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Review Article

Vaginal microbiomes and ovarian cancer: a review

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Abstract: The human microbiome, often termed as "the forgotten organ", is an aggregation of microorganisms and their genomes that forms a mutualistic complex with the host. Recent research has shown the symbiotic merits of a microbiome ecosystem and its crucial role in the hosts' physiological functions. Disruption of this symbiotic relationship is prone to cause a broad spectrum of ailments, including cancer. The compositional and environmental factors that tip the scales from beneficial co-existence to the development of malignancy is actively investigated. Herein we review the latest research in knowledge regarding the association between the vaginal microbiomes and oncogenesis, with a particular focus on ovarian carcinoma.

Keywords: Vaginal microbiome, ovarian cancer, cervical cancer, HPV, immune response

Introduction

The human microbiota is a consortium of bacteria that reside within different body sites and ecological niches, whereas the human microbiome is the collective genomes of all microbial species and their environment [1]. The plurality of these microbiotas subsists in a mutualistic association with their human host. Human microbiota has demonstrated a crucial role in our body's immunity, metabolism, and endocrine [2, 3]. Given the emphasis on microbiomes in gastrointestinal disease development [4], recent studies are beginning to support the interactive role of vaginal microbiomes in gynecological diseases. For example, *Prevotella* species are often associated with bacterial vaginosis and cervicitis [5, 6], while vaginal bacterial communities dominated by *Lactobacillus gasseri* are correlated with increased clearance of HPV infection [7]. *Lactobacillus iners* are linked to an increased risk for *Chlamydia trachomatis* infection and also prevails in the occurrence of HPV infection and cervical intraepithelial neoplasia (CIN) [8-10]. Despite progress in understanding the role of the microbiomes in cervical cancer, investigations regarding the role of microbiome in ovarian cancers are limited. Ovarian cancer is the fifth most commonly diagnosed cancer among women in the United States [11]. Among them, ovarian cancer accounts for more death than any other reproductive cancer, with an estimated 22,530 new cases and nearly 13,980 death in 2019. Despite the high prevalence and public health significance, the etiology of this disease remains largely elusive.

Microbiomes are essential in the prevention of pathogen invasion; therefore disruption of the dynamics between the microbiome and the host vaginal ecosystem is prone to cause vaginal tract infection and cancer [12]. In this review, we discuss the possible roles of vaginal microbiota in carcinogenesis, highlighting the relationship of micro-organisms and viral infection in ovarian cancer.

Vaginal microbiota: an overview

Although the physiological role of the gut microbiota has been explored for decades [13], investigations of microbial compositions have recently extended to the female reproductive system (**Table 1**). Through molecular amplification techniques such as qPCR and DNA sequencing, studies have identified lactic acid-producing *Lactobacillus crispatus, L. gasseri, L.*

Table 1. Summary of major microbiome studies involving gynecological cancers

Author	Disease	Microbiome specimen	Year	Microbiome evaluation	Microbial change
Shannon	HPV infection	Endocervical cytobrushes	2017	16S rRNA sequencing	↑Anaerobes ↓*L. gasseri, Fusobacterium nucleatum*
Brotman	HPV infection	Midvaginal swabs	2014	16S rRNA sequencing	↓*Lactobacillus* spp.
Chao	HPV infection	Posterior vaginal fornix	2019	16S rRNA sequencing	↑*Leptotrichia* and *Prevetella* ↓ *Lactobacillus* spp.
Lee	HPV infection	Endocervical brush	2013	16S rRNA sequencing	↑Sneathia spp.
Paola	HPV persistence	Cervico-vaginal samples	2017	16S rRNA sequencing	↑*Atopobium* spp., *G. vaginalis*
Wu	HPV persistence	Cyto-brush	2018	16S rRNA sequencing	↑*Prevotella*, *Dialister*
Adebamowo	HPV persistence	Mid-vaginal swabs	2017	16S rRNA sequencing	↑*Mycoplasma hominis*
Piyathilake	HSIL	Merocel ophthalmic sponges placed in cervical os	2016	16S rRNA sequencing	↑*L. iners*
Kwasniewski	LSIL	Cervical swabs	2018	16S rRNA sequencing	↑*L. acidophilus, L. iners* ↓*L. crispatus*
Kwasniewski	HSIL	Cervical swabs	2018	16S rRNA sequencing	↑*G. vaginalis , L. acidophilus* ↓*L. crispatus, L. taiwanensis, L. iners*
Oh	CIN	Cervical Sampler Brush	2015	16S rRNA sequencing	↑*A. vaginae, L. iners, G. vaginalis* ↓*L. crispatus*
Godoy-Vitorino	CIN	Cervical samples (posterior fornix)	2018	16S rRNA sequencing	↑*A. vaginae, G. vaginalis*
Bhatla	Cervical cancer	Cytobrush	2013	16S rRNA sequencing	↑*C. trachomatis*
Zhao	Ovarian cancer	Fresh ovarian cancer tissues	2019	16S rRNA sequencing and qPCR	↑*Proteobacteria, Firmicute*
Banerjee	Ovarian cancer	Ovarian cancer tissue	2017	PathoChip Array	↑*Proteobacteria, Firmicutes, Brucella, Chlamydia, Mycoplasma*
Emara	Ovarian cancer	Ovarian cystic fluid and ovarian cancer tissue	2007	ovarian cystic fluid culture	↑*Brucella*
Shanmughapriya	Ovarian cancer	Fresh ovarian tissues	2012	nested PCR-based assay	↑*Chlamydia*
Chan	Ovarian cancer	Human ovarian cancer tissue	2012	PCR-ELISA	↑*Mycoplasma*
Trabert	Ovarian cancer	Serum samples	2018	multiplex, fluorescent bead-based assay	↑*C. trachomatis*
Di Giovanni	Ovarian cancer	Ovarian cancer tissue	2016	Bacterialogical Culture	↑*Mycobacterium* spp.
Nene	Ovarian cancer	Cervical smear samples	2019	16S rRNA sequencing and qPCR	↓*Lactobacillus* spp.
Ness	Ovarian cancer	Blood samples	2003	Serologic testing for IgG antibodies	↑*Chlamydia*
Walther	Endometrial cancer	Vaginal and cervical swabs	2017	16S rRNA sequencing and qPCR	↑*A. vaginae, Porphyromonas* spp.
Hokenstad	Endometrial cancer	Vaginal and cervical swabs	2017	RT-PCR, FISH	↑*Porphyromonas somerae*

iners and *Lactobacillus jensenii*, which dominate the vaginal communities of most reproductive-age healthy women [14]. Besides the Lactobacillus genus, a heterogeneous group of strictly anaerobic bacteria was also reported, including *Prevotella, Atopobium, Gardnerella, Dialister, Sneathia, Megasphaera, Peptoniphilus, Finegoldia, Eggerthella,* and *Aerococcus*. [15]. These microbial florae were classified as five main community state types (CSTs) by Ravel *et al.* [15].

The vaginal microbiota is diverse in population and ethnicity. A healthy vagina microbiome was previously thought to be dominated by *Lactobacillus* species [16]. However, advanced technologies and additional studies using an ethnically diverse cohort of women have revealed a more complex landscape [15, 17]. Some studies indicated that the vaginal microbiomes in some healthy women are composed of *Gardnerella, Atopobium, Prevotella, Pseudomonas*, or *Streptococcus* species rather than *Lactobacillus* [18]. A study evaluated the microbiota composition of the six largest ethnic groups (African Surinamese, Dutch, Ghanaian, Moroccan, South-Asian Surinamese, and Turkish) in Amsterdam, the results showed African Surinamese ethnicity, and Ghanaian ethnicity were correlated with vaginal microbiotas con-

taining *Gardnerella vaginalis*, and African Surinamese ethnicity with vaginal microbiotas dominated by *L. iners* [19]. Ravel *et al.* [20] studied the vaginal microbiome of 396 North American women from four ethnic backgrounds (Asian, Black, Hispanic, and White). Their study showed a significant discrepancy in vaginal microbiome composition. Vaginal bacterial communities dominated by species of *Lactobacillus* were found in 89.7% and 80.2% of White and Asian women, but in only 59.6% and 61.9% of Hispanic and black women. In contrast, a lower prevalence of communities dominated by *Lactobacillus* spp. was seen in Hispanic and black women. Another research showed that *L. iners, L. jensenii,* and *G. vaginalis* were prevalent in Canadian women [21]. Similarly, the study on Belgian women observed a prevalence of *L. crispatus, L. iners,* and *Prevotella* [22]. These observational reports further illuminate the broad spectrum of vaginal microbiota composition across different demographic backgrounds.

A healthy vaginal microbiome is considered the first line of defense. *Lactobacillus* up-regulates tight junction proteins that inhibit pathogen migration and improves epithelial integrity. The possibility of controlling pathogenesis by co-aggregating *Lactobacillus* with pathogens and thereby tying up the latter's ability to spread across surfaces, or by interfering with virulence expression such as the production of toxins, has also been considered [23]. In addition, *L. crispatus* and *L. jensenii* could minimize the effect of inflammation through inhibiting the release of pro-inflammatory mediators from vaginal epithelial cells [24]. The metabolites of the *Lactobacillus* species, besides lactic acid and other acidic compounds, hydrogen peroxide, and bacteriocin-like compounds, may be pivotal to cervicovaginal homeostasis [25].

Changes in vaginal microbiomes are associated with host reproductive fitness. A recent study on sub-Saharan African women found significant differences in vaginal community composition in those developing bacterial vaginosis. Of these women, the composition of the *Lactobacillus* genus and *Lactobacillus vaginalis* were significantly lower, and the composition of *G. vaginalis*, *A. vaginae*, and *P. bivia* were higher after developing bacterial vaginosis [26]. Recent research also identified *Mycoplasma genitalium* as a possible cause for pelvic inflammatory disease (PID), a contributor to epithelial ovarian cancer [27]. Alterations in the vaginal composition are associated with bacterial dysbiosis, which could give rise to cancer-promoting virulence factors.

Vaginal microbiomes and gynecological cancer

Ovarian cancer

Ovarian cancer is a major threat to female health, ranking seventh in the most commonly diagnosed cancer among women worldwide [28]. The early stage of the disease is often asymptomatic, and most patients remain undiagnosed until advanced stages of cancer [29], thus finding specific biomarkers for early diagnosis is of utmost importance. Despite well-characterized risk factors (e.g., family history, age, inflammation, reproductive factors, benign gynecologic conditions, and gynecologic surgery) and genetic susceptibility (e.g., mutations in BRCA1 and BRCA2 genes) [28, 30], its etiology is not fully understood. Recent studies have indicated that many microorganisms are involved in the development of ovarian cancer (**Figure 1A**) [31, 32]. Zhao [33] and colleagues compared ovarian cancer tissue sample (n = 25) with tissues from normal distal fallopian tubes (n = 25) using 16s RNA sequencing. Comparatively, the microbiome diversity and richness was significantly decreased in the ovarian cancer samples. At the phylum level, a significant increase in *Proteobacteria* and *Firmicutes* abundance was seen in ovarian cancer tissue samples, suggesting an association between microbiome compositional change and ovarian cancer development (**Figure 2**). Similarly, in a study by Banerjee *et al.* [34], a microarray-based approach was applied to identify microbial signatures unique to ovarian cancer. Specifically, two predominant bacterial phyla were detected, consisting of *Proteobacteria* (52%) and *Firmicutes* (22%). Additionally, they detected *Brucella, Chlamydia*, and *Mycoplasma* in over 60% of the ovarian cancer sample screened. Their finding is consistent with previous investigations, also highlighting an enrichment of *Brucella* [35], *Chamydia* [36], and *Mycoplasma* [37] in ovarian cancer tissues. Researches investigating the role of *M. genitalium, C. trachomatis*, or *Neisseria gonor-*

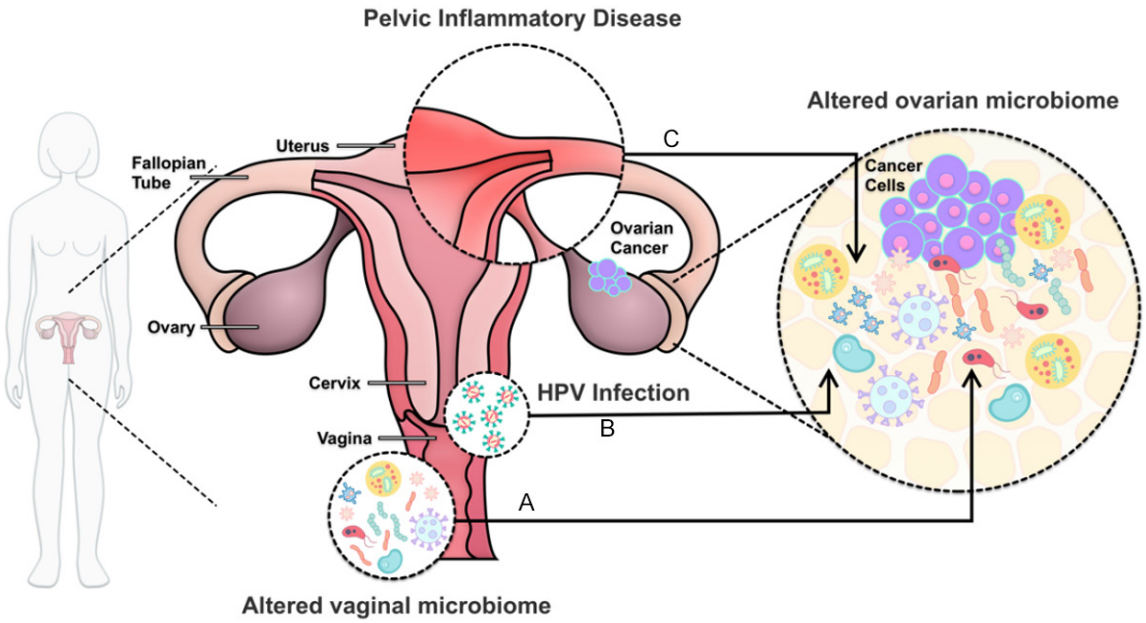

Figure 1. Interactions between female reproductive tract microbiota and the development of ovarian cancer. A. Microbiome compositional alterations in both ovarian and cervicovaginal mircroenvironment have been shown to correlate with the occurence of ovarian cancer. B. HPV genotypes may contribute to ovarian cancer progression. C. PID is related to the etiology of ovarian carcinoma, specifically pathogens like *Chlamydia* are associated with higher risk of developing ovarian cancer.

rhoeae in ovarian cancer suggest a relationship between these microbes and the development of ovarian cancer. A multicenter study evaluated more than 1,100 samples collected from the association of the serologic markers of *C. trachomatis* and ovarian cancer in two separate populations. One is a case-control study in Poland containing 800 subjects, and another is a prospective case-control study in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial consisting of 319 subjects. They found an association between doubled ovarian cancer risk with Pgp3, the antibodies against *Chlamydia* plasmid-encoded protein, also the gold standard in determining *Chlamydia* infection [38]. There is also a report of endometrial tuberculosis stimulating ovarian cancer [39]. Nevertheless, contradictory results were reported, which found no positive indications for the presence of *C trachomatis, N. Gonorrhoeae*, *M genitalium*, and HPV from a cohort of 186 women with ovarian cancer, borderline tumors, or benign conditions [40]. The variance of the sample probably leads to inconsistent results, since ovarian cancer exhibits various histologic types and differs from pathogenesis and tumor microenvironment [41].

More recently, Martin and colleagues, utilizing 16s RNA, compared the cervicovaginal microbial profile of patients with ovarian cancer or patients with BRCA1 mutations with healthy matched controls. Identified microorganism compositions were then classified based on *Lactobacilli* species proportion. Those in which *Lactobacilli* species accounted for at least 50% were labeled community type L, and vice versa community type O. This study demonstrated that ovarian cancer or its risk factors (i.e., age and BRCA mutations) is significantly correlated with a community type O cervicovaginal microbiota [32]. These finds suggest cervicovaginal microbiota dysbiosis may play a role in ovarian cancer tumorigenesis. However, whether such dysbiosis could be altered through the reinstallment of community type L microbiome [32], or whether changes in microbiome composition could translate into ovarian cancer protection, remains to be investigated.

The association between ovarian cancer and hrHPV were extensively studied, despite inconsistent results (**Figure 1B**). HPV was found associated with malignant transformation of mature cystic teratoma (MCT) into primary squamous cell carcinoma (SCC) [42]. A recent

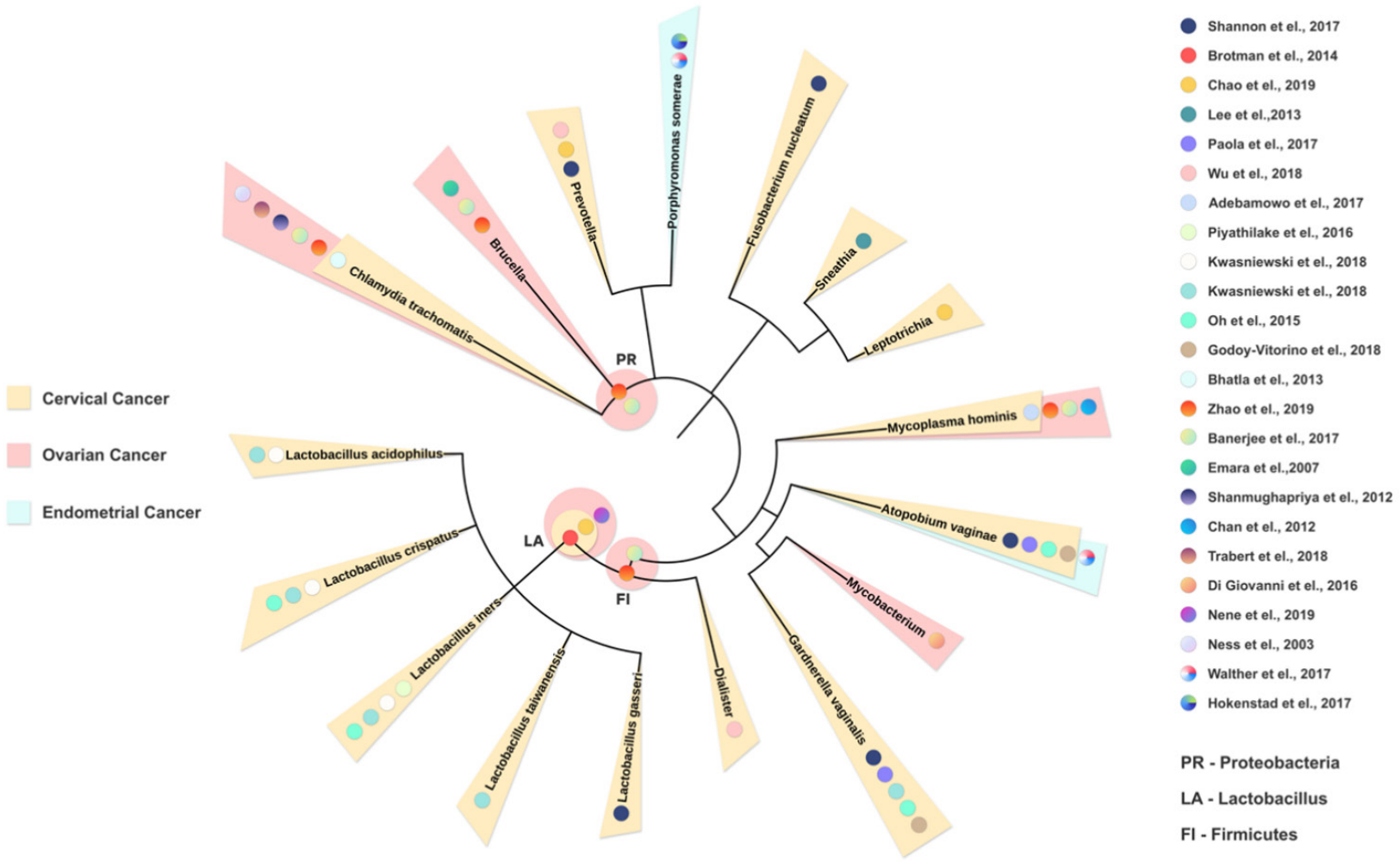

Figure 2. Phylogenetic tree summarizing established links between female reproductive tract microbiome and gynecological cancers. We constructed a phylogenetic tree using evolutionary distance with phyloT software [95] to describe the phylogenetic similarity of all microbiome reported to be associated with gynecological cancer in human studies. From the inside out, representing from the wider (kingdom) to the more specific (species) taxonomy. Based on the phenotype of the various studies included, the bacterial taxonomy is labeled according to the source of publication (colored dots) and shaded (OC = ovarian cancer, light red; CC = cervical cancer, light yellow; EC = endometrial cancer, light blue) based on the cancer types.

study was conducted in Hunan province to investigate the prevalence of HPV infection in epithelial ovarian cancer (EOC). They found HPV18 was positive in 7.76% malignant EOC patients, 9.09% in benign ovarian cancers, while only 1.01% in healthy persons. HPV33 was positive in 12.11% of malignant EOC samples, and 6.06% benign samples, whereas merely 1.51% in healthy people. These results showed a high correlation of HPV 18 and 33 with the ovarian cancer development [43]. Another study achieved similar results that HPV infection was associated with advanced stages of ovarian cancer [44]. In this study, HPV was detected in 10% EOC cases, with the most prevalent genotypes being HPV16 and 18 followed by HPV33. For patients infected with HR-HPV genotypes, they also suggested the CADM1, MAL, PAX1, and ADCYAP1 genes promoter hypermethylation as one of the possible mechanisms contributing to ovarian carcinogenesis. Sangria banerjee [34] detected molecular signatures of both high-risk HPV16 and 18, along low-risk HPVs in the ovarian cancer samples. They found only low-risk HPVs were related to tumor-negative controls, implicating that hrHPV might be the origin of cancer. Interestingly, they observed widespread integration of viral sequences into various intronic regions, or at intergenic regions within 56 kb upstream of numerous cancer-related human genes. HPV integrations of E1 or E2 regions were reported to inactivate the transcription. However, a system-level analysis is further needed to understand the functional interaction between specific phenotypic traits of the microbiome in different ovarian cancer subtypes and HPV infection.

Pelvic inflammatory disease (PID) is widely established as a risk factor for epithelial ovarian cancer (**Figure 1C**) [45]. Among the organisms responsible for PID onset, *Chlamydia* is the most common, followed by *Nesseria gonorrhea* [46, 47]. Previous studies have found that antibodies against *Chlamydia* infection are associated with ovarian cancer [48]. More recently, Trabert [38] and colleagues evaluated the associations between serologic markers and ovarian cancer risk in two independent populations. Specifically, in a Polish case-control study (244 ovarian cancer/566 control subject), antibodies against *Chlamydia* (Pgp3 protein) were associated with an elevation in ovarian cancer risk (OR = 1.63, 95% [CI] : 1.20 to 2.22). Similarly, in another case-control study in the PLCO Cancer Screening Trial (160 ovarian cancers/159 control subjects), Pgp3 antibodies were also found to be associated with increased ovarian cancer risk (OR = 1.43, 95% [CI] : 0.78 to 2.63). However, in both studies, no associations were found between antibodies against other infectious agents and ovarian cancer. Meanwhile, accumulated studies have ascribed PID to mixed infections by organisms in the vaginal, including pathogenic microorganisms responsible for bacterial vaginosis [46, 49-51]. These microbiomes are linked to the etiology of PID and could thus contribute to the development of ovarian cancer.

Cervical cancer

Cervical cancer ranks second in the cause of cancer death in women between 20 to 39 years old [11]. Human Papillomavirus (HPV) persistent infection is pivotal for cervical carcinogenesis. Despite being the most pervasive sexually transmitted infection (STI) worldwide, the majority of 100 subtypes of HPV are non-carcinogenic. However, there are at least 13 high-risk subtypes involved in the pathogenesis of malignancies [52, 53]. Of these subtypes, HPV16 and HPV18 are the most prevalent and account for 70% of the cases [54]. Interestingly, a recent study showed that specific cervicovaginal microbiota composition correlated with the acquisition of high-risk HPV (hrHPV) types [55].

Some studies suggest that dysbiosis occurs in HPV infection, and a broad microbial alteration pattern was revealed a reduction of Lactobacillus spp. The diminution of these commensal microbes is concomitant with a loss of their protective capabilities and could yield a substantial impact on the onset and progression of the disease. The ability of commensal microbes to produce lactic acid is a crucial benefit to the women's genital tract. Lactic acid acidifies the vaginal environment, benefiting the proliferation of Lactobacillus while inhibiting the growth of infection-associated organisms. In a recent study, Shannon and co-worker [7] studied a cohort of 65 African/Caribbean women to assess microbiome composition and structure through 16S rRNA sequencing. They concluded that participants with HPV infection are associated with a vaginal micro-

biome consistent with CST-IV, characterized by a paucity of *Lactobacillus* spp. and a wide array of anaerobes (58.8% vs. 29.4%; P = 0.043). They also observed a step-wise lowering in the relative abundance of *L. gasseri, Fusobacterium nucleatum, Cornybacterium accolens, Anaerococcus tetradius, Finegoldia magna, Peptoniphilus harei,* and *Raoultella planticola*. This result is in accordance with previous observations that women with hrHPV infections have decreased the abundance of *Lactobacillus* spp [56]. Meanwhile, another study carried out on Nigerian women also suggested a correlation between hrHPV infection and a decreased concentration of *Lactobacillus* spp [57]. This association is also linked with an increased correlation of anaerobes, particularly of the genera *Leptotrichia* and *Prevotella* [58]. The relative abundance of *Sneathia* spp. in persons with HPV infection is increased. A Korean twin study by Lee *et al.* [59] found an abundance of *Sneathia* spp. in HPV positive groups, emphasizing its potential as a biomarker for the prediction of HPV infection. Another study also identified *Sneathia* spp. as the most copious species in the cervix of women with squamous intraepithelial lesion and associated the presence of Sneathia spp. with HPV-positive squamous intraepithelial lesion [60]. Alterations of these bacterial populations and concomitant variability of lactic acid production may have profound results on host regulation of inflammation.

Persistent HPV infection could result in precancerous lesions in the female genital tract [61]. A recent study conducted by Paola and colleagues [62] used next-generation sequencing to examine cervicovaginal microbiota in 55 HPV positive women. They found the abundance of CST IV subgroup, including bacterial genera such as *Prevotella, Gardnerella, Atopobium, Megasphoera*, strongly correlated with HPV persistence. This study also identified *Atopobium* spp. and sialidase gene from *G. vaginalis* as feasible microbial markers for HPV persistence. Wu [63] also found 22 taxa to be associated with HPV persistence, and of these, 5 taxa belong to Prevotella and 1 taxon belongs to *Dialister.* Likewise, another study examined 194 Nigerian women and identified a strong correlation between persistent *Mycoplasma hominis* infection and persistent hrHPV (OR 8.78, 95%, P 0.01) [64]. Only small amounts of women would have persistent HPV infection and progress to cervical lesions [65] and the vaginal microbiome might play a role during this process. HPV infection has adversary impact on the host's immune defenses and mucosal metabolism. This leads to the dysbiosis of the vaginal microbiota, and thus promoting viral persistence and disease progression [66]. Further longitudinal studies are needed to investigate whether and how the microbiome helps maintain the persistent infection and develop to CIN or cervical cancer.

Microbiomes also directly linked to cervical cancer (**Figure 2**). Studies to date have documented an overall increase in diversity. Mitra *et al.* [67] suggest that cervical intraepithelial neoplasia (CIN) progression is correlated with increasing vaginal microbiota diversity. This increased diversity was possibly because of the epithelial barrier rupture and the host's immune dysregulation. *Lactobacillus* is the most abundant genus in vaginal microbiotas and can affect the host dichotomously. While the abundance of some *Lactobacillus* spp. is reduced in cervical cancer or precancerous diseases. A cervical microbiome characterized by a prevalence of *L. iners* is associated with high-grade cervical intraepithelial neoplasia in women infected with hrHPVs [8]. A more recent study observed that women with low-grade squamous intraepithelial lesion (LSIL) were characterized by high prevalence of *Lactobacillus acidophilus* and *L. iners*, and no presence of *L. crispatus.* In contrast, women with high-grade squamous intraepithelial lesion (HSIL) were marked with high proportion of *G. vaginalis and L. acidophilus,* and no *L. crispatus*, *Lactobacillus taiwanensis,* or *L. iners* detected [68]. Pathogenic bacteria, including *A. vaginae, G. vaginalis* are increasingly observed in CIN or cervical cancer. Oh *et al.* [69] studied the cervical microbiota of a Korean cohort of 120 women, 70 with CIN and 50 as the control. The investigators observed a predominance of *A. vaginae, L. iners, G. vaginalis* and an accompanied dearth of *L. crispatus* in women with high CIN risk. This result is in accordance with another study that enrichment of *A. vaginae* and *G. vaginalis* was found in patients with CIN3 [70]. Other pathogens, such as *C. trachomatis*, has also been identified as a cofactor of carcinogenesis, with a higher rate of infection in patients with cervical cancer [71,

72]. Though the results are inconsistent among published studies [73], partly owing to specimen variance or analysis methods. Associations were established both for single and multiple hrHPV genotype infections, supporting the hypothesis that a *C. trachomatis* infection contributes to cervical cancer, together with inflammation and HPV [74]. These non-commensal microbiotas may induce inflammatory cytokines, especially in coinfection with HPV. However, how inflammatory cytokines induced by these non-commensal microbiotas are associated with cervical cancer progression needs further elucidation.

Endometrial cancer

Researches have also identified potential microbiotas contributing to the genesis of endometrial cancer (**Figure 2**). A recently identified *A. vaginae* and *Porphyromonas* spp. in the reproductive tract in combination with a high vaginal pH to be statistically related to the occurrence of endometrial cancer [75]. It was further demonstrated that *Porphyromonas* spp. combined with high pH in the vagina could be a promising biomarker for endometrial cancer [76]. These findings are significant, as they put forth a promising biomarker for early detection and pave the way for possible primary preventive interventions. Molecular mechanisms underlying the interaction between microbiome and pathogenesis of endometrial cancer still need elucidation.

Manipulation of the microbiome in gynecological cancer therapeutics

Chemotherapy resistance has long been a problem for patients with ovarian cancer. We previously found Helicase POLQ-like (HELQ) as a promising indicator of cisplatin chemo-resistance for epithelial ovarian cancer [77]. Recently, growing evidence implicates that human microbiomes influence cancer therapy mainly through two aspects: modulating cancer therapeutic response and mediating treatment-related toxity [78]. In preclinical models, the response to oxaliplatin depends on the expression of proinflammatory genes of the microbial flora and the generation of reactive oxygen species by myeloid cells in the tumor microenvironment [79]. Contrarily, the response to gemcitabine can be compromised by *Mycoplasma*, through its pyrimidine nucleoside phosphorylase and cytidine deaminase enzymes, which influences cytostatic activity [80]. For some patients with recurrent or persistent, metastatic gynecological cancer, programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) inhibitors are a possible choice to enhance the clinical outcomes [81]. Recent progress has emphasized the role of the microbiome in regulating tumor responses to chemotherapeutic agents as well as immunotherapies targeting PD-L1 or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) [82]. Furthermore, Routy *et al.* [83] showed that antibiotic usage is associated with abnormal responses to immunotherapeutic PD-1 blockade. Through profiling samples from lung and kidney cancer patients, they found that patients nonresponding to PD-1 inhibitors had lower levels of the bacterium *Akkermansia muciniphila*. After oral supplementation of bacteria in antibiotic-treated mice, response to immunotherapy was restored. Matson *et al.* [84] and Gopalakrishnan *et al.* [85] studied PD-1 blockade in melanoma patients and found a higher concentration of favorable bacteria in the guts of responding patients. They also found an imbalance of gut flora composition in nonresponders, which is associated with impaired immune cell activity. These observations are in conjunction with the hypothesis that the microbiome may play an important role in immunotherapy. However, whether or not cervicovaginal microbiotas could influence the efficacy of chemotherapy in gynecological cancers still needs to be investigated.

Manipulation of the vaginal microbiota is essential for women's health. Probiotic *L. rhamnosus* GR-1 was shown to mediate adhesion to the vaginal epithelium and mediates the attachment of urogenital pathogens [86]. Studies indicate that strains of *Lactobacillus* can inhibit the growth of G*. vaginalis* [87]. *L. crispatus* has also demonstrated potentials as a hopeful probiotic in preventing *N. gonorrhoeae* infections through counteracting *N. gonorrhoeae* viability [88]. Recently, a research implementing *L. rhamnosus* BMX 54 in 57 women for at least 6 months has shown positive outcomes in controlling HPV infection [89]. Additional research shows that supernatants of *L. gasseri, L. jensenii,* and *L. crispatus* could inhibit the activity of cervical cancer cells via regulation of HPV oncogenes [90]. *L. gasseri* and *L.*

crispatus has also been reported to exert cytotoxic effects on cervical tumor cells selectively, but not normal cells [91]. These results are tantalizing, but further studies are necessary to explore the underlying mechanisms for successful applications in humans. Another possible application of probiotics is to restore a healthy genital microbial community after some gynecological procedures.

Conclusion and future direction

Studies to date are encouraging, despite further research on the human microbiome and gynecological cancer are needed. The current studies have given us insights into this field, yet with drawbacks and contradictions. For instance, the studies on the HPV infection, ovarian cancer, and endometrial cancer showed conflicting results. One possible reason is the difference in detection methods. In a meta-analysis mentioned previously [27], the meta-regression suggested that the HPV prevalence was closely related to HPV DNA detection method; racial and local differentiations are another reason behind these discrepancies [18]; individual variances might also account for the different results [92]. Co-factors related to an individual's lifestyle, such as tobacco usage and hormonal contraceptives, as well as multiple sex partners and early sexual activities, are associated with SCC [93]. The difference in location of the analyzed samples also accounts for the inconsistent results, Chen *et al.* [94] has reported the different compositions of microbiota in the cervical canal, uterus, and vagina.

Present researches have focused on the relationship between microbiota and gynecologic malignancy, with little regard for cause and effect. Some microbiome presence may facilitate the HPV infection, but the reverse scenario is also possible that HPV infection harbors a salubrious environment that satisfies microbial needs. Another quandary is although highly pervasive, only a small percentage of women with persistent HPV infection subsequently acquire clinically significant diseases. It remains to be solved what roles these microbiotas could play in HPV persistent infection. Investigation of unique microbiome signature in different cancers paves the way for diagnostic biomarkers as well as provide insights for prognosis, prevention, and the development of treatment.

Current research is promising, owing to technological advances in sequencing technology and improved in vitro models. New knowledge in this field shed light on the diagnosis for early detection and therapeutic potential of cancer. As demonstrated in this review, significant gaps in knowledge remain, especially regarding the microbiome and gynecologic cancers. To have a translational impact, additionally, it is crucial to develop a system-level understanding of health and disease by measuring biological components of a system using a statistical and metagenomics framework. Also, further investigations are needed to improve treatment and develop new interventions for women's health.

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Disclosure of conflict of interest

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