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Potential role of bacterial pathogens in the immunopathogenesis of ovarian cancer

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ABSTRACT

The development of next-generation sequencing (NGS) techniques allowed conducting research with greater efficiency and determining the microbial pattern of niches in the human body that were previously considered sterile. Observed changes in the microbiome composition of patients with cancer lesions increasingly indicate the role of microorganisms in the tumour induction and progression. Overgrowth of certain pathogenic strains within the tissue may cause inflammation, which in its chronic form may lead to destabilization of host genome. Such changes may result in altering the expression of genes encoding proteins involved in significant metabolic pathways and promote pathogenic cell functions such as proliferation stimulation, apoptosis inhibition and modulation of inflammatory response. Consequently, these events may lead to tissue destruction, disruption of physiological processes and development of disease states including cancer. In light of emerging reports on the role of changes in the composition of the microbiota in tumorigenesis induction and the presence of pathogenic strains in the ovarian cancer (OC) tumour microenvironment (TME), the hypothesis of a potential role for bacteria in the pathogenesis of this cancer is also gaining interest. The following review presents a summary of scientific research indicating potential role of TME bacteria in the immunopathogenesis of OC.

KEYWORDS: microbiota, TME, OC microbiota, ovarian microbiota

Introduction

Ovarian cancer is the deadliest disease of the female upper reproductive tract (URT) with a cancerous origin, with more than 300 000 new cases and more than 150 000 deaths reported worldwide each year. Due to the lack of specific symptoms, most patients are diagnosed at an advanced stage of the disease, which, combined with a poor prognosis and frequent drug resistance, translates into a

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high mortality rate. Therefore, it is extremely important to identify potential indicators that would allow early detection and diagnosis of these cancers, which would imply an improved prognosis for patients (Pathak *et al*., 2020). Particular attention of the scientific community has been drawn in recent years to the relationship between the presence of microorganisms in specific locations of the human body and the genesis of neoplastic processes (Łaniewski *et al*., 2020). The presence of certain bacterial species can contribute to altering the expression of genes encoding proteins involved in the inflammatory response, proliferation, apoptosis, cytotoxicity, as well as modifying the secretion of inflammatory factors. Consequently, these events can disrupt physiological processes and promote tumorigenesis (Francescone *et al*., 2014; Di Tucci *et al*., 2023). Relationships between the presence of specific strains and carcinogenesis have previously been demonstrated for several cancers (including gastric, colorectal cancers), and there are also reports of the possible involvement of a dysbiotic microbiota in some female URT cancers, including endometrial cancer (Walther-António *et al*., 2016; Walsh *et al*., 2019; Li *et al*., 2021; Lu *et al*., 2021; Chen *et al*., 2021). In recent years, the role of changes in the composition of patients' microbiota has gained increasing support from the scientific world in terms of potentially promoting carcinogenesis. Also, in the case of ovarian cancer (OC), the first reports of the presence of pathogenic strains in the TME are emerging (Zhou *et al*., 2019; Banerjee *et al*., 2017; Wang *et al*., 2020; Asangba *et al*., 2023). However, determining their potential role in the induction or progression of carcinogenic processes requires further research.

The female URT microbiota

For almost a century female URT was divided into three areas; the non-sterile vagina, the cervical mucus plug, which was believed to form a barrier to microorganisms, and the sterile endometrium, fallopian tubes, and ovaries (Baker *et al*., 2018). That is until the second part of the 20-th century when the first reports of microorganisms isolated from other than the vagina parts of the female reproductive tract (FRT) appeared. However, since these studies were conducted using culture methods, they did not reflect the biodiversity or abundance of microorganisms harbouring these niches, as only 1% of microorganisms residing in the human body are capable of growing on synthetic media. Moreover, the material used in these studies was mostly obtained from swabs, resulting in a high risk of contamination and thus inaccurate results (Tao *et al*., 2017). A breakthrough in case of understanding microbial composition within previously inaccessible areas of the human body was the development of NGS, which not only allowed more precise identification of the material, but also increased the efficiency of the process (Human Microbiome Project Consortium, 2012). Thanks to NGS, today without a doubt, we can say that not only vagina, but also other parts of FRT harbour their own microbiota (Pelzer *et al*., 2011).

Years of research into the origins of microorganisms within the URT indicate that they are most likely bacteria ascending from the vagina (Swidsinski *et al*., 2013). The argument supporting this thesis is the fact that the cervical mucus plug, wrongly considered an impermeable barrier for microorganisms, is not effective against all bacteria (Hansen *et al*., 2014). Transport of microorganisms down the FRT can be supported by the physiological uterine contractions, as well as the peristaltic pump which promotes

the sperm transport. This mechanism can also promote the transport of other associated macromolecules including bacteria, especially during the follicular and luteal phases of the menstrual cycle, which are characterized by increased frequency of contractions (Kunz and Leyendecker 2002; Zeryomanolakis *et al*., 2007).

Regardless numerous research results, we are still unable to conclusively determine the species composition of microorganisms residing in particular parts of the female URT. There are several limitations that we must consider in the context of interpreting the results of studies on the healthy microbial pattern of female URT. First of all, in majority of studies, the analysed material was obtained transcervically which is associated with a high risk of contamination. A potential solution to this problem could be to take material directly from the tissues of patients undergoing hysterectomy or oophorectomy, however, neither of these procedures is performed on healthy patients, so the results obtained could also be inaccurate. Moreover, most studies were performed on relatively small groups of patients, which can lead to false conclusions (Franasiak *et al*., 2020).

Despite the variations in the results obtained and multiple factors that must be considered when interpreting them, we can make some general conclusions based on the known data. While vagina is considered one of the most microbial-rich niches in female body, the available data indicate that the URT differs from it fundamentally in terms of both quantity and biodiversity. Comparing to vagina URT is considered as an environment with a lower bacterial abundance as it harbours up to 10 000 times less microorganisms, yet according to available data it exhibits a higher bacterial biodiversity. The study by Chen *et al*., showed that in the deeper parts of the reproductive tract, the number of *Lactobacillus* bacteria decreases sharply accounting for 97% of the microbiota at the cervical level, 30% at the uterine level and only 1.7% at the ovarian level, while the number of species with more diverse populations increases (Chen *et al*., 2017). However, it is worth noting that the study was conducted on material collected from patients with gynecologic conditions such as: hysteromyoma, adenomyosis, endometriosis and salpingemphraxis, whose microbiota composition may differ from healthy patients.

Ovarian microbiota

While the composition of the vaginal microbiota is considered relatively well understood, and studies of the endometrial microbial pattern provide new reports that bring us closer to determining its composition, we still do not know relatively much about the microorganisms residing in the ovary. Pelzer *et al*., screened follicular fluid collected from 262 *in vitro* fertilized (IVF) women for the presence of microorganisms. Study confirmed that the human follicular fluid contains bacteria, which allows us to hypothesize that the bacteria are also present in the ovary (Pelzer *et al*., 2013). Confirmation of this thesis may be a study conducted by Brewster *et al*. (2022) on a group of 10 postmenopausal women scheduled for salpingo-oophorectomy. Sequencing results confirmed the bacterial presence within proximal fallopian tube, fimbriae, and ovary of examined patients. Moreover, a significant difference between the microbiota of different parts of URT were observed. Microbial composition between the fallopian tube and the ovary differed, which confirmed the thesis that each part of the female URT harbours its own, unique microbiological pattern. Similar observations were made after comparing

the microbiota of the fallopian tube and the fimbriated end. The most abundant species detected in the ovaries were *Proteobacteria* (69%), *Bacteroides* (19%), *Actinobacteria* (10%) and *Firmicutes* (2%) (Brewster *et al*., 2022). In another study Miles *et al*. (2017) collected swabs and tissue samples from 10 patients undergoing total hysterectomy and bilateral salpingo-oopherectomy. The sequencing results obtained indicated greater biodiversity within the URT compared to the vaginal environment which is consistent with the observations of Chen *et al* (Miles *et al*., 2017; Chen *et al*., 2017). In another study Wang *et al*.(2020) compared the microbiome of 6 patients diagnosed with OC and 10 patients diagnosed with noncancerous ovarian conditions, which formed the control group in this study. Results revealed the presence of bacterial LPS both in oncologic and non-cancerous group, which once again confirmed the bacterial presence within the ovaries. Similarly to previous reports *Proteobacteria* was the most abundant phylium both in cancer (67.2%) and the control group (67.1%). Unlikely previous reports the second most abundant phylium in this study was *Firmicutes* (23.77% in the control group and 23.82% in the cancer group) and the third most abundant phylium was *Bacteroidetes* (3.26% in the control group and 3.41% in the cancer group) (Wang *et al*., 2020). The above studies undoubtedly provide us with valuable scientific evidence of microbial presence in the ovaries, but they cannot establish a scientific basis for determining the composition of a healthy ovarian microbiota. The available data comes from studies conducted on relatively small groups of patients who have been diagnosed with gynecological abnormalities such as ovarian cancer or fertility disorders, which can lead to false conclusions.

Role of TME in the immunepathogenesis of cancer

Tumour microenvironment, which is a complex, dynamic, and continuously evolving entity, consists of, among other factors; immune cells, extracellular matrix elements, stroma, and endothelial cells (Anderson and Simon, 2020). Considering recent reports indicating the presence of microorganisms in niches previously considered sterile and their potential role in carcinogenesis, microbes residing in the TME are increasingly being included among other elements forming this specific environment (Niño *et al*., 2022). The presence of microorganisms within a certain niche, can be safe and beneficial for the host, as long as a certain limit of bacterial invasion is maintained. When the microbial load is too high, or enriched in representatives of certain taxa, induction of abnormal pathological processes within the tissue may occur. Disruption of microbal homeostasis may contribute to excessive tissue destruction, immune stimulation, and disruption of key metabolic pathways (Punzón-Jiménez and Labarta, 2021).

Most cases of pathogen-induced inflammation, results in intruders' complete elimination by the immune system and restoration of homeostasis. Unfortunately, in case of some infections, complete elimination of pathogens does not occur, and their presence within the tissue results in the excessive secretion of inflammatory mediators, which over time results in chronic inflammation (Łaniewski *et al*., 2020). Toll-like receptors (TLRs) play a key role in detecting the presence of pathogens within the tissue, through both receptor and activation functions (Janeway and Medzhitov, 2002). Once bacterial molecular patterns are recognized by TLR receptors, the inflammatory cascade within the infected tissue is activated (Fig. 1). The released cytokines stimulate the

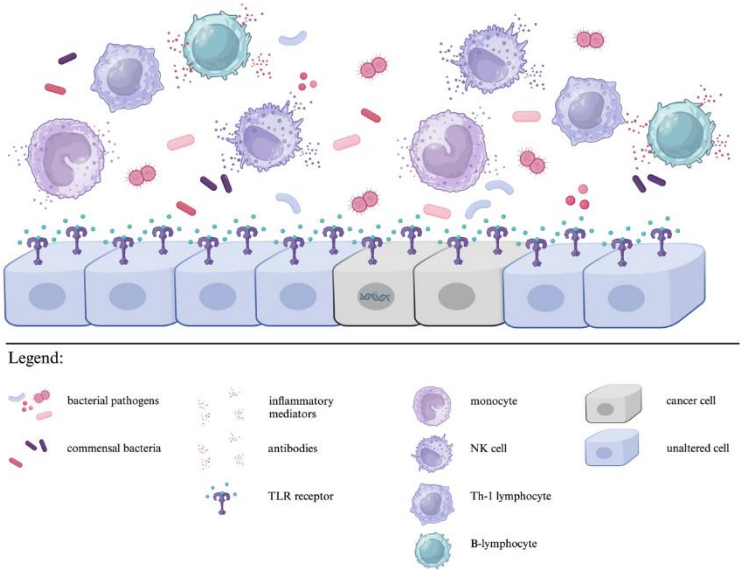

Figure 1. Progression of the inflammatory response in ovarian tissue.

differentiation of type 1 T helper (Th-1) lymphocytes, as well as the activation of monocytes and natural-killer (NK) cells, which secrete further inflammatory mediators including interleukin-2 (IL-2), IL-12, IL-15, IL-18, tumour necrosis factor α (TNF-α), interferons α (IFN-1) and β (IFN-2). Another line of defence activated by TLRs are B lymphocytes, which under activation transform into plasmatic cells secreting antibodies, which are a precise tool to fight foreign microorganisms. TLR receptors thus represent an activating factors for both specific and non-specific immunity (Majewska and Szczepanik, 2006; Bossowska-Nowicka *et al*., 2015).

Under physiological conditions, the inflammatory response persists until the intruder is eliminated, and then undergoes self-silencing. Unfortunately, in the case of a chronic reaction, which may be driven by a persistent bacterial infection, constant activation of TLR receptors, may result in their excessive mobilization, and thus further stimulation of the inflammatory response, or promotion of cellular angiogenesis (Basith *et al*., 2012). TLR receptors may express their role in promoting angiogenesis by stimulating the secretion of vascular endothelial growth factor (VEGF), which is responsible for the vascularization of tumour cells, allowing for significant tumour growth due to its supply in oxygen and nutrients (Carmeliet, 2005). Moreover, TLR activation may result in the activation of protein complex nuclear factor kappa B (NF-κB). NF-κB expresses its role in the inflammatory response by stimulating the production of pro-inflammatory cytokines, which, unfortunately, can also result in inhibition of apoptosis, stimulation of proliferation or enhancement of angiogenesis – processes which are the hallmarks of tumorigenesis (Liu *et al*., 2017). Moreover, TLR activation results in production of oxygen free radicals (ROS), which in excessive amount contributes to

lipids, proteins and nucleic-acid damage. Such changes within cell metabolism may contribute to tumour initiation, development and growth as well as therapy-resistance development (Ding *et al*., 2021).

Presence of certain bacterial taxa within the ovaries and persistent chronic inflammation can also lead to destabilization of host genome. Chronic inflammation, as well as bacterial second metabolites, provide an environment in which damage, resulting in changes to the host genome is more likely to occur. Among other things, the changes can be associated with overexpression of cyclooxygenase 1 (COX1) and 2 (COX2), nitric oxide, and ROS, which in high concentration within the TME may promote damage to the genetic material of host cells, potentially resulting in mutations (Vakkila and Lotze, 2004). Carcinogenesis requires at least two allelic mutations in a single gene, according to model proposed by Knudson. Such single mutations could lead to the development of cancer, but usually more mutations are required. However, chronic inflammation provides a highly mutagenic environment in which DNA damage is more likely to happen. The resulting mutations can lead to abnormalities in cellular metabolism such as proliferation or apoptosis – key processes in the pathogenesis of cancer (Knudson, 1971)

Even up to 13–20% of cancers diagnosed each year may have an inflammatory basis (Shahanavaj *et al*., 2015). However, identification of specific species and the mechanisms responsible for processes underlying carcinogenesis has been a challenge. To date, International Agency for Research on Cancer has classified only one bacterial species as a human carcinogen. Infection with bacteria *Helicobacter pylori*, referred to results in an inflammation of gastric mucosa (Rudnicka *et al*., 2019). The chronic inflammation can lead to peptic ulcer disease which if sustained for a long time, may also lead to stomach cancer (Nomura *et al*., 1991). Similar relationship has been demonstrated in case of colon cancer and the *Fusobacterium sp*. infection, in particular *F. mortiferum*, *F. nucleatum* and *F. necrophorum* species. Activated by *F. nucleatum* NF-κB factor, may result in development of chronic inflammation (Kostic *et al*., 2012).

Similar relationships were observed in case of URT cancers such as endometrial cancer (EC). In experiment conducted by Walther-António *et al*. (2016), presence of *Atopobium vaginae* and *Porphyromonas sp*. bacteria in combination with high vaginal pH was linked with EC. Walsh *et al*. (2019), also identified *Porphyromonas somerae* as the most predative marker for uterine cancer. In another study, Li *et al*. (2021), confirmed that increasing *Prevotella* abundance in endometrial tissue, especially when correlated with elevated serum levels of D-dimers (DD) and fibrin degradation products (FDP), may be associated with carcinogenesis. In study conducted by Lu *et al*. (2021), correlation between increased *Micrococcus sp*. abundance and IL-6 and IL-17 micro-RNA levels in EC patients was observed. In another study enrichment of *Firmicutes*, *Proteobacteria*, *Tenericutes*, *Actinobacteria* and *Bacteroidete* was observed in group of EC patients (Chen *et al*., 2021). The results presented, despite the variation, indicate that the role of changes in the composition of the microbiota not only of the stomach or colon, but also of the female URT should not be underestimated. In addition, some results point to a potentially inflammatory role of pathogens present in the tumour environment. Unfortunately, there is still a lack of evidence indicating whether the presence of microorganisms

in altered tumour tissue is an effect or a cause of neoplastic changes.

Pathogens associated with ovarian cancer

Ovarian cancer is the third most common female cancer with the highest mortality rate worldwide. Every year 300 000 women receive a diagnosis of ovarian cancer, which causes up to 152 000 deaths worldwide each year. The high mortality is caused mostly by the asymptomatic nature of OC, which results in late diagnosis, usually at an advanced stage of cancer (Sung *et al*., 2021). The high mortality rate of this cancer is also influenced by frequent resistance to chemotherapy. OC therapy most often includes optimal surgical reduction of tumour-altered tissue in combination with chemotherapy, as well as neoadjuvant chemotherapy, radiotherapy, and immunotherapy. Unfortunately, for more than 50% of patients with OC, the cancer recurs, usually in a form that is refractory to the chemotherapy used (Ding *et al*., 2021).

Autosomally inherited mutations in the BRCA breast cancer susceptibility (BRCA) genes, also known as hereditary breast and ovarian cancer (HBOC) syndrome, and in the DNA mismatch repair (MMR) genes, also known as hereditary nonpolyposis colorectal cancer (HNPCC) syndrome or Lynch II syndrome, are assumed to be responsible for about 15% of OC cases. However, mutations in the BRCA 1/2 genes, which account for up to 90% of hereditary OC cases, are most often responsible for HBOC syndrome. BRCA genes are a group of cancer suppressor genes, and mutations that are associated with them are predisposed to various cancers, including OC (Pan and Xie, 2017). Among other risk factors the most commonly discussed are high body mass index (BMI), smoking cigarettes, nulliparity and the family history. It is also supposed that a specific factor may be linked to the particular type of ovarian cancer (Fortner *et al*., 2019). Based on the genetic, clinical, and histopathological factors, OC is divided into type I and type II. Type I tumours are characterized by a good prognosis accounting for only 10% OC-related deaths. In this type of tumour cancer cells develop from benign extraovarian precursor lesions which are present in the ovary (Ducie *et al*., 2017). Type II cancers represent the majority of OC cases, characterized as highly aggressive neoplasms. The mortality of type II OC is reaching up to 90% of OC-related deaths, of which high-grade serous carcinoma (HGSC) is considered the most common form of diagnosis, resulting in 70–80% of deaths from OC (Bowtell *et al*., 2017). Scientific evidence over the past decade has challenged the theory that serous ovarian cancer originates in the surface epithelium of the ovary and has identified the fallopian tube as the source of HGSC. The distal region of the fallopian tube, also known as the fimbria, is exposed to the pelvic cavity, which is the most common location of serous carcinoma in BRCA-positive women with p53 mutations (Crum *et al*., 2007). Accordingly, Zhou *et al*. (2019) hypothesized that OC carcinogenesis may be promoted or driven by several factors, including but not limited to pelvic inflammatory disease (PID), local tumour immune microenvironment, hormonal fluctuations, and spontaneous mutations. The research team also points to disruptions in microbial composition as a potential driver of chronic inflammation, and thus perhaps carcinogenesis. In a conducted study, they compared the microbiota composition of tissue samples collected from 25 patients diagnosed with ovarian cancer and 25 normal distal fallopian tube tissue samples. High-throughput sequencing results revealed

that both diversity and richness indexes were significantly reduced in ovarian cancer tissues compared to tissues from normal distal fallopian tubes, which represented a control group in this study. At the phylum level, *Proteobacteria* and *Firmicutes* were the most dominant taxa in both groups. Whereas at the genus level, *Acinetobacter*, *Sphingomonas* and *Methylobacterium* were significantly enriched in the group of cancer patients, while *Lactobacillus* was the outstandingly dominant genus in the control group. Moreover, study revealed that transcriptional profiles of ovarian cancer tissues differed from the normal fallopian tube tissues. Inflammation-associated signalling pathways including NF-κB signalling pathway, cytokine-cytokine receptor interaction and chemokine signalling pathway were dramatically activated in OC tissues. Overall, presented results suggest role of changes within the ovarian microbiome in the stimulation of inflammatory processes, which may have a key role in the induction of carcinogenic processes (Zhou *et al*., 2019). Banerjee *et al*. (2017) screened 99 ovarian cancer samples using pan-pathogen array (PatoChip), combined with capture-next generation sequencing. In contrast to the results of Zhou *et al*., the above study showed greater biodiversity in the material collected from cancer patients, compared to the non-ovarian control group. The predominant bacterial phyla in ovarian cancer samples were *Proteobacteria* (52%), followed by *Firmicutes* (22%). Other phylla including *Bacteroidetes, Actinobacteria, Chlamydiae, Fusobacteria, Spirochaetes* and *Tenericutes* were also present in OC samples (Banerjee *et al*., 2017). Another study conducted by Wang *et al*. (2020) revealed that ovarian bacterial communities in ovarian cancer group were dominated by *Gemmata obscureglobus* (13.89%), followed by *Halobacteroides halobius* (11.99%) and *Methyloprofundus sedimenti* (11.12%). An interesting conclusion was reached by the research team of Asangba and colleagues, they analysed the microbiome of all body sites, with the exception of the stool and peritoneal network, which were not collected from the control group of patients with benign non-cancerous lesions in this study and compared them with the microbiome of patients diagnosed with cancer ovary (Asangba *et al*., 2023). The study showed significant differences between the microbiome of patients with OC, compared to a group of patients with benign gynecological conditions. In addition, the team also observed an overall enrichment of several microbial taxa, including *Dialister*, *Corynebacterium*, *Prevotella* and *Peptoniphilus* in all body sites of patients with unfavourable ovarian cancer outcomes, which may suggest a role for these microbes in modulating the body's response to therapy and provide an indicator in predicting the efficacy of treatment (Asangba *et al*., 2023). This result is also consistent with the thesis that microbial-induced carcinogenesis is often associated with global changes in the microbiome, rather than attributed to individual pathogens (Schwabe and Jobin, 2013).

Conclusions

With the development of research on the human microbiota, more and more evidence point to the undeniable role of microorganisms in the induction and progression of diseases that have posed a challenge to modern medicine for years. Among them, the role of disorders within the microbial composition in the context of cancer is increasingly being discussed. In recent years, numerous studies have shown that female URT, for years wrongly considered sterile, also has its own unique microbiota. Moreover, with

more reports of the presence of pathogenic microorganisms, among patients with established cancerous lesions, there is growing support for the thesis according to which bacteria may play an important role in the induction, and perhaps progression of cancer. Such relationships have previously been well established for gastric cancer, as well as for colorectal cancer. There is also growing evidence supporting the involvement of microorganisms in the induction of neoplastic lesions in the female URT, including the EC. Emerging evidence of the presence of pathogenic strains in the tissues of patients with OC, indicates that a similar relationship may also exist for this cancer. The presence of certain bacterial strains within the tissue can induce inflammation, which, persisting for a long time, develops into a chronic form. Chronic inflammation, in turn, can provoke can provoke changes within the host's genetic material that result in disruption of key metabolic pathways, posing a risk of cancer development.

Ovarian cancer is now one of the most frequently diagnosed and deadliest female cancers in the world. Due to its late diagnosis and lack of specific symptoms, it is most often detected at an advanced stage, when treatment options are severely limited. Determining the microbiological pattern of the ovary, as well as identifying strains with a potentially carcinogenic role, gives hope for the development of specific biomarkers that would allow an early detection of pathological changes in the tissue. However, further research is needed, not only to indicate the composition of the ovarian microbiome in a state of eubiosis and potentially pathogenic strains, but also to detect mechanisms underlying bacterial-induced carcinogenesis.

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