progression of the tumor process. Finally, we note that the factors that contribute to the development of dysbacteriosis are diverse and widely represented in the environment.

Thus, the intestinal microbiota has a significant impact on the development of breast tumors [77]. The mechanisms of such influence are divided into two categories: direct and indirect. The direct ones include the formation of short-chain fatty acids and secondary bile acids, which directly affect tumor cells. In addition, bacteria from the intestine can be transferred to the tumor tissue and then

be a direct factor in the microenvironment of the tumor. Indirect mechanisms are realized under conditions of development of the systemic inflammation induced by a microbiota.

INFLUENCE OF MICROBIOTA ON ESTROGEN METABOLISM

Estrogens are steroid sex hormones, which primarily ensure the passage of sexual function in humans. These hormones are also involved in the normal functioning of many other organs, tissues and physiological systems of the body, such as the functioning of the liver, adipose and bone tissue, nervous, cardiovascular and immune systems [78].

There are four main forms of endogenous estrogen in the human body, which are formed as a result of metabolic transformations of C21-cholesterol. 17-β estradiol (E2) is formed during the reproductive phase of life and is present in the highest concentration in blood plasma. When we talk about estrogen, we usually mean this hormone. Estrone (E1) predominates in menopause and estriol (E3) is produced in women during pregnancy [79]. Estretrol (E4) is exclusively produced by fetal liver cells. This hormone is present during pregnancy from 9 weeks of gestation and only a short time after birth. Its physiological function is not yet fully understood [80].

The main producers of estradiol are gonadal cells (mainly ovaries), adrenal cortex and adipose tissue cells. A small amount of estrogen can be produced by cells of the liver, pancreas and breast, placenta (during pregnancy). Estrogens are formed from cholesterol, especially from the fraction of low-density lipoprotein — cholesterol. Newly formed estrogens in free form enter the circulation, act on sensitive organs and tissues, the cells of which express the corresponding nuclear and membrane estrogen receptors ERα and ERβ [81]. Estrogens undergo various metabolic transformations, mostly in the liver [82]. Initially, estrogens are subject to hydroxylation reactions at positions C2, C4 and C16 of the steroid ring. Members of the superfamily of cytochrome P450 enzymes (CYP 1A1, CYP 1B1, CYP 1A2) catalyze the hydroxylation reactions of estradiol and estrone. In liver cells, the expression of these enzymes is highest, so the main events of estrogen metabolism occur in the liver. However, in the cells of organs and tissues such as kidneys, uterus, brain, pituitary gland, breast, the activity of the enzyme CYP 1B1 is also observed, which indicates their participation in estrogen metabolism. Further estrogens in the liver are subject to conjugation with hydrophilic molecular species with the formation of sulfates and glucuronates that makes their removal from bile possible (Fig. 3). The estrogen metabolites formed have different lifespans and biological effects on sensitive tissues. Estrogen metabolites are excreted from the human body in the bile and then in the feces, as well as in the urine. Studies using radiolabeled estrogen have shown that 65% of administered estradiol, 48% of administered estrone and 23% of administered labeled estriol are excreted in the bile [82].

Recently, a lot of data has been accumulated on the effect of microbiota activity on estrogen metabolism [83]. It has even been shown that in postmenopausal women less diversity of intestinal microbiota is associated with high levels of circulating estrogen, which is also associated with an increased risk of developing tumors of the breast, ovaries, and endometrium [78, 84]. This effect is realized through the involvement of estrogen metabolism.

The set of intestinal bacteria of a macroorganism, the products of which participate in the biochemical transformations of estrogen, is called estrobolome. The concept of estrobolome was first proposed by Plottel and Blaser in 2011 [85]. It is known that the enzymes β-glucuronidase and β-glucosidase, produced by certain types of bacteria, carry out the deconjugation of estrogen [86, 87]. For example, such enzymes have been found in members of the genera *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Lactobacillus*, *Peptococcus*, *Peptostreptococcus*, *Ruminococcus*, *Escherichia* [45, 57, 85, 88]. Free estrogen is reabsorbed into the bloodstream, enters the circulation through the portal vein of the liver, and then acts on all sensitive organs and tissues, including the breast. The consequence of these processes is an increase in the concentration of estrogen in blood plasma. Estrogen metabolism is individual and differs in different women [57, 85]. Growth inhibition of bacterial species with β-glucuronidase or β-glucuronide activity reduces the risk of estrogen-dependent tumors.

In postmenopausal women, the risk of BC development increased with increasing circulating estrogen compared with 2- and 4-hydroxylated estrogen metabolites [89]. The high content of 2- and 4-deoxylated metabolites of estrogen to free estrogen, in contrast, was associated with a high level of diversity of the intestinal microbiota and a reduced risk of developing BC [89].

FEATURES OF THE DIET AND MALIGNANT NEOPLASMS OF THE BREAST

The microbiota of the human body, and especially the most numerous intestinal microbiota, has a significant impact on any metabolic processes in the body, which are the background for health and the development of pathological conditions. When conducting an analytical study, it is impossible not to notice that the development of tumors of the breast is often associated with inflammatory processes, as well as overweight. Approximately 35% of the adult population demonstrate overweight (BMI = 25–30 kg/m^2) or obesity (BMI $\geqslant$ 30 kg/m^2) [82]. Weight gain of 5 kg in postmenopausal women is associated with an increased risk of developing tumors of the breast by 11%, ovaries by 13%, endometrium by 39% [82]. In postmenopausal women, excess adipose tissue is associated with elevated levels of circulating estrogen, which is a risk factor for developing BC.

It is known that changes in the diet significantly affect the composition and functions of intestinal microbiota. Vegetarians have a higher content of conjugated estrogen in the stool and a lower content of estrogen in the blood plasma. It was investigated that the use of dietary fiber affects the composition of the intestinal microbiota in the direction of reducing β-glucuronidase activity, which, in turn, leads to a decrease in the level of deconjugation

and reabsorption of estrogen. Asian women who follow a traditional low-fat diet have 30% lower systemic estrogen levels.

Let us consider the two best-studied diets — "Western type" and "Mediterranean type". For decades, the Western diet (WeD, also called the Standard American Diet, USA) pattern has been known to be rich in refined starches, sugars, red and processed meats, saturated fats and trans fats, low in vegetables, fruits and dietary fiber. It significantly affects the development of diseases such as metabolic syndromes, cardiovascular diseases and tumors of the intestine, prostate, endometrium, breast [90]. Hill *et al.* [91] suggested 50 years ago that the "Western type" diet increases the content and metabolism of fecal bile acids and neutral sterols, which further leads to an increase in the incidence of cardiovascular disease and hormone-dependent tumors. Then the authors noted that increased consumption of dietary fiber by postmenopausal women led to increased excretion of conjugated estrogen in the stool.

It has now been investigated that WeD is associated with an increased risk of developing BC. Adherence to such a diet contributes to the development of inflammation and oxidative stress [92]. The Mediterranean Diet (MeD) is one of the studied healthy diets because it contains more unprocessed plant products. MeD presents vegetables, fruits, whole grains, seeds, nuts, beans, olive oil and animal proteins of fish, poultry and eggs, lactic acid products. Most Mediterranean products are enriched with polyphenolic flavonoids, carotenoids, omega-3 fatty acids, antioxidants, vitamins, and minerals, essential and non-essential amino acids. Many flavonoids and carotenoids have oxidizing properties and protect DNA from damage by reactive oxygen species [93].

Adherence to MeD protects against the development of diabetes, cardiovascular diseases and certain types of cancer. It was also investigated that adherence to the MeD is associated with a decrease in the development of BC of all histological subtypes, and the WeD is associated with an increased risk of BC [92].

It was shown that in the feces of individuals on MeD the number of representatives of the genus *Ruminococcus* is reduced and the content of bacteria of the genera *Lachnospira* and *Prevotella* is increased. MeD is considered to be the closest diet to our ancestors of collectors/hunters. The effect of WeD and MeD on the microbiota of the mammary gland and intestines of animals was studied in an experiment on a model of non-human primates. It was shown that in the breast and feces of monkeys consuming MeD the content of *Lactobacillus* was higher. In addition, the breasts of such animals had higher levels of bile acid metabolites and conjugated phenolic metabolites formed from food due to the activity of the microbiota. It has been shown that monkeys kept on a WeD diet had significantly increased levels of bacteria of the genera *Ruminococcus*, *Lachnospiraceae*, *Oscillospira* and *Coprococcus* in their breast tissue [94]. In the breast tissue of monkeys consuming MeD, the content of bacteria of the genus *Lactobacillus* was 10 times higher. Also, the content of bacteria of the genus *Lactobacillus* was higher in animal feces. Decrease in the number of *Ruminococcus* and *Coprococcus* was observed in the feces of monkeys kept on MeD [94].

Thus, the diet significantly affects the composition of the intestinal microbiota, which, in turn, has a significant impact on the functioning of various organs and systems of the macroorganism. It has been proven that in healthy individuals the intestinal microbiota is characterized by a variety of bacterial species. The intestinal microbiota mostly exhibits anti-inflammatory properties. Dysbiosis, which is a violation of the normal balance of the composition of the microbiota, leads to the development of local inflammation (in Peyer's patches and intestine lamina propria) and systemic inflammation [95]. It is proved that 25% of tumors are formed due to the development of inflammation induced by bacteria.

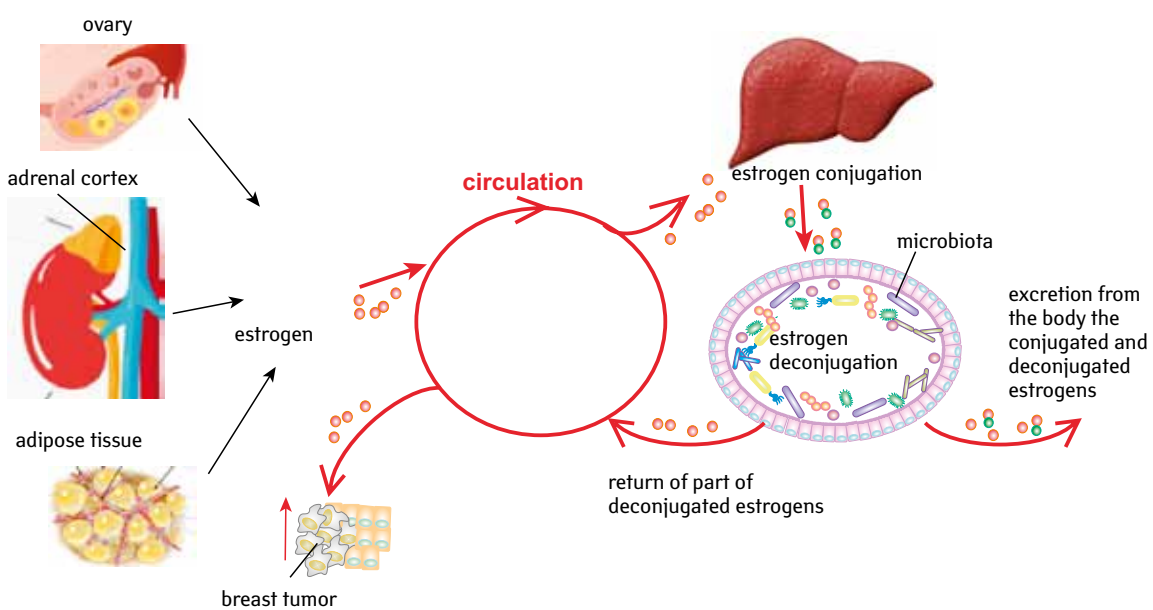

Fig. 3. Schematic representation of the metabolic conversion of estrogen in women.

CONCLUSION

The microbiota is an integral part of the human body. Researchers even call it an additional metabolic organ, which by the number of cells exceeds that of the human body 1.3 times [96], and by the number of genes — 100–150 times. It is now clear that the presence of microbiota in the human body is not limited to the skin and mucous surfaces that contact with the environment. The microbiota is normally present in such tissues and organs that were previously considered sterile: lungs, uterus, breast [97, 98].

Analysis of current research has shown that the human breast has its own unique microbiota, which is closely related to the physiological properties and biological purpose of this organ. With the development of the tumor process in the breast, the microbiota of the organ changes. What is primary in this process still needs further investigation. However, it is shown that histological subtypes of BC are characterized by certain special compositions of microbiota. There is also no doubt that the human intestinal microbiota, which is the most numerous, significantly affects any physiological processes, both in the healthy organism and in pathologies. Intestinal microbiota affects the development of the tumor process in the breast. Representatives of the intestinal microbiota are involved in a number of metabolic transformations that significantly affect physiological and pathological processes in the breast: estrogen metabolism (estrobolome), bile acid metabolism and production of short-chain fatty acids.

How can the composition of microbiota be affected? There are a lot of influencing factors. Genetics, diet, environmental stimuli and infectious agents play an important role in shaping gut microbiota, which can influence immunity. It is also known that respiratory virus infections cause perturbations in the gut microbiota [99]. Such association was found in COVID-19 disease [100]. The associations between gut microbiota composition, levels of cytokines and inflammatory markers in patients with COVID-19 were found [101]. Also it was shown that the gut microbiome is involved in the magnitude of COVID-19 severity possibly via modulating host immune responses. Furthermore, the gut microbiota dysbiosis 30 days after the disease resolution could contribute to persistent symptoms [101]. It is also necessary to mention that cancer is a common comorbidity in COVID-19. Infected cancer patients have much more severe illness and a nearly threefold increase in the death rate compared with COVID-19 patients without cancer [102]. One of the causative factors could be microbiota. Some authors speculate that the estrobolome plays a role in modifying susceptibility to COVID-19 by modulating estrogen levels [103]. It means that it could be the association between the composition of microbiota BC and severity of COVID-19 disease.

Of course, the simplest, most effective and environmentally friendly factor in the release of microbiota composition is the diet [104]. There is currently no exact answer as to the correct, healthy composition of the microbiota [105]. After all, the species composition of the microbiota depends on genetic characteristics (including belonging to a particular race and ethnic group), age, sex, personal hygiene and diet, which depends on the geographical region of residence. In this sense, a thorough analysis of the microbiota of indigenous populations of people in certain areas is needed. Thus, local consumer products (especially of plant and fungal origin) affect the composition of human microbiota. Such studies are now a priority in many developed countries [106, 107]. Obtaining such knowledge is extremely important because it allows you to create approaches to successful personalized medicine. By using microbiota correction approaches based on the diet, it is possible to reduce the risk of life-threatening diseases such as cardiovascular disease, cancer and diabetes.

In addition, manipulating the composition of microbiota can increase the individual sensitivity of patients to drug and immunotherapeutic approaches to treatment and provide support to cancer patients during and after chemotherapy and radiotherapy.

REFERENCES

1. The Integrative HMP (iHMP) Research Network Consortium The Integrative Human Microbiome Project. Nature 2019; **569:** 641–49. doi: 10.1038/s41586-019-1238-8

2. **Plichta DR, Juncker AS, Bertalan M, *et al.*** Transcriptional interactions suggest niche segregation among microorganisms in the human gut. Nat Microbiol 2016; **1:** 1–6. doi: 10.1038/nmicrobiol.2016.152

3. **Lloyd-Price J, Mahurkar A, Rahnavard G, *et al.*** Strains, functions and dynamics in the expanded Human Microbiome Project. Nature 2017; **550:** 61–87. doi: 10.1038/nature23889

4. **Visconti A, Le Roy C, Rosa F, *et al.*** Interplay between the human gut microbiome and host metabolism. Nat Commun 2019; **10:** 1–10. doi: 10.1038/s41467-019-12476-z

5. **Yadav M, Verma MK, Chauhan NS**. A review of metabolic potential of human gut microbiome in human nutrition. Arch Microbiol 2017; **200:** 1–15. doi: 10.007/s00203-017-1459-x

6. **Cong J, Zhang X.** How human microbiome talks to health and disease. Eur J Clin Microbiol Infect Dis 2018; **37:** 1–7. doi: 10.1007/s10096-0183263-1

7. **Schroeder BO, Bäckhed F.** Signals from the gut microbiota to distant organs in physiology and disease. Nat Med 2016; **22:** 1079–90. doi: 10.1038/nm.4185

8. **Cho I, Blaser MJ.** The human microbiome: at the interface of health and disease. Nat Rev Genet 2012; **13:** 260–71. doi: 10.1038/nrg3182

9. **Olive AJ, Sassetti CM**. Metabolic crosstalk between host and pathogen: sensing, adapting and competing. Nat Rev Microb 2016; **14:** 1–16. doi: 10.1038/nrmicro.2016.12

10. **Dzutsev A, Badger JH, Perez-Chanona E, *et al.*** Microbes and cancer. Annu Rev Immunol 2017; **35:** 99–228. doi: 10.1146/annurev-immunol-051116-052133

11. **Eyvazia S, Vostakolaeic MA, Dilmaghanid A, *et al.*** The oncogenic roles of bacterial infections in development of cancer. Microb Pathog 2020; **141:** 1–10. doi: 10.1016/j.micpath.2020.104019

12. **Roesler BM, Rabelo-Gonзalves EMA, Zeitune JMR.** *Helicobacter pylori* and upper gastrointestinal diseases: a review. Health 2014; **6:** 263–73. doi: 10.4236/health.2014.64039

13. **Wu J, Li Q, Fu X.** *Fusobacterium nucleatum* contributes to the carcinogenesis of colorectal cancer by inducing inflammation and suppressing host immunity. Transl Oncol 2019; **12:** 846–85. doi: 10.1016/j.tranon.2019.03.003

14. **Timbang MR, Sim MW, Bewley AF, *et al***. HPV-related oropharyngeal cancer: a review on burden of the disease and op-

portunities for prevention and early detection. Hum Vaccin Immunother 2019; **15:** 1920–8. doi: 10.1080/21645515.2019.1600985

15. **Berman TA, Schiller JT.** Human papillomavirus in cervical cancer and oropharyngeal cancer: one case — two diseases. Cancer 2017; **123:** 2219–29. doi: 10.1002/cncr.30588

16. **Aykut B, Pushalkar S, Chen R, *et al*.** The fungal mycobiome promotes pancreatic oncogenesis via MBL activation. Nature 2019; **574:** 264–7. doi: 10.1038/s41586-019-1608-2

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

18. **Chekhun VF, Lukyanova NYu, Shvets YuV.** Impact of microbiota on development of tumors in human. Onkologiya 2020; **22:** 1–9 (in Ukrainian). doi: 10.32471/oncology.2663-7928.t-22-1-2020-g.8759

19. **Shvets YuV, Lukyanova NYu, Chekhun VF.** Human microbiota and effectiveness of cancer chemotherapy. Exp Oncol 2020; **42:** 1–12. doi: 10.32471/exp-oncology.2312-8852.vol-42-no-2.14611

20. **Sung H, Ferlay J, Siegel RL, *et al*.** Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; **71:** 1–41. doi: 10.3322/caac.21660

21. **Ahmad A.** Breast cancer statistics: recent trends. In: Breast Cancer Metastasis and Drug Resistance. Ahmad A, ed. Second Edition, Springer Nature Switzerland AG, 2019: 1–8.

22. **Wendt C, Margolin S.** Identifying breast cancer susceptibility genes — a review of the genetic background in familial breast cancer. Acta Oncol 2019; **58:** 135–46. doi: 10.1080/0284186X.2018.1529428

23. **Park HL.** Epigenetic biomarkers for environmental exposures and personalized breast cancer prevention. Int J Environ Res Public Health 2020; **17:** 1–18. doi: 10.3390/ijerph17041181

24. **Alizadeh M, Shojaeifarb S, Nedaeiniac R, *et al*.** Association between the microbiota and women's cancers — Cause or consequences? Biomed Pharmacother 2020; **127:** 110203. doi: 10.1016/j.biopha.2020.110203

25. **Younes JA, Lievens E, Hummelen R, *et al*.** Women and their microbes: the unexpected friendship. Trends Microbiol 2018; **26:** 16–33. doi: 10.1016/j.tim.2017.07.008

26. **Hieken TJ, Chen J, Hoskin TL, *et al*.** The microbiome of aseptically collected human breast tissue in benign and malignant disease. Sci Rep 2016; **6:** 1–10. doi: 10.1038/srep30751

27. **Costantini L, Magno S, Albanese D, *et al*.** Characterization of human breast tissue microbiota from core needle biopsies through the analysis of multi hypervariable 16S-rRNA gene regions. Sci Rep 2018; **8:** 1–9. doi: 10.1038/s41598-018-35329-z

28. **Klann K, Williamson JM, Tagliamonte MS, *et al*.** Microbiota composition in bilateral healthy breast tissue and breast tumors. Cancer Causes Control 2020; **31:** 1–12. doi: 10.1007/s10552-020-01338-5

29. **Togo A, Dufour J, Lagier J, *et al*.** Repertoire of human breast and milk microbiota: a systematic review. Future Microbiol 2019; **14:** 623–41. doi: 10.2217/fmb-2018-0317

30. **Ojo-Okunola A, Nicol M, du Toit E.** Human breast milk bacteriome in health and disease. Nutrients 2018; **10:** 1–13. doi: 10.3390/nu10111643

31. **Jost T, Lacroix C, Braegger CP.** Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environ Microbiol 2014; **16:** 2891–904. doi: 10.1111/1462-2920.12238

32. **Katríona E, Lyons KE, Ryan CA, *et al*.** Breast milk, a source of beneficial microbes and associated benefits for infant health. Nutrients 2020; **12:** 1–30. doi: 10.3390/nu12041039

33. **Unar-Munguía M, Torres-Mejía G, Colchero MA, *et al*.** Breastfeeding mode and risk of breast cancer: a dose-response meta-analysis. J Hum Lact 2017; **33:** 422–34. doi: 10.1177/0890334416683676

34. **Johnson HM, Mitchell KB.** Breastfeeding and breast cancer: managing lactation in survivors and women with a new diagnosis. Ann Surg Oncol 2019; **26:** 3032–9. doi: 10.1245/s10434-019-07596-1

35. **Stinson LF, Sindi ASM, Cheema AS, *et al*.** The human milk microbiome: who, what, when, where, why, and how? Nutr Rev 2021; **79:** 1–18. doi: 10.1093/nutrit/nuaa029

36. **Greer FR.** Origins of the human milk microbiome: a complex issue. J Nutr 2019; **149:** 1–3. doi: 10.1093/jn/nxz004

37. **de Andrés J, Jiménez E, Chico-Calero I, *et al*.** Physiological translocation of lactic acid bacteria during pregnancy contributes to the composition of the milk microbiota in mice. Nutrients 2018; **10:** 14. doi: 10.3390/nu10010014

38. **Arroyo R, Martin V, Maldonado A, *et al*.** Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacilli isolated from breast milk. Clin Infect Dis 2010; **50:** 1551–8. doi: 10.1086/652763

39. **Picardoa SL, Coburnb B, Hansen AR, *et al*.** The microbiome and cancer for clinicians. Crit Rev Oncol Hematol 2019; **141:** 1–12. doi: 10.1016/j.critrevonc.2019.06.004

40. **Hieken TJ, Chen J, Hoskin TL, *et al*.** The microbiome of aseptically collected human breast tissue in benign and malignant disease. Sci Rep 2016; **6:** 1–10. doi: 10.1038/srep30751

41. **Urbaniak C, Cummins J, Brackstone M, *et al*.** Microbiota of human breast tissue. Appl Environ Microbiol 2014; **80:** 3007–14. doi: 10.1128/AEM.00242-14

42. **Thyagarajan S, Zhang Y, Thapa S, *et al*.** Comparative analysis of racial differences in breast tumor Microbiome. Sci Rep 2020; **10:** 1–13. doi: 10.1038/s41598-020-71102-x

43. **Urbaniak C, Gloor GB, Brackstone M, *et al*.** The microbiota of breast tissue and its association with breast cancer. Appl Environ Microbiol 2016; **82:** 5039–49. doi: 10.1128/AEM.01235-16

44. **Xuan C, Shamonki JS, Chung A, *et al*.** Microbial dysbiosis is associated with human breast cancer. PLoS One 2014; **9:** 1–7. doi: 10.1371/journal.pone.0083744

45. **Komorowski AS, Pezo RC.** Untapped "-omics": the microbial metagenome, estrobolome, and their influence on the development of breast cancer and response to treatment. Breast Cancer Res Treat 2019; **179**: 1–19. doi: 10.1007/s10549-019-05472-w

46. **Banerjee S, Wei Z, Tan F, *et al*.** Distinct microbiological signatures associated with triple negative breast cancer. Sci Rep 2015; **5:** 15162. doi: 10.1038/srep15162

47. **Banerjee S, Tian T, Wei Z, *et al*.** Distinct microbial signatures associated with different breast cancer types. Front Microbiol 2018; **9:** 951. doi: 10.3389/fmicb.2018.00951

48. **Wang H, Altemus J, Niazi F, *et al*.** Breast tissue, oral and urinary microbiomes in breast cancer. Oncotarget 2017; **8:** 88122–38. doi: 10.18632/oncotarget.21490

49. **Vaiserman A, Romanenko M, Piven L, *et al*.** Differences in the gut Firmicutes to Bacteroidetes ratio across age groups in healthy Ukrainian population. BMC Microbiol 2020; **20:** 1–8. doi: 10.1186/s12866-020-01903-7

50. **Du J, Zhou N, Liu H, *et al*.** Arsenic induces functional re-expression of estrogen receptor a by demethylation of DNA in estrogen receptor-negative human breast cancer. PLoS One 2012; **7:** 1–10. doi: 10.1371/journal.pone.0035957

51. **Karihtala P, Kauppila S, Soini Y, *et al*.** Oxidative stress and counteracting mechanisms in hormone receptor positive, triple-negative and basal-like breast carcinomas. BMC Cancer 2011; **11:** 1–6. doi: 10.1186/1471-2407-11-262

52. **Mani S.** Microbiota and breast cancer. Prog Mol Biol Transl Sci 2017; **151:** 217–30. doi: 10.1016/bs.pmbts.2017.07.004

53. **Saggioro A.** Leaky gut, microbiota, and cancer an incoming hypothesis. J Clin Gastroenterol 2014; **48:** 1–5. doi: 10.1097/MCG.0000000000000255

54. **Magne F, Gotteland M, Gauthier L, *et al.*** The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients 2020; **12:** 1–12. doi: 10.3390/nu12051474

55. **Gunter MJ, Xie XH, Xue XN, *et al.*** Breast cancer risk in metabolically healthy but overweight postmenopausal women. Cancer Res 2015; **75:** 270–4. doi: 10.1158/0008-5472.CAN-14-2317

56. **Yang J, Tan Q, Fu Q, *et al.*** Gastrointestinal microbiome and breast cancer: correlations, mechanisms and potential clinical implications. Breast Cancer 2016; **24**: 1–9. doi: 10.1007/s12282-016-0734-z

57. **Fernández MF, Reina-Pérez I, Astorga JM, *et al.*** Breast cancer and its relationship with the microbiota. Int J Environ Res Public Health 2018; **15:** 1–20. doi: 10.3390/ijerph15081747

58. **Goedert JJ, Jones G, Hua X, *et al.*** Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. J Natl Cancer Inst 2015; **107:** 1–5. doi: 10.1093/jnci/djv147

59. **Zhu J, Liao M, Yao Z, *et al.*** Breast cancer in postmenopausal women is associated with an altered gut metagenome. Microbiome 2018; **6:** 1–13. doi: 10.1186/s40168-018-0515-3

60. **Arumugam M, Raes J, Pelletier E, *et al.*** MetaHIT Consortium, Enterotypes of the human gut microbiome. Nature 2011; **473:** 174–80. doi: 10.1038/nature09944

61. **Luu TH, Michel C, Bard JM, *et al.*** Intestinal proportion of Blautia sp. is associated with clinical stage and histoprognostic grade in patients with early-stage breast cancer. Nutr Cancer 2017; **69**: 1–9. doi: 10.1080/01635581.2017.1263750

62. **Baker JM, Al-Nakkashc L, Herbst-Kralovetz MM.** Estrogen–gut microbiome axis: Physiological and clinical implications. Maturitas 2017; **103:** 45–53. doi: 10.1016/j.maturitas.2017.06.025

63. **Zhao H, Yu Z, Zhao L, *et al.*** HDAC2 overexpression is a poor prognostic factor of breast cancer patients with increased multidrug resistance-associated protein expression who received anthracyclines therapy. Jpn J Clin Oncol 2016; **46:** 893–902. doi: 10.1093/jjco/hyw096

64. **Liu H, Wang J, He T, *et al.*** Butyrate: a double-edged sword for health? Adv Nutr 2018; **9:** 21–9. doi: 10.1093/advances/nmx009

65. **Damaskos C, Valsami S, Konto M, *et al.*** Histone deacetylase inhibitors: an attractive therapeutic strategy against breast cancer. Anticancer Res 2017; **37:** 35–46. doi: 10.21873/anticanres.11286

66. **Biagioli M, Carino A**. Signaling from intestine to the host: how bile acids regulate intestinal and liver immunity. Handb Exp Pharmacol 2019; **256:** 1–14. doi: 10.1007/164_2019_225

67. **Funabashi M, Grove TL, Wang M, *et al.*** A metabolic pathway for bile acid dehydroxylation by the gut microbiome. Nature 2020; **582:** 566–70. doi: 10.1038/s41586-020-2396-4

68. **Chen ML, Takeda K, Sundrud MS.** Emerging roles of bile acids in mucosal immunity and inflammation. Mucosal Immunol 2019; **12:** 851–61. doi: 10.1038/s41385-019-0162-4

69. **Fiorucci S, Biagioli M, Zampella A, *et al.*** Bile acids activated receptors regulate innate immunity. Front Immunol 2018; **9:** 1–17. doi: 10.3389/fimmu.2018.01853

70. **Costarelli V, Sanders TA.** Plasma deoxycholic acid concentration is elevated in postmenopausal women with newly diagnosed breast cancer. Eur J Clin Nutr 2002; **56:** 925–7. doi: 10.1038/sj.ejcn.1601396

71. **Yoshimoto S, Loo TM, Atarashi K, *et al.*** Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013; **499:** 97–101. doi: 10.1038/nature12347

72. **Alasmael N, Mohan R, Meira LB, *et al.*** Activation of the Farnesoid X-receptor in breast cancer cell lines results in cytotoxicity but not increased migration potential. Cancer Lett 2016; **370:** 250–9. doi: 10.1016/j.canlet.2015.10.031

73. **Plaza-Díaz J, Álvarez-Mercado AI, Ruiz-Marín CM, *et al.*** Association of breast and gut microbiota dysbiosis and the risk of breast cancer: a case-control clinical study. BMC Cancer 2019; **19:** 1–9. doi: 10.1186/s12885-019-5660-y

74. **Sheetal S, Sharma D.** The power of small changes: Comprehensive analyses of microbial dysbiosis in breast cancer. Biochim Biophis Acta Rev Cancer 2019; **1871:** 392–405. doi: 10.1016/j.bbcan.2019.04.001

75. **Rao VP, Poutahidis T, Ge ZM, *et al.*** Innate immune inflammatory response against enteric bacteria Helicobacter hepaticus induces mammary adenocarcinoma in mice. Cancer Res 2006; **66:** 7395–400. doi: 10.1158/0008-5472.CAN-06-0558

76. **Rosean CB, Bostic RR, Ferey JCM, *et al.*** Pre-existing commensal dysbiosis is a host-intrinsic regulator of tissue inflammation and tumor cell dissemination in hormone receptor-positive breast cancer. Cancer Res 2019; **79:** 3662–75. doi: 10.1158/0008-5472.CAN-18-3464

77. **Laborda-Illanes A, Sanchez-Alcoholado L, Dominguez-Recio ME, *et al.*** Breast and gut microbiota action mechanisms in breast cancer pathogenesis and treatment. Cancers 2020; **12:** 1–27. doi: 10.3390/cancers12092465

78. **Chen KL, Madak-Erdogan Z.** Estrogen and microbiota crosstalk: Should we pay attention? Trends Endocrinol Metab 2016; **27:** 752–5. doi: 10.1016/j.tem.2016.08.001

79. **Fuentes N, Silveyra P.** Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol 2019; **116:** 135–70. doi: 10.1016/bs.apcsb.2019.01.001

80. **Grandi G, del Savo MC, da Silva-Filho AL, *et al.*** Estetrol (E4): the new estrogenic component of combined oral contraceptives. Expert Rev Clin Pharmacol 2020 **13:** 327–30. doi: 10.1080/17512433.2020.1750365

81. **Hamilton KJ, Hewitt CS, Arao Y, *et al.*** Estrogen hormone biology. Curr Top Dev Biol 2017; **125:** 109–46. doi: 10.1016/bs.ctdb.2016.12.005

82. **Kwa M, Plottel CS, Martin J, *et al.*** The intestinal microbiome and estrogen receptor–positive female breast cancer. J Natl Cancer Inst 2016; **108:** 1–10. doi: 10.1093/jnci/djw029

83. **Chen KL, Madak-Erdogan Z.** Estrogen and microbiota crosstalk: should we pay attention? Trends Endocrinol Metab 2016; **27:** 1–4. doi: 10.1016/j.tem.2016.08.001

84. **Parida S, Sharma D**. The microbiome–estrogen connection and breast cancer risk. Cells 2019; **8:** 1–19. doi: 10.3390/cells8121642

85. **Plottel CS, Blaser MJ.** Microbiome and malignancy. Cell Host Microbe 2011; **10:** 324–35. doi: 10.1016/j.chom.2011.10.003

86. **Raftogianis R, Creveling C, Weinshilboum R, *et al.*** Estrogen metabolism by conjugation. J Natl Cancer Inst Monogr 2000; **27:** 113–24. doi: 10.1093/oxfordjournals.jncimonographs.a024234

87. **Ervin SM, Li H, Lim L, *et al.*** Redinbo. Gut microbiome–derived β-glucuronidases are components of the estrobolome that reactivate estrogens. J Biol Chem 2019; **294:** 18586–99. doi: 10.1074/jbc.RA119.010950

88. **Godoy-Vitorino F.** Human microbial ecology and the rising new medicine. Ann Transl Med 2019; **7:** 1–9. doi: 10.21037/atm.2019.06.56

89. **Fuhrman BJ, Feigelson HS, Flores R, *et al.*** Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. J Clin Endocrinol Metab 2014; **99:** 1–9. doi: 10.1210/jc.2014-2222

90. **Adlercreutz H.** Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest 1990; **201:** 3–23.

91. **Hill MI, Drasar BS, Aries V, *et al*.** Bacteria and aetiology of cancer of large bowel. Gut 1969; **10:** 334–5. doi: 10.1136/gut.10.5.334

92. **Newman TM, Mara Z, Vitolins MZ, *et al*.** From the table to the tumor: the role of Mediterranean and Western dietary patterns in shifting microbial-mediated signaling to impact breast cancer risk. Nutrients 2019; **11:** 1–15. doi: 10.3390/nu11112565

93. **Griffiths K, Aggarwal BB, Singh RB, *et al*.** Food antioxidants and their anti-inflammatory properties: a potential role in cardiovascular diseases and cancer prevention. Diseases 2016; **4:** 1–15. doi: 10.3390/diseases4030028

94. **Shively CA, Register TC, Appt SE, *et al*.** Consumption of Mediterranean versus Western diet leads to distinct mammary gland microbiome populations. Cell Rep 2018; **25:** 47–56. doi: 10.1016/j.celrep.2018.08.078

95. **Schirmer M, Smeekens SP, Vlamakis H, *et al*.** Linking the human gut microbiome to inflammatory cytokine production capacity. Cell 2016; **167:** 1125–36. doi: 10.1016/j.cell.2016.10.020

96. **Sender R, Fuchs S, Milo R.** Are we really wastly outnumbered? revisiting the ratio of bacterial to host cells in humans. Cell 2016; **164:** 337–40. doi: 10.1016/j.cell.2016.01.013

97. **Baker JM, Chase DM, Herbst-Kralovetz MM.** uterine microbiota: residents, tourists, or invaders? Front Immunol 2018; **9:** 1–12. doi: 10.3389/fimmu.2018.00208

98. **Ploton MC, Abakka S, Amouyal E, *et al*.** The lung microbiota. Arch Pediatr 2017; **24:** 667–74. doi: 10.1016/j.arcped.2017.04.007

99. **Dhar D, Mohanty A.** Gut microbiota and Covid-19 — possible link and implications. Virus Res 2020; **285:** 198018. doi: 10.1016/j.virusres.2020.198018

100. **He Y, Wang J, Li F, *et al*.** Main clinical features of COVID-19 and potential prognostic and therapeutic value of the microbiota in SARS-CoV-2 infections. Front Microbiol 2020; **11:** 1302. doi: 10.3389/fmicb.2020.01302

101. **Yeoh YK, Zuo T, Chung-Yan-Lui G, *et al*.** Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut 2021; **70:** 698–706. doi: 10.1136/gutjnl-2020-323020

102. **Dai M, Liu D, Liu M *et al*.** Patients with cancer appear more vulnerable to SARS-COV-2: a multi-center study during the COVID-19 outbreak. Cancer Discov 2020; **10:** 783–91. doi: 10.1158/2159-8290.CD-20-0422

103. **Lasagna A, Zuccaro V, Ferraris E, *et al*.** COVID-19 and breast cancer: may the microbiome be the issue? Future Oncol 2020; **17:** 123–4. doi: 10.2217/fon-2020-0764

104. **Illiano P, Brambilla R, Parolini C.** The mutual interplay of gut microbiota, diet and human disease. FEBS J 2020; **287:** 1–55. doi: 10.1111/febs.15217

105. **Lloyd-Price J, Abu-Ali G, Huttenhower C.** The healthy human microbiome. Genome Med 2016; **8:** 1–11. doi: 10.1186/s13073-016-0307-y

106. **Bashiardes S, Godneva A, Elinav E, *et al*.** Towards utilization of the human genome and microbiome for personalized nutrition. Curr Opin Biotechnol 2018; **51:** 57–63. doi: 10.1016/j.copbio.2017.11.013

107. **Barratt MJ, Lebrilla C, Shapiro H-Y, *et al*.** The gut microbiota, food science, and human nutrition: a timely marriage. Cell Host Microbe 2017; **22:** 134–41. doi: 10.1016/j.chom.2017.07.006

МІКРОБІОТА ОРГАНІЗМУ ЛЮДИНИ ТА РАК ГРУДНОЇ ЗАЛОЗИ

***Ю.В. Швець*[1], *О.О. Лихова*[2], *В.Ф. Чехун*[2]**

[1]ННЦ "Інститут біології і медицини" Київського національного університету ім. Тараса Шевченка

[2]Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького Національної академії наук України, Київ 03022, Україна

Злоякісні новоутворення грудної залози за статистикою в світі випереджають усі пухлинні захворювання у жінок. Нині вже багато відомо щодо молекулярних підтипів цих злоякісних новоутворень, механізмів розвитку лікарської стійкості. Значного успіху досягнено в підходах до ранньої діагностики, що дозволяє визначити пухлинний процес на ранніх стадіях розвитку. Останнім часом активно розвивається галузь досліджень щодо впливу мікробіоти організму людини на розвиток та ефективність лікування пацієнтів з пухлинними захворюваннями, у тому числі і злоякісних новоутворень грудної залози. У цьому огляді представлено аналіз даних літератури з цього питання.

Ключові слова: рак грудної залози, мікробіота.