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Review

The role of microbiota in epithelial ovarian cancer: a scoping review

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Objective: The objective of this review was to examine the comprehensive role of microbiota in epithelial ovarian carcinogenesis. *Methods*: A scoping review method was used, and relevant databases were searched using combinations of key terms. Human and animal studies were selected that met inclusion criteria and critical appraisal tools were used to assess study quality. *Results*: A total of 10 international studies (human n = 8; animal n = 2) were included with total samples sizes varying from 16 to 580. Mean/median ages of women with epithelial ovarian cancer (EOC) were 50.5 to 66 years, and controls were 47.3 to 56 years. Compared to the ovaries and fallopian tubes of women without disease, tissue collected from women with EOC were characterized by differing proportions of bacterial phyla including *Actinobacteria*, *Bacteroidetes*, *Chlamydiae*, *Firmicutes*, and *Proteobacteria*. Intestinal depletions and reduced diversity of genera *Lactobacillus* accelerated ovarian tumor growth in animal models. Cytomegalovirus and human papillomavirus types 6, 16, 18, and 45 had a significantly higher prevalence in women with disease and represented up to 70% of cases with high-grade serous ovarian carcinoma. Colonized bacteria were detected in fallopian tubes, peritoneal fluid, and ovarian tissue similar to that of commensal GI tract and vaginal bacteria. *Conclusion*: The EOC microenvironment harbors diverse microbes. Due to the heterogeneity of microbiota identified between studies, additional research is needed to reconcile findings and ascertain clinical applicability. Future investigations should also examine potential associations between EOC tumor, gut, and vaginal microbiota, patient symptoms throughout disease, chemotherapy response, recurrence, and survival.

Keywords

Microbiota; Epithelial ovarian cancer

1. Introduction

Annually 23,000 women are diagnosed in the United States with ovarian cancer, and 14,000 women die of the disease, contributing to the staggering 185,000 global ovarian cancer deaths [1, 2]. Ovarian cancer is the fifth leading cause of cancer deaths among women following lung, breast, colorectal, and pancreatic cancers [3]. The high national fatality rate has been attributed to failure to identify the disease at an early stage due to inadequate screening methods [4]. Women with ovarian cancer are often asymptomatic in the early stage of disease and are undiagnosed until a more advanced stage [5]. Epithelial tumors account for 90% of ovarian cancers [6]. The high-grade serous ovarian carcinoma (HGSOC) epithelial subtype is characterized by bilateral ovarian involvement, aggressive behavior, late-stage diagnosis, and high mortality [7]. The urgency for timely and effective treatment strategies has led researchers to test and compare therapeutic agents that prove most beneficial for increasing survival rates [8]. Meanwhile, the biological processes surrounding fallopian tube and ovarian susceptibility in early carcinogenesis remains under investigation [9, 10]. Genomic studies have shown heterogeneity within HGSOC tumors, although there are still gaps in definitive regulatory mechanisms that initiate and drive disease [11–13]. Long noncoding RNAs and microRNAs (miRNAs), such as extravascular-derived circulating miRNAs, have emerged as promising biomarkers in early EOC detection [14, 15]. The ongoing search for discovery of new genomic markers that could signal early tumorigenesis has redirected scientific attention to the role of the microbiome in ovarian cancer development and progression.

The human microbiome is the collective genomes of microbes living in the human body [16]. Since the launch of the National Institutes of Health (NIH) Human Microbiome Project (HMP) over a decade ago, scientists have utilized advances in DNA sequencing technology to identify and characterize unique communities of microorganisms residing in the oral cavity, nasal passages, skin, urogenital tract, and gastrointestinal (GI) tract to determine their influence on health and disease [17]. Bacterial microbiota compositional changes have correlated with cancers [18, 19]. The identification of potential molecular pathways linked to microbial signatures has heightened the interest of researchers and clinician scientists. Microbiome research has become an emerging field to address important inquiries concerning the tumor microenvironment; consequently, novel studies have surfaced in the literature that explore both virulent and protective contributions of microbiota in ovarian cancer development [20].

Several ovarian cancer risk factors are well documented such as advancing age, nulliparity, and most importantly genetic mutations that affect homologous recombination and microsatellite instability [21]. Yet this highly heterogeneous disease poses multifactorial challenges for establishing definitive carcinogenic pathways among varying phenotypes. Nonetheless, the immune system is considered to have a valuable role in tumor activity, and inflammation is a key factor in immune response [22]. Microbial invasion promotes inflammatory reactions that mediate immune cells as a defense mechanism, and chronic inflammation is a risk factor for epithelial ovarian cancer (EOC) [23]. Fallopian tube inflammation is believed to contribute to ovarian cancer development, and pelvic inflammatory disease (PID) has been associated with increased disease risk [24]. Some investigators further hypothesize associations of *Chlamydia trachomatis*, cytomegalovirus (CMV), and human papilloma virus (HPV) with EOC while others have found no such relationship [25–33]. In the lower reproductive tract, the vagina is a nonsterile environment dominated primarily by aerobic bacterial colonies (e.g., *Lactobacillus* species) [34]. Consequently, vaginal microbial imbalances trigger immune responses, degrade the host mucosa, and increase vulnerability to the overgrowth of anaerobes causing bacterial vaginosis infection associated with PID [35]. However, only limited research has explored possible contributions of vaginal microbiota in ovarian cancer development [36].

In the absence of infection, microbial communities have been identified within the upper female reproductive tract, once assumed to be a sterile environment [37]. Microbiota composition within these areas may provide an important link between inflammation and ovarian carcinogenesis. Because HGSOC derives in the mucosal epithelium of the fallopian tubes, microbial activity in this region has generated growing interest [38]. Although studies in various literature have concentrated on the role of single microbial pathogens in ovarian cancer development, gaining knowledge of how microbes can coexist in the tumor microenvironment is valuable. While the etiology of ovarian cancer remains inconclusive, discovery of possible microbiota influences can inform research and practice. The objective of this paper is to examine existing evidence on the comprehensive role of microbiota in epithelial ovarian carcinogenesis.

2. Methods

Scoping reviews are undertaken to examine the extent of the evidence surrounding a topic, identify research gaps in the literature, and draw conclusions regarding the overall state of research activity [39]. This review is guided by the Arksey and O'Malley methodological framework for conducting a scoping review that was further refined by Levac and colleagues [39, 40]. The stages of this framework are: (1) identify the research question; (2) identify relevant studies; (3) select studies; (4) chart to data; and (5) summarize and report the results. Data reporting was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist to ensure accuracy, completeness, and transparency.

2.1 Search strategy for relevant studies

The search strategy was developed and conducted in consultation with an experienced librarian. A search was conducted to identify any publications up to April 2021 using electronic databases: PubMed (yielded 141 articles); Cumulative Index of Nursing and Allied Health Literature (CINAHL) (yielded 25 articles); and Embase (yielded 5 articles). Combinations of the following relevant terms were used: "microbiota", "microbiome", "GI tract microbiome", "gastrointestinal microbiome", "GI tract microbiota", "vaginal microbiota", "vaginal microbiome", or "oncobiome", combined with "ovarian cancer", "dysplasia", "ovarian neoplasms", "ovary tumor", and "ovary cancer". Citation searching and reference lists were used to locate any additional primary studies that were not indexed in the original electronic database search. This method yielded six additional studies.

2.2 Study selection

Included were cross-sectional, prospective, retrospective, observational, and experimental human and animal studies that were published in the English language and involved investigation of the relationship between the microbiome consortium and ovarian cancer. Human study inclusion criteria were: (1) subjects ages 18 years and older; (2) EOC histologic subtypes confirmed by tissue biopsy; and (3) EOC tumor stage of any International Federation of Gynecology and Obstetrics (FIGO) classification. Animal study inclusion criteria were EOC mouse model studies (i.e., EOC cell lines, patient-derived xenografts, or genetically engineered models) that examined the relationship between the microbiota and ovarian cancer. Studies were excluded that concentrated on single microbial pathogens. The PRISMA-ScR literature search flow diagram is provided in Fig. 1.

2.3 Data charting

A customized data extraction instrument was developed to investigate the scope of the available literature. Data extraction elements included author(s) year of publication, design, and study location, purpose, study population, human versus animal study, type of tissue sample assessed, microbiota evaluation, and microbial features unique to ovarian cancer that were agreed upon by two independent reviewers (DM and SP). The data extraction tool summarizes study elements, offers expansion of sections pertinent to each study under review, and allows for comparisons across studies. A summary of the extracted data is presented in **Supplementary Table 1**.

2.4 Study quality assessment

The Joanna Briggs Institute (JBI) Critical Appraisal Tools Checklist for Case Control Studies and the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) Risk of Bias Tool for Animal Studies were used to determine the quality of the selected studies (see **Supplementary Ta-**

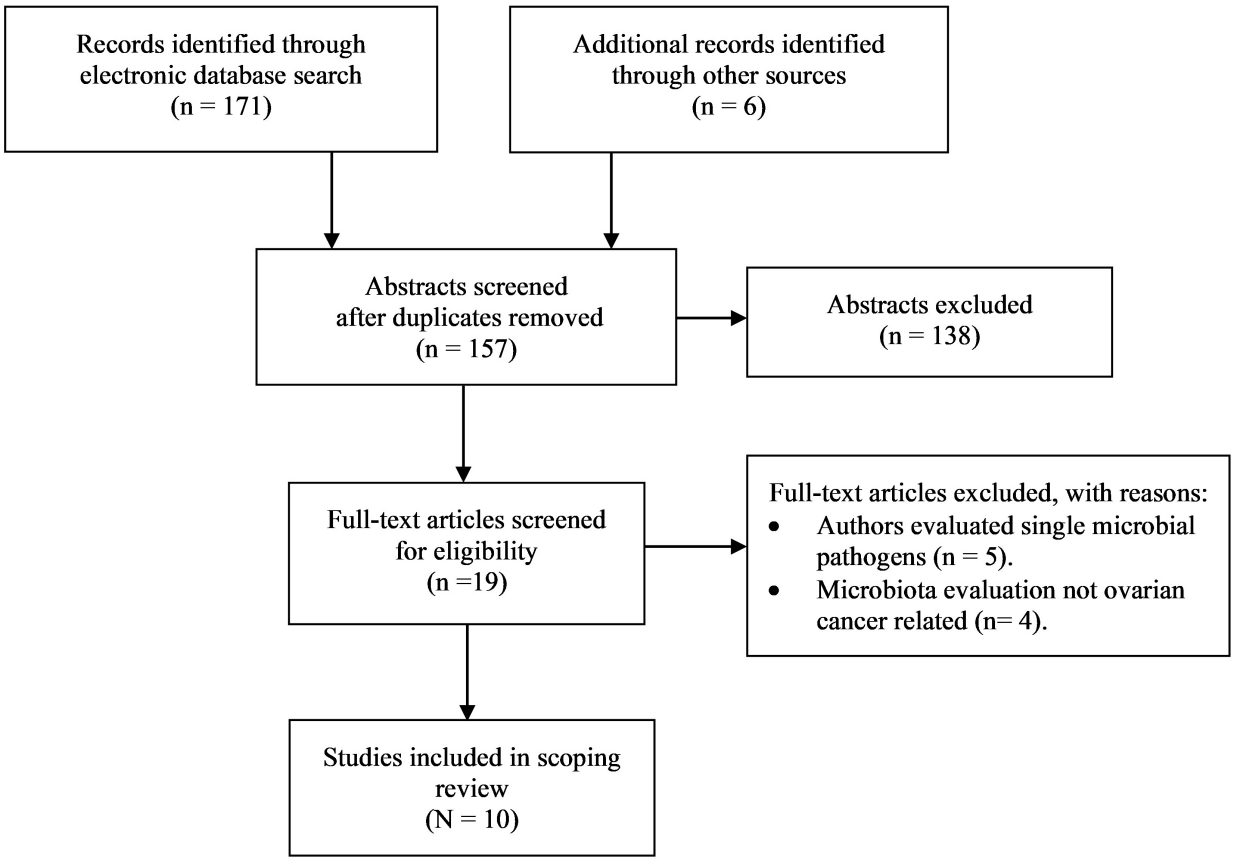

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for Scoping Reviews literature search flow diagram.

bles 2,3). The JBI checklist is a critical appraisal tool assesses methodological quality of the extent to which investigators address the likelihood of bias in study design, conduct, and analysis in their case control studies [41]. The SYRCLE's Risk of Bias Tool was developed and adjusted based on the Cochrane Collaboration Risk of Bias Tool to address varying aspects of bias in animal intervention studies. This includes (1) selection bias; (2) performance bias; (3) detection bias; (4) attrition bias; (5) reporting bias; and (6) other biases [42]. The two authors (DM and SP) who extracted data elements also completed the quality assessment.

3. Results

A total of 10 studies were included with samples sizes varying from 16 to 580 cases. One study was conducted in Denmark [30], Poland [43], and India [26]. Three were conducted in China [44–46] and the United States [47–49]. The remaining study recruited subjects from the Czech Republic, Germany, Italy, Norway, and the United Kingdom [50]. All investigations were cross-sectional, observational, and case control studies involving human tissue samples with the exception of two animal experiments [45, 49]. Technologies used for microbiota evaluation comprised 16S rRNA sequencing, PCR-based assay, PathoChip array, and bacterial culture. Ovarian, fallopian tube, peritoneal, fecal, and cervicovaginal tissues were assessed for microbial expression. Human studies incorporated all EOC histologic subtypes (serous, n = 415; endometroid, n = 38; mucinous, n = 32; clear cell, n = 26; and undifferentiated, n = 1) at varying FIGO Stages of I through IV. A subtotal of 33 cases were reported as Stage I; 26 cases were Stage II; 218 cases were Stage III; and 35 cases were Stage IV. Additionally, some cases were combined as Stage I–II (n = 66), Stage III–IV (n = 108), and not staged (n = 2). One study [47] did not report on the number of cases per histologic subtype, and two studies [26, 47] did not report on FIGO staging. A summary of sample demographics in the human studies is described in Table 1 (Ref. [26, 30, 43, 44, 46–48, 50]).

Although not all studies provided age demographics, reported mean/median ages of women with EOC ranged from 50.5 to 66 years and controls ranged from 47.3 to 56 years. Two studies [48, 50] reported subjects as primarily white although race/ethnicity data were not described in the other studies. Most studies included women who were newly diagnosed with EOC and chemotherapy naïve, and those with recurrent disease or undergoing neoadjuvant therapy were excluded. While one study [47] made microbiota comparisons between EOC tissue samples and non-tumor ovarian tissue from the same cases, others compared EOC case samples to separate ovarian tissue control groups comprised of normal ovarian tissue, [26, 44] benign ovarian conditions [30, 43], and those of positive *BRCA* mutations [47]. In addition, one

Table 1. Summary of sample demographics in human studies.

Author (Year)	Location	Mean age	Epithelial Ovarian Cancer (EOC) case types	Control case types	EOC cases of recurrence?	EOC cases chemotherapy naïve?
Miao *et al.* (2020) [48]	United States	Cancer group: 66.1 years Control group: 56 years	Total n = 10; serous n = 9; endometroid n = 1	Benign ovarian mass n = 20	No. Per exclusion criteria	Yes
Wang *et al.* (2020) [44]	China	Cancer group: 57.3 years Control group: 51.6 years	Total n = 6; serous n = 6	Benign conditions n = 10; Uterine adenomyosis n = 7; Uterine myoma n = 3	No. Per inclusion criteria of new EOC diagnosis	Yes
Ingerslev *et al.* (2019) [30]	Denmark	Cancer group: *55 years Control group: *64 years	Total n = 198; serous n= 163; endometroid n= 15; mucinous n= 11; clear cell n= 9	Benign mucinous cystadenoma tissue n= 176	No. Per inclusion criteria	Yes
Nené *et al.* (2019) [50]	Czech Republic, Germany, Italy, Norway, and United Kingdom	UC Reported by ages $<$ and $\geq$50 years	Total of cancer set n = 176; high grade serous; n = 119; low grade serous n =13; endometroid n = 16; mucinous n= 13; clear cell n = 15	Benign conditions n =69; healthy controls n = 115	No. Per inclusion criteria of suspicion for new EOC diagnosis	Yes
Paradowska *et al.* (2019) [43]	Poland	Cancer group: *53.5 years Control group: *66 years	Total n = 27; high grade serous n = 20; borderline serous n = 2; mucinous n = 2; clear cell n = 2; undifferentiated n = 1	Benign ovarian tumor tissue n = 8	No. Per inclusion criteria of suspicion for new EOC diagnosis	Yes
Zhou *et al.* (2019) [46]	China	Cancer group: 54.5 years Control group: 48.2 years	Total of discovery phase n = 25; high-grade serous n = 25	Benign adenomyoma or myoma of uterus n = 25	UC	UC
Banerjee *et al.* (2017) [47]	United States	UC Not reported	Total n = 99; serous, endometrioid, mucinous; clear cell; transitional cell; mixed types; and carcinosarcoma (number per type not reported)	Matched n = 20 non-tumor tissue from the ipsilateral or contralateral ovary; Unmatched n = 20 benign ovarian tissue of women with *BRCA* mutations	Yes. Three subjects had recurrent disease	UC
Shanmughapriya *et al.* (2012) [26]	India	Combined sample: *55 years	Total n = 24; serous n = 12; endometroid n = 6; mucinous n = 6	Benign ovarian lesion n = 6; healthy controls n = 9	UC	UC

*, Median; EOC, Epithelial Ovarian Cancer; UC, Unclear.

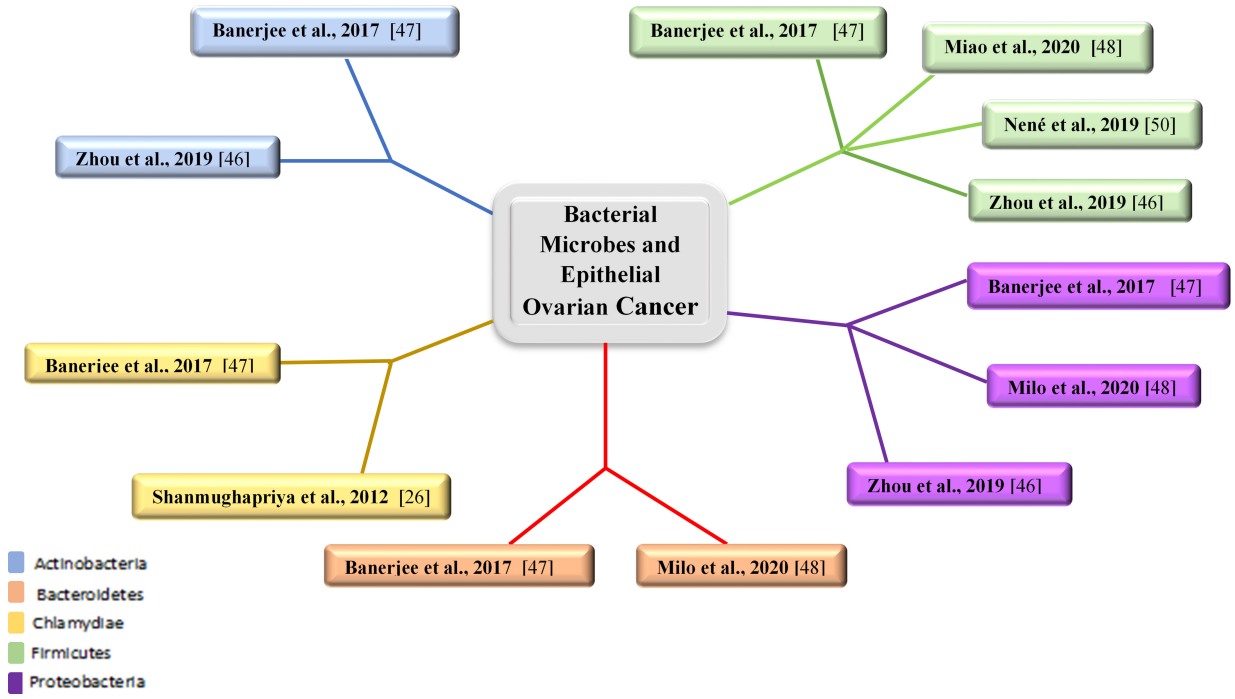

Fig. 2. Bacteria observed in epithelial ovarian cancer human studies.

study [43] assessed for the presence of microbes in fallopian tube tissue of EOC cases, while another study [46] evaluated the normal distal fallopian tube fimbria tissue of uterine adenomyoma and myoma controls. One study [50] compared the proportions of *lactobacilli* species found in the cervicovaginal smears of women from two study sets. The ovarian cancer study set consisted of tissue samples of women with EOC, benign gynecological conditions, and healthy controls. The *BRCA* set included women with *BRCA1* mutations without EOC and women with wildtype *BRCA1* and *BRCA2* mutations who had benign gynecological conditions or were healthy controls. In another study, researchers [48] compared the microbial profile in peritoneal fluid collected from surgical peritoneal washings of women with EOC and those with benign ovarian masses. The animal studies used ovarian cancer (cell line) mouse models to investigate either induced GI bacteria dysbiosis [45] or GI commensal bacterial [49] influences on EOC initiation, progression, and immune response.

3.1 Bacteria and epithelial ovarian cancer

3.1.1 Phylum

Unique proportions of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlamydiae*, *Firmicutes*, *Fusobacteria*, *Spirochaetes* and *Tenericutes* were found in EOC tissue compared to controls [47]. In one study [46], *Proteobacteria* was upregulated while *Firmicutes*, *Candida*, and *Acidobacteria* were downregulated in EOC tissue. Wang and colleagues [44] found no differences in the relative abundance of *Proteobacteria*, *Bacteroidetes*, or *Firmicutes* among groups. However, the authors detected an increase in prevalence of *Aquificae* and *Planctomycetes* and a decreased prevalence of *Crenarchaeota* exclusively in the serous epithelial tissue subtype compared to controls. Eighteen bacterial signature combinations were found in the peritoneal fluid of women with EOC belonging to phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, or *Verruocmicrobia* [48]. A summary of bacteria shared between studies is provided in Fig. 2 and key findings are described in Table 2 (Ref. [26, 46–48, 50–53]).

3.1.2 Genus

Acinetobacter, *Sphingomonas*, and *Methylobacterium* were significantly increased and *Lactococcus* was significantly decreased in the EOC group in one study [46]. Miao *et al.* [48] reported different genera associated with epithelial ovarian tumors consisting of *Prevotella*, *Odoribactor Roseburia*, *Oscillospira*, *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Sutterella*, *Bradyrhizobium*, and *Akkermansia*. Differences were discovered between ovarian cancer and control groups in the relative abundance of *Paenibacillus*, *Haloferula*, *Zavarzinella*, *Photorhabdus*, *Volucribacter*, *Blastococcus*, *Mesotoga*, *Defluviitoga*, and *Dorea* [44]. Furthermore, intestinal depletions *Lactobacillus* significantly accelerated growth of ovarian tumors compared to control groups in tumor xenograft models [45].

3.1.3 Species

The prevalence of *C. trachomatis* was significantly higher [26], *Acinetobacter lwoffii* were more enriched, and *Lactococcus piscium* were less enriched in invasive EOC sample cases [46]. Of 24 EOC cases with *Chlamydia trachomatis* detection, 12 were classified as serous subtype [26]. The relative abundance *of Anoxynatronum sibiricum* and *Methanosarcina vacuolata* was significantly reduced in EOC cases compared to con-

Table 2. Bacterial phyla in epithelial ovarian cancer.

Taxonomy	Function
Actinobacteria	Unique quantities detected in EOC tissues samples compared to controls [46, 47].
	Found in skin, human gut and vagina [51].
	Paired with serum ovarian tumor markers (CA125/HE4), unique peritoneal fluid profiles predicted EOC malignancy [48].
Bacteroidetes	Unique quantities detected in EOC tissues samples compared to controls [47].
	Found in human gut and vagina [52, 53].
Chlamydiae	Higher prevalence in EOC tissues samples compared to controls [26, 47].
	Predominance in EOC tissue samples [46, 47].
Firmicutes	Paired with serum ovarian tumor markers (CA125/HE4), unique peritoneal fluid profiles predicted EOC malignancy [48].
	Reduction of *Lactobacillus* species in ovarian cancer cases compared to control groups [50].
	Found in gut and vagina [51].
	Predominance in EOC tissue samples [47].
Proteobacteria	Reduced in EOC tissue samples [46].
	Paired with serum ovarian tumor markers (CA125/HE4), unique peritoneal fluid profiles predicted EOC malignancy [48].
	Found in gut and vagina [51].

trol cases [44]. In addition, the prevalence of *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus gasseri, and Lactobacillus jensenii* was lower in women with ovarian cancer and those with *BRCA1* gene mutations compared to those without the disease [50]. A variety of additional species were also associated with EOC, including, *Prevotella stercorea*, *Bacteroides ovatus*, *Clostridium colinum*, *Eubacterium dolichum*, and *Akkermansia muciniphila* [48].

3.2 Viruses and epithelial ovarian cancer

Banerjee *et al.* [47] reported that among the viral signatures identified for all cases, 23% were characterized as tumorigenic, yielding a prevalence of greater than 50% in EOC tissue. These viruses included Retroviridae, Hepadnaviridae, Papillomaviridae, Flaviviridae, Polyomaviridae, and Herpesviridae. Although common viral groups were also detected in control tissue samples, specific genetic signatures differed between EOC and control cases. For instance, within the Papillomaviridae family, unique molecular properties of high risk types HPV 16 and HPV 18 were detected in the ovarian cancer samples, whereas molecular signatures of other low risk HPV types were detected in the controls. In another study [43], HPV prevalence was significantly higher in ovarian cancer where HPV 6, HPV 16, and HPV 45 were detected in 74% of all EOC tissues and HPV 16 was detected in 70% of HGSOC subtypes. Moreover, HPV was also observed in the fallopian tube samples of cases with EOC (HPV 6 and HPV 16) and in cases with ovarian metastatic cancer (HPV 16) [43]. To the contrary, Shanmughapriya *et al.* [26] identified HPV 6 in both EOC and control cases with no significant differences in prevalence between groups. Cytomegalovirus (CMV) was detectable in 50% to 70% of EOC case samples representing a significantly higher prevalence in cases compared to controls [26, 43]; among those with HGSOC, CMV was present in 70% of cases [43]. CMV was present in all metastatic ovarian cancer cases [43]. Nonetheless, in a different study, the prevalence of CMV in EOC tissue was insufficient for analysis although Epstein-Barr Virus (EBV) was detected in 5% of EOC cases compared to 0.5% of control cases [30]. A summary of viruses shared between studies is provided in Fig. 3.

3.3 Other microbes and epithelial ovarian cancer

Only one study evaluated additional microbes. Banerjee and colleagues [47] identified distinctive fungi signatures that were significantly detected in either all EOC tissues samples (*Cladosporium*, *Pneumocytis*, *Acremonium*, *Cladophialophora*, *Malassezia*, and *Pheistophora*) or in 95% of EOC cases (*Rhizomucor*, *Rhodotorula*, *Alternaria*, *Geotrichum*). A parasitic signature (*Trichinella*, *Ascaris*, and *Trichomonas*) was also identified in greater than 95% of the EOC cases.

3.4 Immune system response, inflammation, and gastrointestinal microbiota

When comparing human antibacterial-response gene expression profiles among a subset of EOC and normal distal fallopian tube tissues, Zhou *et al.* [46] identified the activation of inflammation-associated signaling pathways (cytokine-cytokine receptor interaction, chemokine signaling, and NF-kappa B signaling) in EOC tissues. In one animal study, TLR5 signaling at regions of bacterial colonization in ovarian tumor-bearing hosts initiated tumor-promoting systemic inflammation and the deployment of myeloid derived suppressor cells (MDSCs) and immunosuppressive gamma delta ($\gamma\delta$) T cells [49]. Moreover, tumors that induced TLR5-dependent systemic interleukin 6 (IL-6) up-regulation responded to exogenous IL-6 by producing additional levels. In the remaining animal study, the presence of macrophages promoted ovarian cancer growth in the tumor xenograft model [45]. Ovarian tumor size and weight assessed in intestinal microbiota dysbiosis mice progressed significantly faster than in the control group, yet this situation resolved with macrophage depletion. Macrophages collected from intestinal microbiota dysbiosis mice were more likely to promote inflammatory cytokine (tumor necrosis factor alpha [TNF-α] and IL-6) production. Additionally, the cytokines secreted by macrophages isolated from intestinal microbiota

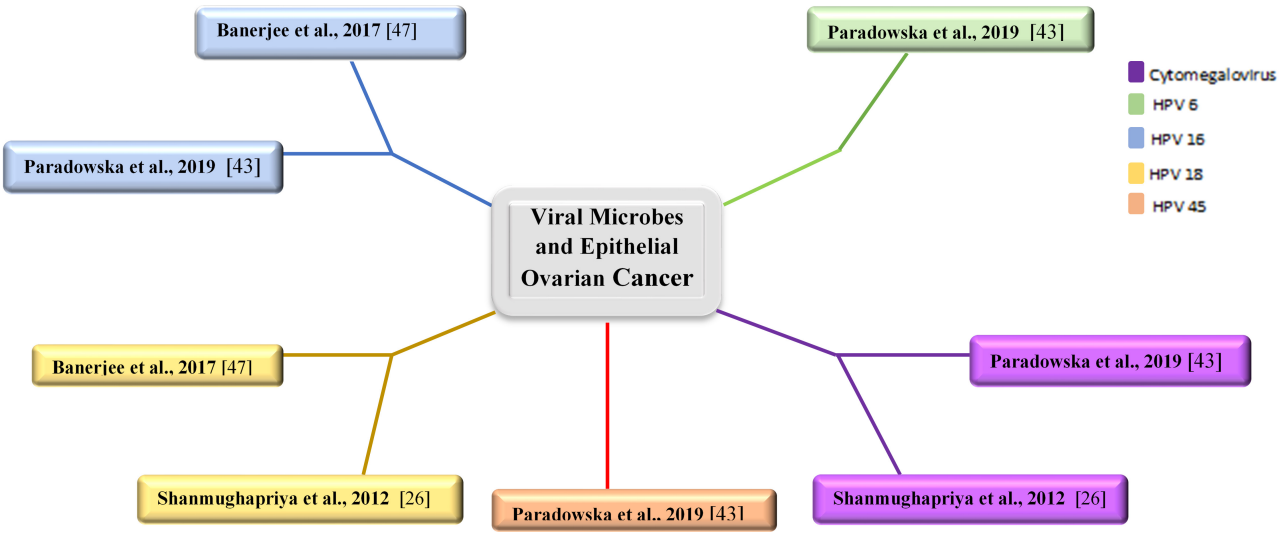

Fig. 3. Viruses observed in epithelial ovarian cancer human studies. HPV, human papillomarvirus.

dysbiosis mice were favorable to epithelial-mesenchymal transition (EMT), epithelial cadherin (E-cadherin) suppression, mesenchymal neural cadherin (N-cadherin) and vimentin overexpression, and proliferation of ovarian cancer cells compared to controls.

4. Discussion

This review examined the association between EOC and a consortium of microbiota harbored within and beyond the female reproductive tract and identified microbial differences with ovarian cancer. While there were commonalities among viral and bacterial phyla classifications, genera and species differed in bacteria groupings between studies. These overall findings suggest a rather convoluted yet dynamic tumor microenvironment involving bacteria and viruses. Although human microbiota encompass the entire genome of microbes, one of 10 studies assessed and used detected widespread signatures across microorganisms including protozoans (parasites) and fungi [47]. However, the few parasites (i.e., *Opisthorchis viverrini*, *Clonorchis sinensis*) known to contribute to cancer were not identified in this review [54]. It is important to acknowledge that Banerjee and colleagues [47] used pan-pathogen array technology to capture parasites and fungi, which would not be detected with methods used in the other studies. While investigation of parasitic and fungi influences on ovarian cancer has received less attention, scientists are exploring the benefit of anti-parasitic therapeutics to suppress ovarian tumors [55].

Infectious microorganisms are known to initiate inflammatory mechanisms and cellular degradation. Several virulent pathogens (i.e., *Helicobacter pylori*, Hepatitis B and C, HPV, and Epstein-Barr virus) are considered global attributers to gastric, liver, cervical, and nasopharyngeal cancers [54]. An important challenge will be differentiating common bacterial species attributable to ovarian tumorigenic states across studies. For example, four of ten studies reported on bacteria at the species level although none of them shared commonalities. Still, reduced amounts of *Lactobacillus* in the GI tract of female mice and *Lactobacillus* species in human cervical smear samples were associated with EOC. *Chlamydia trachomatis* was detected in 80% of EOC cases, which is consistent with prior research [26, 31, 56]. In fact, researchers have found *C. trachomatis* in 84% of high-grade fallopian tube serous cancers and in 17% of HGSOCs [57]. This evidence corroborates the negative impact of chlamydial infection on fallopian tube health particularly as HGSOC, the most lethal EOC histotype, originates within the fallopian tube [38].

Similar to that of the GI tract and vagina, bacteria of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla were detected in the ovaries, fallopian tubes, and peritoneal fluid of women with EOC [52, 58, 59]. *Acinetobacter* and *Sphingomonas* genera belonging to the *Proteobacteria* phylum have been detected in the fallopian tubes and peritoneal fluid of cancer-free reproductive-aged women who were without any known infections [37]. Approximately 150 to 400 bacterial species reside in the GI tract and primarily belong to *Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria* phyla [60]. While exact proportions of GI tract bacteria do vary among individuals, scientists have observed influences of GI microbiota composition shifts in the development of several cancers. With colorectal (CRC) cancer for example, chronic inflammation, intestinal barrier breakage, signaling pathways, and DNA damage are associated with certain bacterial strains [61]. Specifically, *Bacteroidetes* and *Fusobacterium* enrichment are well observed in colorectal cancer tissue and fecal samples [62]. In addition, researchers [63] have observed increases in the richness of *Bacteroidetes* and *Proteobacteria* and reductions of *Actinobacteria* and *Firmicutes* in the stool specimens of patients with preneoplastic colon lesions; other investigators [64] have observed the opposite in CRC tissue samples. While mounting evidence supports the

prevalence of GI tract microbiota dysbiosis in CRC disease, microbial mechanistic pattern associations have not been determined in ovarian cancer research [65]. Unique ecosystem shifts and impaired cell membrane permeability could be important factors and a plausible explanation for bacterial peritoneal migration during ovarian tumorigenesis which may also have consequences in malignant ascites observed in both primary ovarian cancer and recurrent disease [66]. Potential contributory mechanisms could involve disruption of intestinal epithelial adhesion molecule signaling events that compromise normal immune response and cause widespread bacterial expansion [67]. Data from this review substantiate the presence of differing GI commensal bacteria groupings in fallopian tube, peritoneal, and ovarian tissue of women with EOC. Interestingly, primary cancers of the fallopian tubes and peritoneum are staged and managed in the same fashion as EOCs because they are histologically similar [68]. Thus, if initial HGSOC cells that originate in the fallopian tubes also displace to the peritoneum, detection of specific GI tract microbes may signal the presence of early precursor lesions.

Based solely on anatomical location, GI tract microbiota would seem less likely to reside in the reproductive tract than vaginal microbiota. Indeed, one study in this review validated the importance of vaginal commensals by reporting that women with EOC or *BRCA* mutations had reduced quantities of vaginal *Lactobacillus* species compared to women without disease [46]. Increasing literature has demonstrated probiotic effects of *Lactobacillus* against opposing vaginal and GI tract pathogens [69, 70]. Vaginal flora diversity has been associated with several gynecological cancers [36]. For instance, a greater abundance of *Porphyromonas* species was detected in cervicovaginal swabs collected from women with endometrial cancer [71]. *Gardnerella vaginalis*, *Atopobium vaginae*, and *Chlamydia trachomatis* have been enriched in cervical tissue samples of women with cervical intraepithelial lesion abnormalities and cervical cancer [72–75]. Researchers have also observed reductions in vaginal *Lactobacillus* species during HPV infection [76]. Consistent with some existing literature, HPV types 6, 16, 18, 45, and CMV were the most common viruses observed with EOC cases in this review [32]. These findings suggest possible synergistic actions of combined bacterial and viral pathogens during early transformation of epithelial ovarian cells. Yet the correlation between HPV and EOC oncogenesis remains controversial as several studies have reported conflicting findings, which are not seen with other cancers associated with HPV [33, 77]. For example, HPV 16, 18, and 45 types demonstrate the highest invasive cervical carcinogenic potential compared to all other types, and HPV 16 presents the highest risk in the in development of anal cancer [78, 79]. One study in this review reported that CMV was more prevalent in HGSOC cases [43]. In breast carcinogenesis, CMV gene products are proposed to instigate oncogenic infection of macrophages in breast epithelial cells favoring the appearance of tumor-associated macrophages (TAMs) [80]. Although specific pathways linking CMV to EOC are unclear, higher expressions of CMV protein in EOC tissue are linked with shorter survival rates compared to those without infection [81]. Pathogenic viral and bacterial infiltration stimulates inflammatory and immunological responses within the host [82]. Thus identification of microbial modulated immune checkpoints could prove most beneficial for early detection during cellular transformation and for more enhanced treatment targeting.

One animal study in this review reported that GI tract microbiota dysbiosis triggered inflammation, accelerated ovarian tumor growth, and activated TAMs infiltration causing induction of epithelial-mesenchymal transition (EMT) [45]. A hallmark of EMT is the downregulation of E-cadherin to reinforce the deterioration of adherens junctions contributing to the demise of the epithelial barrier function [83]. Investigators also observed microbiota mediated tumor-promoting inflammation locally [45, 46, 49] and systemically [45] through the production of proinflammatory cytokines including IL-6 and TNF-α. IL-6 signaling is shown to regulate cellular proliferation, adhesion and invasion in human ovarian cancer cells [84]. TNF-α is an important mediator of tumor promotion correlated with elevated ovarian cancer risk and advanced EOC tumor grade [85]. Microbiota-proinflammatory interactions have shown cytokine- and stimulus-specific patterns that influence immune response [86]. Thus GI microbiota dysbiosis could be linked to precise immune signaling throughout the tumorigenesis process. MicroRNAs have an important role in tumor cell metabolism and have emerged as key gene regulators to control inflammation [87, 88]. Existing data confirms a relationship between ovarian cancer, vaginal microbiota, miRNAs, and immune response, however, the regulatory mechanisms explaining gut microbiota activity in EOC are much less understood [89].

While chemotherapy influences on gut microbiota and the bidirectional relationship on cancer outcomes was not examined in the studies from this review, GI microbial activity during treatment is of particular importance in gynecologic cancer research [90, 91]. Specifically in women with EOC, fecal samples have shown increases in abundances of *Bacteroidetes* and *Firmicutes* and decreases in *Proteobacteria* after chemotherapy compared to prechemotherapy [92]. Yet, it is uncertain how these fluctuations correlate with overall treatment outcomes. Human investigations are lacking that evaluate tumor and gut microbiota influences on the survival rates of women with EOC. However, in women with cervical cancer who undergo chemoradiation, fecal enrichment of *Escherichia, Shigella, Enterobacteriaceae*, and *Enterobacteriales* are found to be an independent predictor of long term survival and *Porphyromonas*, *Porphyromonadaceae*, and *Dialister* enrichment are shown to be a predictor of short term survival [93]. Additionally, bacterial induced chemotherapy resistance is now broadly recognized across several cancers [94, 95]. Thus probiotics are promising modalities to eliminate the colonization of opposing bacterial species and re-

store healthy microbiota [96, 97]. Immunotherapy has become an exciting option in cancer treatment which includes the administration of immune checkpoint inhibitors that enhance T cell-mediated immune responses to counter tumor activity an improve overall survival of patients [98, 99]. Microbiota are shown to impact the efficacy of immunotherapy in kidney, lung, and melanoma cancers although the role of immunotherapy in EOC treatment remains controversial [100–104]. Consequently, several EOC clinical trials are underway evaluating immunotherapy although new strategies should also incorporate microbial targeted checkpoints.

4.1 Advantages and limitations of study techniques used for microbe detection

This scoping review summarized known associations between microbes. Yet only 10 studies were included that used different techniques of microbiota evaluation with variable sample sizes that could limit the generalizability of findings. Furthermore, none of the studies expanded testing methods to assess for associations between intratumor microbiota functional profiles and subsequent treatment outcomes. Investigators in one study [47] used a pan-pathogen functional gene array (Patho Chip) to capture a broader range of microbes including protozoan and fungi [105], which are not designed for detection with 16S rRNA sequencing. Quantitative PCR (qPCR) assays and 16S rRNA sequencing were the primary methods used to measure microbial genetic expressions in varying tissue samples. Each of these methods has advantages and drawbacks. Although qPCR is highly valid and valuable for detecting specific pathogens, it is not sufficient for large scale genomic detection and quantification. Thus, qPCR methods have been suggested as a validation tool for quantification of gene expression [106]. While advanced genome technology allows high throughput applications for analysis of microbial DNA and RNA, sequencing errors are possible [106]. Included studies varied in the reporting of bacterial taxonomy by phylum, genus, and species levels and used differing quantifiable terms of magnitude (i.e., up/down-regulated, abundance, diversity, proportion, and signatures). Ideally, consistency of species reporting would provide a means to robust interpretations across studies that could be further validated with qPCR. For instance, Zhou *et al.* [46] used qPCR validation in an attempt to recover the bacterial 16S rRNA sequencing results. Meanwhile, the qPCR platform was used for detection of HPV, EBV, and CMV in all the viral studies [26, 30, 43]. While qPCR tests are the traditional approach, a recent meta-analysis [77] reported the prevalence of HPV higher is in ovarian tissues when using two different techniques such as qPCR combined with immunohistochemistry or in situ hybridization. Moreover, expansions in genomic viral sequencing databases are also allowing opportunities to better refine associations between viral genomes and EOC in future investigations.

4.2 Implications for practice and future research

Researchers in each human study in the present review assessed microbial characterization in cervical, ovarian, fallopian tube, and/or peritoneal tissue samples whereas GI tract microbiota was assessed in fecal specimens in the animal studies. Forthcoming animal experiments should integrate analysis of local and systemic host immune response, intestinal epithelial integrity, and tumor tissue microbial markers in the presence of GI tract microbiota dysbiosis. Distinguishing possible direct and indirect microbial-mediated molecular interactions in the tumor microenvironment will prove paramount. Future research should also address chronic inflammation and fecal microbiota profiles in differing ovarian cancer histologic subtypes. Although early lesions are undetectable with current screening methods, women with ovarian cancer often report GI- related symptoms. It is possible that the GI tract commensal microbes signal peritoneal inflammation and GI microbiota dysbiosis becomes a co-factor to many symptoms that women experience. Human investigations are needed to incorporate patient reported symptoms with microbial migration, inflammation, tumor progression, and disease recurrence among ethnically diverse populations for clinical application. Furthermore, research is needed to examine associations between microbiota signatures, treatment tolerance, and tumor response to chemotherapy, and patient survival rates.

5. Conclusions

In this review, we identified a variety of microbes associated with ovarian cancers of epithelial histology. While exact mechanisms remain unknown, the EOC tumor microenvironment harbors diverse microbiota that vary among studies. Based on these findings, we conclude that additional research is needed to replicate study findings and reconcile the literature. The clinical applicability of tumor, GI tract, and vaginal microbiota biosignatures remains indeterminate in this population of women. The detection of distinctive microbial candidates that correlate with existing disease markers could have important implications in the practice setting. While identification of valid and reliable ovarian cancer diagnostic and prognostic biomarkers remains a research priority, differentiating microbiota types that signify early disease would be groundbreaking in clinical gynecologic oncology. EOC research is situated at a pivotal time when investigations are lacking that examine symptom biology and symptom burden on patients. If GI microbiota imbalances are associated with symptom burden, this opens new possibilities for the development of new probiotics that promote healthy GI bacteria and symptom relief. Furthermore, if microbiota profiles unique to EOC could be routinely detected in fecal or vaginal samples, a simple microbial-based test could aid with symptom management, earlier diagnosis, and inform on precise treatment strategies. Nonetheless, important issues must still be considered including uniformity of testing methods and tissue sampling and, most importantly, the practicality of specimen collection in a clinic setting. In particular, the activity of commensal GI tract and vaginal microbiota warrants additional examination in the scientific pursuit of

novel biomarkers that detect early disease, aid prognosis, improve symptom management during treatment, predict disease recurrence, and guide development of therapeutic interventions.

Author contributions

DEM and SP contributed to the conception of the manuscript. BML provided search strategy assistance. DEM and SP wrote the manuscript. LS and JDP reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://ejgo.imrpress.com/EN/10.31083/j.ejgo4205150.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2018 68: 394–424.

[2] Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, *et al.* Ovarian cancer statistics, 2018. CA: a Cancer Journal for Clinicians. 2018 68: 284–296.

[3] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: a Cancer Journal for Clinicians. 2020; 70: 7–30.

[4] Mathieu KB, Bedi DG, Thrower SL, Qayyum A, Bast RC. Screening for ovarian cancer: imaging challenges and opportunities for improvement. Ultrasound in Obstetrics & Gynecology. 2018; 51: 293–303.

[5] Doubeni CA, Doubeni AR, Myers AE. Diagnosis and management of ovarian cancer. American Family Physician. 2016; 3: 937–944.

[6] Desai A, Xu J, Aysola K, Qin Y, Okoli C, Hariprasad R, *et al.* Epithelial ovarian cancer: an overview. World Journal of Translational Medicine. 2014; 3: 1–8.

[7] Lisio M, Fu L, Goyeneche A, Gao Z, Telleria C. High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. International Journal of Molecular Sciences. 2019; 20: 952.

[8] Lee J, Minasian L, Kohn EC. New strategies in ovarian cancer treatment. Cancer. 2019; 125: 4623–4629.

[9] Soong TR, Howitt BE, Miron A, Horowitz NS, Campbell F, Feltmate CM, *et al.* Evidence for lineage continuity between early serous proliferations (ESPs) in the Fallopian tube and disseminated high-grade serous carcinomas. Journal of Pathology. 2018; 246: 344–351.

[10] Kyo S, Ishikawa N, Nakamura K, Nakayama K. The fallopian tube as origin of ovarian cancer: Change of diagnostic and preventive strategies. Cancer Medicine. 2020; 9: 421–431.

[11] Mota A, Triviño JC, Rojo-Sebastian A, Martínez-Ramírez Á, Chiva L, González-Martín A, *et al.* Intra-tumor heterogeneity in TP53 null High Grade Serous Ovarian Carcinoma progression. BMC Cancer. 2015; 15: 940.

[12] Testa U, Petrucci E, Pasquini L, Castelli G, Pelosi E. Ovarian Cancers: Genetic Abnormalities, Tumor Heterogeneity and Progression, Clonal Evolution and Cancer Stem Cells. Medicines. 2018; 5: 16.

[13] Otsuka I. Mechanisms of High-Grade Serous Carcinogenesis in the Fallopian Tube and Ovary: Current Hypotheses, Etiologic Factors, and Molecular Alterations. International Journal of Molecular Sciences. 2021; 22: 4409.

[14] Lucidi A, Buca D, Ronsini C, Tinari S, Bologna G, Buca D, *et al.* Role of Extracellular Vesicles in Epithelial Ovarian Cancer: A Systematic Review. International Journal of Molecular Sciences. 2020; 21: 8762.

[15] Zhou Y, Zheng X, Xu B, Hu W, Huang T, Jiang J. The Identification and Analysis of mRNA-lncRNA-miRNA Cliques From the Integrative Network of Ovarian Cancer. Frontiers in Genetics. 2019; 10: 751.

[16] Weinstock GM. Genomic approaches to studying the human microbiota. Nature. 2012; 489: 250–256.

[17] Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, *et al.* The NIH Human Microbiome Project. Genome Research. 2009; 19: 2317–2323.

[18] Castaño-Rodríguez N, Goh K, Fock KM, Mitchell HM, Kaakoush NO. Dysbiosis of the microbiome in gastric carcinogenesis. Scientific Reports. 2017; 7: 15957.

[19] Ramírez-Labrada AG, Isla D, Artal A, Arias M, Rezusta A, Pardo J, *et al.* The Influence of Lung Microbiota on Lung Carcinogenesis, Immunity, and Immunotherapy. Trends in Cancer. 2020; 6: 86–97.

[20] Massari F, Mollica V, Di Nunno V, Gatto L, Santoni M, Scarpelli M, *et al.* The Human Microbiota and Prostate Cancer: Friend or Foe? Cancers. 2019; 11: 459.

[21] Farolfi A, Gurioli G, Fugazzola P, Burgio SL, Casanova C, Ravaglia G, *et al.* Immune System and DNA Repair Defects in Ovarian Cancer: Implications for Locoregional Approaches. International Journal of Molecular Sciences. 2019; 20: 2569.

[22] Jia D, Nagaoka Y, Katsumata M, Orsulic S. Inflammation is a key contributor to ovarian cancer cell seeding. Scientific Reports. 2018; 8: 12394.

[23] Xie X, Yang M, Ding Y, Chen J. Microbial infection, inflammation and epithelial ovarian cancer. Oncology Letters. 2017; 14: 1911–1919.

[24] Piao J, Lee EJ, Lee M. Association between pelvic inflammatory disease and risk of ovarian cancer: an updated meta-analysis. Gynecologic Oncology. 2020; 157: 542–548.

[25] Idahl A, Le Cornet C, Gonzalez Maldonado S, Waterboer T, Bender N, Tjonneland A, *et al.* Serologic markers of Chlamydia trachomatis and other sexually transmitted infections and subsequent ovarian cancer risk: Results from the EPIC cohort. International Journal of Cancer. 2020; 147: 2042–2052.

[26] Shanmughapriya S, Senthilkumar G, Vinodhini K, Das BC, Vasanthi N, Natarajaseenivasan K. Viral and bacterial aetiologies of epithelial ovarian cancer. European Journal of Clinical Microbiology & Infectious Diseases. 2012; 31: 2311–2317.

[27] Alibek K, Karatayeva N, Bekniyazov I. The role of infectious agents in urogenital cancers. Infectious Agents and Cancer. 2012; 7: 35.

[28] Fortner RT, Terry KL, Bender N, Brenner N, Hufnagel K, Butt J, *et al.* Sexually transmitted infections and risk of epithelial ovarian cancer: results from the Nurses' Health Studies. British Journal of Cancer. 2019; 120: 855–860.

[29] Idahl A, Lundin E, Elgh F, Jurstrand M, Møller JK, Marklund I, *et al.* Chlamydia trachomatis, Mycoplasma genitalium, Neisseria gonorrhoeae, human papillomavirus, and polyomavirus are not detectable in human tissue with epithelial ovarian cancer, borderline tumor, or benign conditions. American Journal of Obstetrics and Gynecology. 2010; 202: 71.e1–71.e6.
[30] Ingerslev K, Høgdall E, Skovrider-Ruminski W, Schnack TH, Lidang M, Høgdall C, *et al.* The prevalence of EBV and CMV DNA in epithelial ovarian cancer. Infectious Agents and Cancer. 2019; 14: 7.
[31] Jonsson S, Oda H, Lundin E, Olsson J, Idahl A. Chlamydia trachomatis, Chlamydial Heat Shock Protein 60 and Anti-Chlamydial Antibodies in Women with Epithelial Ovarian Tumors. Translational Oncology. 2018; 11: 546–551.
[32] Pathak S, Wilczynski JR, Paradowska E. Factors in Oncogenesis: Viral Infections in Ovarian Cancer. Cancers. 2020; 12: 561.
[33] Svahn MF, Faber MT, Christensen J, Norrild B, Kjaer SK. Prevalence of human papillomavirus in epithelial ovarian cancer tissue. A meta-analysis of observational studies. Acta Obstetricia et Gynecologica Scandinavica. 2014; 93: 6–19.
[34] Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. Journal of Physiology. 2017; 595: 451–463.
[35] Muzny CA, Taylor CM, Swords WE, Tamhane A, Chattopadhyay D, Cerca N, *et al.* An Updated Conceptual Model on the Pathogenesis of Bacterial Vaginosis. Journal of Infectious Diseases. 2019; 220: 1399–1405.
[36] Xu J, Peng JJ, Yang W, Fu K, Zhang Y. Vaginal microbiomes and ovarian cancer: a review. American Journal of Cancer Research. 2020; 10: 743–756.
[37] Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, *et al.* The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nature Communications. 2017; 8: 875.
[38] Kim J, Park EY, Kim O, Schilder JM, Coffey DM, Cho C, *et al.* Cell Origins of High-Grade Serous Ovarian Cancer. Cancers. 2019; 10: 433.
[39] Arksey H, O'Malley L. Scoping studies: towards a methodological framework. International Journal of Social Research Methodology. 2005; 8: 19–32.
[40] Levac D, Colquhoun H, O'Brien KK. Scoping studies: advancing the methodology. Implementation Science. 2010; 5: 69.
[41] Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, *et al.* Chapter 7: Systematic reviews of etiology and risk. In Aromataris E, Munn Z (eds.) Joanna Briggs Institute Reviewer's Manual. Australia: Joanna Briggs Institute. 2017.
[42] Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology. 2014; 14: 43.
[43] Paradowska E, Jabłońska A, Studzińska M, Wilczyński M, Wilczyński JR. Detection and genotyping of CMV and HPV in tumors and fallopian tubes from epithelial ovarian cancer patients. Scientific Reports. 2019; 9: 19935.
[44] Wang Q, Zhao L, Han L, Fu G, Tuo X, Ma S, *et al.* The differential distribution of bacteria between cancerous and noncancerous ovarian tissues in situ. Journal of Ovarian Research. 2020; 13: 8.
[45] Xu S, Liu Z, Lv M, Chen Y, Liu Y. Intestinal dysbiosis promotes epithelial-mesenchymal transition by activating tumor-associated macrophages in ovarian cancer. Pathogens and Disease. 2019; 77: ftz019.
[46] Zhou B, Sun C, Huang J, Xia M, Guo E, Li N, *et al.* The biodiversity Composition of Microbiome in Ovarian Carcinoma Patients. Scientific Reports. 2019; 9: 1691.
[47] Banerjee S, Tian T, Wei Z, Shih N, Feldman MD, Alwine JC, *et al.* The ovarian cancer oncobiome. Oncotarget. 2017; 8: 36225–36245.
[48] Miao R, Badger TC, Groesch K, Diaz-Sylvester PL, Wilson T, Ghareeb A, *et al.* Assessment of peritoneal microbial features and tumor marker levels as potential diagnostic tools for ovarian cancer. PLoS ONE. 2020; 15: e0227707.
[49] Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, Perales-Puchalt A, *et al.* Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer Cell. 2015; 27: 27–40.
[50] Nené NR, Reisel D, Leimbach A, Franchi D, Jones A, Evans I, *et al.* Association between the cervicovaginal microbiome, BRCA1 mutation status, and risk of ovarian cancer: a case-control study. Lancet Oncology. 2019; 20: 1171–1182.
[51] Belizário JE, Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. Frontiers in Microbiology. 2015; 6: 1050.
[52] Diop K, Dufour J, Levasseur A, Fenollar F. Exhaustive repertoire of human vaginal microbiota. Human Microbiome Journal. 2019; 11: 100051.
[53] Johnson EL, Heaver SL, Walters WA, Ley RE. Microbiome and metabolic disease: revisiting the bacterial phylum Bacteroidetes. Journal of Molecular Medicine. 2017; 95: 1–8.
[54] de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Global Health. 2020; 8: e180–e190.
[55] Li N, Zhan X. Anti-parasite drug ivermectin can suppress ovarian cancer by regulating lncRNA-EIF4a3-mRNA axes. EPMA Journal. 2020; 11: 289–309.
[56] Zadora PK, Chumduri C, Imami K, Berger H, Mi Y, Selbach M, *et al.* Integrated Phosphoproteome and Transcriptome Analysis Reveals Chlamydia-Induced Epithelial-to-Mesenchymal Transition in Host Cells. Cell Reports. 2019; 26: 1286–1302.e8.
[57] Laban M, Ibrahim EA, Hassanin AS, Nasreldin MA, Mansour A, Khalaf WM, *et al.* Chlamydia trachomatis infection in primary fallopian tube and high-grade serous ovarian cancers: a pilot study. International Journal of Women's Health. 2019; 11: 199–205.
[58] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, *et al.* What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms. 2020; 7: 14.
[59] Amabebe E, Anumba DOC. Female Gut and Genital Tract Microbiota-Induced Crosstalk and Differential Effects of Short-Chain Fatty Acids on Immune Sequelae. Frontiers in Immunology. 2020; 11: 2184.
[60] Davenport ER, Sanders JG, Song SJ, Amato KR, Clark AG, Knight R. The human microbiome in evolution. BMC Biology. 2017; 15: 127.
[61] Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nature Reviews. Gastroenterology & Hepatology. 2019; 16: 690–704.
[62] Xu K, Jiang B. Analysis of Mucosa-Associated Microbiota in Colorectal Cancer. Medical Science Monitor. 2017; 23: 4422–4430.
[63] Mori G, Rampelli S, Orena BS, Rengucci C, De Maio G, Barbieri G, *et al.* Shifts of Faecal Microbiota during Sporadic Colorectal Carcinogenesis. Scientific Reports. 2018; 8: 10329.
[64] Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Frontiers in Microbiology. 2015; 6: 20.
[65] Raskov H, Burcharth J, Pommergaard H. Linking Gut Microbiota to Colorectal Cancer. Journal of Cancer. 2017; 8: 3378–3395.
[66] Smolle E, Taucher V, Haybaeck J. Malignant ascites in ovarian cancer and the role of targeted therapeutics. Anticancer Research. 2014; 34: 1553–1561.
[67] Sumagin R, Parkos CA. Epithelial adhesion molecules and the regulation of intestinal homeostasis during neutrophil transepithelial migration. Tissue Barriers. 2015; 3: e969100.
[68] Halkia E, Spiliotis J, Sugarbaker P. Diagnosis and management of peritoneal metastases from ovarian cancer. Gastroenterology Research and Practice. 2012; 2012: 541842.
[69] Stavropoulou E, Bezirtzoglou E. Probiotics in Medicine: A Long Debate. Frontiers in Immunology. 2020; 11: 2192.
[70] Singh TP, Kaur G, Kapila S, Malik RK. Antagonistic Activity of Lactobacillus reuteri Strains on the Adhesion Characteristics of Selected Pathogens. Frontiers in Microbiology. 2017; 8: 486.

[71] Hokenstad AN, Mariani A, Walther-Antonio M. Vaginal detection of Porphyromonas somerae is indicative of endometrial cancer diagnosis. Gynecologic Oncology. 2017; 145: 76.
[72] Bhatla N, Puri K, Joseph E, Kriplani A, Iyer VK, Sreenivas V. Association of Chlamydia trachomatis infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia - a pilot study. Indian Journal of Medical Research. 2013; 137: 533–539.
[73] Godoy-Vitorino F, Romaguera J, Zhao C, Vargas-Robles D, Ortiz-Morales G, Vázquez-Sánchez F, *et al.* Cervicovaginal Fungi and Bacteria Associated with Cervical Intraepithelial Neoplasia and High-Risk Human Papillomavirus Infections in a Hispanic Population. Frontiers in Microbiology. 2018; 9: 2533.
[74] Kwasniewski W, Wolun-Cholewa M, Kotarski J, Warchol W, Kuzma D, Kwasniewska A, *et al.* Microbiota dysbiosis is associated with HPV-induced cervical carcinogenesis. Oncology Letters. 2018; 16: 7035–7047.
[75] Oh HY, Kim BS, Seo SS, Kong JS, Lee JK, Park SY, *et al.* The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. Clinical Microbiology and Infection. 2015; 21: 674.e1–674.e9.
[76] Chao X, Sun T, Wang S, Fan Q, Shi H, Zhu L, *et al.* Correlation between the diversity of vaginal microbiota and the risk of high-risk human papillomavirus infection. International Journal of Gynecologic Cancer. 2019; 29: 28–34.
[77] Cherif S, Amine A, Thies S, Taube ET, Braicu EI, Sehouli J, *et al.* Prevalence of human papillomavirus detection in ovarian cancer: a meta-analysis. European Journal of Clinical Microbiology & Infectious Diseases. 2021. (in press)
[78] Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, *et al.* Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. International Journal of Cancer. 2012; 131: 2349–2359.
[79] Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: a systematic review and meta-analysis. Lancet Infectious Diseases. 2018; 18: 198–206.
[80] Pasquereau S, Al Moussawi F, Karam W, Diab Assaf M, Kumar A, Herbein G. Cytomegalovirus, Macrophages and Breast Cancer. Open Virology Journal. 2017; 11: 15–27.
[81] Yin M, Chen A, Zhao F, Ji X, Li C, Wang G. Detection of human cytomegalovirus in patients with epithelial ovarian cancer and its impacts on survival. Infectious Agents and Cancer. 2020; 15: 23.
[82] Verhoef J, van Kessel K, Snippe H. Immune Response in Human Pathology: Infections Caused by Bacteria, Viruses, Fungi, and Parasites. Nijkamp and Parnham's Principles of Immunopharmacology. 2019; 360: 165–178.
[83] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nature Reviews Molecular Cell Biology. 2014; 15: 178–196.
[84] Wang Y, Li L, Guo X, Jin X, Sun W, Zhang X, *et al.* Interleukin-6 signaling regulates anchorage-independent growth, proliferation, adhesion and invasion in human ovarian cancer cells. Cytokine. 2012; 59: 228–236.
[85] Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, Fatah R, *et al.* The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. Cancer Research. 2007; 67: 585–592.
[86] Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, *et al.* Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity. Cell. 2017; 167: 1125–1136.e8.
[87] Tahamtan A, Teymoori-Rad M, Nakstad B, Salimi V. Anti-Inflammatory MicroRNAs and their Potential for Inflammatory Diseases Treatment. Frontiers in Immunology. 2018; 9: 1377.
[88] Pedroza-Torres A, Romero-Cordoba SL, Justo-Garrido M, Salido-Guadarrama I, Rodriguez-Bautista R, Montano S, *et al.* MicroRNAs in Tumor Cell Metabolism: Roles and Therapeutic Opportunities. Frontiers in Oncology. 2019; 9: 1404.
[89] Allegra A, Musolino C, Tonacci A, Pioggia G, Gangemi S. Interactions between the MicroRNAs and Microbiota in Cancer Development: Roles and Therapeutic Opportunities. Cancers. 2020; 12: 805.
[90] Muls A, Andreyev J, Lalondrelle S, Taylor A, Norton C, Hart A. Systematic Review: the Impact of Cancer Treatment on the Gut and Vaginal Microbiome in Women with a Gynecological Malignancy. International Journal of Gynecological Cancer. 2017; 27: 1550–1559.
[91] Rizzo AE, Gordon JC, Berard AR, Burgener AD, Avril S. The Female Reproductive Tract Microbiome-Implications for Gynecologic Cancers and Personalized Medicine. Journal of Personalized Medicine. 2021; 11: 546.
[92] Tong J, Zhang X, Fan Y, Chen L, Ma X, Yu H, *et al.* Changes of Intestinal Microbiota in Ovarian Cancer Patients Treated with Surgery and Chemotherapy. Cancer Management and Research. 2020; 12: 8125–8135.
[93] Sims TT, El Alam MB, Karpinets TV, Dorta-Estremera S, Hegde VL, Nookala S, *et al.* Gut microbiome diversity is an independent predictor of survival in cervical cancer patients receiving chemoradiation. Communications Biology. 2021; 4: 237.
[94] Garajová I, Balsano R, Wang H, Leonardi F, Giovannetti E, Deng D, *et al.* The role of the microbiome in drug resistance in gastrointestinal cancers. Expert Review of Anticancer Therapy. 2021; 21: 165–176.
[95] Ma W, Mao Q, Xia W, Dong G, Yu C, Jiang F. Gut Microbiota Shapes the Efficiency of Cancer Therapy. Frontiers in Microbiology. 2019; 10: 1050.
[96] Gagliardi A, Totino V, Cacciotti F, Iebba V, Neroni B, Bonfiglio G, *et al.* Rebuilding the Gut Microbiota Ecosystem. International Journal of Environmental Research and Public Health. 2018; 15: 1679.
[97] Sehrawat N, Yadav M, Singh M, Kumar V, Sharma VR, Sharma AK. Probiotics in microbiome ecological balance providing a therapeutic window against cancer. Seminars in Cancer Biology. 2021; 70: 24–36.
[98] Hayase E, Jenq RR. Role of the intestinal microbiome and microbial-derived metabolites in immune checkpoint blockade immunotherapy of cancer. Genome Medicine. 2021; 13: 107.
[99] Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nature Reviews Immunology. 2020; 20: 651–668.
[100] Palaia I, Tomao F, Sassu CM, Musacchio L, Benedetti Panici P. Immunotherapy For Ovarian Cancer: Recent Advances And Combination Therapeutic Approaches. OncoTargets and Therapy. 2020; 13: 6109–6129.
[101] Pakish JB, Jazaeri AA. Immunotherapy in Gynecologic Cancers: are we there yet? Current Treatment Options in Oncology. 2017; 18: 59.
[102] Shaikh FY, Gills JJ, Sears CL. Impact of the microbiome on checkpoint inhibitor treatment in patients with non-small cell lung cancer and melanoma. EBioMedicine. 2019; 48: 642–647.
[103] Stancu AL. Gut Microbiome and the Response to Immunotherapy in Cancer. Discoveries. 2018; 6: e84.
[104] Gharaibeh RZ, Jobin C. Microbiota and cancer immunotherapy: in search of microbial signals. Gut. 2019; 68: 385–388.
[105] Lee Y, van Nostrand JD, Tu Q, Lu Z, Cheng L, Yuan T, *et al.* The PathoChip, a functional gene array for assessing pathogenic properties of diverse microbial communities. ISME Journal. 2014; 7: 1973–1984.
[106] Git A, Dvinge H, Salmon-Divon M, Osborne M, Kutter C, Hadfield J, *et al.* Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA. 2010; 16: 991–1006.