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ROLE OF MATRIX METALLOPROTEINASES IN THE DEVELOPMENT OF ENDOMETRIOSIS IN WOMEN WITH INFERTILITY

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Abstract

The expression of matrix metalloproteinases 2, 9 and 11 (MMP-2, MMP-9, MMP-11) was investigated in 20 samples of the endometriotic tissue from women with endometriosis-associated infertility. The obtained results are indicative of the enhanced MMP-2 activity against the ground of endometriotic lesion and the peritoneum, as well as in capillaries and in the area of endometrial infiltration in underlying tissue. The elevation of MMP-9 activity was observed in the stroma of the ectopic endometrium bordering with underlying stroma. The intensified MMP-11 expression was found to prevail in outer membranes of endometrial cells. Thus, the obtained findings are indicative of the increased MMP-2, MMP-9 and MMP-11 activity in the sites of endometriotic lesion, that, considering their ability to demonstrate lytic and remodeling action upon underlying connective tissue, may promote endometrial cells infiltration into the underlying tissue (peritoneum), improve capillary permeability and involvement of macrophage and lymphocyte cellular components with further formation of infiltrates. Therefore, the investigation results evidence the role of matrix metalloproteinases in the progression of endometriosis.

Key words: endometriosis, infertility, matrix metalloproteinases

Introduction

Endometriosis, characterized by an ectopic growth of the endometrium outside the uterus, staying viable in the presence of immune control factors despite ectopic location, presents a certain «riddle» for the scientists worldwide, personifying the problem of «uninvited» autotransplant on one side and metastasis – on the other side. Endometriosis affects 10-15% of women of the reproductive age and, according to different sources, from 40% to 50% of women with infertility [1]. There are many theories regarding the pathogenesis of endometriosis, and the theory of retrograde bleeding during menses is considered as the most acknowledged. However, not a single theory has absolute advantages towards alternative theories [2, 3]. As almost all women of the reproductive age are known to have a certain degree of retrograde menses, the existence of other factors, contributing to the development and progression of endometriosis, is suspected. But despite numerous long-term studies and scientific data concerning endometriosis, the pathogenesis of the disease is not known with certainty [4, 5, 6]. The mechanisms of the disease development as well as the aspects of endometrial cells ability for invasion and growth still remain unclear [7]. Current data evidence, that there are specific mechanisms, enabling endometrial cells of women with endometriosis to avoid immune control [8]. Increased viability of endometrial cells in patients with endometriosis, their proliferative activity and invasiveness are given an important role in the development of endometriosis [5, 7, 9, 10]. In order to clarify the mechanisms of enhanced invasiveness of endometrial cells, a special attention is paid to the assessment of expression of integrin molecules and matrix metalloproteinases in the endometrium in case of endometriosis. Existing nowadays data are contradictory [9, 10, 11, 12] and unable to make undoubted decision concerning the role of these factors in pathogenic mechanisms of endometriotic lesions formation [9, 10, 11]. Matrix metalloproteinases (MMPs) belong to the family of Zn^{2+}– and Ca^{2+}–dependent endopeptidases, participating in remodeling of the connective tissue by destruction of its organic components under physiological pH levels. MMPs are known as irreplaceable contributors to numerous physiological processes – morphogenesis, resorption and remodeling of tissues, migration, adhesion, differentiation and proliferation of cells. The breakdown of extracellular matrix by MMPs occurs during normal physiological processes, such as wound healing, angiogenesis, as well as under various stages of reproductive process. The activity of MMPs is regulated by the level of tissue or cellular inhibitors of metalloproteinases (TIMPs). An important role of MMPs has been postulated regarding numerous pathologic conditions, including rheumatic arthritis, osteoarthritis, periodontitis, few autoimmune diseases and etc. [12, 13, 14]. A special function of MMPs is related to the development and generalization of tumor invasion and metastasizing. Positive correlation has been found between the elevation of plasma content of gelatinases (MMP-2, -9) and high rates of metastasizing, and it is considered as a significant predictive factor for many types of tumors. Interestingly, MMP expression changes depending on the type of tissue. The most considerable changes of MMP expression appeared to develop in tissues, undergoing intensive cyclic reconstruction, for instance, such as human endometrium or postpartum uterus of mice. In particular, high activity of MMP (matrilysine) in epithelial cells of the human endometrium is observed during proliferative, late secretory and menstrual phases of menstrual cycle, when the endometrium changes and the level of estrogens increases relatively to progesterone. It is well known, that expression of MMP-1 in endometrial cells is influenced by estradiol and progesterone levels, and MMP-2 and TIMP-1, probably, are synthesized constitutively [13, 14, 15, 16]. In particular, successful embryo implantation in case of extracorporeal fertilization has been demonstrated to depend on corresponding uterus condition and MMPs activity, cytokines, prostaglandins, adhesion molecules. The contents of collagen type 1 transcripts and MMP-2 in uterine epithelium bioptates were higher in women with the diagnosis of «idiopathic infertility» under normal folliculogenesis, menstrual cycle and absence of adhesive process, and particularly elevated in women with repeated abortions. It is well know as well, that the enhance of the content and activity of MMP-2 and the decrease of TIMP level impede the normal process of blastocyst invasion [17, 18]. MMP expression, content and activity are regulated by sex hormones. Progesterone and estrogens reduce the activity of metalloproteinases in endometrial cells culture, but, in case of hormones cancellation, their activity is dramatically augmented, followed by the morphological changes of endometrial cells, typical for the uterine epithelium during menstruation period [19]. Thereby, there is a supposition, that successful modulation of MMP/TIMPs system may restrict the invasive events and prevent the

development of endometriosis [3, 4, 5, 6]. Endometrial ectopies are characterized by the elevation of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9, and the decrease of TIMP-1 and TIMP-2 inhibitors. However, these findings may be interpreted as suggestion, that augmentation of MMP activity, whether due to the increase of MMP expression or the decrease of TIMP expression, will be indicative for the invasiveness of endometrial implants. Based on the mice experimental model of endometriosis, it was shown, that intensification of MMP expression on the endometrial tissue is parallel to the ability of this tissue to develop ectopic lesions. Simultaneous administration of progesterone and MMP inhibitors promotes the decrease of the growth of endometriotic ectopies. This thesis is given a principal significance in deeper understanding of the role of MMP in endometriosis etiology, in the recognition mechanisms, causing the abnormal expression and function of these proteases. Such mechanisms may include the presence of congenital anomalies in the ectopic endometrium of women with endometriosis, changes of functional content of immune and peritoneal cells, comparable with the action of numerous cytokines and growth factors, whose level is known to be elevated in peritoneal fluid of women suffering from this disease [20, 21, 22, 23, 24]. Thus, the detection of mechanisms, determinant for the increased invasiveness of human endometriotic cells, will significantly broaden our notions of the mechanisms of ectopic growth formation in the human body. Definition of new pathogenic causes, regulating invasive abilities of the endometrium, may serve as a foundation to work out new approaches of treatment of endometriosis in humans. Objective of the research was to determine the activity of MMP-2, MMP-9 and MMP-11 in endometriotis lesions of women with endometriosis-associated infertility and to establish their role in the invasiveness of ectopic endometrium.

Materials and Methods

Immunohistochemical investigation of endometriotic samples from 20 women, aged from 23 to 42 years, who've applied to the clinic because of infertility, was conducted. All patients underwent diagnostic-treatment laparoscopy, followed by the determination of macroscopic type of external genital endometriosis with further histological proof. At the moment of admission there weren't other diagnosed disorders, suspected to be causative for infertility.

The activity of matrix metalloproteinases (MMPs) 2, 9 and 11 on ectopic endometrium was studied in women with endometriosis. Fragments of ectopic endometrium (endometriotic tissue), obtained by laparoscopy from the peritoneum of women, served as investigation material. Investigation samples were fixed in formalin, paraffin-embedded in blocks, cut in four-micrometer-thick sections. Detection of MMP-2, 9 and 11 activities was performed by mean of immunohistochemical method according to the standard protocol with the usage of rabbit monoclonal antibodies against MMP-2, MMP-9 and MMP-11 (Dako; Glostrup, Denmark). The results were evaluated by means of light microscopy using Olympus CH20 microscope, connected with Nikon D90 digital camera. Post-processing of the data was performed using Adobe Photoshop (Adobe Systems Incorporated; San Jose, CA), version 3,0.

Results and Discussion

Immunohistochemical analysis has demonstrated the significant enhance of MMP-2 expression in ectopic endometrium of women with endometriosis-associated infertility. The distinctive feature of MMP-2 expression was a considerable increase of its activity precisely on the border of endometriotic lesion and the peritoneum. Elevation of MMP-2 activity was also observed in the stroma of the ectopic endometrium bordering on the underlying stroma (figure 1). Enhanced activity of MMP-2 in the stroma of the ectopic endometrium bordering on the underlying stroma was accompanied by the formation of macrophage-lymphocyte infiltrates (figure 2), that evidenced the implication of immune cellular components into the inflammation zone. Thereby, the obtained research findings allow to suggest, that elevation of MMP-2 activity in the sites of endometriotic lesion on the border with the underlying stroma promotes the invasiveness of ectopic endometrium by remodeling of the underlying stroma and infiltration of endometrial cells into the peritoneum. This process is suggested to be locally associated with macrophages and lymphocytes activation and may unable pregnancy in women with endometriosis. Increased activity of MMP-9 was observed in the stroma of the ectopic endometrium bordering on the underlying stroma as well (figure3, figure 4). The intensified MMP-11 expression was found to prevail in outer membranes of endometrial cells (figure 5, figure 6). Thus, the obtained findings are indicative of the increased MMP-2, MMP-9 and MMP-11 activity in the sites of endometriotic lesion, that, considering their ability to demonstrate lytic and remodeling action upon

underlying connective tissue, may promote endometrial cells infiltration into the underlying tissue (peritoneum), improve capillary permeability and involvement of macrophage and lymphocyte cellular components with further formation of infiltrates. Therefore, the investigation results evidence the role of matrix metalloproteinases in the progression of endometriosis.

Conclusions

Significant elevation of MMP-2, MMP-9 and MMP-11 activity, established on ectopic endometrium of women with endometriosis, is accompanied by macrophage-lymphocyte infiltration.

Immunohistochemical detection of MMP-2, MMP-9 and MMP-11 may serve as the criterion of intensity of endometriotic inflammation invasiveness.

Study of MMP-2, MMP-9 and MMP-11 activities in endometriotic lesions from women with endometriosis-associated infertility is perspective for further investigations in order to determine a possible role of MMP in the development of infertility in case of endometriosis.

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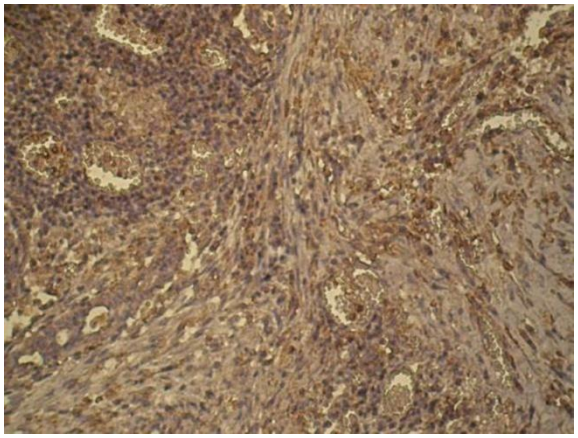

Figure 1. MMP-2 expression on ectopic endometrium of women with endometriosis

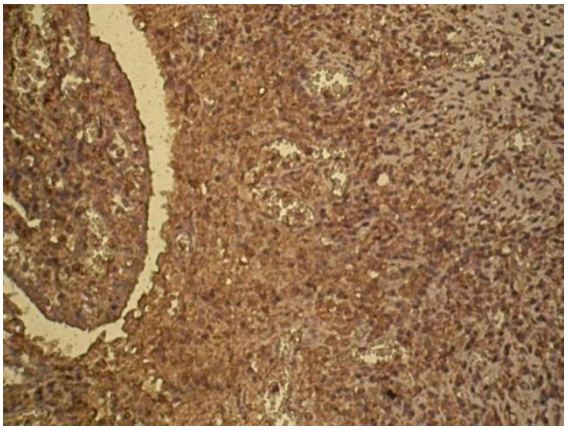

Figure 2. MMP-2 expression on ectopic endometrium of women with endometriosis. Infiltration of endometrial stroma by monocytes and macrophages

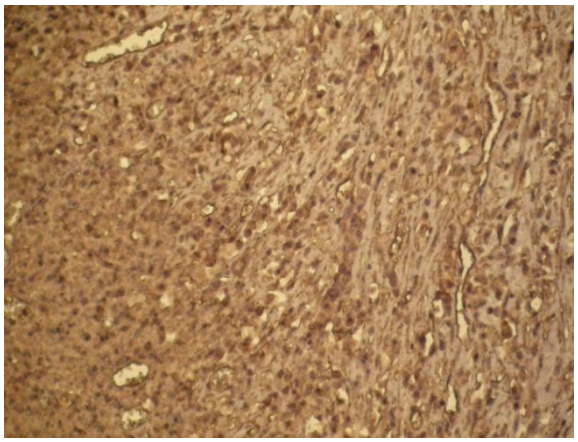

Figure 3. MMP-9 expression on ectopic endometrium of women with endometriosis

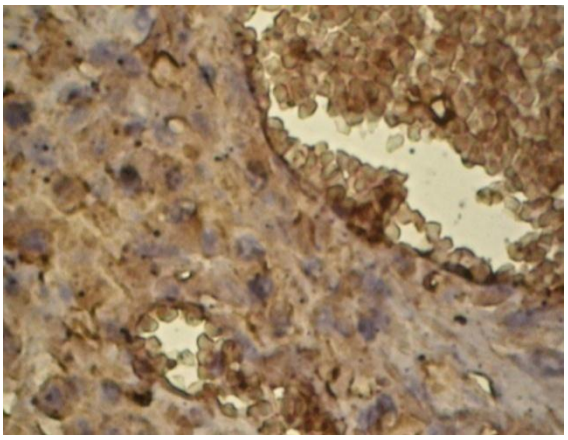

Figure 4. MMP-9 activity in ectopic endometrium of women with endometriosis. Infiltration of endometrial stroma by monocytes and macrophages

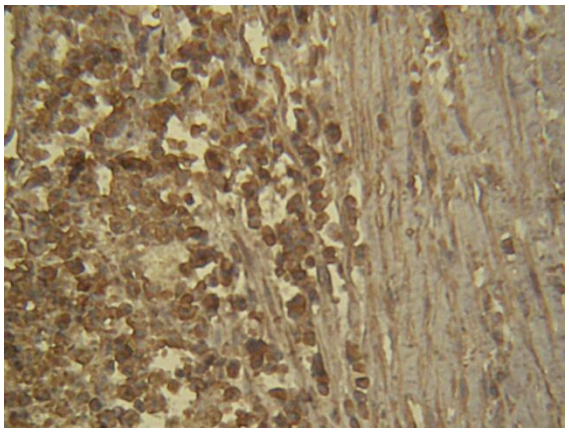

Figure 5. MMP-11 expression on ectopic endometrium of women with endometriosis

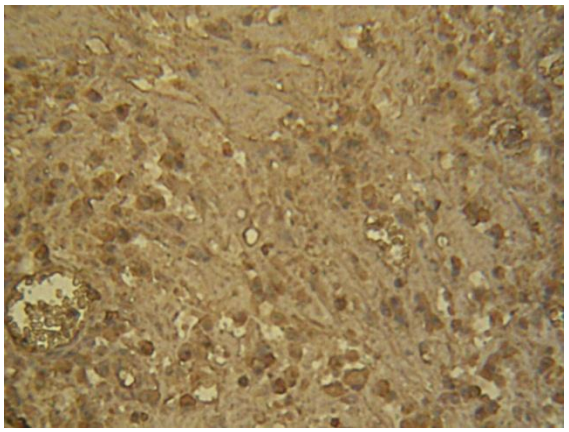

Figure 6. MMP-11 expression on ectopic endometrium of women with endometriosis