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Aberrant Expression of Apoptosis-Related Molecules in Endometriosis: A Possible Mechanism Underlying the Pathogenesis of Endometriosis

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Abstract
Endometriosis, a disease affecting 3% to 10% of women of reproductive age, is characterized by the ectopic growth of endometrial tissue under the influence of estrogen. It is also becoming recognized as a condition in which ectopic endometrial cells exhibit abnormal proliferative and apoptotic regulation in response to appropriate stimuli. Apoptosis plays a critical role in maintaining tissue homeostasis and represents a normal function to eliminate excess or dysfunctional cells. Accumulated evidence suggests that, in healthy women, endometrial cells expelled during menstruation do not survive in ectopic locations because of programmed cell death, while decreased apoptosis may lead to the ectopic survival and implantation of these cells, resulting in the development of endometriosis. Both the inability of endometrial cells to transmit a “death” signal and the ability of endometrial cells to avoid cell death have been associated with increased expression of antiapoptotic factors and decreased expression of proapoptotic factors. Further investigations may elucidate the role of apoptosis-associated molecules in the pathogenesis of endometriosis. Medical treatment with apoptosis-inducing agents may be novel and promising therapeutic strategy for endometriosis.

Keywords
endometriosis, apoptosis, proliferation, pathogenesis, medical treatment

Introduction
Endometriosis, the presence of endometrial tissue containing both glands and stroma outside the uterine cavity, is a persistent disease affecting 10% of the general female population.1 Classic symptoms of endometriosis are dyspareunia, chronic pelvic pain, dysmenorrhea, and infertility. Affected women often have a poor quality of life and the disease is associated with significant health costs.2 Among infertile women, the prevalence of endometriosis is 20% to 40%, and the presence of endometriosis decreases the success rate of in vitro fertilization.3 Moreover, there is an association between untreated endometriosis and development of ovarian cancer.4

Apoptosis plays a critical role in maintaining tissue homeostasis and represents a normal function to eliminate excess or dysfunctional cells. Apoptosis can be initiated by extracellular or intracellular “death signals.” Apoptosis results from a series of related morphologic and biochemical processes. Morphologically, apoptotic cells present with condensed chromatin, multiple membrane-bound organelles (apoptotic bodies), and shrunken appearance. Biochemically, apoptosis is characterized by monomeric or multimeric 180-bp nucleosomal fragments resulting from the cleavage of double-stranded nuclear DNA.5,6 Apoptosis is controlled by the expression of a number of regulatory genes, including c-myc, p53, Fas, nuclear factor (NF)-κB, and members of the B-cell lymphoma/leukemia 2 (Bcl-2) family.7-12

Endometriosis is increasingly being recognized as a condition in which ectopic endometrial cells exhibit abnormal proliferative and apoptotic regulation in response to appropriate stimuli.13 To explain the specific behavior of endometriotic cells, much effort has been devoted to identifying cellular differences among endometriotic lesions, the
The morphology of the eutopic endometrial tissue of women with endometriosis is similar to that of the normal endometrium, but its physiology and biochemistry are different. Recent reports show an abnormal survival capability at the epithelial and stromal levels of the eutopic endometrium of patients with endometriosis that may result in its continuous growth.\textsuperscript{14,15}

**Apoptosis in Endometriotic Lesions and the Eutopic Endometrium of Women With and Without Endometriosis**

The aim of this article is to review the information available on the mechanisms of dysregulated apoptosis in endometriotic lesions and the eutopic endometrium of patients with endometriosis and their possible implications in the pathogenesis of endometriosis. In addition, the role of apoptosis-associated molecules in the treatment of endometriosis is reviewed to link the basic research findings with potential clinical applications.

**Apoptosis in the Normal Endometrium**

The endometrial cycle in regularly menstruating women consists of 3 distinct phases, namely, a proliferative, a secretory, and a menstrual phase. Accumulating evidence suggests that apoptosis helps to maintain cellular homeostasis during the normal menstrual cycle, through the elimination of senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phases of the cycle.\textsuperscript{16-18} This is followed by proliferation of new cells from the basal layer during the proliferative phase of the cycle. In the late secretory phase, cell death by apoptosis increases in the functional layer of the normal endometrium. Specific nuclear DNA fragmentation related to the apoptotic process has been shown in human endometrium.\textsuperscript{19} Hopwood and Levison\textsuperscript{16} reported that some human endometrial cells appear apoptotic when observed by electron microscopy.

**Apoptosis in the Eutopic Endometrium in Endometriosis**

In the late secretory phase, cell death by apoptosis increases in the functional layer of the normal endometrium. However, the...
expected increase of apoptotic cells during the late secretory phase is not observed in the eutopic endometrium of women with endometriosis.20-25 The number of apoptotic cells is lower in both the epithelium and stroma of the eutopic endometrium of women with endometriosis compared to those of normal controls.26 In addition, in the eutopic endometrium of women with endometriosis, both epithelial and stromal cells have higher proliferative capacity than those of the normal endometrium.25,27

Apoptosis in the Endometriotic Lesions

The survival of endometriotic cells at the ectopic site has also been investigated from the viewpoint of susceptibility of endometriotic tissues to apoptosis.20-22,28-32 It has been demonstrated that apoptosis in endometriotic lesions is lower than that in the endometrium of the same patients and that of control women.20-22 At present, decreased susceptibility of endometriotic epithelial and stromal cells to apoptosis is considered to contribute to the pathogenesis of endometriosis.20-22,28,29,31,32 The resistance of endometriotic cells to apoptosis is considered to be either intrinsic or brought about by environmental factors.

Endometriotic cells are resistant to macrophage-mediated cytotoxicity,21 interferon-γ-induced apoptosis,31 and staurosporine-induced apoptosis.32 Endometriotic stromal cells have greater proliferative capacity than eutopic endometrial stromal cells.33 The survival of endometriotic cells may antagonize caspase-3-mediated apoptosis.34

Jones et al25 reported that there was no apoptosis in the endometriotic stromal cells from peritoneal endometriotic tissue. No phenomenon of apoptosis has been demonstrated in endometriotic epithelial cells from ovarian endometriosis.36 On the other hand, Harada et al28 found that apoptosis was increased in ovarian endometriosis. Beliard et al15 also demonstrated reduced apoptosis in endometriotic lesions. A meaningful evaluation of these conflicting data is hampered by their use of different methodologies and different endometriotic lesions (ovarian vs peritoneal endometriosis). Ovarian and peritoneal endometriosis are thought to have different pathogenetic origins. Therefore, these endometriotic lesions could have different biological features. The manipulation of cell lines derived from endometriotic lesions at different locations may provide a valuable experimental system to study the molecular and cellular processes underlying the pathogenesis of the disease.

Aberrant Expression of Proapoptotic Factors, Anti-Apoptotic Factors, Cell Cycle Regulators, and Other Molecules Related to Proliferation, Differentiation, Inflammation, and Homeostasis in Endometriosis

Apoptosis is tightly regulated by a variety of regulatory proteins. Of these modulators, the Bcl-2 family proteins are key regulators of apoptosis that include both antiapoptotic proteins (eg, Bcl-2, Bcl-XL, Mcl-1, and A1) and proapoptotic proteins (eg, Bak, Bax, Bad, and Bid). The Bcl-2 protein has been extensively studied in human endometriotic tissues. In contrast, few data have been reported on the expression of other apoptosis-related proteins, such as Bax and Fas.

In a study using complementary DNA (cDNA) microarray analysis, several apoptosis-related genes were shown to be downregulated in endometriotic tissues.37 This finding is consistent with the decreased spontaneous apoptosis observed in the eutopic and ectopic endometria of women with endometriosis. This observation could provide useful information for finding candidate genes whose products might regulate the apoptotic machinery in endometriosis and, additionally, could be used as molecular targets for diagnosis or treatment of endometriosis (Figure 1).

As described below, widely different expression levels of apoptosis-related protein have been reported at different sites of endometriotic lesions.13,28,36,38 These differences in the expression of apoptosis-related proteins according to the locations of endometriosis suggest the involvement of different apoptotic pathways and could be explained by different etiopathologies.39

Apoptosis-regulatory molecules

Bcl-2

The Bcl-2 protein is probably the best characterized of the apoptosis-related molecules. The Bcl-2 gene defines a new class of proto-oncogenes that block cell death by regulating mitochondrial membrane function without promoting cell proliferation.40,41 It is now clear that the action of Bcl-2 depends on the concentration of, and interaction with, a potential antagonist protein, Bax. Bax is a 21-kDa protein of the Bcl-2 gene family that shares high amino acid homology with Bcl-2, heterodimerizes with Bcl-2, and homodimerizes with itself. When Bcl-2 is overexpressed, Bcl-2 heterodimerizes with Bax and cell death is repressed.42 Thus, the ratio of Bcl-2-to-Bax is important in determining susceptibility to apoptosis.43

Early studies provided conflicting data regarding the difference in Bcl-2 expression between normal endometrial tissue and eutopic/ectopic endometrial tissue in women with endometriosis: Bcl-2 was negative in almost all samples from ovarian endometriosis,28,36 but it was significantly increased in both endometriotic epithelial and stromal cells in the other studies.35,44 Several detailed studies demonstrated that Bcl-2 expression was detected exclusively in glandular epithelial cells and stromal cells of endometriotic tissue and the eutopic endometrium with or without endometriosis, with a peak expression in the proliferative phase.14,45 Previous reports have described a reduced Bcl-2 expression in ovarian endometriosis.36,45 whereas peritoneal endometriosis has been reported to show high Bcl-2 expression.15 Other studies have shown that Bcl-2 expression is lower in cystic than in noncystic endometriotic lesions,36,38 suggesting that Bcl-2 expression differs according to the location of endometriotic lesions.13,46 In primary cultures, upregulated expression of
Bcl-2 protein has been observed in endometriotic stromal cells of ovarian endometriosis in comparison with eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31} Peritoneal macrophages from women with endometriosis are resistant to apoptosis, based on their increased expression of the antiapoptotic protein Bcl-2.\textsuperscript{45}

Estrogen receptor expression has been shown to be higher in endometriotic lesions than in eutopic endometrial tissue from the same patients.\textsuperscript{47} The elevated concentrations of estrogen receptors in endometriosis could lead to an upregulation of Bcl-2, thereby preventing apoptosis of stromal or epithelial cells.\textsuperscript{47,48}

**Bcl-X**

Bcl-X is another member of the Bcl-2 family of genes and provides an interesting example of a single gene that, via alternative splicing mechanisms, encodes either a positive or a negative regulator of apoptosis.\textsuperscript{49} The long form of Bcl-X (Bcl-X\textsubscript{L}) contains an open reading frame of 233 amino acids with 2 domains homologous to Bcl-2, whereas Bcl-X\textsubscript{S} contains 170 amino acids truncated from Bcl-X\textsubscript{L} in which the region with highest homology to Bcl-2 has been deleted.\textsuperscript{49} These 2 forms of Bcl-X have opposing functions in that Bcl-X\textsubscript{L} renders cells resistant to apoptotic cell death upon deprivation of growth factors, whereas Bcl-X\textsubscript{S} counters the resistance to apoptotic cell death conferred by Bcl-2.\textsuperscript{42,49} Upregulated expression of the Bcl-X\textsubscript{L} protein has been observed in endometriotic stromal cells from ovarian endometriotic tissue in comparison with eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31} Whereas, Zubor et al\textsuperscript{50} demonstrated the increased Bcl-X\textsubscript{S} messenger RNA (mRNA) expression in the eutopic endometrium of women with endometriosis compared to those of healthy controls. Recently Braun et al\textsuperscript{51} analyzed the expression of apoptosis-regulating genes with expression not only as a single gene but also through the evaluation of the Bcl-X\textsubscript{L}/Bcl-X\textsubscript{S} ratio and revealed substantially higher levels in eutopic endometrial cells from women with endometriosis compared to endometrial cells in women without endometriosis.

**Bax**

Bax is a Bcl-2 family member that promotes cell death susceptibility, possibly by countering the effect of Bcl-2 on cellular survival through heterodimer interaction.\textsuperscript{42} Bax expression has been detected exclusively in the glandular epithelial cells of endometriotic tissue and eutopic endometrium with or without endometriosis, throughout the menstrual cycle without cyclic changes.\textsuperscript{45,52} Bax is highly expressed in the epithelial cells of ovarian endometriosis.\textsuperscript{53} Goumenou et al\textsuperscript{54} found a strong correlation between high Bax and low Bcl-2 expression in ovarian endometriosis. Although the difference is not statistically significant, Bax expression tends to be lower in colorectal than in ovarian endometriosis.\textsuperscript{13} In primary cultures, Bax protein expression in endometriotic stromal cells of ovarian endometriotic tissue was similar to that in eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31}

**Bad**

Bad is a preapoptotic factor of the Bcl-2 family protein. Bad is phosphorylated and sequestered in the cytosol by the 14-3-3 protein.\textsuperscript{55} Upon dephosphorylation, Bad translocates to the mitochondria, where it associates primarily with Bcl-X\textsubscript{L}, but also with Bcl-2, and thereby promotes apoptosis.\textsuperscript{56,57} The levels of the Bad protein in endometriotic stromal cells from ovarian endometriotic tissue have been shown to be similar to those in eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31}

**Fas-Fas ligand (FasL)**

Fas, also called APO-1 or CD95, is a type I membrane protein of 45 kDa that belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family.\textsuperscript{59} Fas ligand, a type II membrane protein of 37 kDa, belongs to the TNF superfamily.\textsuperscript{58} The Fas-FasL system is a major pathway for the induction of apoptosis in a variety of cells and tissues.\textsuperscript{59} Fas-bearing cells undergo apoptotic cell death when they interact with Fas ligand.\textsuperscript{9} The Fas-FasL system has been suggested to be the mediator of the direct action of gonadotropin-releasing hormone (GnRH) analogues on endometriotic cells.\textsuperscript{72}

Fas expression has been observed in eutopic endometrial cells.\textsuperscript{28} It was also detected in the endometriotic epithelial and stromal cells from ovarian endometriotic tissue.\textsuperscript{31,36} By immunohistochemistry, eutopic endometrial stromal cells from women with endometriosis demonstrated higher Fas expression compared with those from women without endometriosis.\textsuperscript{60} Fas expression is higher in peritoneal endometriosis than in the normal eutopic endometrium.\textsuperscript{13} However, Fas expression in ovarian and colorectal endometriosis is lower than that in the normal eutopic endometrium.\textsuperscript{13} In primary culture, the levels of Fas protein in endometriotic stromal cells of ovarian endometriotic tissue were similar to those in eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31}

In primary culture, the levels of Fasl protein in endometriotic stromal cells of ovarian endometriotic tissue were found to be similar to those in eutopic endometrial stromal cells from women with and without endometriosis.\textsuperscript{31} Upregulation of Fasl expression by endometriotic cells could be induced after the adhesion of these cells to the extracellular matrix proteins laminin, fibronectin, and collagen IV.\textsuperscript{60} Fasl expressed on endometriotic cells may induce apoptosis of the local immune cells, including activated T lymphocytes, thereby reducing attacks by host immune surveillance and promoting the survival of endometriotic cells.

Fas and Fasl are proteins that exist in both transmembrane and soluble forms. Soluble Fasl can be proteolytically cleaved from membrane-bound Fasl by metalloproteinases.\textsuperscript{61,62} Transmembrane Fas triggers apoptosis when bound by Fasl, whereas soluble Fas (sFas) inhibits Fas-mediated apoptosis.
by preventing death signal transduction. Hence, Fas-mediated apoptosis is a result of receptor-ligand interactions, whereas sFas acts as a functional antagonist of FasL-mediated apoptosis. Serum levels of sFas have been shown to be significantly lower in patients with endometriosis. Further, women with moderate-to-severe endometriosis have elevated serum and peritoneal fluid concentrations of sFasL.

**Tumor Necrosis Factor-α and TNF Receptor**

Tumor necrosis factor-α has a wide variety of biological activities, possessing noticeably proinflammatory, antitumor, and apoptotic actions. Tumor necrosis factor-α derived from the normal human endometrium has been shown to induce apoptosis in endometrial glandular epithelial cells, suggesting that this cytokine plays a role in menstrual shedding. Tumor necrosis factor-α promotes the proliferation of endometriotic stromal cells through induction of interleukin (IL)-8 expression. These receptors are encoded by 2 independent genes, and each receptor is capable of mediating distinct intracellular signals. Cytotoxic, antiproliferative, and apoptosis-inducing effects of TNF-α are mediated by TNF-RII.

Several studies have shown that TNF-α concentrations in the peritoneal fluid are higher in women with endometriosis than in normal women. Tumor necrosis factor-α levels in the peritoneal fluid and the stage of endometriosis have been found. Tumor necrosis factor-α levels in the peritoneal fluid and the stage of endometriosis have been shown to be significantly lower in patients with endometriosis.

**Inhibitor of Apoptosis Proteins**

Survivin, cellular inhibitor of apoptosis protein (cIAP) 1, cIAP2, and X chromosome-linked IAP (XIAP) are members of the IAP gene family. Inhibitor of apoptosis proteins directly inhibit the terminal effector caspases 3 and 7, and thus protect the cells from apoptosis.

Endometriotic cells express more survivin genes than normal endometrial cells from women without endometriosis. Endometriotic cells also show enhanced expression of survivin-2B and survivin-EX3, splice variants of survivin. Interestingly, staurosporine induces the survivin expression in both endometriotic stromal cells and eutopic endometrial stromal cells.

**Caspase 1**

Caspase 1, also known as interleukin 1 converting enzyme (ICE), proteolytically processes the immature IL-1β from a 33-kDa precursor to the 17-kDa mature form. Caspase 1 mediates the programmed cell death which is distinct from other forms of "classical apoptosis." Caspase-1-dependent apoptosis do not require the key elements of the mitochondrial pathway, such as caspase 3, Bcl-2, and Bcl-XL, death pathway.

**Caspase 3 and DNA fragmentation factor (DFF) 40/DFF45**

DFF is a dimer consisting of 40 kDa subunit (DFF40/CAD) with nuclease activity and 45 kDa subunit (DFF45/ICAD), which acts as a DFF40 chaperone and is a substrate for caspase 3. DFF45 cleavage by caspase 3, the DFF40 is released and joins H1 histone, causing DNA fragmentation. In viable cells, there is a stoichiometric 1:1 ratio of DFF40/DFF45 and DFF45 is required.
for proper DFF40 synthesis. Thus, DFF45 acts not only as an inhibitor but also as a chaperone allowing the DFF40 to properly fold and gain nuclease activity.91 Banas et al92 demonstrated a decreased level of DFF45 observed in ovarian endometriosis.

**Cell Cycle Regulators**

**p53**

The *p53* gene and its encoded protein are related to cell cycle regulation, cellular growth, and apoptosis and are gatekeepers or guardians of cell division.93,94 *p53* expression is higher in ovarian and colorectal endometriotic tissue than peritoneal endometriotic tissue or the normal eutopic endometrium.95 In contrast, weak *p53* expression has been detected in peritoneal endometriotic and the eutopic endometrium from women with and without endometriosis.15,95,96 Eutopic and ectopic endometrial cells from women with endometriosis expressed decreased *p53* mRNA expression compared to endometrial cells from women without endometriosis.51 On the other hand, Zubor et al97 demonstrated the increased *p53* mRNA expression in the eutopic endometrium of women with endometriosis compared to those of healthy controls. These findings suggest that the regulation of apoptosis may be site-specific, with different regulation occurring among the endometriotic lesions in different locations. Recently, Chang et al97 reported that endometriosis was associated with *p53* polymorphism and that heterozygotes and proline homozygotes had a higher risk of endometriosis.

**p21**

The *p53*-inducible gene, *p21*, encodes an inhibitor of cyclin-dependent kinases involved in G1 arrest.98-100 *p21* is highly expressed in the endometriotic epithelial cells of ovarian endometriosis.101 *p21* expression is higher in ovarian endometriotic tissue than in peritoneal and colorectal endometriotic tissue and the normal eutopic endometrium.13 There is a positive correlation between *p53* and *p21* expressions in endometriosis.13

**c-myc**

c-*myc* is a key element in controlling cell proliferation, differentiation, and apoptosis.101,102 This oncoprotein heterodimerizes with partner Max to form a complex that binds to DNA in a sequence-specific manner, leading to cell cycle progression and transformation by triggering transcriptional activation of its downstream genes, such as *c-fos* and *Bcl-2*.103,104 *c-myc* expression is upregulated in the eutopic endometrium from women with endometriosis compared with the level in the normal endometrium.25

**Nuclear Transcriptional Factor**

**Nuclear Factor-κB**

The pleiotrophic transcription factor NF-κB has been identified as a critical component of several signal transduction pathways.85 One important function of NF-κB is its ability to protect cells from apoptosis by activating antiapoptotic genes.10,11 Recent studies indicate that NF-κB is constitutively active in a number of malignant tumors, such as breast cancer and prostate cancer, and that it plays an antiapoptotic role in the survival of these tumor cells.105-107 Wieser et al108 have demonstrated the constitutive activation of NF-κB in endometriotic cells. It is suggested that NF-κB may play a significant role in the proliferation of endometriotic lesions.109,110

Tumor necrosis factor-α and estradiol induce NF-κB activation in endometriotic stromal cells, whereas progesterone and danazol inhibit NF-κB activation in endometriotic stromal cells.111 Activation of NF-κB by LPS induces proliferation of endometriotic stromal cells.112 Nuclear factor-κB inhibitors, such as N-tosyl-L-phenylalanine chloromethyl ketone and BAY 11-7085, have been shown to significantly block the proliferation of endometriotic stromal cells.112,113 BAY 11-7085 induces apoptosis and G0/G1 phase cell cycle arrest of endometriotic stromal cells.113 Additionally, downregulation of the Bcl-2 and Bcl-XL expression with simultaneous activation of caspase 3, caspase 8, and caspase 9 was observed in endometriotic stromal cells after treatment with BAY 11-7085.113 Suppression of NF-κB activity by proteasome inhibitors also suppresses proliferation of endometriotic cells in vitro.110 Nuclear factor-κB inhibitors, BAY 11-7085 and SN-50, significantly reduce the development of endometriotic lesions in a nude mice model.114

**Serine/Threonine Kinases**

**Mitogen-Activated Protein Kinases**

To date, 3 types of mitogen-activated protein kinases (MAPKs) have been well characterized, that is, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK. Phosphorylation of MAPKs induces their activities to phosphorylate downstream substrates, thereby regulating various cellular functions, including gene expression, mitosis, movement, metabolism, and apoptosis.115 It has been reported that ERK, JNK, and p38 MAPK are expressed in endometriotic cells.116 The p38 MAPK phosphorylation rates in the endometriotic tissues are significantly higher than those in the eutopic endometrium of the same patients with endometriosis.116 Mitogen-activated protein kinases have been suggested to act as pivotal intracellular signal transducers in endometriotic cells and thus have a pathophysiological role in the development of this disease.116 Estrogen induces ERK1/2 phosphorylation in eutopic endometrial stromal cells from patients with endometriosis but not in endometrial stromal cells from women without endometriosis.117 ERK1/2 inhibition reduces proliferation and increases apoptosis of endometrial stromal cells.117

**Protein Kinase B/Akt**

Protein kinase B/Akt, a serine/threonine kinase, regulates the function of many cellular proteins involved in apoptosis and
proliferation. The main pathway for Akt phosphorylation is the phosphatidylinositol-3-kinase (PI3K) secondary messenger system. After the activation of certain growth factor receptors, protein tyrosine kinases result in autophosphorylation of tyrosine residues, triggering phosphatidylinositol-4,5-biphosphate to generate phosphatidylinositol-3,4,5-triphosphate, which is a secondary messenger. This in turn induces Akt phosphorylation on serine 473 and/or threonine 308, transforming it to its active form, phosphorylated-Akt. Akt phosphorylation has an antiapoptotic role by inhibiting Bad, procaspase 9, forkhead family transcription factors, and TRAIL. Higher Akt phosphorylation was revealed in ectopic and eutopic endometrium from patients with endometriosis compared with normal endometrium. It is suggested that increased Akt phosphorylation may be related to the altered apoptosis/proliferation harmony in endometriosis. Estrogen is considered to be one of the factors responsible for the high Akt activation in endometriotic cells.

**Other Molecules**

**Calpain 5**

Calpains are cytoplasmic calcium-dependent cysteine proteases that have been implicated in several different cellular activities including the regulation of apoptosis and cell differentiation. Penna et al demonstrated that Calpain 5 protein expression was decreased in both stromal and glandular cells from women with endometriosis compared with that of fertile controls. The authors also reported that Calpain 5 expression is regulated by HOXA10 and that decreased Calpain 5 expression in endometriosis is considered as a result of decreased HOXA10 expression.

**Ubiquitin**

Ubiquitin is a 76-amino acid protein that is involved in the degradation of short-lived, regulatory, or misfolded proteins, thereby maintaining cellular homeostasis. Ubiquitin tags these proteins to be taken to the proteasome and in some instances also to the lysosomal machinery to prevent damage of cells. Immunohistochemical analysis has indicated that ubiquitin is predominantly expressed in the endometriotic stromal cells. It is suggested that ubiquitin may contribute to a reduced sensitivity to apoptosis in the endometriotic tissue.

**Cyclooxygenase 2**

Cyclooxygenase 2 (COX-2), a rate-limiting enzyme in the biosynthesis of prostaglandin E2 (PGE2), is highly expressed in endometriotic tissues and results in increased concentrations of peritoneal PGE2 in women with endometriosis than in disease-free women. ProstaglandinE2 modulates expression of stromal cell HOXA10 expression and that decreased Calpain 5 expression was decreased in both stromal and glandular cells from women with endometriosis compared with that of fertile controls. The authors also reported that Calpain 5 expression is regulated by HOXA10 and that decreased Calpain 5 expression in endometriosis is considered as a result of decreased HOXA10 expression.

**Apoptosis-Inducing Properties of Current Medical Treatment for Endometriosis**

Medical therapies historically have included contraceptive steroids, progestogens, and agonists of GnRH, as well as androgens and nonsteroidal anti-inflammatory agents. Current medical treatment aims to inhibit the growth of endometriotic implants by suppressing ovarian steroids and inducing a hypoestrogenic state, of the medical agents mentioned above, GnRH agonists have gained predominance in the medical treatment of endometriosis.

New therapies are likely to be based on the numerous molecular targets, including progesterone receptors, estrogen receptors, aromatase, angiogenic factors, metalloproteinases, cytokines and chemokines, haptoglobin, peroxisome proliferator-activated receptor-γ, and antioxidants. Some of these agents have apoptosis-inducing properties.

**Gonadotropin-Releasing Hormone Agonists**

Gonadotropin-releasing hormone agonists suppress the release of follicle-stimulating hormone and luteinizing hormone from...
the pituitary gland, and inhibit ovarian steroidogenesis, resulting in a hypoestrogenic state that is suitable for the remission of endometriotic lesions. In addition, both eutopic and ectopic endometrial tissue in women with endometriosis have apparent GnRH receptors, suggesting that GnRH may function as a direct regulator of their growth. Gonadotropin-releasing hormone agonist increases the apoptotic rate of ectopic and eutopic endometrial cells in endometriosis by upregulating the expression of Bcl-2. This agent also reduces the cell proliferation of eutopic endometrial epithelial cells in women with endometriosis, while endometrial cells from women without endometriosis are not affected. Gonadotropin-releasing hormone agonist inhibits the expression of phosphorylated inhibitor-kB in endometriotic stromal cells, indicating the suppression of NF-kB inactivation in these cells.

**Progesterone**

Progesterone and other synthetic progestins commonly used in the clinical management of endometriosis can suppress the NF-kB pathway. Combination oral contraceptives significantly diminish cell proliferation and induce apoptosis of eutopic endometrial tissue from patients with endometriosis. These agents induce Bax expression in the eutopic endometrium from women with endometriosis, whereas they reduce Bcl-2 expression in these tissues. Cell proliferation was significantly reduced in the epithelium and stroma of both the eutopic and the ectopic endometrium after treatment with the levonorgestrel-releasing intrauterine system. The use of levonorgestrel-releasing intrauterine system induces Fas expression in the epithelial cells of the ectopic and eutopic endometrium.

**Danazol**

Recent evidence suggests that danazol can act directly on endometriotic tissue in vitro to inhibit DNA synthesis and induce apoptosis.

**Aromatase Inhibitors**

Aromatase expression is consistently found in endometriotic lesions and in the eutopic endometrium from patients with endometriosis, whereas it is absent in the eutopic endometrium from women without endometriosis. Zeitoun and Bulun demonstrated that aromatase was a key molecule in the pathophysiology of endometriosis and that its inhibition may be a novel therapeutic strategy. Aromatase inhibitors inhibit proliferation and induce apoptosis of eutopic endometrial epithelial cells in patients with endometriosis. Using murine model, Bilotas et al demonstrated that aromatase inhibitors decrease cell proliferation and increase apoptosis of endometriosis.

**Conclusions**

Based on the above findings, apoptosis plays a major role in the pathophysiology of endometriosis. Altered expression of apoptosis-related genes in women with endometriosis may explain individual susceptibility to the disease and may answer the question of why only some women develop endometriosis. Although the principal mechanisms by which endometriotic cells acquire antiapoptotic properties have not been elucidated, there might be 2 possible mechanisms: (1) endometriotic cells are genetically resistant to some apoptotic stimuli, or (2) intrauterine environments in endometriotic patients cause resistance to apoptotic stimuli in endometriotic tissues. Otherwise, our present review could not prove the Sampson’s theory that endometriotic cells originate from the refluxed menstrual endometrial cells. Further studies may establish a cause–effect relationship between the dysregulated apoptosis and the pathogenesis of endometriosis.

The data presented in this review may indicate that apoptosis plays a role in the pathophysiology of endometriosis. Manipulation of the cell death processes in endometriosis would be useful for treating the disease. Recently, a variety of compounds that can regulate apoptosis in endometriotic cells have been examined in experimental studies. Some of these apoptosis-inducing agents may be utilized in the clinical treatment for endometriosis in the future. Additional detailed studies focusing on the process of apoptosis should be undertaken in order to clarify the pathogenesis of endometriosis in the different sites and to determine appropriate therapeutic modalities.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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