[Frontiers in Bioscience S4, 1201-1212, June 1, 2012]

Proteinase-activated receptors in the endometrium and endometriosis

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1. ABSTRACT

Proteinase-activated receptors (PARs) are G protein-coupled receptors activated by various proteinases. PARs play important roles in haemostasis, thrombosis, and inflammation. PAR_1 and PAR_2 are expressed in endometrial cells from the eutopic endometrium and endometriotic cells derived from endometriotic lesions. A typical activator of PAR_1, thrombin, and a typical activator of PAR_2, tryptase, are produced in the endometrium as well as endometriotic lesions. PAR_1 activation in endometrial stromal cells induces production of vascular endothelial growth factor and matrix metalloproteinases, and increases activities of tissue-type and urokinase-type plasminogen activator. PAR_2 activation in endometrial stromal cells stimulates interleukin (IL)-8 and stem cell factor production and proliferation of the cells. PAR_1 activation in endometriotic stromal cells induces production of IL-8, monocyte chemotactic protein-1, and cyclooxygenase-2, and proliferation of the cells. PAR_2 activation in endometriotic stromal cells increases secretion of IL-6 and IL-8, and the number of the cells. These findings indicate a wide range of function of PAR_1 and PAR_2 in the endometrium and endometriosis, and suggest PAR_1 and PAR_2 as possible therapeutic targets for endometriosis.

2. PROTEINASE-ACTIVATED RECEPTORS: OVERVIEW

The family of proteinase-activated receptors (PARs) is comprised of four members: PAR_1, PAR_2, PAR_3 and PAR_4. PARs are G protein-coupled receptors (GPCRs) that are uniquely activated by proteinases. PARs are expressed in various cells including vascular, immune and epithelial cells, astrocytes and neurons and transmit cellular responses to coagulant proteinases as well as other proteinases expressed in distinct tissues (1-4). PARs are critical mediators of haemostasis, thrombosis, inflammation, and have been implicated in cancer progression, indicating this receptor class as an important drug target.

Activation of PARs is induced through an irreversible proteolytic mechanism. Proteinases bind to and cleave the extracellular N-terminal domain of PARs at specific sites to unmask a new N-terminus that acts as a tethered ligand that binds to the receptor and triggers intracellular signaling (Figure 1). Synthetic peptides that mimic the first six amino acids of the newly formed N-terminus can activate PARs independent of proteinase and receptor cleavage, except for PAR_3(5). Viewed more

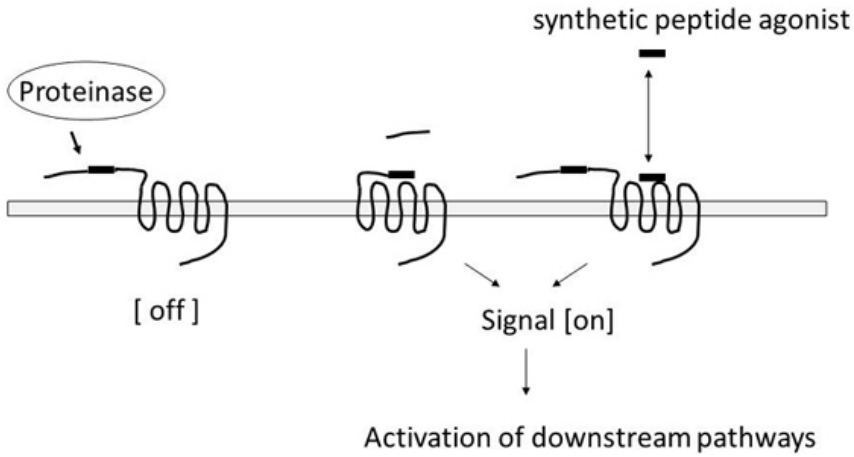

Figure 1. PAR is a member of a group of seven transmembrane G protein-coupled receptors. On the activation of PARs, proteases such as thrombin and trypsin cleave at a point within the extracellular N-terminal domain and, thereby, unmask a new amino terminus that functions as a tethered ligand to bind back to the receptor. This property of PAR enables the usage of a specific agonist comprised of the amino-terminal peptides to study distinct PARs.

closely, the activation of PARs is thought to occur through peptide ligand interactions with residues residing in the second extracellular loop (6), unlike most classic GPCRs where ligand binding occurs in a pocket formed by the transmembrane helices. Once activated, GPCRs function as guanine nucleotide exchange factors and promote exchange of GDP for GTP on the α subunit leading to βγ subunit dissociation. Both the GTP bound α subunit and βγ subunits signal to various effectors to promote diverse cellular responses. Activated PARs also interact with various adaptor proteins, such as β-arrestins, that facilitate signal transduction independent of heterotrimeric G protein coupling(5). Activation of PARs also induces the activation of mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNKs) also known as stress-activated protein kinases (SAPKs), and p38 MAPK (7, 8).

Proteinases that cleave and activate PARs are various, including proteinases from the coagulation cascade, inflammatory cells, and the digestive tract. Serine proteinases of the coagulation cascade are the best characterized activators of PARs (9, 10). Proteinases that mediate coagulation and anticoagulation by cleaving zymogens or active enzymes themselves can also signal to several cell types by cleaving and activating PARs. Thrombin activates PAR_1, PAR_3, or PAR_4 at the surface of platelets, resulting in aggregation, which contributes to hemostasis (9). Tissue factor (TF)-VIIa complex and TF-VIIa-Xa complex signal by cleaving PAR_1 and PAR_2 on a range of cell types, including endothelial cells, playing an important role in inflammation (11-13).

Mast cell tryptase has been noted as an activator of PAR_2. Tryptase is the most abundant proteinase of human mast cells; it comprises up to 25% of the total cellular proteins and is expressed by almost all subsets of human mast cells (14). Many of the proinflammatory and mitogenic effects of tryptase are mimicked by PAR_2 agonist peptides (APs), suggesting that tryptase exerts its effects through this receptor. Human tryptase from a variety of sources (purified from human lung, skin, and mast cell lines) can cleave PAR_2 to expose the tethered ligand domain and signals to PAR_2 transfected cells as well as many cell types that naturally express PAR_2 at physiological levels(4).

3. PROTEINASE-ACTIVATED RECEPTORS IN THE ENDOMETRIUM

3.1. Role of proteinase-activated receptor 1 in the endometrium

The human endometrium is a dynamic tissue that, in response to the prevailing steroid environment of sequential ovarian estrogen and progesterone exposure, undergoes well-characterized cycles of proliferation, differentiation, and tissue breakdown on a monthly basis. If pregnancy fails to be established, the endometrium is then shed and regenerates. Menstruation is the reproductive process whereby the upper two thirds of the endometrium is shed and regenerated repeatedly. The endometrium is accordingly a site of recurrent physiological injury and repair (15). The remodelling of the endometrium occur naturally not only menstruation but also parturition, endometrial regeneration and uterine involution and have features in common with events of tissue injury and repair in other tissues, where many proteinases play important roles.

Decidualization of human endometrial stromal cells is initiated in the midluteal phase and spreads throughout the late luteal phase endometrium. Decidualized cells express high levels of TF (16-18), which binds to plasma factors VII/VIIa to generate factor Xa. The resulting formation of thrombin from prothrombin initiates hemostasis by converting fibrinogen to fibrin. Moreover, thrombin mediates various cellular effects by binding to a family of PARs. The luteal phase is accompanied by increased vascular permeability and stromal edema, which would generate thrombin via enhanced access of circulating

plasma clotting factors to TF derived from perivascular decidual cell. It is thus feasible that thrombin play a role in the decidualized endometrium.

Throughout the menstrual cycle, angiogenesis is tremendously significant process to support the function and the architecture of the endometrium(19, 20). In nonfertile menstrual cycles, progesterone withdrawal appears to elicit ischemia, hypoxia, and endometrial sloughing. After that, ruptured endometrial spiral arterioles and venules are suggested to serve as an angiogenic nidus that restores the vasculature in the next cycle. According to this notion, endothelial cells sprout from the disrupted vessels and recruit or induce pericytes or smooth muscle cells to form capillaries, venules, or arterioles. However, the mechanisms that contribute to post-menstrual repair and secretory phase remodelling have not clearly understood while stereological data has identified vessel elongation as a major endometrial angiogenic mechanism in the mid-late proliferative phase(20). From the molecular point of view, angiogenic factors identified in human endometrium includes epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and TGF-ß, angiopoietin and VEGF, and VEGF is noted as the most likely mediator of endometrial angiogenesis (19, 21, 22).

VEGF expression is induced by thrombin in several cell types (23-25). Thrombin induces VEGF secretion from human endometrial stromal cells that are decidualized by estradiol and medroxyprogesterone acetate. In addition, a synthetic thrombin receptor activating peptide stimulates PAR_1 to increase VEGF secretion in decidualized human endometrial stromal cells (26). These findings indicate that thrombin mediates angiogenesis indirectly by enhancing VEGF expression in human endometrial stromal cells. Interestingly, thrombin also stimulate angiogenesis directly by enhancing endothelial proliferation and by elevating levels of the KDR receptor in endothelial cells (27). Collectively, decidual cell-derived thrombin and VEGF may act via their respective receptors to synergistically enhance luteal phase angiogenesis. Moreover, thrombin and VEGF each enhance endothelial permeability by different pathways (28, 29). This would increase access of clotting factors to decidual cell-expressed TF to promote a feed-forward cycle of enhanced thrombin and VEGF production. These findings appear to be consistent with the persistence of angiogenesis, as well as the peak in VEGF expression in the luteal phase endometrium.

Thrombin may play not only physiological but pathological roles in the endometrium. For example, thrombin may elicit abnormal uterine bleeding by promoting aberrant angiogenesis and/or vessel maintenance. In contrast to ovarian steroid withdrawal-mediated menstrual bleeding, the chronic, erratic, and prolonged bleeding occurs from compromised surface microvessels in users of long-term progestin-only contraception (21). The endometrium from such patients show increased expression of TF (18, 30) as well as of VEGF (31). As VEGF would elicit a prolonged increase in vascular permeability, chronic TF-derived thrombin generation may further stimulates VEGF production with resultant sustaining aberrant angiogenesis. Aberrant angiogenesis would produce the enlarged, distended, fragile vessels and overcomes TF-thrombin mediated hemostasis.
Extracellular matrix degradation is also essential for the regulation of angiogenesis (32). This process is controlled by several enzymes, specifically by a family of matrix metalloproteinases (MMPs), which possess a zinc molecule at their catalytic site and are divided into three subclasses according to their substrate specificity (33, 34). Thrombin and a PAR_1 agonist SFLLRN increase the production of MMP-1 and active MMP-2 from cultured secretory phase endometrial stromal cells. The increase is suppressed by an MEK inhibitor, suggesting that thrombin stimulates the production of MMPs via activation of PAR_1 and the MAPK system. In order to stimulate angiogenesis and sustain integrity of the endometrium, PAR_1 activation by thrombin may coordinate vessel growth and tissue degradation via the production of VEGF and MMPs.

PAR_1 also plays an important role in lysis of blood clots, which is an essential phenomenon to maintain the homeostasis of the endometrium where both physiological and pathological bleeding occurs. Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are serine proteinases involved in the breakdown of blood clots. As an enzyme, it catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown. Plasminogen activator inhibitor (PAI-1) inhibits the serine proteinases tPA and uPA, and hence inhibits fibrinolysis, the process that degrades blood clots (35). Thrombin, by activating PAR_1, stimulates the secretion of uPA, tPA, and PAI-1 in cultured endometrial stromal cells. This might be somewhat complicated in that both agonistic and antagonistic substances for plasminogen activating system are upregulated. However, the consequent net activity induced by thrombin is the increase in tPA and uPA activity (36). Progesterone works towards the adverse direction, suppressing uPA and tPA activitiy by increasing the production of PAI-1 and decreasing the production of uPA and tPA in endometrial stromal cells (17, 37). Given these findings, perivascular decidualized stromal cells may modulate the conflicting requirements for hemostasis during placentation and hemorrhage during menstruation. In the midluteal phase, stroma edema and transudation of plasma proteins are expected to facilitate thrombin generation at perivascular sites. However, a high progestational miliu would minimize thrombin enhancement of PA activity and may support implantation of the blastocyst. In contrast, the reduction of circulating progesterone that occurs at the end of the luteal phase impedes this effect and increase thrombin enhancement of PA activity. As a result, the increased PA activity would promote fibrinolysis and endometrial stromal ECM degradation, facilitating the sloughing of the functional layer of the endometrium.

3.2. Role of proteinase-activated receptor 2 in the endometrium

PAR_2 as well as PAR_1 play various roles in the endometrium during the menstrual phase. As described

above, decidualized endometrial cells express high levels of TF, which binds to plasma factors VII/VIIa to generate factor Xa. TF-VIIa complex and TF-VIIa-Xa complex are produced in this process and function as an activator of PAR_2. Mast cell tryptase also activates PAR_2 in the endometrium. It is well known that a variety of lymphomyeloid cells are present in the endometrium and modulate the endometrial functions (15). Mast cells do not alter in number during the cycle, but dramatic mast cell activation occurs immediately before and during menstruation. Extensive mast cell activation/degranulation, as judged by extracellular tryptase, was a common feature of the functional layer of the endometrium just prior to and during menstruation(38). It is thus plausible that PAR_2 is highly activated by TF-VIIa complex, TF-VIIa-Xa complex, and mast cell tryptase in the decidualized endometrium around menstruation period.

Expression of PAR_2 in the endometrial tissues is observed throughout the menstrual cycle and in the decidual tissues. The expression levels appear to be low from the midproliferative phase to the midsecretory phase and increase from the late secretory phase to the early proliferative (menstrual) phase (39). The expression levels are also increased in the decidual tissues. This expression pattern of PAR_2 seems to be in proportion to the production of TF-VIIa complex, TF-VIIa-Xa complex and tryptase in the endometrium and to be appropriate for PAR_2 to exert its effects efficiently in the endometrium.

We have shown that PAR_2 is expressed in endometrial stromal cells and epithelial cells, with some interindividual variation. PAR2 activation increase the production of IL-8 in both endometrial stromal and epithelial cells and the proliferation of endometrial stromal cells (39). IL-8 is a CXC chemokine produced by a range of cells. Primary function of IL-8 is the induction of chemotaxis of neutrophils. It also functions as a potent angiogenic factor. In addition, IL-8 stimulates the proliferation of several cell types, including endometrial stromal cells. Interestingly, IL-8 antisense oligonucleotide treatment decrease IL-8 production as well as cell proliferation compared with scrambled oligonucleotide treatment (40, 41), suggesting that IL-8 may act as an autocrine growth factor in the endometrium. The expression of IL-8 is low during the midproliferative to midsecretory phase and high around menstruation (42-44). It is speculated that IL-8 is responsible for leukocytes accumulation in the endometrium around the time of menstruation where leukocytes are involved in the degradation and scavenging of endometrial tissue. IL-8 in the uterus is also suggested to play unique roles in endometrial angiogenesis (45), which is crucial for appropriate repairing of the endometrium after menstruation. Moreover, IL-8 increases matrix metalloproteinases and adhesion to fibronectin in endometrial stromal cells (46-48). Given a wide variety of functions of IL-8 in the endometrium, PAR2 activation would influence the endometrial function through enhancing IL-8 expression in the endometrium.

It is notable that PAR_2 activation increases the expression of stem cell factor (SCF) also known as kit-ligand (KL), or steel factor in the endometrium (39). SCF is a cytokine that is known to play an important role in hematopoiesis, spermatogenesis, and melanogenesis. With regard to PAR_2 in the endometrium, functions of SCF on mast cells should be noticed. Mast cells are the only terminally differentiated hematopoietic cells that express SCF receptor. Mice with SCF mutations have severe defects in the production of mast cells, having less than 1% of the normal levels of mast cells. Conversely, the injection of SCF increases mast cell numbers near the site of injection by over 100 times. SCF also promotes mast cell adhesion, migration, proliferation, and survival. Moreover, it promotes the release of histamine and tryptase, which are involved in the allergic response (49). As mentioned above, extracellular tryptase indicative of mast cell activation and degranulation is remarkably increased in the endometrium around menstrual period (38). Given the possible significance of mast cell-derived proteinases, such as tryptase and chymase, in extracellular matrix degradation and tissue remodeling of the endometrium (50), the increase in PAR_2 expression at the menstrual period may upregulate SCF production in endometrial stromal cells and consequently activate mast cells to repair the shed endometrium. It is interesting to speculate that the function of mast cells may be potentiated by SCF via the feed-forward loop comprised of tryptase, PAR_2 and SCF in the endometrium.

Matrix metalloproteinase 7 (MMP-7), or matrilysin, is a secreted proteinase expressed by glandular and mucosal epithelial cells, keratinocytes, fibroblasts and macrophages (51). As with other MMPs it can act on the extracellular matrix and thereby regulate cell migration and tissue repair. MMP-7 is secreted as an inactive protein pro-MMP-7 which is converted to the active form MMP-7 when cleaved by extracellular proteinases. PAR_2 activation increases MMP-7 production in endometrial epithelial cells (39). MMP-7 is expressed in the endometrium in the perimenstrual and proliferative phase but not the early to midsecretory phase (52). As this expression pattern is similar to that of PAR_2, it is speculated that the PAR_2 activation-induced MMP-7 production contributes to the MMP-7 expression in the endometrium. This expression pattern also implies possible roles of the enzyme in tissue breakdown and regeneration of the endometrium around the menstrual period. Accordingly, PAR_2 activation in endometrial epithelial and stromal cells may be an integral event for the tissue modification to restore the disrupted endometrium during the menstrual cycle.

PAR_2 activation is mediated via different sets of MAPKs, depending on the cell type (53-55). PAR_2 activation stimulates the phosphorylation of ERK1/2, JNK, and p38 MAPK in endometrial stromal cells (39). This finding may explain the pleiotropic effects of PAR_2 in endometrial stromal cells as the MAPK signal transduction pathways are among the most widespread mechanisms of eukaryotic cell regulation. Interestingly, inhibitors of all the three MAPKs inhibited PAR_2-dependent IL-8 secretion from endometrial stromal and epithelial cells. In addition,

the PAR_2-induced proliferation of endometrial stromal cells is suppressed with these inhibitors. Consequently, it is suggested that all the three MAPKs are involved in the signal transduction in the endometrium.

4. PROTEINASE-ACTIVATED RECEPTORS IN ENDOMETRIOSIS

4.1. Role of proteinase-activated receptor 1 in endometriosis

Endometriosis is defined as the presence of endometrial glands and stroma outside the endometrial cavity and uterine musculature. These ectopic endometrial implants are usually located in the pelvis, but can occur nearly anywhere in the body. Endometriosis can be associated with many distressing and debilitating symptoms, such as pelvic pain, severe dysmenorrhea, and dyspareunia (56, 57). It also often causes infertility and deteriorates quality of life of women in reproductive ages. Despite numerous studies, considerable controversy remains regarding the incidence, pathogenesis, natural history, and optimal treatment of this disorder. As a pathogenesis of the disease, the implantation theory is most widely believed which proposes that endometrial tissue from the uterus is shed during menstruation and transported through the fallopian tubes, thereby gaining access to and implanting on pelvic structures (58). However, substantial molecular mechanisms that corroborate the theory have not been clearly elucidated. In addition, how the implanted endometriotic lesion grows or regresses is not well understood either.

Studies on its pathogenesis for long years suggest that endometriosis has features of a chronic inflammatory disease. For instance, elevated concentrations of proinflammatory mediators (59-62) and increased concentrations of inflammatory cells (63, 64) have been reported in the peritoneal fluid of women with endometriosis. In addition, inflammatory responses occurring in endometriotic cells are suggested to accelerate endometriosis (65-67). The inflammatory changes observed in the peritoneal cavity are assumed to be induced by endometriotic cells, local inflammatory cells (68), and secretory products such as prostaglandins(62) and proinflammatory cytokines (57, 69, 70). Proteinases activated in the coagulation cascades are also possible inducers of inflammation in endometriosis (71).

Another specific feature of endometriosis is recurrent bleeding (72). Early peritoneal lesions frequently represent one or more small endometriotic polyps emerging from glandular structures located in the subperitoneal tissue (red lesions) (73). These early lesions are highly vascularized implants and bleed at menstruation (74). The puckered, pigmented lesion is characterized by a variable amount of fibrosis, a moderately vascularized stroma, glands with intraluminal debris, and the presence of hemosiderin-laden macrophages (black lesions). Endometrial cyst is a pseudocyst likely to be formed by a hematoma that causes invagination of the ovarian cortex, and is colonized by endometriotic epithelium and stroma. On histologic examination evidence of extravasation of red blood cells or the presence of hemosiderin-laden macrophages has been found in a large proportion of biopsies of histologically verified endometriosis (75). Once bleeding occurs, hemostasis follows by activating coagulation cascade. It is thus quite feasible that endometriotic lesions are exposed to various substances produced in the coagulation process. Thrombin is one of the typical products of the process which not only catalyzes plasminogen to plasmin but stimulates PAR_1.

PAR_1 is expressed in endometriotic stromal cells as well as eutopic endometrial cells. Thrombin and SFLLRN increase gene expression and secretion of IL-8 and MCP-1 from endometriotic stromal cells (71). MCP-1 and IL-8 are typical chemokines involved in the pathogenesis of endometriosis. Concentrations of MCP-1 and IL-8 are increased in the peritoneal fluid of women with endometriosis (41, 76, 77). They are expressed in endometriotic lesions, and their expressions are increased with IL-1ß treatment (65, 78, 79). MCP-1, also known as CCL2, is an inflammatory cytokine belonging to the CC chemokine family, and has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, like psoriasis, rheumatoid arthritis and atherosclerosis. MCP-1 is suggested to recruit and activate macrophages in endometriosis, and these macrophages are supposed to play a central role in the pathophysiology of the disease. As observed in the eutopic endometrium, IL-8 plays pleiotropic functions in endometriotic lesions, such as chemoattraction and activation of neutrophils, angiogenesis, stimulation of proliferation, and survival of endometriotic cells, and are suggested to contribute to the development of endometriosis (80, 81). With these backgrounds, the finding that activated PAR_1 stimulated productions of MCP-1 and IL-8 in endometriotic cells supports the notion that the thrombin not only plays a role in a coagulation cascade but regulates the production of significant chemokines responsible for the development of endometriosis.

Thrombin and SFLLRN induce cyclooxygenase-2 (COX-2) expression in endometriotic stromal cells, indicating the involvement of PAR_1 in COX-2 expression in endometriosis (71). Prostaglandins produced by the enzyme activity of COX-2 stimulate the inflammation and promote the disease. In particular, locally produced prostaglandin E_2 stimulates the expression of steroidogenic genes that enable the endometriotic stromal cells to synthesize estradiol (82), which exacerbate the disease.

PAR_1 activation in endometriotic stromal cells has another remarkable feature: induction of TF expression (71). IL-8 also stimulates TF expression in endometriotic stromal cells. TF localizes initial part of extrinsic pathway of coagulation cascade and make complex with factor VIIa to activate downstream coagulation process. Taken together, a feed-forward loop to enhance inflammation and coagulation is possibly present at bleeding site of endometriosis, where PAR_1 activation could link inflammation with coagulation, and confers self-sustaining mechanisms for the progression of endometriosis (Figure 2). PAR_1 activation may also contribute to the development

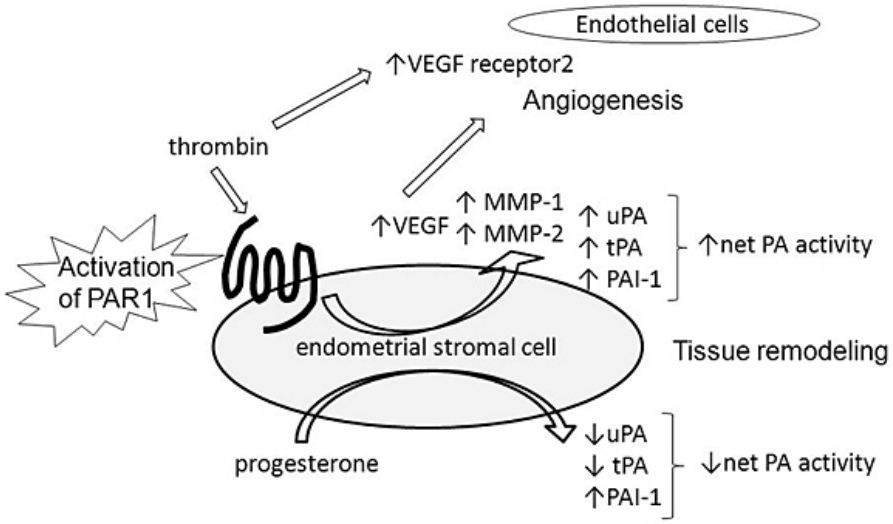

Figure 2. Possible roles of thrombin in endometriosis. Thrombin stimulates production of IL-8, MCP-1, tissue factor and COX-2 via PAR_1 activation. Thrombin also stimulates proliferation of endometriotic stromal cells via PAR_1 activation. Thrombin stimulates production of MMP2 in a PAR_1-independent manner. In combination, these events may stimulate the development of endometriosis.

of endometriosis as thrombin stimulates proliferation of endometriotic stromal cells through PAR_1 activation (71).

Interestingly, thrombin, but not PAR_1 agonist SFLLRN, increased the production of pro-MMP-9 and MMP-2 in endometriotic cells, indicating that this effect is independent from PAR_1. This response may be mediated by thrombin binding to thrombomodulin which increase MMP-2 production by activating protein C (83, 84). It is known that MMP-2 and MMP-9 are increased in endometriotic tissues (85, 86). Given that the increased MMPs are involved in the development of endometriosis by promoting breakdown and remodeling of endometriotic tissues, the effect of thrombin to increase the MMPs also contribute to the progression of the disease.

Assuming that thrombin activation promotes the development of endometriosis, inhibition of PAR_1 is a promising therapeutic approach for the treatment of the disease. Because a PAR_1 antagonist is specific for the cellular actions of thrombin and does not interfere with fibrin generation, it is expected to have less adverse effects on bleeding than the currently available thrombin inhibitors. In fact, chemicals with selective antagonistic effects on PAR_1 did not perturb coagulation parameters in animal models (87, 88). Hence, it would be interesting to examine whether these substances have the potential for endometriosis therapy.

4.2. Role of proteinase-activated receptor 2 in endometriosis

Accumulating evidence supports that endometriosis has characteristics of an allergic disease in which mast cells play an essential role in allergic inflammation (89-94). High numbers of activated mast cells are present in endometriosis sites that are strongly positive for corticotropin-releasing hormone and urocortin both of which can activate mast cells (95, 96). Concentrations of SCF, which stimulates mast cells, are increased in peritoneal fluid of women with endometriosis (60). Invasions of mast cells and degranulation as well as proliferation of interstitial component have been documented in endometriotic lesions from patients with endometriosis (90, 95). In addition, a relation between endometriosis and allergy is also supported by the finding that interstitial hyperplasia, which is a major step of infiltration and lesion of mast cells, is significantly inhibited by the administration of a leukotriene (LT) antagonist that has antiallergic action in the rat endometriosis model (97-100). Collectively, endometriosis has the typical characteristics of type I allergy.

Tryptase is a representative serine proteinase released by activated mast cells in endometriotic lesion (89, 95). We demonstrated that PAR_2, which is activated by mast cell-derived tryptase, is expressed in endometriotic stromal cells (7). This finding indicates possible involvement of tryptase-induced activation of PAR_2 in endometriosis. PAR_2 in endometriotic stromal cells may also be activated by TF-VIIa complex and TF-VIIa-Xa complex in view of the specific feature of the endometriosis, recurrent bleeding. Activation of PAR_2 induces an increase in IL-6 and IL-8 secretion by endometriotic stromal cells (7). IL-8 is suggested to promote the development of endometriosis as described above. Multiple lines of evidence indicate that IL-6 is also involved in the progression of the disease (101). The expression of IL-6 is increased in both endometriotic tissue and in peritoneal fluid of women with endometriosis (102, 103). IL-6 increases aromatase activity, haptoglobin production, and hepatocyte growth factor production in endometriotic cells and/or endometriotic cells (104-107). Furthermore, IL-6 directly stimulates the proliferation of endometriotic stromal cells (104). These findings indicate

that IL-6 stimulate the progression of endometriosis by various mechanisms such as cell proliferation, angiogenesis, and immunomodulation. Given the promoting roles of IL-6 and IL-8 in endometriosis, mast cell tryptase, TF-VIIa complex, and TF-VIIa-Xa complex could contribute to the development of the disease by activating PAR_2 which in turn increase IL-6 and IL-8 production. Viewed in a wider framework, PAR_2 appears to translate an allergic response and a coagulating response into an inflammatory response that develop the endometriosis. Another function of PAR_2 is that PAR_2 activation stimulates proliferation of endometriotic stromal cells (7). This also underscores the importance of PAR_2 in the pathogenesis of endometriosis. Interestingly, inhibitors of ERK1/2, JNK, and p38 MAPK suppress the PAR_2 activation-induced proliferation in endometriotic stromal cells (7), which is similar to the finding observed in endometrial stromal cells.

4.3. Proteinase-activated receptor 2 in mouse model of endometriosis

There are several model of endometriosis in mouse (108). We have developed an autotransplantation-type model. In this model, the uterus of donor mouse that is ovariectomized and treated with estradiol is removed and its endometrium is minced. The minced endometrium is injected using needle into the abdominal cavity of the recipient mice that is also ovariectomized and treated with estradiol. The advantage of this model is that the injected endometrium mimics the endometrium in retrograde menstruation in women. The characteristics of our method were demonstrated by the model using green fluorescent mouse and we have reported several studies using our method (109-112).

In view of the *in vitro* study showing the possible roles of PAR_2 in the establishment of endometriosis, we performed an an *in vivo* study using our endometriosis model with PAR_2-deficient mice (kindly provided by Kowa Co. Ltd.). As expected, both the number and the total weight of endometriotic lesions were significantly decreased in the PAR_2-deficient mice compared to the wild type mice (109). At the same time, concentrations of IL-6 and MCP-1 were decreased in the peritoneal fluid and the serum of the PAR_2-deficient mice. As described above, IL-6 and MCP-1 are suggested to promote endometriosis via inflammatory responses. Therefore, the decreased concentrations of IL-6 and MCP-1 in the PAR_2-deficient mice imply that endometriosis-associated inflammation is alleviated in the peritoneal cavity of the mice. Collectively, PAR_2 is suggested to be involved in the development of experimental endometriosis in mice and that the anti-inflammatory environment in PAR_2-deficient mice might hinder the progression of the disease. Combined with the findings of the *in vitro* study, PAR_2 would be argued as a target for the treatment of endometriosis. It is also noticed that p38 MAPK inhibitor impede the development of endometriosis in the similar mouse model (110). In light of the finding that p38 MAPK inhibitor suppress the PAR_2 activation-induced proliferation of human endometriotic stromal cells, the inhibitory effect of the p38 MAPK inhibitor on endometriosis observed in the mouse model may partly dependent on the inhibition of the PAR_2 function.

5. PERSPECTIVE

PAR_1 and PAR_2 appear to play multiple roles in the physiologies and pathologies of the endometrium. They are also suggested to play important functions in the pathogenesis and pathophysiology of endometriosis. Therefore, it is expected to modulate the endometrial function and treat endometriosis by regulating the expression and the function of PAR_1 and PAR_2. Stated differently, the PARs themselves, their activating serine proteinases and their associated signaling pathways can be considered as attractive target for therapeutic drug development. In this context, it is remarkable that several agonists and antagonists for PAR_1 and PAR_2 have been developed (113) and an oral PAR_1 antagonist is under clinical trial for the treatment and prevention of atherothrombosis (114). It is thus warranted to study more on PARs in the endometrium and endometriosis in order to find PAR targeting therapies as well as to understand the mechanism of the disease.

6. ACKNOWLEDGEMENT

This work is partly supported by grants from the Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology.

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Key Words: Endometrium, Endometriosis, Proteinase-activated receptor, Review

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