

Review

Involvement of resistance to apoptosis in the pathogenesis of endometriosis

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Summary. Endometriosis, a disease affecting 3-10% of women of reproductive age, is characterized by the ectopic growth of endometrial tissue. Increasingly, endometriosis 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 anti-apoptotic factors and decreased expression of pre-apoptotic factors.

This paper is a review of the recent literature focused on the differential expression of apoptosis-associated molecules in the normal endometria of women without endometriosis, and in the eutopic and ectopic endometria of women with endometriosis. The role of apoptosis in the pathogenesis of endometriosis and the basic and clinical research on the current medical treatment for endometriosis from the view of apoptosis will be discussed.

Key words: Endometriosis, Apoptosis, Proliferation, Pathogenesis, Medical treatment

Introduction

Endometriosis, the growth of functional endometrial tissue containing both glands and stroma outside the uterine cavity, is a chronic recurrent disease affecting 10% of the general female population (Strathy et al., 1982). 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 (Garry et al., 2000). Among infertile women, the prevalence of endometriosis is 20 to 40%, and the presence of endometriosis decreases the success rate of *in vitro* fertilization (Yanushpolsky et al., 1998). Moreover, there is an association between untreated endometriosis and development of ovarian cancer (Brinton et al., 1997).

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-base pair nucleosomal fragments resulting from the cleavage of double-stranded nuclear deoxyribonucleic acid (DNA) (Kerr et al., 1972; Oberhammer et al., 1993). Apoptosis is controlled by the expression of a number of regulatory genes, including *c-myc*, p53, Fas, nuclear factor (NF)- κ B, and members of the B-cell lymphoma/leukemia-2 (Bcl-2) family (White, 1993; Osborne and Schwartz, 1994; Nagata and Golstein, 1995; Beg and Baltimore, 1996; Van Antwerp et al., 1996; Sattler et al., 1997).

Endometriosis is increasingly being recognized as a condition in which ectopic endometrial cells exhibit abnormal proliferative and apoptotic regulation in

response to appropriate stimuli (Dufournet et al., 2006). To explain the specific behavior of endometriotic cells, much effort has been devoted to identifying cellular differences among endometriotic lesions, the eutopic endometrium of women with endometriosis, and the normal endometrium of women without endometriosis.

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

Apoptosis in endometriotic lesions and the eutopic endometrium of women with and without endometriosis

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 (Meresman et al., 2000; Beliard et al., 2004).

Apoptosis in the normal endometrium

The endometrial cycle in regularly menstruating women consists of three 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 (Hopwood and Levison, 1976; Kokawa et al., 1996; Shikone et al., 1996). 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 (Tabibzadeh, 1996). Hopwood and Levison (1976) 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 (Dmowski et al., 1998, 2001; Gebel et al., 1998; Imai et al., 2000; Braun et al., 2002; Johnson et

al., 2005). 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 (Szymanowski, 2007). 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 (Wingfield et al., 1995; Johnson et al., 2005).

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 (Harada et al., 1996; Dmowski et al., 1998; Gebel et al., 1998; Imai et al., 2000; Selam and Arici, 2000; Harada et al., 2004; Nishida et al., 2005; Izawa et al., 2006). 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 (Dmowski et al., 1998; Gebel et al., 1998; Imai et al., 2000). At present, decreased susceptibility of endometriotic epithelial and stromal cells to apoptosis is considered to contribute to the etiology of endometriosis (Harada et al., 1996; Dmowski et al., 1998; Gebel et al., 1998; Imai et al., 2000; Selam and Arici, 2000; Nishida et al., 2005; Izawa et al., 2006). 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 (Gebel et al., 1998), interferon- γ -induced apoptosis (Nishida et al., 2005), and staurosporine-induced apoptosis (Izawa et al., 2006). Endometriotic stromal cells have greater proliferative capacity than eutopic endometrial stromal cells (Klemmt et al., 2006). The survival of endometriotic cells may antagonize caspase-3-mediated apoptosis (Peiro et al., 2001).

Jones et al. (1998b) 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 (Suganuma et al., 1997). On the other hand, Harada et al. (1996) found that apoptosis was increased in ovarian endometriosis. Beliard et al. (2004) 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 endometrioses 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.

Apoptosis-related molecules

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 anti-apoptotic proteins (e.g., Bcl-2, Bcl-X_L, Mcl-1, and A1) and pro-apoptotic proteins (e.g., 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 cDNA microarray analysis, several apoptosis-related genes were shown to be downregulated in endometriotic tissues (Arimoto et al., 2003). 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.

As summarized in Table 1, widely different expression levels of apoptosis-related protein have been reported at different sites of endometriotic lesions (Harada et al., 1996; Suganuma et al., 1997; Nezhat et al., 2002; Dufournet et al., 2006). 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 (Bontis and Vavilis, 1997).

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 (Korsmeyer, 1992; Reed, 1997). 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 (Oltvai et al., 1993). Thus, the ratio of Bcl-2 to Bax is important in determining susceptibility to apoptosis (Chao and Korsmeyer, 1998).

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 (Harada et al., 1996; Suganuma et al., 1997), but it was significantly increased in both endometriotic epithelial and stromal cells in the other studies (Jones et al., 1998a,b). 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 (McLaren et al., 1997; Meresman et al., 2000). Previous reports have described a reduced Bcl-2 expression in ovarian endometriosis (McLaren et al., 1997; Suganuma et al., 1997), whereas peritoneal endometriosis has been reported to show high Bcl-2 expression (Beliard et al., 2004). Other studies have shown that Bcl-2 expression is lower in cystic than in non-cystic endometriotic lesions (Suganuma et al., 1997; Nezhat et al., 2002), suggesting that Bcl-2 expression differs according to the location of endometriotic lesions (Nezhat and Kalir, 2002; Dufournet et al., 2006). In primary cultures, upregulated expression of Bcl-2 protein has been observed in endometriotic stromal cells of ovarian endometriosis in comparison with eutopic

Table 1. Summary of the expression of apoptosis-related molecules in endometriotic lesions.

	Eutopic endometrium with endometriosis	Ovarian endometriotic cyst	Peritoneal endometriotic lesion
Bcl-2	Unaffected	Downregulated	Upregulated
Bcl-X _L	N.D.	Upregulated	N.D.
Bax	Unaffected	Upregulated	N.D.
Bad	N.D.	Unaffected	N.D.
Fas	Upregulated	Downregulated	Upregulated
TNF-RII	Downregulated	N.D.	N.D.
NF-κB	N.D.	Upregulated	N.D.
p38 MAPK	N.D.	Upregulated	N.D.
Survivin	N.D.	N.D.	Upregulated
Ubiquitin	N.D.	Upregulated	N.D.
p53	Unaffected	Upregulated	Unaffected
p21	N.D.	Upregulated	Unaffected
c-myc	Upregulated	N.D.	N.D.
COX-2	Upregulated	Upregulated	Upregulated
Caspase-1	Downregulated	Downregulated	Downregulated

N.D.: not described.

endometrial stromal cells from women with and without endometriosis (Nishida et al., 2005). Peritoneal macrophages from women with endometriosis are resistant to apoptosis, based on their increased expression of the anti-apoptotic protein Bcl-2 (McLaren et al., 1997).

Estrogen receptor expression has been shown to be higher in endometriotic lesions than in eutopic endometrial tissue from the same patients (Jones et al., 1995). The elevated concentrations of estrogen receptors in endometriosis could lead to an upregulation of Bcl-2, thereby preventing apoptosis of stromal or epithelial cells (Jones et al., 1995; Fujishita et al., 1997).

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 negative regulator of apoptosis (Boise et al., 1993). The long form of Bcl-X (Bcl-X_L) contains an open reading frame of 233 amino acids with two domains homologous to Bcl-2, whereas Bcl-X_S is a 170 amino acid truncated form of Bcl-X_L in which the region with highest homology to Bcl-2 has been deleted (Boise et al., 1993). These two forms of Bcl-X have opposing functions in that Bcl-X_L renders cells resistant to apoptotic cell death upon deprivation of growth factors, whereas Bcl-X_S counters the resistance to apoptotic cell death conferred by Bcl-2 (Boise et al., 1993; Oltvai et al., 1993). Upregulated expression of the Bcl-X_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 (Nishida et al., 2005).

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 (Oltvai et al., 1993). 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 (McLaren et al., 1997; Goumenou et al., 2004). Bax is highly expressed in the epithelial cells of ovarian endometriosis (Fauvet et al., 2003). Goumenou et al. (2001) 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 (Dufournet et al., 2006). 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 (Nishida et al., 2005).

Bad

Bad is a pre-apoptotic factor of the Bcl-2 family protein. Bad is phosphorylated and sequestered in the cytosol by the 14-3-3 protein (Downward, 1999). Upon dephosphorylation, Bad translocates to the mitochondria, where it associates primarily with Bcl-X_L, but also with Bcl-2, and thereby promotes apoptosis (Yang et al., 1995; Zhou et al., 2000). 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 (Nishida et al., 2005).

Fas-Fas ligand (FasL)

Fas, also called APO-1 or CD95, is a type I membrane protein of 45 kDa that belongs to the TNF/nerve growth factor receptor family (Nagata and Golstein, 1995). Fas ligand, a type II membrane protein of 37 kDa, belongs to the TNF superfamily (Suda et al., 1993). The Fas-FasL system is a major pathway for the induction of apoptosis in a variety of cells and tissues (Nagata, 1994). Fas-bearing cells undergo apoptotic cell death when they interact with Fas ligand (Nagata and Golstein, 1995). The Fas-Fas ligand system has been suggested to be the mediator of the direct action of GnRH analogues on endometriotic cells (Imai et al., 2000).

Fas expression has been observed in eutopic endometrial cells (Harada et al., 1996). It was also detected in the endometriotic epithelial and stromal cells from ovarian endometriotic tissue (Suganuma et al., 1997; Nishida et al., 2005). By immunohistochemistry, eutopic endometrial stromal cells from women with endometriosis demonstrated higher Fas expression compared with those from women without endometriosis (Selam et al., 2002). Fas expression is higher in peritoneal endometriosis than in the normal eutopic endometrium (Dufournet et al., 2006). However, Fas expression in ovarian and colorectal endometriosis is lower than that in the normal eutopic endometrium (Dufournet et al., 2006). 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 (Nishida et al., 2005).

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 (Nishida et al., 2005). 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 (Selam et al., 2002). 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 (Kayagaki et al., 1995; Powell et al., 1999). 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 (Ueno et al., 1999). Serum levels of sFas have been shown to be significantly lower in patients with endometriosis (Onalan et al., 2006). Further, women with moderate to severe endometriosis have elevated serum and peritoneal fluid concentrations of sFasL (Garcia-Velasco et al., 2002).

Tumor necrosis factor (TNF)- α and TNF-receptor

TNF- α has a wide variety of biological activities, possessing noticeably proinflammatory, anti-tumor, and apoptotic actions (Bazzoni and Beutler, 1996). TNF- 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 (Tabibzadeh, 1996). TNF- α promotes the proliferation of endometriotic stromal cells through induction of interleukin (IL)-8 expression (Iwabe et al., 2000). TNF- α exerts its biological effects via two cell-surface receptors, the TNF receptor type 1 (TNF-RI) (50-60 kDa) and the TNF receptor type 2 (TNF-RII) (75-80 kDa). These receptors are encoded by two independent genes, and each receptor is capable of mediating distinct intracellular signals. Cytotoxic, anti-proliferative and apoptosis-inducing effects of TNF- α are mediated by TNF-RII (Heller et al., 1992; Innins et al., 1992; Higuchi and Aggarwal, 1993; Tabibzadeh et al., 1995). TNF-RII expression is observed mainly in the endometrial glandular cells of the endometrium (Kharfi et al., 2003). High levels of TNF-RII expression have been reported in the normal endometrium (Chegini et al., 1999).

Several studies have shown that TNF- α concentrations in the peritoneal fluid are higher in women with endometriosis than in normal women (Halme et al., 1983; Mori et al., 1991; Harada et al., 1997; Iwabe et al., 2000). TNF- α levels are significantly elevated in the peritoneal fluid of patients with endometriosis (Iwabe et al., 2000). A possible correlation between TNF- α levels in the peritoneal fluid and the stage of endometriosis has been found (Mori et al., 1991).

Both TNF-RI and TNF-RII expressions have been demonstrated in endometriotic stromal cells (Iwabe et al., 2000; Nishida et al., 2004). A significant decrease in TNF-RII expression in endometrial glandular cells of patients with endometriosis compared to normal subjects has also been demonstrated (Kharfi et al., 2003). Based

on these observations, it is suggested that dysregulation of apoptosis in response to TNF- α in endometriosis may produce cyclical enhancement of endometriotic cell proliferation.

NF- κ B

The pleiotrophic transcription factor NF- κ B has been identified as a critical component of several signal transduction pathways (Harada et al., 1997). One important function of NF- κ B is its ability to protect cells from apoptosis by activating anti-apoptotic genes (Beg and Baltimore, 1996; Van Antwerp et al., 1996). Recent studies indicate that NF- κ B is constitutively active in a number of malignant tumors, such as breast cancer and prostate cancer, and that it plays an anti-apoptotic role in the survival of these tumor cells (Barnes and Karin, 1997; Nakshatri et al., 1997; Palayoor et al., 1999). Wieser et al. (2005) have demonstrated the constitutive activation of NF- κ B in endometriotic cells. It is suggested that NF- κ B may play a significant role in the proliferation of endometriotic lesions (Sakamoto et al., 2003; Guo, 2007).

TNF- α and estradiol induce NF- κ B activation in endometriotic stromal cells, whereas progesterone and danazol inhibit NF- κ B activation in endometriotic stromal cells (Horie et al., 2005). Activation of NF- κ B by lipopolysaccharide (LPS) induces proliferation of endometriotic stromal cells (Iba et al., 2004). NF- κ B 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 (Iba et al., 2004; Nasu et al., 2006). BAY 11-7085 induces apoptosis and G0/G1 phase cell cycle arrest of endometriotic stromal cells (Nasu et al., 2006). Additionally, down-regulation of the B-cell lymphoma/leukemia-2 (Bcl-2) and Bcl-X_L expression with simultaneous activation of caspase-3, caspase-8, and caspase-9 was observed in endometriotic stromal cells after treatment with BAY 11-7085 (Nasu et al., 2006). Suppression of NF- κ B activity by proteasome inhibitors also suppresses proliferation of endometriotic cells in vitro (Guo, 2007).

Mitogen-activated protein kinases (MAPKs)

To date, three types of MAPKs have been well-characterized, i.e., 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 (Pearson et al., 2001). It has been reported that ERK, JNK, and p38 MAPK are expressed in endometriotic cells (Yoshino et al., 2004). The p38 MAPK phosphorylation rates in the endometriotic tissues are significantly higher than those in the eutopic endometrium of the same patients with endometriosis (Yoshino et al., 2004).

MAPKs 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 (Yoshino et al., 2004). Estrogen induces ERK1/2 phosphorylation in eutopic endometrial stromal cells from patients with endometriosis, but not in endometrial stromal cells from women without endometriosis (Murk et al., 2008). ERK1/2 inhibition reduces proliferation and increases apoptosis of endometrial stromal cells (Murk et al., 2008).

Survivin

Survivin is a member of the inhibitor of apoptosis (IAP) gene family (Ambrosini et al., 1997). IAP proteins directly inhibit the terminal effector caspases 3 and 7, and thus protect the cells from apoptosis (Devereaux et al., 1997). Endometriotic cells express more survivin genes than normal endometrial cells from women without endometriosis (Ueda et al., 2002). Endometriotic cells also show enhanced expression of survivin-2B and survivin-EX3, splice variants of survivin (Fujino et al., 2006). These findings suggest that upregulated expression of survivin in endometriotic tissues may be a reflection of the resistance against apoptosis and the development of endometriosis.

Ubiquitin

Ubiquitin is a 76 amino acid protein (Schlesinger et al., 1975) that is involved in the degradation of short-lived, regulatory or misfolded proteins, thereby maintaining cellular homeostasis (Glickman and Ciechanover, 2002). Ubiquitin tags these proteins to be taken to the proteasome and in some instances also to the lysosomal machinery to prevent damage of cells. Immuno-histochemical analysis has indicated that ubiquitin is predominantly expressed in the endometriotic stromal cells (Ilad et al., 2004). It is suggested that ubiquitin may contribute to a reduced sensitivity to apoptosis in the endometriotic tissue.

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 (Lane, 1992; Levine, 1997). p53 expression is higher in ovarian and colorectal endometriotic tissue than peritoneal endometriotic tissue or the normal eutopic endometrium (Arimoto et al., 2003). In contrast, weak p53 expression has been detected in peritoneal endometriotic tissue and the eutopic endometrium from women with and without endometriosis (Schneider et al., 1998; Nakayama et al., 2001; Beliard et al., 2004). Eutopic and ectopic endometrial cells from women with endometriosis expressed decreased p53 mRNA expression compared to endometrial cells from women without endometriosis

(Braun et al., 2007). 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 al. (2002) 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 (Brugarolas et al., 1995; Deng et al., 1995; Attardi et al., 1996). p21 is highly expressed in the endometriotic epithelial cells of ovarian endometriosis (Fauvet et al., 2003). p21 expression is higher in ovarian endometriotic tissue than in peritoneal and colorectal endometriotic tissue and the normal eutopic endometrium (Dufournet et al., 2006). There is a positive correlation between p53 and p21 expressions in endometriosis (Dufournet et al., 2006).

c-myc

c-myc is a key element in controlling cell proliferation, differentiation, and apoptosis (Albaro et al., 1999; Adachi et al., 2001). 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 (Miyazaki et al., 1995; Smith et al., 2004). c-myc expression is upregulated in the eutopic endometrium from women with endometriosis compared with the level in the normal endometrium (Johnson et al., 2005).

Cyclooxygenase (COX)-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 suffering from endometriosis than in disease-free women (Karck et al., 1996). PGE2 modulates expression of steroidogenic acute regulatory protein and aromatase in endometriotic stromal cells and thereby regulates estrogen metabolism (Ebert et al., 2005). COX-2 is more abundantly expressed in ectopic endometrium than in the eutopic endometrium during similar phases of the menstrual cycle in women (Ota et al., 2001; Chishima et al., 2002). COX-2 is expressed in both glandular epithelium and stroma of endometriotic tissues in women (Ota et al., 2001; Chishima et al., 2002). Moreover, expression of COX-2 protein is higher in eutopic endometria from women with endometriosis compared with women without endometriosis. Inhibition of COX-2 induces apoptosis in endometriotic cells through caspase-3 pathways (Laschke et al., 2007).

Caspase-1

Caspase-1, also known as interleukin 1 converting enzyme (ICE), proteolytically processes the immature IL-1 β from a 33 kDa precursor to the 17 kDa mature form (Cerretti et al., 1992). 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-X $_L$ death pathway (Yasuhara, 1997). Eutopic and ectopic endometrial cells from women with endometriosis expressed decreased caspase-1 mRNA expression compared to endometrial cells from women without endometriosis (Braun et al., 2007).

Apoptosis-inducing properties of current medical treatment for endometriosis

Medical therapies historically have included contraceptive steroids, progestogens, and agonists of gonadotropin-releasing hormone (GnRH), as well as androgens and non-steroidal anti-inflammatory agents (Practice Committee of the American Society for Reproductive Medicine, 2004). Current medical treatment aims to inhibit the growth of endometriotic implants by suppressing ovarian steroids and inducing a hypoestrogenic state (Practice Committee of the American Society for Reproductive Medicine, 2004); 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.

GnRH agonists

GnRH 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 (Bergqvist et al., 1998). In addition, both eutopic and ectopic endometrial tissue in women with endometriosis have apparent GnRH receptors (Imai et al., 1994; Chatzaki et al., 1996; Janovick and Conn, 1996), suggesting that GnRH may function as a direct regulator of their growth. GnRH agonist increases the apoptotic rate of ectopic and eutopic endometrial cells in endometriosis by upregulating the expression of Bax and FasL and by downregulating the expression of Bcl-2 (Imai et al., 2000; Meresman et al., 2003; Bilotas et al., 2007). This agent also reduces the cell proliferation of eutopic endometrial epithelial cells in women with

endometriosis (Meresman et al., 2003), while endometrial cells from women without endometriosis are not affected (Imai et al., 2000). GnRH agonist inhibits the expression of phosphorylated inhibitor- κ B in endometriotic stromal cells, indicating the suppression of NF- κ B inactivation in these cells (Sakamoto et al., 2003).

Progesterone

Progesterone and other synthetic progestins commonly used in the clinical management of endometriosis can suppress the NF- κ B pathway (Kalkhoven et al., 1996; McKay and Cidlowski, 1999). Combination oral contraceptives significantly diminish cell proliferation and induce apoptosis of eutopic endometrial tissue from patients with endometriosis (Meresman et al., 2002). These agents induce Bax expression in the eutopic endometrium from women with endometriosis, whereas they reduce Bcl-2 expression in these tissues (Meresman et al., 2002).

Danazol

Recent evidence suggests that danazol can act directly on endometriotic tissue in vitro to inhibit DNA synthesis and induce apoptosis (Surrey and Halme, 1992; Braun et al., 1994).

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 (Bulun et al., 2000, 2004). Zeitoun and Bulun (1999) 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 (Meresman et al., 2005).

Conclusions

Based on the above findings, apoptosis plays a major role in the pathophysiology of endometriosis (Jones et al., 1998b; Lebovic et al., 2001). 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 two possible mechanisms: 1) endometriotic cells are genetically resistant to some apoptotic stimuli, or 2) intraperitoneal environments in endometriotic patients cause resistance to apoptotic stimuli in endometriotic tissues.

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 (Nasu et al., 2005, 2006; Nishida et al., 2006). 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.

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