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Metallothionein and RCAS1 expression in comparison to immunological cells activity in endometriosis, endometrial adenocarcinoma and endometrium according to menstrual cycle changes

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Abstract

Objective. Endometrium is a specialized organ in which phenomena controlling the level of cell proliferation and apoptosis are marked. The aim of our study was to determine the presence of proteins involved in apoptosis and proliferation: RCAS1, MT and the number of CD56-positive cells and their activity to elucidate their possible role in the development of adenocarcinoma and endometriosis.

Materials and methods. MT, RCAS1, CD56-positivity and CD69 expression were assessed in 55 tissue samples by Western blot and immunohistochemistry methods.

Results. We found that endometrium during secretory menstrual cycle phase is characterized by significantly higher RCAS1 and higher MT expression than in proliferative phase. The number of CD56-positive cells and the CD69 antigen expression was significantly increased. Endometrial adenocarcinoma was characterized by significantly increased RCAS1 expression, while MT expression was comparable to the level found in the secretory phase. The number of CD56-positive cells was significantly decreased and their activity was comparable to the level found in the secretory phase. Endometriosis was accompanied by significantly lower RCAS1 and MT expressions, with lower number of CD-56 positive cells and lower expression of CD69 antigen in comparison to the secretory phase.

Conclusions. The ability of endometrium to determine cytotoxic activity (RCAS1 expression changes) and high protection against DNA damage (MT expression) with concomitant changes in the number of immune cells and their activity, observed in normal endometrium during the menstrual cycle phases seems to be fundamental for pathological features of endometrial adenocarcinoma and endometriosis.

Keywords: RCAS1; Metallothionein; Endometriosis; Endometrial adenocarcinoma; NK cells

Introduction

Endometrium is not just a simple tissue, but a complex organ which proper function enables the realization of a fundamental aim—reproduction [1]. The phenomena controlling the number of cells – proliferation and apoptosis – are marked in endometrium. Secretory phase of the menstrual cycle is characterized by the changes enabling the implantation of a blastocyst. However, growing pregnancy does not conclude decidualization every cycle and the menstrual bleeding occurs, beginning a new cycle. Thus, decidualization is continued in early pregnancy and is basic for the development of immune tolerance during pregnancy, which is stopped with the beginning of the labor [2]. These changes are unique and enabled by the coexistence of lymphoid tissue and endometrial cells. The alterations observed during the whole normal menstrual cycle concern

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both morphological changes of epithelium and uterine glands, and lymphoid tissue [3–5].

Dynamic changes observed physiologically in endometrium seem to determine the pathology. The presence of lymphoid tissue within endometrium is linked to the ability of the regulation of cytotoxic response. Changes of regulation of cytotoxic response influenced by hormonal alterations indicate the proliferative potential and resistance to apoptosis of endometrial cells [6]. Endometrial adenocarcinoma and endometriosis are basic endometrium-derived pathologies. Endometriosis is regarded as a dissemination of normal endometrial cells. The theory of endometriosis which is based on retrograde menstruation phenomenon was described by Samson in 1925 [7]. A retrograde menstruation is observed in 90% of laparoscopic procedures performed during menstruation.

Avoiding of immune recognition by various human cancer cells was until now seen as main RCAS1 function [8–13]. RCAS1 expression was also detected in placenta, endometrium, lymphoid tissue, bone marrow and glandular epithelium which might suggest that it is not only the marker of malignant disease but could also be involved in the maturation of lymphocytes [10,14–17]. RCAS1 induces apoptosis not only in activated T cells but was also shown to participate in the maturation of erythroid progenitor cells [16]. Monocytes and macrophages were demonstrated to express RCAS1 and were able to induce apoptosis of activated T lymphocytes in histiocytic necrotizing lymphadenitis, characterized by proliferating macrophages and T lymphocytes undergoing apoptosis in necrotic lesions [17]. The presence of RCAS1 expression in Waldeyer's ring lymphoid tissue was also demonstrated [18].

Metallothionein (MT) is a metal-binding low molecular weight protein with functional roles in cell growth, repair and differentiation [19]. MT immunoreactivity was prominent in cancer tissue, presenting a nuclear pattern of staining being inversely correlated with apoptotic index [20]. It is also known that perinuclear MT localization is important for protective function of MT against DNA damage and apoptosis induced by external stress stimuli [21,22]. According to this study, it was suggested that MT overexpression might protect the tumor cells from entering the apoptotic process and thereby to contribute to tumor expansion. MT expression was increased in endometrial carcinoma cases compared with benign hyperplastic endometrial lesions. In endometrial adenocarcinoma, the elevated MT content was associated with high cancer grade and stage [23].

The aim of our study was to evaluate the ability of endometrium to regulate the cytotoxic activity and high protection of DNA damage with concomitant changes in the number of immune cells and their activity in normal endometrium during the menstrual cycle phases, endometrial adenocarcinoma and endometriosis by the analysis of the expression of RCAS1, metallothionein and the estimation of infiltrating cytotoxic cells (by the number of CD56-positive cells) and their activity (the expression of CD69 antigen).

Material and methods

The subjects

Informed consent for the use of endometrial tissue was obtained from all patients. The approval from the Ethical Committee of the Jagiellonian University in Krakow (KBET/379/13/2003) for this research program was also granted. We assessed the expression of RCAS1, MT protein in tissue specimens. We also determined the number of CD56-positive cells and their activity by the expression of CD69 antigen. Above described factors were established in 55 tissue samples as follows: in 13 endometrial adenocarcinomas, 12 ovarian endometriosis and 30 healthy endometrium. The patients in our study were randomly selected.

Eutopic endometrial tissue

Eutopic human endometrium tissues were obtained from non-menopausal fertile women, aged 25–45 years. These patients underwent hysterectomy because of benign gynecological indication (leiomyomas) or laparoscopic or hysteroscopic diagnostic procedures. All the patients did not receive any hormonal treatment. The surgical procedure was performed in Gynecology and Infertility Clinic of Jagiellonian University in Krakow and in Gynecology and Obstetrics Ward of General Hospital in Sucha Beskidzka, Poland. Tissue samples were classified according to the menstrual cycle phases, which are determined on the basis of Beier H. reports and on the biochemical analysis of uterine secretion, which divides the cycle into three phases: proliferative, periovulatory and secretory [1]. Groups of patients were as follows: 16 were in proliferative phase and 14 in secretory phase, we excluded patients in periovulatory phase.

Endometriosis

Ovarian endometriosis tissue samples were obtained from women aged 28–36 and treated in Gynecology and Infertility Clinic of Jagiellonian University, by a laparoscopic cyst enucleation. The diagnosis of endometriosis was proved by the histopathological examination in the Department of Pathomorphology of Jagiellonian University.

Endometrial adenocarcinoma

The study group consisted of 13 patients with endometrioid carcinoma. All women underwent radical hysterectomy with pelvic lymphadenectomy in the Department of Gynecology, Obstetrics and Oncology of Jagiellonian University. The women age ranged between 48 and 72