Body Microbiota and Its Relationship With Benign and Malignant Breast Tumors: A Systematic Review

Ali A. Samkari 1, Meaad Alsulami 2, Linah Bataweel 1, Rozan Altaifi 2, Ahmed Altaifi 2, Abdulaziz M. Saleem 3, Ali H. Farsi 3, Omar Iskanderani 3, Nouf Y. Akeel 1, Nadim H. Malibary 1, Mai S. Kadi 5, Emad Fallatah 1, Mahmoud Fakiha 6, Alaa A. Shabkah 7, Nora H. Trabulsi 1

1. Department of Surgery, King Abdulaziz University Faculty of Medicine, Jeddah, SAU 2. Faculty of Medicine, King Abdulaziz University, Jeddah, SAU 3. Department of Radiation Oncology, King Abdulaziz University Faculty of Medicine, Jeddah, SAU 4. Visceral and General Surgery, Hautepierre Hospital, Strasbourg, FRA 5. Department of Community Medicine, King Abdulaziz University Faculty of Medicine, Jeddah, SAU 6. Department of Surgery, University of Jeddah Faculty of Medicine, Jeddah, SAU 7. Department of Surgery, International Medical Center, Jeddah, SAU

Corresponding author: Ali A. Samkari, ali.samkari.md@gmail.com

Abstract

Breast cancer is the most frequent type of cancer as well as one of the main causes of cancer-related mortality in women. Human microbial dysbiosis, which has been related to a range of malignancies, is one of the variables that may impact the chance of developing breast disorders. In this review, we aimed to investigate the relationship between breast cancer and benign breast tumors with dysbiosis of the microbiome at different body sites. We performed a systematic review of MEDLINE, Scopus, Ovid, and Cochrane Library to identify original articles published until July 2020 that reported studies of breast disease and microbiota. Twenty-four original articles were included in the study, which looked at the features and changes in breast, gut, urine, lymph node, and sputum microbial diversity in patients with benign and malignant breast tumors. In breast cancer, the breast tissue microbiome demonstrated changes in terms of bacterial load and diversity; in benign breast tumors, the microbiome was more similar to a malignant tumor than to normal breast tissue. Triple-negative (TNBC) and triple-positive (TPBC) types of breast cancer have a distinct microbial pattern. Moreover, in breast cancer, gut microbiota displayed changes in the compositional abundance of some bacterial families and microbial metabolites synthesis. Our review concludes that breast carcinogenesis seems to be associated with microbial dysbiosis. This information can be further explored in larger-scale studies to guide new prophylactic, diagnostic, and therapeutic measures for breast cancer.

Categories: Gastroenterology, General Surgery, Oncology
Keywords: breast disease, microbial dysbiosis, microbiome, breast tissue, breast cancer

Introduction And Background

Breast cancer is the most frequent type of cancer in women and is one of the main causes of cancer-related mortality in women [1,2]. Older age, prolonged exposure to female hormones, BRCA1 and BRCA2 genes mutations, and the presence of a personal or family history of breast and other cancers are all well-known risk factors for developing breast illnesses [3,4].

One of the factors that might influence the risk of the development of breast diseases is human microbial dysbiosis [5,6]. The microbiome, as defined by Lederberg and McCray, is the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body spaces [7]. In 1960, it was difficult to understand the role of microbiota. Our understanding of microbiota has improved as genome-analyzing tools for complex microorganisms have advanced, but we still do not know much about its clinical significance [8].

Microbial dysbiosis results when maladaptation or abnormal composition occurs within the microbial community of a given organ or tissue [9]. The literature has reported a link between microbial dysbiosis and the development of a variety of cancers [10-12]. The microbiota have also been shown to help increase drug efficacy, decrease drug toxicity, and prevent cancer [13]. Other studies have concluded that microbiota could be used in the diagnosis, prediction of risk and course, and prevention of disease [14].

The association between different types of microbiota and gastrointestinal pathological conditions has been well studied. Some investigators propose an association between colorectal cancer and certain microbiota detected by fecal and oral swabs [15]. In a new field of research, recent studies have suggested an association between inflammatory bowel diseases and microbiota [16,17]. Research on different breast pathological conditions and their links to microbiota that inhabit breast tissue and other organs is limited. In this comprehensive review, we, therefore, aimed to study the characteristics and changes (dysbiosis) in breast,
gut, and other body site microbiomes in relation to breast cancer and benign breast tumors.

**Review**

**Study protocol and registration**

The study protocol is available at https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=187358. The registration code is CRD42020187358.

**Search strategy**

From May 20 to June 5, 2020, we performed a comprehensive search of databases (MEDLINE, Cochrane Library, Ovid, and Scopus) and retrieved the literature published to June 2020, as well as the relevant reference lists of research discovered through an electronic search. The following were the MEDLINE database search terms: ("bacteria"[mesh] OR "viruses"[mesh] OR "fungi"[mesh] OR "archaea"[mesh] OR "Microbiota"[mesh]) AND ("breast diseases"[mesh] OR "breast"[mesh]). We focused our search on studies on adults and humans.

**Study selection**

The primary screening of the studies was done by two authors based on the title and abstract by using the search terms described above independently, and duplicate studies were removed. A full-text review was undertaken independently. When the inclusion and exclusion criteria were not met during the initial review, the articles were discarded. When uncertainty existed, a third senior author resolved the disagreement. We included all studies that matched all of the following criteria.

**Study Design**

We included retrospective cohort studies and secondary analyses in which the main purpose was to evaluate the microbial diversity and characteristics of breast or gastrointestinal tissue or any other body site in patients with breast cancer or benign breast tumors.

**Study Subjects**

Studies included adult females, 18 years and over, with breast malignancy or benign breast tumors.

**Outcomes**

The outcomes were the characteristics and changes (dysbiosis) in breast, gut, and other body microbiomes in relation to benign and malignant breast tumors.

Studies considered by the authors to be unrelated to the subject, non-human studies, and non-English studies were excluded.

**Breast microbiota**

A total of 5616 articles were screened after the initial search, cross-referencing, and removal of duplicate studies. Inclusion and exclusion criteria were applied by reading the title and abstract. Following this step, 58 studies were included in the full-text reading. After a full-text review, 34 more studies were excluded, and a final 24 studies were selected and included in this review (Figure 1). All included studies and their main characteristics and findings are summarized in Table 1.
FIGURE 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

| Reference         | Type of study | Aim                                                                 | Sample size | Sample site within the breast | Sample assessment methods | Main findings                                                                                                                                 |
|-------------------|---------------|----------------------------------------------------------------------|-------------|--------------------------------|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Xuan et al. [18]  | Cross-sectional | To investigate the potential role of microbiota in breast cancer     | 20 patients with estrogen receptor-positive breast cancer | Normal adjacent tissue and tumor tissue | 16S rDNA pyrosequencing | The most numerous phyla in breast tissue are Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria. Sphingomonas yanoikuyae was more prevalent in the normal surrounding tissue, while Methylobacterium radiotolerans were more numerous in tumor tissue. The breast cancer stage was shown to be inversely associated with bacterial burden in tumor tissue. |
| Urbaniak et al. [19] | Cross-sectional | To investigate the presence of microbiome within the mammary gland | Canadian samples: 11 benign, 27 cancer, 5 healthy Irish samples: 33 cancer, 5 healthy | From the patients, Samples were taken from outside the tumor marginal zone | 16S rRNA sequencing and culture | Bacillus species, Micrococcus luteus, Propionibacterium acneus, and Propionibacterium granulosum were the most abundant species in the case as well as control tissue. |
| Goedert et al. [20] | Cross-sectional | To investigate the difference in gut microbiota among patients with breast cancer with regard to menopausal status. | 48 postmenopausal patients with breast cancer, pretreatment, vs 48 control patients. Urine (without preservative) and feces | NA | 16S rRNA and Fecal DNA gene sequencing | Faecalibacterium, Clostridialesaceae, and Ruminococcaceae were found in higher concentrations in breast cancer patients. On the other hand, Lachnospiraceae and Dorea species were found in lower concentrations in breast cancer patients. |
| Authors | Design | Objective | Samples | Methods | Results |
|---------|--------|-----------|---------|---------|---------|
| Banerjee et al. [21] | Case-control, cross-sectional | To discover the microbiota linked to TNBC. | 100 TNBC, as well as 20 non-matched and 17 matched controls | Matched-controls were collected from the same patient’s normal neighboring tissue. Breast tissues from healthy people were used as non-matched controls. | PathoChip technology |
| Yazdi et al. [22] | Cross-sectional | To evaluate bacterial dysbiosis in sentinel lymph nodes from breast cancer patients. | 123 frozen sentinel lymph nodes from breast cancer patients were collected, as well as 123 normal neighboring breast tissue and 5 normal mastectomies. | Normal adjacent breast tissue | RT-PCR and pyrosequencing |
| Chan et al. [23] | Experimental | To characterize the microbiome present in NAF. | 23 healthy control women and 25 with a history of breast cancer NAF, nipple/areola skin swap | Aliotis species was present only in NAF from breast cancer, while Sphingomonadaceae was found to be more prevalent in healthy samples. | 16S rRNA sequencing |
| Urbaniak et al. [24] | Case-control, cross-sectional | To investigate the possible involvement of breast microbiota in the development of breast cancer. | 71 fresh breast tissue samples were collected from women, 13 of whom were benign, 45 were cancer, and 23 were healthy. | From women with cancer, tissue samples were taken from outside the marginal zone | 16S rRNA gene sequencing |
| Haiken et al. [25] | Cross-sectional | To explore the microbiome of breast cancerous adjacent tissue and its relationship to breast cancer development. | 33 patients: 16 benign, 17 cancer for breast tissue, buccal swap, skin swap, full-thickness skin biopsy. | From women with tumors, the tissue was obtained from normal adjacent breast tissue. | 16S rRNA gene sequencing |
| Luu et al. [26] | Cross-sectional | To investigate the association between microbiota composition and clinical and biological parameters of breast cancer patients. | A stool sample from 31 with early-stage breast cancer: 23 patients had a normal body mass index (BMI), and 8 were overweight or obese | NA | Quantitative PCR (qPCR) targeting 16S rRNA |
| Wang et al. [27] | Case-control, cross-sectional | To explore the microbiome of breast tissue and its association with the tumor expression profile. | 78 patients: 57 with invasive breast cancer, 21 healthy controls mid-stream clean-catch urine samples, and samples of tumor and nearby normal breast tissue were obtained. | Control breast tissue samples were taken on the right and left sides. In addition, tumor tissue and ipsilateral neighboring normal tissue were taken from patients. | DNA extraction 16S rRNA gene sequencing |
| Thompson et al. [5] | Cross-sectional | To study the breast microbiota and its association with the tumor expression profile. | 668 breast tumor tissues 72 normal adjacent tissue | Tumor tissue and non-cancerous adjacent tissue | 16S rRNA gene sequencing |
| | Case-control, cross-sectional | To study the postmenopausal breast cancer associations with urinary levels of estrogens. | 48 postmenopausal breast cancer cases and 48 controls | Alpha diversity is drastically diminished in... | |
| Authors          | Design   | Objective                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Methods                                                                                                                                                                                                                                                                                                                                                              | Findings                                                                                                                                                                                                                                                                                                                                                           |
|------------------|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Goedert et al.  | Cross-sectional | To identify the population of pathogenic bacteria residing within the Mycobacterium avium complex species in NTM-infected women.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Sputum samples from 5 healthy women, 5 women with NTM, and 15 women with both NTM and breast cancer (NTM-BCa); sera, DNA extractions from fecal samples and qPCR were collected.                                                                                                                                                                                                                     | Presence of diverse microbial community in the sputum and the extracellular vesicles in women with NTM and in women with NTM-BCa. These microbiota were dominated by Fusobacterium, Bacteroides, and Alistipes, which have estrobolome activity and are associated with breast and other type of cancers. |
| Banerjee et al. | Cross-sectional | To investigate the association between diversity of the gastrointestinal microbiome and breast cancer.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Normal and tumor tissues were obtained from 9 core needle biopsies and 6 surgical excisional biopsies.                                                                                                                                                                                                                                                                                                                                                                      | Proteobacteria was the most numerous phylum among all areas, followed by Firmicutes, Actinobacteria, and Bacteroidetes. DNAAs from Enterobacter cloacae, CadA E. coli, and LdcC E. coli, were identified less often in cancer patients. In stage 0 breast cancer patients, levels of CadA and LdcC were found to be lower than their levels in other individuals. In stage 1 breast cancer patients, fecal samples showed lower levels of E. coli LdcC protein as compared to healthy females. |
| Shi et al.       | Cross-sectional | To assess the association between the diversity of the gastrointestinal microbiome with the patterns of expression TILs in patients with breast cancer.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Tumor tissues from 80 patients with breast cancer were analyzed.                                                                                                                                                                                                                                                                                                                                                                           | Among different TIL expression groups in a patient with breast cancer, the gut microbiome diversity was distinct and compositionally different.                                                                 |
| Mikó et al.     | Experimental | To explore the microbiome diversity among the different types of breast cancer.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Pan-pathogen microarray (PathoChip) strategy and DNA sequencing were performed.                                                                                                                                                                                                                                                                                                                                                                                         | Gut bacterial species composition seems to be different between postmenopausal patients and postmenopausal healthy control.                                                                 |
| Costantini et al.| Cross-sectional | To examine the 16S-rRNA genes for the hypervariable region that best represents the microbiome in breast tissue.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 16S rRNA gene sequencing was performed on paired normal and tumor tissues.                                                                                                                                                                                                                                                                                                                                                                                | Micrococcaceae, Propionicimonas, Rhodobacteraceae, Caulobacteraceae, Methylobacteriaceae, and Nocardioidoaceae families were found in breast tissues from patients with malignant tumors. |
| Kovács et al.   | Experimental | To assess the ability of cadaverine to influence breast cancer cell behavior.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Fresh fecal samples, 16S ribosomal RNA genes were analyzed.                                                                                                                                                                                                                                                                                                                                                                                                           | Among different TIL expression groups in a patient with breast cancer, the gut microbiome diversity was distinct and compositionally different.                                                                 |
| Zhu et al.      | Cross-sectional | To compare the gut microbial community and its functional capabilities between patients with breast cancer and healthy controls.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | DNA sequencing was performed on fecal samples from 25 healthy controls, 44 postmenopausal healthy controls 5 patients with breast cancer, 25 postmenopausal healthy controls, 46 postmenopausal healthy controls.                                                                                                                                                                                                                                           | Gut bacterial species composition seems to be different between postmenopausal patients and postmenopausal healthy controls.                                                                 |
| Meng et al.     | Cross-sectional | To examine the microbiome of breast tissue from individuals with benign and cancers of various histological grades.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 16S rRNA gene sequencing was performed on 10 patients with breast cancer.                                                                                                                                                                                                                                                                                                                                                                                             | Micrococcaceae, Propionicimonas, Rhodobacteraceae, Caulobacteraceae, Methylobacteriaceae, and Nocardioidoaceae families were found in breast tissues from patients with malignant tumors. |
| Banerjee et al. | Cross-sectional | To explore the association between changes in the microbiome, microbiome-derived metabolites, and breast cancer.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | DNA extraction from fecal samples and qPCR were performed.                                                                                                                                                                                                                                                                                                                                                                                                           | Patients with early-stage breast cancer versus control women had reduced serum LCA levels, a reduced chenodeoxycholic acid to LCA ratio, and a lower abundance of Bahl of Clostridium sordellii, Staphylococcus aureus and Pseudomonas putida. |
| Phikey et al.   | Cross-sectional | To examine the functional capabilities of breast cancer tissues and control breast samples from healthy individuals.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Pan-pathogen microarray (PathoChip) strategy and DNA sequencing were performed.                                                                                                                                                                                                                                                                                                                                                                                   | Gut bacterial species composition seems to be different between postmenopausal patients and postmenopausal healthy control.                                                                                                                                                                                                 |

For the full text, please refer to the original article.
Breast Tissue Microbiota

The existence of microbes in breast tissue has recently been explored. Different types of microbiota have been found that are distinct and clearly distinguishable from those found in the overlying skin tissue [18,19]. Proteobacteria were the most prevalent bacterial phyla identified in the included articles among breast tissue samples taken from benign, malignant, and healthy breasts, followed by Actinobacteria and Firmicutes [5,18,19,32].

Benign vs. Malignant vs. Healthy

The microbiota appears to have a role in both benign and malignant disorders, as the profiles of benign tumors were more similar to those of normal neighboring tissue from women with malignant tumors than to those of tissue from healthy patients [6]. Nevertheless, studies have shown some differences in microbial profiles between the two-disease status [5,19].

Normal adjacent tissues from patients with breast cancer exhibited a considerably greater number of certain bacteria, including Bacillus, Staphylococcus, and members of the Enterobacteriaceae family, as compared to patients with benign breast tumors and healthy women [6]. Moreover, proteobacteria relative abundance was found to be significantly higher in malignant disease than in benign disease [5].

Furthermore, the fluid aspirated from the nipple in patients with breast cancer (ductal carcinoma) and from healthy controls differed significantly in beta diversity [23], suggesting a possible role of bacterial dysbiosis.
in cancer formation.

**Malignant vs. Normal Adjacent**

Several studies showed that tissue from malignant tumors and normal adjacent tissue of the same breast shared similar bacterial diversity [6,26,32]. However, other studies found differences in microbial profiles in terms of the relative abundance of some bacteria. At the genus level, Xuan et al. found the bacterium *Sphingomonas yanoikuyae* was most prevalent in adjacent normal tissue, and the bacterium *Methyllobacterium radiotolerans* was most prevalent in tumor tissue [12,18]. In contrast, Wang et al. [26] found that the genus *Methyllobacterium* was considerably lower in cancer patients compared to non-cancer individuals. This difference between studies might be due to different extraction techniques or types of sample preparation.

When compared to non-cancerous surrounding samples, *Mycobacterium phlei* and *Mycobacterium fortuitum* were shown to be the most abundant species in tumor tissues [5].

On quantitative analysis, Xuan et al. found that the tissue of estrogen receptor-positive breast cancer has 10-fold more bacteria when compared to paired normal tissue from the same patients [18]. Nevertheless, despite this increase in microbial load in breast cancer tissue, antibacterial responses within cancer tissues were found to be significantly downregulated compared to normal breast tissue [12]. These data suggest a possibility that microbiota has the ability to influence the local immune microenvironment of the breast.

**Role in carcinogenesis**

The bacterial load in breast tissue seems to be inversely correlated with the stage of breast cancer, as the highest copy numbers of bacterial DNA were found in tumor tissues from patients with early-stage cancer, and it decreased with the more advanced stage. However, this association was not found with adjacent normal tissue from the same patients as no difference in bacterial load was found across stages [18]. These observations have significance in guiding new diagnostic implications for breast cancer.

The histological grade of breast cancer has also shown some effects on the tumor microbial profile [31]. Meng et al. discovered that when tumor grade increased, the compositional abundance of the Bacteriodaceae family was reduced. Furthermore, as malignancy grows, so does the prevalence of the genus Agrococcus [31].

Luminar type of breast cancer may have an influence on the microbial community of tumor tissue. Banerjee et al. discovered unique patterns of bacterial, viral, fungal, and parasitic profiles in triple-negative (TNBC) and triple-positive (TPBC) breast cancer samples. The microbial profiles of human epidermal growth factor receptor 2 (HER2/neo)-positive and estrogen receptor (ER)-positive samples, on the other hand, were similar [21,29]. When hormone receptor-positive breast cancer samples were compared to hormone receptor-negative samples, Wang et al. discovered a substantial drop in *Methyllobacterium* [26]. Furthermore, samples with histopathologic evidence of lymphovascular invasion contained fewer methylobacterium than those without lymphovascular invasion.

It has not yet been fully elucidated whether there is a specific microbial species that plays a role in breast cancer development. However, *Escherichia coli* isolated from adjacent normal tissue of patients with breast cancer has been shown to generate DNA double-stranded breaks, the most harmful sort of DNA damage [6].

16S rRNA sequencing data of breast tumors from untreated patients and from those treated with neoadjuvant chemotherapy indicates that chemotherapy increases the tumor proportional abundance of *Pseudomonas* by 85%, suggesting that chemotherapy induces preferential growth or survival of some types of bacteria [38].

**Gut microbiota**

The gut microbiota also seems to undergo changes in the presence of breast cancer. The number of these species in postmenopausal patients with breast cancer is higher than that in healthy controls, with a higher abundance of specific species, including *E. coli*, *Prevotella amnii*, and *Enterococcus gallinarum* [30].

The prevalence of *Blautia* species in the gut microbiota increased substantially with breast cancer grade. The number of *Clostridium cocoïdes*, *Clostridium leptum*, and Bacteroides clusters was significantly higher in clinical stage II/III breast cancer than in clinical stage 0/I breast cancer [25].

In early-stage breast cancer, bacterial cadaverine biosynthesis is decreased in the gut, resulting in less production of anti-cancer bacterial metabolites [33]. Lithocholic acid (LCA), another bacterial metabolite that can slow the growth of breast cancer, was also shown to be decreased in the guts of individuals with early-stage breast cancer [28]. The level of expression of tumor-infiltrating lymphocytes (TIL), an indicator of tumor immunity in solid cancers with prognostic value, was associated with gut microbial diversity in patients with breast cancer [54].
Obesity was linked to a considerably decreased total number of *Eggerthella lenta* bacteria, Blautia, *Faecalibacterium prausnitzii*, and Firmicutes species in early-stage breast cancer as a probable cofounder [25]. Furthermore, the proportions of Akkermansia muciniphila (AM) in the gut microbiota were lower in individuals with breast cancer who had increased body fat [39].

**Microbiota of other body sites**

The difference in diversity of the urinary microbiome between patients with breast cancer and healthy women was largely related to menopausal status, with peri/postmenopausal samples being significantly more diverse than premenopausal samples [26].

Oral rinse microbiomes have also been investigated. There was no significant difference between breast cancer patients and healthy women [26].

The presence of *Methylobacterium radiotolerans*, a species that was more abundant in tumor tissue than in normal paired tissue [18], was investigated in pathologically negative sentinel lymph nodes and found to be significantly associated with a higher stage of breast cancer [22].

No significant difference was observed in alpha and beta diversity in the microbiome of sputum samples in a comparison of samples from patients with nontuberculous mycobacterial lung disease with and without breast cancer [35].

**Discussion**

In benign and malignant breast cancer, the role of microbiota is poorly understood in the literature. Several hypotheses have been proposed, including its ability to induce chronic inflammation and regulate immunity, induce DNA damage, and modulate estrogen metabolism [40,41]. This review summarized the currently available data on the role that human microbiota plays in benign and malignant breast tumors.

A number of characteristics of breast microbiota have been discovered in the last few years. A wide diversity of microorganisms is specific to breast tissue and distinct from the microbiota of other body sites [29,24]. The most numerous microbial phyla detected in breast tissue, according to the evaluation of these microbiomes, are Firmicutes and Proteobacteria [5,18,19,32]. However, in one study, bacteria from the phylum Bacteroidetes were the most prevalent, with very few Proteobacteria [24]. Possible explanations for the presence of a microbiome within breast tissue include the passage of skin microbiome through the nipple, during lactation or sexual contact through nipple–oral contact, and finally, translocation of the gut microbiota to breast tissue [36], with the data suggesting more support for the latter hypothesis [42–44].

Microbial dysbiosis, which occurs when the abundance of some species within the microbial community changes relative to others, can result in the microbial community’s typical function being lost [9]. Some authors reported dysbiosis of the breast tissue microbiome in patients with breast cancer [5,6,18,21,33,29,31,32,36]. Moreover, the bacterial load also differed according to the clinical stage of breast cancer [18]. These findings demonstrate an association between dysbiosis and breast cancer. However, it is unclear whether microbial dysbiosis is merely secondary to cancer development or a causative agent in breast carcinogenesis.

We have described the potential role of some types of bacteria in carcinogenesis, including *E. coli* and *Staphylococcus* species. Both were shown to be more common in breast tissue from breast cancer patients and had the ability to produce DNA double-stranded breaks, a condition known to possibly cause cancer [6,45]. Benign breast tumors, unexpectedly, have a microbial profile similar to that of malignant tumors [6]. Urbaniak et al. [6] hypothesized that a lower level of DNA-damaging bacteria in benign tumors could be a possible factor in preventing malignant transformation.

The gut microbiota and its relationship with estrogens have been well investigated in the literature. β-glucuronidase secreted by gut bacteria allows gut microbiota to bind to estrogen receptors (ER) [10]. ER activation promotes cell proliferation, which is a well-defined process in breast cancer [46].

The studies in our review showed that the gut microbiomes display changes among breast cancer patients. Postmenopausal women with breast cancer had an altered gut microbial composition in both alpha and beta diversity metrics [20,50]. Moreover, although not statistically significant, this change was associated with a higher level of urinary estrogens in patients with breast cancer [35]. Furthermore, a significant correlation between estrogen-independency and immunoglobulin A IgA+/IgA− gut microbiota was found in postmenopausal patients with breast cancer [27]. These data suggest a relationship between gut microbiota, estrogen levels, and breast cancer.

Gut bacterial metabolites play a major role in microbiome-to-host signaling [47–50]. According to one study, the levels of LCA, one of the gut microbial metabolites, are lower in patients with breast cancer, especially in the early stages 0/I [28]. According to the same study, LCA performs an antineoplastic function in breast
cancer cells. It specifically inhibits vascular endothelial growth factor production, epithelial-to-mesenchymal transition, and metastasis development. Furthermore, in animal models, it stimulates antitumor immunity and alterations in metabolism [28]. These findings are concordant with previous reports in which the same metabolite was found to be inversely correlated with Ki-67, a proliferation index in breast cancer [51].

Cadaverine is another microbial metabolite generated by the LdcC and CadA enzymes during the decarboxylation of lysine [52,53]. It exerts its effects through the trace amine–associated receptors (TAARs) TAAR1, TAAR8, and TAAR9. Previously, TAAR1 was also found to be related to inhibition of breast cancer growth and with a favorable effect on the overall survival of patients with primary breast cancer [54]. In our review, we described the results of a study by Kovács et al. [33], who showed that DNA coding for bacterial enzymes responsible for cadaverine production is decreased in the fecal microbiome of patients with early breast cancer. Moreover, prolonged survival among patients with early-stage breast cancer was associated with higher expression of lysine decarboxylase [33]. These findings suggest that gut microbial dysbiosis in patients with breast cancer leads to a decreased production of antineoplastic bacterial metabolites.

In breast cancer, immune cell infiltration, specifically cytotoxic CD8+ T cells, has predicted an improved prognosis [55-57]. Additionally, tumor-infiltrating lymphocytes (TILs) found in breast cancer prior to chemotherapy can predict pathological complete response and improve prognosis [58]. Shi et al. [54] investigated the TILs expression in breast cancer in relation to the gut microbiome and found that microbial diversity is different among different expression levels of TILs. Higher TILs expression was associated with a higher diversity of gut microbes. Moreover, the compositional abundance of some microbiota species was different according to TILs expression levels. These findings suggest a potential role for gut microbiota in the prognosis of patients with breast cancer.

To the best of our knowledge, this is the first systematic review to examine the relationship between human microbiota and benign and malignant breast tumors. The limitations of this review are the inclusion of small retrospective studies and the heterogeneity of the results due to the use of different sampling techniques; the description of different body site microbiomes, and the study of different pathological characteristics. Further large-scale studies, including clinical trials, are needed to confirm this association and to discover the possible clinical applications of microbiota in the prevention and diagnosis of breast cancer, as well as therapeutic interventions. Furthermore, studies are required that focus more on specific members of the microbiome, such as E. coli and Staphylococcus, which could contribute to a better understanding of the pathophysiological characteristics of breast cancer.

Conclusions

Current data have linked microbial dysbiosis with breast cancer. Across different histological types, stages, and grades of breast cancer, both the breast and the gut microbiome display changes. However, the exact mechanism underlying these clinical observations is poorly understood. Moreover, yet to be fully identified is whether this microbial alteration causes cancer or is one of the consequences of carcinogenesis, and whether there are specific microbial agents that contribute to the pathophysiological characteristics of this disease.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

The authors would like to acknowledge Dr. Hisham Nomaan for his valuable contribution in providing access to different databases.

References

1. Ghoncheh M, Pournamdar Z, Salehiniya H: Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev. 2016, 17:43-6. 10.7314/apjcp.2016.17.s5.43
2. Senkus E, Kyriakides S, Ohno S, et al.: Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015, 26:Suppl 5:v8-30. 10.1093/annonc/mdv298
3. Breast cancer statistics. (2021). Accessed: May 30, 2021: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer#headin...
4. Valencia OM, Samuel SE, Viscusi RK, Riall TS, Neumayer LA, Aziz H: The role of genetic testing in patients with breast cancer: a review. JAMA Surg. 2017, 152:589-94. 10.1001/jamasurg.2017.0552
37
36
35
34
33
32
31
30
29
28
27
26
25
23
22
21
20
19
17
16
15
14
13
12
11
10
7
6
5
4
3
2
1
194x96
17
16
15
14
13
12
11
10
7
6
5
4
3
2
1
194x96
Chiba A, Bawaneh A, Velazquez C, et al.: Neoadjuvant chemotherapy shifts breast tumor microbiota populations to regulate drug responsiveness and the development of metastasis. Mol Cancer Res. 2020, 18:150-9. 10.1158/1541-7786.MCR-19-0451

Frugé AD, Van der Pol W, Rogers LQ, Morrow CD, Tsutaya Y, Demark-Wahnefried W: Fecal Akkermansia muciniphila is associated with body composition and microbiota diversity in overweight and obese women with breast cancer participating in a presurgical weight loss trial. J Acad Nutr Diet. 2020, 120:650-9. 10.1016/j.jand.2018.08.164

Fernández MF, Reina-Pérez I, Astorga JM, Rodríguez-Carrillo A, Plaza-Díaz J, Fontana L: Breast cancer and its relationship with the microbiota. Int J Environ Res Public Health. 2018, 15: 10.3390/ijerph15081747

Parida S, Sharma D: The microbiome-estrogen connection and breast cancer risk. Cells. 2019, 8:10.3390/cells8121642

Arroyo R, Martín V, Maldonado A, Jiménez E, Fernández L, Rodríguez JM: Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacillus isolated from breast milk. Clin Infect Dis. 2010, 50:1551-1. 10.1086/652765

Fernández L, Cárdenas N, Arroyo R, Manzano S, Jiménez E, Martín V, Rodríguez JM: Prevention of infectious mastitis by oral administration of Lactobacillus salivarius PS2 during late pregnancy. Clin Infect Dis. 2016, 62:568-73. 10.1093/cid/cwv974

Lakrite JR, Pouthadiño T, Mirabal S, et al.: Gut bacteria require neutrophils to promote mammary tumorigenesis. Oncotarget. 2015, 6:9587-96. 10.18632/oncotarget.3528

Khanna KK, Jackson SP: DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet. 2001, 27:247-54. 10.1038/s38579

Doisneau-Sixou SF, Sergio CM, Carroll JS, Hui R, Musgrove EA, Sutherland RL.: Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. Endocr Relat Cancer. 2003, 10:179-86. 10.1077/erc.0.0100179

Burcelin R, Serino M, Chabo C, et al.: Metagenome and metabolism: the tissue microbiota hypothesis. Diabetes Obes Metab. 2015, 17 Suppl 5:61-70. 10.1111/dom.12157

Dumas ME: The microbial-mammalian metabolic axis: beyond simple metabolism. Cell Metab. 2011, 13:489-90. 10.1016/j.cmet.2011.04.005

Puertollano E, Kolida S, Yaqoob P: The microbial-mammalian metabolic axis: beyond simple metabolism. Cell Metab. 2011, 13:489-90. 10.1016/j.cmet.2011.04.005

Vattai A, Akyol E, Kuhn C, et al.: Remaining mysteries of molecular biology: the role of polyamines in the cell. J Mol Biol. 2015, 427:5389-406. 10.1016/j.jmb.2015.06.020

Seiler N: The microbial-mammalian metabolic axis: beyond simple metabolism. Cell Metab. 2011, 13:489-90. 10.1016/j.cmet.2011.04.005

Rajendran S, Sharma D, Dutt A, et al.: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A. 2009, 106:3698-703. 10.1073/pnas.0812874106

Tang X, Lin CC, Spasojevic I, Iversen ES, Chi JT, Marks JR: A joint analysis of metabolomics and genetics of breast cancer. Breast Cancer Res. 2014, 16:415. 10.1186/s13058-014-0415-9

Miller-Fleming L, Olin-Sandoval V, Campbell K, Ralser M: Catabolism of polyamines. Amino Acids. 2004, 26:217-33. 10.1007/s00726-004-0070-z

Vattai A, Akyol E, Kuhn C, et al.: Increased trace amine–associated receptor 1 (TAAR1) expression is associated with a positive survival rate in patients with breast cancer. J Cancer Res Clin Oncol. 2017, 143:1637-47. 10.1007/s00432-017-2420-8

Seo AN, Lee HI, Kim EL, et al.: Tumor-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. Br J Cancer. 2013, 109:2705-13. 10.1038/bjc.2015.654

Lee S, Foulkes WD, Leung S, Gao D, Lau S, Kos Z, Nielsen TO: Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. Breast Cancer Res. 2014, 16:432. 10.1186/s13058-014-0432-8

Mahmoud SM, Paish EC, Powe DG, et al.: Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011, 29:1949-55. 10.1200/JCO.2010.30.5037

Denkert C, Loibl S, Nenke A, et al.: Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010, 28:105-13. 10.1200/JCO.2009.23.7370