mgr farm. Alicja Sztokfisz-Ignasiak

Rola chromograniny A w patogenezie endometriozy

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki medyczne

Promotor: dr hab. n. med. Izabela R. Janiuk

Katedra i Zakład Histologii i Embriologii Warszawskiego Uniwersytetu Medycznego

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The role of chromogranin A in the pathogenesis of endometriosis

Endometriosis is a chronic, estrogen-dependent, inflammatory gynecological disorder associated with pelvic pain and infertility. It affects not only women of reproductive age, but also those post-menopause. Endometriosis is characterized by the presence of endometrial-like tissue outside its physiological location. Those ectopic locations include the ovaries and/or the pelvic viscera. This disorder affects ca. 10% of women worldwide. Among women suffering from infertility, this number increases to 50% of cases. The pathogenesis of endometriosis is still unclear. There are three classic theories, currently considered only as a "starting point" for more advanced research. Although they present probable pathways of endometrial cell migration to ectopic sites, they do not explain the method of their implantation, proliferation, and activation. Further research has been conducted worldwide for decades to explain these mechanisms. It is estimated that the diagnosis of endometriosis is delayed by 10 years. From 2022, the European Society of Human Reproduction and Embryology (ESHRE) recommends branching off from laparoscopy as "a gold standard" for the diagnosis of endometriosis on behalf of empirical pharmacological therapies and medical imaging tests.

Chromogranin A (CgA) is an acidic hydrophilic glycoprotein belonging to the granin family, which was initially identified in the chromaffin granules of the bovine adrenal medulla. It is a prohormone for several biologically active peptides, the most important and most widely studied of which are: vasostatin I (VS-I), vasostatin II (VS-II), chromofungin (CHR), chromacin, prochromacin, pancreastatin (PST), catestatin (CST), serpinin and WE-14. CgA is not only found in chromaffin cells, but it is also expressed in keratinocytes, cardiac muscle cells, endothelial cells, and pancreatic islet cells in many animal species. It has various intra- and extracellular functions. Along with hormones, transmitters, and amines it is involved in the sorting and formation of secretory granules or synaptic vesicles. It is one of the driving forces inducing membrane budding of the trans-Golgi network (TGN) to form secretory vesicles and participates in the fusion of secretory granules with the plasma membrane. CgA is a unique molecule because the peptides derived from its degradation exhibit antagonistic effects on maintaining homeostasis in the body. For instance, full-length CgA is a regulator of angiogenesis and a precursor of peptides that act proangiogenically (e.g. CST, VS-II) and anti-angiogenically (VS-I). In turn, glucose homeostasis is maintained by PST, an anti-insulin peptide and CST, which has pro-insulin activity. CgA is expressed in many endocrine and

neuroendocrine tumors and its elevated levels in the blood may also accompany non-cancerous conditions, e.g. organ failure, inflammation, autoimmune disease, or cardiovascular diseases.

So far, the connection between CgA and its derivatives and endometriosis has not been described.

The aims of the study included:

- immunohistochemical (IHC) identification of CgA and examination of the *CHGA* gene expression by using polymerase chain reaction with real-time analysis (RT-PCR) in endometrial lesions and eutopic endometrium collected from healthy patients,
- determination of the presence, concentration, and cross correlation of CgA and its degradation products: CST, PST and VS-II in serum and peritoneal fluid collected from both group of patients: 1) diagnosed with endometriosis and 2) healthy controls, using immunoenzymatic ELISA tests,
- an attempt to determine the potential mechanism responsible for CgA expression using cell culture studies.

The study group consisted of patients suffering from endometriosis, in which the disease was confirmed both laparoscopically and histopathologically. The control group consisted of patients hospitalized and operated on for reasons other than endometriosis in whom disease was not diagnosed.

In *in vitro* studies two cell lines (12Z and Ishikawa) were incubated with cytokines IL-1, IL-6, TNF and TGFβ at concentrations 10 ng/mL and 20 ng/mL for 24 hours. After that time, in cells collected from cell culture, the expression of *CHGA* (RT-PCR) was determined.

Immunohistochemical reactions showed groups of chromogranin-positive cells in fragments of endometrioid lesions, whereas in eutopic endometrium only single cells revealed CgA expression. However, no increased mRNA expression of the *CHGA* gene was observed in endometrial cysts compared to eutopic endometrium collected from the control group of women. Higher concentrations of CgA, CST and PST in serum and peritoneal fluid were detected in patients with endometriosis compared to the controls. In the above-mentioned fluids, no statistically significant differences in VS-II levels were observed. Cell culture studies showed higher mRNA expression for the *CHGA* gene in 12Z cells after IL-1 stimulation and in

Ishikawa cells after TNF stimulation. On the other hand, reduced expression of this gene was observed in the Ishikawa cell line after TGFβ stimulation.

In conclusion, based on the obtained results, it can be assumed that CgA and its derivatives – CST and PST play a crucial role in the pathogenesis of endometriosis and may become biomarkers in minimally invasive diagnostics of this disease, and furthermore, targeting the CgA pathway might constitute a promising, novel therapeutic approach to endometriosis.