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Review

REVIEW

The Intestinal Microbiome and Estrogen Receptor–Positive Female Breast Cancer

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Abstract

The huge communities of residential microbes, including bacteria, viruses, Archaea, and Eukaryotes, that colonize humans are increasingly recognized as playing important roles in health and disease. A complex populous ecosystem, the human gastrointestinal (GI) tract harbors up to 10^{11} bacterial cells per gram of luminal content, whose collective genome, the gut metagenome, contains a vastly greater number of individual genes than the human genome. In health, the function of the microbiome might be considered to be in dynamic equilibrium with the host, exerting both local and distant effects. However, 'disequilibrium' may contribute to the emergence of disease, including malignancy. In this review, we discuss how the intestinal bacterial microbiome and in particular how an 'estrobolome,' the aggregate of enteric bacterial genes capable of metabolizing estrogens, might affect women's risk of developing postmenopausal estrogen receptor–positive breast cancer. Estrobolome composition is impacted by factors that modulate its functional activity. Exploring variations in the composition and activities of the estrobolome in healthy individuals and in women with estrogen-driven breast cancer may lead to development of microbiome-based biomarkers and future targeted interventions to attenuate cancer risk.

Humans become colonized at birth by microbiota, primarily bacteria (which are the focus of this review) (1,2), and over 90% reside within the gastrointestinal (GI) tract. The GI tract harbors more than 500 different bacterial species, and estimates of the number of bacteria we carry reach 10^{11} per gram of luminal content (3–5). Bacterial load, along with species diversity, increases from the stomach to the colon, creating a complex microbial ecosystem (6). The composition of the GI tract microbiota reflects host variables, such as delivery mode, genetics, diet, alcohol intake, environmental exposures, and medications, in particular antibiotics. Investigation of bacterial microbiome composition, function, and assessment of the aggregate of its genes (the metagenome) is now possible via advances in 16S ribosomal RNA (rRNA) sequencing and informatics (7–10). Humans and microbes have coevolved a complex intricate relationship to benefit the host while allowing the intestinal microbiota to thrive in a mutually advantageous equilibrium (11–13).

Microbiome perturbation can, however, be associated with risk of developing inflammatory, autoimmune, and malignant disease (14–17). Microbial community dysbiosis, a pathologic disequilibrium, could potentially favor oncogenesis and tumor progression and affect responses to cancer therapy and toxicity profiles of chemotherapeutics (18–20). The human gut microbiome is functional and exerts both local and long-distance effects involving hormonal intermediates, metabolites, and immunologic messengers (21,22). Host-microbe interactions thus have the potential to influence carcinogenesis through mechanisms such as chronic inflammation, induction of genotoxic responses, alteration of the microenvironment, and metabolism (23,24). This could be mediated by the microbial ecosystem as a whole or via specific microbes such as the bacterium *Helicobacter pylori*, which is associated with increased risk of adenocarcinoma of the stomach in humans (25–27).

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The Gut Bacterial Microbiome Includes an Estrobolome

Plottel and Blaser define 'estrobolome' as "the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens" (28). Estrogens are C-18 steroid hormones derived from the stepwise reduction of C-27 cholesterol. The main forms of endogenous estrogens are estradiol (E2, predominant in nonpregnant women prior to menopause), estrone (E1, predominant after menopause), and estriol (E3, highest during pregnancy) (29). Estrogens circulate in the blood in free or protein-bound form and exert diverse biological effects. Parent estrogens (E2, E1) undergo first-pass hepatic metabolism; irreversible hydroxylation at the C-2, C-4, or C-16 positions of the steroid ring result in estrogen metabolites that vary in hormone potency and half-life (30). In the liver, estrogens and their metabolites (including cathechol estrogens via hydroxylation and subsequent methylation) are conjugated through glucuronidation or also through sulfonation to allow for biliary excretion (31). Conjugated estrogens are excreted in bile, urine, and feces (32,33). Studies of injected radioactively labeled estradiol, estrone, and estriol in women indicate that approximately 65% of injected estradiol, 48% of injected estrone, and 23% of injected estriol are recovered in bile (34). As approximately 10% to 15% of injected radiolabeled estradiol, estrone, and estriol are found in conjugated form in feces (35,36), a biologically significant proportion of estrogens are reabsorbed in the circulation. Hepatically conjugated estrogens excreted in the bile can be deconjugated by bacterial species with ß-glucuronidase activity in the gut, leading to their reabsorption into the circulation. Especially relevant are gut bacteria possessing ß-glucuronidases and ß-glucosidases, hydrolytic enzymes involved in the deconjugation of estrogens (Figure 1) (37–41).

Bacterial ß-glucuronidases catalyze the hydrolysis of endogenous ß-glucuronides produced in the liver and exogenous ß-glucuronides found in the diet, such as complex carbohydrates (42–44). Many metabolites, steroid hormones, and xenobiotics are excreted into the intestinal tract by bile after hepatic glucuronidation. The removal of glucuronic acid from conjugated substrates (deconjugation) by intestinal bacterial ß-glucuronidases promotes reabsorption of their respective aglycones into the enterohepatic circulation. Distinct bacterial ß-glucuronidase genes from the human gut microbiota have been described (39,40). The well-characterized *gus* gene is commonly found in gut bacteria (40,45) whereas the BG gene has more recently been described by metagenomic analysis (39). In the human GI tract, the BG gene is well represented in the bacterial phyla Bacteroidetes and Firmicutes whereas *gus* is more common in Firmicutes (41).

ß-glucuronidase activity can be modulated by diet and by bacterial context. Increased fecal ß-glucuronidase activity has been reported in healthy humans consuming diets high in fat or protein whereas fiber consumption decreases activity (46–48). In *Escherichia coli* grown in culture, ß-glucuronidase activity was controlled by bacterial population density, suggesting that quorum sensing in vivo affects enzyme levels (49).

Importantly, bacterial ß-glucuronidases are potential drug targets, as recently shown using ß-glucuronidase inhibitors (19,50). The unique structure of bacterial ß-glucuronidases, containing asparagine and lysine (N-K) residues in the active site modulating functional activity, has been elucidated (19,50). This N-K motif is conserved in ß-glucuronidase but not in ß-galactosidase, a homologue differing at the C-4 and C-5 positions; both catalyze the hydrolysis of similar glycoside substrates (51). Small changes in inhibitor structure can alter conformation within the ß-glucuronidase active site, leading to differences in catalytic activity and pharmacologic inhibition; this was shown by the alleviation of GI toxicity in mice from the chemotherapeutic drug irinotecan (CPT-11) (50). In that study, inhibition of ß-glucuronidase did not affect the serum pharmacokinetics of the drug or its metabolites.

Many bacterial genera and species in the human gut contain genes encoding ß-glucuronidase in humans (Table 1; Supplementary Table 1, available online), underscoring the prevalence of the enzyme and extending earlier observations of individual bacteria obtained from human feces that express ex vivo sulfatase (deconjugative) activity (40,41,52). Circulating inactive steroids are also converted to biologically active estrogens by the hepatic sulfatase pathway (32,52,53). Presumably, gut bacteria expressing sulfatase activity would be capable of hydrolyzing estrogen molecules that had undergone hepatic sulfation and biliary excretion into the GI tract. As such presumed bacterial enzymatic activities and their impact on steroid metabolism and the enterohepatic circulation of estrogens have not been investigated, they remain to be determined.

Studies of fecal samples from healthy volunteers confirm β-glucuronidase and β-glucosidase bioactivity in humans and define optimal collection and processing techniques (54,55). Additional potential enzymatic activities of the estrobolome include dehydrogenation; studies of ex vivo human fecal extracts from the pregenomic era demonstrated both oxidative and reductive reactions affecting several estrogens (56,57). Intestinal bacterial β-glucuronidase and β-glucosidase activity have also been shown to be induced by a high pH, which may also increase colon cancer the risk (58,59). The role of bacterial β-glucuronidase and β-glucosidase activity in breast cancer risk (or risk reduction) is currently unknown.

The series of reductive reactions that allows synthesis of estrogens from cholesterol precursors is catalyzed in part by hydroxysteroid dehydrogenases (HSD), a group of alcohol oxidoreductases (60,61). Bioinformatic annotation of HSDs, present in essentially all complete bacterial genomes, indicate that HSDs are widely found in the normal human gut microbiota (62). An estrobolome enriched in bacterial hydroxysteroid deconjugative activity may contribute to the modulation of the interconversion of conjugated forms of estrogens as well as androgenic molecules, all relevant to the overall estrogenic host milieu (56). Thus, the reach of estrobolome enzymatic functions may extend beyond deconjugation.

Estrobolome Physiology and Perturbation

Experimental evidence for a central role of gut bacteria in estrogen metabolism was noted decades ago. Germ-free rats excreted amounts of free steroid hormones too small to have been characterized by a gas chromatography mass spectrometry technique whereas conventional rats excreted greater, quantifiable amounts of free steroid hormones, reflecting the deconjugative activity of their residential bacteria (63). Introduction of bacteria into the gut of such germ-free mice shown to exhibit impaired reproductive parameters led to normalization of the estrous cycle in females and increased sperm counts in males, all resulting in restoration of overall fertility and reproductive capacity (64).

Goedert and colleagues evaluated the association of fecal microbiome composition and diversity with urinary levels of estrogens and estrogen metabolites in a cross-sectional study of

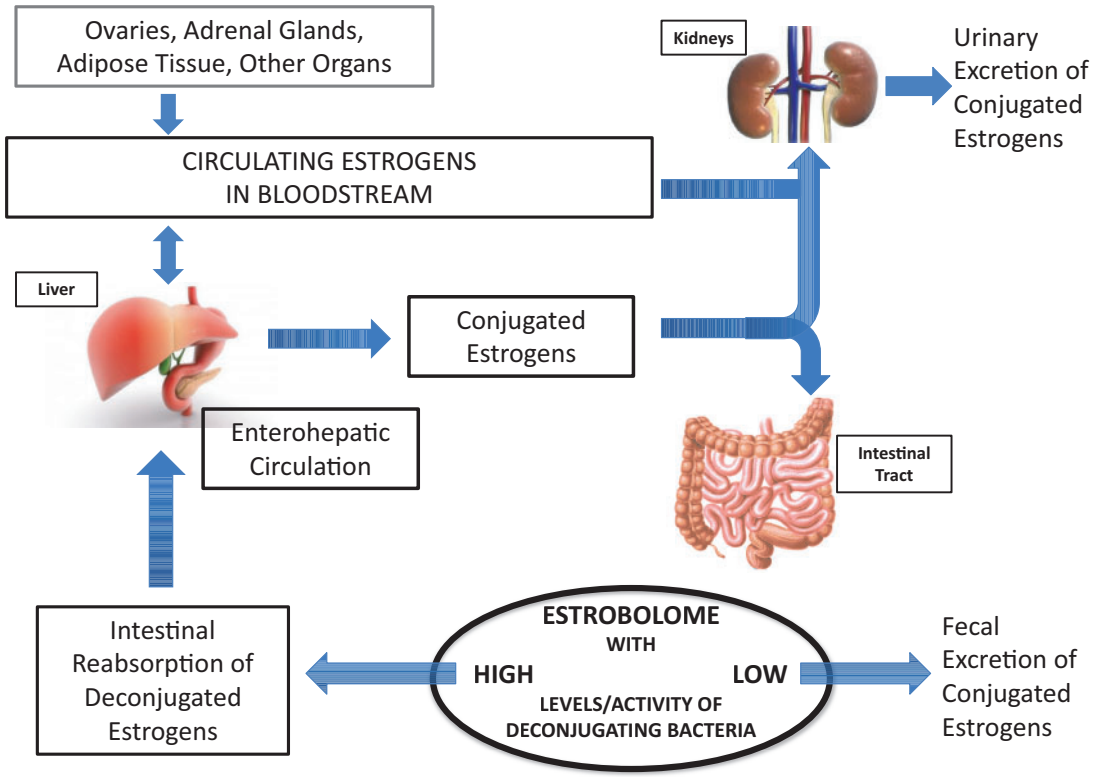

Figure 1. The estrobolome and enterohepatic circulation of estrogens. Estrogens are primarily produced in the ovaries, adrenal glands, and adipose tissue and circulate in the bloodstream in free or protein-bound form and first undergo metabolism in the liver, where estrogens and their metabolites are conjugated. Conjugated estrogens are eliminated from the body by metabolic conversion to water-soluble molecules, which are excreted in urine or in bile into the feces. The conjugated estrogens excreted in the bile can be deconjugated by bacterial species in the gut with beta-glucuronidase activity (constituents of the 'estrobolome'), subsequently leading to estrogen reabsorption into the circulation. Circulating estrogens exert effects on target tissues including breast, which stimulate cellular growth and proliferation. By modulating the enterohepatic circulation of estrogens, the estrobolome affects both the excretion and circulation of estrogens. In turn, the composition of the estrobolome can be shaped by factors such as antibiotics, other drugs, and diet that modulate its functional activity. Adapted from Plottel SC, Blaser MJ. Microbiome and malignancy. *Cell Host Microbe*. 2011;10(4):324-335.

60 healthy postmenopausal women (65). Urinary estrogens (E_2 and E_1) and their metabolites were measured using a standardized approach (66,67), and the fecal microbiome was assessed using bacterial 16S rRNA gene sequencing. Microbial diversity in the fecal specimens was statistically significantly associated with the relationship of estrogen metabolites to parent estrogens (E_2 and E_1); importantly, the ratio of metabolites to parent estrogens was increased with phylogenetic diversity. These observational findings support the hypothesis that differences in estrogen metabolism and levels are associated with variability in gut microbial diversity. Goedert and colleagues also demonstrated a relationship between gut microbial richness, systemic and fecal estrogens, and beta-glucuronidase activity in healthy postmenopausal women and men (68).

Estrogen Exposure and Breast Cancer Risk

In the United States, breast cancer affects one in eight women and is the second leading cause of female cancer death after lung cancer (69). The most common breast cancer subtype is hormone receptor (HR)–positive/human epidermal growth factor (HER) 2–negative, comprising more than 70% of patients (70), with the majority occurring in postmenopausal women (71).

Based on the Surveillance, Epidemiology, and End Results (SEER) database and projections from the United States Census Bureau, the number of new breast tumors, both in situ and invasive, is expected to increase (72). The incidence of HR-positive invasive tumors is projected to increase by 4% annually between 2011 and 2030 in women age 70 to 84 years (from 47 800 to 95 300 patients/year) and 1.6% among women age 50 to 69 years (from 98 000 to 125 700 patients/year). The number of in situ HR-positive tumors is also expected to increase among all age groups combined (from 53 900 to 127 400 patients/year).

Estrogen is recognized as a causal factor in the etiology of HR-positive breast cancer and plays an important role in initiation and promotion of neoplastic growth (73,74) (Figure 2). Decades ago, Trichopoulos advanced the hypothesis that greater concentrations of estrogens in pregnancy increase the probability of breast cancer in the offspring years later, drawing attention to the potential oncogenic significance of estrogens (75). States of relative estrogen excess in pregnancy, such as twin gestation, are associated with increased relative risks of breast cancer in daughters born of those pregnancies (76), as with intra-uterine exposure to the synthetic estrogen diethylstilbestrol (77).

States of relative estrogen excess contribute to hormone-driven breast cancer in postmenopausal women (78), confirmed by a meta-analysis of nine prospective studies (n = 663 women with breast cancer and 1765 women without breast cancer) demonstrating a statistically significant association between endogenous sex hormone levels (including E2 and E1) and breast cancer in postmenopausal women (79). For example, the relative risk of developing breast cancer for women who had estradiol levels in the highest quintile compared with women with levels in the lowest quintile was 2.0 (95% confidence interval [CI] = 1.47 to 2.71).

The ratio of estrogen metabolites to parent estrogens has also been linked to postmenopausal breast cancer risk. The risk

Table 1. 60 bacterial genera colonizing the human intestinal tract that encode ß-glucuronidase and/or ß-galactosidase + (157)*

Genus	ß-glucuronidase	ß-galactosidase
Collinsella	+	—
Edwardsiella	+	—
Alistipes	+	+
Bacteroides	+	+
Bifidobacterium	+	+
Citrobacter	+	+
Clostridium	+	+
Dermabacter	+	+
Escherichia	+	+
Faecalibacterium	+	+
Lactobacillus	+	+
Marvinbryantia	+	+
Propionibacterium	+	+
Roseburia	+	+
Tannerella	+	+
Actinomyces	—	+
Alistipes	—	+
Anaerostipes	—	+
Bacteroides	—	+
Barnesiella	—	+
Bifidobacterium	—	+
Blautia	—	+
Butyricicoccus	—	+
Butyrivibrio	—	+
Catenibacterium	—	+
Cedecea	—	+
Cetobacterium	—	+
Citrobacter	—	+
Clostridium	—	+
Collinsella	—	+
Coprobacillus	—	+
Coprococcus	—	+
Dorea	—	+
Dysgonomonas	—	+
Enterobacter	—	+
Enterococcus	—	+
Eubacterium	—	+
Fusobacterium	—	+
Hafnia	—	+
Holdemania	—	+
Klebsiella	—	+
Lactobacillus	—	+
Megamonas	—	+
Mitsuokella	—	+
Odoribacter	—	+
Paenibacillus	—	+
Parabacteroides	—	+
Paraprevotella	—	+
Pediococcus	—	+
Porphyromonas	—	+
Prevotella	—	+
Pseudoflavonifractor	—	+
Roseburia	—	+
Ruminococcus	—	+
Staphylococcus	—	+
Streptococcus	—	+
Subdoligranulum	—	+
Turicibacter	—	+
Weissella	—	+
Yokenella	—	+

*+= Human Microbiome Project (HMP) gut-associated microbial genomes (n = 517) were indexed for the presence of ß-glucuronidase (EC 3.2.1.31) or ß-galactosidase (EC 3.2.1.23) using the Integrated Microbial Genomes database (1). Taxa were classified at the genus level.

increased with higher circulating concentrations of the parent estrogens (E2 and E1). Risk decreased with increasing ratios of 2- and 4-pathway estrogen metabolites to parent estrogens via hydroxylation by hepatic cytochrome P450 (80) and metabolic pathways favoring estrogen 2-hydroxylation over 16α-hydroxylation (81–83). Furthermore, 2-, 4-, and 16α-hydroxyestrogens may be considered carcinogenic (84).

Administration of exogenous estrogens for five to seven years in the placebo-controlled Women's Health Initiative trials (n = 27 347 postmenopausal women) demonstrated a statistically significant increase in breast cancer during and after the intervention period for combined estrogen and progestin treatment but not for treatment with estrogen alone (85).

How the Estrobolome May Affect Estrogen Levels and Breast Cancer Risk

An important role of the intestinal microbiome is the modulation of systemic estrogens (38–41) as it affects the enterohepatic circulation of estrogens and their reabsorption. We have postulated that, in theory, an estrobolome enriched in enzymes favoring deconjugation would promote reabsorption of free estrogens and thus increase relative total estrogen burden, potentially contributing to the risk of development of hormone-driven malignancies such as breast cancer (Figure 3). The bacterial composition of the estrobolome in turn is likely affected by host factors such as age and ethnicity, as well as lifetime environmental influences including diet, alcohol, and antibiotic use, which may exert selective pressures on bacterial populations (86–88). Some of these factors have also been independently linked to breast cancer risk. Possible overlapping or additive modulators are discussed below.

Antibiotics

A large body of evidence, dating back several decades, indicates that antibiotics perturb bacterial gut populations. Ampicillin administration causes large increases in fecal conjugated estrogens and reduction in urinary estrogen in women (89–91). Healthy men given oxytetracycline had increased fecal conjugated estrogen excretion in parallel with a decrease in urinary estrogen excretion (92). Experimental data point to a decrease in fecal beta-glucuronidase enzyme activities when rats were administered antibiotics (93). These studies suggest that certain antibiotics, by promoting increased excretion of (conjugated) estrogens, reduce the overall deconjugative activity of intestinal bacteria, at least in the short-term and could hypothetically lower breast cancer risk. However, long-term consequences of antibiotics on estrogen excretion have not been studied.

Several epidemiologic studies, in contrast, have suggested a possible association of antibiotic use and breast cancer risk (94–98). In a North American case-control study involving 2266 women with breast cancer and 7953 healthy controls, increased prior antibiotic use was associated with an increased risk of breast cancer, with an estimated odds ratio of 2.07 (95% CI = 1.48 to 2.88) in women who had received long-term antibiotic treatment (94). In the study, all classes of antibiotics were associated with increased breast cancer risk, and the association remained after adjustment for factors including premenopausal or postmenopausal status, age at menarche, and family history. Other studies showed a possible association (94), but this was not universally observed (96,97). In a nine-year follow-up of 2.1 million women, a slightly increased risk of breast cancer with antibiotic

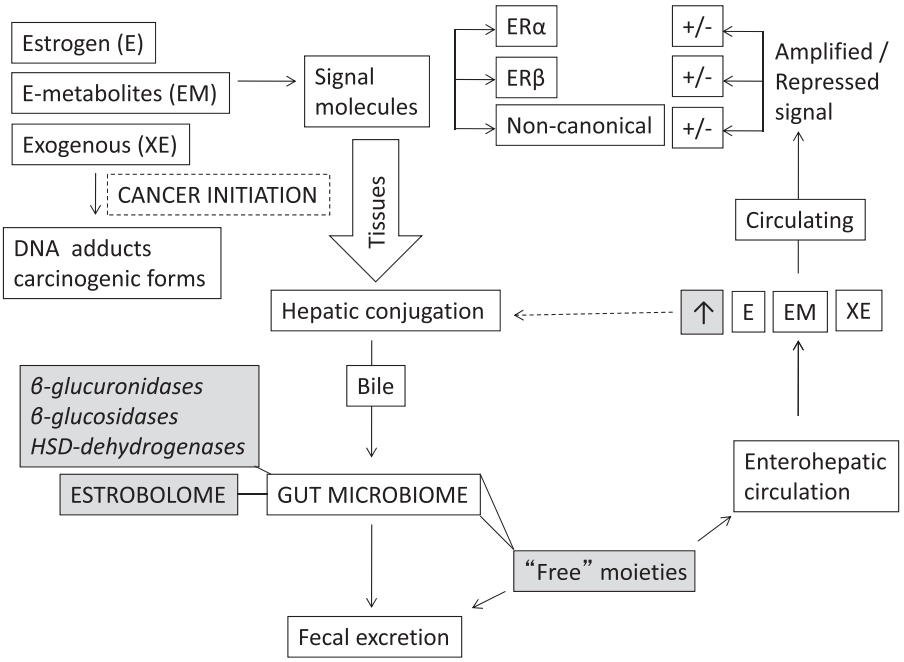

Figure 2. Potential impact of the estrobolome on estrogens. Forms of endogenous estrogen (E) including E2 (estradiol) and E1 (estrone), estrogen metabolites such as hyroxylated moieties, and exogenous estrogens are relevant to breast tissue carcinogenesis. Estrogens act as signaling molecules via several pathways including canonical paths (alpha and beta receptors) and noncanonical mechanisms. Receptors for estrogen are widely distributed in tissues. Estrogen circulates both freely and protein bound. In the liver, estrogens undergo E2 and E1 interconversion and first pass metabolism. Hepatic conjugation of estrogen allows for biliary excretion of conjugated estrogen and conjugated estrogen metabolites into the gastro-intestinal tract where beta-glucuronidases, glucosidases, and hydroxysteroid dehydrogenases of bacterial origin (the estrobolome fraction of the microbiome) regenerate "free" forms of those molecules. The entero-hepatic circulation therefore contributes to plasma levels of estrogens and their metabolites. An estrobolome enriched in bacteria whose enzymatic activity is higher in deconjugative and hydroxylating function would lead to greater relative levels of circulating free estrogens.

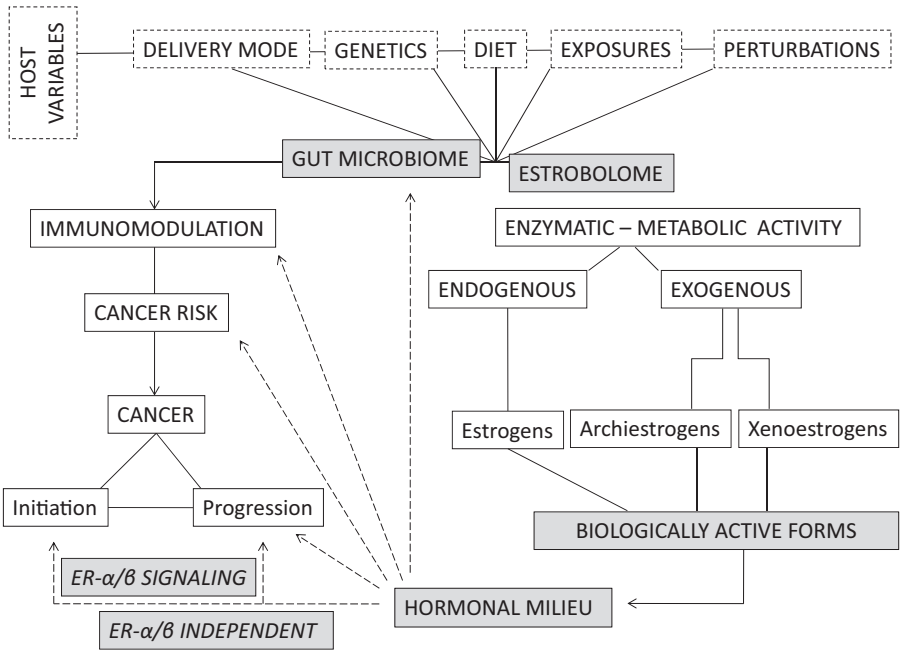

Figure 3. Potential link of the gut estrobolome and breast cancer risk. The estrobolome/microbiome modulates levels of circulating estrogens and thereby the endogenous hormonal milieu, which can affect the risk of hormone-driven malignancies including breast and endometrial cancer directly or indirectly, for instance via immunomodulation. Higher deconjugating activity in the gut may lead to higher relative plasma levels of estrogen and metabolites, potentially increasing cancer risk, whereas either lower deconjugating activity may have opposite effects. Conversely, the microbiome is modulated by factors such as the environment, host variables, and possibly sex steroid levels.

exposure (hazard ratio [HR] = 1.14, 95% CI = 1.10 to 1.18) was observed, but there was little evidence of a dose response, with a hazard ratio of 1.17 (95% CI = 0.97 to 1.42) for long-term use (98). A recent population based study involving 31 131 women with breast cancer found, after multivariable adjustment for body mass index (BMI), smoking, alcohol use, diabetes, and hormone replacement therapy use (99), only a moderate increase in breast cancer risk with the subset that received antibiotics (100). These studies therefore suggest a possible but small increase in breast cancer risk with use of some antibiotics, but it is important to keep in mind that antibiotic use is so ubiquitous (101) that unaccounted exposures could substantially affect epidemiologic findings. As the microbiome may be most prone to modulation by antibiotics during the first years of life (102), additional information and adjustment for antibiotic exposure in childhood may be needed.

Adiposity and Diet

Adiposity has been associated with higher circulating estrogen levels in postmenopausal women, as well as with increased breast cancer risk (103,104). Approximately 35% of the adult world population is either overweight (BMI = 25–30 kg/m^2) or obese (BMI $\geq$ 30 kg/m^2), with a rising incidence (105). A meta-analysis of 50 prospective observational studies confirmed a relationship between adult weight gain in women and risk for cancer; each 5 kilogram increase in weight was associated with increases in postmenopausal breast (+11%), ovarian (+13%), and endometrial cancers (+39%) (102). In postmenopausal women, obesity and excess adiposity may lead to increased circulating estrogens through the peripheral aromatization of androgens but also can induce insulin resistance, increase insulin-like growth factor (IGF)–1, and suppress production of hepatic hormone-binding proteins, thereby increasing total and bioavailable estrogens (106).

Changes in diet are known to affect overall gut microbiome composition and function (107). Strict vegetarians have increased fecal excretion of conjugated estrogens compared with nonvegetarians, leading to decreased plasma estrogen concentrations (108,109). Diets rich in fats and red meat promote the production of bile acids necessary for fat digestion and absorption. Microbiota then metabolize these compounds into secondary bile acids (110,111). An American study compared 10 premenopausal women consuming a "Western diet" that included high fat (40% of calories) and low fiber with 10 age-matched vegetarians consuming a high fiber and moderate fat (30%) diet; the vegetarians had triple the estrogens in feces and 15% to 20% lower serum estrogen levels (108). Fecal bacterial ß-glucuronidase activity was also statistically significantly lower in the vegetarians than in the omnivores ($P < .05$). Immigrants from Asia consuming a low fat diet (20%-25% of calories) had systemic estrogen levels 30% lower than American women eating a high-fat diet (109) . Diet is possibly associated with estrogen metabolism and levels, possibly via the estrobolome, although additional factors such as lifestyle, exercise, and supplements may contribute. In addition, the potential impact of complex dietary fibers that might induce bacterial enzyme selectivity in the estroblome is currently unknown and remains investigational.

Hypercholesteremia is a risk factor for ER-positive breast cancer (112). The cholesterol metabolite 27-hydroxycholesterol (27HC) has been shown to possess estrogenic activities and to promote breast tumor growth in xenograft mouse models by binding to the ER on epithelial cells of mammary glands and stimulating cellular proliferation (113). In humans, 27HC is enriched in ER-positive breast tumors although its role in the pathogenesis of breast cancer remains to be defined (114,115).

Alcohol

Alcohol consumption increases the risk of breast cancer, in particular ER-positive tumors in postmenopausal women (116–120). A recent study showed a positive association between alcohol consumption and endogenous estrogen levels and mammographic density in premenopausal women (121). Similarly, alcohol intake after breast cancer diagnosis is associated with both increased risk of recurrence and death (122). While the pathophysiology is likely multifactorial (120), daily alcohol consumption increases serum estrogen levels, particularly E1 (123,124) but also E2 (125) and other forms of estrogen (126–128). Several biological mechanisms may account for the association of alcohol consumption with the development of breast cancer: 1) alcohol may increase the activity of ER signaling in breast tumors or may increase endogenous steroid hormone levels (123,128–130); 2) ethanol may stimulate the transcriptional activity of ER-α ligand in human breast cancer cell lines in a dose-dependent manner while downregulating expression of BRCA1, an inhibitor of ER-α transcriptional activity (129); and 3) among healthy postmenopausal women who were not on HRT and who consumed 15 to 30 g of alcohol per day, concentrations of serum estrone sulfate were increased by 7.5% and 10.7%, respectively, compared with levels in postmenopausal women who did not consume alcohol (123).

One mechanism contributing to the observed association of alcohol and elevated circulating estrogens could be mediated via gut microbial populations although precise interactions require definition. Alcohol consumption may lead to small intestinal bacterial overgrowth (SIBO) (131). Both gut anaerobic and aerobic bacteria are present at higher levels in subjects with chronic alcohol abuse and alcoholic cirrhosis compared with healthy controls (132–134). SIBO has also been observed in animal models of alcoholic liver disease. Alcohol consumption alters the composition of the colonic microbiome in rats (135). Ethanol may affect the metabolism of intestinal bacteria although it has not been well studied. In a rat model of metabolic alterations of GI tract luminal contents following chronic ethanol consumption (136), pathways that were altered affected fatty, bile, and amino acids, as well as steroids, including 4-hydroxyestrone. In animal models, 4-hydroxyestrone, a catechol estrogen metabolite, has biologically significant estrogenic activity and has been shown to be involved in estrogen-induced tumorigenesis (137). The interactions between alcohol, estrogens, and breast carcinogenesis in humans need greater definition.

Probiotics and fermented foods containing lactic acid bacteria have been explored for anticancer properties, which may involve modulation of the intestinal microbiome and the host immune response (138). Human subjects who consumed oral supplements of *Lactobacillus acidophilus* demonstrated a reduction in the activity of fecal enzymes, including β-glucuronidase (139,140). In several epidemiologic studies, consumption of fermented milk products was associated with decreased risk of breast cancer (141,142), possibly through changes in the intestinal microbiota altering the enterohepatic cycling of estrogenic compounds.

Microbiome of Human Breast Tissue

Decades ago, a link between the bile acids in the gut and cystic breast tissue in humans was identified (143,144). The bile salt

lithocholate, proven to be originating from the intestines, was found in the aspirate of cyst fluid from the breast in women with fibrocystic disease at much higher concentrations than in the serum (143). While the mechanism for maintenance of high bile concentrations in the breast cysts remains to be studied, the farnesoid X receptor (FXR), a bile acid receptor, has been identified in the benign and malignant breast microenvironment (145). Bile acids such as deoxycholic acid (DCA) have been shown to stimulate the growth and metastases of breast cancer cells through FXR, suggesting that bile acids may play a role in breast tumor carcinogenesis.

While beyond the scope of this review of the gut microbiome and breast cancer, microbiota of human breast tissue has also been studied and revealed differences between breast tumor tissues compared with paired normal tissue (146–148). It is unknown if these findings reflect local dysbiosis creating an environment that favors breast tumor formation, ie, oncogenic triggers, or reflect host selection for microbes adapted to the fatty acid-rich environment in the breast tissue. This, however, suggests that, as with other parts of the body, breast tissue has a unique microbiome.

Conclusion and Further Directions

Interest is growing in the dynamic roles of the microbiome in human health and disease. Investigations exploring the potential interactions of the gut microbiome and breast cancer span the translational research spectrum and require collaborations between basic scientists, including immunologists, cell biologists, and microbiologists, clinicians such as oncologists and endocrinologists, animal researchers, epidemiologists, biostatisticians, and bioinformaticians. The ongoing Microbiome Quality Control Project (149) and the International Human Microbiome Standards (IHMS) Project (150) address sample collection, sample storage, standardization, and reproducibility (151–153); microbiome researchers should follow their recommendations in planning studies. In particular, fecal sample biobanking should be incorporated in the study design of longitudinal epidemiologic investigations.

Much of our understanding of the enterohepatic circulation of estrogens is informed by studies performed before 1970. Recent advances in analytical methods, including accurately and sensitively measuring different conjugated and unconjugated estrogens in serum and urine should provide tools for meaningful comparisons between individuals (67,154). Animal models serve to test hypotheses under controlled conditions. Observational large-scale human studies are needed to identify and confirm associations while controlling for other (genetic, epigenetic, dietary, and environmental) variables affecting the microbiome and confounders of cancer risk. A recent study investigating differences in gut microbiome composition among postmenopausal women showed a less diverse fecal microbiome and a statistically significantly altered composition in newly diagnosed breast cancer patients (87% had ER-positive tumors) compared with healthy controls (155), and this finding deserves follow-up.

Further studies are needed to evaluate the estrobolome hypothesis and to define the impact of the microbiome's metabolic capacity on estrogen metabolism and host physiology. Determining the "functionality" of differing microbiomes vis-à-vis deconjugative activity is essential. We are currently conducting a prospective case-control study in postmenopausal women with recently diagnosed ER-positive breast cancer as well as healthy controls to assess the relationship between the gut bacterial microbiome/β-glucuronidase activity, circulating estrogen levels, and breast cancer. If the estrogen metabolism–gut microbiome axis is functional with underlying individual variations in estrogen levels, it is plausible that the estrobolome could contribute to the risk of hormone-driven malignancies including breast cancer and as such could serve as a potential biomarker (156). Furthermore, interventions that may include use of prebiotics, probiotics, or antimicrobial agents could be designed specifically to target gut bacterial species with β-glucuronidase activity (19,50) to decrease estrogen-related cancer risk or become components of future therapies. In conclusion, links between the microbiome and estrogen-driven breast cancer are growing, and we hope that research will identify specific characteristics of the gut microbiome that can be used to develop novel approaches for breast cancer risk assessment, prevention, and treatment.

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