

*Forum Minireview***Basic and Translational Research on Proteinase-Activated Receptors: Proteinase-Activated Receptors in Female Reproductive Tissues and Endometriosis**Yutaka Osuga^{1,*}, Yasushi Hirota¹, and Yuji Taketani¹¹*Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan**Received August 19, 2008; Accepted November 5, 2008*

Abstract. During the menstrual cycle, dynamic morphological changes are observed in the ovarian follicle and the endometrium. These changes are associated with the onset of the inflammatory response in which many proteinases play various roles. Thrombin-induced activation of PAR₁ (proteinase-activated receptor 1) stimulates the production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) in human granulosa cells, suggesting a possible role for PAR₁ in the ovulatory process. In the endometrium, PAR₂ expression increases during the menstrual period. PAR₂ activation induces IL-8 production and cell proliferation in human endometrial stromal cells. PAR₁ also stimulates proinflammatory cytokine production in human endometrial stromal cells. Thus, the PARs may be important in directing the dynamic changes of the endometrium. PARs also appear to play a role in endometriosis, a common gynecological disease, since activation of PAR₁ and PAR₂ induces the secretion of inflammatory cytokines and the proliferation of stromal cells in endometriotic lesions. Taken together, PARs appear to play diverse roles in the human reproductive organs.

Keywords: ovary, endometrium, endometriosis, proteinase-activated receptor (PAR), reproduction

Introduction

During the menstrual cycle, dynamic morphological changes are observed in the ovarian follicle and the endometrium. These changes, which are pivotal in reproduction, are associated with the onset of the inflammatory response. Endometriosis, an endometrium-related disease, is also accompanied by inflammatory responses. Emerging findings have suggested that proteinase-activated receptors (PARs) play important roles in these inflammatory reactions. PARs are seven-transmembrane G protein-coupled receptors that are activated by proteinases. For example, thrombin can activate PAR₁, PAR₃, and PAR₄, while trypsin can activate PAR₁, PAR₂, and PAR₄. Cleavage of the PARs occurs within the extracellular N-terminal domain, thereby unmasking a new amino terminus, and the

cleaved N-terminal domain functions as a tethered ligand by binding back to the receptor (1, 2). In this review, the findings on the role of PARs in female reproductive tissues and endometriosis are presented, and the pathophysiological significance of PARs in these tissues is discussed.

PAR₁ in human luteinized granulosa cells

Ovulation is an inflammation-like process in which a mature ovarian follicle ruptures and discharges an oocyte that participates in reproduction. At ovulation, various morphological changes are observed in the follicle, including the extravasation of erythrocytes and fibrin deposition in the extracellular space of the follicular wall and in the follicular fluid. After expulsion of the oocyte, a fibrin clot forms in the remnant antral cavity. These findings suggest an involvement of thrombin, a proteinase essential for fibrin formation, in the ovulatory process. Indeed, the generation of thrombin and its functional activity in the follicular fluid has been

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demonstrated (3).

Granulosa cells form the inner layer of the ovarian follicle wall. These cells undergo luteinization at ovulation and subsequently become a component of the corpus luteum. To address the possible role of thrombin in ovulation, the expression of PAR₁ in human luteinized granulosa cells, as well as the effects of thrombin and a PAR₁ agonist on the production of inflammation-related molecules in luteinized granulosa cells, was investigated (4). Luteinized granulosa cells were collected at the time of oocyte pick-up from patients undergoing in vitro fertilization. Expression of PAR₁ mRNA was detected in luteinized granulosa cells by RT-PCR analysis. Thrombin and SFLLRN (Ser-Phe-Leu-Leu-Arg-Asp), a PAR₁-agonist peptide, stimulated the production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) in cultured luteinized granulosa cells. The stimulatory effects of thrombin were inhibited by inhibitors of thrombin [hirudin and L-prolyl-L-arginine chloromethyl ketone (PPACK)] and the protein kinase C inhibitor calphostin C. As IL-8 and MCP-1 are typical chemoattractants for leukocytes, these findings suggest involvement of PAR₁ in leukocyte infiltration into the extravascular spaces in the ovulatory follicle. In addition, thrombin and SFLLRN stimulated the gelatinase activities of luteinized granulosa cells, the effect of both being inhibited by hirudin and PPACK. Therefore, in luteinized granulosa cells, PAR₁ may also participate in the dissolution of the granulosa cell basement membrane and fragmentation of the extracellular matrix of the follicular wall that precedes rupture of the follicle.

PARs in the endometrium

Decidualization is a process of endometrial differentiation that is essential for successful implantation of the embryo and maintenance of pregnancy. Decidualization occurs under the influence of progesterone secreted from the corpus luteum. Interestingly, the tissue factor content in endometrial stromal cells is increased in the decidualization process, and therefore, thrombin may play a role in the decidualized endometrium. Thrombin-induced PAR₁ activation increases VEGF expression in decidualized endometrial stromal cells (5). Thus, PAR₁, via up-regulation of VEGF, may be involved in angiogenesis and vascular permeability in the decidualized endometrium. Furthermore, PAR₁ activation by thrombin increases the production of IL-8, matrix metalloproteinase (MMP)-1, and active MMP-2 in endometrial stromal cells (6, 7), implying multiple roles of PAR₁ in the endometrium.

Expression of PAR₂ mRNA in the human endometrium is increased during the menstrual phase and in

early pregnancy (8). This finding is consistent with a remarkable increase in the number of leukocytes in the endometrium during menstruation since PAR₂-activating proteinases are secreted by the resident leukocytes such as mast cells and neutrophils. In vitro, the PAR₂-agonist peptide (PAR₂AP, Ser-Leu-Ile-Gly-Lys-Val) stimulates IL-8 production in both endometrial epithelial cells and endometrial stromal cells (8). PAR₂AP also stimulates the mRNA expression of stem cell factor, a known activator of mast cells, in endometrial stromal cells and protein expression of activated MMP-7, an epithelial cell-specific matrix metalloproteinase, in endometrial epithelial cells. These findings indicate the involvement of activated PAR₂ in upregulating molecules important for endometrial remodeling in the tissue modification process during the menstrual cycle. In addition, PAR₂AP significantly increased the incorporation of 5-bromo-2'-deoxyuridine into DNA in endometrial stromal cells. This mitogenic effect underscores the possible involvement of PAR₂ in repair of the endometrium undergoing shedding during menstruation. Recent studies have demonstrated that the tissue factor-FVIIa complex activates PAR₂. Such signaling is an emerging role for tissue factor (9). Since tissue factor is produced in the endometrium, it is currently speculated that tissue factor-stimulated PAR₂ also plays a role in the endometrium.

Possible relevance of PARs to the pathogenesis of endometriosis

Endometriosis, defined by the presence of viable endometriotic tissue outside the uterus, is an enigmatic disease. It impairs the health of women of reproductive age, causing pelvic pain and infertility. Implantation and growth of endometrial cells from the overflow of menstrual blood into the peritoneal cavity is a widely accepted hypothesis for the pathogenesis of endometriosis, in which peritoneal inflammation is thought to play a pivotal role (10). PAR₁ and PAR₂, which play roles in many inflammatory events, might be involved in the development of the disease. In addition, the disease is characterized by recurrent ectopic bleeding, and the resultant generation of thrombin is expected to activate PAR₁. Mast cells and neutrophils present in endometriotic tissues might activate PAR₂ by producing specific proteinases. The expression of PAR₁ and PAR₂ in endometriotic cells, as well as the effects of PAR-activating molecules on these cells, has, therefore, been investigated.

Possible function of PAR₁ in endometriotic cells

Using endometriotic stromal cells from surgically

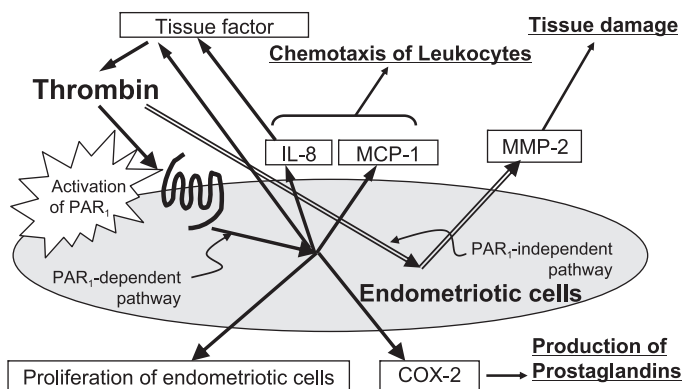


Fig. 1. Possible roles of thrombin in endometriosis. Thrombin stimulates production of IL-8, MCP-1, tissue factor, and COX-2 via PAR₁ activation. Thrombin also stimulates proliferation of endometriotic stromal cells via PAR₁ activation. Thrombin stimulates production of MMP-2 in a PAR₁-independent manner. In combination, these events may stimulate the development of endometriosis.

removed endometrioma (11), several findings have been obtained (Fig. 1). PAR₁ mRNA was expressed in endometriotic stromal cells. Thrombin and SFLLRN increased the mRNA expression of IL-8, MCP-1, and cyclooxygenase-2 (COX-2) and the protein secretion of IL-8 and MCP-1 in endometriotic stromal cells. The concurrent addition of the thrombin inhibitor PPACK inhibited the thrombin-induced secretion of IL-8 and MCP-1. Since MCP-1, IL-8, and prostaglandins are involved in the pathogenesis of endometriosis, PAR₁ is thought to function in the development of endometriosis. IL-8, as well as thrombin, stimulated the expression of tissue factor in endometriotic stromal cells. The increased level of tissue factor may stimulate the coagulation cascade to produce thrombin, which subsequently enhances IL-8 production through PAR₁ activation in endometriotic lesions. Viewed this way, PAR₁ activation could link inflammation with coagulation, thus conferring self-sustaining mechanisms for the progression of endometriosis. Moreover, thrombin and SFLLRN stimulate the proliferation of endometriotic stromal cells. Therefore, thrombin-induced PAR₁ activation might be involved in the pathophysiology of endometriosis, stimulating inflammatory responses of endometriotic cells and their mitogenic activity. In contrast to PAR₁-dependent upregulation of MMP-2 by thrombin in endometrial stromal cells (6), MMP-2 production was increased by thrombin without PAR₁ activation in endometriotic stromal cells (11). Thrombin may stimulate the development of endometriosis both dependently and independently of PAR₁.

Possible function of PAR₂ in endometriotic cells

Activation of PAR₂ stimulated the proliferation of endometriotic stromal cells and the secretion of IL-6 and IL-8 from these cells in a dose-dependent manner (12). Since IL-8 is a chemoattractant of neutrophils, it can be speculated that PAR₂ activation in endometriotic stromal

cells may promote the migration of neutrophils via IL-8 secretion. Neutrophils can secrete PAR₂-activating proteinases, and thus PAR₂ activation may cause self-perpetuating inflammation at endometriotic lesions. In addition, activation of PAR₂ stimulated the phosphorylation of mitogen-activated kinases (MAPKs), such as p38 MAPK, p42/44 MAPK, and the stress-activated protein kinase/c-jun N-terminal kinase; and this finding may imply pleiotropic functions of PAR₂ in endometriotic tissues. Activation of PAR₁ and PAR₂ in endometriotic stromal cells may, therefore, result in the pathophysiology observed in endometriosis by inducing the growth and inflammation of endometriotic lesions.

Endometriosis model in PAR₂ deficient mouse

In view of the possible significance of PAR₂ in the establishment of endometriosis demonstrated in the *in vitro* study, an *in vivo* study was performed using PAR₂-deficient mice (kindly provided by Kowa Co., Ltd., Tokyo). A mouse model of endometriosis was developed according to the method previously described (13). Both the number and the total weight of endometriotic lesions were significantly decreased in the PAR₂-deficient mice compared to the wild type mice (Fig. 2A). Concentrations of IL-6 and MCP-1 were decreased in the peritoneal fluid and the serum of the PAR₂-deficient mice (Fig. 2B), suggesting alleviated inflammation in the peritoneal cavity of the mice. These findings indicate that PAR₂ is involved in the development of experimental endometriosis in mice and that the anti-inflammatory environment in PAR₂-deficient mice might hinder the progress of the disease. Combined with the findings of the *in vitro* study, it can be argued that PAR₂ would be a target for the treatment of endometriosis.

Conclusion

PARs may play diverse roles in female reproductive

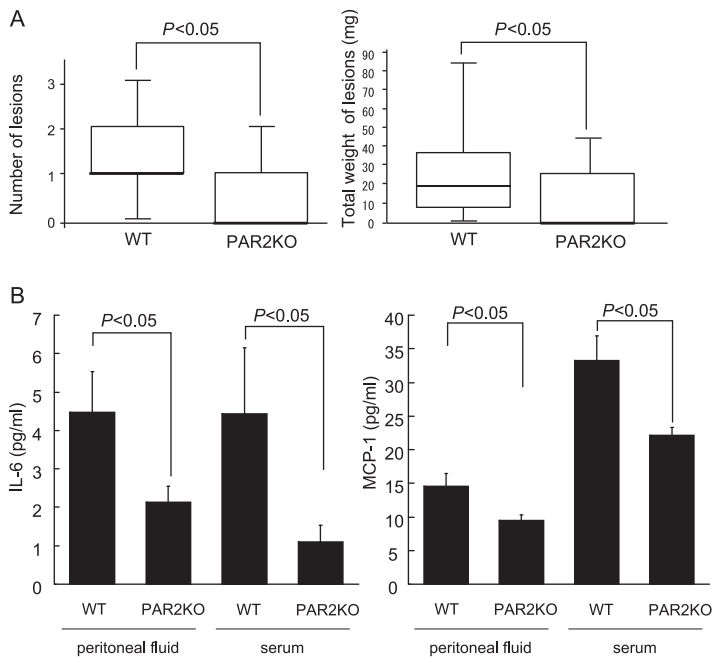


Fig. 2. Endometriotic lesions and cytokines in peritoneal fluid in a model of endometriosis using PAR₂-deficient mice. Number and total weight of endometriotic lesions (A) and concentrations of IL-6 and MCP-1 in peritoneal fluids and serum (B) are shown. Endometriosis was induced in wild type (WT, n = 20) and PAR₂^{-/-} (PAR₂KO, n = 21) C57BL/6 mice by injecting syngenic endometrial fragments into the peritoneal cavity. Three weeks later, the mice were sacrificed and endometriotic lesions in the peritoneal cavity were measured. At the same time, peritoneal fluid and serum were collected from each mouse. IL-6 and MCP-1 concentrations in the peritoneal fluid and serum were measured using specific ELISA. Boxes represent the distances between the first (25%) and third (75%) quartiles, the horizontal lines in the boxes represent the medians, and the whiskers represent the 10th percentile at the lower limit and the 90th percentile at the upper limit (A). Values are shown as the mean ± S.E.M. (B).

tissues and endometriosis. Their roles appear to be essential for normal physiological events in reproduction, such as ovulation, endometrial changes, and menstruation, as well as the pathogenesis of endometriosis. Further understanding of the function of PARs in these tissues is necessary for the development of reproductive medicine.

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