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The role of estradiol-induced microRNAs in the pathogenesis of endometrial cancer

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Abstract

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Endometrial cancer is the most common cancer of the female reproductive tract and the fourth most common cancer in women. It is diagnosed mainly in postmenopausal patients. Due to the increasing prevalence of obesity in the population, this cancer is increasingly being diagnosed. Risk factors include metabolic syndrome, menstrual disorders and unbalanced estrogen therapy. Historically, two types of endometrial cancer were distinguished - type I endometrioid adenocarcinoma (estrogen-dependent) and type II (non-endometrioid adenocarcinoma, most often serous, independent of hiperestrogenism), which is characterized by a worse prognosis. Currently, molecular tests play an important role in diagnostics, the results of which are included in the latest FIGO 2023 classification of endometrial cancer.

MicroRNAs are short non-coding RNA molecules whose mechanism of action is the post-transcriptional regulation of gene expression by silencing translation or degradation of mRNA. By affecting their targets, they participate in the pathogenesis of many diseases, including cancers. In cancer cells, they can act as tumor supressors or oncomiRs. MicroRNA expression is regulated by multiple factors. Previous studies have shown the influence of estrogens on microRNA expression and neoplastic transformation in breast cancer. The aim of the presented doctoral thesis, consisting of a series of three review articles and two original papers, was to investigate the role of estradiol-induced microRNAs in the pathogenesis of endometrial cancer.

The first article in the series is a review article entitled *"Regulators at Every Step-How microRNAs Drive Tumor Cell Invasiveness and Metastasis"*, published in *Cancers*. The article discusses in detail the mechanism of action and the role of microRNA molecules as inhibitors or promoters of each stage of cancer progression. It describes the influence of microRNAs on cancer cell migration, local invasion, epithelial-mesenchymal transition, angiogenesis, and distant metastasis. The tables summarize and organize the existing data on the role of individual microRNA molecules and their target mRNAs in the above-mentioned processes. In addition, the potential use of microRNAs as cancer biomarkers and therapeutic agents is discussed.

The second article in the series is a systematic review entitled *"The Role of miRNAs in the Regulation of Endometrial Cancer Invasiveness and Metastasis-A Systematic Review"*,

which was published in *Cancers*. The manuscript, prepared according to PRISMA guidelines, analyses 163 articles that met the inclusion criteria. The paper collected data on the expression of 106 microRNA molecules and their role in the regulation of endometrial cancer cell invasiveness and migration *in vitro*, as well as tumor growth and metastasis in *in vivo* studies. Of these, 63 microRNA molecules acted as inhibitors, and 38 promoted the development of endometrial cancer. Data on the mechanisms of action of microRNAs in endometrial cancer cells, their targets and their effect on various signaling pathways were also organized. Moreover, the study summarized the correlation of the expression of individual microRNAs with clinical parameters of patients. This study showed the complexity of the processes in which microRNAs are involved in the pathogenesis of endometrial cancer.

A special group of microRNA molecules is the microRNA-200 family, which includes microRNA-200a, microRNA-200b, microRNA-200c, microRNA-141, and microRNA-429. They regulate all hallmarks of cancer described by Hanahan and Weinberg, as discussed in the third publication entitled *"The role of miR-200 family in the regulation of hallmarks of cancer"*. The microRNA-200 family can act as oncomiRs and as inhibitors of proliferation, tumor growth, migration, invasion, and angiogenesis in various types of cancer, including endometrial cancer.

The literature review was the basis for the original research, whose results were collected in two manuscripts. The article entitled *"Decreased expression of miR-23b is associated with poor survival of endometrial cancer patients"* was published in *Scientific Reports* journal. The aim of this study was to evaluate the expression level of a panel of 16 miRNAs in both types of endometrial cancer and healthy endometrium. A total of 45 patients participated in the study: 18 patients with type 1 endometrial cancer, 12 patients with type 2 endometrial cancer, and 15 patients with healthy edometrium as controls. Using the laser microdissection method (LCM), tumor tissues and healthy endometrial tissues were dissected from FFPE slides. RNA was isolated from the tissues, and the expression of selected miRNAs was determined using real-time qPCR. The expression levels of miR-23b, miR-125b-5p, miR-199a-3p, miR-221-3p, and miR-451a were decreased in endometrial cancer compared to healthy endometrium. The expression of miR-34a-5p and miR-146-5p was increased in type I endometrial cancer compared to type II endometrial cancer. Analysis of The Cancer Genome Atlas (TCGA) database confirmed the decreased

expression levels of miR-23b, miR-125b-5p, and miR-199a-3p in EC. Reduced expression of miR-23b was associated with poorer survival of endometrial cancer patients.

The article *"Estradiol induces global changes in miRNA expression in endometrial cancer cells and upregulates oncogenic miR-182"* has been published as a preprint in *bioRxiv*. The aim of this study was to evaluate the role of the miRNA-estrogen axis in endometrial cancer cells. The study used the Ishikawa endometrial cancer cell line, which is estrogen-dependent. Cells were incubated with estradiol and then RNA was isolated. A microarray method was used to identify estradiol-induced miRNAs in endometrial cancer cells. Then, tissues from 45 patients (18 patients with type I endometrial cancer, 12 patients with type II endometrial cancer, and 15 patients with healthy edometrium) were cut using LCM. The expression of selected miRNAs and their targets was assessed using RT-qPCR. Ishikawa cells were transfected with miRNA-mimic, miRNA inhibitor (anti-miRNA), and corresponding controls. We identified 66 miRNAs whose expression was upregulated after incubation of endometrial cancer cells with estradiol. Among them, the expression level of miR-182 was increased in type I endometrial cancer tissues compared with healthy endometrium. Additionally, miR-182 acted as an oncomiR in endometrial cancer, as its increased expression promoted endometrial cancer cells proliferation, and decreased miR-182 expression was associated with inhibition of cancer cell proliferation. Inhibition of miR-182 increased the expression of SMAD4, an important member of the TGFβ signaling pathway, which may be a potential mechanism of action of this microRNA.

In summary, the results of the conducted studies indicate an important role of microRNAs in the pathogenesis of endometrial cancer, including estradiol-induced microRNAs. Therefore, microRNAs may become promising biomarkers of the disease. Potentially, microRNAs can be used for therapeutic purposes, but their clinical application requires further translational studies.