CERVICAL CANCER AND THE MICROBIOME

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Abstract

Gynecological cancers have been associated with microbiome formation. Cervical cancer (CC) in frequency is the fourth most common cancer in women worldwide. The human microbiota represent the constellation of microorganisms that inhabit our bodies. The microbiome is able to modulate immune responses, alter the physiology of the human organism and increase the risk of viral infections and development of diseases such as cancer.

The microbiota plays an important role controlling viral infections, such as those caused by human papillomavirus (HPV) infection or HIV. Microbiomes are also directly linked to cervical cancer (CC). Microbiomes are essential in preventing the invasion of pathogens; therefore, disruption of the dynamics between the microbiome and the vaginal ecosystem, as a host, is prone to cause infections and acquired lesions that cause copulation disorders and cancer.

Cervical cancer is almost invariably caused by HPV genotypes, 16 and 18, responsible for 65-80% of cervical cancers. HIV-positive women show an increased risk of HPV infection and development of cervical intraepithelial neoplasia (CIN).

In this review, we summarize the current knowledge concerning changes in the cervical microbiota in the context of HPV infection. Also, the discussion about the probiotics intervention on the microbiota is integrated.

Keywords:
cervical cancer, microbiome, HPV, koilocytes

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Introduction

The human microbiome represents the multitude of microorganisms in the body, that exist in a mutualistic relationship with the host. Recent studies indicate that perturbations in the microbiome may be implicated in a number of diseases, including cancer.

The concept of microbiome was first used by Lederberg and McCray to designate a set of commensal, symbiotic, or pathogenic microorganisms which share the same living space and develop a complex interaction with certain human tissues.

Our knowledge of the human microbiome and its implications in health and disease has grown exponentially in recent years, largely due to advances in DNA sequencing technology and the establishment of several international consortia to characterize and understand the role of the human microbiome in health and in several types of diseases.

The microbiome is able to modulate the immune responses, alter the physiology of the human body and increase the risk of viral infections and development of diseases such as cancer. The meta-analyses provide evidence that sexually active women with vaginal dysbiosis may have anogenital infection, associated with cervical cancers, with lesions precursor to HPV infection with low-risk genotypes and high-risk oncogenic genotypes, in which 16 HPV genotype is present in over 50% squamous cell carcinomas.

In HPV and cervical cancer research, vaginal dysbiosis is often diagnosed when clue cells are seen on Papanicolaou smears, and the reported accuracy of this approach is inconsistent. The microbiome of the healthy female genital tract is characterized by the presence of one or more types of lactobacilli. The composition of the cervico-vaginal microbiome (CVM) is influenced by various factors, such as ethnicity, hormonal alterations, sexual activity and hygiene habits, as well as lactation, diabetes mellitus, stress and dietary factors.

Vaginal acidity prevents colonization by anaerobes, maintains the cervical epithelial barrier through production of bacteriocins, and acts against mucin degradation, keeping away opportunistic infections.

Recently, the vaginal microbiome has emerged as a new variable that could greatly influence the natural history of HPV infections and their clinical impact. Therefore, understanding the impact of the vaginal microbiome (VM) composition and its alterations (dysbiosis) on HPV infection/persistence may contribute to a better prediction of the outcomes of infections by this virus. HPV carcinogenesis is mediated by its E6 and E7 oncoproteins, which force differentiating epithelial cells to re-enter the cell cycle to grow and increase viral production.

Cervical cancer (CC) represents the fourth most frequent malignancy among women worldwide and it is a serious public health problem. In the 1980s, HPV was identified in cervical cancer tissue. HPV infections are a major etiological agent for cervical cancer (CC).

However, not all women with HPV infection develop cervical cancer. A cancer that occurs in the tissues of the cervix, the lower part of uterus that connects to vagina, develops slowly over a period of time.

There may be warning signs and symptoms: metrorrhagia, postmenopausal bleeding, bleeding during intercourse, dyspareunia, vaginal discharge. HPV infection is one of the most common sexually transmitted infections,
but it is unclear why only a small proportion of infections with high-risk viral strains progress to cervical cancer (Fig.1).

Factors associated with viral persistence and progression to CC are not yet evident, however, recent work has shown a possible role of the epithelial microbiome in viral infections and cancer progression. In this context, changes in the vaginal microbiome were detected in women infected with HPV and in women with HPV-associated lesions and cancer.

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections. However, only a small percentage of high-risk HPV (HR) infections progress to premalignancy and cancer. HPV infections are acquired through multiple sexual intercourse, sexually active adolescents and young adult women at the highest risk of acquisition.

During HPV infection, low-grade cervical abnormalities may be clinically detectable during screening by the presence of koilocytes. The abundance of Lactobacillus iners has been associated with the elimination of high-risk HPV infections. The vaginal microbiota dominated by Lactobacillus crispatus is associated with a low risk of HIV infection and, in HIV-positive women, a low risk of HPV infection. (Fig.2).

Dysbiosis of the cervico-vaginal microbiota, mainly involving sexually transmitted infections, is associated with increased cervical cancer risk. On the other hand, cervico-vaginal environment modifications may act together with HPV infection, negatively contributing from the early stages of CC and creating, for instance, a local immunosuppression state. Some studies have suggested that certain cervico-vaginal microbiota species can modulate the local inflammatory immune response, possibly promoting the expression of immunosuppressive cytokines, and that abnormal vaginal microbiota is related to HPV infection and persistence.

Persistent infection with high-risk human papillomavirus can lead to cervical dysplasia and cancer. More than 200 different types of HPV that infect epithelial cells are currently described.
These are further divided into **low-risk** and **high-risk** HPV (lr-HPV and hr-HPV, respectively), depending on their carcinogenic potential (20, 21, 22).

Persistent infection with high-risk oncogenic human papillomavirus (HPV) (hr-HPV) is the main factor for the development of CC and it has been found in 99.7% of CC samples.

HrHPV infection is necessary, but not sufficient for the development of CC, and additional factors are involved in the establishment, progression or regression of the disease. Infection with hr-HPV is widespread in sexually active women with multiple partners. Of hr-HPV strains, HPV-16 and HPV-18 are responsible for approximately 70% of CC cases worldwide. Although HPV infection per se is not enough to promote CC, the development of a form of persistent infection is a major factor for cervical lesion progression and cancer outcome.

Most HPV-infected women do not develop cervical cancer because the immune response controls the infection, preventing cervical lesion development and its progression to cancer. Thus, only a small proportion of infected women are unable to control the infection and develop CC.

This fact suggests that additional factors might influence the progression of **cervical intraepithelial neoplasia (CIN)** to CC or, conversely, its regression. The cervico-vaginal microbiome is a dynamic network of microorganisms able to modulate a host’s immune responses and promote an environment susceptible to viral infection acquisition and development of CIN (3, 4), (Fig.3).

It has been shown that, in hrHPV-infected cells, tobacco smoke induces an increase in oncogene E6 transcription, leading to a decrease in p53 activity and levels, which

![Fig. 2 Schematic model of the population-level natural history of human papillomavirus infection and cervical cancer. Purple boxes indicate well-accepted natural history model parameters; blue boxes represent uncertainties (1)](image-url)
**Fig. 3** The impact of microbiota dysbiosis in carcinogenesis (5)

**Fig. 4** Classification of human vaginal microbial communities
may facilitate the development of squamous cell carcinoma. It is worth noting that CST IV (from the classification of the types of community states (23), was increased in women who smoked (Fig.4).

Microbiome’s dysbiosis can have important effects on overall health and has recently been linked to cancer progression and treatment responses.

Recently, some studies have shown an association between the cervicovaginal microbiome and HPV infection, as well as CIN and CC. HPV cancers may be uniquely affected by the microbiome, as these solid tumors appear in the mucosa of the urogenital tract, which each have unique and diverse microorganisms. I

nsights into the potential influence of the microbiome on viral persistence, immune response, host-mucosal environment, and cancer treatments for HPV-related cancers are just beginning to emerge.

The microbiome of a healthy female genital tract is characterized by the presence of one or few types of lactobacilli.

Changes in the vaginal microbiome have been detected in women infected with HPV and in women with HPV-associated lesions and cancer (6).

Several studies have correlated different vaginal microbiomes (VMs) with HPV infection, different degrees of CIN and CC. Microorganisms can produce toxins able to alter host cells and even modulate the immune system affecting its functionality (Fig.5).

The composition of the cervico-vaginal microbiota is dynamic, changing due to the hormonal fluctuations that occur during women’s reproductive cycle, use of oral contraceptives, sexual activity, vaginal douching, lactation, diabetes mellitus and stress.

Proteomic studies have been performed on cervicovaginal fluid to analyze and understand the role of the microbiota on the cervical region metabolism. A study using proteomic data showed that dysbiosis causes cervicovaginal inflammation and detrimental changes within the mucosal barrier.

It is known that HPV is necessary but not sufficient to cause CC. Among the cofactors in CC development, the vaginal microbiome (VM) may play an important role (7).

VM has been associated with higher HPV infection rates, suggesting that an increase in the diversity of vaginal bacteria together with a reduction in lactobacilli may contribute to the persistence of HPV infection (8).

Persistent infection with some types of mucosal human papillomavirus (HPV) is the etiological factor for the development of cervical cancer and its precursor lesions.

On the other hand, cervicovaginal environment modifications may act together with HPV infection, contributing since the early stages of CC and creating, for instance, a local immunosuppression state (9).

Some studies have suggested that certain cervicovaginal microbiota species can modulate the local inflammatory immune response, possibly promoting the expression of immunosuppressive cytokines, and that abnormal vaginal microbiota is related to HPV infection and persistence (10).

Recent studies have assessed the potential relationship between the vaginal microbiome (VM) and gynecological cancer.
The sex hormones interfere with VM composition, regulating the release of pro-inflammatory cytokines, chemokines, and antimicrobial peptides (AMP) contributing towards the selection of vaginal microbial species.

Estrogen, in particular, has been implicated in the passage to a lactobacillus-rich microbiome during puberty and inversely poor during menopause. Estrogen in the vaginal epithelium leads to its maturation and proliferation, as well as to the accumulation of glycogen, which is needed for a lactobacillus-rich environment. After menopause, the decline in estrogen production is accompanied by a decrease in lactobacilli and a predominance of anaerobes (14).

The VM composition is dynamic (12). Interaction between environmental and genetic factors with gut microbiota influences gut immunity. Sex hormones influence autoimmunity via gut microbial composition. Modulation of gut microbial composition may provide a novel target for treatment for autoimmune diseases. Variations in the vaginal flora composition during the menstrual cycle as a consequence of variations in estrogen and progesterone levels have been demonstrated.
The VM composition is influenced by various factors, such as ethnicity, hormonal alterations, sexual activity, and hygiene habits, as well as lactation, diabetes mellitus, stress, and dietary factors (13).

Variations in the vaginal flora composition during the menstrual cycle as a consequence of variations in estrogen and progesterone levels have been demonstrated (15).

Probiotics have also been investigated for their beneficial role in the clearance of HPV, a virus associated with the development of cervical cancer.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Probiotics containing species of lactobacilli have been used in the treatment of urogenital infections to improve the vaginal flora.

Another possible application of probiotics is to restore a healthy genital microbial community after some gynecological procedures. Additional longitudinal studies are needed to understand whether CC and lesions outcomes are associated with the constitution of the vaginal microbiome (16).

Modulation of the vaginal microbiota is essential for women’s health. An association between bacterial vaginosis (BV) and CIN has long been suggested.

Studies suggest a variety of mechanisms in which BV may result in HPV persistence and CIN. Women with BV expressed increased levels of cytokine interleukin (IL)-1β and decreased levels of anti-inflammatory molecule SLPI (secretory leukocyte protease inhibitor). Gut dysbiosis has been associated with tumorigenesis through inflammation and cytokine modulation, but its role in HPV clearance and cervical carcinogenesis is still unclear. It was suggested that both the cervical and gut microbiome are associated with treatment response in cervical cancers.

The use of probiotics is demonstrated in vivo and in vitro for HPV clearance and significant CIN regression. Further longitudinal studies are needed to investigate whether and how the microbiome helps maintain the persistent infection and develop to CIN or cervical cancer.

Therefore, the manipulation of the microbiota by the use of probiotics or by vaginal microbiota transplant may be a feasible option to induce HPV infection clearance, CIN regression, and stop progression to cervical cancer (17).

A key area of controversy is whether serum antibodies from natural HPV infection protect against re-infection with the same HPV type.

It is possible that a lack of protection associated with lower HPV antibody titers may be the result of misclassification of baseline HPV serostatus, rather than a lack of protection associated with lower antibody titers.

Other studies suggest that antibodies may offer protection against type-specific re-infection that wanes with age.

According to the World Health Organization, probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host”.

The species of Bifidobacterium, Lactobacillus and Streptococcus are capable of altering the host’s microbiome, improving the immune response and the inflammatory state.

Probiotics containing species of lactoba-
cilli have been used in the treatment of urogenital infections to improve the vaginal flora.

The mechanism of action would involve vaginal acidification, prevention of bacterial adhesion, and synergistic action with the host’s immune system (18).

The potential of probiotics as a single therapy has also been documented. The effects of probiotics on cytological alterations of the cervix and on HPV infection were evaluated (19).

Additional longitudinal studies are needed to understand whether CC and lesion outcomes are associated with the constitution of VM. Evidence linking probiotics to HPV clearance has been published (15).

However, the mechanisms involved in the role of the microbiota on the promotion of, or protection to those conditions are yet to be fully elucidated.

Conclusions

Vaginal dysbiosis likely is a largely understudied yet important risk factor in HPV infection and cervical cancer epidemiology. Differences in microbiota composition were found between normal cytology, cervical lesions and cancer.

Recent studies showed the association between high-diversity cervical microbiota and HPV infection, CIN and cervical cancer. Whether microbes could act as carcinogenic agents leading to neoplasia or the tumor microenvironment modulates its surrounding microbial community still remains to be elucidated.

It remains to be solved what roles these microbiotas could play in HPV persistent infection. It is known that HPV is necessary but not sufficient to cause CC.

Persistent HPV infection with hr-HPV types is the main risk factor for the development of almost all CC and a significant proportion of vaginal and vulvar malignancies.

However, the mechanisms involved in the role of the microbiota on the promotion of, or protection to those conditions are yet to be fully elucidated. Also, further investigations are needed to improve treatment and develop new interventions for women’s health.

Improved HPV vaccination coverage and vaginal dysbiosis prevention and management will likely reduce cervical cancer disease burden significantly.

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Competing interests: The author stated that there are no competing interests.
Bibliographical references:

1. Gravitt PE, Winer RL. Natural History of HPV Infection across the Lifespan: Role of Viral Latency. Viruses 2017; 9(10):267.
2. Kyrgiou M, Mitra A, Moscicki A-B. Does the vaginal microbiota play a role in the development of cervical cancer? Transl. Res. 2017; 179: 168-82.
3. Velicer C, Zhu X, Vuocolo S, Liaw KL, Saah A. Prevalence and incidence of HPV genital infection in women. Sex. Transm. Dis. 2009; 36: 696-703.
4. Liu SH, Brotman RM, Zenilman JM, Gravitt PE, Cummings DA. Menstrual cycle and detectable human papillomavirus in reproductive-age women: A Time Series Study. J. Infect. Dis. 2013, 208, 1404-15.
5. Curty G, de Carvalho PS, Soares MA. The Role of the Cervicovaginal Microbiome on the Genesis and as a Biomarker of Premalignant Cervical Intraepithelial Neoplasia and Invasive Cervical Cancer. Int. J. Mol. Sci. 2020; 21(1): 222.
6. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clin Sci (Lond). 2017; 131(17): 2201-21.
7. Guillon F, Paraskevas M, Rand F, Heywood E, Brunham R, McNicol P. Vaginal microbial flora as a cofactor in the pathogenesis of uterine cervical intraepithelial neoplasia. Int J Gynaecol Obstet. 1992; 37(3): 185-91.
8. Kwasniewski W, Wolun-Cholewa M, Kotarski J, Warchol W, Kuzma D, Kwasniewska A, et al. Microbiota dysbiosis is associated with HPV-induced cervical carcinogenesis. Oncol Lett. 2018; 16(6): 7035-47.
9. Audirac-Chalifour A, Torres-Poveda K, Bahena-Román M, Téllez-Sosa J, Martínez-Barnetche J, Cortina-Ceballos B, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: A pilot study. PLoS One. 2016; 11(4): e0153274.
10. Gao W, Weng J, Gao Y, Chen X. Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. BMC Infect Dis. 2013; 13(1): 271.
11. Chase D, Goulder A, Zenhausern F, Monk B, Herbst-Kralovetz M. The vaginal and gastrointestinal microbiomes in gynecologic cancers: a review of applications in etiology, symptoms and treatment. Gynecol Oncol. 2015; 138(1): 190-200.
12. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014; 2(1): 4.
13. Amabebe E, Anumba DO. The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. Front Med (Lausanne). 2018; 5: 181.
14. Torcia MG. Interplay among Vaginal Microbiome, Immune Response and Sexually Transmitted Viral Infections. Int J Mol Sci. 2019; 20(2): 266.
15. Mitra A, Maclntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome. 2016; 4(1): 58.
16. Castanheira CP, Sallas ML, Rafaela Almeida Lima Nunes, Noely Paula Cristina Lorenzi, Lara Termini. Microbiome and Cervical Cancer. Pathobiology. 2020; 1-11.
17. Reid G. Therapeutic opportunities in the vaginal microbiome. Microbiol Spectr. 2017; 5.
18. Cojocaru M.: Intestinal microbiota in the PCOS. Journal of Clinical Sexology. Vol 3: No.1,19-24, DOI:10.37072/ JCS.2020.01.02, 2020.
19. Verhoeven V, Renard N, Makar A, Van Royen P, Bogers JP, Lardon F, et al. Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. Eur J Cancer Prev. 2013; 22(1): 46-51.
20. Nițescu Vasile, Ramba Doina: Sexuality, sexually transmitted diseases by oncogenic viral genotypes and importance of molecular screening methods, ISSN Online 2668-0394, 2018, DOI:10.37072/JCS.2018.01.02.
21. Nițescu Vasile, Nițescu Valentin, Treaty of Clinical Sexology, The Publishing House of the Romanian Academy,2018, 261-262.
22. Băcanu F.,Nițescu V.:The incidence of penile cancer, Journal of Clinical Sexology, Vol.4,No.1/2021,31-38, ISSN Online 2668-0394.
23. Ravel et al. (2011) Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(Suppl. 1):4680–4687. doi:10.1073/pnas.1002611107
24. https://wrda.net/2019/06/10/focus-on-cervical-cancer-awareness/