Seminars in Cancer Biology 70 (2021) 112–127

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Significance of human microbiome in breast cancer: Tale of an invisible and an invincible

Jatin Chadha, Deeptashree Nandi, Yama Atri, Alo Nag *

Department of Biochemistry, University of Delhi South Campus, New Delhi, 110021, India

ARTICLE INFO

Keywords:
Microbiome
Breast cancer
Microbial dysbiosis
Anticancer therapy
Probiotic therapy

ABSTRACT

The human microbiome is a mysterious treasure of the body playing endless important roles in the well-being of the host metabolism, digestion, and immunity. On the other hand, it actively participates in the development of a variety of pathological conditions including cancer. With the Human Microbiome Project initiative, metagenomics, and next-generation sequencing technologies in place, the last decade has witnessed immense explorations and investigations on the enigmatic association of breast cancer with the human microbiome. However, the connection between the human microbiome and breast cancer remains to be explored in greater detail. In fact, there are several emerging questions such as whether the host microbiota contributes to disease initiation, or is it a consequence of the disease is an irrevocably important question that demands a valid answer. Since the microbiome is an extremely complex community, gaps still remain on how this vital microbial organ plays a role in orchestrating breast cancer development. Nevertheless, undeniable evidence from studies has pinpointed the presence of specific microbial elements of the breast and gut to play a role in governing breast cancer. It is still unclear if an alteration in microbiome/dysbiosis leads to breast cancer or is it *vice versa*. Though specific microbial signatures have been detected to be associated with various breast cancer subtypes, the structure and composition of a core "healthy" microbiome is yet to be established. Probiotics seem to be a promising antidote for targeted prevention and treatment of breast cancer. Interestingly, these microbial communities can serve as potential biomarkers for prognosis, diagnosis, and treatment of breast cancer, thereby leading to the rise of a completely new era of personalized medicine. This review is a humble attempt to summarize the research findings on the human microbiome and its relation to breast cancer.

1. Chronicles of the human microbiome

The human body is one such super-creature harbouring about 10 folds as many microbial cells as its own body cells [1]. It may come as a surprise to many that only a small proportion of our genetic material is inherited from our parents whereas a gargantuan portion of our genetic blueprint is actually shared with the microbial communities! In other words, the human body successfully functions due to the combined efforts from our visible organs and our invisible microbial dwellers. The Nobel laureate Joshua Lederberg in 2001 coined the term "Microbiome" for this consortium of symbiotic microflora [2]. Accordingly, for humans, it came to be known as the "Human Microbiome". This conglomerate, or more specifically, the "microbiota", constitutes of the microbial taxa associated with complex organisms like human beings. The human microbiota is as unique as one's fingerprint and undergoes dynamic changes over the course of life. Speaking of the human microbiota, the first thing that sparks in our minds is the gut microflora. A multiplex of bacteria, viruses, fungi, archaea, and small protozoa resides within our gut. Notably, there exists a deep interplay between this community and the host mucosal epithelial cells and immune cells in a reciprocal fashion. Microbiota of the gut plays a cardinal role in multiple cellular and metabolic functions, a few of which include digestion, metabolism of bile acids, synthesis of essential growth factors and vitamins B and K, protection against systemic infiltration and expulsion of intestinal pathogens, and boosting of the host immune system through activation of immune cells [3]. In this manner, the microbiota maintains homeostasis in the gut. However, disruption in this equilibrium facilitated by repeated courses of antibiotics, unhealthy diet, stress, and countless other factors results in a state of "dysbiosis", leading to an impaired microbiota. Consequently, a perturbed microbial ecosystem

* Corresponding author at: Department of Biochemistry, University of Delhi South Campus, Biotech Building, 2nd Floor, Benito Juarez Road, Dhaula Kuan, New Delhi, 110021, India.
E-mail address: anag@south.du.ac.in (A. Nag).

https://doi.org/10.1016/j.semcancer.2020.07.010
Received 18 June 2020; Received in revised form 19 July 2020; Accepted 20 July 2020
Available online 24 July 2020
1044-579X/

within the host is linked to numerous human pathologies including antibiotic-induced diarrhea, obesity, inflammatory bowel disease, rheumatoid arthritis, asthma, type-2 diabetes, and even cancer [4–10]. Although this complex relationship between the host microbiome and human disorders has been a hot topic of research in the last decade, considerable knowledge gaps still exist. This demands a detailed understanding of the optimal microbial composition of a healthy gut and how it influences the local and systemic immune responses. Moreover, such investigations will be extremely useful in the management of diseases and maintenance of health and fitness of the body.

Regardless of the fact that microbes have been an integral member of our landscape since the beginning of life itself, their recognition did not befall us before the 1680s. This was precisely when Antonie van Leeuwenhoek noticed a striking variation in the microbial composition of healthy *versus* diseased individuals under the microscope. However, it took almost two centuries before a relationship could be established between microbes dwelling within humans and various pathological conditions. Research carried out by Louis Pasteur and Robert Koch in the 19th century played an instrumental role in this realization. The late 1800s was a remarkable period that heralded new techniques and devices for the cultivation and identification of micro-organisms. These include the advent of Petri-dishes and agar that allowed *in vitro* growth of bacteria, propagation of viruses using fertile hen eggs and the improvements in staining techniques. Robert Koch, in 1876, discovered that the bacteria *Bacillus anthracis* was the culprit behind the deadly disease, anthrax. This established the first direct link between a microbe and a specific disease. Later on, different microbes were uncovered as the causative agents for various ailments, including tuberculosis, cholera, gonorrhea, dysentery, and whooping cough. In the early 1900s, Arthur I Kendall determined the conditions feasible for the maintenance and survival of the intestinal microflorae. He further demonstrated the outcome of diet on the gut microbiota and subsequently, the health of primates. Later in the 1950s, Rene Dubos along with his colleagues discovered several indigenous florae of the human gut, using germ-free animals. His group concluded that external and internal factors could modulate the host microbiota and cause different disorders. Later on, the emergence of high-throughput techniques, like, DNA amplification and sequencing technologies along with computational methods enabled better characterization of a huge array of microbes. Carl Woese and Norman Pace pioneered the use of ribosomal RNA as molecular markers, which meant scientists could now measure and interrogate the functional relationships among the microbial communities. These communities were referred to as alpha diversity based on the number of distinct species and their distribution. On the contrary, the variation of abundance of different taxa among different samples came to be known as beta diversity. Both these features could now be evaluated by analysis of the 16S ribosomal RNA. Whole-genome sequencing, on the other hand, has been the most routinely employed approach for sequencing the entire genome of all the microbes present in a given specimen. Recent times have witnessed the use of meta-transcriptomics and metabolomics in conjunction with metagenomics for unraveling host-microbial connection with human health. With recent advancements in microbiology, a large proportion of commensal microbes are now culturable [11]. Culturomics has enabled elucidation of several aspects of microbiota functions or its association with the illness which were not possible by mere computational analysis. An extensive impact was associated with the Human Microbiome Project, undertaken by the National Institutes of Health, spanning nine years from 2008 till 2017. This project made the microbiome an area to focus on for the scientific community. It also set the momentum for ever-expanding research and industrial ventures in the field of the human microbiome. In current times, this arena forms an indispensable sector of the biotechnology industry. Importantly, this project managed to identify a dramatic 81–99 % of the huge number of microbial species known to inhabit humans. Multiple studies were also conducted for determining the association of human microbiota with disease conditions, including Crohn's disease, oesophageal cancer, acne, psoriasis, atopic dermatitis, colitis, and immunodeficiency. A second phase of the project was also initiated in 2014 for mapping the role of microbiome in pregnancy, inflammatory bowel disorder, and type-2 diabetes. Several labs have now proposed microbes as the underlying etiology for cardiovascular problems, autism, duodenal ulcers, and stomach cancer. The mysterious relationship between microbiota and cancer surfaced in the late 19th century following the attempts by William Coley to cure sarcomas. He did partially succeed in his venture as injecting heat-inactivated *Streptococci* into cancer patients led to effective antitumor responses [12]. A landmark event in the association of microbes with cancers was the discovery of a virus as the causative player of sarcomas in chickens by Peyton Rous in 1911. Additionally, bacteria mediated tumors were identified with *Helicobacter pylori* induced gastric cancer being one of the best-studied examples. In the 1920s, an intravesicular injection of *Mycobacterium bovis* in superficial bladder cancer patients not only generated an anticancer response but also increased their survival rates [13]. Such findings hinted at the probability of microbe-mediated response in cancer cells. Recent studies have provided fertile pieces of evidence to prove that gut microbiome is an effective contributor in combating cancer by modulating the efficacy of several anticancer therapies [14–18]. Despite the exciting advancements and discoveries, most of the research has been focussed on the association between microbiota and human disorders. Nevertheless, more evidence is required to actually ascertain the microbiome as a pivotal determinant of tumorigenesis. Understanding the mechanisms employed by the microbiota in influencing cancer initiation, progression, and metastasis and response to various treatments can yield novel opportunities for diagnostic and therapeutic strategies.

2. Breast Cancer: the grim reaper

Breast Cancer is the second most prevalent carcinoma worldwide and the most frequently diagnosed malignancy in women [19]. It is estimated to affect one in every eight women during their lifetime. It is the leading cause of mortality from cancer in women with more than 0.6 million deaths in 2018 worldwide (6.6 % of global cancer-associated deaths) [20,21]. Such staggering numbers persist despite breakthrough advancements in research, diagnostics, and treatment. If we hope for the mortality rates to decline in the near future, we need to understand in detail the precise etiology of breast cancer and design novel approaches to target them.

Recent studies have illustrated the risk of diverse genetic elements and biomarkers in breast cancer progression. These comprise of inherited loss and mutations in the BReast CAncer (*BRCA1/2*) genes [22,23], expression of Estrogen Receptor (*ER*), Progesterone Receptor (*PR*) and Human Epidermal growth factor Receptor 2 (*HER2*) [24]. Interestingly, the interplay of several environmental and lifestyle components have been closely associated with an increased incidence of breast cancer. The factors range from an unhealthy diet, sedentary routine, smoking and obesity to exposure to radiation, consumption of alcohol, tobacco, and hormonal contraceptives as well as hormone replacement therapy [25,26]. The risk of breast cancer positively correlates with increasing age in women and the majority of breast cancer cases are diagnosed following menopause. Nevertheless, the present risk factors and genetic-epigenetic determinants could only be corroborated with a slender amount of global breast cancer cases. Hence, this has fuelled interest in recognizing the potential triggers for breast cancer and the subsequent development of preventive and remedial strategies against this catastrophic disease.

Contemporary schemes for breast cancer treatment such as surgery (lumpectomy), radiotherapy, chemotherapy, and targeted therapy may have improved overall survival [26]. Sadly, however, these methods have their limitations with a low therapeutic index [27,28]. Unfortunately, these practices non-specifically target all cells, both healthy and tumorous. Consequently, prompting severe side effects, including

infertility, fatigue, hair loss, heart damage, leukemia, lowered body immunity, and thrusting one into depression. In totality, the side effects surpass the effectiveness of cancer therapy and leads to poor prognosis of cancer patients. In this view, the development of novel strategies that are natural and selectively cytotoxic for cancer cells alone are imperative. The potent health benefits of microorganisms, their ease of large-scale production, and the capability to synthesize a wide range of powerful tumor-targeting bioactive molecules render their use as a possible anticancer therapeutics.

In this regard, emerging studies have implicated the involvement of the gut microbiome in affecting malignancy and response to breast cancer treatment [29–31]. These bacterial communities within the host micro-niche may act as auxiliary environmental factors for the establishment and progression of breast cancer. Untangling this complex interplay between the host microflora, breast cancer progression and therapeutic response can open new avenues for breast cancer detection and management. This raises the need to explore a scientific frontier and answer some questions, like; can we identify the microbial communities that increase predisposition to breast cancer? Is there a direct relationship between dysbiosis of the human microbiome and breast cancer development? Can we engineer our microbiome to better respond to therapies? How can we modulate our diet to aid in breast cancer management? Can such comparatively natural approaches complement conventional radio- and chemotherapy to minimize the side effects that we face in current times? Is it possible to manage pain in breast cancer patients by exploiting the role of gut microbiota in the functions of the central nervous system? This review examines the quintessential evidences which ornament the role of the human microbiome in breast cancer development, its ability to modulate host metabolism and immune responses. Additionally, we have outlined the recent developments in this direction to provide revolutionary insights into the dynamic functioning of the human microbiome against breast cancer.

3. The missing link between the human microbiome and breast carcinoma: connecting the dots

When we talk about the breast microbiome, it does not necessarily mean just the microbiome of the breast tissue alone. Multiple studies conducted over the years have identified that the microbes residing within the breast tissue actually originate from (i) the gut, (ii) the mammary gland, and (iii) breast milk during the early phases of life. This complex pool of microbes, in turn, is modulated by a number of factors throughout the life of the person. The factors may be external (environmental and lifestyle players), which can be therefore modified, and internal (host genetics, host immunity, *etc.*), which are unique to each person and are not under our control. We have tried to highlight a few of such essential key modulators of the breast microbiome in Fig. 1, which may be hijacked and altered during breast carcinogenesis. In this section, we have discussed the contribution of each component that makes up the breast microbiome towards breast cancer risk.

3.1. The Gut feeling: Microbial dysbiosis in breast carcinoma

The human gastrointestinal (GI) tract is an exquisite ecological niche colonized by complex and innumerable communities of bacteria, fungi, and yeasts. It has been appraised as the “Second Genome” of the body [1]. It is estimated to comprise of about 1014 heterogeneous microbial species accommodating approximately 150-fold as many genes as the human genome [3]. The abundance and diversity of the gut microflora vary with multiple parameters including age, race, diet, hygiene, host genetics, environment subjection to antibiotics/drugs, and maternal colonization. Metagenomics and cultural surveys have reported the presence of the diverse genera in the GI tract [32]. These constitute *Lactobacillus, Bifidobacterium, Bacteroides, Enterococcus, Faecalibacterium, Ruminococcus, Eubacterium, Peptococcus, Streptococcus, Peptostreptococcus, Streptomyces*, and *Clostridium.* Instead of being a passive dweller, this intricate ecosystem of commensal and symbiont microbiota forms a mutualistic association with the host. Moreover, this ecosystem

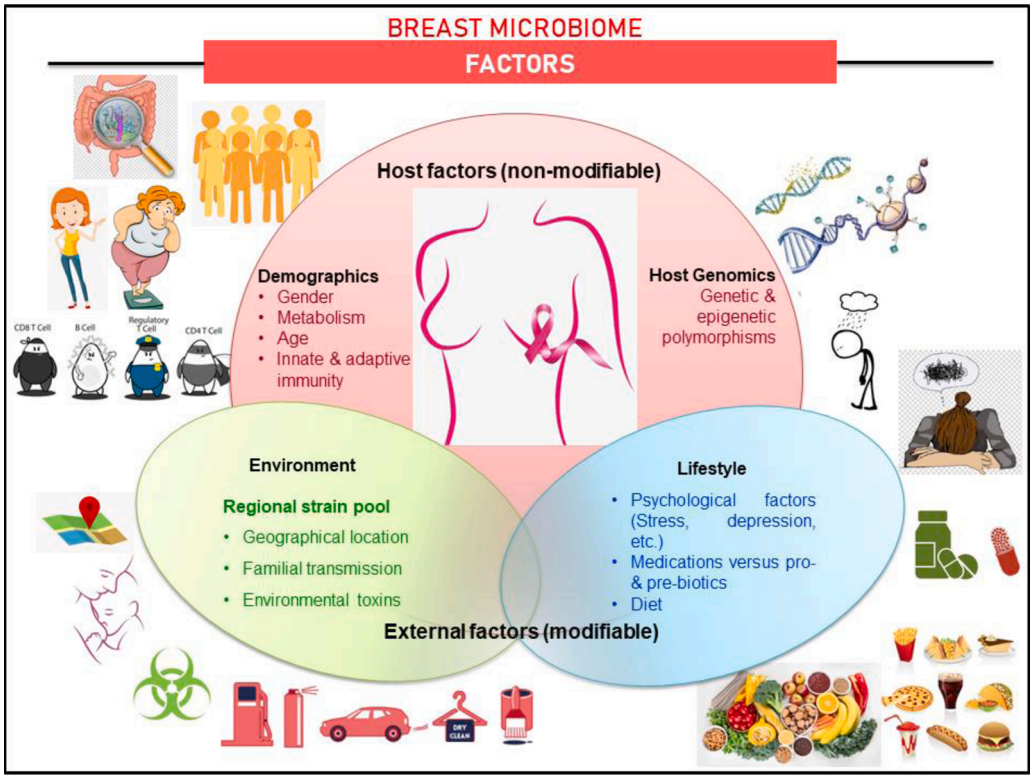

Fig. 1. A multitude of factors affect the human breast microbiota and differentially modulate the outcome on breast tumorigenesis.

functions as a multidimensional "microbial organ" [33] by supporting nutritional absorption, metabolism, biosynthesis of essential amino acids and vitamins, inactivation of toxins and carcinogens and development and maintenance of innate and cell-mediated immunity [34]. The major microbial metabolites through which the gut microbiota maintains such a vast array of functions constitute of short-chain fatty acids, ammonia, hydrogen, methane, carbon dioxide, *etc.* [35]. However, a perturbation in this relationship and balance may lead to a state of 'dysbiosis' or loss of gut homeostasis. This may evidently result in deleterious consequences for the host, including several pathological conditions. Therefore, though the host-gut microbiome interaction is extremely sensitive it undoubtedly serves as an ideal model for studying host-microbiota and disease interrelation [36].

Consumption of prebiotics and probiotics and fecal microbiota transplantation (FMT) of healthy bacteria under pathological conditions reportedly alter the number of commensal bacteria of the intestines. Consequently, they aid in improving the condition of the host. Studies on animal models have revealed the association between the microbiome and development of cancer. They also show how external factors can maneuver cancer outcomes by affecting the microbiome [37,38]. For the first time in 1976, a study found increased fecal excretion of conjugated estrogens in subjects consuming ampicillin experienced implying active estrogen metabolism by the gut microbes [39]. In 1990, consumption of whole-grain products was hypothesized to increase the fecal bulk and alter *β-glucuronidase* (BGUS) activity, enhancing the risk of breast cancer [40]. Microbial *β-glucuronidase* is responsible for de-conjugating estrogen. This enables the reabsorption of free estrogen in the blood, which transports them to other organs, thereby promoting metastasis. Studies have established the association of breast cancer with estrogen level and metabolism in patients as a consequence of distinct richness in their gut microbial communities [41]. Moreover, gut dysbiosis has been associated with a higher level of circulating estrogen in postmenopausal breast cancer [42]. Microbial species of *Clostridia, Ruminococcaceae*, and *Escherichia* families have been reported to be involved in estrogen metabolism. Goedert *et al.* in a case-controlled study compared the fecal microbiota between postmenopausal breast cancer patients and healthy matched subjects. It was concluded that breast cancer patients exhibit less microbial diversity and distinct composition. Breast cancer patients possessed elevated levels of *Clostridiaceae, Ruminococcaceae* and *Faecalibacterium*, whereas reduced levels of *Lachnospiraceae* and *Dorea* [43]. Another study conducted by Bard *et al.* assessed the difference of gut microbiome composition among breast cancer patients with distinct clinical stages by DNA isolation and 16S rRNA sequencing. Patients with Grade III breast cancer displayed enrichment of *Blautia* sp. compared to Grade I individuals. Moreover, the absolute number of *Bifidobacterium* varied significantly according to the clinical stages of breast cancer [3]. A similar study in 2017 showed the abundance of *Firmicutes* and *Bacteroidetes* in fecal samples of breast cancer patients. A significantly higher richness of *Bacteriodetes, Clostridium, Faecalibacterium,* and *Blautia* sp. was particularly observed in stages II and III compared to stage I [29]. Enrichment of *Staphylococcus, Enterobacteriaceae, Bacillus, Fusobacterium, Gluconobacter, Hydrogenphaga, Atopobium*, *and Lactobacillus* has been correlated with breast tumors in another study [44]. Rosean and his colleagues demonstrated, in mice model of hormone receptor-positive breast cancer, that the administration of antibiotics resulted in a shift in the bacterial communities. They noted an abundance of *Akkermansia, Escherichia, Alistipes*, and *Shigella* and a concomitant reduction in the number of *Bacteroides, Lactobacillus*, and *Lachnospiraceae* in the mice cecum [45]. Additionally, pre-existing dysbiosis in these mice was associated with increased inflammation and ultimately promote metastasis. In this regard, biopsies of colon cancer patients have associated *Fusobacterium,* a commensal with tumor metastasis [46]. The enrichment of this bacterium in breast cancer patients points towards the possible contribution of *Fusobacterium* in breast tumor metastasis as well. Strikingly, supplementing mice with an antibiotic cocktail was accompanied by an aberrant gut microbiota and significant acceleration in breast tumor growth. Similarly, administration of Cephalexin, an antibiotic routinely used in breast cancer, led to alterations in *Bacteroides, Anaeotruncus*, and *Odoribacter* that dramatically affected the rate of breast cancer [47]. Additionally, overexploitation of antibiotics has been shown to reduce the levels of lignan enterolactone in the plasma. This reduction directly affected the gut microbiome and increased the risk of breast cancer [48]. Unsurprisingly, intestinal dysbiosis is involved in multiple types of cancers including colorectal cancer [49], breast cancer [10], and lung cancer [50]. Moreover, recent evidences have attributed global changes in intestinal microbiota to contribute towards gastric carcinogenesis [3]. These findings suggest that the gut microbiome may play a significant role in breast cancer initiation and tumorigenesis. However, discrete and precise investigations need to be pioneered into this avenue.

3.2. The breast microbiome and breast cancer interplay: the kiss of Judas

The notion that the human breast is a sterile tissue is no longer true. The recent race to characterize the human microbiome indicated that the breast tissue has its unique microbial structure, distinct from other tissues and distal microbiomes [51]. Now we have come to know about the presence of microbes in human milk [52]. To add to this, the infant oral and skin microbes are also reported to have access to the mammary ducts during breastfeeding [53]. Such emerging concepts give support to the concept of microbial existence in the breast tissue that has persisted right from the beginning. In subsequent years, 16S rRNA sequencing and culturing methods have detected diverse communities of bacteria in the mammary tissue. These include *Bacillus, Pseudomonas, Propionibacterium, Prevotella, Acinetobacter, Listeria,* and members of the class *Enterobacteriaceae, Gammaproteobacteria,* and family *Comamonadaceae* [51]. Such microbial communities are seen to associate as commensals and contribute towards breast health by activating the immune-surveillance pathways and degrading potent carcinogens [10].

The role of mammary microbiota in regulating the risk of breast cancer evolution has been questioned by the scientific community. It is also unknown whether breast cancer corresponds to a specific microbial fingerprint *i.e.* the presence or absence of specific commensal or pathogenic species. In this view, studies focussing on potent carcinogenic viruses have established a positive correlation between breast cancer with Human Papilloma Viruses (HPV) [54,55] and with Epstein Barr virus (EBV) [56]. More recently, additional viral signatures likely to be associated with breast cancer, such as *Adenoviridae, Retroviridae, Coronaviridae* and *Herpesviridae,* have also been reported [64]. Interestingly, *Nodaviridae* was observed primarily in *HER2+* subtype of breast cancer [65], pointing towards its potential clinical translation as a unique biomarker for such patients. However, due to the lack of reproducibility, such studies have ignited debates and demand thorough validation [57–59]. A recent study in 2016 established the relationship between HPV, STAT3 activity, and Interleukin-17 (IL-17) in breast cancer progression. HPV infection was shown to induce STAT3 signaling which elevated the levels of IL-17, a pro-inflammatory cytokine, ultimately aiding in breast cancer progression [60]. Literature indicates the range of prevalence of HPV, EBV, and Mouse Mammary Tumor Virus (MMTV) in 0–86 %, 0–68 %, and 0–57 % of breast cancer cases, respectively [61]. These studies have raised fundamental questions over the putative association of viral infections with breast cancer and have polarised this debate with the use of Next Generation Sequencing (NGS) technologies. Therefore, more studies should be pioneered to confirm the plausible role of "breast virome" and viral communities in breast cancer.

Studies pertaining to the differential microbial composition of the breast tissue between healthy and cancer individuals are still at an initial stage. Most of the available data has emphasized on the predominance of *Sphingomonas yanoikuyae* in breast specimens of healthy women. The phyla *Proteobacteria* and *Firmicutes* have been repeatedly reported as the highly prevalent representatives of a normal breast microbiota. Additionally, healthy women were characterized with enriched existence of

Prevotella, Lactococcus, Streptococcus, Corynebacterium and *Micrococcus* while very low amounts of *Staphylococcus, Bacteroidetes* and *Enterobacteriaceae* were found in such samples [3]. The presence of *Thermoanaerobacterium thermosaccharolyticum, Candidatus Aquilina* sp., *Anoxybacillus, Leuconostoc, Geobacillus* and *Turicella otitidis* has also been attributed to the healthy breast specimens [74]. Moreover, there exists a certain degree of variation in the absolute percentage prevalence of each of the constituent members within the normal breast tissue across different studies. Interestingly, a report had indicated the presence of *Lactobacillus* in 2.2 % of healthy breast tissues while its occurrence was reduced to 1.4 % in cancerous lesions [74]. This is in agreement with the well known protective functions associated with this bacterium. A separate study showed the abundance of *Sphingomonas* in 50 % of healthy tissue [74]. Another scientific group had identified *Ralstonia,* which was previously reported only in breast milk, as one of the most abundant genera in the breast tissue [69]. Nonetheless, its correlation with tumorigenesis is yet to be uncovered. The possibility that we may encounter more similarities than differences among tumorous and adjacent healthy tissues has been suggested as well. Emerging research has only begun to identify some important quantitative changes in the microbiota as the healthy breast evolves into a more cancerous phenotype. Investigations on mammary tissues from breast cancer patients have indicated a reduction in diversity and abundance of bacterial genera such as *Clostridium leptum, C. coccoides, Faecalibacterium*, and family *Ruminococcaceae* [29,43]. In concordance, 16S rRNA analysis has shown a relative abundance of *Bacillus, Staphylococcus*, and members of *Enterobacteriaceae* in patients with breast cancer [62]. Quantification of bacterial DNA in breast cancer subjects by Xuan *et al.* disclosed the abundance of *Methylobacterium radiotolerans* in tumor tissues and that of *Sphingomonas yanoikuaye* in normal adjacent tissues [10]. Moreover, a differential antibacterial response against tumor tissues was connected with a reduced number of *S. yanoikuaye*, pointing towards its probiotic role in breast physiology. Furthermore, total bacterial content was significantly lower in tumor tissues than the adjacent healthy tissues.

Notably, the variation in breast microbiota of patients with different stages of breast cancer has also been confirmed [3]. Heiken *et al.* showed that the breast microbiome of malignant and benign breast carcinomas differ dramatically. The taxonomic profiles of invasive and benign breast cancer established the dominance of *Firmicutes* and *Bacteroides*. However, malignant tissues were correlated with enrichment of low abundance taxa including *Fusobacterium, Gluconacetobacter, Atopobium, Hydrogenophaga,* and *Lactobacillus* [62,63]. More recently in 2018, Banerjee and colleagues discovered predominant microbial genomic signature sequences of bacteria, viruses, and fungi in Triple-Negative breast cancer samples (TNBC). Bacterial probes included members of the families *Caulobacteriaceae, Actinomycetaceae, Enterobacteriaceae, Prevotellaceae, Sphingobacteriaceae, Brucellaceae, Flavobacteriaceae,* and *Bacilliaceae* [64], many of which have previously been associated with various other cancers [65]. Amongst fungal families, *Aspergillus, Candida, Coccidiodes, Geotrichum* and *Rhodotorula* have found prominence due to their abundance in different breast cancer subtypes [64]. However, more cohort based clinical trials are needed for their validation as subtype specific signatures.

The few studies related to the microbial composition in healthy *versus* cancerous breast do not provide any microbial signature or consensus. Such lack of consistency may be attributed to the differences in geographical locations, cohort size, microbial-identification technologies, type and stage of breast cancer, subject age, diet, ethnicity, *etc.* Based on these findings, it would be fair to say that we still do not fully understand the breast microbiota composition. Studies in this avenue are still in their infancy and it appears premature to declare a microbial signature at this juncture. Considering the significance of breast microbiome in breast cancer, these potential microbial signatures hold great promise in identifying individuals susceptible to developing a particular type of breast malignancy. As mentioned in our review, Banerjee *et al.* has reported some potential microbial signatures related to different breast cancer subtypes. However, in order to accentuate their clinical translation into an effective prognostic or diagnostic biomarker, more intensive investigation and thorough validation through high-throughput technologies and large- scale clinical trials are much needed. These can prove to be extremely promising and path-breaking to meet our goal of conquering breast cancer, by drawing out a potential microbial consensus. Yet more peculiar microbes specific for a subtype have to be identified for their bench-to-bedside translation as diagnostic biomarkers for breast cancer. Also, studies on their mode of familial transmission can help in predicting susceptible patients within a family and consequent generations. Though these evidences advocate the connection of the breast microbiome to breast cancer, more research and clinical confirmations for determining how these microbial signature sequences affect breast cancer development are required.

3.3. *The far-reaching effects of breast milk microbiota on breast tumorigenesis*

Following its birth, a neonate infant has to adjust to an extra-uterine environment, which contains potential pathogenic micro-organisms. This happens to be in stark contrast to a safer and almost sterile intra-uterine condition. The trophic factors ingested by the baby from the maternal colonic and vaginal microbiota serve as an initial shield for the baby immediately after its exposure to the external world. This is followed by colonization in its own gut that begins with exposure to oral feeding over the subsequent time course. The best example of protection imparted through bacterial colonization is breastfeeding. Such a practice continuously supplies beneficial bacteria to the infant's gut, allowing maturation of its immune system. Techniques like Next Generation Sequencing (NGS) and quantitative PCR (qPCR) have successfully characterized the breast milk microbiome. Strikingly, several similarities were found between the breast milk microbiome and the breast microbiome composition. The microflora of the milk arises from the microbes of the GI tract *via* the entero-mammary path, from the infant's mouth through the maternal skin during breastfeeding [66] and through mammary intercourse [63]. Nevertheless, investigations on the biodiversity of the human breast milk microbiome and its changes are numbered. Additional parameters that influence the microbial composition and diversity of human milk constitute diet, consumption of antibiotics, pregnancy, childbirth, postpartum period, and even geographic distribution [34]. Recently, cohort studies have elaborately demonstrated the notable variation in the human milk microbiota across different geographical locations [67,68]. One such study accounts for the relative abundance of *Firmicutes, Proteobacteria, Streptococcus, Propionibacterium*, and *Pseudomonas* in breast milk samples of Finnish, South African, Chinese and Spanish women [67]. These have been validated by other studies which also lay emphasis on the presence of phyla *Bacteroidetes, Actinobacteria,* and bacterial strains of *Bifidobacterium, Corynebacterium, Serratia,* and *Staphylococcus* in human milk [69–71]. A metagenomics study conducted on ten donor milk samples estimated that the bacterial community in human milk comprises of over 360 genera pertaining to the phyla *Proteobacteria* (65 %) and *Firmicutes* (34 %) and the genera of *Pseudomonas* (61.1 %), *Staphylococcus* (33.4 %) and *Streptococcus* (0.5 %) [72]. Functional metagenomic analysis of the human milk by Ward *et al.* identified that the microbial sequences along with Open Reading Frames (ORFs) of genes involved in membrane transport, metabolism of nitrogen, and stress response were associated with colonization of infant's gut and development of immunity in the human intestine [72].

Studies relating the milk microbiome to the risk of breast cancer are very few. Preliminary findings from an investigation in 2005 demonstrated that the nipple aspirate fluid of breast cancer patients *versus* healthy controls contained significantly different microbiota profiles [73]. A similar study pinpoints microbiome variation in nipple aspirate of benign and malignant tissues in breast cancer individuals [74,75]. Another study cited the essential role of the ductal microbiome in breast

cancer development. The major phyla *Proteobacteria, Bacteroidetes, Firmicutes*, and *Actinobacteria* constituted 89.1 % and 82.9 % of the total microflora in healthy subjects and breast cancer survivors respectively [75]. Bacteria of the genera *Neisseria, Corynebacterium durum, Ruminococci, Lachnospiraceae, F. prausnitzii, Mycobacterium kansasii, Rothia mucilaginosa*, and *Clostridium baratii* were abundant in breast cancer individuals. *Lactobacillus salivarius* predominated in healthy women. Chemotherapy is also found to significantly deviate healthy microbial populations and their metabolomic profiles. This was marked by a significant decrease in *Bifidobacterium, Eubacterium*, and *Clobacterium* species and an increase in *Stenotropomonas, Acinetobacter*, and *Xanthomonadaceae* species in milk samples of healthy subjects and those undergoing chemotherapy for Hodgkin's lymphoma [76]. Nonetheless, further insights and investigation on this precious fluid can help uncover its role in breast cancer progression and malignancy.

3.4. Distal microbiomes in breast cancer: distance no bar

The study of the importance of gut, breast, and breast milk microbiota in breast cancer has garnered much interest in the scientific community. Even so, emerging studies that are still in their infancy are analyzing the likely contributions of other microbiomes like urine, fecal, oral, and vaginal in breast carcinogenesis. In this context, urine microbiome analysis presents itself as a non-invasive method for the evaluation of breast cancer risk. A study on the urine microbial diversity of breast cancer patients disclosed elevated levels of gram-positive bacteria in these patients [77]. Furthermore, a relatively greater abundance of *Corynebacterium*, *Staphylococcus*, *Actinomyces*, and *Propionibacteriaceae* was detected in cancer samples as opposed to non-cancer ones regardless of their menopausal status and Body Mass Index (BMI) levels. Notably, the study identified postmenopausal breast cancer females to exhibit incremented diversity of *Varibaculum*, *Porphyromonas*, *Prevotella*, *Bacteroides*, and members of the class *Clostridia* with diminished levels of *Lactobacillus* in contrast to premenopausal breast cancer patients. As stated before, the group emphasized that the observed microbial diversity depended on the status of menopause, age, and BMI but not so much on the demography of patients.

Similar to urine microbiome, fecal microbiome analysis also confers the benefit of non-invasive assessment of microbial diversity. Importantly, this may also be the reason why many of the gut microbial contributions to breast cancer are established by investigating the microbial composition of feces. Numerous studies point towards alterations in fecal microbiota in breast cancer patients and add weight to the notion of its scrutiny in breast cancer. Incidentally, analysis of fecal samples from postmenopausal healthy females demonstrated a greater number of *Clostridia* class and decreased abundance of *Bacteroides* as compared to the control group. Notably, these alterations were seen in females with a higher ratio of estrogen metabolite to parent estrogen. Thus, emphasizing the importance of this ratio in dictating diversity of the fecal microbiome [30]. In a separate study, assessment of urine and fecal samples of postmenopausal breast cancer cases pointed towards differences in IgA-coated and non-coated fecal microbiome independent of the estrogen levels. The alpha diversity was also found to be low in these patients. Moreover, post-menopausal breast cancer patients displayed reduced count of *HPA0247*, *Salmonella enterica*, *Fusobacterium nucleatum*, *Eubacterium eligens*, and *Roseburia inulinivorans*. Inversely, the number of species related to *Acinetobacter radioresistens*, *Actinomyces, Citrobacter koseri*, *Escherichia coli*, *Enterococcus gallinarum*, *Erwinia amylovora*, and *Shewanella putrefaciens* was observed to be more in these patients [31]. Examination of BaiH ORFs from different bacterial species in postmenopausal breast cancer patients revealed marked differences in the levels of several bacteria. For instance, the ORF for the gene from *Bacteroides thetaiotaomicron* and *Pseudomonas putida* was identified to be more abundant than BaiH ORF belonging to *Clostridium sordelli*, *Pseudomonas putida,* and *Staphylococcus aureus* [78]. Additionally, qPCR analysis of CadA and LdcC genes in the fecal samples from postmenopausal breast cancer females revealed a reduced prevalence of CadA in *Escherichia coli* and LdcC DNA in *Escherichia coli*, *Enterobacter cloacae* and *Hafniaalvei* [79]. The changes in the fecal microbial diversity are also identified to correlate with different grades of breast carcinoma. For example, grade III breast cancer patients were seen to have more of *Blautia* sp. in contrast to grade I cases. Moreover, *F. prausnitzii, Firmicutes, Blautia*,and *Egerthella* also displayed altered abundance in breast cancer *vs* healthy samples [3]. Fecal microbial diversity was also seen to be modified by the BMI status of breast cancer patients. The abundance of *Firmicutes*, *Faecalibacterium prausnitzii*, *Blautia* sp., and *Eggerthella lenta* was detected to be on the higher side in overweight breast cancer patients [29]. In a similar context, body fat was found to be a determinant of *Akkermansia muciniphila* (AM) abundance in breast cancer patients. This, in turn, correlated with altered frequency of *Prevotella, Clostridium, Lactobacillus, Campylobacter, and Helicobacter* [80]. Such exciting observations were in agreement with established literature that depicts obesity as an important player in breast tumor development. These studies substantiate our hypothesis that the axis between the host microbiome and the host system plays a vital role in the occurrence of breast cancer.

A plethora of analyses on periodontal diseases have suggested that it may have a positive correlation with an increased incidence of breast cancer development in postmenopausal women [81–83]. In 2008, Jensen and colleagues discovered elevated levels of cariogenic oral microbes such as *S. mutans* and *Lactobacillus* in breast cancer patients who were undergoing chemotherapy. These patients also exhibited more plaque formation having higher inflammation in comparison to the control group which was not receiving chemotherapy [84]. In another pilot study, 16S rRNA analysis of buccal cavity samples collected from breast cancer patients post-chemotherapy indicated significant enhancement in the microbial diversity [85]. On the contrary, a separate study found no considerable difference in the microbial taxa of oral rinse samples in breast cancer and non-cancer patients [77]. The lack of consistency in data in this area necessitates deeper interrogation and clinical validation of more diverse cohorts to come to a unified conclusion.

Investigations on the vaginal microbiome in pre- and postmenopausal women demonstrate greater diversity of bacteria in the vaginal tract in postmenopausal women along with lower levels of *Lactobacilli* [86]. Treatment regimens such as chemotherapy and estrogen deprivation therapy may cause a drop and even lack of *Lactobacilli* sp. in the vaginal microbiome in breast cancer patients. The levels of this species may be restored either through its supplementation [87,88] or by the administration of estrogen exogenously [89,90]. On similar lines, a pilot study by Julian Marschalek *et al.* reports that by orally administering *Lactobacilli* sp. to postmenopausal women undergoing chemotherapy, the Nugent score may be improved for these patients [91]. These studies are encouraging but one cannot undermine the fact that extensive investigation is still warranted using larger cohorts for determining the wider net of taxa fluctuations. Such identification may prove invaluable for the diagnosis and prognosis of breast cancer patients.

4. Comprehensive analysis on the role of multifaceted human microbiome in breast cancer : evidences from studies

The disclosure of the human microbiome project and the emergence of advanced molecular techniques have uncovered a wealth of information to the scientific community. The last decade has seen unprecedented research that has successfully established the role of this "forgotten organ" with diabetes, obesity, and neurodegenerative diseases. Presently, its functional relationship with organ-specific cancers is being unearthed, more importantly as a cancer-promoting factor or as a novel anticancer therapeutic. Studies pertaining to propose the probable functional pathway and related mechanism are underway to delineate the impact of the human microbiome on breast cancer.

4.1. Role in estrogen metabolism: disrupting the homeostasis

As discussed before, apart from traditional risk factors, the endogenous burden of free circulating estrogen is a causative risk factor for breast tumorigenesis, particularly in postmenopausal women [92–94]. Circulating estrogens undergo phase II conjugation reactions in the liver *via* an enzyme, UDP-glucuronosyltransferase that attaches either a glucuronic acid residue and/or sulfate. Once in the intestine, conjugated estrogens are fated for excretion [44]. The "estrobolome" includes a collection of enteric bacterial products involved in estrogen metabolism and reabsorption [95]. Under physiological conditions, a healthy estrobolome allows optimal conjugation of the estrogen within the hepatocytes, maintaining standard levels of estrogen within the body. Alteration in the estrobolome results in the secretion of higher levels of BGUS. As a result, this leads to enhanced removal of the glucuronic moiety from conjugated estrogens, thereby promoting its reabsorption into the enterohepatic circulation. This exerts a cumulative effect and promotes aberrant levels of free estrogen in circulation over time, which substantially increases the risk of developing estrogen receptor (*ER*) positive breast cancer (Fig. 2) [44]. Numerous evidences attest to this notion. The notable mechanism by which the microbiome influences the levels of circulating estrogen majorly involves the de-conjugation process. Barbara *et al.* investigated the role of gut microbiome diversity and composition in regulating the urinary estrogens and estrogen metabolites in healthy women. The ratio of estrogen metabolites to parent estrogen directly correlated with the abundance of microflora, specifically, the order *Clostridiales* and family *Ruminococcaceae,* whereas the genus *Bacteroides* was negatively correlated [30]. In a similar case-control study involving men and postmenstrual women, the richness and diversity of gut microbiome was positively correlated with the levels of urinary estrogens and inversely with the levels of fecal estrogens. Alpha-diversity influenced estrogen metabolism, including the taxa *Clostridia* and members of the family *Ruminocaccaceae* [96].

The estrobolome comprises commensal bacterial communities that harbor the BGUS genes which have been characterized lately [97,98]. Interestingly, BGUS is also detected to be prevalent in the nipple aspirate fluid of breast cancer survivors [75]. Microbial BGUS activity plays a quintessential role in governing the dynamics of the estrobolome [99]. Many bacteria of the human gut have been established to encode this enzyme including the phyla *Bacteroidetes* and *Firmicutes* [100]. Functional activity of fecal BGUS has been directly associated with urinary estrogen and negatively correlated with total fecal estrogens in women [41]. Similar findings have been reported in rodent models [101]. Additionally, the enzyme's activity is known to be dependent on diet and bacterial context [44]. It has been shown that healthy individuals consuming high fat and/or protein-rich diet exhibit increased fecal BGUS activity [102,103]. On the contrary, a high fiber diet significantly reduced BGUS activity [104]. Diets rich in fat and protein stimulate commensal bacteria to metabolize bile acids into deoxycholic and lithocholic acids, which favor the growth of *Fusobacterium nucleatum, Proteobacteria, Escherichia coli, Enterobacter, Citrobacter,* and *Klebsiella.* This effect has shown to be detrimental to members of *Firmicutes* and *Bacteroidetes* and the latter outnumbers them. This induces a state of intestinal dysbiosis [105]. As a consequence, *Proteobacteria* dominate and produce BGUS, thereby de-conjugating estrogens and increasing the estrogen burden in the body [106]. Such *β-Glucuronidase* producers can, therefore, regulate the bioavailability of estrogen and impact the development of breast cancer. Additionally, the consumption of antibiotics stimulates gut dysbiosis, which again actively contributes to an increased risk of breast cancer. However, this still remains controversial [99]. *Bacillus cereus*, found to be elevated in breast cancer tissue, has been shown to metabolize progesterone to 5-α-320-dione. This, in turn, is found in increased levels in breast tumors and stimulates breast cancer cell proliferation *in vitro* [62,107]. These evidences highlight the

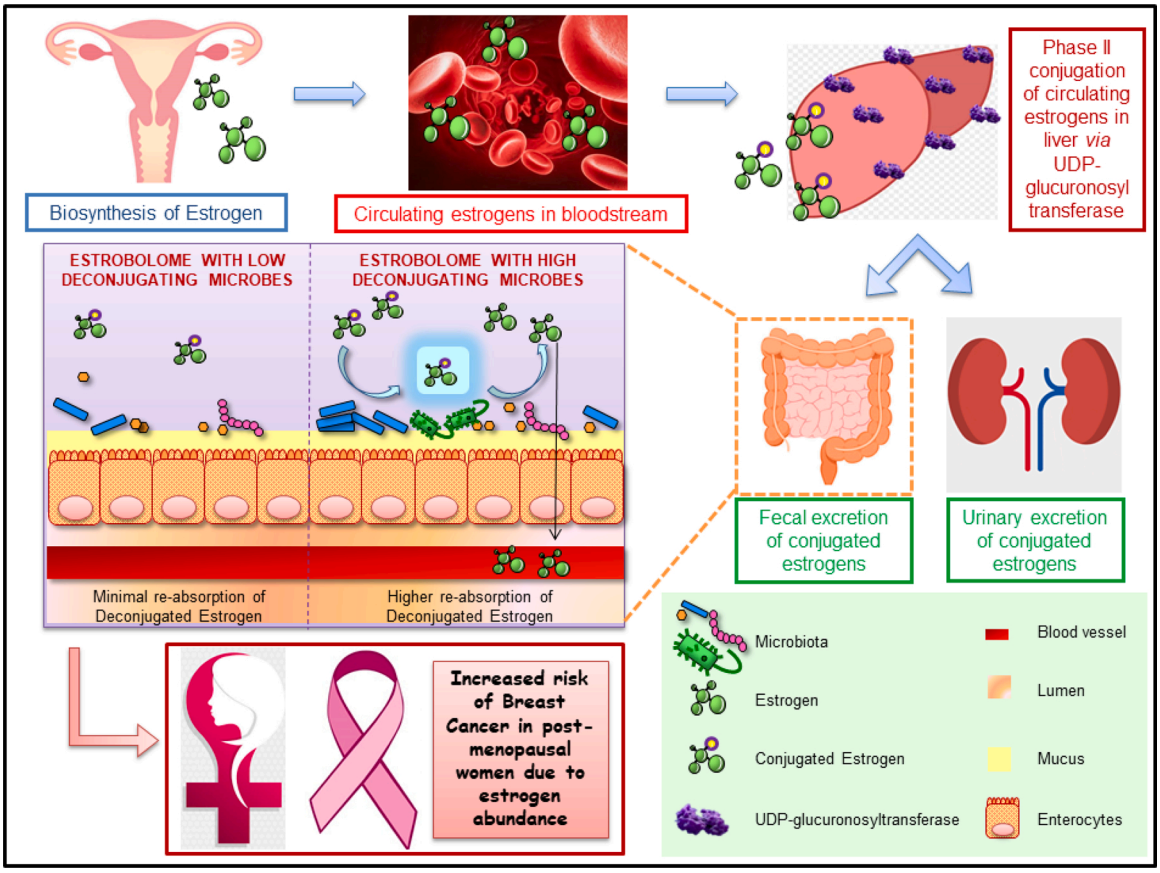

Fig. 2. The estrobolome plays a pivotal role in governing the risk of breast cancer development.

underestimated significance of the gut microbiome in estrogen metabolism which directly implies the risk of developing breast cancer in postmenopausal women.

4.2. Role in genomic stability and DNA damage: oncogenic drivers

Genomic instability is an underlying hallmark of cancer that generates genomic diversity by random mutations and chromosomal rearrangements [108]. Studies in the past have provided confirmatory evidence of the role of specific commensal and pathogenic microorganisms in fuelling DNA damage, thereby introducing genomic instability in mammalian cells. Members of *Enterobacteriaceae*, along with Proteobacteria, secrete microbial toxins that induce DNA double-strand breaks (DSBs) in the host genome. On these lines, a commensal strain of *E. coli* (phylogenetic group B2) was reported to cause DNA damage in CHO and HCT-116 cells by producing a polyketide-peptide genotoxin, Colibactin [109]. This resulted in gross nuclear defects such as aneuploidy, the formation of micronuclei, anaphase bridges, and ring chromosomes that persisted in dividing cells up to 21 days. Anchorage-independent colony formation was also exhibited by infected cells. Later, a similar study demonstrated the carcinogenic potential of the bacterium *Helicobacter pylori*, the causal agent of gastric ulcers. It was shown to introduce DSBs in DNA, contributing to chromosomal aberrations both *in vitro* and *in vivo* [110]. *E. coli* has been confirmed as a predominant bacterial species in the healthy and cancerous breast tissue by multiple studies [62,108]. Recently, Urbaniak *et al.* isolated three *E. coli* (phylotype B2) and one *Staphylococcus epidermidis* strains from normal tissue of breast cancer patients and examined their ability to introduce DNA DSBs in HeLa cell lines. Interestingly, these isolates were able to introduce DNA damage *in vitro*. However, clinical isolates of *Bacillus, Micrococcus,* and *Propionibacterium* did not introduce such defects [62]. On the contrary, *Lactobacillus* and *Streptococcus thermophilus* have been shown to counter DNA damage in HT-29 colorectal cells. They confer such protection possibly by the production of antioxidants that scavenge and nullify ROS like peroxide and superoxide radicals [111]. This clearly underscores the dual role of the host microbiota in cancer initiation and reveals that the microbiome is a key player to determine whether it will exert oncogenic or tumor-suppressive functions within the tissues.

4.3. Role of microbiota in host immune system: a sentinel at our disposal

The mucosal immune system is highly specialized and complex and it undergoes critical changes soon after bacterial colonization of the intestine [112]. The gut microbiota confers numerous benefits to its host, particularly in regulating the immune homeostasis [113] and by governing the maturation and functioning of the Gut-Associated Lymphoid Tissue (GALT), including Peyer's patch, lymphoid follicles, and the mesenteric lymph nodes [114,115]. The gut microbiome aids in the production of interferon (IFN)-γ and granzyme by Helper ($CD4^+$) and Cytotoxic ($CD8^+$) T cells. This promotes the recruitment of macrophages, activation and activation and maintenance of Natural Killer (NK) cells, lymphoid cells, B cells, and both cytotoxic as well as helper T lymphocytes [116]. These cells, in turn, produce a diverse array of immune-signaling molecules like interferons, cytokines, defensins, lysozyme, AntiMicrobial Peptides (AMPs), granulysin, and various antibodies which together regulate the microbial ecology and immune surveillance [117,118]. T-cell mediated host adaptive immunity is critical for recognition and clearance of tumor cells and immune checkpoints play an indispensable role in the activation of these lymphocytes. The composition of the gut microbiota can significantly influence the outcome of therapeutic inhibitors used against immune checkpoints, thus implying the key role of the host microflorae in immune regulation.

Persistent and dysregulated inflammation has been linked with an increased risk of breast cancer [119]. A cohort study of 10 years involving 1300 breast cancer patients established a relationship between patient survival and the number of $CD8^+$ effector T cells infiltrating breast cancer tumors. It was shown that patients whose breast tumors house more than 24 $CD8^+$ cells per field of the tumor had significantly increased survival to breast cancer (75 % *versus* 45 %) as opposed to those having 5 or fewer $CD8^+$ cells [120]. In compliance with these results, a separate study with over 170 triple-negative breast cancer patients spanning 8 years confirmed that patients with a greater (more than 36 lymphocytes/mm^2) number of lymphocytes infiltrating their tumors were accompanied with a three-fold higher rate of relapse-free survival than those infiltrated with fewer (20 cells/mm^2) lymphocytes [121]. Cytotoxic T lymphocytes or $CD8^+$ T cells are known to be the most potent immune cells in eliminating tumors [122]. Maturation of these lymphocytes is found to occur after contact with *Sphingomonas* sp. [123]. Interestingly, the proportion of *Sphingomonas* undergoes a significant reduction during inflammation, thereby preventing the conventional development of $CD8^+$ antitumor cytotoxic T cells [124,125].

Effector Lymphocyte count is associated with poor cancer-related outcomes upon diagnosis. Studies have also related the ratio of neutrophils to lymphocytes at the time of diagnosis to predict long-term cancer outcomes. A study on early-stage breast cancer revealed that a neutrophil-to-lymphocyte ratio of more than 2.5 was linked with a 4-fold risk of breast cancer relapse in about 10 years, as opposed to patients exhibiting a lower ratio [126]. An analogous study on 316 breast cancer patients additionally divulged that neutrophil-to-lymphocyte ratio of more than 3.3 led to a 44 % higher death risk within 5 years of diagnosis than those exhibiting a ratio of less than 1.8 [127]. It is quite evident that neutrophils and lymphocytes are influenced by host microbiota and inflammation. Recently, Jessica *et al.* demonstrated that neutrophil-associated immune responses to gut microbes can remarkably impact carcinogenesis in tissues like mammary glands [128]. Interestingly, systemic interplay has been reported between gut microbes, Interleukin-6 (IL-6), and neutrophils in breast cancer patients. It was demonstrated that Toll-like Receptor - 5 (TLR-5) dependent malignant progression was accelerated by commensal bacteria in IL-6 responsive tumors [129]. Moreover, a potential inflammatory biomarker Immunoglobulin A (IgA) has been linked with breast cancer [31]. Thus, there are clear indications for the involvement of the human microbiome in regulating chronic inflammation and the host immune system during breast carcinogenesis. A critical mechanism of triggering oncogenesis may be intestinal microbiome induced damage of the immune guardians, the lymphocytes. Previously it was noted that protein and fat-rich diets induce intestinal dysbiosis and favours the growth of *F. nucleatum* [105]. Surprisingly, this bacterium has been shown to kill maturing lymphocytes by interacting with Multifenestrated epithelial cells (M cells) in Peyer's patch, thereby reducing the number of circulating systemic lymphocytes [130,131]. Altogether, these findings point towards the crucial role of the microbial community in regulating host immunity. While some microbes expedite the maturation and activation of the immune surveillance, others act in far more dangerous ways and annihilate the immune cells. More detailed investigations are in need to detect and classify the microbial members associated with the host immune system and elucidate their exact mechanisms. This can lead to a successful, effective, and targeted response in patients subjected to immunotherapies.

5. Orchestrating the host microbiome as breast cancer therapeutics: still a long way to go

"*Let food be thy medicine and medicine be thy food.*"- *Hippocrates*

The relationship between breast cancer and the human microbiome has opened up new horizons for preventive and therapeutic strategies. Such innovative regimes can be employed for lowering the incidence of breast cancer globally. Microbial- mediated cancer therapy is a novel

mode of treatment that is being exploited to address the primary resistance mechanisms delimiting the prevalent therapies. This approach may prove to be exceptionally effective, particularly for breast tumor patients. Nobel laureate Elie Metchnikoff postulated over a century ago that health could be improved and life prolonged by consuming the host-friendly bacteria present in sour milk and yogurt. His theory flourished for a time and re-emerged again in the late 20th century, establishing a completely new vision of perceiving microbes as probiotics. The World Health Organization (WHO) defines probiotics as live microorganisms that confer a health benefit on the host when administered in adequate amounts in food or as a dietary supplement [132]. Probiotics have been shown to modulate and boost immunity and even display preventive and therapeutic potential against different diseases including various cancers [133–135]. Furthermore, dietary prebiotics which include selectively fermented ingredients upon consumption is utilized by the resident microbiota of the host. This results in altered composition/functional implications of the host microflora that ultimately benefit the host. Therefore, focusing on the therapeutic application of pro- and prebiotics in breast cancer might be a rational approach. This section reviews the potential role of such microbial supplements in breast cancer prevention and treatment in various *in-vitro, in vivo*, and human studies.

5.1. Evidences from cell-culture based studies

Preliminary investigations regarding the possible efficacy of probiotics on breast cancer treatment were performed on various breast cancer cell lines. The effect of probiotics on the oncogenic properties of breast cancer cell lines has been assessed by cytotoxicity assays and analysis of proliferative, inflammatory, and apoptotic biomarkers. An example is Kefir extract which is a probiotic beverage prepared from fermentation of milk with kefir grains composed of bacterial and yeast consortium. A study performed in 2006 found exposure to kefir extract decreased the growth of MCF-7 breast cancer line in a dose-dependent manner, while no such effect was exerted on normal mammary epithelial cells [136]. Similarly, Zamberi *et al.* showed that kefir water exerts cytotoxicity against 4T1 breast cancer cells, reduces cell migration and invasion, and also induces apoptosis [137]. A probiotic strain isolated from the vagina, *Lactobacillus plantarum* was also found to exhibit a dose- and time-dependent cytotoxicity against MCF-7 cells, but not normal cells (HUVEC). Moreover, it also led to apoptosis in the HeLa cell line [138,139]. The same group also demonstrated another vaginal probiotic strain, *Enterococcus faecalis* to inhibit proliferation of MCF-7 cells up to 41.27 %, without any significant differences in the growth of HUVEC cells [139]. Similarly, live, heat-killed and cytoplasmic formulations of *E. faecalis* and *Staphylococcus hominis* isolated from human breast milk were evaluated for their effect on MCF-7 cells. All three formulations of the bacteria led to a significant decrease in the proliferation of MCF-7 cells in a concentration- and time-dependent manner. Interestingly, morphological signs of apoptosis including membrane blebbing and cell shrinkage were reported in 34.6 % of apoptosing MCF-7 cells, whereas MCF-10A (breast epithelial cells) did not show significant differences following treatment with the formulations [140]. In a study by Lee *et al.*, *Lactococcus lactis KC24* isolated from Kimchi, led to a remarkable 91.89 % reduction of MCF-7 cell growth [141].

Bacterial metabolites have also proven to exhibit promising anticancer activities. MDA-MB-231 breast cancer cells were found to undergo apoptosis by down-regulation of the NF-kB pathways as a consequence of conjugated linoleic acid produced by *L. plantarum* [142]. A surfactin-producing probiotic strain of *Bacillus subtilis CSY191* has been shown to dose-dependently hinder the growth of MCF-7 cells with IC_{50} of 9.65 μM at 24 h [143]. Additionally, Azurin, a redox protein of *Pseudomonas aeruginosa* attenuated MCF-7 proliferation and induced apoptosis by increasing the intracellular levels of p53 and Bax [144]. Bacteriocins produced by bacteria as an antagonistic factor to eliminate competition by distinctly-related microbes have also been tested for antitumoral activity. Nisin, produced by *L. lactis*, has been recently certified GRAS status and approved by WHO. Nisin exerts considerable cytotoxic effects on MCF-7 cells [145]. Similarly, Colicin E secreted by *E. coli* has been shown to arrest MCF-7 cells in the G1 phase and increase apoptosis by 58 % [146]. *Streptococcus bovis* secretes the lantibiotic Bovicin HC5, which demonstrated noticeable antitumor activity in MCF-7 breast cancer cells [42]. Besides, Silva *et al.* have indicated that a *Bifidobacterium* sp. synergistically with *L. acidophilus* converts Laphacol, an anticancer drug, into a more cytotoxic compound (naphthoquinone). This formed compound displays greater toxicity against SKBR-3 breast cancer cells than Laphacol itself, without affecting normal fibroblast cells [147]. Bacterial polysaccharides have also been tested for the delivery of drugs to cancer tissues. The sulfated polysaccharide of *Halomonas maura,* Mauran, in combination with chitosan nanoparticle formulation has been used for sustained and prolonged release of 5-fluorouracil, leading to more effective killing of breast adenocarcinoma cells [148]. Some bacterially generated peptides have additionally displayed potent anticancer activities against various breast cancer cell lines, such as MDA-MB231, MCF-7, and others. A few examples include ohmyungsamycins A and B, Pep27anal2, Entap, and proximicins from *Streptomyces* sp., *Streptococcus pneumoniae, Enterococcus* sp., and *Verrucosispora* sp., respectively [149]. Apart from this, bacterial toxins, like, endotoxins A and B, hyaluronidases, and diphtheria toxins secreted from *Pseudomonas aeruginosa, Streptococcus pyogenes* and *Corynebacterium diptheria* reportedly alter breast cancer cell proliferation, differentiation, and apoptosis [149]. These pieces of evidence, put together in Table 1, point in a positive direction for the exploitation of probiotics in breast cancer therapy. However, these warrant further validation in animal models.

5.2. Evidences from experimental animal models

Animal studies are considered as “gold standards” for studying the effects of an agent on the physiology, metabolism, or responses of an organism. They provide the best insights since various biological entities are tested on a whole, living organism under ideal physiological conditions. Researches establishing the relationship of probiotics with breast cancer treatment are endless and majorly performed on gnotobiotic rodent models.

Given the fact that kefir water induced apoptosis in 4T1 breast cancer cells *in vitro*, Zamberi *et al.* tested the impact of kefir water on breast tumor-bearing female BALB/c mice. Interestingly, orally-administered kefir water induced multiple significant changes including reduced tumor size and weight, lung metastasis, and pro-inflammatory cytokines parallel to increased DNA fragmentation/apoptosis of tumor sections and number of $CD4^+$ and $CD8^+$ T lymphocytes [137]. Two distinct studies involving oral administration of *L. acidophilus* in similar breast tumor models of mice showed enhanced survival with a marked decrease in IL-4 and an increase in INF-γ levels. This led to a stronger NK cell-mediated T helper 1 (Th1) immune response [150,151]. Similarly, the immunomodulatory effect of *L. acidophilus ATCC 4356* on BALB/c mice with breast tumor revealed a significantly slower tumor growth rate, an increase in the level of IL-12 and a decrease in tumor growth factor-β (TGF-β) level, suggesting a heightened Th1-mediated delayed-type hypersensitivity response [152]. Likewise, Maroof et al. concluded that *L. acidophilus ATCC 4356* reduced tumor growth rate and angiogenic potential corresponding to a decrease in IL-4 levels and an increase in IFN-γ levels in 4T1 breast cancer cells -bearing murine models [153]. An oral dose of *L. casei* was shown to significantly slow down tumor growth and increase the survival of breast cancer mice models. Moreover, the levels of IL-12 and INF-γ were elevated leading to enhanced NK cell activity [154]. In two distinct studies by Aragon *et al.*, breast tumor-possessing BALB/c mice fed with *L. casei CRL 431* fermented milk exhibited lowered rates of tumor growth, metastasis, and angiogenesis with higher rates of survival. Cytokine profile revealed decreased levels of IL-6 and increased monocyte chemoattractant

Table 1
In vitro research using breast cancer cell lines has provided efficient insights into the role of microbiota in tumor suppression.

In vitro studies conducted with Probiotics against Breast Cancer					
Serial No.	Probiotic strain/ Neutraceutical	Cell Line tested	Effects observed	Proposed Mechanism	Reference
1	Kefir Water	MCF-7	Cytotoxic against cell line	Not indicated (Bioactive compounds promote Apoptosis)	[136]
2	Kefir Water	4T1	Cytotoxic to cells, reduced cell invasion	Cancer cell apoptosis	[137]
3	*plantarum 5BL*	MCF-7	Reduced cell proliferation	Induction of apoptosis	[138]
4	*faecalis*	MCF-7	Inhibition of cell proliferation	Induction of cancer cell apoptosis	[139]
5	Live, Heat-killed, Cytoplasmic fractions of *E. faecalis* and *S. hominis*	MCF-7	Reduced cell proliferation	Induction of apoptosis	[140]
6	*Lactococcus lactis KC24*	MCF-7	Reduced cell proliferation	Cancer cell apoptosis	[141]
7	Conjugated Linoleic acid from *L. plantarum*	MDA-MB-231	Reduced cell proliferation	Induction of apoptosis by down-regulation of NF-kB pathway	[142]
8	*Bacillus subtilis CSY191*	MCF-7	Reduced cell proliferation	Surfactin production induces cancer cell apoptosis	[143]
9	Azurin from *Pseudomonas aeruginosa*	MCF-7	Cancer cell apoptosis	Increase in intracellular levels of p53 and Bax	[144]
10	Nisin	MCF-7	Reduced cell proliferation	Cancer cell lysis	[145]
11	Colicin E	MCF-7	Reduced cell proliferation and G1 cell-cycle arrest	Cancer cell apoptosis	[146]
12	*Bifidobacterium* sp. & *L. acidophilus*	SKBR-3	Cytotoxic against cell line	Synergistic conversion of Lapachol to antitumor Naphthoquinone	[147]

protein -1 (MCP-1), thereby maintaining a heightened antitumor response associated with $CD8^+$ T cells [155]. In the follow-up study, they analyzed the cytokine profile and a discovered reduction in the levels of IL-6, IL-10, and Tumor Necrosis Factor-α (TNF-α) corresponding to less tumor growth and angiogenesis [156]. An elaborate study by LeBlanc *et al.* evaluated the effects of milk fermented by two strains of *L. helveticus R389,* and protease-deficient *L89* on tumor-harboring BALB/c mice over a 7-day clinical regimen. They detected a reduction in tumor size and in the levels of IL-6 and $Bcl2^+$ cells with both the strains. However, mice fed with *L89* strain exhibited IL-10 upsurge, which boosted the levels of INF-γ and TNF-α in mammary glands resulting in apoptosis. On the other hand, *R389* fed mice exhibited a remarkable increase in IgA^+ and $CD4^+$ cells, thereby leading to an increase in the ratio of $CD4^+/CD8^+$ cells [157]. In a similar study, oral administration of milk fermented by *L. helveticus* lowered the secretion of IL-6, IL-10, IFN-γ, and TNF-α and inhibited tumor growth in 4T1 breast cancer cells-bearing female BALB/c mice [158]. Diet modifications have also been associated with breast cancer in mice. A study was conducted on two groups of mice, both of which were engineered to develop human breast tumors using two separate diets. One group was fed on a Western diet and the other FVB strain erbB2 (*HER2*) mutant mice was fed on the standard animal facility mouse chow. Both the groups were routinely administered *L. reuteri.* It was found that *L. reuteri* diminished mammary neoplasia at early stages in both the groups *via* microbially-triggered $CD4^+$ $CD25^+$ lymphocytes [159].

Combinational therapies for breast cancer clearance have also been tested in mice models. Yazdi *et al.* orally-administered a co-formulation of *L. plantarum* enriched with selenium nanoparticles (SeNP) to delineate the effects on 4T1 breast cancer cells-bearing female BALB/c mice. Astonishingly, these test mice displayed boosted levels of splenic levels of pro-inflammatory cytokines IL-2, INF-γ, and TNF-α which correlated with a significant increase in NK cell activity. Additionally, they also showed decreased tumor volume and increased the survival rate [160]. Interestingly, the Nobel prize-winning cancer intervention strategies by negative immune regulation have also been tested with probiotic co-administration. Administration of *Bifidobacterium* along with Programmed cell Death protein 1 ligand L1 (PD-L1) specific antibody abolished tumor outgrowth by augmenting dendritic cell function resulting in enhanced $CD8^+$ T cell priming in mice model [15]. Likewise, the antitumoral efficacy of Ipilimumab, a Cytotoxic T Lymphocyte Antigen 4 (CTLA4)-specific monoclonal antibody was found to be dependent on *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* [161].

These studies report about the exciting interaction of probiotic microorganisms with the human immune system, stimulating it to mount an enhanced antitumoral response, which have been depicted in Fig. 3. The normal breast tissue houses a healthy consortium of microbes that maintain optimal immune surveillance. On the other hand dysbiosis in this microbiome is associated with an anomaly in the immune system and ultimately increases the chances of breast tumor development. We have discussed how the microbiome influences the activation and regulation of the host immune system in our earlier section. Together with the animal-based observations, we reason that introduction of beneficial microbes such as *Bifidobacterium* sp. and *Lactobacillus* sp. may replenish and re activate the immune system in the cancerous tissue, for destroying tumor cells and promoting their clearance. At the same time, there are several menacing bacteria like *E. coli* and viruses like HPV that act as oncogenic stimulators by evading and destroying the immune cells. Table 2 summarizes the important *in vivo* studies carried out in the mice models for a deeper understanding of the association between the host microbiota and breast carcinoma.

The blockade of tumors suggests that the human microbiome can be exploited or repurposed as a novel and precise medicine strategy for anticancer therapeutics. However, more studies are essential to understand how these microbes interact with the immune repertoire to alter the tumor environment. Moreover, how such interactions can be revolutionized in predicting the treatment response in different breast cancer patients is a critical aspect of interrogation.

5.3. Findings from clinical trials/human studies: lessons for the future

A promising role of probiotics in inhibition of tumor growth, modulation of immune system, and induction of apoptosis has been unravelled in *in vitro* and *in vivo* experimental setups. Nonetheless their use as a potential breast cancer therapy has not been established in clinical trials. Correlative studies have shown that consumption of yogurt containing *Streptococcus thermophilus* and *L. delbrueckii* significantly decreased the risk of colon cancer [162] and relapse of superficial bladder cancer [163]. In a case-controlled study in Japan, 306 breast cancer patients and 662 healthy female subjects aged between 40–55 years were probed about their diet, lifestyle, and other breast cancer risks. Their study concluded that regular consumption of *L. casei* Shirota strain and soy isoflavones since adolescence correlated with decreased incidence of breast cancer in Japanese women [164]. In a separate study, it was shown that infants fed with prebiotic supplemented milk demonstrated similar levels of *Bifidobacterium* and *Lactobacillus* spp. as was found in infants who had normal breastfeeding. These infants

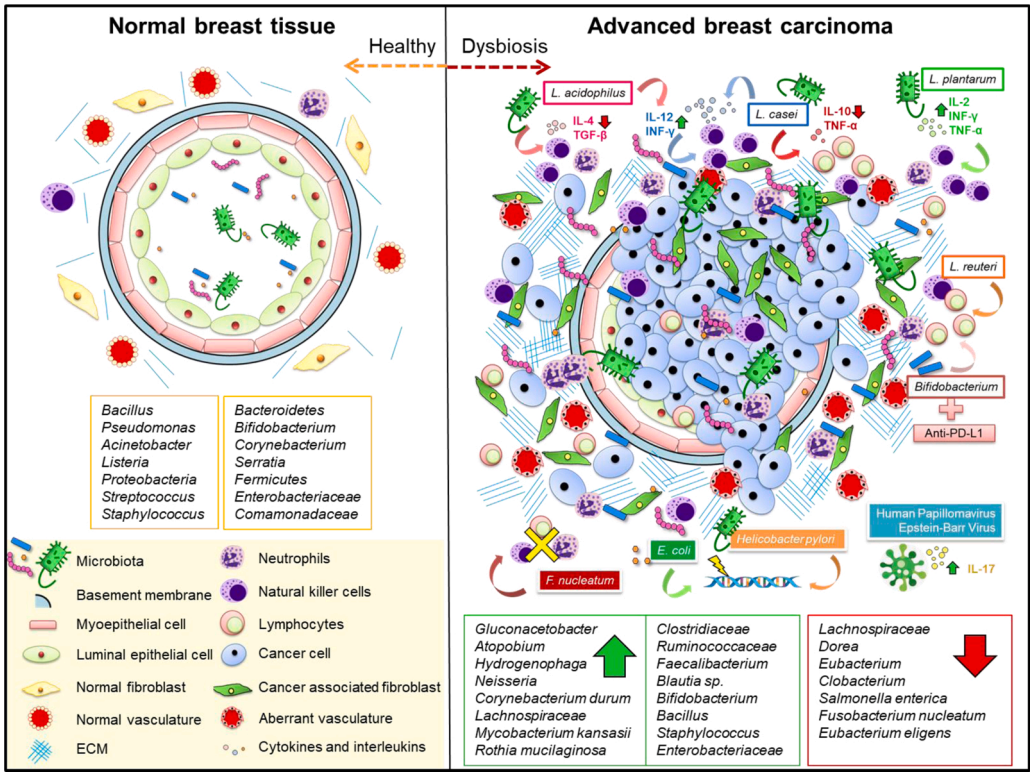

Fig. 3. Comparative insight into the interplay between the human breast microbiota and host immune system under physiological and pathogenic conditions.

Table 2
In vivo experiments conducted on mice models harbouring breast tumor has elaborately validated the immune modulation by microbiota and its consequences in breast cancer development.

Animal Studies conducted with probiotics for treatment of breast cancer					
Serial No.	Probiotic strain/ Neutraceutical	Animal model employed	Effects observed	Mechanism proposed	Reference
1	Kefir Water	4T1-breast cancer bearing BALB/c mice	Reduced tumor size, pro-inflammatory and pro-carcinogenic markers	Increase in $CD4^+$ and $CD8^+$ T lymphocytes & apoptosis	[137]
2	*L. acidophilus ATCC 4356*	BALB/c mice bearing breast cancer	Heightened antitumoral response and increased survival rate	Increase in IFN-γ and activation of NK cells	[150]
3	*L. acidophilus ATCC 4356*	BALB/c mice bearing breast cancer	Increased immune response and survival rate	Th1 cytokine bias with increase in IFN-γ and decrease in Th2 cytokines	[151]
4	*L. acidophilus ATCC 4356*	BALB/c mice with transplanted breast tumor	Reduced tumor growth rate	Increase in IL-12 leading to Th1-mediated delayed hypersenstivity response	[152]
5	*L. acidophilus*	4T1-breast cancer bearing BALB/c mice	Reduced tumor growth rate with increased lymphocyte proliferation	Th1 cytokine-mediated antitumoral immunity	[153]
6	*L. casei ATCC* 39392	BALB/c mice with transplanted breast tumor	Increased survival rate and reduced tumor growth rate	Increased levels of IL-12 and IFN-γ with heightened NK cell toxicity and delayed hypersensitivity response	[154]
7	Milk fermented with *L. casei CRL 431*	4T1-breast cancer bearing BALB/c mice	Reduced tumor growth, vascularity and angiogenesis	Decrease in IL-6 and increase in $CD4^+$ lymphocytes	[155]
8	Milk fermented with *L. casei CRL 431*	4T1-breast cancer bearing BALB/c mice	Delayed tumor growth and reduced angiogenesis	Decrease in IL-6 and TNF-α levels	[156]
9	Milk fermented with *L. Helveticus R389*	4T1-breast cancer bearing BALB/c mice	Reduced tumor growth	Cancer cell apoptosis by elevated levels of IL-10 and reduced IL-6	[157]
10	Milk fermented with *L. Helveticus R389*	4T1-breast cancer bearing BALB/c mice	Reduced or blocked tumor growth	Induction of apoptosis by increased levels of IL-4, IL-10 and diminished IL-6 levels	[158]
11	*L. Reuteri ATCC-PTA-6475*	FVB strain erbB2 (*HER2*) mutant mice	Inhibition of mammary neoplasma	Antitumoral activity conferred by microbially-stimulated $CD4^+$ $CD25^+$ lymphocytes	[159]
12	*L. plantarum* enriched with SeNP	4T1-breast cancer bearing BALB/c mice	Decreased tumor volume and increased survival rate	Elevated levels of pro-inflammatory cytokines IL-2, IFN-γ, TNF-α and increased NK cell activity	[160]

developed a more effective immune response against allergic episodes and infectious manifestations till up to 5 years of age [165]. However, infants given standard milk formula exhibited a poorer immune response to allergies due to the reduced population of such microflora. Such studies clearly denote the importance of breast milk microbiota in immune regulation against pathological conditions.

Currently, the clinicaltrial.gov web page lists four on-going clinical trials that are focussed on assessing the health benefits of probiotics in breast cancer patients. The first trial aims to study $CD8^+$ T lymphocyte numbers in breast cancer patients consuming probiotics thrice a day [1]. The second study is a randomized-controlled pilot study to investigate the role of physical exercise along with probiotic supplementation

(*L. rhamnosus, L. paracasei, L. acidophilus,* and *B. bifidum*) on gut microbial balance, gut immune system and the quality of life in 30 breast cancer survivors [2]. The third group is evaluating the impact of the gut microbiome in shaping the immune system to fight against breast cancer [3]. The fourth study is investigating whether the dominance of particular organisms of the microbiome is related to a complete pathological response in breast cancer patients undergoing neoadjuvant chemotherapy [4].

These studies can lay the foundation for deciphering the precise role of probiotics in breast cancer therapeutics. Therefore, to delineate and determine if probiotics can be used as a strategy for breast cancer intervention, large-scale animal studies, and clinical trials are the need of the hour.

6. Possible future directions and clinical therapies: A logical fallacy?

Breast cancer is a heterogeneous illness with multiple subtypes. Although recent scientific advances have paved an era of "personalized treatment", most breast tumor patients end up with surgery as the curative option. Unfortunately, this means a complete mastectomy for many women and an endless phase of antibiotic treatment. Antibiotics, though a necessity, end up killing even the good microbiota beneficial for the host system. The difficulty in treating breast carcinoma has emphasized the importance of research centralized around its multifactorial etiology, where there exists a strong link between genetic and environmental factors. The high rate of resistance of breast cancer patients to traditional therapies has weakened the efficacy of chemo-, radio- and immunotherapy, thereby imparting a negative impact on the quality of life of these women. Furthermore, the long-term side effects, the life- long duration of treatments, and the exorbitant financial impact associated with them add to the dismay of the patients.

We have reiterated the importance of the breast microbiota in the homeostasis of a dense network of physiological processes of the host, including priming of the immune surveillance. Disruption of this beautiful ecosystem inevitably makes the host susceptible to a long list of ailments, making the host a regular visitor at the clinic almost all her life. The microbiome of the host gut and breast play a vital role in disease progression and response to therapy. Also, there is a close link between the risk of breast tumor and dietary regimes, implying that a diet that keeps the microbiome in a healthy shape can regulate the outcome of treatment strategies. This has been exemplified by the positive correlation between estrogen metabolism and breast cancer initiation in *ER*-positive postmenopausal women. The possible role of a western diet and the consequent elevation of estrogen levels has also been closely linked to the microbial BGUS activity [99]. Therefore, a novel but unexplored intervention strategy can be the selective inhibition of microbial BGUS. This can effectively reduce the likelihood of reabsorption and recirculation of de-conjugated estrogens, thereby, suppressing *ER*-positive breast cancer risk.

There are multiple ongoing projects to decipher the interaction between the microbiome and the prevalent drugs being clinically used for breast cancer treatment. One study suggests that the microbiome can be modulated by the synthesis of anthracyclines by *Streptomyces* strains. Anthracyclines are bacteriostatic to different bacterial species [47]. Estrogen receptor modulators like Tamoxifen are toxic to *Klebsiella, Pseudomonas, Acinetobacter, Bacillus* among other beneficial strains of microbes. Taxanes have been shown to impede the immunomodulatory functions of the microbiota. Such results necessitate further research in this sphere to ensure the success of the therapeutic modules.

The role of microbes in breast cancer is like a double-edged sword. While certain bacteria have been shown to promote breast tumors, others have exhibited promising tumor-suppressive actions. The application of bacterial therapy has witnessed a considerable increase in the past decade. Undoubtedly, since the inclusion of bacterial therapy could mean the usage of lower doses of radiation and chemotherapeutics, minimal damage to healthy cells and prolonged better life of breast cancer individuals Bacteria promote tumor regression by prompting a strong activation of the immune system. Moreover, certain microbial communities selective target and colonize tumor tissues but not adjacent healthy cells. This can work in favor of the host by helping it to overcome the cytotoxicity connected with conventional chemo and radiotherapy. Recent advances in synthetic biology have made it possible for microbes to carry out more complex and co-ordinated functions as a "living therapeutic". The relative ease of manipulation in *E. coli, Salmonella typhimurium*, and *S. enterica* has made them the most extensively studied microbes in anticancer therapy. Clearly, it is vital to differentiate the notorious cancer-promoting bacteria from the beneficial classes of bacteria as the latter can be applied as a therapeutic option for the management of cancer. Poor tumor penetrance, low cancer cell toxicity, and inadequate targeting of the core of the tumor can be improved to a substantial extent using genetically modified bacterial therapy. The inability to control the bacterial infection is a concern associated with bacterial therapy. Nonetheless, genetic engineering technologies have aided in overcoming this challenge. Lack of well-designed clinical trials, innate bacterial toxicity, short half-life, and DNA instability are a few shortcomings of bacterial therapy. Applications of preclinical models have started to address the potential underlying outcomes of microbiota modulations. Overall, a combinatorial approach using conventional modules in adjunct with alternative regimes can provide the best solution for the treatment of breast cancer patients.

A fundamental mechanistic association between the occurrence of therapeutic resistance in the cancer cells and the evolution of the bacterial communities within those malignant tissues have been an enigma to the scientific societies for a long time. A number of microbes, including *Bacteroides fragilis, Fusobacterium nucleatum*, and *Enterococcus faecalis* have been demonstrated to cause aberrations to host cell adhesions and promote epithelial-to-mesenchymal transitions, which is intimately connected to metastasis [166]. Bacterially induced inflammatory response, production, and secretion of bacterial toxins, enzymes, and oncogenic peptides have been deemed as important contributors to oncogenesis. Secretion of quorum-sensing peptide PhrG by *Bacillus subtilis, Streptococcus mitis*, and *E. coli* have shown to stimulate competency (CSP), and extracellular death factors (EDF), respectively. These alterations accelerated tumor invasion, angiogenesis and finally promoted anomalous metastasis of breast cancer cells [167].

Dietary alterations, pro- and prebiotics, antibiotics, and FMT can influence the gut microbiota constituents and thereby impact breast cancer outcomes. Prominently, probiotics can help modulate and boost the host immune system in fighting the tumor. In this context, oral administration of probiotic formulations together with Fecal Microbiota Transplantation (FMT) from healthy individuals can prove worthy to fight against breast cancer. Currently, clinical trials deploying FMT in cancer subjects are in their infancy, but, the results from pre-clinical studies have prompted much enthusiasm and excitement among scientists. Understandably, because chemotherapy and radiotherapy are noted to adversely affect the GI tract microbiome. Chemotherapy-induced diarrhea (CID) causes toxicity and about 20–45 % of all chemotherapy patients experience loss of intestinal microflora. This contributes to the worsened quality of patient life, more frequent hospital visits, longer stay in the hospitals, and treatment interruption or discontinuation [168]. Therefore, supplementation of probiotic beverages/supplements in the course of chemotherapy can prove to be beneficial for recovery. Besides, this so-called "Microbe-Chemo therapy" can further help in supporting and escalating the antitumoral responses in breast cancer patients.

The composition and structure of the human microbiome may also be considered as a predictive biomarker for breast cancer prognosis and to follow treatment outcomes. Alterations and fluctuations of microbial communities in the gut microbiome can increase the risk of breast cancer [105]. Metagenomic analysis of breast tissue-specific microbiome prior to therapy and during treatment course can delineate the

community richness, species diversity, and the relative proportion of reputed "valuable" or "detrimental" microorganisms. These signatures may be suggestive of cancer severity, treatment outcome, and future medication. In the future, validating these associations would aid exploiting the human microbiome as another parameter that correlates with cancer treatment. The use of antibiotics is prevalent among cancer patients to reduce the risk of infection during immune-compromised situations. However, such antibiotics perturb the normal functions of the host microbiota, which orchestrates a vast array of immune responses within the host. Therefore, making use of antibiotics that specifically target a spectrum of the microbiome can help in regulating the gut microflora to reduce the risk of breast cancer. Unearthing the exact mechanisms behind the exacerbation of breast tumors in response to antibiotic-induced disturbances in the microbiota is an important field of research. Hence, it is vital to clearly comprehend the fate of the clinical use of antibiotics on the network/ pathways being regulated by the host-microbiome for better efficacy of antitumor response. In this aspect, the emergence of antibiotic-resistant pathogens is of serious concern in current times. Antibiotic resistance in breast cancer patients, for whom surgery is the single option, can prove fatal if not counteracted. Thus, how different antibiotic regiments may influence breast microbiota and breast cancer progression is an important question that needs to be answered. However, the microbiome diversity across populations in different environmental conditions, geographical locations, and consuming distinct diet makes it a complex and challenging task to identify the specific-microbial cancer signatures, rather transforming the avenue into personalized medicine.

Interestingly, epigenetic modifications and reprogramming of host cells by commensal bacteria have also been associated with chronic diseases including cancer [61,169]. Overproduction of bacterial metabolites/by-products can stimulate epigenetic reprogramming that affects cancer cell viability, migration, and induces apoptosis [77]. Epigenetic hallmarks of cancer include hypermethylation of gene-specific promoters, and global hypomethylation of repetitive sequences, thus activating or silencing important pathways in breast cancer development [30]. For instance, promoter-methylation of *ERα* gene has a characteristic feature of TNBC types associated with poor prognosis in women lacking a family history of breast cancer [78]. Another gene affected by epigenetic reprogramming is the *BRCA1*, which predisposes women to ovarian and breast cancers [30]. Thus, it would be compelling to connect these dots and evaluate whether and how commensal bacteria perform epigenetic remodeling in breast cancer.

There is a dearth of knowledge in the area of the relation between the breast microbiome and metastasis of the patient as well as how breast tumor resistance to conventional approaches is related to breast microbiome. More detailed research towards these directions can add to our existing knowledge about this important association and help in the design of more specific and efficient therapeutic regimes. Also, we should not think that what works for breast cancer can be extrapolated to other cancers as it would require separate clinical authentication.

7. Conclusion

The relationship between the human microbiome and breast cancer is yet to be explored in great detail. Breast cancer diagnosis is often equivalent to suffering, toxic therapies, and impending fatality. Efficacy of conventional treatment modalities are limited as a scalpel cannot target every last cancer tissue and the other methods do not distinguish between malignant and healthy cells. The human microbiome has been recently recognized to influence the status and pathogenesis of breast cancer. There is considerable debate about the efficacy and accuracy of formulations in off-the-shelf probiotics and the variation in their effects on the host microbiota. There remains a mammoth part of our evolving microbiome that is still not characterized. The identification of such consortium can extend our knowledge of the existing interactions and interplay between the immune system and the human microflora. Today we live in the "omics era" and the rise of the age of microbiome is upon us. With the emergence of advanced and high-throughput techniques, it is foreseeable that the role of the human microbiome in breast cancer shall be put to justice. To end these speculations, large-scale studies on appropriate animal models as well as clinical trials are needed to validate the bench-based investigations. More translational research is needed from both industry and academia to establish a relationship between the current understanding of probiotics and breast cancer treatment. This remains an unexplored peak, if scaled, might just help us win the war against cancer.

Funding source

This work was supported by research grants from Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), Department of Science and Technology (DU-DST), and University Grants Commission (under UGC-SAP). Financial support from ICMR, DBT and UGC for providing fellowship to JC, DN and YA, respectively is also appreciated.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

AN lab is supported by research grants from Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), Department of Science and Technology (DU-DST), and University Grants Commission (under UGC-SAP). Financial support from ICMR, DBT and UGC for providing fellowship to JC, DN and YA, respectively is also appreciated.

References

[1] B. Zhu, X. Wang, L. Li, Human gut microbiome: the second genome of human body, Protein Cell 1 (8) (2010) 718–725.
[2] J. Peterson, et al., The NIH human microbiome project, Genome Res. 19 (12) (2009) 2317–2323.
[3] M.F. Fernandez, et al., Breast Cancer and its relationship with the microbiota, Int. J. Environ. Res. Public Health 15 (8) (2018).
[4] F. Barbut, J.L. Meynard, Managing antibiotic associated diarrhoea, BMJ 324 (7350) (2002) 1345–1346.
[5] C.M. Velicer, et al., Antibiotic use in relation to the risk of breast cancer, JAMA 291 (7) (2004) 827–835.
[6] R.E. Ley, et al., Microbial ecology: human gut microbes associated with obesity, Nature 444 (7122) (2006) 1022–1023.
[7] J. Vaahtovuo, et al., Fecal microbiota in early rheumatoid arthritis, J. Rheumatol. 35 (8) (2008) 1500–1505.
[8] C. Manichanh, et al., The gut microbiota in IBD, Nat. Rev. Gastroenterol. Hepatol. 9 (10) (2012) 599–608.
[9] S.L. Russell, et al., Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma, EMBO Rep. 13 (5) (2012) 440–447.
[10] C. Xuan, et al., Microbial dysbiosis is associated with human breast cancer, PLoS One 9 (1) (2014) p. e83744.
[11] H.P. Browne, et al., Culturing of' unculturable' human microbiota reveals novel taxa and extensive sporulation, Nature 533 (7604) (2016) 543–546.
[12] W.B. Coley, The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases, Clin. Orthop. Relat. Res. 1991 (262) (1893) 3–11.
[13] H.W. Herr, A. Morales, History of bacillus Calmette-Guerin and bladder cancer: an immunotherapy success story, J. Urol. 179 (1) (2008) 53–56.
[14] M. Vetizou, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, Science 350 (6264) (2015) 1079–1084.
[15] A. Sivan, et al., Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy, Science 350 (6264) (2015) 1084–1089.
[16] N. Iida, et al., Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment, Science 342 (6161) (2013) 967–970.
[17] E. Zagato, et al., Endogenous murine microbiota member Faecalibaculum rodentium and its human homologue protect from intestinal tumour growth, Nat. Microbiol. 5 (3) (2020) 511–524.
[18] H. Armstrong, et al., The complex interplay between chronic inflammation, the microbiome, and Cancer: understanding disease progression and what we can do to prevent it, Cancers (Basel) 10 (3) (2018).

[19] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1) (2019) 7–34.
[20] J. Ferlay, et al., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (5) (2015) E359–86.
[21] F. Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (6) (2018) 394–424.
[22] J. Zamborszky, et al., Loss of BRCA1 or BRCA2 markedly increases the rate of base substitution mutagenesis and has distinct effects on genomic deletions, Oncogene 36 (6) (2017) 746–755.
[23] K.B. Kuchenbaecker, et al., Risks of breast, ovarian, and contralateral breast Cancer for BRCA1 and BRCA2 mutation carriers, JAMA 317 (23) (2017) 2402–2416.
[24] M. Arnedos, et al., Precision medicine for metastatic breast cancer–limitations and solutions, Nat. Rev. Clin. Oncol. 12 (12) (2015) 693–704.
[25] M. Dieterich, et al., Influence of lifestyle factors on breast cancer risk, Breast Care Basel (Basel) 9 (6) (2014) 407–414.
[26] H.J. Senn, St. Gallen consensus 2013: optimizing and personalizing primary curative therapy of breast cancer worldwide, Breast Care Basel (Basel) 8 (2) (2013) 101.
[27] A.K. Jain, et al., Recent developments and biological activities of thiazolidinone derivatives: a review, Bioorg. Med. Chem. 20 (11) (2012) 3378–3395.
[28] I.A. Khawar, J.H. Kim, H.J. Kuh, Improving drug delivery to solid tumors: priming the tumor microenvironment, J. Control. Release 201 (2015) 78–89.
[29] T.H. Luu, et al., Intestinal proportion of Blautia sp. Is associated with clinical stage and histoprognostic grade in patients with early-stage breast Cancer, Nutr. Cancer 69 (2) (2017) 267–275.
[30] B.J. Fuhrman, et al., Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women, J. Clin. Endocrinol. Metab. 99 (12) (2014) 4632–4640.
[31] J.J. Goedert, et al., Postmenopausal breast cancer and oestrogen associations with the IgA-coated and IgA-noncoated faecal microbiota, Br. J. Cancer 118 (4) (2018) 471–479.
[32] D. Rea, et al., Microbiota effects on cancer: from risks to therapies, Oncotarget 9 (25) (2018) 17915–17927.
[33] F. Baquero, C. Nombela, The microbiome as a human organ, Clin. Microbiol. Infect. 18 (Suppl 4) (2012) 2–4.
[34] S.Z. Eslami, et al., Microbiome and breast Cancer: new role for an ancient population, Front. Oncol. 10 (2020) 120.
[35] A.M. Valdes, et al., Role of the gut microbiota in nutrition and health, BMJ 361 (2018) k2179.
[36] R.F. Schwabe, C. Jobin, The microbiome and cancer, Nat. Rev. Cancer 13 (11) (2013) 800–812.
[37] H. Tlaskalova-Hogenova, et al., The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases, Cell. Mol. Immunol. 8 (2) (2011) 110–120.
[38] C. Jin, et al., Commensal microbiota promote lung Cancer development via gammadelta t cells, Cell 176 (5) (2019), p. 998-1013 e16.
[39] H. Adlercreutz, et al., Intestinal metabolism of estrogens, J. Clin. Endocrinol. Metab. 43 (3) (1976) 497–505.
[40] H. Adlercreutz, Western diet and Western diseases: some hormonal and biochemical mechanisms and associations, Scand. J. Clin. Lab. Invest. Suppl. 201 (1990) 3–23.
[41] R. Flores, et al., Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: a cross-sectional study, J. Transl. Med. 10 (2012) 253.
[42] M.S. Roberts, et al., Enterohepatic circulation: physiological, pharmacokinetic and clinical implications, Clin. Pharmacokinet. 41 (10) (2002) 751–790.
[43] J.J. Goedert, 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. 107 (8) (2015).
[44] S. Mani, Microbiota and breast Cancer, Prog. Mol. Biol. Transl. Sci. 151 (2017) 217–229.
[45] W.V. Ingman, The gut microbiome: a new player in breast Cancer metastasis, Cancer Res. 79 (14) (2019) 3539–3541.
[46] A.D. Kostic, et al., Genomic analysis identifies association of Fusobacterium with colorectal carcinoma, Genome Res. 22 (2) (2012) 292–298.
[47] E. Miko, et al., Microbiome-microbial metabolome-cancer cell interactions in breast cancer-familiar, but unexplored, Cells 8 (4) (2019).
[48] P. Knekt, et al., Does antibacterial treatment for urinary tract infection contribute to the risk of breast cancer? Br. J. Cancer 82 (5) (2000) 1107–1110.
[49] L. Zitvogel, et al., Cancer and the gut microbiota: an unexpected link, Sci. Transl. Med. 7 (271) (2015) 271ps1.
[50] C. Carbone, et al., Lung and gut microbiota as potential hidden driver of immunotherapy efficacy in lung Cancer, Mediators Inflamm. 2019 (2019) 7652014.
[51] C. Urbaniak, et al., Microbiota of human breast tissue, Appl. Environ. Microbiol. 80 (10) (2014) 3007–3014.
[52] C. Urbaniak, J.P. Burton, G. Reid, Breast, milk and microbes: a complex relationship that does not end with lactation, Womens Health Lond. (Lond) 8 (4) (2012) 385–398.
[53] D.T. Ramsay, et al., Ultrasound imaging of milk ejection in the breast of lactating women, Pediatrics 113 (2) (2004) 361–367.
[54] N. Akil, et al., High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study, Br. J. Cancer 99 (3) (2008) 404–407.
[55] B. Heng, et al., Human papilloma virus is associated with breast cancer, Br. J. Cancer 101 (8) (2009) 1345–1350.
[56] F. Fina, et al., Frequency and genome load of Epstein-Barr virus in 509 breast cancers from different geographical areas, Br. J. Cancer 84 (6) (2001) 783–790.
[57] S. Bonlokke, et al., Evidence of No association between human papillomavirus and breast Cancer, Front. Oncol. 8 (2018) 209.
[58] S.L. Glaser, et al., Absence of Epstein-Barr virus EBER-1 transcripts in an epidemiologically diverse group of breast cancers, Int. J. Cancer 75 (4) (1998) 555–558.
[59] K. Herrmann, G. Niedobitek, Lack of evidence for an association of Epstein-Barr virus infection with breast carcinoma, Breast Cancer Res. 5 (1) (2003) R13–7.
[60] N. Zhang, et al., Human papillomavirus infection correlates with inflammatory Stat3 signaling activity and IL-17 expression in patients with breast cancer, Am. J. Transl. Res. 8 (7) (2016) 3214–3226.
[61] O.M. Gannon, et al., Viral infections and breast cancer - A current perspective, Cancer Lett. 420 (2018) 182–189.
[62] C. Urbaniak, et al., The microbiota of breast tissue and its association with breast Cancer, Appl. Environ. Microbiol. 82 (16) (2016) 5039–5048.
[63] T.J. Hieken, et al., The microbiome of aseptically collected human breast tissue in benign and malignant disease, Sci. Rep. 6 (2016) 30751.
[64] S. Banerjee, et al., Distinct microbial signatures associated with different breast Cancer types, Front. Microbiol. 9 (2018) 951.
[65] S. Banerjee, et al., Distinct microbiological signatures associated with triple negative breast cancer, Sci. Rep. 5 (2015) 15162.
[66] M.K. McGuire, M.A. McGuire, Got bacteria? The astounding, yet not-so-surprising, microbiome of human milk, Curr. Opin. Biotechnol. 44 (2017) 63–68.
[67] H. Kumar, et al., Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations, Front. Microbiol. 7 (2016) 1619.
[68] K.A. Lackey, et al., What's normal? Microbiomes in human milk and infant feces are related to each other but vary geographically: the INSPIRE study, Front. Nutr. 6 (2019) 45.
[69] L. Costantini, et al., Characterization of human breast tissue microbiota from core needle biopsies through the analysis of multi hypervariable 16S-rRNA gene regions, Sci. Rep. 8 (1) (2018) 16893.
[70] K.M. Hunt, et al., Characterization of the diversity and temporal stability of bacterial communities in human milk, PLoS One 6 (6) (2011) e21313.
[71] R. Martin, et al., Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR, Appl. Environ. Microbiol. 75 (4) (2009) 965–969.
[72] T.L. Ward, et al., Human milk metagenome: a functional capacity analysis, BMC Microbiol. 13 (2013) 116.
[73] T.M. Pawlik, et al., Significant differences in nipple aspirate fluid protein expression between healthy women and those with breast cancer demonstrated by time-of-flight mass spectrometry, Breast Cancer Res. Treat. 89 (2) (2005) 149–157.
[74] S. Parida, D. Sharma, The power of small changes: comprehensive analyses of microbial dysbiosis in breast cancer, Biochim. Biophys. Acta Rev. Cancer 1871 (2) (2019) 392–405.
[75] A.A. Chan, et al., Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors, Sci. Rep. 6 (2016) 28061.
[76] C. Urbaniak, et al., Effect of chemotherapy on the microbiota and metabolome of human milk, a case report, Microbiome 2 (2014) 24.
[77] H. Wang, et al., Breast tissue, oral and urinary microbiomes in breast cancer, Oncotarget 8 (50) (2017) 88122–88138.
[78] E. Miko, et al., Lithocholic acid, a bacterial metabolite reduces breast cancer cell proliferation and aggressiveness, Biochim Biophys Acta Bioenerg 1859 (9) (2018) 958–974.
[79] T. Kovacs, et al., Cadaverine, a metabolite of the microbiome, reduces breast cancer aggressiveness through trace amino acid receptors, Sci. Rep. 9 (1) (2019) 1300.
[80] A.D. Fruge, et al., 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. 120 (4) (2020) 650–659.
[81] J.L. Freudenheim, et al., Periodontal disease and breast Cancer: prospective cohort study of postmenopausal women, Cancer Epidemiol. Biomarkers Prev. 25 (1) (2016) 43–50.
[82] N.N. Nwizu, et al., Periodontal disease and incident Cancer risk among postmenopausal women: results from the women's health initiative observational cohort, Cancer Epidemiol. Biomarkers Prev. 26 (8) (2017) 1255–1265.
[83] X. Mai, et al., Periodontal disease severity and cancer risk in postmenopausal women: the Buffalo OsteoPerio Study, Cancer Causes Control 27 (2) (2016) 217–228.
[84] S.B. Jensen, et al., Oral mucosal lesions, microbial changes, and taste disturbances induced by adjuvant chemotherapy in breast cancer patients, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 106 (2) (2008) 217–226.
[85] J.J. Napenas, et al., Molecular methodology to assess the impact of cancer chemotherapy on the oral bacterial flora: a pilot study, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 109 (4) (2010) 554–560.
[86] J. Ravel, et al., Vaginal microbiome of reproductive-age women, Proc Natl Acad Sci U S A 108 (Suppl 1) (2011) 4680–4687.
[87] G. Donders, et al., Effect of ultra-low-dose estriol and lactobacilli vaginal tablets (Gynoflor(R)) on inflammatory and infectious markers of the vaginal ecosystem in postmenopausal women with breast cancer on aromatase inhibitors, Eur. J. Clin. Microbiol. Infect. Dis. 34 (10) (2015) 2023–2028.

[88] M.L. Krychman, et al., Chemotherapy-induced dyspareunia: a case study of vaginal mucositis and pegylated liposomal doxorubicin injection in advanced stage ovarian carcinoma, Gynecol. Oncol. 93 (2) (2004) 561–563.
[89] R.M. Brotman, et al., Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy, Menopause 21 (5) (2014) 450–458.
[90] J. Shen, et al., Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis, Sci. Rep. 6 (2016) 24380.
[91] J. Marschalek, et al., Influence of Orally Administered Probiotic Lactobacillus Strains on Vaginal Microbiota in Women with Breast Cancer during Chemotherapy: A Randomized Placebo-Controlled Double-Blinded Pilot Study, Breast Care Basel (Basel) 12 (5) (2017) 335–339.
[92] B.J. Fuhrman, et al., Estrogen metabolism and risk of breast cancer in postmenopausal women, J. Natl. Cancer Inst. 104 (4) (2012) 326–339.
[93] C.M. Dallal, et al., Estrogen metabolism and breast cancer risk among postmenopausal women: a case-cohort study within B~FIT, Carcinogenesis 35 (2) (2014) 346–355.
[94] H. Samavat, M.S. Kurzer, Estrogen metabolism and breast cancer, Cancer Lett. 356 (2 Pt A) (2015) 231–243.
[95] C.S. Plottel, M.J. Blaser, Microbiome and malignancy, Cell Host Microbe 10 (4) (2011) 324–335.
[96] R.T. Falk, et al., Relationship of serum estrogens and estrogen metabolites to postmenopausal breast cancer risk: a nested case-control study, Breast Cancer Res. 15 (2) (2013) R34.
[97] M. Dabek, et al., Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria, FEMS Microbiol. Ecol. 66 (3) (2008) 487–495.
[98] D. Beaud, P. Tailliez, J. Anba-Mondoloni, Genetic characterization of the beta-glucuronidase enzyme from a human intestinal bacterium, Ruminococcus gnavus, Microbiology 151 (Pt 7) (2005) 2323–2330.
[99] M. Kwa, et al., The intestinal microbiome and estrogen receptor-positive female breast Cancer, J. Natl. Cancer Inst. 108 (8) (2016).
[100] F.M. McIntosh, et al., Phylogenetic distribution of genes encoding beta-glucuronidase activity in human colonic bacteria and the impact of diet on faecal glycosidase activities, Environ. Microbiol. 14 (8) (2012) 1876–1887.
[101] R.J. Santen, W. Yue, J.P. Wang, Estrogen metabolites and breast cancer, Steroids 99 (Pt A) (2015) 61–66.
[102] B.S. Reddy, et al., Effect of high-fat, high-beef diet and of mode of cooking of beef in the diet on fecal bacterial enzymes and fecal bile acids and neutral sterols, J. Nutr. 110 (9) (1980) 1880–1887.
[103] L. Domellof, et al., Fecal sterols and bacterial beta-glucuronidase activity: a preliminary metabolic epidemiology study of healthy volunteers from Umea, Sweden, and metropolitan New York, Nutr. Cancer 4 (2) (1982) 120–127.
[104] B.S. Reddy, et al., Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer, Gastroenterology 102 (5) (1992) 1475–1482.
[105] H. Tilg, J.R. Marchesi, Too much fat for the gut's microbiota, Gut 61 (4) (2012) 474–475.
[106] K. Gloux, et al., A metagenomic beta-glucuronidase uncovers a core adaptive function of the human intestinal microbiome, Proc Natl Acad Sci U S A 108 (Suppl 1) (2011) 4539–4546.
[107] J.P. Wiebe, et al., The 4-pregnene and 5alpha-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion, Cancer Res. 60 (4) (2000) 936–943.
[108] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[109] G. Cuevas-Ramos, et al., Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells, Proc Natl Acad Sci U S A 107 (25) (2010) 11537–11542.
[110] I.M. Toller, et al., Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells, Proc Natl Acad Sci U S A 108 (36) (2011) 14944–14949.
[111] V.J. Koller, et al., Impact of lactic acid bacteria on oxidative DNA damage in human derived colon cells, Food Chem. Toxicol. 46 (4) (2008) 1221–1229.
[112] A.M. Kabat, N. Srinivasan, K.J. Maloy, Modulation of immune development and function by intestinal microbiota, Trends Immunol. 35 (11) (2014) 507–517.
[113] H.J. Wu, E. Wu, The role of gut microbiota in immune homeostasis and autoimmunity, Gut Microbes 3 (1) (2012) 4–14.
[114] E.M. Brown, M. Sadarangani, B.B. Finlay, The role of the immune system in governing host-microbe interactions in the intestine, Nat. Immunol. 14 (7) (2013) 660–667.
[115] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, Nat. Rev. Immunol. 9 (5) (2009) 313–323.
[116] J.E. Belizario, J. Faintuch, M. Garay-Malpartida, Gut microbiome dysbiosis and immunometabolism: new frontiers for treatment of metabolic diseases, Mediators Inflamm. 2018 (2018), p. 2037838.
[117] K. Honda, D.R. Littman, The microbiota in adaptive immune homeostasis and disease, Nature 535 (7610) (2016) 75–84.
[118] C.A. Thaiss, et al., The microbiome and innate immunity, Nature 535 (7610) (2016) 65–74.
[119] S.M. Crusz, F.R. Balkwill, Inflammation and cancer: advances and new agents, Nat. Rev. Clin. Oncol. 12 (10) (2015) 584–596.
[120] S.M. Mahmoud, et al., Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer, J. Clin. Oncol. 29 (15) (2011) 1949–1955.
[121] N.R. West, et al., Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer, Br. J. Cancer 108 (1) (2013) 155–162.
[122] A.D. Gritzapis, et al., Identification of a novel immunogenic HLA-A*0201-binding epitope of HER-2/neu with potent antitumor properties, J. Immunol. 181 (1) (2008) 146–154.
[123] B. Wei, et al., Commensal microbiota and CD8+ T cells shape the formation of invariant NKT cells, J. Immunol. 184 (3) (2010) 1218–1226.
[124] B.C. Mercier, et al., NOD1 cooperates with TLR2 to enhance T cell receptor-mediated activation in CD8 T cells, PLoS One 7 (7) (2012) e42170.
[125] L. Franchi, et al., Function of Nod-like receptors in microbial recognition and host defense, Immunol. Rev. 227 (1) (2009) 106–128.
[126] B. Azab, et al., Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients, Ann. Surg. Oncol. 19 (1) (2012) 217–224.
[127] H. Noh, M. Eomm, A. Han, Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients, J. Breast Cancer 16 (1) (2013) 55–59.
[128] J.R. Lakritz, et al., Gut bacteria require neutrophils to promote mammary tumorigenesis, Oncotarget 6 (11) (2015) 9387–9396.
[129] M.R. Rutkowski, et al., Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation, Cancer Cell 27 (1) (2015) 27–40.
[130] C.W. Kaplan, et al., Fusobacterium nucleatum outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes, Infect. Immun. 78 (11) (2010) 4773–4778.
[131] B.J. Shenker, J.M. DiRienzo, Suppression of human peripheral blood lymphocytes by Fusobacterium nucleatum, J. Immunol. 132 (5) (1984) 2357–2362.
[132] C. Hill, et al., Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic, Nat. Rev. Gastroenterol. Hepatol. 11 (8) (2014) 506–514.
[133] E. Motevaseli, A. Dianatpour, S. Ghafouri-Fard, The role of probiotics in Cancer treatment: emphasis on their in vivo and in vitro anti-metastatic effects, Int. J. Mol. Cell. Med. 6 (2) (2017) 66–76.
[134] A. Gorska, et al., Probiotic Bacteria: a promising tool in Cancer prevention and therapy, Curr. Microbiol. 76 (8) (2019) 939–949.
[135] Y. Nazir, et al., Probiotics and their potential preventive and therapeutic role for Cancer, High serum cholesterol, and allergic and HIV diseases, Biomed Res. Int. 2018 (2018), p. 3428437.
[136] C. Chen, H.M. Chan, S. Kubow, Kefir extracts suppress in vitro proliferation of estrogen-dependent human breast cancer cells but not normal mammary epithelial cells, J. Med. Food 10 (3) (2007) 416–422.
[137] N.R. Zamberi, et al., The antimetastatic and antiangiogenesis effects of kefir water on murine breast Cancer cells, Integr. Cancer Ther. 15 (4) (2016) NP53–NP66.
[138] Y. Nami, et al., Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium Lactobacillus plantarum 5BL, Microbiol. Immunol. 58 (9) (2014) 492–502.
[139] Y. Nami, et al., A newly isolated probiotic Enterococcus faecalis strain from vagina microbiota enhances apoptosis of human cancer cells, J. Appl. Microbiol. 117 (2) (2014) 498–508.
[140] Z. Hassan, et al., Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of Enterococcus faecalis and Staphylococcus hominis isolated from human breast milk, In Vitro Cell. Dev. Biol. Anim. 52 (3) (2016) 337–348.
[141] N.-K. Lee, et al., Multifunctional effect of probiotic Lactococcus lactis KC24 isolated from kimchi, Lwt - Food Sci. Technol. 64 (2) (2015) 1036–1041.
[142] R.H. Kadidrareddy, V. Mathakala, U.M. Devi Palempalli, Therapeutic activity of conjugated linoleic acids synthesized by lactobacillus plantarum, Int. J. Pharma Bio Sci. 7 (4) (2016).
[143] J.H. Lee, et al., The production of surfactin during the fermentation of cheonggukjang by potential probiotic Bacillus subtilis CSY191 and the resultant growth suppression of MCF-7 human breast cancer cells, Food Chem. 131 (4) (2012) 1347–1354.
[144] T. Yamada, et al., Apoptosis or growth arrest: modulation of tumor suppressor p53's specificity by bacterial redox protein azurin, Proc. Natl. Acad. Sci. U.S.A. 101 (14) (2004) 4770–4775.
[145] A. Avand, V. Akbari, S. Shafizadegan, In vitro cytotoxic activity of a Lactococcus lactis antimicrobial peptide against breast Cancer cells, Iran. J. Biotechnol. 16 (3) (2018) e1867.
[146] J. Chumchalova, J. Smarda, Human tumor cells are selectively inhibited by colicins, Folia Microbiol (Praha) 48 (1) (2003) 111–115.
[147] E. Oliveira Silva, et al., Cytotoxicity of lapachol metabolites produced by probiotics, Lett. Appl. Microbiol. 59 (1) (2014) 108–114.
[148] S. Raveendran, et al., Bacterial exopolysaccharide based nanoparticles for sustained drug delivery, cancer chemotherapy and bioimaging, Carbohydr. Polym. 91 (1) (2013) 22–32.
[149] A. Yaghoubi, et al., Bacteriotherapy in breast Cancer, Int. J. Mol. Sci. 20 (23) (2019).
[150] A.A. Imani Fooladi, et al., Th1 cytokine production induced by Lactobacillus acidophilus in BALB/c mice bearing transplanted breast tumor, Jundishapur J. Microbiol. 8 (4) (2015) e17354.
[151] Soltan-Dallal MM, et al., Effect of oral administration of lactobacillus acidophilus on the immune responses and survival of BALB/c mice bearing human breast cancer, Tehran University Medical Journal 67 (11) (2010) 753–758.
[152] M.H. Yazdi, et al., Oral administration of Lactobacillus acidophilus induces IL-12 production in spleen cell culture of BALB/c mice bearing transplanted breast tumour, Br. J. Nutr. 104 (2) (2010) 227–232.

[153] H. Maroof, et al., Lactobacillus acidophilus could modulate the immune response against breast cancer in murine model, J. Clin. Immunol. 32 (6) (2012) 1353–1359.
[154] M.M. Soltan Dallal, et al., Lactobacillus casei ssp.cAsei induced Th1 cytokine profile and natural killer cells activity in invasive ductal carcinoma bearing mice, Iran. J. Allergy Asthma Immunol. 11 (2) (2012) 183–189.
[155] F. Aragon, et al., Inhibition of growth and metastasis of breast Cancer in mice by milk fermented with Lactobacillus casei CRL 431, J Immunother 38 (5) (2015) 185–196.
[156] F. Aragon, et al., The administration of milk fermented by the probiotic Lactobacillus casei CRL 431 exerts an immunomodulatory effect against a breast tumour in a mouse model, Immunobiology 219 (6) (2014) 457–464.
[157] A. de Moreno de LeBlanc, et al., Effects of milk fermented by Lactobacillus helveticus R389 on a murine breast cancer model, Breast Cancer Res. 7 (4) (2005) R477–86.
[158] M. Rachid, et al., Effect of milk fermented with a Lactobacillus helveticus R389(+) proteolytic strain on the immune system and on the growth of 4T1 breast cancer cells in mice, FEMS Immunol. Med. Microbiol. 47 (2) (2006) 242–253.
[159] J.R. Lakritz, et al., Beneficial bacteria stimulate host immune cells to counteract dietary and genetic predisposition to mammary cancer in mice, Int. J. Cancer 135 (3) (2014) 529–540.
[160] M.H. Yazdi, et al., The preventive oral supplementation of a selenium nanoparticle-enriched probiotic increases the immune response and lifespan of 4T1 breast cancer bearing mice, Arzneimittelforschung 62 (11) (2012) 525–531.
[161] K. Dubin, et al., Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis, Nat. Commun. 7 (2016) 10391.
[162] V. Pala, et al., Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort, Int. J. Cancer 129 (11) (2011) 2712–2719.
[163] Y. Aso, et al., Preventive effect of a Lactobacillus casei preparation on the recurrence of superficial bladder cancer in a double-blind trial, The BLP Study Group. Eur Urol 27 (2) (1995) 104–109.
[164] M. Toi, et al., Probiotic beverage with soy isoflavone consumption for breast Cancer prevention: a case-control study, Curr. Nutr. Food Sci. 9 (3) (2013) 194–200.
[165] S. Arslanoglu, et al., Early neutral prebiotic oligosaccharide supplementation reduces the incidence of some allergic manifestations in the first 5 years of life, J. Biol. Regul. Homeost. Agents 26 (3 Suppl) (2012) 49–59.
[166] S. Gaines, et al., How the microbiome is shaping our understanding of cancer biology and its treatment, Semin. Colon Rectal Surg. 29 (1) (2018) 12–16.
[167] B. De Spiegeleer, et al., The quorum sensing peptides PhrG, CSP and EDF promote angiogenesis and invasion of breast cancer cells in vitro, PLoS One 10 (3) (2015) p. e0119471.
[168] A.C. Miller, E.M. Elamin, Use of probiotics for treatment of chemotherapy-induced diarrhea: is it a myth? JPEN J. Parenter. Enteral Nutr. 33 (5) (2009) 573–574.
[169] J. Yang, et al., Gastrointestinal microbiome and breast cancer: correlations, mechanisms and potential clinical implications, Breast Cancer 24 (2) (2017) 220–228.