УДК 618.146-006.6
https://doi.org/10.20538/1682-0363-2023-2-145-155

Modern concepts in cervical carcinogenesis

Naumova L.A., Starodumova V.A.

Surgut State University
1, Lenina Str., Surgut, Khanty-Mansiysk Autonomous Area – Yugra, 628412, Russian Federation

ABSTRACT

The article discusses modern ideas about cervical carcinogenesis as a multi-stage process of multifactorial genesis. Currently, ideas about the pathogenesis of cervical cancer (CC) are based not only on understanding the role of high-risk oncogenic human papillomavirus (HPV) in this process and accumulation of genetic changes caused by it, but also on formation of a complex HPV interactome, or a network of intermolecular interactions of HPV oncoproteins with host cell proteins. Carcinogenesis also involves a wide range of epigenetic events and, above all, impairment of the regulatory function of miRNAs. An important role in cervical carcinogenesis is attributed to the concept of cancer stem cells (CSCs) formulated in recent years, which is closely related to the explanation of disease recurrence and treatment resistance, as well as to new approaches to treatment. The cervicovaginal microbiome and cervical microenvironment, which are responsible for natural clearance of HPV, regression of epithelial lesions, and modeling of the immune response, are becoming promising objects for research.

The aim of the review was to present up-to-date information on the most important mechanisms of cervical carcinogenesis, as well as on new approaches to the treatment of CC, based, in particular, on the use of knowledge about regulatory miRNAs, CSC markers, and the state of the cervicovaginal microbiota.

Keywords: cervical carcinogenesis, cervical cancer genomics, miRNA, cancer stem cells, microbiota

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Naumova L.A., Starodumova V.A. Modern concepts in cervical carcinogenesis. *Bulletin of Siberian Medicine.* 2023;22(2):145–155. https://doi.org/10.20538/1682-0363-2023-2-145-155.

Современные представления о цервикальном канцерогенезе

Наумова Л.А., Стародумова В.А.

Сургутский государственный университет (СурГУ)
Россия, 628412, Ханты-Мансийский автономный округ (ХМАО) – Югра, г. Сургут, ул. Ленина, 1

РЕЗЮМЕ

Рассматриваются современные представления о цервикальном канцерогенезе как многостадийном процессе мультифакторного генеза. В настоящее время представления о патогенезе рака шейки матки (РШМ) базируются не только на понимании роли в этом процессе вируса папилломы человека (ВПЧ) высокого онкогенного риска и накоплении обусловленных им генетических изменений, но и формировании сложного интерактома ВПЧ, или сети межмолекулярных взаимодействий онкобелков ВПЧ с белками клетки-хозяина. Также в процессе канцерогенеза принимают участие эпигенетические модификации широкого спектра и, прежде всего, нарушения регуляторной функции микроРНК. Важное место в пред-

✉ *Naumova Lyudmila A.,* naumovala@yandex.ru

ставлениях о цервикальном канцерогенезе занимает сформулированная в последние годы концепция раковых стволовых клеток (CSCs), с которой тесно связано объяснение рецидивов заболевания и устойчивости к лечению, а также понимание новых подходов к лечению. Перспективным объектом для исследования становится также цервиковагинальный микробиом и цервикальное микроокружение, ответственные за естественный клиренс ВПЧ, регрессию эпителиальных повреждений и моделирование иммунного ответа.

Цель обзора – изложение актуальной информации о важнейших механизмах цервикального канцерогенеза, а также новых подходах к лечению РШМ, базирующихся в частности на использовании знаний о регуляторных микроРНК, маркерах CSCs и состоянии цервиковагинальной микробиоты.

Ключевые слова: цервикальный канцерогенез, геномика рака шейки матки, микроРНК, раковые стволовые клетки, микробиота

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Наумова Л.А., Стародумова В.А. Современные представления о цервикальном канцерогенезе. *Бюллетень сибирской медицины.* 2023;22(2):145–155. https://doi.org/10.20538/1682-0363-2023-2-145-155.

INTRODUCTION

All over the world, cervical cancer (CC) is one of the most common malignant gynecological diseases, for which morbidity and mortality rates tend to worsen. In 2020, more than 600,000 new CC cases were detected globally and 342,000 deaths were registered [1]. CC ranks fourth among cancer types in women after breast cancer, colorectal cancer, and lung cancer [2–4] and is the second cause of death from cancer in women aged 20–39 years [1, 5–7]. In underdeveloped countries, with high CC prevalence (ranks second in cancer incidence in women) and late diagnosis, the 5-year survival rate of patients with CC is less than 50% [2–4]. Despite existing screening programs (human papillomavirus (HPV) tests, cytology, colposcopy) and vaccination leading to a decrease in mortality from CC, there is a growing need for screening for precancer and CC at early stages, allowing for the use of organ preserving therapy that ensures fertility preservation [5, 6, 8].

Ideas about cervical carcinogenesis are closely related to the modern understanding of the features of the transition zone between the endo- and exocervix, which is known as the squamo-columnar junction of the cervix (SCJ) and is characterized by the presence of cervical stem cells (SCs) [2, 4, 5, 9]. The discovery of SCs provides an understanding of not only the mechanisms of physiological and reparative regeneration of cervical epithelium, but also of the appearance of cancer stem cells (CSCs) that are the origin of carcinogenesis [2, 4, 5, 9, 10].

CSCs determine the malignant potency of the tumor that is the rate of development and metastasis and the degree of regression during treatment [4, 5, 9, 11].

Although persistent high-risk HPV infection (HR-HPV) is of particular importance in tumor transformation [1, 5], CC is a multifactorial disease caused not only by the accumulation of genetic changes, but also by a wide range of epigenetic changes: impaired DNA methylation and modification of histones and non-coding RNA (ncRNA) [6, 12–15]. The oncogenic potency of HPV infection is determined by a large number of additional factors including the influence of hormones, multiple sexual partners, obesity, smoking, alcohol abuse, poor nutrition, immunosuppressive cervical microenvironment, abnormal vaginal microbiota, coinfections with *Chlamydia trachomatis* or human immunodeficiency virus [1, 5, 7, 16–19].

Lactobacillus spp. are the main representatives of the vaginal flora and the first line of defense against pathogenic microflora in HPV infection, and as the severity of cervical intraepithelial neoplasia (CIN) increases, the prevalence of an increasingly toxic flora including *Fusobacterium, Sneathia,* and *Streptococcus* is diagnosed [5, 16, 20]. Researchers are accumulating more and more data on the critical role of the cervicovaginal microbiome (CVM) in the dynamics of HPV infection: the natural clearance of HPV, CIN regression, and the immune response modeled by the CVM [1, 5, 7, 16].

The study of molecular mechanisms of CC pathogenesis based on the methods of systems biology (genomics, transcriptomics, proteomics, metabolomics of CC) is crucial for obtaining a large amount of data on prognostic and important for treatment CC biomarkers and for developing effective CC therapies [3, 21].

ONCOGENIC HPV STRAINS AND MOLECULAR GENETIC MECHANISMS OF CERVICAL CANCER PATHOGENESIS

Currently, more than 200 HPV and animal genotypes have been described and sequenced, of which approximately 30 types affect the anogenital tract, 15 of these types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) were classified as HR-HPVs associated with CIN2/3 and cancer *in situ* or high-grade intraepithelial lesion (HSIL) and invasive cervical cancer. HPV 16 and 18 cause about 70% of squamous cell carcinomas and more than 90% of adenocarcinomas [2, 12–14, 22, 23].

At the same time, it was proven that in almost 90% of HPV-infected women the virus is no longer detected (transient infection) 6–18 months after infection, in one tenth of infected women the infection persists (persistent infection), and precancerous cervical lesions may develop [2, 14, 22] which progress to invasive cervical cancer in 0.3–1.2% of cases. The question of the possibility of complete elimination of the virus, with it remaining latent in basal cells with the preservation of the reactivation potential is still to be discussed. Persistent HPV infection is diagnosed in the presence of viral DNA of the same type upon re-examination after 6–12 months [2, 22, 23].

The life cycle of HPV penetrating through a microtrauma into basal cells of the exocervical epithelium is closely related to the differentiation of epitheliocytes, depends on a number of cellular factors and sequentially expressed viral proteins, and is described in detail in numerous reviews [4, 5, 12, 14, 15]. The virus can exist in an episomal, integrated, or mixed (cooccurrence of an integrated and episomal form) forms in infected cells [14, 24].

Integration of viral DNA into the host cell genome can have different signatures (more than 3,500 breakpoints have been described) and cause amplification of oncogenes, inactivation of tumor suppressor genes, inter- or intrachromosomal rearrangements, and genomic instability [14, 24]. Changes are more often detected in the *PIK3CA, TP53, KRAS*, and *PTEN* genes, often in STK*11, POU5F1B, FHIT, KLF12, KLF5, LRP1B, LEPREL1*, etc. [3, 6, 24, 25]. The integration of viral DNA into the host genome is accompanied by the destruction of the *E2* viral gene, which regulates the E6 and E7 oncoproteins, which leads to their overexpression [14].

During integration, the viral genome incorporates into the host cell genome at sites of damage or double-strand breaks in DNA due to the deactivation of the normal DNA damage response (DDR) by oncoproteins E6 and E7, which consists of ATR and P53 activation [3, 12]. The E7 protein affects the ATR protein kinase and inactivates Rb, disrupting the cell cycle inhibitors p21, p27.The E6 protein inhibits DNA repair, causes uncontrolled proliferation of infected cells through disruption of apoptosis (ubiquitin – proteasome degradation of the p53 tumor suppressor and binding to procaspase-8), and suppresses the response of many genes to interferon [3, 12, 14]. The E6 protein activates the PI3K / Akt pathway, promotes the degradation of the transcriptional repressor NFX1, and induces the activation of hTERT (human telomerase reverse-transcriptase), leading to the immortalization of transformed cells [3, 5, 12, 14].

Currently, the existence of a complex HPV interactome or a network of E6 and E7 intermolecular interactions with host cell proteins has been established [12, 14, 15]. The E6 and E7 proteins can modulate the gene expression profile, host cell proteome, and intracellular signaling pathways, including MAPK-, Wnt-, Akt-, Notch-, mTORC-, and STAT-dependent cascades, which leads to changes in the epithelial phenotype [12, 14].

Integration of viral DNA into the genome of basal cells with stem-like properties in the transformation zone leads to their transformation into CSCs [2, 5, 15], the population of which is maintained by activation of Wnt/β-catenin, Notch, and Hedgehog [3, 12, 14], Oct3/4, NANOG, and SOX2 signaling pathways by oncoproteins [26]. A study of CC (whole exome sequencing) carried out as a part of the Cancer Genome Atlas (TCGA) revealed a huge number of mutations. So, 192 “tumor – control” pairs in the aggregate contained 43,324 somatic mutations; 11 samples containing more than 600 mutations per sample were identified as hypermutated [25].

Integrative clustering of CC samples using data from the analysis of the genome (DNA), transcriptome (mRNA), DNA methylation, microRNA expression (miRNA), and copy number variations (CNV) of genes revealed pronounced molecular heterogeneity of CC. In particular, three clusters of CC were identified: clusters of squamous CC with high and low expression of keratin genes and a cluster that includes predominantly adenocarcinomas. The three clusters identified were associated with features of CC histology, HPV types, HPV integration status, UCEC-like status, APOBEC mutagenesis level, evaluation of the epithelial – mesenchymal transition (EMT) and mRNA, expression of KRAS, ERBB3, and HLA-A. The isolated clusters differed not only in the expression of the *SPRR* and *TMPRSS* genes involved in keratinization, but also of *SMGs, ARID1A, NFE2L2*, and *PIK3CA* genes responsible for various metabolic pathways in the cell [25, 27].

For the first time, mutations in ERBB3, CASP8, HLA-A, SHKBP1, and TGFBR2 were identified in CC, and ERBB3 (HER3) was immediately identified as a

therapeutic target due to aberrant signaling between mutant HER3 and activated HER2. Mutations of HLA-A, HLA-B, NFE2L2, MAPK1, CASP-8, SHKBP1, and TGFBR2 have been found exclusively in squamous CC [25]. The detected amplifications of CD274 (PD-L1) and PDCD1LG2 (PD-L2), which significantly correlate with the expression of granzyme A and perforin, key genes of cytolytic effector cells, do not exclude the effectiveness of immunotherapy in some types of CC [25].

Also, 1,026 epigenetically silent genes were identified including zinc-metalloproteinases ADAM and ADAMATS modeling the extracellular matrix and collagen genes (COL), which were methylated to a greater extent in CC than in the control samples [25].

In the *PIK3CA* gene, there were mainly activating mutations of the helical domain E542K and E545K, in which the main nucleotide substitution pattern was associated with the mutagenesis of APOBEC, a family of cytidine deaminases that play the main role in the deamination of cytidine to uridine in DNA and RNA and control various biological processes (embryogenesis, innate immunity, regulation of protein expression). Currently, APOBEC mutagenesis, which causes tumor mutations due to the aberrant DNA editing mechanism, is considered as a molecular driver of CC pathogenesis [23, 25]. In HPV-negative CC, a lower rate of APOBEC mutagenesis and high expression of miRNAs responsible for EMT were noted [27].

In general, the differences were found in CC pathogenesis in the presence and absence of its association with HPV in terms of three important characteristics including cancer driver genes, the severity of genomic instability, and mitotic pathways in cancer pathogenesis [28]. It is the features of molecular mechanisms and genetic disorders that underlie cancer pathogenesis in each specific case that determine various outcomes of CC [28, 29].

EPIGENETIC MODIFICATIONS IN CERVICAL CARCINOGENESIS

Recent studies have shown that the integration of the viral genome into the host cell genome is not enough for the development of CC [12–14]. CC pathogenesis is a multi-stage process associated not only with the accumulation of genetic changes, but, above all, with the accumulation of a wide range of epigenetic modifications in the viral and host cell genomes. These modifications are associated with impaired DNA methylation and modification of histones and ncRNA [12–15, 30]. Epigenetic changes referring to reversible modifications of gene function are natural mechanisms of cellular adaptation and occur under the influence of a wide range of factors including age, lifestyle, endocrine profile, chronic inflammation, and cellular stress. These factors, in turn, form a cellular microenvironment with a complex network of interactions of cytokines, chemokines, free radicals, growth factors, and enzymes, which determines the effect on critical signaling pathways involved in maintaining cellular homeostasis [12–14, 17, 18, 30, 31].

In CC, cytosine methylation (the replacement of a hydrogen atom by a methyl group) catalyzed by DNA methyltransferases (DNMTs) is predominantly observed in the CpG motif. Methylation of the gene promoter usually leads to gene silence, while demethylation causes an increase in its expression, and it is also possible when oncogenes are expressed due to demethylation of their promoters (shown on the example of serine / threonine kinase 31 (STK31)), as well as variation in the effects of DNA methylation depending on ethnicity [13, 14].

The E6 and E7 oncoproteins can induce the expression of DNMTs and histone-modifying enzymes, including histone deacetylases (HDACs), acetyltransferases (HATs), methyltransferases (HMTS), and demethylases. They can also alter the activity of proteins associated with chromatin remodeling complex and miRNA processing [13]. The E6 oncoprotein activates *DNMT1* expression through degradation of p53 and release of transcription factors Sp1, which bind to the *DNMT1* gene promoter, while the E7 oncoprotein activates the expression of a stable complex with pRB and the release of the transcription factor E2F [12–14]. In CC, *CADM1, CDH1, DAPK1, EPB41L3, FAM1A4, MAL, PAX1,* and *hTER* are the most frequently methylated genes [13]. At the early stage of CC pathogenesis, the retinoic acid receptor β (*RARβ*) gene associated with cell differentiation and the Wnt/β inhibitory factor gene, the product of which inhibits the Wnt/β-catenin signaling pathway, are often methylated [14].

The HPV genome also undergoes epigenetic modification, as during the virus life cycle, methylation of late L1 and L2 oncoproteins and the LCR regulatory region changes [12, 13]. LCR hypermethylation prevents the E2 protein from regulating the expression of viral *E6* and *E7* oncogenes, causing its overexpression [14].

Post-translational modifications of histones (acetylation, methylation, phosphorylation, SUMOylation, and ubiquitination) affecting the structural state and transcriptional activity of chromatin are crucial in the regulation of the cell cycle, cell differentiation, maintenance of SCs, and epigenetic inheritance of transcriptional programs [14]. In normal cells, the balance between HDACs and HATS ensures the control over cell proliferation and death; E6 and E7 oncoproteins disrupt this balance, causing uncontrolled proliferation of cancer cells [12, 14]. Inhibition of HDACs, which reduce the level of histone acetylation and cause the silence of tumor suppressor

genes, is becoming a promising approach in cancer therapy. So, the inhibitor of HDAC all-trans retinoic acid (ATRA) in combination with suberoylanilide hydroxamic acid (SAHA) increases the content of acetylated histones in the promoter of the tumor suppressor genes *RARβ2*, E-cadherin and β-catenin, the deacetylation of promoters of which is reduced or absent in CC. The combination of valproic acid (VPA) and ATRA demonstrates additional antitumor effects due to reactivation of the expression of RARβ2, E-cadherin, P21CIP1, and P53 and a decrease in the expression of the *STAT3* gene, which activates transcription of genes responsible for proliferation. These results do not exclude the use of HDAC inhibitors and RARβ2 agonists as a new approach to the CC treatment [14].

Due to advances in biotechnology and high-throughput sequencing, the leading role of the aberrant expression of ncRNAs, single-stranded RNA transcripts, which include miRNAs, long ncRNAs (lncRNAs), and circular ncRNAs (circRNAs) in cancer pathogenesis has become clear. MiRNAs are of particular importance in this process, since they can participate in the regulation of both the most important biological processes (proliferation, differentiation, apoptosis) and molecular pathways of cancer pathogenesis (cell cycle, Wnt / β-catenin pathway, P53, E6-p53, E7 -pRb, PI3K-Akt, Notch) [3, 12–15, 30, 32–34]. MiRNA dysregulation may be a consequence of genomic mutations, abnormal modification of DNA methylation at miRNA gene loci or single-nucleotide polymorphisms (SNPs), as well as dysregulation of proteins involved in miRNA biogenesis [15, 32, 35].

Based on their effect on proto-oncogenes and oncosuppressors, miRNAs are respectively divided into oncomiRNAs and tumor suppressor miRNAs (TSMIRs); the content of the latter is significantly reduced in most types of cancer [15]. MiRNAs can also affect viral DNA replication; in turn, E6 and E7 oncoproteins can catalyze aberrant methylation of genes encoding miRNAs through the induction of DNMT overexpression [32]. Continuous E6 / E7 expression is associated with a decrease in intracellular concentrations of miR-23a, miR-23b, miR-206, miR-143, miR-15, and miR-16, all of which are associated with antitumor activity [12, 14].

Almost every stage of CC development has its own miRNA signature, which theoretically can be of great importance for the diagnosis, treatment, and monitoring of the process [15, 33, 36]. So, increased expression of miR-16, miR-21, miR-34a, and miR-143 was found in LSIL. In HSIL, increased expression of miR-21 and a decrease in expression of miR-143 were found [36] along with an increase in miR-205-5p, miR-130a-3p, and miR-3136-3p expression and suppression of miR-381-3p and miR-4531, while miRNA expression patterns did not depend on HR-HPV infection [33]. MiR-499 and miR-18a are described as markers of invasive CC; miR-125, which suppresses VEGF and migration and invasion of tumor cells (already used as TSMIR in the treatment of CC), and miR-375, acting through regulation of MELK and increased apoptosis, are described as oncosuppressors [15].

Downregulation of let-7c, miR-124, miR-126, miR-143, and miR-145 regulates oncogene expression. Destabilization of p53 by the E6 oncoprotein leads to suppression of miR-34a expression, which affects several components of the cell cycle, including CDK4, cyclin E2, E2F-1, hepatocyte growth factor MET receptor, and Bcl-2 [14]. Overexpression of miR-21 and downregulation of miR-29 were found useful for the diagnosis of invasive CC [15]. Activation of miR-31 and miR-9 expression and overexpression of miRNA-21 under the influence of E6 / E7 are associated with the progression of CC and poor prognosis [14].

Currently, several hundred miRNAs have been identified that are differentially expressed in CC [15, 34, 36]. Participation of ncRNAs in cancer pathogenesis significantly complicates the understanding of the molecular biology of cancer and makes these transcripts the subject of numerous studies aimed at revealing their diagnostic and prognostic value [14]. Their use in diagnosis has not yet been successful due to methodological problems and pronounced variability in the composition of miRNAs in different patients with the same type of cancer. In treatment based on miRNAs, it is also necessary to overcome the problem of their degradation by nucleases, inaccurate delivery to target cells, and side effects in the form of activation of immune responses [15].

CANCER STEM CELLS

In accordance with the hypothesis on cancer stem cells (**CSCs**), a tumor is characterized by a special hierarchy of cells, in which a small part of the subpopulation, called CSCs, has stem cell-like properties, including the ability to undergo asymmetric division, self-renewal, oncogenesis, and resistance to chemo- and radiation therapy due to the presence of detoxification or elimination systems [4, 5, 8, 9, 30, 37]. As a result of asymmetric division of CSCs, one daughter cell remains a stem cell, while another one losing its stemness gives rise to descendants that form the bulk of tumor cells of various degrees of differentiation [9]. The CSC paradigm allows to approach the understanding of the genesis of cancer, including CC, the explanation of the mechanisms of its metastasis, relapses, and resistance to chemo- and radiation therapy [4, 9, 11, 30, 38].

There are several theories on the origin of CSCs. Mutations leading to the appearance of CSCs can occur

in SCs and in already transformed (tumor) cells, causing the selection of cells with high oncogenic activity [5, 30]. Researchers cannot rule out the presence of a subpopulation of resident CSCs that ensure the development and maintenance of the initial tumor and are dormant in the niches of CSCs [4, 5, 8, 30], as well as circulating in the blood and disseminating CSCs responsible for metastasis. The CSC niche is a microenvironment of cells formed by the extracellular matrix and stromal cells (fibroblasts, macrophages, etc.) and which, through call – cell and cell – matrix interactions involving a large number of various signaling molecules (cytokines, miRNAs, hormones), determines the regulation of the dormant state of CSCs, activation of CSCs in response to stress (hypoxia, chemotherapy), as well as proliferation and differentiation of their descendants [4, 39]. Cancer cells are able to transit stochastically between these states, which also supports the hierarchy of differentiation and occurs, in particular, during treatment [9].

The existence of CSCs determines the heterogeneity of most tumors, including CC, which is manifested both through intertumor heterogeneity that refers to different aggressiveness and different clinical outcomes in the same type of tumor and through intratumor heterogeneity which is manifested by biological and molecular differences between tumor cells in the same tumor in one patient. The tumor is a combination of CSCs with mixed mesenchymal / epithelial phenotypes and cancer non-stem cells, which can acquire stem cell-like properties when exposed to EMT [4, 9]. EMT characterized by the induction of transcription factors SNAI1, SNAI2, TWIST1, and BMI1 is considered as the main source of CSCs [4, 5]. CSCs are capable of transdifferentiating into vascular endothelial cells, as well as into other tumor-associated stromal cells, which can also contribute to tumor heterogeneity [4, 9].

CIN and subsequent events of CC pathogenesis develop in the cells of the transition zone between the endo- and exocervix, which is known as SCJ of the cervix or the transformation zone [4, 5]. The cells at the cervical SCJ have unique morphology and their specific markers include keratin 7 (Krt7), anterior gradient 2 (AGR2), CD63, matrix metalloproteinase 7 (MMP7), and guanine deaminase (GDA). The persistence of the SCJ genetic profile in CIN and CC cells indicates the origin of numerous CC subtypes from the cells at cervical SCJ and suggests that SCJ may be a niche for cervical CSCs [4, 9, 26].

Existing methods of treatment (chemo- and radiation therapy) eliminate only cancer non-stem cells, CSCs survive in niches that protect them and lead to cancer relapse [4, 5, 8, 9, 39], which allows them to be considered as a core of the malignant tumor [39] and the most likely target for treatment [8, 9, 39]. The molecular mechanisms that determine the future of CSCs, cell – cell interactions, and the molecular profile of the cervical CSC niche are still largely unclear, and laboratory protocols have not been developed to isolate CSCs in a sufficient amount, which makes their identification a difficult task [9, 11].

Currently, there is no set of universal markers for the identification and isolation of CSCs. The main source of material for studying CSCs is the so-called side population, which is a small (up to 20%) subpopulation of cells inside the tumor, exhibiting the properties of CSCs [4, 5]. Identification of multipotent CSCs can be based on the ability of these cells to secrete the Hoechst fluorescent dye and under certain conditions to form spheroids that are cells of the epithelial / mesenchymal phenotype. The existing method of isolating CSCs is based on determining the expression of surface markers using fluorescence-activated cell sorting (FACS), confocal microscopy, immunohistochemistry, reverse transcription polymerase chain reaction (RT-qPCR), as well as subsequent testing of the oncogenic efficiency of the obtained cells in an animal experiment and analyzing the resulting tumor subpopulation [5].

A number of cytokeratins (CK-5, 8, 13, 17, 18 and 19) of CSCs and SCs of the cervix have common expression, but only CK-19 and CK-17 are considered as possible CSC markers, since their high expression in CC is associated with high malignancy and metastasis, while the side population of cells expressing CK-19 and CK-17 demonstrates high oncogenicity [5, 11]. Np63, a key protein of the Sonic-Hg signaling pathway, is also considered as a putative CSC marker, and it may be responsible for stemness through the induction of the Bmi-1 protein required for SC proliferation [11].

Common CSC markers in cancers of various body locations include high expression of CD44, CD90, CD44, CD133, CD271, CD49f, ALL+ [4, 5, 26] and OCT4, NANOG, β-catenin, and aldehyde dehydrogenase (ALDH1). ALDH1 is associated with detoxification of chemicals through the oxidation of aldehydes and protection from high concentrations of reactive oxygen species. Expression of ALDH1 correlates with high tumor activity and radioresistance [3–5, 11, 26]. The resistance of CSCs to death is provided by active repair of DNA damage through phosphorylation of ATM and CHK1 / CHK2 or activation of anti-apoptotic WNT / β-catenin, PI3K / Akt, and Notch signaling pathways [4]. The development of therapies that prevent DNA repair in cancer cells or target anti-apoptotic proteins of the Bcl-2 family has proven to be more difficult than expected; studies of PARPi inhibitors (poly(ADP-ribose)

polymerase), which is involved in DNA repair, mainly concern ovarian cancer [15].

The SOX2-positive cell population in CC demonstrates radioresistance and expresses higher levels of genes related to stemness (OCT4 and ALDH1) and EMT, which confirms that SOX2 is a marker for cervical CSCs. As a key transcription factor, SOX2 plays an important role in SCs and may be involved in the initiation of the tumor process [9, 26, 40].

The resistance of CSCs to treatment and the occurrence of relapses are associated with increased activity in CSCs in carrier proteins, including MDR1 (ABCB1), ABCC1 (MRP1,) and ABCG2. ABCG2 is an ATP-binding cassette transporter that blocks absorption, pumps a wide range of chemical compounds out of cells, and plays an important role in the development of multiple drug resistance in a number of cancer types [4, 5, 9, 11, 26]. ABCG2 activity is also associated with an increase in the expression of HIF-2α, a transcription factor induced by hypoxia 2, which, in turn, is associated with the expression of Oct4, a transcription factor that maintains CSC stemness [11]. In CC, the Nrf2 transcription factor plays an important role in regulating the expression of some endogenous antioxidants and detoxification enzymes [9].

Osteopontin (OPN), a chemokine-like protein of the extracellular matrix, which is secreted by tumor and stromal cells, is also considered as a potential CSC marker. Overexpression of OPN in CC correlates with resistance to hypoxic cell injury during radiation exposure and poor survival rate. OPN also induces tumor angiogenesis by modulating HIF1α-dependent VEGF expression in response to hypoxia, regulates CD44-mediated p38 phosphorylation, affects the nuclear factor kappa-B (NF-kB) in activated B cells and NF-kB-dependent furin expression, which are involved in the response to HPV. The self-renewal ability of CSCs mediated by OPN can be suppressed by a decrease in NF-κB expression [9].

More and more data are accumulated on epigenetic programming of stemness, in particular, the involvement of miRNAs in the regulation of the cell cycle of both SCs and CSCs by direct or indirect influence on its various components (*RB*, p53, p21, *LATS2, PTEN*, cyclins, CDKs, and CDKIs) [4, 30], while differences in the transcription level of individual miRNAs in resident SCs and CSCs were revealed [30]. In CSCs of various tumors, a decrease in the expression of the let-7 miRNA family with oncosuppressive properties was revealed, which leads to overexpression of the MYC, RAS, HMGA2, and BLIMP oncogenes. Members of the let-7 family also downregulate PTEN, cause inactivation of the PI3K / AKT / MTOR pathway, and suppress EMT [30].

Therapeutic strategies targeting CSC signaling pathways and the niche of CSCs are under development [4]. As a component of the CC treatment, DNMT inhibitors, which are anti-CSC compounds, are already used. The use of a dual delivery system with sequential release of drugs suppressing tumor cell proliferation can be potentially effective. The use of drugs (antibodies) that affect CSCs can be also effective along with targeting molecular mechanisms that maintain the dormant state of CSCs indefinitely or eliminate a subpopulation of such cells, in particular, ablation of cytosolic phospholipase A2 alpha (CPLA2a). CPLA2a is a key mediator of inflammation and the pathophysiology of cancer. It markedly improves the sensitivity of CSCs to chemotherapy by suppressing β-signaling catenin. Targeting CSCs using nanoparticles can be an effective approach [4].

EPITHELIAL – MESENCHYMAL TRANSITION

Mortality in CC is largely determined by the recurrence of the tumor process. At the same time, EMT plays the most important role in invasion, metastasis, and metastatic recurrence of a tumor [4, 41]. EMT is characterized by transdifferentiation, or the acquisition of a mesenchymal phenotype by an epithelial cell due to a loss of expression of epithelial markers (E-cadherin, β-catenin, occludin, claudin, plakophilin, cytokeratins, desmoplakins) and increased expression of mesenchymal markers (N-cadherin, vimentin, fibronectin) [30, 41–43].

EMT can be initiated by dysregulation of various signaling pathways, which include transcriptional regulators of oncogenes and oncosuppressors, miRNAs, and growth factors [30, 41]. The transcriptional regulators of EMT in CC include Snail / Slug / Smug; Zeb1 / Zeb2; Twist1 / Twist2; E47; among growth factors – TGF, EGF; among oncogenes – HVP16; E6 / E7; Sam68; AEG1; FTS, among oncosuppressors – Klotho, SFRPs; and a wide range of miRNAs – miR200, the miR-155 and miR361-5p family [25, 41], as well as a number of other molecular factors – β1-integrin receptors, MMPs7 / 9, IL-6, TNF-α, etc. [41]. EMT is also determined by the regulatory function of the chemokine network, in particular, by the level of CXCL6. In CC, a decrease in the level of miR-101-5p was found, which normally suppresses cancer progression through inhibition of CXCL6 promoting EMT [32]. Twist1 / Twist2 are the main regulators of EMT; they are responsible for the activation of the AKT and β-catenin pathways and the preservation of CSCs [41]. EMT-exposed CSCs can enter a dormant state, evading traditional therapies that target actively dividing cells [4].

In the EMT cluster in CC, studies revealed high immunoreactivity of the stroma with high expression of caveolin-1, MYH11, and RAB11, as well as YAP and

ZEB1 dysregulation, which may be associated with lower levels of miR-200A-3p expression in this cluster. These data suggest a potential role of YAP and stromal reactivity in the regulation of EMT and progression of CC [25]. Understanding the molecular aberrations characteristic of EMT and the underlying cellular signaling of EMT is important for the development of treatment leading to reduction of mortality from CC [4, 41].

VAGINAL MICROBIOTA AND CERVICAL MICROENVIRONMENT

In recent years, studies have accumulated data on the significant role of the cervicovaginal microbiome (CVM) in the dynamics of HPV infection, including the natural clearance of HPV, CIN regression, and the immune response modeled by the CVM [7, 16, 19]. The protective role of the normal vaginal microbiota is associated with certain types of lactobacilli: *Lactobacillus (L.) crispatus*, *L. gasseri*, *L. jensenii*, *L. iners*, which produce a number of antimicrobial compounds and lactic acid, providing low vaginal pH (≤ 4.5) necessary to suppress colonization by pathogenic species (*Chlamydia trachomatis, Neisseria gonorrhoeae, and G. Vaginalis)* and maintain vaginal homeostasis [2, 7, 16, 19, 22, 44–46].

In dysbiosis, or bacterial vaginosis, lactobacilli are depleted and replaced by a polymicrobial consortium of microaerophilic and anaerobic bacteria [2, 7, 22, 44, 47]. HPV infection is more often associated with non-lactobacillus dominance in the CVM, when *Atopobium*, *Mycoplasma hominis, Haemophilus, Gardnerella, Sneathia and Megasphaera, Prevotella* dominate. The development of CIN is caused by the presence *of Sneathia, Atopobium, Parvimonas, Fusobacterium, Anaerococcus, Peptostreptococcus, Mycoplasmateles, Pseudomanales* [7, 19, 22]. CC is associated with an increase in aerobic bacterial flora and the presence of *Corynobacterium spp.* and *Staphylococcus spp*. [20]. A high risk of HR-HPV infection and the development of CIN is associated with a small number of *L. crispatus* producing peptidoglycans that activate Langerhans cells, which are the most important antigen-presenting cells in the cervical epithelium. A strong relationship was established *in vivo* between Langerhans cell activity and the clearance of HPV, while *L. crispatus* was the most common *Lactobacillus* species in individuals with the natural clearance of HPV [7, 5, 46]. The clearance of HPV is also associated with *L. gasseri* [22].

Vaginal dysbiosis is accompanied by an alteration of the epithelial barrier due to the production of sialidase by anaerobes, alters immune and metabolic signaling, supporting the production of proinflammatory cytokines and chemokines, activating a number of cell signaling pathways, in particular the NF-κB pathway. It also induces genomic instability of epithelial cells, angiogenesis, impaired proliferation, and apoptosis [16, 22, 46]. It also plays an active role in the progression of the tumor process in both CC, endometrial and ovarian cancer, including through participation in the modulation of estrogen metabolism [22].

A comprehensive analysis revealed signatures associated with the development of the tumor process and the composition of the cervicovaginal microbiota and identified features of the cervicovaginal microenvironment (immune mediators, vaginal pH), which may contribute to the persistence of HPV, the development of precancerous lesions, and cancer progression [16, 19, 22, 47–50]. A number of studies demonstrated the critical role of CVM in the modulation of cervicovaginal immune responses and even host susceptibility to HIV [7]. Potencies of LSIL (low-grade squamous intraepithelial lesion) and HSIL are closely correlated with the functional state of the immune system, in particular T- and B lymphocytes, dendritic cells, NK cells, and macrophages [6, 46]. The depletion of T cells that helps tumors escape the host immune system is phenotypically characterized by co-expression of immune inhibitory receptors or immune checkpoints, a gradual loss of proliferation, cytokine secretion, and cytolytic activity.

In CC, the most promising immune checkpoints are PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T lymphocyte antigen-4) [6]. In HSIL, the immune microenvironment is characterized by the absence of intraepithelial CD3+, CD4+, and CD8+ T cell infiltrates and Langerhans cells compared to normal epithelium and an increase in CD25+FoxP3+ regulatory T cells (Tregs) and CD163+ M2 macrophages. Spontaneous regression of HSIL is associated with a low Treg count, an increasing number of intraepithelial CD8+ T cells, and a high ratio of CD4+/CD25+ T cells [46].

L. crispatus can suppress anaerobic growth and activate Langerhans cells by lowering factors associated with epithelial barrier disruption (sialidase and anaerobic biopellicle) and immune suppression through the effect on Treg, Th2, Th17 cells, IL-10, IL-17, and TGFß. At the same time, the expression of biomarkers of activated Langerhans cells, which include antigen-binding langerin and TLR9, antigen-presenting CD1a and MHC-I, co-stimulatory CD80 / 86 and CD40 molecules, and CMI-associated molecules (cytotoxic T lymphocytes, Th1, IFNγ, TNFα), increases [7].

In general, the effect of CVM on the CC pathogenesis is a complex and not yet fully clear process. It is probably associated with the implementation of various enzymatic mechanisms and metabolic pathways involved in protecting the epithelium, removing toxins, and stimulating and regulating the immune system, which makes

CVM a target for the development of innovative therapeutic approaches based on the use of microbial products as immune activators, in particular in HPV infection [7, 16, 44, 46].

CONCLUSION

Knowledge obtained in the field of cancer biology suggests that, despite the close relationship between CC and HR-HPV, a variety of epigenetic events play an important role in CC pathogenesis, as they regulate the cell cycle, determine chromosome stability, and activate telomeres and apoptosis. These epigenetic events are functionally closely related to the state of the cervicovaginal microbiota and immunity. Expanding the understanding of the most important mechanisms of CC pathogenesis determines both the variety of diagnostic and prognostic markers and potentially possible ways of treating CC, in particular, based on the use of knowledge about regulatory miRNAs, CSC markers, and the state of the cervicovaginal microbiota.

REFERENCES

1. Wilailak S., Kengsakil M., Kehoe S. Worldwide initiatives to eliminate cervical cancer. *Int. J. Gynecol. Obstet*. 2021;155(1):102–106. DOI: 10.1002/ijgo.13879.
2. Bhatla N., Aoki D., Sharma D.N., Sankaranarayanan R. Cancer of the cervix uteri. *Int. J. Gynaecol. Obstet*. 2018;143(2):22–36. DOI: 10.1002/ijgo.12611.
3. Lin M., Ye M., Zhu X. Recent advances on the molecular mechanism of cervical carcinogenesis based on systems biology technologies. *Comput. Struct. Biotechnol. J.* 2019;17:241–250. DOI: 10.1016/j.csbj.2019.02.001.
4. Fiore R.D., Suleiman S., Drago-Ferrante R., Subbannayya Y., Pentimalli F., Giordano A. et al. Cancer Stem Cells and Their Possible Implications in Cervical Cancer: A Short Review. *Int. J. Mol. Sci.* 2022;23(9):5167. DOI: 10.3390/ijms23095167.
5. Mendoza-Almanza G., Ortíz-Sánchez E., Rocha-Zavaleta L., Rivas-Santiago C., Esparza-Ibarra E., Olmos J. Cervical cancer stem cells and other leading factors associated with cervical cancer development. *Oncol. Lett*. 2019;18(4):3423–3432. DOI: 10.3892/ol.2019.10718.
6. Guo L., Hua K. Cervical cancer: emerging immune landscape and treatment. *Onco. Targets Ther.* 2020;12(13):8037–8047. DOI: 10.2147/OTT.S264312.
7. Dai W., Du H., Li S., Wu R. Cervicovaginal microbiome factors in clearance of human papillomavirus Infection. *Front. Oncol.* 2021;11:722639. DOI: 10.3389/fonc.2021.722639.
8. Chhabra R. Cervical cancer stem cells: opportunities and challenges. *J. Cancer Res. Clin. Oncol.* 2015;141(11):1889–1897. DOI: 10.1007/s00432-014-1905-y.
9. Huang R., Rofstad E.K. Cancer stem cells (CSCs), cervical CSCs and targeted therapies. *Oncotarget.* 2017;8(21):35351–35367. DOI: 10.18632/oncotarget.10169
10. Zhao Z., Wang Y., Wu Y., Li D., Zhang T., Ma Y. et al. Single-cell analysis defines the lineage plasticity of stem cells in cervix epithelium. *Cell Regen.* 2021;10(1):36. DOI: 10.1186/s13619-021-00096-2.
11. Ortiz-Sánchez E., Santiago-López L., Cruz-Domínguez V.B., Toledo-Guzmán M.E., Hernandez-Gueto D., Muñiz-Hernández S. et al. Characterization of cervical cancer stem cell-like cells: phenotiping, stemness, and human papilloma virus co-receptor expression. *Oncotarget.* 2016;7(22):31943–31954. DOI: 10.18632/oncotarget.8218.
12. Senapati R., Senapati N.N., Dwibedi B. Molecular mechanisms of HPV mediated neoplastic progression. *Infect. Agent. Cancer*. 2016;11(1):59. DOI: 10.1186/s13027-016-0107-4.
13. Soto D., Song C., McLaughlin-Drubin M.E. Epigenetic alterations in human papillomavirus-associated cancers. *Viruses.* 2017;9(9):248. DOI: 10.3390/v9090248.
14. Da Silva M.L.R., De Albuquerque B.H.D.R., Allyrio T.A.M.F., De Almeida V.D., Cobucci R.N.O., Bezerra F.L. et al. The role of HPV-induced epigenetic changes in cervical carcinogenesis (Review). *Biomed. Rep*. 202115(1):60. DOI: 10.3892/br.2021.1436.
15. Bañuelos-Villegas E.G., Pérez-yPérez M.F., Alvarez-Salas L.M. Cervical cancer, papillomavirus, and miRNA dysfunction. *Front. Mol. Biosci*. 2021;8:758337. DOI: 10.3389/fmolb.2021.758337.
16. Castanheira C.P., Sallas M.L., Nunes R.A.L., Lorenzi N.P.C., Termini L. Microbiome and cervical cancer. *Pathobiology.* 2021;88(2):187–197. DOI: 10.1159/000511477.
17. Lee S.A., Baik S., Chung S.H. Functional roles of female sex hormones and their nuclear receptors in cervical cancer. *Essays Biochem*. 2021;65(6):941–950. DOI: 10.1042/EBC20200175.
18. Baik S., Mehta F.F., Unsal E., Park Y., Chung S.H. Estrogen inhibits epithelial progesterone receptor-dependent progestin therapy efficacy in a mouse model of cervical cancer. *Am. J. Pathol.* 2022;192(2):353–360. DOI: 10.1016/j.ajpath.2021.10.008.
19. Klein C., Gonzalez D., Samwel K., Kahesa C., Mwaiselage J., Aluthge N. et al. Relationship between the Cervical Microbiome, HIV Status, and Precancerous Lesions. *mBio*. 2019;10(1):e02785-18. DOI: 10.1128/mBio.02785-18.
20. Manzanares-Leal G.L., Coronel-Martínez J.A., Rodríguez-Morales M., Rangel-Cuevas I., Bustamante-Montes L.P., Sandoval-Trujillo H. et al. Preliminary identification of the aerobic cervicovaginal microbiota in Mexican women with cervical cancer as the first step towards metagenomic studies. *Front. Cell Infect. Microbiol*. 2022;12:838491. DOI: 10.3389/fcimb.2022.838491.
21. Martinez-Rodriduez F., Limones-Gonzalez J.E., Mendoza-Almanza B., Esparza-Ibarra El., Gallegos-Flores P.I., Ayla-Lujan J.A. et al. Understanding cervical cancer trough proteomics. *Cells.* 2021;10(8):1854. DOI: 10.3390/cells10081854.
22. Łaniewski P., Ilhan Z.E., Herbst-Kralovetz M.M. The microbiome and gynaecological cancer development, prevention and therapy. *Nat. Rev. Urol*. 2020;17(4):232–250. DOI: 10.1038/s41585-020-0286-z.
23. Revathidevi S., Murugan A.K., Nakaoka H., Inoue I., Munirajan A.K. APOBEC: A molecular driver in cervical cancer pathogenesis. *Cancer Lett.* 2021;496:104–116. DOI: 10.1016/j.canlet.2020.10.004.

24. Kamal M., Lameiras S., Deloger M., Morel A., Vacher S., Lecerf C. et al. Human papilloma virus (HPV) integration signature in Cervical Cancer: identification of MACROD2 gene as HPV hot spot integration site. *Br. J. Cancer*. 2021;124(4):777–785. DOI: 10.1038/s41416-020-01153-4.
25. Salvesen H.B. The cancer genome atlas research network. Integrated genomic and molecular characterization of cervical cancer. *Nature*. 2017;543(7645):378–384. DOI: 10.1038/nature21386.
26. Organista-Nava J., Gómez-Gómez Y., Garibay-Cerdenares O.L., Leyva-Vázquez M.A., Illades-Aguiar B. Cervical cancer stem cell-associated genes: prognostic implications in cervical cancer. *Oncol. Lett.* 2019;18(1):7–14. DOI: 10.3892/ol.2019.10307.
27. Kecheryukova M.M., Snezhko A.V., Verenikina E.V., Mensheninina A.P., Adamyan M.L., Arja A.Yu., et al. Complex molecular characterization of cervical cancer: metastatic markers. *Modern Problems of Science and Education.* 2020.2 (in Russ.). URL:https://science–education.ru/ru/article/view?id=29769 (accessed 23.09.2021).
28. Yu X., Xu J., Xu D., Bi X., Wang H., Lu Y. et al. Comprehensive analysis of the carcinogenic process, tumor microenvironment, and drug response in HPV-positive cancers. *Front. Oncol*. 2022;12:842060. DOI: 10.3389/fonc.2022.842060.
29. Olusola P., Banerjee H.N., Philley J.V., Santanu Dasgupta S. Human papilloma virus-associated cervical cancer and health disparities. *Cells.* 2019;8(6):622. DOI: 10.3390/cells8060622.
30. Mens M.M.J., Ghanbari M. Cell cycle regulation of stem cells by microRNAs. *Stem. Cell Rev. Rep*. 2018;14(3):309–322. DOI: 10.1007/s12015-018-9808-y.
31. Li Y., Tollefsbol T.O. Age-related epigenetic drift and phenotypic plasticity loss: implications in prevention of age-related human diseases. *Epigenomics.* 2016;8(12):1637–1651. DOI: 10.2217/epi-2016-0078.
32. Shen S., Zhang S., Liu P., Wang J., Du H. Potential role of microRNAs in the treatment and diagnosis of cervical cancer. *Cancer Genet.* 2020;248–249:25–30. DOI: 10.1016/j.cancergen.2020.09.003.
33. Causin R.L., Silva L.S., Evangelista A.F., Leal L.F., Souza K.C.B., Pessôa-Pereira D. et al. MicroRNA biomarkers of high-grade cervical intraepithelial neoplasia in liquid biopsy. *Biomed Res. Int*. 2021;2021:6650966. DOI: 10.1155/2021/6650966.
34. Zheng M., Hou L., Ma Y., Zhou L., Wang F., Cheng B. et al. Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. *Mol. Cancer.* 2019;18(1):76. DOI: 10.1186/s12943-019-0999-x.
35. Babion I., Jaspers A., van Splunter A.P., van der Hoorn I.A.E., Wilting S.M., Steenbergen R.D.M. miR-9-5p exerts a dual role in cervical cancer and targets transcription factor TWIST1. *Cells.* 2019;9(1):65. DOI: 10.3390/cells9010065.
36. Babion I., Miok V., Jaspers A., Huseinovic A., Steenbergen R.D.M., van Wieringen W.N., Wilting S.M. Identification of deregulated pathways, key regulators, and novel miRNA-mRNA interactions in HPV-mediated transformation. *Cancers (Basel).* 2020;12(3):700. DOI: 10.3390/cancers12030700.
37. Zhao Z., Wang Y., Wu Y., Li D., Zhang T., Ma Y. et al. Single-cell analysis defines the lineage plasticity of stem cells in cervix epithelium. *Cell Regen*. 2021;10(1):36. DOI: 10.1186/s13619-021-00096-2.
38. Lim J.R., Mouawad J., Gorton O.K., Bubb W.A., Kwan A.H. Cancer stem cell characteristics and their potential as therapeutic targets. *Med. Oncol.* 2021;38(7):76. DOI: 10.1007/s12032-021-01524-8.
39. Oshimori N. Cancer stem cells and their niche in the progression of squamous cell carcinoma. *Cancer Sci.* 2020;111(11):3985–3992. DOI: 10.1111/cas.14639.
40. Sudhalkar N., Rathod N.P., Mathews A., Chopra S., Sriram H., Shrivastava S.K. et al. Potential role of cancer stem cells as biomarkers and therapeutic targets in cervical cancer. *Stem. Cell Rev. Rep*. 2018;14(3):309–322. DOI: 10.1007/s12015-018-9808-y.
41. Campo L., Zhang C., Breuer E.-K. EMT-inducing molecular factors in gynecological cancer. *Biomad. Res. Int*. 2015;2015:420891. DOI: 10.1155/2015/420891.
42. Gonzalez D.M., Medici D. Signaling mechanisms of the epithelial–mesenchymal transition. *Science Signaling*. 2014;7(344):re8. DOI: 10.1126/scisignal.2005189.
43. Ye X., Weinberg R.A. Epithelial–mesenchymal plasticity: a central regulator of cancer progression. *Trends Cell Biol.* 2015;25(11):675–686. DOI: 10.1016/j.tcb.2015.07.012.
44. Tango C.N., Seo S.S., Kwon M. et al. Taxonomic and functional differences in cervical microbiome associated with cervical cancer development. *Scic. Rep.* 2020;10(1):9720. DOI: 10.1038/s41598-020-66607-4.
45. Kaelin E.A., Skidmore P.T., Łaniewski P., Holland L.A., Chase D.M., Herbst-Kralovetz M.M. et al. Cervicovaginal DNA virome alterations are associated with genital inflammation and microbiota composition. *mSystems*. 2022;7(2):e0006422. DOI: 10.1128/msystems.00064-22.
46. Muntinga C.L.P., Vos van Steenwijk P.J., Bekkers R.L.M., van Esch E.M.G. Importance of the immune microenvironment in the spontaneous regression of cervical squamous intraepithelial lesions (CSIL) and implications for immunotherapy. *J. Clin. Med*. 2022;11(5):1432. DOI: 10.3390/jcm11051432.
47. Lin W., Zhang Q., Chen Y., Dong B., Xue H., Lei H. et al. Changes of the vaginal microbiota in HPV infection and cervical intraepithelial neoplasia: a cross-sectional analysis. *Sci. Rep.* 2022;12(1):2812. DOI: 10.1038/s41598-022-06731-5.
48. Bokulich N.A., Łaniewski P., Adamov A., Chase D.M., Caporaso J.G., Herbst-Kralovetz M.M. Multi-omics data integration reveals metabolome as the top predictor of the cervicovaginal microenvironment. *PLoS Comput. Biol.* 2022;18(2):e1009876. DOI: 10.1371/journal.pcbi.1009876.
49. Curty G., de Carvalho P.S., Soares M.A. The role of the cervicovaginal microbiome on the genesis and as a biomarker of premalignant cervical intraepithelial neoplasia and invasive cervical cancer. *Int. J. Mol. Sci*. 2019;21(1):222. DOI: 10.3390/ijms21010222.
50. Yang L., Yang Y., Meng M., Wang W., He S., Zhao Y. et al. Identification of prognosis-related genes in the cervical cancer immune microenvironment. *Gene.* 2021;766:145119. DOI: 10.1016/j.gene.2020.145119.

Authors' information

Naumova Lyudmila A. – Dr. Sci. (Med.), Professor, Department of Pathophysiology and General Pathology, Medical Institute, Surgut State University, Khanty-Mansiysk Autonomous District – Yugra, Surgut, naumovala@yandex.ru, https://orcid.org/0000-0003-1145-3710

Starodumova Valentina A. – Post-Graduate Student, Department of Pathophysiology and General Pathology, Medical Institute, Surgut State University, Khanty-Mansiysk Autonomous District – Yugra, Surgut, starodumovav@list.ru, https://orcid.org/0000-0001-8362-0608

(✉) **Naumova Lyudmila A.,** naumovala@yandex.ru

Received 07.06.2022;
approved after peer review 30.08.2022;
accepted 08.12.2022