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The importance of epigenome research in the diagnosis and treatment of endometriosis

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ABSTRACT

The causes of endometriosis remain unexplained. Studying the molecular mechanisms at the origin of the lesions leads to conclusions about the important role of the epigenome. This mini-review is a summary of the current state of knowledge about the processes of epigenetic control of gene expression involved in the pathogenesis of endometriosis.

Keywords: endometriosis, gene expression, methylation.

Endometriosis, defined as the presence of endometrial cells outside the uterine cavity (ectopic endometrium), affects up to 10% of women of reproductive age and is one of the most common causes of infertility, and gynecological hospital ward admission and one of the main causes of hysterectomy [1]. In endometriosis, the ectopic endometrium occurring intraperitoneally and extraperitoneally is sensitive to hormonal changes characteristic of the sexual cycle and maintains its secretory activity, resulting in a chronic inflammatory reaction. This process leads to internal bleeding, the development of painful nodules, inflammation, scar formation and adhesions and anatomical changes in the pelvic [2]. Endometriosis is most frequently found in women suffering from pelvic pain and/or infertility. Due to its non-specific symptoms, early diagnosis of endometriosis is difficult, and often incidental. Laparoscopic examination is standard in the diagnosis and treatment of endometriosis [3]. The etiopathogenesis of endometriosis is also not fully explained; however amongresearchers, the most popular hypothesis concerns retrograde transport of menstrual blood through the fallopian tubes and into the peritoneal cavity [4]. However, hereditary, environmental, autoimmune, allergic and epigenetic factors are a key influence in the implantation of endometrial cells, and the ectopic formation, metastasis and recurrence of the disease [5]. Among the mentioned factors, epigenetic ones are the subject of intense research aimed at elucidating the etiopathogenesis of the disease.

Epigenetics can be defined as a branch of science investigating inherited traits introduced into the genome through methylation of the nucleotides in the DNA sequence, histone modification, and interaction between microRNA (miRNA). Such modifications alter the expression of genes without interfering in the nucleotide sequences in the DNA strand. Characteristics included within the study of epigenome affected prenatal development, hormonal factors, age, gender, and environmental factors such as diet, exposure to chemical and physical factors. The etiology of many diseases correlates with specific patterns of the epigenome, which are manifested in disorders of gene expression, and thus in impaired function of cells and tissues [6, 7].

The most widely investigated element of epigenetic gene modifications is methylation of the cytosine residues of cytosine-guanine dinucleotides (CpG). CpG

dinucleotide present throughout the genome once per 80 base pairs. However, in some areas of the genome specifically related to gene promoter sequences, CpG appear sequentially in lengths of approx. 200 base pairs. These places are called CpG islands. The entire genome outside the CpG island cytosines in dinucleotides is constitutively methylated. However, CpG islands located in the promoter site of a gene are generally not the targets of methylation. In this way, they are accessible to transcription factors, initiating the process of gene expression [9].

Advances in our understanding of the methylation patterns of the genes in endometriosis occurred after the publication of the results of two projects focusing on a genome – wide DNA methylation analysis. Working independently of each other and using slightly different strategies, these authors reached similar results. Their results are consistent in terms of different methylations of promoter regions of the 21 genes in the ectopic endometrium compared to the eutopic endometrium[10, 11]. Both studies confirmed observed also in our studies, gene promoter hypermethylation of *HOXA10* and *HOXA11*, and as a result – reduced expression of these genes in the ectopic or eutopic endometrium [12]. Among genes with different methylations of CpG islands in the ectopic endometrium, which appears to be unquestionably associated with endometriosis, are genes encoding steroid hormone receptors: i.e. the *NR5A1* gene encoding transcription factor SF-1, which is responsible for the expression of genes encoding enzymes of steroidogenesis pathway; the *CYP-19* gene encoding the aromatase; and *COX-2* encoding cyclooxygenase-2, a key enzyme in the conversion of arachidonic acid to prostaglandins and one which triggers an inflammatory response.

The process of CpG methylation involves DNA methyltransferase (DNMT), while demethylation occurs with the participation of other enzymes such as TETs, AID and GAAD45. Also, the expression of these enzymes appears to have a different effect on the DNA methylation pattern in the ectopic endometrium [13].

Another mode of epigenetic gene regulation involved in the etiopathogenesis of the endometriosis is histone code post-translational modification. Histone proteins are responsible for chromatin organization. There are 130 known variants of the post-translational modification of histone proteins, of which the most important seem to be: trimethylation of the lysine 4 of histone H3 (H3K4-ME3); and acetylation of histones H3 and H4 (H3 / H4Ac). Both of these changes lead to a loosening of the chromatin structure and consequently, to the initiation of transcription. On the other hand, methylation of the lysine 27 on histone H3 leads to chromatin condensation and silencing of the gene expression associated with modified histones [14, 15]. Compared to the eutopic endometrium, which is dependent on histone deacetylase (HDAC), research into post-translational modification of the histone code in the ectopic endometrium indicates a lower level of the acetylation of histones H3 / H4 covered with promoter regions of *p16, p21, Bcl2, BclX* genes critical for apoptosis [16]. While the results of other studies into the expression and activity of HDAC compounds with endometriosis are still contradictory, the existence of differences in the histone code seems to be of indisputable importance in the etiology of endometriosis [17].

Further important regulatory molecules able to modify gene expression are miRNAs. They are single-stranded, non-coding, short (21–23 nucleotide) RNA sequences. By pairing with a homologous sequence of the mRNA, microRNAs can silence geneits expression [18]. Specific miRNA also plays a role in transcriptional gene silencing by inducing structural changes in a complementary locus of the chromatin [19]. A detailed review of published results of research into miRNA expression in endometriosis was published by Borghese *et al*. (2016) [20]. Similarly to the previously discussed mechanisms, miRNA acts in a multi-dimensional manner, also by modification of the expression of genes responsible for other epigenetic mechanisms: DNA methylation and histone modification [21, 22].

The mechanisms for the epigenetic control of gene expression associated with endometriosis presented in this review require further research to answer many questions. There is still no clear answer as to whether the many instances of methylation observed in the ectopic endometrium are an effect or a cause of endometriosis. Another problem is the patchy research methodology, which often precludes a comparison of any obtained results. Despite these difficulties, the development of our understanding of the functioning of the epigenome allows us to be optimistic about the prospects for the diagnosis and treatment of endometriosis. While the repair of the genetic code is still a matter for the distant future, epigenome modifications are still possible. There have been successful results from therapy with DNMT inhibitors [23]. Promising results have also been provided by HADAC inhibitors [24]. However, the greatest hope is offered by the therapeutic use of miRNAs [25]. The obtained results probably can be used to develop and introduce new patterns of diagnosis and treatment for endometriosis.

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