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REVIEW

Prospects of NSAIDs administration as double-edged agents against endometrial cancer and pathological species of the uterine microbiome

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ABSTRACT

Many types of cancers, including endometrial cancer, were found to have cyclooxygenase-2 (COX-2) overexpression. Because this enzyme belongs to the group of pro-inflammatory enzymes, so-called NSAIDs (non-steroidal anti–inflammatory drugs) directly inhibit its activity. An increasing number of reports on COX-2 involvement in cancer, as well as on the role of microbiota in abnormal metabolism and signaling of cells, forces the development of new NSAID types. Besides, NSAIDs can affect some bacteria, which are vaginal/endometrial microbiome members. The overgrowth of those species was found to be a major cause of some uterus diseases. Those infections can lead to chronic inflammatory response and suppress anti-tumorigenic cell pathways. The purpose of this review is to highlight the COX-2 enzyme role in endometrial cancer, the potential effect of the endometrial microbiome on COX-2 enzyme overexpression, and the prospects of NSAIDs use in terms of this type of cancer.

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Introduction

Cyclooxygenases, COX-1 and COX-2 isoforms particularly, are extremely important for essential and accurate cellular prostaglandin (PG) production. However, from the perspective of tumor progression, prostaglandin E2 (PGE2), synthesized in the result of COX-2-activated arachidonic acid transformation seems to be crucial. Production of PGE2 initiates signaling cascade, leading to the intensification of various metabolic processes and apoptosis inhibition or prevention. Hence, the overexpression of COX-2 can play a key role not only in inflammatory processes but also in misbalance of cell division, apoptosis, normal metabolism, and angiogenesis, in turn leading to cancer formation.[1]

Endometrial cancer (EC), one of the most common types of cancer among women, may be induced by various initiation factors, but the molecular base of these processes is still poorly understood. Reports, including those on the overexpression of COX-2 in EC, are controversial but indicate the dependency of enzyme expression and stage of women's lifetime (pre-menopausal or post-menopausal period of life). The results of Jeon et al. demonstrated the overexpression of COX-2 in the post-menopausal period, however, according to the authors, this phenomenon was not associated with malignancy of tumors.[2,3] In contrast, Lambropoulou et al. noticed a positive correlation between COX-2 expression and cancer FIGO (International Federation of Gynecology and Obstetrics) stage. Besides, they suggested that the enzyme level may serve as a prognostic factor of treatment and indicates the degree of myometrial invasion.[4] COX-2 overexpression in endometrial cancer tissue samples was confirmed in other studies. Ma et al.[5] based their notes on observations of correlated up-regulation of COX-2, glucose transporter GLUT-1, common for EC, and metastasis factor VEGF (vascular endothelial growth factor), which biosynthesis is PGE2-dependent. These studies demonstrated also the dependency of COX-2 transcription on HIF-1α (hypoxia-inducible factor) induction, as observed in the case of GLUT-1 and VEGF overexpression.[6-8] Moreover, Huang et al.[9] presented the possible contribution of COX-2/PGE2/HIF-1α/VEGF pathway to the process of tumor angiogenesis in gastric carcinoma. *In vitro* studies showed that the reduced PGE2 levels effectively suppressed HIF-1α protein accumulation, which resulted in a similar inhibitory effect on VEGF production. This mechanism may be possible also in the case of endometrial cancer.

Apoptosis blockage and neoplastic transformation can be caused by COX-2. As it was identified, the exposure of cancer cells on selective COX-2 inhibitors led to apoptosis induction by anti-apoptotic Bcl-2 protein down-regulation. Vice versa, the overexpression of COX-2 led to increased production of Bcl-2, which signifies the importance of Bcl-2 and COX-2 cross-talk in pathological conditions. Moreover, COX-2 may affect Akt signaling regulation, increasing the pathological effect. Linked with excessive activity of kinase phosphorylation, COX-2 indirectly leads to NF-κB overexpression, which is an essential apoptotic suppressor. As reported by St-Germain et al. COX-2 silencing in EC cell lines (HEC-1-A, RL 95–2, and Ishikawa) inhibits NF-κB activity and restore apoptosis sensitivity. A growing body of evidence confirms the impact of COX-2 on tumor progression.[10,11]

It is well known that COX-2-induced NF-κB expression may significantly increase the intracellular NO production. NF-κB regulates expression of nitric oxide synthase (iNOS),

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by catalyzing the NO production, which serves as a signal molecule of cellular pathways, such as angiogenesis, apoptosis, immunological, and anti–inflammatory response. High-level expression of iNOS is common for increased ROS (reactive oxygen species) environment, related to almost all types of cancer.[12] Furthermore, Li et al.[13] observed the significant correlation between COX-2 and iNOS levels in endometrial cancer. Results suggest the combined COX-2 and iNOS modulation of angiogenesis, caused by local NO overproduction. Those interactions were investigated further, in the presence of provoking factors. Hussain et al.[14] presented the example of induced chronic inflammation (defined by COX-2 overexpression) in a murine model, showing the effect of increased NO levels after *Cryptosporidium parvum* infection. The level of Ki-67 protein, a marker of most cancer types, was significantly enhanced. Poljakovic et al.[15] studies revealed the correlation between iNOS and COX-2 levels in mouse kidney cells. Initial infection by *Escherichia coli* AD 110 strain was leading to spontaneous DNA mutations. This defines the importance of the inflammatory state induced by a cell environment in uncontrolled cancer development.

MMPs (extracellular matrix metalloproteinases), became a subject of interest in terms of their pathological role in cancer cell metabolism, including invasion and metastasis. Studies of Byun et al.[16] proved the correlation between COX-2 and MMP-2 expression in the human lung A549 cancer cell line. Following studies by Pan et al.[17] indicated the reduction of *MMP-2* gene expression after the treatment of the mentioned cells with COX-2 inhibitors. MMP-2, belonging to Zn-dependent endoproteinases, seems to play a crucial role in metastasis development of endometrial cancer. The meta-analysis carried out by Liu et al.[18] confirmed other reports – overexpression degree of MMP-2 in endometrial cancer samples was the independent indicating factor of disease malignancy. It may also help to assess the effectiveness of treatment. Besides, an increased MMP-2 level and pathological angiogenesis are associated with ovarian endometriosis. Historical biochemical data reported a high level of PGE2, COX-2, and VEGF in the case of disease, suggesting the relationship between those units.[19]

Potential role of microbiome in endometrial cancer initiation

The coexisting microbiome and its role in cancer initiation and progression grow in interest. Well-studied *Helicobacter pylori*, which infection promotes the development of MALT lymphoma and gastric adenocarcinoma, has been known as carcinogen factor for many years.[20] About 60% of intestinal-type gastric cancers are associated with *H. pylori* infections. These bacteria down-regulate the host immune responses driving the immunity toward tolerance rather than to the protective response. Moreover, during the acute phase of colonization *H. pylori* resists the oxidative stress caused by an inflammatory response and in the chronic phase of infection. Also, it was shown that due to the oxidative stress, damage of the DNA of epithelial cells increases during *H. pylori* infection. Colonization by these bacteria led to genomic instability and inhibition of the DNA mismatch repair (MMR) system protein expression. In consequence, the accumulation of numerous DNA mutations of gastric epithelial cells, activation of oncogenes and/or inactivation of tumor suppressor genes occurs, which promotes the development of gastric cancer.[21] Chronic inflammation, caused by *Helicobacter* colonization, can induce abnormal Wnt/β-catenin signaling pathway activation, which plays a critical role in cell differentiation, proliferation, growth, and survival.[22,23] *Fusobacterium nucleatum* exists in the oral cavity of healthy people, however, it may also promote human colorectal adenomas and carcinomas (CRCs) tissue development. Using the similar carcinogenic mechanism as *H. pylori*, this species is associated with CRC risk.[24,25] It was also noted that Wnt/β-catenin signaling down-regulated inflammatory response, by pro-inflammatory cytokine production, such as IL-1β, IL-6, IL-8, and TNF-α, as well as repression of NF-κB activity.[26] Nuñez et al.[27] reported the role of Wnt/β-catenin signaling as an enhancer of *COX-2* gene expression in gastric cancer. This fact indicates the possibility of the indirect role of COX-2 in cancer progression caused by bacterial colonization.

Another bacterial species, which can stimulate cancer progression but also may support cancer therapy, is *Salmonella ssp*. On one hand, *Salmonella enterica* serovar Typhimurium is well known as a cancer promoter and a possible cause of hepatobiliary cancer in the case of chronic infection.[28] On the other hand, attenuated strains of *S. enterica* ser. Typhimurium, which are opportunistic bacterial pathogens, have the potential as a cancer therapy tool – inflammasome activation caused by those strains resulted in tumor growth suppression.[29] Thus, bacteria may be considered as double-edged agents in cancer initiation and progression.

The role of endometrial and uterine microbiome in pathophysiological processes is still unclear. The empowerment of metagenomics and the improvement of sequencing methods create the opportunity to find the answers to those questions. The microbiome of a normal uterine tract of healthy women is dominated by *Lactobacillus* species – *L. crispatus, L. gasseri, L. iners*, and/or *L. jensenii*. The major role of those bacteria is to support normal pH (3.8–4.5) by lactic acid production and inhibit the growth of pathogenic species.[30] Moreover, *Lactobacillus* prevents cervical cancer and the development of HPV-related diseases. The desired quantity of those species and their metabolites effectively inhibit the growth of cervical cancer cells, mainly by immunological mechanisms involved in the regulation of cancer-related genes. Additionally, *lactobacilli* representatives can be used as a tool to construct the HPV-related protein vaccine, which can also provide anti-cancer effects. *Lactobacillus* displays the signal peptide of S-layer on a cell membrane monolayer, which is easy to combine with exogenous target proteins. This determines those bacteria as the desired vector for recombinant protein vaccine and potential candidates as gene therapy and target therapies vector.[31] As a natural barrier to defense pathological uterus species, *lactobacilli* were found to inhibit the growth of pathological vaginal species (eg. *Candida albicans*), exhibited probiotic and anti-cancer properties, induce apoptosis and inhibit proliferation of cancer cells, and be involved in cell immunological anti–inflammatory responses.[32-34]

Based on 16 S rDNA sequencing of endometrial cancer samples, a low cell count of *Lactobacillus spp*. was detected,

which also correlated with an increased vaginal pH (>4,5). Moreover, the results suggested that the detection of *Atopobium vaginae* and *Porhyromonas* sp. (90% match to *P. somerae*, a pathogen present in bone infections) in the gynecologic tract is statistically associated with EC. At the same time, the *in situ* hybridization method showed the presence of *Lactobacillus* spp., *Gardnerella vaginalis, Enterobacter* sp., *Streptococcus agalactiae, E. coli*, and *Enterococcus faecalis* in women group with uterine pathologies. In the case of CIN (cervical intraepithelial neoplasia) *L. crispatus, L. iners, A. vaginae, G. vaginalis*, and *Fusobacterium* were dominating.[35-37] Moreover, differences between microbial profiles of healthy women group vs. women with endometrial polyps and chronic endometritis were reported. The latter ones were characterized by increased abundance of *Lactobacillus, Gardnerella, Bifidobacterium, Streptococcus, Alteromonas*, and *Prevotella*.[38] The most common pathological bacterial species – *A. vaginae* and *G. vaginalis*, are also associated with a risk of preterm labor, which underlines the importance of those species in pathological conditions.[39,40] The proportion of those bacterial strains in BV-positive vaginal smear slides was 54,1% and 82,0%, respectively, suggesting the synergistic role of bacteria in a biofilm formation. Data published by Brooks et al.[41] presented the significant reduction of BV-associated bacteria in a group of women using oral hormonal contraception. Additionally, women using that kind of contraceptives were more likely to be colonized by *Lactobacillus* species compared with women using condoms. This can mean the impact of the sex hormone synthesis process on women's microbiome balance.[42] EC is associated with aberrant estrogen synthesis, which overproduction causes mitotic activity in endometrial cells, thus increasing the risk of malignant transformation as well.[43] This information suggests the potential role of the endometrial and vaginal microbiome in endometrial cancer initiation and progression. Furthermore, *G. vaginalis* mentioned previously as a marker of BV produces cholesterol-dependent cytolysin (CDC) called vaginolysin (VLY), the representative of pore-forming toxin (PFT) group.[44] CDCs were identified in 23 taxonomically related species of gram-positive representatives. Structurally these toxins are antigenically related proteins, of about 50–60 kDa. Some of them, eg. listeriolysin O, pneumolysin, perfiringolysin, and pyolysin, are the major virulence factors of their producers.[45]

VLY activates p38 MAPK (mitogen-activated protein kinases), which suggests its important role in some cancer types (prostate, breast, bladder, liver, lung cancers). In addition, VLY induces pro-inflammatory signaling, by IL-8 production in human epithelial cells.[44,46,47] Bacterial VLY, like most of CDCs, uses cholesterol from the cholesterol-rich membrane for integration and recognizes specifically 3-β-hydroxy group by threonine/leucine pair of cholesterol recognition motif, located in the 1st loop.[48] Results of Gelber et al.[44] showed that VLY binds indirectly to cholesterol, involving hCD59 protein as the receptor. Then VLY recruits membrane complement regulatory proteins (mCRPs) to initiate the pore formation in a cell membrane. Cholesterol supports and stabilizes toxin-receptor binding. As mentioned, CD59 is overexpressed in many types of tumors, including breast cancer.[49] Abdelmaksoud et al.[50] observed a dose-dependent reduction of *G. vaginalis* and normalizing the level of *Lactobacillus* spp. in vaginal flora after treatment with statins, known as lipid-decreasing mediators. Statins affect plasma membrane cholesterol content, preventing pore formation by bacteria. Moreover, BV–isolated *G. vaginalis* strains demonstrated high adherence activity during cultivation *in vitro* with HeLa and ME-180 vaginal epithelium cells. As the co-culture method showed, under these conditions BV-associated bacteria were able to disrupt the protective layer of *lactobacilli*, causing the cytotoxic effect.[51,52] Patterson et al.[52] also reported the ability of *G. vaginalis* and *A. vaginae* BV–isolates to adhere to the ME-180 cell line. Quantitative estimation was ascertained by confocal microscopy. The adherence score was about 75% and 25% for mentioned strains respectively. At the same time, *A. vaginae* did not show the cytotoxic effect against this type of cells, when *G. vaginalis* strains were strongly affecting cell viability after 24-hour incubation. Adherence activity was presented on the human 3-dimensional endometrial epithelial cell model (EEC) as well. Construct of 3D EEC was based on the HEC-1A human endometrial carcinoma cell line, which additionally suggests the potential contribution of presented bacterial species with endometrial cancer. In this case, *G. vaginalis* was able to form clusters of bacterial cells attached to the surface of the cell aggregates, as it was clearly demonstrated by SEM (scanning electron micrographs) images.[53]

Generally, bacterial toxins are known to be involved in cancer initiation and progression. Pro-tumorigenic toxin activity is typical for some types of *Escherichia coli* species. So-called *E. coli pks*$^{+}$, harboring the *pks* genomic island was found to be highly enriched in colon cancer patients. The *pks* encoded colibactin toxin attaches to colon epithelial cells, causing inflammation and host-cell DNA damage such as double-strand breaks, chromosomal aberrations, and G2/M cell cycle arrest.[54-56] Another example of the virulence effect caused by *E. coli* colonization is the cytotoxic necrotizing factor 1 (CNF1) toxin. This toxin binds the epithelial cells and leads to increased cell proliferation, inflammation, and host DNA damage. CNF1 activates the Rho GTPase family proteins, involved in the actin cytoskeleton organization. In the first stage, NF-κB is activated and then CNF1 increases the level of anti-apoptotic proteins, immortalizing the damaged cell, which potentially leads to cancer development.[57,58]

Bacterial dysbiosis can also induce endometriosis – estrogen-dependent inflammatory disease, characterized by the ectopic growth of endometrial glands stroma outside the uterine cavity. Endometriosis is associated with pH changes and the reduction of the abundance of *Lactobacillus* spp. as well as the increase of the number of specific gram-negative and facultatively anaerobic bacterial species, particularly *A. vaginae* and *G. vaginalis*. Moreover, the disease is related to high estrogen receptor (ER)-positive EC risk. This type of cancer is characterized by increased estrogen production and COX-2 overexpression. In this case, COX-2 up-regulation was caused by pro-inflammatory mediators (IL-1β and TNF-α) activation of HIF-1α and NF-κB pathways.[59-61] Evaluation of intra-uterine microbial colonization revealed an increased number of pathogens, such as *Gardnerella, α-Streptococci, Enterococci*, and *E. coli* in women with endometriosis, in comparison with healthy individuals. A similar shift was also observed in GnRHa (gonadotropin-releasing hormone

agonist) treated women, regardless of whether they had endometriosis or not.[62] Besides, *Gardnerella* is one of the main causes of chronic endometritis, which characterizes by the persistent inflammatory state, and found to be a risk factor of endometriosis occurrence.[63,64]

Bacterial infections could be also involved in the regulation of host cell epigenetic mechanisms, affecting chromatin structure and transcriptional programming. In recent years, the involvement of bacterial pathogens and their toxins in acetylation, methylation, and mimicking host's chromatin-regulatory factors was found. For example, *Mycobacterium tuberculosis, H. pylori, Listeria monocytogenes*, and their metabolites are manipulating the expression of histone acetyltransferases (HATs) and histone deacetylases (HDACs), leading to suppression of transcription of host defense genes through epigenetic changes in histone acetylation marks.[65] Uropathogenic *E. coli* affects DNA methylation and down-regulates the *CDKN2A* gene expression, which plays a key role in coding tumor suppressor proteins.[66] *Bacillus anthracis* is known as MAPK kinase inhibitor and *IL-8* gene down-regulator.[67,68] Moreover, the permanent exposition to bacterial toxins could provide tolerance, repressing *TNF-α* and *IL1-β* gene expression. This phenomenon relies on a change of composition of NF-κB transcription factor in the proximal promoters of the mentioned genes.[69] Interestingly, bacterial toxins and metabolites found to be affecting host epigenetics in a specific manner. As was observed by Draisma et al.[70] in clinical trials based on the administration of LPS, tolerance was demonstrated by the attenuated release of pro-inflammatory cytokines, including tumor necrosis factor (TNF-α), but not transforming growth factor β (TGF-β). This could lead to undergo cell proliferation without an efficient immunological response.

Potentially, a disorder of epigenetic regulation in EC may be tied to bacterial pathogens' presence. For example, the down-regulation of a tumor suppressor gene encoding human mismatch repair hMLH1 protein was found in sporadic endometrial carcinoma but also many other types of cancer, including ovarian tumors, sporadic colorectal cancer, acute myeloid leukemia, and others. It is suggested that this process may be induced by the presence of *Yersinia enterocolitica* and *H. pylori* which leads to the hypermethylation of the hMLH1 promoter region. Similar hypermethylation in promoter regions of 24 tumor suppressor genes was found in endometrioid carcinomas, however, in this case, microsatellite instability in methylated regions was so far suggested as the main reason for epigenetic changes.[71-73]

Bacterial infection, causing chronic inflammation, attributes up to 10–20% of cancers. However, the initiation of the inflammatory state by the presence of bacteria seems to be not a limited aspect and should be examined more thoroughly.[54]

NSAIDs as antimicrobial agents

The antibacterial property of some NSAIDs was recently discovered. The reason is the increased resistance to the commonly used antimicrobial drugs. Since 90′, diclofenac sodium has been studied as a *Salmonella* Typhimurium growth inhibitor. Moreover, diclofenac, aspirin, and etodolac can prevent biofilm formation.[74] Chan et al.[75] tested the effects of drug treatment on gram-positive and gram-negative strains. The wide-spectrum activity of aspirin was noted. Minimal inhibitory concentrations (MICs) were 2,5 mg/mL and 5 mg/mL, respectively. At the same time, ibuprofen was found to be a powerful agent against gram-negative bacterial species (MIC 0,625–2,5 mg/mL). These concentrations are much lower than normal therapeutic doses used during anti–inflammatory therapy. Similarly, a prevalence rate of *H. pylori*, the gastric cancer inducer, was decreased in specimens collected from indomethacin and ibuprofen users.[76] Sodium salicylate (aspirin salt) treatment reduces the capsular polysaccharide formation of *Klebsiella pneumoniae* hypermucoviscosity phenotype (HV-KP). It is particularly interesting, because capsular polysaccharide plays a protective role, helping bacteria to avoid phagocytosis. Besides, it has been identified, that capsular polysaccharide, as a determinant of *K. pneumoniae* infection, is ominous for patients with type 2 diabetes mellitus.[77]

The mechanisms of a preventive antimicrobial activity of NSAIDs are unclear. The study of Yin et al.[78] presented the ability of these drugs to bind *E. coli* DNA polymerase III β subunit, also known as a sliding champ (SC). The compounds used during studies were mainly vedaprofen, carprofen, and bromfenac, which are routinely used in veterinary protocols. NSAIDs are blocking bacterial protein-protein interactions, DNA replication, and repair. Studies of Cai et al.[79] described the impact of PGE2 on enhanced biofilm formation by methicillin-resistant *Staphylococcus aureus* (MRSA). Bacterial biofilm formation by those strains can cause toxic shock syndrome, sepsis, pneumonia, endocarditis, and impetigo. MRSA-positive patients are particularly difficult to treat. PGE2 found to be attenuating the bactericidal effects of kanamycin or ampicillin, commonly used against *S. aureus* infections. Potentially, PGE2 causes drug resistance, by enhancing efflux pump activity, which is why biofilm formation further continues. Celecoxib, aspirin or naproxen inhibited the PGE2 activity in combined therapy, decreasing bacterial growth ratio.

An important aspect from the vaginal and endometrial microbiome perspective is the selectiveness of some NSAIDs against gram-negative bacteria. Milani and Iacobelli[80] reported the effect of ibuprofen and ibuprofen isobutanolammonium (Ib-isob, Ginenorm) to interfere with *G. vaginalis* adhesion. In addition, antifungal effect against *Candida albicans*, vulvovaginal candidiasis (VVC) etiological agent, was noticed. Combinatorial treatment with econazole stopped the germ-tube formation, preventing penetration into mucous cells.

NSAIDs and their potential role in endometrial cancer prevention and therapy

NSAIDs, COX-2 selective inhibitors particularly, seem to be promising anticancer agents, however, the reports regarding their effectiveness seem to be contradictory. The regular administration of aspirin led to about 20% lower risk of breast cancer occurrence in a tested women group. In other studies, more than two years of the administration of celecoxib or rofecoxib, used in chronic inflammatory states, remarkably

reduced risk of cancer development: 71% breast, 70% colon, 55% prostate and 79% lung cancer.[81,82] However, there are also reports indicating opposite effects, e.g. administration of ibuprofen in HER2- breast cancer group, increased risk of cancer development in about 27%.[83] Brasky et al.[84] reported aspirin and non-aspirin NSAIDs (ibuprofen, naproxen, indomethacin, piroxicam, and sulindac) association with the extent of endometrial carcinoma-specific mortality within I-staged patients. Nevertheless, the reported results were not validated, the dose and frequency of NSAID use were not precise, which means that analysis has some limitations to consider.

Positive NSAID therapy effects in the case of some EC types were mentioned. Nevadunsky et al.[85] presented the positive effect of aspirin and statins in combined therapy of non-endometrioid endometrial cancer group, manifesting in improved low disease-specific mortality. Research of Matsuo et al.[86] suggested the association of low-dose aspirin consumption with good survival outcomes in endometrial cancer women group. Therapy was efficient for women younger than 60 years old, with body mass index (BMI) of 30 or greater, in the first stage of the disease and after postoperative radiotherapy. Generalizing, a meta-analysis of Verdooth et al.[87] reported about an 11% reduction of risk of endometrial cancer development in case-control studies after regular aspirin use. For cohort studies, about 8% risk of EC development reduction was observed. For non-aspirin drugs, this percentage was smaller – about 9% and 6% respectively. Additionally, body mass index analysis was assessed. BMI higher than 30 was found to be an adverse factor, avoiding the positive preventing effect.

Induced dose-dependent growth repression of endometrial cancer cells was observed *in vitro*. Acetylsalicylic acid led to about 21% – 88% growth inhibition of Ishikawa cells, apoptosis induction, and reduced Bcl-2 expression in a dose-dependent manner.[88,89] Indomethacin affected the HEC-1-B cancer cell line by up-regulation of PTEN tumor suppressor.[90] Celecoxib, a selective COX-2 inhibitor, reduced HEC-1-A and HEC-1-B cell lines growth, assisting the inhibition of tumor cell proliferation.[91,92]

The mechanism responsible for NSAIDs' tumor preventive effect is characterized by multiple responses of cancer cells signaling pathways. Chemopreventive properties of these drugs could act through the combined action of various cell proteins at the molecular, as well, as epigenetic and post-transcriptional levels. Some of NSAIDs (aspirin, indomethacin) provide the enhanced expression of *NAG-1* (non-steroidal anti–inflammatory drug-activated gene-1), which encodes the member of anti-tumorigenic and pro-apoptotic TGF-β protein superfamily.[93] Baek et al.[94] presented the effect of increased NAG-1 production in the human colorectal cell line (HCT-116) in response to NSAIDs treatment. The consequence was the apoptosis initiation, which was induced in concentration and time-dependent manner.

Arousing interests around NSAIDs as chemopreventive agents persuade to create new derivatives of these compounds. Non-steroidal anti–inflammatory hybrid nitrate drugs (NO-NSAIDs) are conjugates of NO-donating moiety and well-known NSAIDs, which enhances the activity of such compounds. NO-NSAIDs, NO-ASA (nitroaspirin) particularly, inhibit cellular β-actin /TCF signaling pathway, NOS2 expression and binding of NF-κB transcription factor to DNA regulatory sequences.[95,96] Moreover, NO-derivative suppresses the microsatellite instability (MSI) of nonpolyposis colorectal cancer (HNPCC). The effective NO-ASA dose was from 300- to 3,000-fold lower than ASA.[97] NO-indomethacin was reported to affect invasive and noninvasive adenocarcinomas in azoxymethane treated rat model. Moreover, drug use did not create a threat of gastrointestinal ulcers or other side effects.[98]

Other derivatives were designed to be COX–independent targets and to involve other mechanisms of anti–inflammatory response. One of those is cyclic guanosine monophosphate phosphodiesterase, or cGMP PDE, which is responsible for negative cGMP signaling regulation. The example is sulindac, which found its place in colon cancer prevention and treatment.[99] Pro-NSAIDs drugs, such as acetylcholinesterase (AChE) inhibitors, phospho-NSAIDs, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), 4-hydroxy-TEMPO (TEMPOL) and hydrogen sulfide (HS) NSAIDs, represent an effective approach in cancer prevention and treatment supporting, but none of them were examined from the endometrial cancer perspective.[100]

Conclusions

Nowadays, endometrial cancer is one of the most common cancer types in women's population. Molecular bases of the disease are depending on various factors, women's lifecycle, and lifestyle. Bacterial features and balanced microbiome are relatively new factors to consider from the perspective of cancer initiation, prevention and the success of treatment. The importance of normal bacterial uterine/endometrial flora was proved in relation to bacterial vaginosis, endometriosis, and vaginitis. However, bacterial endometrial flora as a cancer-promoting agent is still one of the most omitted and poorly investigated items. As was noted, some bacteria are well known for their tumorigenic effect, which should be also examined thoroughly in endometrial cancer cases.

G. vaginalis and *A. vaginae* presence in the endometrial samples seems to be a new factor to estimate cancer initiation risk. The overgrowth of those strains potentially leads to cancer development, by inducing COX-2 overexpression, which is common in EC cases. The COX-2 enzyme is involved in various cell signaling pathways, proliferation, angiogenesis, and apoptosis – the crucial mechanisms in a normal life cycle. The reason for *G. vaginalis* participation in cancer initiation and/or progression could be a bacterial toxin, so-called vaginolysin (VLY). The presence of VLY can serve as a pro-inflammatory factor, increasing the COX-2 expression. Moreover, *G. vaginalis* has a high pore-forming activity to epithelium cells. Bacterial toxins and metabolites may also be engaged in host epigenetic chromatin remodeling, down- or up-regulating of crucial genes products, which are involved in normal cell cycle and tumor suppression. Current approaches to study that coexistence have some limitations but open wide prospects in the understanding of endometrial cancer development and research of new ways of treatment.

Meanwhile, the cancer-preventive and antimicrobial effects of some NSAID derivatives were examined. Regular consumption of well-known drugs, mainly aspirin, contributes to cancer risk reduction. Moreover, some of them are

demonstrating to be gram-negative antibacterial agents, which is an important aspect from the perspective of the endometrial and vaginal microbiome. Inhibition of bacterial growth, as well as cancer cells, was shown to be dose-dependent. Nevertheless, mechanisms of those interactions are unclear, illustrating the ground for further research.

NSAIDs seem to be a perfect double-edged tool in antimicrobial and antitumor assistance. For enhancing those properties, NSAIDs were designed in conjunction with other molecules. NO-NSAIDs and cGMP PDE were reported to have positive effects on the gastrointestinal tract. AChE inhibitors, phospho-NSAIDs, TEMPO, TEMPOL, and HS NSAIDs were found to be beneficial as cancer preventative agents. However, none of them were examined on EC cell lines or uterine/endometrial microbiome representatives.

Described reports suggest the potential role of bacteria in EC. NSAIDs could be a powerful therapy tool from both – bacterial and cancer cell perspectives. These drugs can be also useful in case of other pro-inflammatory diseases, which can be initiated by bacterial infections. This aim can lead to the manifestation of new therapeutic strategies, new NSAIDs modifications and have big potential for chemical and biological research study.

Disclosure of Interest

The authors have no conflicts of interest to declare.

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