Abstract

Background: The human body is colonized by millions of bacteria, with the permanent colonizers referred to collectively as the microbiome. The microbiome, microbial by-products of metabolism affect the human body in numerous ways, providing a protective and often symbiotic relationship with the human host. Nevertheless, through environmental fluctuations, individual’s genetic predisposition and/or unknown factors, dysbiosis or a state of imbalance may develop, leading to the development of diseases, including cancer. The effects of microbial dysbiosis are largely focused on the gastrointestinal system and development of gastric-related disorders.

Aims: This research review extends the scope of the microbiome’s role outside of the gastrointestinal domain, specifically investigating pathways by which the microbiome modulates breast cancer risks.

Method: CINAHL and PubMed databases, journals, and citation indices were searched using the keyword “microbiome” in combination with breast cancer, dysbiosis, estrogen, immune system, obesity, epigenetics, nursing.

Results: The microbiome plays an active role in modulating estrogenic levels, in establishing and stimulating the immune system, and contributes to the development of obesity affecting breast cancer development through epigenetic dysregulation. Many epigenetic changes are reversible through either pharmacotherapy or through behavioral changes, and therefore are amenable to nursing interventions. Knowledge of the microbiome’s systemic effects can shed light on individualized breast cancer prevention and treatment care plans, improve dietary guidelines, and bolster the immune system.
Exploring the Role of the Microbiome in Breast Carcinogenesis

Breast Cancer Background

It is predicted that 1 out of 8 women will develop invasive breast cancer, the most common cancer that affects women. Greater than two hundred thousand new cases are expected to develop this year alone, claiming the lives of more than 40,000 women per year, and half a million annually worldwide (Siegel, Miller, & Jemal, 2015; Stewart & Wild, 2014).

The breast is composed of multiple cell types that give rise to at least five breast cancer subtypes. These subtypes are termed: luminal A; luminal B; HER2-enriched; normal-like, and basal-like (Rattani & Swift-Scanlan, 2014). Gene expression refers to the process whereby the instructions in DNA are copied to RNA and translated to their final protein product. Not everyone who is genetically pre-disposed to breast cancer develops breast cancer, pointing to the role environmental factors play in carcinogenesis. Modifiable risk factors in breast cancer development include: parity, breastfeeding, body mass index, oral contraceptive use, menopausal hormone therapy use, consumption of alcohol, cigarette smoking, and physical activity (Nickels et al., 2013). These environmental risk factors may be modifying an individual’s susceptibility to cancer by decreasing genetic expression at the epigenome level (Rattani & Swift-Scanlan, 2014; Bardowell et al., 2013).
Exploring the Role of the Microbiome in Breast Carcinogenesis

Epigenetics refers to processes by which gene expression and function are altered and modified without changing the actual DNA sequence (Aguilera, Fernandez, Munoz, & Fraga, 2010). One epigenetic change, DNA methylation, can lead to alterations that influence the way proteins (histones) package DNA. If the histones that comprise chromatin are packaged too tightly, then the DNA is not accessible to being copied to mRNA and ultimately gene expression is silenced. An individual’s epigenome can accumulate alterations over a lifetime due to intrinsic (genetic), environmental, and/or undetermined factors (Aguilera et al., 2010). In individuals with breast cancer, tumor suppressor genes are often inactivated or deleted through changes in epigenetic regulation in response to environmental stimuli (Toland, 2012).

One frequently overlooked contributor to epigenetic dysregulation that interacts at the physiologic and environmental level is the microbiome. Fluctuations within microbial communities of the human body (collectively referred to as the “microbiome”), microbial interaction with the human host immune system, and downstream chemical changes influenced by bacterial metabolic by-products, may all be involved in pathways that can impact epigenetic alterations. Ultimately, microbiome research may help individualize cancer treatments by identifying new therapeutic targets. Of notable interest to nursing, is that many epigenetic changes are reversible through either pharmacotherapy or through behavioral changes, and therefore are amenable to clinical interventions. For example, the microbiome can be influenced by diet and other modifiable exposures, and therefore hold promise for improving health outcomes in general, and cancer prevention in particular.
The Human Microbiome

The human body is host to more than 10 trillion microbial cells. In humbling contrast, the human genome contains ~20,000 protein-coding genes. Most of the human microbial cells reside in the lumen of the GI tract, but different communities find suitable residences in different anatomical sites (Bultman, 2014). The symbiotic coexistence of microbes benefits the human hosts in a number of ways including: carbohydrate metabolism, nutrient reclamation from food, absorption of minerals, and breakdown of dietary toxins (Shapira, Sultan, Lee, & Taioli, 2013). In turn, the human gut provides an optimal habitat, supplying the bacteria with a steady stream of nutrients, and protecting the microbes from nematodes, roundworms and other bacterial adversaries (Bultman, 2014). This symbiosis points to a historical process of the microbes’ co-evolution with humans, with more and more researchers describing humans as a “supraorganism”—that is, a single organism system composed of multiple organisms.

Therefore, the Human Microbiome Project (HMP) was initiated to better understand the complex molecular and environmental impacts of the microbiome on human health. The HMP is a worldwide joint scientific venture, designed to gain further understanding of microbiome structure and function, by characterizing the microbial communities of various body sites and testing correlations between microbiome fluctuations over time and its relation to human health. A clearer picture of variations in human microbiota has been accelerated by the use of advanced tools, epidemiological studies, whole genome sequencing, gnotobiotic (germ-free) mouse models, next-generation sequencing, and phylogenetic microarray (Aguilera et al., 2010; Bultman, 2014; Rajilic-Stojanovic, Heilig, Tims, Zoetendal, & de Vos, 2012).
Exploring the Role of the Microbiome in Breast Carcinogenesis

HMP researchers group and classify the human gut microbiota that is composed of viruses, fungi, and bacteria (Robinson & Pfeiffer, 2014). This paper narrows its focus on the bacterial role in the microbiome. Due to diversity and sheer number, finding bacterial communities that were consistently similar between individuals, and across cultural, national and continental boundaries was thought to be an onerous task. However, investigations of the “core” human microbiome repeatedly find more than 90% of all bacterial phylogenetic types belong to 2 of the 70 known divisions (phyla) – *Bacteriodetes* and *Firmicutes* (Rajilic-Stojanovic et al., 2012; Turnbaugh et al., 2007). Recent findings further indicate environmental fluctuations (including use of antibiotics, dietary cleanses and regimes, compositional changes from traveling), may affect the abundance, but not necessarily the presence and composition of specific core microbial species (Rajilic-Stojanovic et al., 2012).

A unified classification framework and nomenclature for all bacteria has recently been introduced (Yarza et al., 2014). The lack of standardization up until recently, produced disagreement amongst which phylotypes are considered “permanent colonizers,” categories that varied depending on the assay method used (Rajilic-Stojanovic et al., 2012; Shapira et al., 2013). Using 16S Ribosomal RNA sequencing, Shapira et al. lists 7 major phyla that are consistently found amongst children, adults and elderly as: Actinobacteria, Bacteriodetes, Verrucomicrobia, Firmicutes, Tenericutes, Proteobacteria, and Cyanobacteria (Shapira et al., 2013). Meanwhile, Rajilic-Stojanovic et al. (2012), used human intestinal tract chip (HITChip) comprehensive phylogenetic microarray to identify the following core phyla: Bifidobacterium, Bacteroidetes, Faecalibacterium, Blautia, Dorea, and Ruminococcus. These discrepant findings indicate
Exploring the Role of the Microbiome in Breast Carcinogenesis

the need for continued investigation in what constitutes the microbiome core, and the use of standard framework for nomenclature/classification (Rajilic-Stojanovic et al., 2012; Yarza et al., 2014).

Conversely, Arumugam et al. (2011) sought to classify the microbiome through a higher level of consolidated genomic studies. Using new “metagenome” derived functional biomarkers from four countries’ fecal data and comparing these data with 1,511 phylogenetic profile reference genomes from around the world, the study proposed that microbial species can be classified into one of three distinct groups or enterotypes that is neither nation nor continent specific. Though there were methodological limitations, including the fact that fecal samples are not representative of the entire gut microbiome, the study suggests that the metagenomic classification of three biomarker molecules (two of which are ATP complexes that corresponds with the body’s Body Mass Index) may be more important than phylogenetic differences (Arumugam et al., 2011). Despite varied classification methods, there is growing support of a “core” microbial community across individuals that remain fairly constant and invariable over long periods of time (Rajilic-Stojanovic et al., 2012). Therefore, it is recommended that testing associations of specific microbiota compositions with environmental exposures and/or disease risk should be limited to the numerically most dominant phylotypes (Durban, Abellan, Jimenez-Hernandez, Latorre, & Moya, 2012).

Applications of knowledge gained from studying microbial communities have been largely focused on obesity, gastrointestinal cancer development, and other gastric problems. More recently, the scope of study has expanded into other areas, including the
Establishment of Ecosystem and Dysbiosis

The bacterial communities in the intestine are inherited at birth through the birth canal and skin to mouth contact. The bacteria first to arrive establish numerical dominance. Subsequently, they can either cooperate with newcomers or exclude competitors (Durban et al., 2012). In addition to the first-comer’s established presence, however, other factors play a role in the composition of an individual’s microbiome ecosystem, including variations relative to genetic conditions, age, medication, and diet. Such changes may ultimately lead to unique pH, temperature, or secretions, offering microenvironments favorable to select bacterial communities (Shapira et al., 2013). When outside influences such as disease, medications, and altered diet disturb the established ecosystem, the result is described as a state of dysbiosis. Dysbiosis leads to abnormalities of the host immune system, continued inflammation, leaves the host vulnerable to pathogens, and encourages rapid replication of pathogenic bacteria that further propagate the continued disequilibrium (Arthur et al., 2012; Shapira et al., 2013; Sheflin, Whitney, & Weir, 2014).

Microbiome Dysbiosis and Increased Estrogenic Burden

In the case of breast cancer, dysbiosis is implicated in increased production of metabolites that raise circulating levels of estrogen. In the 70s, a study proposed that Lecithinase-negative clostridia have the ability to stimulate production of estradiol, estrone, and 17-methoxy-extradiol from androstenedione (precursor of male and female
sex hormones. These are carcinogens that act on mammary tissue or promote tumor growth (Hill, Goddard, & Williams, 1971). Since then, more evidence for the role of estrogen in tumor formation has been uncovered. Studies of postmenopausal women have consistently produced results indicating that higher levels of estradiol, estrone, and estrone sulfate circulating in the body are positively correlated with an increased risk of breast cancer (Fuhrman et al., 2012). In fact, approximately 80% of breast cancers express the estrogen receptor, making estrogen receptor (ER) positive breast cancer the most prevalent breast cancer type (Al-Hussaini, Subramanyam, Reedijk, & Sridhar, 2011; Cancer Genome Atlas Network, 2012).

Recently, linkages between circulating levels of estrogen, breast cancer risk, and microbial activity in the human gut have also been described. Fuhrman et. al (2012) found that higher circulating levels of estrogen may depend on individual’s microbial composition. Drawing on data from the multi-center Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, which followed 39,116 female participants, ages 55-74 year in a period of 8 years, the authors concluded that nearly all estrogen, estrogen metabolites, and particularly the serum concentration of unconjugated estradiol, is associated with increased risk of breast cancer (Fuhrman et al., 2012). Estrogen circulates bound to protein or free in the blood and is deactivated by the liver and transported to the intestinal lumen as bile acids. Individuals that have bacterial colonies favorable to the growth of Proteobacteria species (E.coli, Klebsiella, Enterobacter, Citrobacter), that outcompete commensal bacteria such as Firmicutes and Bacteriodetes, have increased estrogenic burden. E.coli has been shown to produce beta-glucuronidase that acts to deconjugate estrogen in the intestinal lumen, recycling the estrogen back into
Exploring the Role of the Microbiome in Breast Carcinogenesis

the bloodstream rather than being secreted in feces or urine (Plottel & Blaser, 2011; Shapira et al., 2013).

Interestingly, another study found that women with higher urinary excretion of parent estrogens are at lower risk for breast cancer, thus supporting the hypothesis that once circulating estrogens are removed, breast cancer risk decreases (Eliassen et al., 2012). Subsequently, a different follow-up study collected both urine and fecal samples from 60 postmenopausal subjects and found urinary excretion of estrogen was linked to bacterial activity. Their findings suggest that women with a more diverse gut microbiome demonstrated an elevated urinary [excretion] ratio of hydroxylated estrogen metabolites to parent estrogen (Fuhrman et al., 2012).

Microbiome and the Immune System

The microbiome also plays an active role in enhancing or undermining the host immune system, contributing to tumorigenesis and metastasis, that holds promise for potential cancer prevention and treatment strategies. The microbiome modulates and contributes to chronic inflammation, helps to maintain gut epithelial cells, influences the number of circulating neutrophils, and can enhance or suppress the immune system in both human and animal models (Shapira et al., 2013; Turnbaugh et al., 2007).

All bacteria, including bacteria with established beneficial roles, have the potential to become pathogens. In fact, up to 20% of all cancers worldwide are related to microbial infection and subsequent inflammation (Elinav et al., 2013). Chronic inflammation, a dysregulated protective mechanism of continuous tissue repair and growth response, is firmly established as a risk factor for tumorigenesis. Chronic
Exploring the Role of the Microbiome in Breast Carcinogenesis

Inflammatory processes produce toxins and free radicals that damage host DNA, and can accelerate the development of mammary tumors (Caygill, 2012; Elinav et al., 2013; Iyengar, Morris, Hudis, & Dannenberg, 2013; Rakoff-Nahoum, 2007; Rao et al., 2006; Schwabe & Jobin, 2013).

Additionally, specific bacterial interactions with the immune cells can result in structural differences of the immune system. The bacterial role can be seen in a neonate’s Peyer’s patches that function to stimulate the B cells and T cells, isolated lymphoid follicles (IFLs) and mesenteric lymph nodes (MLN). The development of these immune sentinels is spurred by microbial colonizers (Maynard, Elson, Hatton, & Weaver, 2012). In contrast, gnotobiotic or germ free rodents, have considerably decreased neutrophil number and function, and significantly smaller or reduced Peyer’s patches, spleen and mesenteric lymph nodes (MSN) – all of which were attributed to the absence of microbiota (Reading & Kasper, 2011; Shapira et al., 2013). In fact, the introduction of “as little as” one commensal bacterial species, can lead to better immunity, reduces the rate and susceptibility to infection and chronic inflammation (Reading & Kasper, 2011).

*See Table 1 for Bacterial Role in the Immune System.*

**Dysbiosis of the Microbiome, Obesity and Carcinogenesis**

The importance of the microbiome in breast carcinogenesis and breast cancer prognosis is especially evident when looking into the growing trends in obesity. Obesity is a risk factor for the initiation of breast cancer, and is associated with larger tumors, poor treatment prognosis, metastasis, and cancer recurrence (Iyengar et al., 2013; Protani,
Exploring the Role of the Microbiome in Breast Carcinogenesis

Coory, & Martin, 2010). A study comparing obese individuals with those classified as overweight found that obesity, along with increased hip to waist ratio, doubles the breast cancer incidence in both pre-and post-menopausal women (Amadou et al., 2013; Biglia et al., 2013; Simpson & Brown, 2013).

**Mechanism linking Obesity to Breast Cancer**

Obesity is not only associated with metabolic dysfunction such as Type 2 Diabetes, insulin resistance, vascular disorders and kidney problems, but is also linked to breast cancer by several metabolic pathways. Adipose tissue is now recognized as an endocrine organ. Adipose cells have estrogen receptors, and produce leptin, that stimulates the body’s metabolic function as well as other important hormonal functions (Galic, Oakhill & Steinberg, 2010). In adipose tissue, low grade-inflammation and dysregulated metabolism increase aromatase expression, an enzyme responsible for the synthesis of estrogen (Simpson & Brown, 2013). Observed histologically in actual tumor sites, increased aromatase enzyme contributes to chronic inflammation. Adipocytes of breast tissue are filled with lipids and saturated fatty acids that can circulate in the blood stream, and cause activation of inflammatory complexes such as tumor necrotic factor (TNF)-α. In addition, adipocyte tissues are often hypoxic, with few blood vessels that recruit activated macrophages (M1) into the surrounding fat cells. Infiltrated with M1, adipose cells releases cytokines and TNF-α, creating a chronic state of low-grade inflammation and increasing breast cancer risk (Harford, Reynolds, McGillicuddy, & Roche, 2011; Olefsky & Glass, 2010; Simpson & Brown, 2013; Galic, Oakhill &
Exploring the Role of the Microbiome in Breast Carcinogenesis

Steinberg, 2010.

Microbiome’s Role in Obesity

Obesity is an epidemic in the US and worldwide. Obesity statistics reveal more than a third of Americans are currently obese, with as much as 65% of the American population expected to be obese by 2030 (Iyengar et al., 2013). Considering this rising trend, the negative health effects, and the cost of obesity-related treatments, uncovering the microbiome’s role in obesity has generated a lot of excitement.

When comparing obese to lean microbiomes between twins, obesity is associated with reduced bacterial diversity, altered representation of bacterial genes and metabolic pathways. Additionally, the microbiota of obese individuals harvest more energy from diet than non-obese individuals by extracting energy from non-digestible polysaccharides (Turnbaugh et al., 2009b). An earlier study demonstrated that microbial diversity alterations in the Bacteriodetes and Firmicutes/Actinobacteria ratio, may depend on a low-calorie diet (Ley, Turnbaugh, Klein, & Gordon, 2006). Applying this concept into humanized gnotobiotic mouse models transplanted with fecal microbial communities, Turnbaugh et al. 2009 found that switching to a “Western” diet, high in fat and sugar, altered the microbiome metabolic pathways and gene expression in one day (Turnbaugh et al., 2009a).

Furthermore, microbiota interacts with epithelial cells to influence energy expenditure or storage (Tilg & Kaser, 2011; Turnbaugh et al., 2009b). For example, the amount of complex carbohydrates metabolized into Short-Chain Fatty Acids (SCFA), a nutrition and energy source for colonocytes that maintain the integrity of gut epithelial
tissue, is dependent on microbial composition. Specifically, contingent on the whether the predominant microbial community is co-colonized by the prominent archaeon *Methanobrevibacter smithii* and *Bacteroides thetaiotaomicron*, influences the efficiency of carbohydrate fermentation and energy absorption, resulting in overproduction of SCFA, and increased production of adipocytes (Samuel & Gordon, 2006).

Furthermore, microbes affect obesity through production of signaling molecules that directly affect the feeling of fullness after eating, increase the storage of lipids in adipocytes, and decrease fatty acid oxidation in muscle tissue (Furet et al., 2010; Tremaroli & Backhed, 2012; Turnbaugh et al., 2006).

**Microbiome in the Breast Tissue**

Given the distal effect of the gut microbiome on organ systems, a new study has emerged examining the microbiota closer to home—looking into the colonizers of breast tissue. Because microbiota exists in many different parts of the body, and have been consistently found in breast milk, Urbaniak et al. (2014) postulated that bacteria use the nipple to access the breast ducts, thus creating a breast microbiome. The primary phylum of bacteria found in the breast tissue of women with or without cancer was *Proteobacteria*, followed by *Firmicutes*—a composition that is significantly different from the GI tract or other microbial colonies found in other parts of the body. The authors suggest that *Proteobacteria* may have been selected due to their ability to acclimate to the high fatty acid content in the breast tissue (Urbaniak et al., 2014). Certain pathogenic bacteria that are able to metabolize fat such as *Enterobacteriaceae*, *Psuedomonas*, and *Streptococcus agalactiae*, were also found in the breast tissues,
Exploring the Role of the Microbiome in Breast Carcinogenesis

though they have not multiplied or induced infections (Urbaniak et al., 2014). The absence of infection and minimized population of these pathogenic microbes underscores the importance of maintaining bacterial-to-host homeostasis.

Using next-generation sequencing, Xuan et. al. (2014) examines the dysbiosis of the breast microbiome by comparing the microbes present in ER+ breast tumor tissue with adjacent normal tissue from the same patient. Their findings show that breast cancer tissue generally correlates with increased presence of Methylobacterium radiotolerans, and a decrease in Sphingomonas yanoikuyae numbers. However, in normal tissue the inverse was true; there were more S. yanoikuyae compared to M. radiotolerans. Xuan et al. (2014) postulates that these two bacterial colonies balance each other’s population in healthy tissue. Furthermore, a decrease in the strength of antibacterial response against tumor tissues was also observed as a result of decline in numbers of S. yanoikuyae, suggesting that this organism may have probiotic functions in the breast (Xuan et al., 2014). Microbial studies describe that S. yanoikuyae is a gram-negative bacteria that express glycosphingolipid (GSLs), compounds that activate cells that play a role in innate immunity such as natural killer cells (NKCs), dendritic cells and macrophages. NKCs are important mediators of cancer immunosurveillance and have been reported to have an integral role in controlling breast cancer metastasis. For example, NKCs are involved in direct killing of tumors cells in culture media and in vivo models, and inhibit active tumor growth in mice that lack endogenous protective lymphocytes (Bassiri, Das, & Nichols, 2013; Kubota, Takimoto, Kaneko, Inoue, & Kumazawa, 2009; Xuan et al., 2014). The extent the breast microbiota help to shape the local and systemic immune
Exploring the Role of the Microbiome in Breast Carcinogenesis

response, provide protective function and play a role in tumorigenesis is an area that needs further investigation.

**Bacterial Influences on Epigenetic Reprogramming of Host Cells**

Bacterial mechanisms that induce physiologic changes to the host signaling pathways, tissues, and cells also influence epigenetic processes. Both bacteria and the human host are responsive to their environment through multiple pathways that impact the epigenome. In bacteria, methylation of chromosomal DNA can lead to DNA fragmentation, recombination, translocation and can silence gene expression of a protein that is responsible for bacterial communication, replication, and host tissue invasion. This bacterial protein also contributes to anti-bacterial resistance crucial to bacterial survival and inter-bacterial competition. For instance, bacterial protein expression regulated by DNA methylation, influences basic bacterial growth, migration and gene expression for protective polysaccharide production or stimulation of the host’s T-cell response (Zautner, 2014). Over production of bacterial metabolic by-products can induce epigenetic reprogramming that affects gene expression, cancer cell viability, and migration and induces apoptosis or programmed cell death (Bryan et al., 2010; Kumar, Bryan, & Kumar, 2014; Zautner, 2014). In terms of bacterial survival, epigenetic programming has also been implicated in the bacteria’s ability to resist antibiotics, as demonstrated by antibiotic-resistant of *Streptococcus pneumoniae*. Additionally, lipopolysaccharides (LPS), a major component of gram-negative bacteria’s outer membrane, can be regulated/dysregulated at the epigenetic level, resulting in immunosuppression and poor disease prognosis (Angrisano et al., 2010; Zautner, 2014).
Exploring the Role of the Microbiome in Breast Carcinogenesis

In higher-level eukaryotes, hotspots for methylation occur at cytosine residues 5’ to a guanine (CpG), as opposed to adenine and cytosine nucleotides in bacteria (Zautner, 2014). Epigenetic hallmarks of cancer include hypermethylation of gene-specific promoter and global hypomethylation of patterned/repeated sequences, which silences or activates pathways important in cancer development such as cell communication, tissue invasion/metastasis, cell cycle, DNA repair, and hormone signaling (Toland, 2012). Epigenetic reprogramming has been implicated in many different facets of breast cancer, including breast cancer subtypes. For example, promoter-methylation of the Estrogen receptor alpha gene (ERα) has been noted in triple negative, basal-like breast cancer types associated with poor prognosis in women without family history of breast cancer (Jing et al., 2011). Another area affected by epigenetic activation or silencing is the BRCA1 gene, which predisposes women to ovarian or breast cancer (Toland, 2012).

The unifying theme between the epigenetic processes of bacteria and host systems is that bacterial influences can induce hypermethylation and epigenetic reprogramming in host cells that can ultimately lead to cancer. However, in terms of direct cause of breast cancer, bacterial epigenetic activation that produces breast tumors remains to be definitely described. Nevertheless, bacterial epigenetic reprogramming has been demonstrated to have a direct impact on intrauterine growth restriction after infection with oral bacteria Campylobacter rectus, and also directly involved in the formation of gastric cancer by H. Pylori (Zautner, 2014).

IV-Nursing Implications:
Exploring the Role of the Microbiome in Breast Carcinogenesis

Triggers that activate the cancer oncogenes may involve many systems at disequilibrium. Deregulation at the epigenetic level, fueled by environmental factors and the activities of the microbiome can activate breast cancer development. The risk of cancer can be mitigated through awareness of bacterial actions, the establishment of a healthy microflora, and through specific dietary choices that affect estrogenic levels, chronic inflammation, and obesity. Health professionals can make lifelong positive impacts by promoting adoption of healthy behaviors. Encouraging new mothers to breastfeed establishes microbiome diversity, introduces commensal bacteria Bifidobacterium, guards against the risk of obesity, and stimulates the proper development of the immune system (Reading & Kasper, 2011; Shapira et al., 2013; Turroni et al., 2012). Breastfeeding is also critical for establishing mucosal tolerance to immunosurveillant NKCs that is important in establishing a balanced immune system and in breast cancer prevention (Olszak et al., 2012). Additionally, women with deleterious BRCA1 mutations, who breast-fed for a cumulative total of more than one year, have a statistically significant reduced risk in breast cancer compared to those who did not breastfeed (Kotsopoulos et al., 2012).

Awareness of the microbiome and how dysbiosis can occur are also important teaching points that could help patients navigate conflicting dietary advice and promote adherence to dietary recommendations that decrease breast cancer risk and/or enhance treatment response. Long-term dietary choices have an effect on an individual’s enterotypes, and the abundance of certain bacterial colonies. Furthermore, changes in the microbiome are reversible through dietary choices (Zhang et al., 2012). For example, numerous studies have documented how high fat and high sugar diet alters microbiota
Exploring the Role of the Microbiome in Breast Carcinogenesis

composition leading to increased risks in obesity, and subsequently breast cancer (Carmody et al., 2015; Hildebrandt et al., 2009; Minicozzi et al., 2013; Zhang et al., 2010). In response to the recommendation against high sugar intake, non-caloric artificial sweeteners (NAS) have gained popularity as an alternative sweetener. However, a recently published study suggests that artificial sweetener, saccharine, may directly alter the microbiome, leading to dysbiosis, and subsequently to glucose intolerance. Using multiple strategies, including 16rRNA analysis and metagenomic mapping, the study demonstrated that saccharine-fed mice, along with healthy human volunteers, had enhanced energy harvesting glycan-degradation pathways, associated with metabolic disorders and obesity (Suez et al., 2014). Though this is the first study on NAS and microbiome, the epidemiological rise in obesity that coincides with the increased used in NAS underscores the need to correlate food production, dietary guidelines and nutritional recommendations to studies of the microbiome.

Saturated fat has also been in the news recently after a meta-analysis concluded that the evidence of cardiovascular harm from high saturated fat intake was statistically not significant (Chowdhury et al., 2014). Within the same year, a study that followed almost ninety thousand women over a period of 20 years, revealed that though there was a lack of positive association between overall total fat intake and the risk of breast cancer, there was evidence indicating breast cancer risk increases with early adult intake of animal fat (Farvid, Cho, Chen, Eliassen, & Willett, 2014). The ongoing controversy over saturated fat, whether it is good or bad, adds to the plethora of confusing dietary advice. Accordingly, GI studies may help guide best dietary practices because they reveal that diets high in fat alter the microbiome diversity by decreasing the abundance of
Exploring the Role of the Microbiome in Breast Carcinogenesis

*Bacteriodetes*, while increasing both *Proteobacteria* and *Firmicutes*, independent of whether or not the subjects were obese (de Wit et al., 2012; Hildebrandt et al., 2009; Wu et al., 2011).

The impact of alcohol consumption on health outcomes in general is another important area of research. Specific investigation into the gut microbiome of those who frequently consume alcohol reveal a gut flora in dysbiosis, with higher number of *Proteobacteria* and lower average abundance of *Bacteroidetes*, higher levels of serum endotoxins, as well as decreased connectivity in bacterial networks (Mutlu et al., 2012).

Furthermore, continuing to be knowledgeable about the role of microbiome, as well as actions of specific bacterial communities, can shed light on how the microbiome enhances response to chemotherapeutic agents. For instance, identification of chemical inhibitors of bacteria’s B-glucuronidase, demonstrated potential to intensify chemotherapeutic regimen by delaying the side-effect of diarrhea (Wallace et al., 2010).

In terms of breast cancer treatments, methods of staging tumors are currently investigating not only the actual cancer cells, but also corresponding immune-balancing factors, based on bacterial stimulation of CD8+ effector T cells (Mlecnik, Bindea, Pages, & Galon, 2011). The gut microbiome is also serving as a biomarker and therapeutic target for obesity through microbiome transplants (Turnbaugh, Ley, Mahowld, & Gordon, 2013; Turnbaugh et al., 2006). Additionally, probiotic treatments are another area by which bacteria can play a protective role by excluding pathogenic colonization, simulating the host immune system, and enhancing mucosal barriers to invasion (Reading & Kasper, 2011). Likewise, microbial secondary metabolites and bacterial mechanisms
Exploring the Role of the Microbiome in Breast Carcinogenesis

have long been useful in the development of antibiotics and antitumor agents, such as Cisplatin (Joyce et al. 2010; Vaishnav, 2011).

The above examples of how the knowledge of the microbiome can and is being utilized in breast cancer treatments and preventative education is not comprehensive, but rather indicative of the emerging importance of this area of research and highlights the potential health gains that will likely result from further study of the microbiome and its systemic effects.

Table 1—Bacterial Role in the Immune System

<table>
<thead>
<tr>
<th>Bacteria/ Bacterial Actions</th>
<th>Immune and Cancer Effects</th>
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| Bacterial by-products of metabolism | • Stimulates increased inflammatory signals, such as IL-17 cytokines  
• Damages host intestinal wall;  
• Leads to dysbiosis; impaired homeostasis |
| Ex: Nitric Oxide synthase | • Used only by pathogenic bacteria for rapid replication of pathogens (Sheflin et al., 2014). |
| Infection by *Helicobacter hepaticus* in mouse models | • Increased proinflammatory CD4+ CD45RB (hi) lymphocytes; |
Exploring the Role of the Microbiome in Breast Carcinogenesis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Effects</th>
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| **Fusobacterium nucleatum** in cell culture studies | • Activates apoptotic pathways;  
• Directly kill lymphocytes;  
• May promote metastasis and tumor growth  
(Shapira et al., 2013; Kaplan et al., 2010) |
| **Sphingomonas**         | • Stimulates CD8+ effector T cells;  
• Improved response to adjuvant chemotherapy,  
• Better cancer survival rates;  
• Decreased metastasis, recurrence rates  
(Denkert et al., 2010; Shapira et al., 2013; Mlecnik et al., 2011) |
| **Bacteriodes fragilis** | • Induces systemic T-cell regulatory response:  
• Improves immune defects of gnotobiotic mice after monocolonization  
(Ivanov et al., 2009; Reading & Kasper, 2011) |

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Exploring the Role of the Microbiome in Breast Carcinogenesis


Exploring the Role of the Microbiome in Breast Carcinogenesis

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Exploring the Role of the Microbiome in Breast Carcinogenesis


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Exploring the Role of the Microbiome in Breast Carcinogenesis


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Exploring the Role of the Microbiome in Breast Carcinogenesis


Exploring the Role of the Microbiome in Breast Carcinogenesis


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