Review Article

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Interaction of cervical microbiome with epigenome of epithelial cells: Significance of inflammation to primary healthcare

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Abstract: One pillar of the predictive, preventive, and personalized medicine framework strategies is the female health. The evaluation of women's lifestyle and dietary habits in context with genetic and modifiable risk factors may reflect the prevention of cervical cancer before the occurrence of clinical symptoms and prediction of cervical lesion behavior. The main aim of this review is to analyze publications in the field of precision medicine that allow the use of research knowledge of cervical microbiome, epigenetic modifications, and inflammation in potential application in clinical practice. Personalized approach in evaluating patient's risk of future development of cervical abnormality should consider the biomarkers of the local microenvironment characterized by the microbial composition, epigenetic pattern of cervical epithelium, and presence of chronic inflammation. Novel sequencing techniques enable a more detailed characterization of actual state in cervical epithelium. Better understanding of all changes in multiomics level enables a better assessment of disease prognosis and selects the eligible targeted therapy in personalized medicine. Restoring of healthy vaginal microflora and reversing the outbreak of cervical

abnormality can be also achieved by dietary habits as well as uptake of prebiotics, probiotics, synbiotics, microbial transplantation, and others.

Keywords: cervical cancer, microbiome, epigenome, inflammation, human papillomavirus, 3P-medicine

Introduction

A female health is one medical area of the framework strategies in predictive, preventive, and personalized (3P) medicine. This innovative approach evaluates patient's lifestyle and dietary habits in context with suboptimal health status and the risks strongly associated with the formation, treatment, and progression of cervical lesion to cancer. The concept reflects the view that chronic diseases can be effectively predicted and prevented before clinical symptoms occur [1].

High-risk human papillomaviruses (hrHPVs) are responsible for almost 5% of all cancers worldwide [2]. Cervical cancer is the fourth most common cancer in women; an estimated 570,000 new cases were diagnosed, and an estimated 311,000 women died of the disease in 2018. Cervical cancer is preventable and successfully treatable at early stages [3] that makes the disease as an ideal candidate applicable in the context of 3P medicine. More than 200 HPV types are recognized, of which around 40 types are specific to mucosal surfaces with stratified squamous epithelia, affecting the anogenital tract and the oral cavity [4]. HPV infections are the most common sexually transmitted infection in the United States, and approximately 80% of all sexually active individuals are infected with HPV at least once during their lifetime [5,6]. More than 90% of cervical HPV infections are spontaneously cleared [7]. Therefore, the factors and mechanisms involved in regression, persistence, and progression are the focus of intense research. Persistent, hrHPV infections are a major risk factor for subsequent cancer development [8,9].

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The mechanisms involved in the regulation of HPV infection include modifiable and nonmodifiable factors, with promising results in the latest research regarding genetic and epigenetic regulation and the inflammation process.

The factors that influence the development of cervical lesions include smoking, woman's age, behavioral factors (number of sexual partners, age at menarche, age at onset of sexual activity, and number of pregnancies and deliveries), hormonal factors (substitution therapy and oral contraceptives), and coinfection with other sexual transmitted diseases (herpes simplex virus, *Chlamydia trachomatis*, etc.) [10]. The presence of pathogens is usually accompanied by inflammation and endogenous factors including genetic and immunological conditions involved in the host's response to eliminate the infection [11]. The vaginal microbiome plays a significant role in the clearance of HPV infection and may be associated with reactivation of latent HPV infection, the formation of precancerous cervical lesions, and the progression to cancer [12]. HPV infection often triggers the vaginal dryness influenced by a vaginal pH, microbiome composition, and microbiota, depending on the hormonal status and phase of menstrual cycle. Vaginal and vulvar dryness should be considered as an important risk factor in currently applied diagnostics and treatment of women with gynecological dysharmonia [13]. These modifiable risk factors together with microbial biomarkers present in high-grade and low-grade cervical dysplasia could help in triage of patients with accentuated chances of lesion progression or regression. The excessive surgical treatment of cervical abnormality could negatively affect women's sexual life and obstetrical outcomes [14].

The microbiome has been studied in relation to the gut epithelium and inflammatory bowel disease. Various model organisms have made it possible to study the microbiome in the context of other human diseases, including autoimmune diseases (asthma, atherosclerosis, psoriasis, etc.), obesity, cardiovascular disease, and cancer [15]. Early findings from colorectal cancer research suggest that the bacterial microbiome plays a role in carcinogenesis, and one of the proposed pathways is the interaction of the microbiome with the host, presumably through epigenetic changes in the intestinal epithelium [16]. Altered host defense response to pathogens or a dysbiotic microbiome can indirectly result in carcinogenesis. This theory has been verified in animal models of liver and colorectal cancer and is supported by findings that antibiotic treatment of the germ-free organism has been reported to result in a reduction in the number of tumors [17]. Many authors believe that the precise classification/taxonomy/genetic sequencing of the bacteria could potentially be used as a biomarker for prevention and disease screening [18].

According to one hypothesis, the specific microbiota and their genes are beneficial to humans at an early age but can be harmful later in life. An inflammatory response in the host can control an infection or allergy at an early stage of life; however, the same inflammation can promote atrophy and oncogenesis later in life [15]. It has been speculated whether the cervical microbiome affects the host's cells directly or indirectly through alterations of the host's cells, their genomes, or altering access to chromatin [19]. Other theories are based on the fact that ethnic, environmental, and developmental factors contribute to epigenetic changes in the host genome, leading to slight alterations in host cell function and preferential growth of specific microbial communities [20].

Determining the influence of the microbiome on the epigenome of epithelial cells is a challenge because of the simultaneous influence of environmental factors such as nutrition, health status, and the use of medications [21]. Novel technologies, such as *in vitro* 3D epithelial cocultures, make it possible to study the interaction between microbial agents and their hosts [22]. The main aim of this review is to analyze publications in the field of cervical cancer research to examine the interaction of the cervical microbiome with epithelial cells in relation to inflammation and to assess direct evidence of epigenetic changes related to the cervical microbiome. Association with HPV infection is common in cervical cancer, although other genetic and epigenetic factors are required for disease progression [23]. The formation of a squamous intraepithelial lesion is indicative of the precancerous stage of cervical cancer; however, the lesion often heals spontaneously and the persistence or progression of cervical lesions can take years to decades [24]. Consequently, the long time to the development of cancer gives a wide scope for the implementation of 3P medicine and its pillars in targeted preventive, early predictive, and therapeutic strategies, thus enabling individualized approach to the patient. Improved management of cervical cancer (CC) patients requires the identification of novel biomarkers from clinical material, with respect to the local microenvironment, which represents an innovative approach to state-of-the-art medicine.

The structure of the cervical epithelium: a key place in the issue

The cervix uteri or only "cervix" is the lower part of the uterus in the women's reproductive system, the lower vaginal

portion, known as ectocervix covered with nonkeratinized stratified squamous epithelium is exposed to microorganisms including commensal flora, probiotics, and pathogens [25]. The HPV viral life cycle is closely connected to the process of cellular differentiation in squamous stratified epithelium (Figure 1), with the primary infection occurring in basal keratinocytes, which are the only local cells engaged in cell division [26,27].

The concept of a TZ is crucial for understanding HPV-induced cervical carcinogenesis. Cervical precancerous lesions are typically localized to the area of the TZ [28]. The metaplastic epithelium of the TZ, including the SCJ, is the prime target of HPV infection and cervical carcinogenesis [29]. The susceptibility of this region to HPV infection is possibly due to decreased immune responsiveness and increased estrogen responsiveness associated with an increased level of estrogen receptors [30].

Reserve cells of embryonic origin localized to the SCJ are involved in the metaplastic process, and their role has been a subject of intense research because of their role has been a subject of intense research because of their vulnerability to HPV-driven neoplastic process [31]. These cells express multiple immunohistochemical markers, including cytokeratin 7, anterior gradient protein 2 homolog,

cluster differentiation 63, matrix metalloproteinase-7, and guanine deaminase. The same markers are also typical for high-grade squamous intraepithelial lesions (HSIL) and carcinomas [32]. The tumor microenvironment (TME) also plays a crucial role in carcinogenesis and includes fibroblast growth factors, interleukin 1 (IL-1), IL-1B, IL-8, microRNA-126, matrix metalloproteinase (MMP), T helper 17 cells, and vascular endothelial growth factor (VEGF) [33].

HPV infection is also responsible for morphological changes in the squamous epithelium that are detectable visually and by cytological staining, as in the Papanicolaou (Pap) test/smear. Pap staining is an effective method for the detection of precancerous changes or histological cervical intraepithelial neoplasia (CIN), which has further classification depending on its maturation, migration, and localization in the epithelium: CIN I or low-grade squamous intraepithelial lesion (LSIL); CIN II or HSIL; CIN III or carcinoma *in situ*. Other uncertain changes in cervical epithelium are classified as either atypical squamous cells of undetermined significance (ASC-US) or ASC that cannot rule out HSIL [34]. Pap smears alone can be detrimental, and supplementation with colposcopy is recommended to avoid overdiagnosis and consider the risk-benefit ratio of the follow-up or immediate treatment of cervical lesion. The cervical cancer

Figure 1: Schematic illustration and colposcopy of the uterine cervix showing the types of epithelia, transformation zone (TZ), original, and new squamocolumnar junction (SCJ). (A) The original squamous epithelium is a glycogenated epithelium with a smooth surface without any glands in the underlying stroma. (B) The endocervical epithelium consists of one layer of cylindrical cells covering the grape-shaped mucosa. (D) The original SCJ represents the border between the original squamous epithelium and the metaplastic squamous epithelium. (C) The TZ is the area between (D) the original SCJ and (E) the new SCJ. The uterine cervix consists of three distinct epithelial types: tall, mucin-secreting columnar cells of the endocervix in a single layer; glycogenated, stratified squamous cells in the ectocervix; and a TZ in between, which results from gradual metaplastic replacement of columnar cells by squamous cells.

screening in the future integration of precision cancer prevention regimes should match individual's risk of cancer in context with genomic and environmental factors [35].

Cervical microbiome profiling is the basis of predictive diagnosis

The human body is at any moment inhabited by 500–1,000 bacterial species [36]. The human vaginal microbiome, in the right composition, contributes to the prevention of urological tract infections [37], urogenital diseases, and sexually transmitted diseases [38]. The lactic acid-producing genus *Lactobacillus* plays an important protective role [39] consisted of several mechanisms such as biofilm formation on the vaginal mucosa [14], the production of H₂O₂, and the acidic vaginal environment created by the *Lactobacillus* bacteria through sugar metabolism [40]. In addition to lactobacilli, the vaginal microbiome may contain other bacteria such as *Actinomyces*, *Aerococcus*, *Atopobium*, *Bifidobacterium*, *Enterobacter*, *Escherichia*, *Facklamia*, *Gardnerella*, *Haemophilus*, *Sneathia*, and others [41].

Bacterial vaginitis was first described by Gardner and Dukes in 1955 and was referred to as a *Haemophilus vaginalis* infection [42]. Bacterial vaginosis (BV) was considered as a common but serious bacterial infection due to its potential to cause inflammation of the internal genitalia [43]. In 1983, Amsel et al. [44] defined the first criteria for BV: white, milky discharge; pH > 4.5; fishy odor, especially after reaction with 10% KOH; and the presence of at least 20% key cells. At least three of the criteria had to be met for the diagnosis of BV [44]. In 1991,

Nugent et al. changed the criteria for diagnosis of BV based on a vaginal swab plated on a microscopic slide evaluated by a scoring system after Gram staining [45].

Ravel et al. [39] examined the vaginal microbiome and vaginal pH of 396 asymptomatic, sexually active women. This study divided the vaginal microbiome into five groups called community state types (CSTs). The dominant microbe in group I was *Lactobacillus crispatus*, predominating in 26.2% of women. Group II, characteristic for 6.3% of women, was dominated by *L. gasseri*. Group III, present in 34.1% women, was dominated by *L. iners*. and *L. jensenii* was dominant in group V. Vaginal swab examinations in the remaining 27% showed dominance of strict anaerobes including *Gardnerella*, *Prevotella*, *Megasphaera*, and *Sneathia*. The lowest pH was found in group I, whereas the highest pH was found in group IV (also called BV) [39]. Moreover, a more diverse vaginal microbiome represented by *Gardnerella*, *Sneathia*, *Megasphaera*, *Dialister* spp., and *L. gasseri*, without cervical dysplasia, was found to be more susceptible to HPV infection [46]. *L. iners* was discovered to produce a cholesterol-dependent cytolysin, known as inerolysin. Inerolysin is a pore-forming toxin like the vaginolysin protein secreted by *Gardnerella*. These cytotoxins form pores in the vaginal epithelium to impair its integrity and promote viral infection [47]. A summary of CST classes with dominant and additional bacterial strains is given in Table 1 [48,49].

The process of cervical carcinogenesis tends to be more apparent in cases of high bacterial diversity of the vaginal microbiome [50]. Higher bacterial diversity was also observed in patients with invasive cervical cancer as compared with a healthy group [51–54]. Bacterial species like *Gardnerella* and *Streptococcus* may play an important

Table 1: Description of the microbial composition of CST classes with dominant and additional bacterial strains, as reported in the literature [48,49]

Vaginal CSTs	Dominant bacterial strain(s)	Additional bacterial strain(s)
CST I	<i>L. crispatus</i>	<i>L. iners</i> , <i>Gardnerella</i> spp., <i>L. jensenii</i> , <i>Klebsiella</i> spp., <i>Ureaplasma</i> spp.
CST II	<i>L. gasseri</i>	<i>L. acidophilus</i> , <i>L. delbrueckii</i> , <i>L. crispatus</i> , <i>L. jensenii</i> , <i>A. vaginae</i> , <i>Ureaplasma</i> spp.
CST III	<i>L. iners</i>	<i>L. acidophilus</i> , <i>L. crispatus</i> , <i>L. gasseri</i> , <i>L. jensenii</i> , <i>Klebsiella</i> spp., <i>Prevotella</i> spp., <i>Gardnerella</i> spp., <i>Ureaplasma</i> spp.
CST IV	Strictly anaerobic bacteria: <i>Gardnerella</i> spp., <i>Prevotella</i> , <i>Megasphaera</i> , <i>Atopobium</i> , <i>Sneathia</i> spp., <i>Streptococcus</i> , <i>Staphylococcus</i>	<i>L. crispatus</i> , <i>L. gasseri</i> , <i>L. iners</i> , <i>L. jensenii</i>
CST V	<i>L. jensenii</i>	<i>L. crispatus</i> , <i>L. gasseri</i> , <i>L. iners</i>
Bifidobacteria	<i>B. breve</i>	<i>L. delbrueckii</i> , <i>G. vaginalis</i>

Abbreviations: CST, community state type; *L.*, *Lactobacillus*; *B.*, *Bifidobacterium*; *A.*, *Atopobium*; *G.*, *Gardnerella*; spp., species.

role in the progression of cervical precancerous lesions to invasive disease [50]. The presence of specific bacteria in the vaginal microbiome, such as *Prevotella bivia* and *P. disiens*, could be used as a biomarker of CIN 2 + lesions [54]. Another article by Chao et al. [55] evaluated the potential for the vaginal microbiome to be used as a marker for HSIL. A study of 272 women revealed an increasing abundance of *Stenotrophomonas*, *Streptococcus*, *Pseudomonas*, and a paucity of *Bifidobacterium* and other genera such as *Prevotellaceae* and *Faecalibacterium* in patients with HSIL. These findings implicated these genera as potential biomarkers of HSIL and their possible use in cervical screening [55].

Mitra et al. [56] confirmed the depletion of *Lactobacillus* spp., high diversity of the vaginal microbiome, and high levels of proinflammatory cytokines in women with CIN. The study also evaluated the vaginal microbiome of women after excisional treatment for CIN at their 6-month follow-up visit; they suggested that failure to reestablish a *Lactobacillus*-enriched CST may lead to higher risk of development and persistence of cervical precancerous lesions, even after surgical treatment [56]. This leads to the question of whether classical surgical treatment is sufficient, and if this approach addresses the cause of the development of cervical lesions. The use of probiotics significantly improved cytological results in women with HPV and ASC-US/LSIL during follow-up in a study by Ou et al. [57]. However, probiotic use did not cause a significant improvement in the clearance of HPV infection [57].

Many studies indicated the role of the vaginal microbiome in the development of cervical lesions. CST IV, characterized by a low proportion of lactobacilli and wide bacterial diversity, plays an important role in this process. Some authors suggest the possibility of using the vaginal microbiome as a biomarker of squamous intra-epithelial lesions, and its potential for influencing the composition of the vaginal microbiome as a therapeutic tool. However, microbial diversity among individuals should be better understood. The differences between individuals and the frequency of microbial population leading to the information related to the development of cancer or outcome of anticancer therapy that could be useful in personalized therapeutics with integrated individual genetic structure and disease history [58].

Dominance of pathogens associated with vaginal dysbiosis in the vaginal microbiome leads to changes in the function of immunity and epithelial homeostasis. The production of proinflammatory cytokines and chemokines that lead to the development of chronic inflammation is increased, immune mechanisms are activated, and

the viscosity of cervicovaginal secretions is reduced by the production of mucin-degrading enzymes. These changes, by damaging the mucosal barriers, increase the risk of infection with sexually transmitted diseases including hrHPV [59]. For example, vaginal dysbiosis associated with *Megasphaera elsdenii* and *P. timonensis* leads to increased production of proinflammatory cytokines by dendritic cells [60]. CST IV is associated with the production of interferon γ (IFN- γ) and IL-17. In the *Lactobacillus*-dominant CST groups, increased expression of these cytokines was not demonstrated [61].

All these studies demonstrate that identification of microbiome population might be one of the key aspects of precision medicine in the future. Microbial composition may early identify the potential risk of precancerous lesion formation or permanent BV. A diagnostic test identifying bacterial strains can be applied as a screening tool among healthy people or various clinical groups, although more confirmatory tests and metagenomic *in vitro* and *in vivo* studies should be achieved to get a corresponding microbiome signature.

Epigenetic alterations as useful clinical markers in early diagnosis and prognosis of cancer, and prediction of treatment responses

Recent studies have shown that epigenetic alterations are common in tumorigenesis, cervical carcinogenesis, and metastasis because they can modify the cellular micro-environment. Epigenetic alterations are heritable changes that do not alter the DNA sequence. The most important regulators of gene expression are posttranslational histone modification and related chromatin remodeling, DNA methylation, and noncoding RNAs [62]. There is current growing interest in the study of these modifications as biomarkers to determine disease progression and/or for use in targeted therapy in personalized medicine. Today, epigenetic changes can be better studied by new sequencing techniques such as whole genome bisulfite sequencing, chromatin immunoprecipitation combined with massive parallel sequencing, assay for transposase-accessible chromatin using sequencing, and RNA-sequencing. It is necessary to analyze the huge amount of sequencing data usually by artificial intelligence (AI) utilization to integrate epigenetic data and other omics data. Solving the issues of AI-based techniques, collection of high-quality annotations with right clinical information in

multiomics context will be important for the realization of precision medicine [63].

Histone modifications in preventive and targeted therapy

Histone modification is one of the most studied epigenetic alterations. Histone proteins attach to DNA via attraction to negatively charged phosphate groups [64]. Chromatin is comprised of nucleosomes that consist of 146 base pairs of DNA wrapped around a core of two copies each of H2A, H2B, H3, and H4 histone proteins [65]. Chromatin structure is determined by nucleosome spacing and can be categorized as either transcriptionally inactive heterochromatin or transcriptionally permissive euchromatin. Heterochromatin is condensed which prevents DNA transcription [66]. Chromatin remodeling involves covalent histone modification that occurs at the N-terminal tails of histones, affecting the accessibility of the DNA. Histones are modified by the well-known processes of acetylation, methylation, and phosphorylation, and the lesser-known processes of citrullination, ubiquitination, ADP-ribosylation, deamination, N6-formylation, O-GlcNAcylation, propionylation, butyrylation, crotonylation, and proline isomerization. These modifications are regulated by specific writer and eraser enzymes, which in many cases have yet to be identified [67,68]. Acetylation is usually associated with active transcription of euchromatin, whereas deacetylation is associated with heterochromatin [69]. Histone acetylation was found to regulate intracellular pH. Many tumors have low pH and reduced levels of histone acetylation, which correlates with a poor clinical outcome [70]. These modifications make up the “histone code.” The balance between acetylation and histone deacetylation is essential to optimize gene expression and posttranslational modification [71]. In tumors, hyperacetylation often occurs in protooncogenes, whereas hypoacetylation in tumor suppressor genes occurs with DNA methylation to enhance gene silencing [72]. Aberrant histone modification may help oncogenic drivers accelerate cancer progression, metastasis, and therapeutic resistance [73].

Histone deacetylase (HDAC) inhibitors and several epigenetic drugs have been considered as therapeutic targets in precision medicine and may be useful in cases with the absence of pathological mutations. The application of epigenetic cancer treatment is influenced by individual genetic background, organ-specific microbiome, and pharmacogenomics of the drug. Some challenges also should be overcome, such as the delivery and penetration of

the drug to cancer tissue, the specificity of epigenetic changes in a complex disease, and the evaluation of epigenetic drugs on larger clinical trials [74].

DNA methylation biomarkers for early detection and prediction of cancer risk

DNA methylation is another important epigenetic modification with a role in cervical carcinogenesis. It is the most studied epigenetic alteration. DNA methylation includes hypermethylation and hypomethylation. Methylation is the covalent attachment of a methyl group to adenine or cytosine. Enzymes such as DNA methyltransferases (DNMTs) transfer a methyl group to alternate cytosines, thereby generating 5-methylcytosine (5-mC) [75]. Methylation is most often concentrated in short CpG regions called “CpG islands” and around large, repetitive sequences. CpG islands are CpG-rich areas of ~1 kb that are usually located in the vicinity of genes, often near the promoters of widely expressed genes [76].

The maintenance of all methylation processes in the genome is mediated by DNMT1. Establishment of DNA methylation patterns during embryogenesis and adjustment of genomic fingerprints during germ cell development is achieved by the *de novo* methyltransferases DNMT3A and DNMT3B. A *de novo* DNMT3C methyltransferase, found in murine germ cells, specializes in the methylation of young retrotransposons. Currently, DNMT2 is not considered a DNA methyltransferase because of its methylation activity on small transfer RNAs and regulator DNMT3L forms DNMT3L–DNMT3A heterotetramers and eases the methylation of cytosine residues [77]. 5-mC can be oxidized into 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine, and 5-carboxylcytosine by ten-eleven translocation (TET) proteins in a step-by-step process, leading to demethylation of cytosine [78–80]. It was proven that TET-dependent reprogramming *in vivo* can restore youthful gene expression patterns and restore the function and regenerative capacity of tissues [81].

S-Adenosylmethionine (SAM) is a methyl group donor that plays an essential role in the survival of organisms. Of the many functions of SAM, the most important are its contribution to mitochondrial activity, intracellular and extracellular communication, and the formation and maintenance of the microbiome [82]. SAM assists histone modifications and noncoding RNAs (ncRNAs) in the regulation of gene expression without altering the DNA sequence. Maintenance of the DNA methylation pattern of the genome is also critical in replication and

DNA damage repair, with subsequent chromatin rearrangement in response to DNA damage [83].

Aberrant DNA methylation is an epigenetic change in tumors that leads to tumor development and progression by silencing tumor suppressor genes and activating oncogenes [84]. Several studies have shown that cellular methylation interfaces with HPV. During HPV infection, epigenetic changes caused by oncoproteins (E5, E6, E7) induce the expression of DNMTs that lead to aberrant DNA methylation and disruption of normal epigenetic processes [85]. It influences the methyltransferase activity of enzymes and subsequently leads to altered gene expression. DNA methylation can also be used as a biomarker for disease diagnosis and prognosis prediction and for targeted therapy by reactivating tumor suppressor genes [86]. It is believed that genome-wide studies may help to find the most relevant methylation biomarkers useful in the clinical practice in the next few years [87].

ncRNAs as biomarkers for wide use in precision medicine

Deregulated expression of microRNAs, ncRNAs, and circular RNAs plays a key role in the cervical cancer development. They influence carcinogenesis through transcriptional, translational, and posttranslational modifications associated with proteins, RNA, and DNA. ncRNAs are divided into two groups: small ncRNAs (20–200 nucleotides) and long ncRNAs (lncRNAs; >200 nucleotides). LncRNAs regulate gene expression by cis-regulation and trans-regulation mechanisms [88]. miRNAs are small, ncRNAs, 21–25 nucleotides in length, that regulate gene expression at the posttranscriptional level by binding to sequence motifs located within the 3' untranslated region of mRNA transcripts [89]. Processing of miRNAs starts via transcription by RNA polymerase II to a long, capped, polyadenylated primary miRNA transcript. This primary transcript is cleaved by nuclear RNase III (Drosha) into precursor micro-ribonucleic acids (pre-miRNAs), and the pre-miRNA is then transformed into a mature miRNA by RNase III (Dicer), and subsequently, the translation is suppressed [90]. Expression profiling of small RNAs in cervical neoplasia revealed upregulated “oncogenic” miRNAs, such as miR-10a, miR-21, miR-19, and miR-146a, and downregulated “tumor-suppressive” miRNAs (tumor suppressor miRs) such as miR-29a, miR-372, miR-214, and miR-218 [89]. miR-21 is able to advance invasion of cervical cancer through suppressing tissue inhibitors of metalloproteinases 3 (TIMP3) and miR-20a, and miR-106a suppressed invasion through TIMP2 [91].

miR-21 is also a negative regulator of expression of the programmed cell death 4 tumor suppressor gene.

miRNAs regulate gene expression by influencing important regulatory genes and modulating the process of epithelial-to-mesenchymal transition (EMT). In cervical cancer, they also have a role in signaling pathways, including Wnt/ β -catenin, Notch, and phosphoinositide-3 kinase (PI3K)-Akt pathways. Transcription of ncRNAs can modulate gene activity in response to external oncogenic stimuli and DNA damage [92]. Some miRNAs can also be down-regulated by epigenetic modifications, such as DNA hypermethylation.

miRNAs are more stable in body fluids, such as serum and plasma and in exosomes and tissue samples. Therefore, miRNAs have a potential to serve as biomarkers of the disease, which has been demonstrated in studies where authors used them as markers for clinical diagnosis, prognosis, prediction of therapy response and disease monitoring. The use of miRNAs in precision medicine should provide possible solutions to overcome some existing challenges and advance emerging opportunities [93].

Microbiome-mediated modulation of epigenetic pattern of cervical epithelial cells

How can the specific microbiota influence the epigenome of cervical squamous epithelium? This question has yet to be answered to transfer this knowledge to precision medicine. A recent investigation evaluated the influence of the microbiome on gene expression and alteration of chromatin structure via epigenetic modification. It has been shown that gut or intestinal microbiota can also influence the cervical microbiota [94]. The intestinal microbiota regulates approximately 10% of host genes, including genes regulating immunity, cell proliferation, and metabolism. Epigenetic regulatory mechanisms are involved in host defense against invading pathogens or pathogen persistence via gene expression changes in different types of immune cells [95].

The implication of microflora in intestinal homeostasis has been studied more extensively. The microbiome impacts on homeostasis through histone modification and chromatin remodeling in intestinal immune cells [96]. In a study on colonic epithelial cells, the authors treated a cell culture with live microbiota from healthy individuals and confirmed alteration of gene expression in more than 5,000 host genes through changes in host

chromatin accessibility and transcription factor binding. For example, opening of the chromatin resulted in upregulation of *tropomyosin 4* gene expression responsible for actin filament binding which might have a role in cytoskeletal regulation that affects the host cell response to the microbiome [19]. Probiotic bacteria, such as *B. breve* and *L. rhamnosus* GG, inhibit lipopolysaccharide (LPS)-induced expression of interleukins IL-17 and IL-23 by suppressing histone acetylation [97]. The members of the genera *Bifidobacterium* and *Lactobacillus* produce lactic acid and/or short-chain fatty acids (SCFAs) that act as histone deacetylation inhibitors and mediate the favorable effect of probiotics [98]. Production of SCFAs directly affects peripheral tissues beyond the gut and the gut–brain axis through the regulation of neuroendocrine and neuroimmune functions [99].

DNA methylation is an essential epigenetic mechanism for maintaining intestinal homeostasis and differentiation of intestinal stem cells [100–102]; there is also evidence that the intestinal microbiota affects DNA methylation [100,103,104]. A study regarding the impact of the commensal microbiota on host gene expression and DNA methylation in murine intestinal epithelial cells revealed that the microbiota regulated transcription through induction of DNA methylation changes in regulatory regions, especially in the case of acute inflammation where the microbiota induced TET2/3-dependent enhancer demethylation and chromatin remodeling [104]. *In vitro* infection of human dendritic cells (professional antigen-presenting cells [APCs]) with *Mycobacterium tuberculosis* induced DNA demethylation at distal enhancer elements, predominantly in genes expressing immune transcription factors. Hypomethylated regions were associated with increased levels of 5-hmC which implicated TET enzymes in the process of bacteria-induced demethylation [105].

Epigenetic changes, mainly DNA methylation, are important factors in the induction and regulation of the immune response to bacteria [106]. There is a paucity of studies focusing on the relationship between the cervicovaginal microbiota and DNA methylation. Nené *et al.* provided evidence of a strong local interaction between the cervicovaginal microbiome and the host epigenome, in which methylation pattern determined the specific composition of microbial communities [20]. The methylation status of 819 CpGs was used to discriminate between nonlactobacilli-dominant communities associated with hypomethylation and lactobacilli-dominant cervicovaginal samples [20].

Pathogenic bacteria are able to induce DNA methylation changes directly through the regulation of TET and DNMT enzymes or indirectly through the production of inflammatory mediators in response to infection [105,107].

One study, through immunohistochemical analysis of cervical tissues, found that expression of TET1 dioxygenase was upregulated in precancerous lesions and downregulated in invasive cancers. TET1 interaction with chromatin-modifying suppressors (lysine-specific histone demethylase and enhancer of zeste homolog 2) subsequently led to the silencing of EMT and a reduction of ZEB1 and VIM expression [108].

L. crispatus and *L. jensenii* were reported to modulate cell proliferation genes by downregulating HDAC4 and upregulating histone acetylase (HAT) EP300. Lower epithelial proliferation is mediated through an increase in cyclin-dependent kinase inhibitor 1A (CDKN1A) expression and connected inhibition of cyclin-dependent kinase 4 activity [109]. HAT EP300 also regulated estrogen receptor 1 (ESR1), mediating vaginal cell proliferation via ligand binding to the receptor. Exposure of cervical epithelial cells to *L. crispatus* and *L. jensenii* but not to *L. iners* or *G. vaginalis* resulted in lower expression of ESR1 by EP300, which could be a possible mechanism for controlling cell homeostasis [109].

A cervicovaginal microbiome with predominant *Lactobacillus* spp. was found to be protective against *C. trachomatis* infection through lactic acid D(–) and L(+) isoforms in a pH-dependent manner [109]. A cervicovaginal environment with nonlactobacilli (O-type) communities was found to be less supportive for *Lactobacillus* colonization due to CpG island methylation mirroring gastrointestinal differentiation [20]. Cervical cancer is frequently compared to head and neck squamous cell cancer (HNSCC) because of the similarities of the squamous epithelium and its susceptibility to HPV infection. A recent study on HNSCC tissues reported that *Fusobacterium* and *Peptostreptococcus* were the most frequent pathogenic bacteria in HNSCC cancer tissues and in oral rinse samples. *Fusobacterium nucleatum* was also identified with host gene promoter hypermethylation and hypomethylation, presumably through gene dysregulation related to the inflammatory response and cell proliferation through epigenetic silencing [18]. *F. nucleatum* was present in highly differentiated cervical cancers containing cells with characteristics of cancer stem cells [110].

Lactobacillus spp. communities in cervical epithelium also play a protective role through microbial modulation of human miRNAs. *In vivo* experiments on cervical epithelial cells and small RNA transcriptome sequencing (small RNA-seq) of cervical specimens showed miR-183, miR-193b, and miR-223 expression in dominance of *Lactobacillus* spp. associated with signaling, cell cycle, development, transcription, and hypoxia. Other amended miRNAs (miR-203b and miR-320b-1) had no experimentally validated targets [109]. Elevated levels of miR-23a-3p and miR-130a-3p have been

described as being specific to non-*Lactobacillus*-dominant microbial communities in young women [111]. Another *in vivo* study exposed ectocervical cells to *L. iners* or *G. vaginalis* bacteria-free supernatants. The authors found significantly increased expression of miR-143, miR-145, miR-193b, miR-146, miR-223, miR-148b, and miR-15a. Bacterial supernatants from *L. crispatus* did not alter miRNA expression. Interestingly, differentially expressed miRNAs were significantly reduced following exposure of ectocervical cells to *L. crispatus* bacteria-free supernatant [112]. The exact downstream targets of these miRNAs remain unknown.

It is certain that the microbiome and microbially secreted metabolites are capable of epigenetic modulation of gene activity and through these mechanisms can influence the host responses. The composition of the microbiome can be influenced by dietary composition, which will also affect the epigenetic background of microbiome. However, food forms the microbiome through epigenetic mechanisms, and it is necessary to clarify how cancer risk is increased due to food-related microbially produced metabolites. The answer to this question will significantly affect the use of diet in association with the microbiome and epigenetic changes in individualized personalized medicine and facilitate a special approach in preventive and targeted therapy [113]. The multidisciplinary knowledge from the intersection of metabolomics, epigenomics, metagenomics, and foodomics could contribute to the clarification of the issue, which will enable to overcome barriers of 3P medicine between scientific knowledge and clinical practice.

Inflammatory response of cervical epithelium in reaction to microbial colonization

The microbiome is composed of pathogenic or commensal bacteria that help to maintain homeostasis in the tissues in which they grow. The cervical epithelial barrier retains the microbiota in a mucosal layer and prevents the penetration of pathogenic bacteria and viruses into the cervical stroma. Bacterial colonies affect the epithelial cells by the metabolites and secreted factors that they produce, such as LPS, and can induce an inflammatory response and disruption of the epithelial barrier [112,114]. Therefore, the breakdown of the cervical epithelial barrier could be the mechanism by which inflammation induces remodeling of the cervix [114]. Epithelial cells detect pathogens by the recognition of bacterial LPS through Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors, nucleotide-binding

and oligomerization domain (NOD)-like receptors (NLRs), and C-type lectin receptors (CLRs) [115,116]. In innate immune cells, the detection of bacteria triggers signaling pathways that lead to the production of cytokines, chemokines, and antimicrobial peptides that constitute defensive and inflammatory reactions. Adaptive immunity contributes to the immune response through the presentation of bacterial antigens by APCs that activate T and B cells [106]. Excreted *Lactobacillus* biosurfactants are important in protecting the epithelium against the growth of anaerobic bacteria and to form a biofilm that prevents the adhesion of pathogenic bacteria [117].

The impact of the cervicovaginal microbiota on cervical function and permeability of the epithelial barrier has been studied *in vitro* by Anton et al. [112]. They showed that bacterial supernatants derived from *L. crispatus*, *L. iners*, and *Gardnerella vaginalis* use various mechanisms to alter the integrity of the epithelial barrier. The results of their study indicate that both *L. iners* and *G. vaginalis* increase the permeability of the epithelial barrier by various mechanisms, including aberrant miRNA expression, altered inflammatory mediator profile (IL-6 and IL-8), and *L. iners*-induced cleavage of E-cadherin, leading to disruption of adherens junctions. Conversely, *L. crispatus* has a protective effect on the epithelial barrier in the presence of LPS that induces increased cell permeability and inflammation [112]. Łaniewski and Herbst-Kralovetz studied the complex impact of cervicovaginal bacteria (*L. crispatus* as a commensal associated with healthy microflora and *G. vaginalis*, *P. bivia*, *Atopobium vaginae*, and *Sneathia amnii* associated with BV) on cervical epithelial cells in a 3D cervical cell model [118]. The immune-metabolic analysis revealed that *A. vaginae* and *S. amnii* had the highest proinflammatory potential due to their induction of proinflammatory cytokine and chemokine production, iNOS, and oxidative stress. Their results also showed that *G. vaginalis*, *P. bivia*, and *S. amnii* negatively affect the integrity of the epithelial barrier by decreasing levels of mucins and MMPs. Conversely, *L. crispatus* was reported to produce an antimicrobial compound, phenyl-lactic acid, which contributed to a protective microenvironment [118].

Microbial imbalance can modulate the inflammatory response through the production of proinflammatory cytokines, chemokines, and induction of oxidative stress [59]. Cervical epithelium-associated APCs and epithelial cells sense highly diverse communities in the genital tract [119]. A study by Anahtar et al. [120] showed that local immune response and proinflammatory cytokine production is species-specific and is associated predominantly with highly diverse bacterial communities in CST IV. Based on transcriptional analysis, they identified differentially expressed genes in cervical

APCs in women with *Prevotella*-dominant communities compared with women with *Lactobacillus*-dominant communities. Upregulated genes are implicated in nuclear factor kappa B (NF- κ B), TLR, NLR, and tumor necrosis factor alpha (TNF- α) signaling pathways. The results of the study indicate that endocervical APCs sense microbial LPS and activate immune response through TLR-4 signaling and NF- κ B pathways, leading to secretion of chemokines and cytokines that initiate inflammation [120]. Moreover, the production of the cytokines TNF- α and IFN- γ can negatively affect endocervical epithelial barrier function by tight junction disruption leading to bacterial and viral translocation [121,122]. Elevated expression of anti-inflammatory IL-4 and transforming growth factor β 1 (TGF β 1) in cervical cells was associated with the presence of *Fusobacterium* spp., which was found to be involved in the development of an immunosuppressive microenvironment [123]. *Fusobacterium* spp. can also activate proinflammatory pathways and inhibit immunocytotoxicity to promote cervical carcinogenesis [124]. Figure 2 represents a schematic difference between the epithelial homeostasis and BV.

A study focusing on CST classification and local immune response profiling concluded that a different type of inflammation is activated and controlled by both the dominant *Lactobacillus* spp. and by non-*Lactobacillus* bacterial colonies. One method is through balance between the anti-inflammatory (IL-1ra) and proinflammatory (IL-1 α and IL-18) response. The other, nondependent immune method is specific to *L. iners* in CST IV, with increased inflammation and production of proinflammatory factors such as macrophage migration inhibitory factor and TNF- α [48].

The long-term inflammation and metabolites produced by immune cells also cause DNA damage and activate epigenetic changes that may induce carcinogenesis. Cancer cells have similar DNA methylation alterations to epithelial cells at sites of chronic inflammation [125]. In gastric cancer, there is direct evidence that the presence of *Helicobacter pylori* and chronic inflammation lead to CDH1 promoter methylation and aberrant methylation in other CpG islands of genes encoding components of intracellular junctions (vezatin, adherens junctions transmembrane protein (VEZT), Connexin 32, and Cx43), cell cycle regulation (CDKN2A), DNA repair (the human homolog of *E. coli* MutL (hMHL-1) and O-6-methylguanine-DNA methyltransferase), inflammation (transcription termination factor 2 and cyclooxygenase-2 [COX-2]), transcription factors (RUNX family transcription factor 3, Forkhead Box D3, upstream transcription factor 1 (USF1) and USF2, and GATA binding protein 4 (GATA-4) and GATA-5), and other tumor suppressor genes [126]. In the prevention of chronic inflammation, some studies revealed the positive effect of antiinflammatory drug on

gut microbiota with significant reduction of bacterial strains associated with inflammation and cancer [127]. The results have been confirmed also in double-blind, randomized, placebo-controlled clinical trial that has found changes induced by the antiinflammatory drug that influenced also microbiome-induced metabolites [128].

As bacterial infection and inflammation are accompanied by metabolic changes leading to the production of reactive oxygen and nitrogen species (ROS/NOS) and oxidative stress [129–131], it can be assumed that oxidative stress is one of the mechanisms that induces changes in DNA methylation status. Typical oxidative damage of DNA involves the formation of 8-OHdG. In addition to its mutagenic effect, 8-OHdG can also negatively influence the DNA methylation of nearby cytosine [132,133]. ROS are also able to induce oxidation of 5-mC to 5-hmC, contributing to active DNA demethylation [134]. Furthermore, oxidative stress can catalyze DNA methylation through upregulation of DNMT expression by superoxide anion formation or by the formation of a new, DNMT-containing complex [135,136]. The exact role of oxidative stress as a mediator in the interaction between the microbiome, inflammation, and the epigenome of the cervical epithelium remains to be elucidated.

Based on earlier studies, there is strong evidence that the microbiome plays a crucial role in maintaining cervicovaginal homeostasis and inflammatory response, but the underlying mechanisms have not been fully described. It has been proved that all cancers are also associated with inflammation, especially with chronic condition. In cancer treatment, the chronic inflammation should be eliminated including exogenous factors supporting inflammation, biomarkers based on chronic inflammation pathways should be established and proinflammatory factor networks should be extensively studied to identify therapeutic targets and to prevent the occurrence and development of cancer [137]. An examination of metabolites during inflammation of the cervical epithelium and BV may improve the precise identification of inflammatory-induced biomarkers that could aid in the precision medicine in prediction of the risk of cervical dysplasia development.

Molecular pathways activated in inflammatory response to microbiome that should be targeted in precision medicine

Current evidence supports the apparent relationship between the cervical microbiome, inflammation and various pathologies

Figure 2: The difference between the epithelial homeostasis and BV. Bacterial colonies affect the epithelial cells by the metabolites and secreted factors as LPS. Epithelial cells detect pathogen by recognition of bacterial LPS through TLRs, RIG-1-like receptors, NLR, and CLR. Vaginal epithelial homeostasis is specific with *Lactobacillus* spp., which produces biosurfactants in a biofilm that prevents the adhesion of pathogenic bacteria. The local epithelial immune response is based on the innate immune cells, such as macrophages, NK cells, and neutrophils, which produce cytokines, chemokines, and antimicrobial peptides that form defense and inflammatory responses. Adaptive immunity contributes to the immune response through the presentation of bacterial antigens by APCs that activate T and B cells. BV is specific with nonlactobacilli species, as strictly anaerobic bacteria and *L. iners*, and higher pH. *L. iners* and *G. vaginalis* increase the permeability of the epithelial barrier by aberrant miRNA expression, altered inflammatory mediator profile (IL-6 and IL-8), and cleavage of E-cadherin leading to disruption of adherens junctions. *L. crispatus* has a protective effect on the epithelial barrier with production of phenylactic acid. BV-associated microflora classified in CST IV including *G. vaginalis*, *P. bivia*, *Atopobium vaginae*, and *Sneathia amnii* have a higher production of proinflammatory cytokines and chemokines, iNOS, and oxidative stress; decreased levels of mucins and MMPs and upregulated genes are implicated in NF- κ B, TLR, NLR, and TNF- α signaling pathways. Elevated expression of antiinflammatory IL-4 and TGF β 1 in cervical cells was associated with the presence of *Fusobacterium* spp. creating the immuno-suppressive microenvironment. Abbreviations: *A. vaginae*, *Atopobium vaginae*; APC, antigen presenting cells; CLR, C-type lectin receptors; *G. vaginalis*, *Gardnerella vaginalis*; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; iNOS, inducible nitric oxide synthase; *L. crispatus*, *Lactobacillus crispatus*; *L. iners*, *Lactobacillus iners*; LPS, lipopolysaccharide; miRNA, microRNA; MMPs, matrix metalloproteinases; mRNA, messenger RNA; NK, natural killer; NLR, NOD-like receptors; *P. bivia*, *Prevotella bivia*; RIG-I, retinoic acid-inducible gene I; RISC, RNA-induced silencing complex; *S. amnii*, *Sneathia amnii*; TGF β 1, transforming growth factor β 1; TLRs, Toll-like receptors.

such as HPV infection and cancer. The disturbed microbial balance results in an augmented release of proinflammatory cytokines and chemokines promoting immune dysregulation in female reproductive tract and providing a suitable condition for the tumor development [138]. The several molecular pathways are activated in response to microbial colonization. In personalized approach is possible to identify the patient in greater risk of long-term chronic inflammation, who should be treated with a specific drug to improve the patient's quality of life and to evade the precancerous condition.

The inflammation is characterized by tissue remodeling and alterations of functions of epithelial, vascular, and immune cells affected by various molecular pathways involving specific mediators such as cytokines, chemokines, or growth factors. Immune evasion is important for HPV persistence and is thus essential for the development of cervical cancer [139]. Therefore, chronic inflammation provides a tumor-supporting environment for the

development and progression of cervical cancer. Proinflammatory mediators produced by activated immune cells link infection, inflammation, immunity, and cancer [140]. Still, circulating inflammatory markers, such as interleukins (IL-6, IL-1 β), TNF- α , or IFN- β are significantly increased in HPV + cervical cancer patients [141]. Indeed, TNF contributes to the regulation of cellular processes including differentiation, proliferation, apoptosis, and inflammation [142]. TNF- α is defined as a proinflammatory cytokine defending the host against various pathogens including HPV. However, the systemic increase in TNF- α could result in or promote carcinogenesis [143]. Moreover, proinflammatory IL-17 secreted by various cells of TME activates the Janus kinase 2/signal transducer and activator of transcription 3 (STAT3), PI3K/Akt, and NF- κ B to promote the progression of cervical cancer [144].

Moreover, the NF- κ B family involves transcription factors that are essentially associated with the regulation of immune responses, inflammation, viral replication, and

Table 2: Selected studies exemplifying the epigenetic regulation of inflammation, cervical microbiome, and tumor pathogenesis

Targeted pathway	Cell line (or study design)	Effect	Reference
miRNA			
TNF- α	HeLa	miRNA-21 regulates the proliferation and apoptosis of cervical cancer cells via TNF- α	[160]
NF- κ B, TNF- α	HeLa and C33A	TNF- α activate NF- κ B activity that can reduce miR-130a expression, and miR-130a targets and downregulates TNF- α	[161]
NF- κ B	HeLa, C33A, and cervical cancer tissues	miR-429 involved in regulation of NF- κ B pathway (by targeting inhibitor of nuclear factor kappa-B kinase subunit beta) and functions as a tumor suppressor	[162]
Notch	HeLa, Caski, ME-180, C33A, SiHa, and SW756	miRNA-873-5p promotes progression of cervical cancer by regulating ZEB1 via Notch signaling	[163]
Wnt/ β -catenin	HeLa	miR-142-5p promotes cervical cancer progression by targeting LMX1A through Wnt/ β -catenin pathway	[164]
COX-2	HeLa	miRNA-101 inhibits cell proliferation, invasion, and promotes apoptosis by regulating COX-2	[165]
TNF	Cervical cancer tissue specimens and cell lines	MMP14 is characterized as a promoter of TNF signaling while miR-484/MMP14 could suppresses proliferation and invasion of cervical cancer cells by TNF signaling inhibition; DNMT1-mediated silencing of miR-484	[142]
Oxidative stress pathway	Cervical cancer cells	E6 disrupts the expression of miR-23b, miR-218, and miR-34a; the increased expression of miR-15a/miR-16-1 induces the inhibition of cell proliferation, survival, and invasion	[155]
Histone modifications			
Wnt/ β -catenin	C33A, MS751, HeLa, SiHa, and End1/E6E7	HDAC10 shows antitumor effects on cervical cancer via a novel microRNA-223/TXNIP/Wnt/ β -catenin pathway	[166]
NF- κ B	In silico prediction with <i>in vitro</i> assays	HDAC1 interacts with the p50 NF- κ B subunit via its nuclear localization sequence to constrain inflammatory gene expression	[167]
DNA methylation			
Notch	SIHA, CASKI, HeLa, C4-1, and C33A, and Ect1/E6E7	Gene <i>MSX1</i> acts as a tumorsuppressor in cervical cancer (exerts effects via Notch signaling); the promoter methylation of MS1 detected in 42% of primary cervical tumor tissues	[168]
Notch	110 cervical cancer patients	Altered promoter methylation of Notch1 and Notch3 receptor genes is observed in cervical cancer	[169]

cancer [138]. The HPV infection induces NF- κ B downregulation to allow its replication triggered by the immune system, leading to a status of persistent HPV infection. However, NF- κ B is again activated during the progression

to high-grade intraepithelial neoplasia and cervical cancer. Eventually, an increase in NF- κ B signaling is characterized by mutations in upstream signaling molecules [145]. Above all, NF- κ B can be considered as a link between cancer and

Figure 3: Signaling pathways and epigenetic changes associated with inflammation and cervicovaginal imbalance. Abbreviations: HPV, human papillomavirus; miRNA, microRNA; TSG, tumor suppressor gene; 5mC, 5-methylcytosine; ROS, reactive oxygen species; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; JAK, the Janus kinase; STAT3, signal transducer and activator of transcription 3; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; IL, interleukin; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor- β ; TLR-4, Toll-like receptor 4; MAPKs, mitogen-activated protein kinases; 8-oxodG, 8-hydroxy-2'-deoxyguanosine; IFN- β , interferon β ; MMP14, matrix metalloproteinase 14; and MSX1, Msh homeobox 1.

inflammation. NF- κ B activation results from the underlying inflammation or as a consequence of inflammatory microenvironment typical for carcinogenesis. Moreover, NF- κ B is capable of upregulating proinflammatory and tumor-promoting mediators such as IL-6 or TNF- α , and survival genes, such as B-cell lymphoma-extra large (*Bcl-XL*) [146]. In addition to the induction of IL-6 by E6 through NF- κ B, IL-6 promotes the activation of STAT3 that induces TGF- β , IL-6, and IL-10 and supports the aggregation of regulatory T cells and induces the immunosuppressive microenvironment [138].

Among others, Wnt/ β -catenin signaling contributes to the inflammatory functions of the cell, at least partly, through repressing or enhancing NF- κ B pathway while NF- κ B pathway also regulates Wnt/ β -catenin either positively or negatively [147]. In fact, aberrant Wnt/ β -catenin signaling components are associated with tumorigenesis including cervical cancer [148]. Furthermore, the evidence supports the role of Notch signaling in the regulation of inflammation. Notch has been also found to be associated with NF- κ B pathway in cancer and thus reveals the ability to regulate inflammatory cytokines including TNF- α and IL-1 β as well as TLR agonists [149].

Moreover, TLRs could recognize either endogenous or exogenous pathogens and thus mobilize immune responses. In response to the stimulation, for example, by exogenous pathogens (bacterial LPS), TLR-4 regulates immune responses. Activated TLR-4 contributes to the stimulation of its downstream signaling pathways MAPKs and NF- κ B and upregulation of genes encoding proinflammatory cytokines. Indeed, TLR-4 plays an essential role in carcinogenesis. TLR-4 facilitates the local immunosuppressive microenvironment and promotes HPV-positive cervical cancer growth [150]. The expression of TLR-4 is high in cervical cancer cells [151].

Oxidative stress, caused by the imbalance of free radicals and antioxidants in the body, leads to cell and tissue damage. Uncontrolled oxidative stress causes chronic inflammation with potential cancer development [152]. Excessive ROS generation enhances the oxidative damage of DNA resulting in inflammation, DNA mutations, genomic instability, and cancer promotion [153]. Inflammation-related cancers are characterized by increased 8-*oxo*-7,8-dihydro-2'-deoxyguanosine and 8-nitroguanine, the biomarkers of oxidative damage, that are commonly found in the tissues of cancer and precancerous lesions due to infection (e.g., human papillomavirus-related cervical cancer) [154]. ROS trigger DNA damage and regulate the viral life cycle through pro-survival and antiapoptotic effects on infected cells and increase *E6* and *E7* gene expression. Overexpression of *E6* and *E7* also leads to deregulation of oncogenes and miRNAs expression [155].

Moreover, COX-2 is a key enzyme in the metabolism of prostaglandin (PG) that is closely associated with inflammation and carcinogenesis. Increased COX-2 expression is observed in epithelial cancer cells including cervical cancer. In a milieu of viral inflammation typical for cervical cancer, COX-2 represents an important trigger of carcinogenesis [156]. HPV E5, E6, and E7 could upregulate COX-2 and PG E2 resulting in the activation of COX-PG pathway that is considered as the main cause of HPV-induced inflammation [157]. Finally, TME is a complex ecosystem while inflammatory cells represent one of the most important components. Tumor-associated macrophages contribute to the carcinogenesis through releasing various mediators such as proinflammatory platelet-derived growth factor or proangiogenic VEGF. Cyclin-dependent kinases (CDKs) are crucial regulators of cell cycle processes; for example, cyclin-dependent kinase 12 (CDK12) is markedly elevated in cervical cancer while its knockdown promotes the infiltration of macrophages and regulates the immune microenvironment in cervical cancer cells [158].

The cancer-associated inflammation pathways can be influenced by phytochemicals with antiinflammatory effects on immune cells, suppression of proinflammatory transcription factors, cytokines, and chemokines. The antiinflammatory properties of flavonoids revealed their potential application in preventive and therapeutic medicine to improve individual outcomes in cancer linked chronic inflammation. The biological balance between uncontrolled chronic inflammation and controlled inflammation is essential for cancer prevention, prediction, and prognostication [159].

Indeed, above discussed pathways linking cervical microbiome, inflammation, and cancer can be epigenetically regulated. Therefore, Table 2 provides an overview of studies exemplifying the possible epigenetic modulation of selected pathways that can be also associated with inflammation and cervical cancer. Figure 3 illustrates the above-mentioned signaling pathways and epigenetic changes that are associated with inflammation and cervicovaginal imbalance.

Conclusions

The maintenance of the cervical epithelial barrier and a beneficial vaginal microbiome is a major implication on women's health, including healthy pregnancy and resistance to sexually transmitted diseases. In this review, we highlighted the pivotal contribution of cervical microbiome, epigenetic changes, and inflammation to the formation of cervical intraepithelial lesion and progression

to cervical cancer. In the modern precision medicine, a dilemma of whether changes in microbial composition in individual tissues lead to dysregulation of cancer promoting physiological functions, or whether an increase in proinflammatory condition in a specific tissue can modify the environment and promote the growth of some microbial strains over others, should be resolved and the answer could be subsequently applied in therapeutic bacterial or bacterial metabolites administration to exploit their unique and immunosuppressive properties in remodeling of TME and promote a beneficial local microbiome [170].

Currently, there are few studies investigating the effect of the cervical microbiome on epigenetic changes in the cervical epithelium and also in relation to the host inflammatory response. Novel techniques, such as next generation sequencing, allow us to better characterize the cervical microbiome and predict how cervical lesions will develop. The huge data produced in collection of sequencing results use the AI to find the intersection between multiomics disciplines. The antitumor influence of microbial metabolites together with the activation of the local immune system may serve in preventive medicine to regress precancerous lesions. The intervention of new types of microbiome supports the expansion of favorable over the harm genera of microorganisms. In modern precision medicine, reconstruction and restoring of healthy vaginal microflora will reverse the process of disease outbreak. Identification of inflammatory-induced biomarkers often found in cervical cancer may help in the prediction of cervical precancerous lesion development in case of vaginal dysbiosis. Currently, practice of precision medicine is in uptake of prebiotics, probiotics, and synbiotics as a part of diet and other ways in phage therapy and antibiotics as well as microbiota transplantation [171] may prevent gynecological cancers and reduce vaginal toxicity related to cancer treatment.

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