Vaginal and tumor microbiomes in gynecological cancer (Review)

MENGZHEN HAN[1], NA WANG[1], WENJIE HAN[1], MENG BAN[2], TAO SUN[3] and JUNNAN XU[3]

[1]Department of Breast Medicine, Cancer Hospital of China Medical University, Liaoning Cancer Hospital; [2]Liaoning Microhealth Biotechnology Co., Ltd.; [3]Department of Breast Medicine, Cancer Hospital of Dalian University of Technology, Liaoning Cancer Hospital, Shenyang, Liaoning 110000, P.R. China

Received September 27, 2022; Accepted February 14, 2023

DOI: 10.3892/ol.2023.13739

Abstract. Cervical, ovarian and endometrial cancer are the three most common types of gynecologic cancer. As a hub, the vagina connects the site of gynecological cancer with the external environment. Lactobacilli participate in the formation of a healthy vaginal microenvironment as the first line of defense against pathogen invasion; a dysbiotic vaginal microenvironment loses its original protective function and is associated with the onset, metastasis, poor efficacy and poor prognosis of gynecological cancer. The early diagnosis of cancer is the key to improve the survival time of patients with cancer. The screening of *Porphyromonas*, *Sneathia* and *Atopobium vaginae,* and other microbial markers, can assist the diagnosis of gynecological cancer, and screen out the high-risk population as early as possible. With the in-depth study of the microbes in tumor tissues, reasearchers have analyzed the immunological associations of microorganisms in tumor tissues. Due to the structural-functional interconnection between the organ of gynecological tumorigenesis and the vagina, the present study aims to review the relationship between vaginal and tumor microorganisms and gynecological cancer in terms of occurrence, screening, treatment and prognosis.

Contents

Correspondence to: Professor Junnan Xu, Department of Breast Medicine, Cancer Hospital of Dalian University of Technology, Liaoning Cancer Hospital, 44 Xiaoheyan Road, Dadong, Shenyang, Liaoning 110000, P.R. China
E-mail: 15040292193@126.com

Key words: vaginal microbe, lactobacilli, cervical cancer, ovarian cancer, endometrial cancer, tumor microbiome

1. Introduction

With the development of high-throughput sequencing technology and biotransformation technology, and the decreasing cost of sequencing, the understanding of the microbiome has been deepened. The homology and diversity of microorganisms at target sites can be known through sequence comparison and statistical analysis (1). The dynamic balance and stability of the microbiome is an important indicator of the stability of human internal environments (2). Common gynecological cancer types include cervical caner, ovarian and endometrial cancer, which occur in women at all ages, but mostly in postmenopausal women (3,4). The vagina is a structure with open physiological features that allow easy invasion and colonization by microbes. Invading pathogenic microbes can disrupt the ecological balance of the vagina and cause inflammation, which can lead to the risk of cancer (5,6). Recently, more and more studies have focused on the impact of vaginal microorganisms on gynecological malignancies. The present review first discusses the vaginal microbial environment to elucidate the vaginal steady state and the role of lactobacilli and their metabolites in maintaining stability. Most studies are limited to phenomenological findings and there is a lack of research on the underlying mechanisms, and no method for the early screening of gynecological malignancies is widely recognized. In addition, the results obtained vary depending on the sampling method, the geographical location of the subjects and the clinical indicators. The present review pooled the latest research advances and compared the consistency and differences between studies in order to obtain more generalized results. Moreover, the microbes discovered recently in cancerous tissues and their role in tumor immunity may be a key to future cancer treatment (7), but the research is still in its infancy and further study is required to confirm the importance of these microbes. Tumor tissue microbes are in direct contact with cancer cells and immune cells (8). Microbes are not only the 'cause' of gynecologic cancer, but also the 'consequence' of a series of adverse effects brought about by cancer. The current review presents the association between microbes and gynecological malignancies in terms of cancer susceptibility, marker prediction, subsequent treatment and prognosis, and the disorders and changes of microbes due to gynecological carcinogenesis from both cause and consequence perspectives. The aim is to elucidate the association between gynecological malignancies and microbes in vaginal and tumor tissues, and

to provide a theoretical basis for future cancer interventions and in-depth studies using flora.

2. Vaginal microbes and the acidic vaginal environment

Vaginal microbes. Vaginal microbes are mainly anaerobes and facultative anaerobes (9,10). Under normal conditions, lactobacilli are the dominant bacteria in the vagina (11). Vaginal microbiota is homeostatic in a healthy state and is mainly regulated by estrogen levels (12). Vaginal microbiota changes during pregnancy, menstruation, menopause, hormonal use and environmental age changes (13). Currently, vaginal flora is mainly classified using the community state type (CST) proposed by Ravel *et al* (14). Vaginal flora is divided into five groups: CST I, II, II, IV and V. The dominant bacteria in CST I, II, III and V are lactobacilli, dominated by *L. iners*, *L. crispatus*, *L. gasseri* and *L. jensenii*, respectively. CST IV is the most diverse and contains more anaerobic bacteria, including *Prevotella*, *Dialister*, *Atopobium*, *Gardnerella* and *Megasphaera* (14). Although the importance of lactobacilli in the vagina cannot be ignored, the CST definition reverses the previously accepted one-sided conclusion that a healthy vaginal environment must be dominated by lactobacilli, allowing for a more accurate and comprehensive understanding of vaginal homeostasis (14). Scholars are increasingly aware that vaginal homeostasis is an integrated outcome and that the vaginal microbiome is dynamic (15). Based on current studies, it cannot be determined what composition is necessarily homeostatic or necessarily risky. Attention should be focused on the changes in vaginal microbial trends in diseased and healthy populations, determining the key and characteristic bacteria, and developing probiotic and flora interventional methods for clinical translation.

Acidic vaginal environment. *Lactobacillus* creates an acidic environment in the vagina, which is not conducive to the growth, metabolism and reproduction of pathogenic bacteria and opportunistic pathogens (11,16,17), and the invasion of viruses and other pathogens (18,19). In addition, metabolites such as lactic acid, H_2O_2, antibacterial molecules and bacteriocins, play antibacterial, antiviral and immunomodulatory roles (20). The dysbiosis of vaginal microbes is closely associated with carcinogenesis, immunosuppression and drug resistance (21-24). First, dysbiosis is involved in the progression of gynecological cancer (25,26). A 2020 study included women who were healthy (n=68), infected with human papillomavirus (HPV) (n=78), presented with low-grade squamous intraepithelial lesions (SILs) (n=51), presented with high-grade SILs and diagnosed with cervical cancer (n=9). 16S ribosomal RNA-sequencing (rRNA-seq) analysis of vaginal swabs collected from the subjects showed that increased vaginal microbial diversity was associated with HPV infection and precancerous progression. The α diversity index (an index that assesses the diversity of species within a particular ecosystem) of the cervical cancer group was the highest and significantly different from other groups (25). Secondly, in recent years, numerous *in vitro* and *in vivo* studies have shown that the dysbiosis of the vaginal microbiome affects local microecological homeostasis and local immune parameters (including immune cells and cytokines) (27), and induces pro-inflammatory responses (28,29). An *in vitro* experiment by Doerflinger *et al* found that *Atopobium vaginae* increased the expression of membrane-associated mucins and induced a robust proinflammatory response [i.e., expression of chemokine (C-C motif) ligand 20, human β-defensin 2, interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor α (TNF-α)] (29). Finally, despite the lack of mechanistic exploration, the results of next-generation sequencing technology and multi-omics studies also point to the potential association between an unconventional vaginal microbiome and drug resistance (to chemotherapy drugs and antiviral drugs, among others) (30,31). Wang *et al* (30) found that there was a significant difference in vaginal microbiome α diversity between platinum responders and non-responders in women with cervical cancer, with the diversity of non-responders being significantly higher.

Lactobacilli and related metabolites adversely affect the growth and survival of cervical cancer cells. A study has shown that different concentrations of lactobacilli supernatant can inhibit the activity of cervical cancer cells by regulating the expression of genes (32). Motevaseli *et al* (33) also found that lactobacilli supernatant and normal vaginal lactobacilli had cytotoxic effects on cervical tumor cells, but not on normal cervical epithelial cells, and were not affected by pH and lactic acid in the vaginal environment. Moreover, the exopolysaccharides secreted by the *L. gasseri* strains (G10 and H15), which were isolated from the vagina of healthy women, could both inhibit the proliferation of cervical cancer cells and also affect apoptosis (34). Palma *et al* (35) showed that long-term (6 months) use of *Lactobacillus rhamnosus* BMX 54 was twice as likely to resolve HPV-associated cytological abnormalities than short-term use (3 months). Based on this feature, vaginal *Lactobacillus*-like probiotic supplementation for patients with cervical cancer may be a future aid to delay the progression of cervical cancer.

The decline of the dominance of lactobacilli is associated to the occurrence of gynecological diseases. The decreased abundance of lactobacilli facilitates the invasion of pathogenic bacteria and promotes the development of sexually transmitted diseases, and precancerous inflammation and lesions. It has been revealed that South African women with a low vaginal lactobacilli abundance are more than four times more likely to be infected with human immunodeficiency virus than other women (36). This phenomenon is associated with interference of immune regulation by high-risk flora (36). In turn, local inflammation and infection caused by pathogen invasion and lesions can affect the abundance of lactobacilli, further causing microenvironmental dysregulation and promoting disease deterioration (37). Using 16S rRNA-seq, Borgogna *et al* (38) found that women with HPV infections had a lower lactobacilli content and lost the dominance of vaginal flora compared to women who were HPV^-. An increase in the abundance of bacteria associated with bacterial vaginosis, provides favorable conditions for the development of precancerous lesions (39).

The detection of cancer biomarkers (e.g., protein molecules and microRNA) can be used for cancer diagnosis (40), drug administration (41) and prognosis prediction (42), and its potential role has important implications for controlling the cancer burden. A 2019 study showed that proinflammatory cytokines (IL-6 and TNFα), apoptosis-related proteins [soluble (s)Fas, sFas ligand (sFasL) and TNF-related apoptosis-inducing

Table I. Potential biomarkers and effects in gynecological cancer.

Cancer type	Microbes	Location	Predictability and potential effects
Cervical	*Sneathia, Pseudomonas, Ureaplasma urealyticum, Ureaplasma parvum, Chlamydia trachomatis, Trichomonas vaginalis*	Vagina	HPV and hrHPV infections
	Atopobium	Vagina	HPV persistence
	Bifidobacteria\Lactobacillus	Vagina	HPV clear
	Bacteroides	Vagina	Chemotherapy curative effect
	F. necrophorum	Vagina	Diagnosis of cervical cancer
Ovarian	Proteobacteria/Firmicutes	Ovary	Occurrence and development of ovarian cancer
	Chlamydia trachomatis, Lactococcus	Ovary	Diagnosis of ovarian cancer
Endometrial	*Porphyromonas, Atopobium vaginae*	Vagina	Inflammatory response; diagnosis of endometrial cancer
	Pelomonas, Prevotella	Uterus	Prediction of endometrial cancer

hrHPV, high-risk human papilloma virus.

ligand], growth and angiogenesis factors (hepatocyte growth factor, stem cell factor and vascular endothelial growth factor) and others (α-fetoprotein and osteopontin), were elevated in the local cervicovaginal microenvironment of patients with cervical cancer, and were negatively correlated with the abundance of lactobacilli and positively correlated with vaginal pH (43). Changes in lactobacilli and the acidic environment they create may cause activation of pathways related to inflammation and apoptosis within the vaginal cervical epithelium, acting as a pro-cancer factor (44). Additionally, lactobacilli can regulate the vaginal microecological system and exogenous lactobacilli supplementation can reverse the dysregulation to some extent (45,46). One study displayed reduced Nugent scores and improvement in vaginal dysbiosis by oral administration of a pertinent lactobacilli strain mixture (*Lactobacillus crispatus* LbV 88, *Lactobacillus gasseri* LbV 150N, *Lactobacillus jensenii* LbV 116 and *Lactobacillus rhamnosus* LbV96) (47). Other researchers have found that vaginal microbiome transplantation can improve dysbiosis when transplanting vaginal microorganisms from healthy women to treat bacterial vaginosis (48). However, the feasibility and safety of vaginal microbiome transplantation cannot be determined, as the number of subjects in which this has been performed is too small and the potential risks remain elusive. The exploration of the beneficial effects of vaginal lactobacilli will contribute to the future development of vaginal targeted probiotic products, the macroscopic control of vaginal flora through flora transplantation and the modification of vaginal antibiotics.

3. Vaginal microbes in gynecological malignancies

Vaginal-cervical microorganisms in cervical cancer

HPV infection. High-risk HPV (hrHPV), such as HPV-16 and HPV-18, are recognized oncogenic factors in cervical cancer (49). Although most hrHPV infections are spontaneously cleared (50), persistent infection is capable of causing cervical intraepithelial neoplasia, which can eventually lead to cancer (51). Firstly, linear discriminant analysis effect size analysis showed that increased vaginal microbial diversity is strongly associated with HPV infections (52), and Fusobacteria, including *Sneathia spp*., may be microbial markers associated with HPV infections (Table I) (53). In addition, HPV infections also cause fluctuations in the diversity and abundance of vaginal microorganisms (25). The composition and functions of vaginal microorganisms are altered in women with hrHPV infections (54), and *Pseudomonas* is more likely to be detected in hrHPV+ patients and patients with cervical cancer than in HPV- subjects (55). In addition, the pathogenicity of different HPV types varies significantly, with hrHPV being carcinogenic and low-risk HPV tending to cause only benign lesions (56). Not only that, but the incidence of cervical cancer likewise varies among different types of hrHPV infection (57). Huang *et al* (58) found that combinations of Oribacterium, Lachnobacterium and Thermus among the cervicovaginal microbiota were more likely to be associated with HPV-16, while combinations of Motilibacter were more likely to be associated with HPV-52, and combinations of Litorilinea and Paludibaculum, and the absence of L. iners, were more likely to be associated with HPV-58. It was predicted that cervicovaginal flora may also be associated with specific types of HPV infection, which in turn may affect carcinogenesis (58). Artificial interventions with cervicovaginal microbes may affect the cervical cancer burden by suppressing hrHPV infections. In addition, the varying cervicovaginal microbiota have differences in the persistence and elimination rate of HPV *in vivo* (59). Moreover, CST IV, dominated by bacteria associated with bacterial vaginosis, is a risk factor for the persistence of HPV, and Atopobium spp. may be a microbial marker for the persistence of HPV (60). In addition, a 16S RNA-seq analysis of 28 women with persistent HPV infection and 30 women with HPV clearance showed the proportion of bifidobacteria and lactobacilli may play a role in clearing HPV to a certain extent (61). It has been speculated that inflammation may

be involved in the interaction between microorganisms and HPV infections. Lv *et al* (62) compared the cervicovaginal microbiota of hrHPV- and hrHPV+ subjects and found that Ureaplasma urealyticum, Ureaplasma parvum, Chlamydia trachomatis and Trichomonas vaginalis were risk factors for hrHPV infections. In addition, it was hypothesized that microbes promoted hrHPV infections by triggering inflammation and thereby affecting the protective effect of the immune system. Other similar studies indicated that this conjecture was correct (23,63). However, there is no definite conclusion about the underlying mechanism.

Precancerous lesions and treatment. Control of precancerous lesions can prevent them from deteriorating to cervical cancer. A decrease in the dominance of lactobacilli and an increase in the cervicovaginal microbial diversity facilitate the development of cervical cancer (64-68). Researchers adjusted the dysregulated cervicovaginal flora by excising the precancerous lesions. For vaginal flora, resection of the cervical intraepithelial neoplasia (CIN) had no ameliorative effect (69), whereas, for cervical microorganisms, resection of the CIN using a loop electrosurgical excision procedure (70) decreased the cervical microbial diversity and restored the lactobacilli dominance. In terms of treatment, chemotherapy efficacy varies among patients (30). One study revealed significantly lower vaginal microbial α diversity and enrichment of *Bacteroides* in patients with non-responding cervical cancer than in patients with significant responses to platinum drugs (30), suggesting that manual intervention of vaginal-cervical microorganisms may facilitate the responsiveness of patients to chemotherapy and enhance the therapeutic effect. The accurate control of microbial changes and roles at the CIN stage plays an important role in the construction of future non-invasive early screening models to reduce global cervical cancer mortality disparities and reduce the disease burden.

Biomarker prediction. The regional difference in cervical cancer incidence is associated with regional financial level (71). Early screening for cervical cancer is an effective way to reduce the global burden of cervical cancer. Therefore, using microbial markers to identify patients in the early stages is one of the current research focuses and is important in cervical cancer prevention and treatment. Previous studies found that *Sneathia* was a hallmark of patients with HPV^+ SILs and that it was enriched in all women with precancerous lesions, cervical cancer and abnormal vaginal pH (67,72). The abundance of *Fusobacterium* was significantly higher in advanced cervical cancer compared with that in the early stages, and *F. necrophorum* was observed only in cervical cancer (72). In addition, according to a Korean study, significant abundance variations were found in vaginal *Lactobacillus* and *Gardnerella* in women with cervical cancer compared with that in healthy women, while *Streptococcus* abundance in women with CIN was significantly different from that in healthy women (73). Thus, it is speculated that fusobacteria, *Sneathia* and *Streptococcus* may be microbial markers of precancerous lesions and cervical cancer, and may promote the development of cervical cancer via maintenance of an immunosuppressive microenvironment by increasing the levels of relevant cytokines *in vivo*. The aforementioned exploration has limitations, such as small sample sizes and no assessment of environmental factors. However, it provides a relatively reliable direction for early screening and treatment of cervical cancer in the future. Moreover, due to different study focuses and research methods, some differences exist in the prediction of cervical cancer biomarkers. However, in general, it is a consensus among scholars that a microbial environment with a higher proportion of anaerobic bacteria and a lower proportion of lactobacilli is more prone to HPV infection.

Vaginal microbes in ovarian cancer. Ovarian cancer is a severe threat to women's health, as due to the lack of symptoms in the early stage and the deficiency of obvious screening effects, the majority of cases are already in the terminal stages when diagnosed (74). Therefore, although the incidence of ovarian cancer is lower than that of cervical and endometrial cancer, the mortality rate ranks top among the gynecological malignancies (75). Due to differences in sequencing methods, inclusion populations and analytical methods, there is some variation in the identification of microbial markers. However, in general, the dominance of the vaginal microorganism lactobacilli is lower in patients with ovarian cancer than in healthy women and is accompanied by a rise in diversity (76,77).

The studies on vaginal flora have indicated that bilateral salpingectomy reduces the risk of ovarian cancer by 42 to 78%, which is more protective than bilateral tubal ligation (13 to 41% risk reduction) (78). It is hypothesized that a salpingo-oophorectomy or ligation reduces the exposure of the ovary to microorganisms, thus reducing the risk of ovarian cancer (79,80). Regular physical examination and bilateral tubal ligation for fertile women with high risks of ovarian cancer may help reduce the short-term incidence of ovarian cancer. In addition, cervicovaginal microbes with <50% lactobacilli are significantly associated with ovarian cancer and known risk factors, such as age or BRCA1 germline mutations (76). However, more experiments in this area are needed to further validate such ideas and action mechanisms.

The sensitivity of patients to platinum drugs is the key to the outcomes of ovarian cancer treatment. Jacobson *et al* (81) concluded that the vaginal microbiota of patients with ovarian cancer was statistically associated with platinum sensitivity. Vaginal flora with a predominance of *Escherichia* was likely in patients with platinum-resistant tumors. According to these findings, we hypothesize that artificially aligning a patient's vaginal flora to platinum-sensitive flora through probiotics, vaginal flora transplantation and other methods could improve the efficacy of platinum drugs and prolong the platinum-free interval. However, this speculation needs to be verified by more in-depth studies in the future.

Vaginal microbes in endometrial cancer. Alterations in the microorganism and microenvironment have been demonstrated to associate with carcinogenesis in a variety of cancer types, such as lung, gastrointestinal and skin cancer (82). Although the vaginal microbiome associated with endometrial cancer has been less studied, the correlation between the two cannot be denied. First, epidemiology shows that endometrial cancer is more frequent in postmenopausal women (83), and changes in vaginal microbiology in postmenopausal women (e.g., a lower proportion of *Lactobacillus* and a higher proportion of *Prevotella* and *Gardnerella*) (84-86) may be involved in endometrial carcinogenesis, which in turn affects its incidence.

Second, scholars are now beginning to accept the presence of microorganisms inside the uterus, and evidence points to the possibility that vaginal microorganisms may rise during uterine peristalsis and sperm transport through the cervical canal (87-89). With advances in next-generation sequencing technology, the characterization of the *in utero* microbiome is becoming increasingly clear. Although the biomass of microorganisms in the uterus is low (90-92), bacteria, viruses (such as adeno-associated virus, human herpes viruses and human cytomegalovirus) (93-95), *Chlamydia* (*Chlamydia trachomatis*) (96,97), *Mycoplasma* (*Mycoplasma hominis*) (98) and fungus (*Candida albicans*) (99) have been detected. In 2015, Mitchell *et al* (91) evaluated the endometrial flora of 58 patients by quantitative PCR, among which *L. iners*, *Prevotella* and *L. crispatus* were the most common. However, a 2017 16s rRNA-seq analysis showed that *Lactobacillus*, *Pseudomonas*, *Acinetobacter* and *Vagococcus* were the most abundant genera in the endometrial environment (92). Some scholars believe that vaginal microorganisms may be the source of abnormal microbial composition in the uterus. Evidence for this speculation was provided in a study by Walther-António *et al* (100). The study included 16S RNA-seq experiments on collected samples from the vagina, cervix, fallopian tubes, ovaries, peritoneum and urine, and found a significant correlation between the microbial composition of the vagina, cervix, fallopian tubes and ovaries in patients with endometrial cancer (100). Since the vagina is the only channel through which the aforementioned organs are connected to the outside, it is scientific and relevant to study the microbial environment within the organs of gynecological cancer and the tumor tissue, starting with the vaginal flora. Firmicutes, spirochaetes, actinobacteria, bacteroidetes and proteobacteria in patients with endometrial cancer were significantly increased compared with those in controls; the presence of *Porphyromonas* and *Atopobium vaginae* at a vaginal pH of >5, with a sensitivity and specificity of 100 and 60%, respectively, for the diagnosis of endometrial cancer (100), provides a potentially effective way to diagnose endometrial cancer. In another study a few years later, *Porphyromonas* was also used as a biomarker for endometrial cancer; female menopausal status, body mass index and vaginal pH were demonstrated to influence the vaginal microbial composition, with the three factors independently increasing microbial diversity (101).

Cancer is a malignant lesion caused by multiple pathological factors, in which microbiome changes may be a combination of bacterial, viral and other alterations. Despite the association between HPV and endometrial cancer being hypothesized as early as 1991 (102), there is general agreement that there is little association between HPV infection and common endometrial cancer (103,104), with only one study belatedly opposing this (105). The study by Abu-Lubad *et al* (105) used polymerase chain reaction to detect HPV DNA in 144 formaldehyde-fixed paraffin-embedded tissues and found that the infection rate of the endometrial cancer group was higher. However, no definite conclusions can be drawn. Notably, however, some studies have found associations between endometrial cancer and hepatitis B virus infections (106), viral antigens and RNAs of measles viruses (107). Jiang *et al* (106) analyzed the statuses of HBV serum markers in 398 women with endometrial cancer and compared them with those of 788 healthy women, and found that the hepatitis B surface antigen-positive rate and the hepatitis B carrier rate in women with endometrial cancer were both significantly higher than those in the controls. In addition, Benharroch *et al* (107) used immunohistochemistry to find the presence of measles virus antigen in tumor cells in 72% of patients with endometrial cancer. Although this does not prove a causal association between endometrial cancer and the virus, all such findings provide a new direction for endometrial cancer research, and the etiology of endometrial cancer may be complex.

In terms of treatment, Tsementzi *et al* (108) demonstrated that endometrial and cervical carcinogenesis, and subsequent treatment, were associated with marked changes in the vaginal microbiota, including enrichment of pathogenic bacteria, and identified *Sneathia* as a potential biomarker for endometrial or cervical cancer in postmenopausal women. This suggests that the microbial environment altered by cancer treatment also affects the development of cancer, and that maintaining microbial homeostasis in a cancerous state is equally important.

4. Tumor microbiome in gynecological malignancies

Presence, role and origin of the tumor microbiome. There is a relatively clear understanding of the microbes within the human environment that are in close contact with the outside world. However, there are questions with regard to whether microbes exist within the tumor tissues that form later in life, and whether the microbes have similar effects to vaginal intestinal microbes on host health and tumor development. Due to the superior geographic location of bacteria within tumor tissues, studying such microbes may play a key role in future tumor control.

Microbes do exist in tumor tissues, and this fact was supported by a microbiome analysis of 1,526 samples from 7 tumor types, with some differences in the microbial composition of various tumor types (8). Nejman *et al* (109) used 5R 16S rRNA-seq technology to detect the microbiome in breast, lung, ovarian and pancreatic cancer, melanoma, and bone and brain tumors, and found that there were significant differences in microbial diversity and richness, among which breast cancer samples had the highest microbial diversity, higher than in neighboring tissues and healthy subjects. No higher bacterial load was found in ovarian cancer than in adjacent tissues. Moreover, the dominant bacteria of different cancer types were also different. For example, firmicutes and bacteroidetes phyla were dominant in colorectal cancer, while proteobacteria dominated in pancreatic cancer. In general, proteobacteria, firmicutes, actinobacteria, bacteroidetes, fusobacteria and cyanobacteria occupied a high proportion in the aforementioned seven types of cancer. Intratumoral microbes were mainly found within tumor cells, in the periphery and interior of tumor tissues, and in the surrounding blood vessels, most of which were localized in cancer cells and immune cells (109), and their secretions could act directly. In addition, the microbiome in the same tumor tissue differs at different sites in different stages and staging (110). The role of tumor microbes in tumors can be divided into two aspects; the presence of related microbes affect the proliferation and apoptosis of cancer cells, expression of biomarkers (111), gene

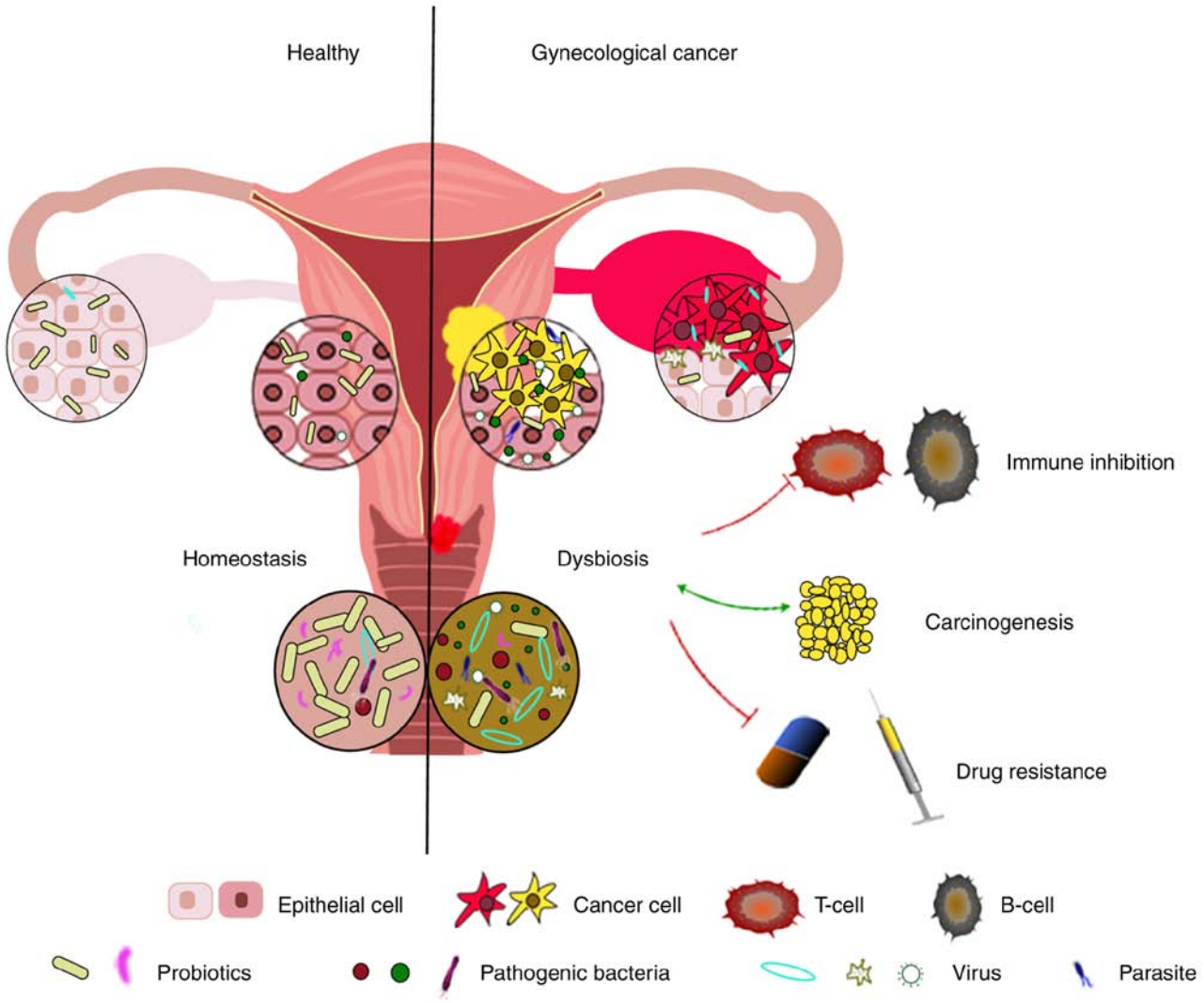

Figure 1. Microbial dysbiosis and gynecological cancer. The homeostasis of microbes in the vagina and reproductive organs has no harmful effect on the host. With the decrease in the dominance of *Lactobacillus* and the increase in pathogenic bacteria and virus invasion, the dysbiotic microbes in the vagina, cervix, uterus and ovary are associated with the immune inhibition, carcinogenesis and increased drug resistance of the host.

expression levels and control of immune cell activity (111,112), thus affecting cancer immunity, treatment and prognosis (113) (Fig. 1). Microorganisms within tumor tissues may be associated with inflammation. For example, Kostic *et al* (114) revealed marked enrichment of fusobacterium in colorectal cancer tissues through genomic and histological analyses, and hypothesized that these microbes might be involved in tumorigenesis via inflammation-mediated mechanisms.

The source of microbes within the tumors also deserves consideration. Various types of human flora are known to play a role in the production and development of cancers, so we speculate that intestinal, vaginal and other human flora may be the main source of microbes within the tumors. In a study exploring the microbial origin of pancreatic cancer tissues, the bacterial DNA profiles of both pancreatic and non-pancreatic cancer tissues were found to be similar to those of duodenal tissues, suggesting that bacteria present in the pancreas might migrate from the intestine to the pancreas (115). Due to the structural and functional connectivity of the reproductive organs, we speculate that the source of microorganisms within gynecological malignancies may be closely associated with the vagina. However, few studies have been conducted. Research on the presence and role of tumor tissue microbes is still in its infancy, and most speculations have not been confirmed. Future research on tumor microbes should focus on the source and role of microbes to improve the tumor microenvironment and achieve tumor control by understanding the relevant mechanisms.

Gynecological cancer microbiome

Ovarian cancer microbiome. The presence and variability of microorganisms have been found in ovarian cancer samples. The viruses, bacteria, fungi and parasites in ovarian cancer samples and the viral integration sites in the host genomes of tumor samples (116) may play a role in the development of cancer. The relevance of viruses, *Chlamydia* and *Mycoplasma*, among others, to ovarian carcinogenesis is controversial. Some scholars believe that despite the low percentage of detection in ovarian cancer samples, there are still differences in species diversity and composition compared with healthy samples and this may be involved in ovarian cancer progression in concert with other oncogenic

factors (117-120). By contrast, the results of a 2010 study showed that *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, HPV and polyomavirus were not detectable in either benign diseases, borderline tumors or ovarian cancer samples (121). The bacterial composition of ovarian cancer tissues has been found to be different from that of non-cancerous tissues. Zhou *et al* (122) found that ovarian cancer tissues had a significantly lower diversity and abundance of microflora, and a higher ratio of proteobacteria/firmicutes than normal tissues, suggesting that changes in the microbial composition might be associated with the development of ovarian cancer. Not only is there a significant difference in the diversity and composition of the microbiomes between women with and without cancer (122,123), but the flora of different parts of the upper genital tract of women with ovarian cancer are also significantly different. A recent study that collected tissue flora of the proximal fallopian tube, fimbriae and ovaries in women with ovarian cancer found significant differences between the microbiomes of the different sites through high throughput sequencing of the 16S gene in the V1-V3 region (124). In terms of the early prediction of ovarian cancer, one study indicated a significant decrease in *Lactococcus* in ovarian cancer tissue, which may be a potential biomarker for identifying the disease. However, due to the lack of studies targeting the ovarian microbiota, the role of microbes in the ovaries remains ambiguous.

Endometrial cancer microbiome. Scholars used to regard the uterus as a sterile environment, but current research points to the existence of microbiota in the uterus as well. Studies on the uterine flora are still in their initial stages, so there are different views on the composition and variation of microbes in the uterus. Some scholars believe that lactobacilli are most abundant in the endometrium (125,126). However, others hold the opposing view that microbes in the uterus are dominated by *Acinetobacter*, *Pseudomonas*, *Cloacibacterium* and *Comamonadaceae*, while lactobacilli are less common (21). Carcinogenesis may be able to cause local microbiome variation. Wang *et al* (127) revealed that endometrial cancer tissues differed significantly from the non-cancerous fraction of the microbiome, accompanied by elevated α diversity and enrichment of *Prevotella*, *Atopobium*, *Anaerococcus*, *Dialister*, *Porphyromonas* and *Peptoniphilus*, similar to the dysbiotic state of the vaginal flora (127). The differences in results are related to the method of tissue sampling and the magnitude of contamination potential, the scientific nature of which needs to be confirmed by numerous studies. Dysbiosis of the intrauterine flora may be associated with inflammation and may impact the development of cancer. Lu *et al* (128) demonstrated that dysbiosis of the endometrial flora and inflammatory factors in patients with endometrial cancer was associated with possible *Micrococcus*. Additionally, it has been suggested that the specific presence of *Atopobium vaginae* and *Porphyromonas somerae* can target endometrial cells to express pro-inflammatory cytokines and chemokines (129), which potentially provides a reference to further explore the mechanism between endometrial flora and inflammatory response. Furthermore, viruses that invade and parasitize the uterus may have oncogenic effects. A study on the association between the endometrium and human mammary tumor viruses showed that 23.2% of human mammary tumor viruses and proteins were detected in patients with endometrial cancer, while none were detected in the normal endometrium (130). It is hypothesized that human mammary tumor viruses influence endometrial development. However, the exact mechanism is unknown. In terms of biomarker prediction, scholars indicated *Pelomonas* and *Prevotella* enrichment in cancerous tissues, found *Prevotella* to be associated with elevated serum D-dimer and fibrin degradation products, and hypothesized that the microbial marker of *Prevotella,* along with D-dimer and fibrin degradation products, might predict endometrial cancer (131). However, this study has the problem of a small sample size. There is still a great lack of research on microbes in the uterus. Choosing the correct sampling enables a study to determine the microbial composition of the normal endometrium, then compare it with that of diseased endometrial samples for analysis, and finally investigate the mechanisms by which microorganisms within tumor tissues affect endometrial cancer, thus providing a unique direction for preventing and treating endometrial cancer.

5. Prospects

In summary, the associations between vaginal microbes and gynecological malignancies are broadly divided into three aspects: i) The interaction of vaginal flora with infection by pathogenic microorganisms such as HPV and *Chlamydia trachomatis*; ii) the significance of landmark microorganisms for the early prediction of gynecological malignancies; and iii) the interaction of vaginal microbes with the treatment effect and prognosis of gynecological malignancies. Vaginal microbes exist in almost all stages of the development and control of gynecological malignancies. Additionally, the presence and role of microorganisms within the tumor should not be ignored, especially their link with tumor immunity. Further research should focus on the connection between the tumor microbiome of gynecological malignancies and the composition and structure of vaginal microbes, to study the microbial composition at the level of the reproductive tract as a whole and make macroscopic regulation of the microecological stability of the reproductive tract to promote the prevention and treatment of gynecological cancer. Finally, strengthening the popularization of knowledge about gynecological malignant tumors and other diseases to enable the majority of women to understand the risk of gynecological diseases deeply, avoid risks in daily life, take physical examinations on time and improve their awareness of vaccinations related to gynecological diseases, will play a huge role in reducing the incidence of gynecological cancer in the future. In further studies, analyses should be conducted in larger sample sizes and more complex populations, taking into account the differences in clinical indicators of the women tested and focusing on the dynamic changes in cancer occurrence, in order to grasp the overall disease development and a general trend of microbiome changes in gynecological cancer. In addition, the development of personalized screening and treatment should also be on the agenda, and through the study of population differences, screening and treatment plans can be formulated more precisely and with better results.

Acknowledgements

Not applicable.

Funding

This study was supported by Shenyang Breast Cancer Clinical Medical Research Center (grant no. 2020-48-3-1), LiaoNing Revitalization Talents Program (grant no. XLYC1907160), Beijing Medical Award Foundation (grant nos. YXJL-20 20-0941-0752 and CORP-239-N27), Wu Jieping Medical Foundation (grant no. 320.6750.2020-12-21,320.6750.2020-6-30) and the Fundamental Research Funds for the Central Universities (grant nos. 2022029 and 2022030).

Availability of data and materials

Not applicable.

Authors' contributions

JX and TS conceived and designed the review. MH and NW wrote the draft of the paper. WH and MB created the figure and edited the manuscript. All authors read and approved the final manuscript and submission of this manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

MB is employed by Liaoning Microhealth Biotechnology Co., Ltd.

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