Chapter 2

Microenvironment in Vagina as a Key-Player on Cervical Cancer: Interaction of Polymorphic Genetic Variants and Vaginal Microbiome as Co-Factors

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Abstract

Current knowledge point to persistence of risk factors for the development of cervical intraepithelial neoplasia. The infection with a high-risk oncogenic Human Papillomavirus (HPV) subtypes, most commonly 16 and 18, is a necessary, although not sufficient, condition for development of invasive cervical cancer (ICC) and its precancerous precursor, cervical intra-epithelial neoplasia (CIN). It has been suggested that CIN disease severity and the diversity of vaginal microbiota are associated and this may determine viral persistence and disease behaviour. Our work focuses on the genetic variability associated to the modulation of genotoxicity induced by vaginal microbiota diversity. Relatively little is known about the mechanisms associated with clearance or persistence of HPV infection, therefore we hypothesized that may be under the influence of the genetic background.

Keywords: factors of persistence, genetic variation, microbiome, onco-microbiota, cervical cancer

1. Introduction

The vaginal microenvironment plays an important role in reproductive health. Human microbiome research has shown commensal bacteria to be a major factor in both wellness and disease pathogenesis. Interest in the microbiome has recently expanded beyond the gut to include a multitude of other organ systems for which the microbiome may have health implications. Here, we review the role of the vaginal microbiome in health and disease, with a particular focus on gynecologic malignancies, specifically cervical cancer. Further research is

required to understand the molecular mechanisms involved in the complex role that bacterial communities can play in the development of cancer.

Cervical cancer is one of the most preventable cancers. However, its progression and above all, the progress towards prevention is often frustrating. Moreover, and despite the continuously growing body of knowledge, the role of factors that affect the human papillomavirus (HPV) persistence are not yet fully understood.

Indeed, the oncogenic HPVs are a necessary cause of cervical cancer; however, they are not a sufficient cause, being other cofactors implicated in the increase of risk. We have also to consider external factors to the host, such as smoking habits, nutritional and behavioural factors (number of partners and their characteristics, age at onset of sexual activity), hormonal therapies-sexual steroids (oral contraceptives and post-menopausal substitution therapy), herpes simplex infections, *Chlamydia trachomatis* or other sexually transmitted infectious diseases and also nonspecific inflammatory diseases. Genetic and immunological factors and other endogenous co-factors may induce initiation and progression associated with genotoxicity, mutagenicity and irreversible cell proliferation [1, 2].

Dysbiosis results from the disruption of equilibrium of the microbiome. Given that the vaginal microbiome composition has been shown to play a role in the HPV infection and the rate of HPV clearance, the vaginal microbiome structure may be associated with the development of cervical cancer secondary to a persistent HPV infection.

Nevertheless, recent and concise data show that composition of the early-life microbiota is critical in the development of the immune system, and how deviations from homeostasis can induce disease later in life [3].

Our group has been presenting data that reflects mainly the influence of genetic, epigenetic and environmental including the vaginal microbiota-derived factors in the natural history of HPV associated lesions leading to cervical cancer as a multifactorial disease process.

In this scenario, the microbiota and its genome (microbiome) fulfils part of the natural history of cervical cancer. In the last years, it has been characterized HPV-genotypes profile, and bacterial vaginosis (BV) leading to its association with the prevalence of HSIL and progression to invasive cervical cancer (ICC) in adult women.

Despite the risk factors status knowledge, we may consider the need of a more proactive behaviour, namely, a strategy for improving the local fora with topic therapy. In this chapter, we will focus in the role of genetic susceptibility associated to the development of cervical cancer. Furthermore, we will discuss opportunities for interventions that modify the microbiome for therapeutic purpose.

2. Factors of persistence

2.1. Vaginal microbiota, HPV and co-infections

More than ever, the association of a disrupted microbiota and the increasing incidence of chronic human diseases have been addressed [4]. Locally, the microbiota affects the functions

and regulates the immunity of epithelial barrier. Therefore, the vaginal microbiome plays an essential role not only in health and dysbiosis, but also in modulation of immune response and, possible, in the carcinogenic process. Additionally, the persistence of risk factors, namely, HPV and other co-infections, may be associated to the disruption of these barriers [5].

The carcinogenic process in cervical cancer results in systemic and persistent damages, with important changes in immune checkpoints of the involved microenvironment [6]. From the key-players involved in this process, the microbiota influences, locally, physiological functions from the maintenance of barrier homeostasis to the regulation of metabolism, hematopoiesis, inflammation, immunity and other functions systematically [4, 7]. This barrier is supported by immune cells, for example, B cells, which produces IgA that helps to neutralize pathogenic bacteria (**Figure 1**) [8]. When this barrier locally fails it is created a favourable environment for carcinogenesis, the dysregulation of the integrity of vaginal epithelial cells will lead to more susceptibility for infections, causing low-grade chronic inflammation that leads to disease.

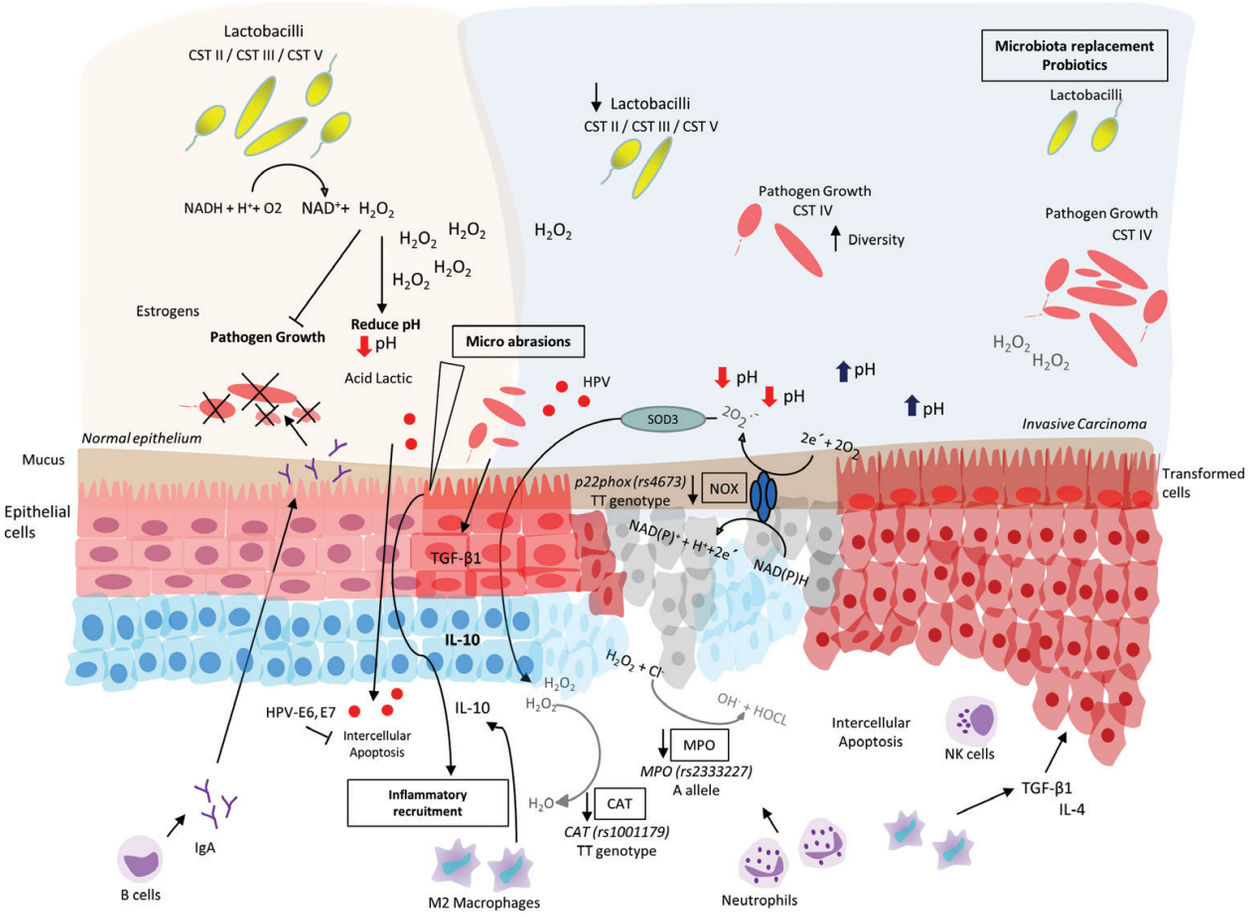

Figure 1. The presence of certain types of lactobacillus lowers pH and induces H_2O_2, which may contribute to the formation of HOCL by myeloperoxidase. CSTs IV are associated to bacterial vaginosis and the other microorganisms, to pre-cancerous lesions and cervical cancer. The types of groups of bacillus may be preponderant for maintaining the vaginal balance. Four species are most important for the balance of the vagina ecosystem: *L. gasseri* (II) *L. crispatus* (I), *L. jensenii* (V) and *L. iners* (III). The inter-individual genetic polymorphic variations should be integrated in a complex model, since a compromise vaginal microflora and inefficient genetic profile may contribute to the development of cervical cancer; CSTs, community state types; CSTs I, *Lactobacillus crispatus*; CSTs II, *Lactobacillus gasseri*; CSTs III, *Lactobacillus iners*; CSTs IV, *Lactobacillus, Sneathia amnii* and *Fusobacterium*; CSTs V, *Lactobacillus jensenii*.

The collection of microorganisms (or microbiota) populates complex ecosystems where genome is called microbiome and the implications on women health, from conception to the next generation, has been recently discussed [9]. A healthy vaginal microbiome is apparently dominated mainly by Community State Types (CST): *Lactobacillus crispatus* (*CSTs I*), *Lactobacillus gasseri (CSTs II), Lactobacillus iners (CSTs III)* and *Lactobacillus jensenii* (*CSTs V*), which, regulate for instance, the balance of reactive oxygen species (ROS) (**Figure 1**) [7]. Although vaginal dysbiosis presents biological plausibly by influencing host's innate immune response, susceptibility to infection, and the development of cervical disease, the underlying cause is not yet well understood. Nevertheless, greater diversity in the vaginal microbiota was associated in women with HPV-positive with cervical intra-epithelial neoplasia (CIN) [10].

There is a strong relationship between infection with HPV, pre-neoplastic lesions and cervical cancer. Moreover, the prevalence of high-risk HPV genital infection (HSIL) and cervical cancer in adult women has been documented. Therefore, the determination of this specific environment correlated with demographic, behavioural and clinical parameters will contribute to a better knowledge of key-players that triggers the carcinogenic pathways in cervical cancer. Other different risk factors including early age at first intercourse, multiple sex partners and low socioeconomic status also have significant role in disease initiation [11].

The HPV infects only epithelial cells, firstly, throughout the basal layer of the epithelium, probably via microabrasions in the epithelial surface, then the viral DNA is released from the capsid and transported into the nucleus as free genetic material or extrachromosomal episomes (**Figure 1**) [12]. Environmental factors might locally influence the initiation of this invasion, among other co-factors, HPV allows potential tumour cells to escape from lactobacilli-mediated control and interfering with intracellular induction of apoptosis [7].

Recently, changes from pro-inflammatory to anti-inflammatory signals - the cytokine milieu - may affect whether or not an infection is cleared [13] and hypothetically an environment favourable or not for tumour growth, might follow a change in regulation of the expression of pro-inflammatory cytokines. After infection takes place, the microbiome changes and its diversity increases. HPV proteins E2, E6 and E7 enhance IL-10 expression secondary to macrophages type 2 presence. These latter are also enhanced in its activity by TGFβ-1 in cytokines expression and its phagocytic efficacy, which is in turn stimulated by the microbiota present. The increase of diversity in the microbiota, through its toxins (FadA from *Fusobacterium* spp.) will promote a metastasis phenotype similar to what happens in cervical cancer [13].

Moreover, polymorphic genetic variants used as surrogate markers might explain the inter-individual variations and the differential immune response causing the persistence and the progression of HPV effects. We had studied some polymorphisms that are associated with genetic susceptibility to cervical infection and increase for risk of acquiring and transmitting HPV infection. These polymorphisms are involved in several pathways, throughout the production of metabolites or other carcinogenic substances, by increasing the susceptibility of the inflamed epithelium or by changing the immune system equilibrium (**Figure 1**).

2.2. Lactobacilli enhance reactive oxygen species and the role of genetic susceptibility in the vaginal microbiome

The ROS, comprise a group of oxygen derivatives from distinct oxidation status of O_2, such as, superoxide radical anion ($O_2^{\cdot-}$), and hydroxyl free-radical (OH^{-}) and as well as non-radical forms, namely H_2O_2 [14]. The latter has a role as a second messenger molecule in signalling cascades that regulates gene expression and fundamental cellular processes such as proliferation, differentiation and migration [15]. Lactobacilli's H_2O_2 production in the absence of peroxidase may result in toxic concentrations of H_2O_2 and cause damage of the mucosa [16]. The presence of peroxidases guarantees the generating of hypochlorous acid (HOCL) in the vagina inducing a steady removal of excess of H_2O_2 and generation of HOCL [17].

H_2O_2-producing lactobacilli strains, use a NADH oxidase that directly generates H_2O_2 in a two-electron reduction of O_2 (**Figure 1**). Klebanoff et al. proposed that hydrogen peroxide H_2O_2, product of lactobacilli and peroxidase, in the vagina of healthy women might be responsible for the prevention of vaginosis and also might exert an antitumour effect [18]. The antimicrobial effect of H_2O_2-generating lactobacilli is efficiently enhanced in the presence of peroxidases (such as myeloperoxidase and eosinophil peroxidase) and halides [18]. This points to a role of HOCL as superior antimicrobial compound. The vaginal fluid of the majority of healthy women contains sufficiently high concentration of peroxidase to allow biologically significant HOCL synthesis in the presence of H_2O_2-generating lactobacilli [18].

Bauer proposed that peroxidase, which converts H_2O_2 into HOCL, is responsible for creating a microbicial vaginal milieu by maintaining a balanced, non-toxic [18]. The papers of Bauer had highlighted the role of lactobacilli in the vaginal flora of healthy premenopausal women pointing to the beneficial effects for the predominance of microorganisms. Lactobacilli adhere to epithelial cells and thus cause sterile prevention of cell infections with undesirable microorganisms. Lactobacilli cause low pH through production of lactate and also release bactericidal compounds (**Figure 1**) [7]. Others ROS are the highly reactive and toxic by-products of oxygen metabolism, which can damage bacterial nucleic acids, proteins and cell membranes [19].

Recent work of Kruger and Baeur 2017, confirms that the lactobacillus-derived H_2O_2 per se is not likely to be beneficial for the vaginal epithelium, because it causes nonselective lesions in nontransformed as well as transformed cells. The combination of lactobacillus and peroxidase is more favourable. Moreover, the lactobacilli in this system can be completely mimicked in vitro by H_2O_2 generated by glucose oxidase, indicating that its contribution for potential tumour prevention is fully explained by bacterial generation of H_2O_2 [17].

This idyllic scenario is considered for normal cells or untransformed cells, which have a wide antioxidant regulatory defence system that serves to prevent the oxidative stress and the development of neoplasms [20]. Nevertheless, the papillomavirus infected cells (in particular by oncogenic types HPV 16, 33, 31) are resistant to this pathway of apoptosis induction. In transformed cells caused by damages induced by HPV, cells lose control of senescence and p53 activity is abrogated [21].

The combination of the host genome and microbiome increases genetic variation and phenotypic plasticity, enabling the holobiont to increase its overall fitness [22]. Genome-wide association studies identified cervical cancer susceptibility variants across different populations [23]. Therefore, the input of these and other polymorphic variants, may reflect the interindividuality of response in women with cervical cancer. In this chapter, we will focus on some these polymorphisms involved in the modulation of ROS production.

2.2.1. NAD(P)H oxidase (NOX)

The production of $O_2^{\cdot -}$ through NAD(P)H oxidase (NOX) by transformed cells or cervical cancer cells, can be specifically targeted with production of OH^- that induces apoptosis of these cells. The spontaneous dismutation of superoxide anions produce H_2O_2, at low pH, causes mutagenic effects that initiate malignant transformation (**Figure 1**). The high local concentrations of ROS through expression of SOD and catalase, it has also the potential to prevent elimination of transformed cells trough ROS/Reactive Nitrogen Species (RNS)-dependent intercellular apoptosis-inducing signalling [14].

The changes in the gut influences the vaginal microbiome, for instance the expression of NOX is modulated by, for example, the presence of *Helicobacter pylori*, which induces an indirect prooxidative mechanism through recruitment of neutrophils and by assembling of their NOX2 components to the cell membrane [1, 24].

NOX are membrane-associated oligomeric proteins that produce $O_2\cdot$ for host defence and other functions. Generation of extracellular $O_2\cdot$ through NOX is associated with oncogene activation and seems to be required for the control of cell proliferation and maintenance of the transformed state [25]. This protein consists of among other peptides by a regulatory 22-kDa α- subunit (p22phox) and a 91-kDa catalytic β-subunit (gp91phox). The p22phox protein is the NOX element responsible for the regulation of electron transfer to gp91phox [26]. The *p22phox* (*CYBA* gene) polymorphism with rs4673 (C-242 T) causes a functional non-conservative substitution from histidine-72 to a tyrosine residue that decreases its activity [27] (**Figure 1**). Our previous work unravels the association between *CYBA* polymorphism in women with ICC, having been observed a heterosis phenomenon with a protective profile in ICC [14]. This U type curve reflects, on one hand, the homozygote genotype CC leaded to increased ROS production, mainly H_2O_2 resulting from dismutation of O2·, which in turn results in excessive cell growth; on the other hand, the homozygote genotype TT lowers ROS production, mainly decreasing O2· mediated apoptosis cell capacity, resulting in a higher risk for the development of tumours in both cases [14]. Updated data from our cohort, we found that the TT genotype of *ph22pho*x polymorphism was a tendency for increased risk in ICC (**Figure 1**) (OR = 3.57, 95% [0.85–13.48], P = 0.057), being age and smoking habits dependent factors.

Women with cervical cancer will have a lower induction of O2· and, consequently, compromising the dismutation by SOD3 of this apoptotic factor into H_2O_2. The continuous modification of vaginal microbiota throughout depletion of lactobacillus or infections with HPV, contribute to increase of pH, influencing the concentrations of ROS in vaginal milieu. Moreover, women with TT genotype of *p22phox* polymorphisms will have a worse response to these important modifications (**Figure 1**).

2.2.2. Catalase

The NOX and catalase (CAT) proteins work in sequence in a metabolic pathway. Transformed cells, spontaneous and enzymatic dismutated O_2^- into H_2O_2 by SOD occurs at right density to allow optimal velocity of the ROS interactions [28]. The CAT is a heme enzyme that plays a predominant role in controlling H_2O_2 and O2· protecting in this way cells from deleterious effects of oxidative stress. In healthy women, this protector effect rises from the conversion of H_2O_2 into H_2O and O_2, but in cervical cancer transformed cells, the ROS signalling is inhibited by a membrane associated catalase and causing control system failure that ultimately results in cell apoptosis failure [26, 29]. Notwithstanding, women with a genetic variant of *CAT* associated with a decrease activity will not contribute to this control system (**Figure 1**).

In humans, the *CAT* gene is located on chromosome 11p13 and its rs1001179 polymorphism (C-262 T) is located on the promoter region and influences transcription and consequent expression of this enzyme and hence the oxidative status of cells and its microenvironment [30]. In a case–control study, we observed a greater risk for developing ICC associated with the homozygote genotype TT of *CAT* polymorphism C-262 T polymorphism of the CAT gene (OR = 3.03, 95% CI 1.46–6.29, P = 0.003) [31]. Similarly to other cancers types, the T variant of this polymorphism is associated to a decreased enzyme activity, generating high levels of ROS [32–34]. The interaction of CC genotype of *p22phox* polymorphism and the TT genotype of *CAT* leads to a higher risk for ICC (OR = 3.95, 95% CI 1.07–14.52, P = 0.032) [31].

Moreover, recent reports have suggested a connection between oestrogen exposure, CAT activity and polymorphism in breast cancer [35]. These findings suggest that CAT genotype modifies the effect of hormone replacement therapy (HRT) use on breast cancer risk and that HRT may affect risk by affecting oxidative stress. This scenario, also might be important in cervical cancer, namely, women with *CAT* TT genotype (associated to a decreased catalase activity), will deficiently protects cells from ROS.

2.2.3. Myeloperoxidase

The oxidative stress conditions are generated by the release of ROS at the infection site by host immune cells such as neutrophils and monocytes. Additionally, resistance of oncogenic papilloma virus-expressing cells to apoptosis induction by the HOCL/hydroxyl anion pathways is likely, as papilloma virus-containing cells are also resistant to intercellular induction of apoptosis [36].

The toxic concentrations of H_2O_2 could be converted by myeloperoxidase (MPO). MPO, a lysosomal enzyme expressed in polymorphonuclear neutrophils, has the potential to kill HPV transformed cells, as a component of an intercellular induced-apoptosis pathway. The MPO was also being pointed as a key-player on controlling of vaginal microenvironment, namely, the H_2O_2-generating *Lactobacillus acidophilus*. In healthy women, this production inhibits the overgrowth of potentially pathogenic organisms; in fact, it can be toxic to other bacteria, fungi, viruses, spermatozoa, or tumour cells [37].

Supposedly, there are no resident neutrophils and macrophages on vaginal microenvironment, nevertheless the persistence of risk co-factors and ROS may lead to the recruitment of

inflammatory cells. The presence of MPO on vaginal milieu is activated. Probably, the persistence of death cells recalls of neutrophils to vagina, being MPO important for the control of excess production of HOCl. In the vaginal microenvironment, the MPO catalyzes the reaction between H_2O_2 and either thiocyanate ions or a halide, such as iodide, bromide or chloride ions, yielding HOCl, which participates in the oxidative burst during the innate host defence [38]. MPO may act in synergy with other proteins. Therefore, an imbalance between oxidants/antioxidants could mean a higher chance for mutations and oncogenesis leading to diseases, including cancer—since MPO produces ROS secondary derivatives can be involved in the neoplastic transformation of cells through this pathway.

H_2O_2, and a halide form a powerful antimicrobial system in phagocytes and tissue fluids, which certain microorganisms can serve as the source of H_2O_2 for this system. The equilibrium of the production of H_2O_2 by *Lactobacilli* in the vagina appears to be a nonspecific host defence mechanism, which can be potentiated by myeloperoxidase that produces HOCl (**Figure 1**).

The production of superoxide through NADPH oxidase from cervical cancer cells, can be specifically targeted with production of OH^- radicals that induces selective apoptosis of these cells [16].

The polymorphism in the *MPO* gene induce a transition G463A (rs2333227), in the promotor region of the gene, where the wild-type G allele promotes the binding of transcription factors leading to a higher transcriptional activity than the A allele [39].

We found that women with the GG genotype had lower risk for cervical cancer than the women who displayed the heterozygous genotype GA (OR = 0.546, 95% CI = 0.315–0.939, P = 0.028, OR = 2.210, 95% CI [1.257–3.886], p = 0.008, respectively). The genotype that leads to a higher concentration of ROS (GG) presents itself as a protection factor in comparison to the homozygous genotype (AA) [39]. Moreover, recently, we observed that the A carriers of MPO polymorphism were about 5-fold of increased risk for cervical cancer (OR = 5.41, 95% CI [2.15–13.64], P < 0.0001) (**Figure 1**), being dependent of age (OR = 3.38, 95% CI [0.85–13.48], P = 0.085) and independent of smoking habits (OR = 3.85, 95% CI [1.33–11.11], P = 0.013). The interaction of HOCl and superoxide of transformed cells will generate apoptosis-inducing hydroxyl radicals.

We suggest that there is an association between the H_2O_2-producing strains found in the vaginal microbial flora and high activity of MPO leading to a clearance of the HPV-infected cells, the relation may also lead to the apoptosis of the transformed cells, producing $O2^{\cdot}$ and $OH^{\cdot}$, acting as a protective factor for a cervical cancer [16, 17, 26].

2.2.4. Reactive nitrogen species

Nitric oxide is generated by nitric oxide synthase (NOS) and presents 3 isoforms: neuronal (NOS1), endothelial (NOS3) and inducible (NOS2). High local concentrations of ROS through expression of SOD and catalase might be associated to prevention of elimination of transformed cells trough ROS/RNS-dependent intercellular apoptosis-inducing signalling [14, 16]. Conversely the excess of NO inhibits the apoptosis induction associated to H_2O_2 and reversely NO-mediated apoptosis induction was inhibited by excess of H_2O_2 [29].

The NOS3 gene is located in the 7q35–36 region of chromosome 7 and the genetic polymorphism with a great clinical relevance is the 27 bp-VNTR 4b/a intron 4 [40]. In addition, NO produced by endothelial and epithelial cells, also modulates the regulation of vascular endothelial growth factor and is possibly associated to increase in processes of invasiveness and metastasis [41]. Preliminary results from our group, although only with a trend, identify that the A variant of NOS3 polymorphism, which is associated with higher activity of this enzyme, predisposes to ICC OR = 7.50, 95%CI [0.88–63.9], P = 0.066).

2.2.5. Catechoestrogens and cytochrome P450 (CYP1A1)

There is a clear association between the excessive and cumulative exposure to oestrogens and the development of cancer in hormone-sensitive tissues, such as the cervix. Therefore, we found that CYP1A1 and Catechol-o-methyltransferase (COMT) work in a metabolic sequence and their interaction could lead to an alternative pathway of oestrogen metabolism with production of 16-OH-estrone that is more proliferative and less apoptotic [42, 43]. The role of oestrogen and the association of *CSTs* favourable for balance vaginal milieu, was previously debated [44].

Aryl hydroxylase (AhR) the transcription factor of CYP1A1 is also associated with immunosuppression after activation of IL-22 pathway, and the maintenance of intraepithelial in innate lymphocytes leading to the mucosal protection from inflammation [45].

Recently, a very interesting work unlighted the mechanism, where the Lactobacillus-derived H_2O_2 suppress host kynurenine metabolism, by inhibiting the expression of the metabolizing enzyme, indoleamine 2,3-dioxygenase (IDO1), in the intestine [46]. Moreover, maintaining elevated kynurenine levels during Lactobacillus supplementation diminished the treatment benefits.

3. Treatment and prevention: microbiota-derived factors

Finally, as suggested by other authors, it may be possible to expand the use of probiotics in the treatment of gynecologic cancers. The study of the role of probiotic bacteria for the prevention of colon and cervical cancer has led to the conclusion the tumour preventive effects of probiotic bacteria might be due to their control of the microbial flora, establishment of beneficial metabolic effects and stimulation of the immune system [37, 47]. Therefore, we can act in the prevention, specifically, the relapses.

Genetic analysis based on single nucleotide polymorphisms identified genetic variants associated with tumour rejection in mice, which could potentially affect ROS production and NK cell activity. That results also supports that B cells play a detrimental role in antitumour immunity and suggest that targeting B cells could enhance the antitumour response and improve the efficacy of therapeutic cancer vaccines [8].

The conventional photon radiotherapy for cervical cancer irradiates parts of the healthy tissue. This treatment perturbs the vaginal microbiome and disrupt the epithelial barrier function, permitting translocation of pathogenic bacteria and causing an inflammatory response [48]. The role of probiotic bacteria for the prevention of colon cancer has led to conclusion of the

tumour preventive potential by the microbes. Additionally, the genetic polymorphism might be related to genetic susceptibility to infections and so, the implementations of probiotics may reinforce the immune system. A better understanding of this line will allow for the development of therapies that can manipulate the microbiome to reinstate homeostasis.

The application of probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 concomitantly with specific anti-infective agents provides more reliable cytological diagnostics, reduces the number of false positive and false negative findings on cervical malignancy and normalizes vaginal microflora in higher percentage of patients with vaginal infections compared with therapy including anti-infective agents only [49].

The use of probiotics, such as L. acidophilus, concomitant with the subsequent use of antibiotics, helps to restore the natural bacteria in the digestive tract that eventually are killed by antibiotics.

Recently, due to changes in the sexual behaviour of the general population, especially in developed countries, there has been an increase in the incidence of HPV infection in other parts of the body, including oropharynx and anus, among others.

In summary, a personalized clinical / therapeutic approach is suggested to avoid unnecessary treatments, based on previous history (onset of sexual activity, number of partners, anovulatory, parity, nutrition, alcohol, tobacco, genetics-immunity, etc.), in vaginal pH, Lactobacillus, in the diagnosis of HPV, viral load, mRNA, HSV, CMV, HSIL, AGC, CINI and CINII / III. *Chlamydia trachomatis, Mycoplasma, Ureaplasma, Neisseria gonorrhoeae*) and in the immunohistochemical study (p16 and Ki-67) of dysplasia [50, 51].

4. Conclusions

The HPV is not a sufficient cause for developing of cervical cancer, therefore other factors may be involved in this susceptibility, namely the microenvironment in vagina and inter-individual genetic polymorphic variations. These variables must be integrated in a complex model that integrates other co-factors, such as, smoking, diet and oral contraceptives. According to this review based on recent data, it seems that a deficiency of an antioxidant mechanism associated to a compromise vaginal microflora and inefficient genetic profile may contribute to the development of cervical cancer. We hypothesis that the genetic background and dysbiosis may contribute to increase risk for gynecologic advanced cancer.

Therefore, the equilibrium of gut/vaginal microbiota and adequate supplementation for a homeostasis of oxidant and antioxidant species may contribute to the regression of the persistence of factors associated with cervical cancer.

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Conflict of interest

The authors declare that they have no competing interests.

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