Apoptosis and endometriosis

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1. ABSTRACT

Apoptosis is a distinctive form of programmed cell death resulting in the efficient elimination of cells without eliciting an inflammatory response. Endometriosis is characterized by the presence of endometrial cells with capacity to avoid apoptosis outside the uterus. Apoptosis plays a fundamental role for the pathogenesis of endometriosis. Eutopic endometrium from women with endometriosis has increased expression of anti-apoptotic factor and decreased expression of pro-apoptotic factors compared with endometrium from healthy women. These differences could contribute to the survival of regurgitating endometrial cells into the peritoneal cavity and development of endometriosis. Increased apoptosis of Fas-bearing immune cells in the peritoneal cavity may leads to their decreased scavenger activity that eventually results in prolonged survival of ectopic endometrial cells in women with endometriosis. This study is a current review of the literatures focused on the physiological role of apoptosis in normal endometrium and alterations in regulation of apoptosis in eutopic and ectopic endometrium from women with endometriosis. The role of apoptosis in the treatment of endometriosis is also reviewed.

2. INTRODUCTION

Endometriosis is defined by the presence of endometrium-like glandular tissue and stroma outside the uterus. It is a common disease affecting 5% to 15% of women in the general population and 40% of women seeking infertility evaluation (1). Several theories have been proposed to explain the pathogenesis of endometriosis, including development by metaplasia, development from Müllerian remnants, and after implantation and growth of endometrium following retrograde menstrual reflux. Two new theories have recently proposed. The tissue injury and repair theory suggests that local production of estrogens by ectopic endometrial tissue results in infiltration of basal endometrium into the myometrium and the peritoneal cavity, and eventually leads to the development of endometriosis (2). Moreover, Quinn's "denervation-reinnervation" theory postulates that nerve injuries results in denervation and reinnervation, making possible deposits of viable endometrial cells in injured ectopic sites to provoke pain of various intensities (3).

Nearly all women of reproductive age exhibit

some degree of reflux of endometrial debris (4). Menstrual effluents retrogradely shed into the peritoneal cavity were observed to contain viable endometrial cells (5-9). These mechanisms are necessary but insufficient to explain why only some patients develop the disease. It is possible that an altered eutopic endometrium resists to normal peritoneal means of cleaning. It is also possible that the disease is secondary to alterations of the cellular and humoral immunity that induce excessive receptivity of the peritoneal mesothelium, hyperactivated macrophages, and abnormalities of NK cells (10). An excess of refluxing endometrium or altered endometrium has the potential to form a proinflammatory or hormonal environment favorable to establish the disease (10). The peritoneal environment may alter a genetic predisposed endometrium, which then becomes favorable for invasion. It is well known that hundreds of genes are dysregulated in endometriosis (11,12).

The fact that the eutopic endometrium of women with endometriosis shares changes with ectopic tissue and that these changes are not found in the eutopic endometrium of disease-free women has advanced the view that the primary defect in endometriosis is to be found in the eutopic endometrium (13). Cells and tissue elements, derived from such an altered eutopic endometrium and shed into the peritoneal cavity, have been proposed to have a higher potential for implantation and growth on peritoneal surfaces and development into endometriosis (14-16). On the other hand, the fact that many differences observed between eutopic endometrium of disease-free women and ectopic tissue of a patient with endometriosis can be explained as the direct influence of the different environment of peritoneal fluid (PF) (17-20). One of the endometrial alterations appearing in eutopic and ectopic endometrium from women with endometriosis refers to the regulation of apoptosis. In particular, decreased susceptibility of endometrial tissue to apoptosis may contribute to the pathogenesis of endometriosis.

3. MOLECULAR BASIS OF APOPTOSIS

3.1. Induction and execution of apoptosis

Apoptosis is a specific mode of cell death with a characteristic pattern of morphological, biochemical and molecular changes such as, nuclear chromatin condensation, cytoplasmic blebbing, and internucleosomal DNA degradation (21). Apoptosis can result from activation of three major pathways: the first starts with binding of death receptors with the cognate death ligands, leading to the activation of caspase-8, and the second involves the mitochondrial release of cytochrome c resulting in the activation of caspase-9. As the third, recent studies have revealed that induction of endoplasmic reticulum (ER) stress and the subsequent activation of the unfolded protein response (UPR) play a critical role in the induction of apoptosis. All of these pathways eventually activate caspase-3 as the effector of apoptosis (22-25).

Fas and tumor necrosis factor receptor (TNFR) are belonging to tumor necrosis factor/ nerve growth factor receptor superfamily. Fas and TNFR1 contain a cytoplasmic domain called the "death domain" (26). Upon receptor activation following the binding of cognate death ligands (e.g., FasL and TNFalpha), the death domain interacts with the adaptor protein (e.g., FADD) leading to the formation of death inducing signaling complex (DISC), and initiates the execution process of apoptosis (27). Following their activation, caspase-8 and -9, the initiator caspases, cleave and activate the effector caspases, caspase-3, -6 and/or -7.

Caspase-9 activation starts with the mitochondrial release of cytochrome c in response to apoptotic stimuli. The cytochrome c binds to apoptotic protease activating factor 1 (Apaf-1) in the presence of dATP, and the Apaf-1/cytochrome-c/dATP complex forms an oligomeric "Apoptosome" (28, 29), which recruits and activates the pro-caspase-9 leading to the activation of effector caspases such as caspase-3 (Figure 1). Caspase-3 plays a central role in proteolytic cleavage of ICAD (DFF45) to activate CAD (DFF40) leading to DNA fragmentation into nucleosomal units.

Inhibitors of apoptosis interact with multiple cellular partners and inhibit apoptosis induced by a variety of stimuli. Although caspase activity is regulated by the zymogen activation, direct inhibition of active caspase by inhibitors of apoptosis is important. Eight human inhibitors of apoptosis have been identified so far (30), among them, XIAP, c-IAP1, and c-IAP2 are thought to directly inhibit caspase-3, caspase-7 and caspase-9 (31-34).

3.2. Bcl-2 family regulates the mitochondrial pathways through organelle morphogenesis

In mammalian cells, mitochondria have a central role in apoptosis that is regulated by members of the Bcl-2 family (35). The mitochondrial pathway integrates stress and some developmental apoptotic stimuli, and is triggered by the translocation of proapoptotic Bcl-2 family member, such as Bax and Bak, to the surface of mitochondria where the anti-apoptotic proteins are located. The interaction between pro-apoptotic (Bak, Bax) and anti-apoptotic (Bcl-2, Bcl-X_L) proteins disrupts the normal function of Bcl-2 and Bcl-X_L and can lead to the pore formation at the mitochondrial membrane, release of cytochrome c from mitochondria into the cytosol, which induces pro-caspase-9 activation (Figure 1) (36,37). Although Bcl-2 family proteins are potent regulators of apoptosis, their intracellular localization to mitochondria has been observed, but their exact function still remains unclear. It is possible that their action can be mediated via direct interactions between members of the Bcl-2 family and core components of the mitochondrial fission/fusion machinery, such as, dynamin-related protein 1 (Drp1) and mitofusin 2 (Mfn2) GTPases (38, 39). Two members of the Bcl-2 family, Bax and Bak, have the ability to change intracellular localization during the course of apoptotic process and concentrate into foci at sites of mitochondrial scission (40), suggesting that Bcl-2 family members may also regulate apoptosis through mitochondrial morphogenesis. Nowadays, it is a matter of debate whether the role of Bcl-2 proteins in mitochondrial morphogenesis is functionally distinct from their role in apoptosis (39).
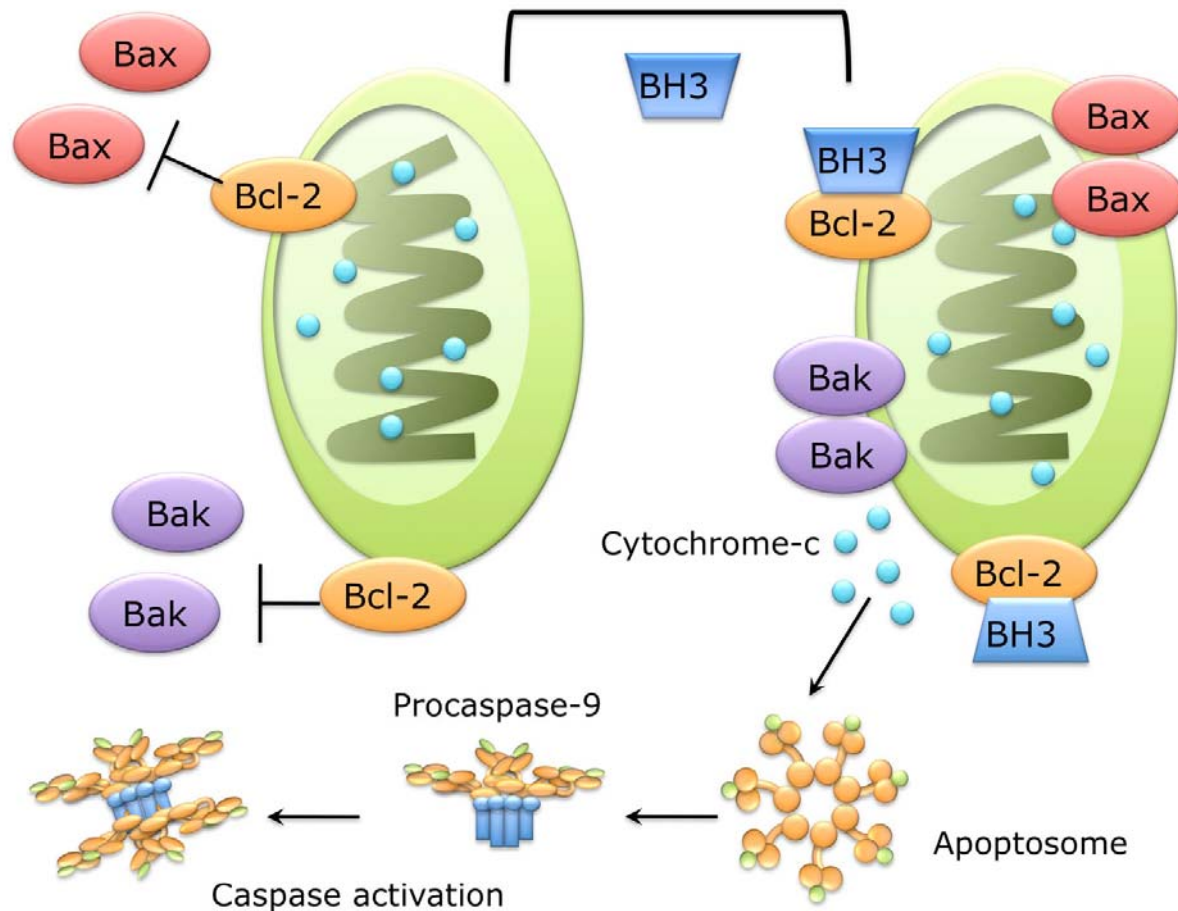
Figure 1. Specific apoptotic stress signals trigger the activation of particular BH3-only proteins which then interact with anti-apoptotic members on the outer mitochondrial membrane resulting in the release of Bax-like pro-apoptotic factors. Bax undergo a conformational change, insert into the outer mitochondrial membrane where they provoke pore formation and release of cytochrome c, apoptosome formation and activation of pro-caspase-9.

Some investigators suggest that Bax/Bak-induced mitochondrial fission promotes apoptosis-associated cytochrome c release (40-43), while others suggest that mitochondrial fission may promote cytochrome c release and therefore, act to drive caspase activation during apoptosis (44, 45). Further studies are required to explore the interactions between Bcl-2 family members and mitochondrial morphogenesis proteins and to clarify the functional impact of these interactions.

4. APOPTOSIS IN THE NORMAL ENDOMETRIUM

Accumulated evidences suggest that apoptosis helps to maintain cellular homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium (46-48). In normal endometrium, apoptotic cells were identified in the glandular epithelium of late secretory and menstruating endometrium, while very little apoptosis was detected during the proliferative phase or at the beginning of the secretory phase (49, 50). Vaskivuo *et al*., showed that the pattern of apoptosis negatively correlated to serum estradiol concentrations in the proliferative phase (50). Upon progesterone withdrawal, two days before the onset of menstruation, endometrial epithelial cells show a high degree of apoptosis (51).

4.1. Bcl-2 family in normal endometrium

The Bcl-2 protein is probably the best well characterized of apoptosis-related molecules, and data now support a role for the Bcl-2 protein as a cell death repressor (52). Bcl-2 has been considered to inhibit apoptosis in the human endometrium during the proliferative phase (53). Bcl-2 cyclically expressed in endometrial glandular and stromal cells, peaks during the late proliferative phase, while decreased dramatically in the early and mid-secretory phase to reappear in the late secretory phase (53-58). The basal layer of the endometrium showed increased expression of Bcl-2, whereas, death receptor Fas and caspase-3 were showed higher expression in the functional layer of the endometrium (60).

The *Bcl-2* gene is only one member of this multigene family, consisting of numerous proteins

homologous to Bcl-2 (61-63). Other members of the *Bcl-2* gene family likely play important roles in controlling apoptosis by mechanisms that are opposing or complementary to the action of Bcl-2. The susceptibility of any given cell to a potential apoptotic stimulus may be determined by the ratio of pro- and anti-apoptotic Bcl-2 family members presented in the cell at that time (63).

Bcl-X_L and Bcl-X_S are members of the Bcl-2 family with opposing functions (Bcl-X_L anti-apoptotic; Bcl-X_S pro-apoptotic). The ratio Bcl-X_L/Bcl-X_S is thought to play a major role in setting the "apoptotic threshold" in various cell types. This ratio is increased at the secretory phase in normal endometrial cells (64). Bax is another Bcl-2 family member that promotes cell death susceptibility, possibly by countering the effect of Bcl-2 on cellular survival through heterodimer interaction (62). In contrast with the expression pattern of Bcl-2, Bax is up-regulated in the glandular epithelial cells during the secretory phase of the normal menstrual cycle (65). Bak (Bcl-2 homologous antagonist/ killer) is localized almost exclusively to the glandular epithelial cells, especially in the functional layer of the secretory endometrium (63). Showing similar expression pattern with Bax, immunoreactive Bak was absent from most of the endometrial cells of the proliferative phase (66). These data imply the existence of a dynamic interplay among many members of the Bcl-2 family in triggering apoptosis.

Several scientists have suggested that ovarian steroids may control endometrial apoptosis by up- and down-regulation of Bcl-2 and Bax expression (67, 68). The cyclic pattern of Bcl-2 expression in endometrial glandular cells was related to changes in estrogen receptor and progesterone receptor patterns throughout the cycle (54). Critchley *et al* reported an increase in Bcl-2 protein expression in glandular and surface epithelium of antiprogestin-treated endometrium (69), suggesting that Bcl-2 expression may be stimulated by estrogen and down-regulated by progesterone.

4.2 Fas/FasL in normal endometrium
Fas expression seems to be unchanged in the different phases of the menstrual cycle (70). However, another group of authors have found that Fas immunostaining on human endometrial glandular cells was stronger during the secretory than during the proliferative phase (54). This discrepancy could be attributed to the different endometrial cell type examined by the authors. Similarly, Peng *et al* demonstrated that FasL exhibits peak expression during the secretory and menstrual phases (71).

It is possible in normal human endometrium, caspase-8 is initially activated by the Fas/FasL signal, resulting in the caspase cascade. Activated caspase-8 can switch on both the death receptor pathway and the mitochondrial pathway via Bid degradation (72). It is possible that both the mitochondrial and the death receptor pathways are involved in apoptosis of human endometrial cells.

5. APOPTOSIS IN ENDOMETRIOSIS

5.1. Apoptosis of eutopic and ectopic endometrium in endometriosis
Differences in apoptosis between eutopic endometrium from women with endometriosis and normal endometrium could contribute to the survival of regurgitating endometrial cells into the peritoneal cavity and the development of endometriosis.

Gebel *et al* reported that the percentage of apoptosis in sloughed endometrial cells was greatly reduced among women with endometriosis implying that the number of surviving cells that enter the peritoneal cavity is greater in women who develop endometriosis (73). The apoptosis indices in the eutopic endometrium of women with endometriosis were lower compared to women without endometriosis (73, 74). This difference was caused primarily by a significant decrease in apoptosis during the late secretory/menstrual and early proliferative phases in women with endometriosis. The cyclic variability of apoptosis may be lost in these women. The eutopic endometrium of women with endometriosis (epithelial/stromal cells) appeared with increased proliferation compared with the one in women without the disease (75).

One can speculate that if the decrease in apoptosis facilitates ectopic survival and implantation of the endometrial cells then there may be an inverse correlation between the level of apoptosis and the severity of the disease. However, this hypothesis was not confirmed (76,77). This may be attributed to the fact that various locations of endometriosis are presented with different expression patterns of apoptotic molecules.

5.2. Bcl-2 family in endometriosis
The expression of Bcl-2 in endometrial glandular cells has a cyclic pattern in eutopic endometrium in patients with endometriosis, but that cyclic changes were not apparent in peritoneal and ovarian endometriotic tissues (54). Jones *et al* did not detect apoptosis in stromal cells from peritoneal endometriotic tissues (78). In accordance with these findings, Bcl-2 is expressed to a greater extent in stromal cells from ectopic tissues (78). This overexpression may be directly correlated to the increase in the number of estrogen receptors expressed by ectopic stroma (79).

An increased expression of Bcl-2 protein was found in proliferative eutopic endometrium from women with endometriosis when compared with normal endometrium from healthy women (80). In contrast, Bax expression was absent in proliferative endometrium, whereas there was an increase in its expression in secretory endometrium from women with endometriosis and healthy women. The altered expression of Bcl-2 in eutopic endometrium of women with endometriosis resulted to a decreased number of apoptotic cells and consequently to their abnormal survival in the ectopic locations (80).

The expression of Bcl-2 varied according to the location of endometriosis, suggesting the involvement of
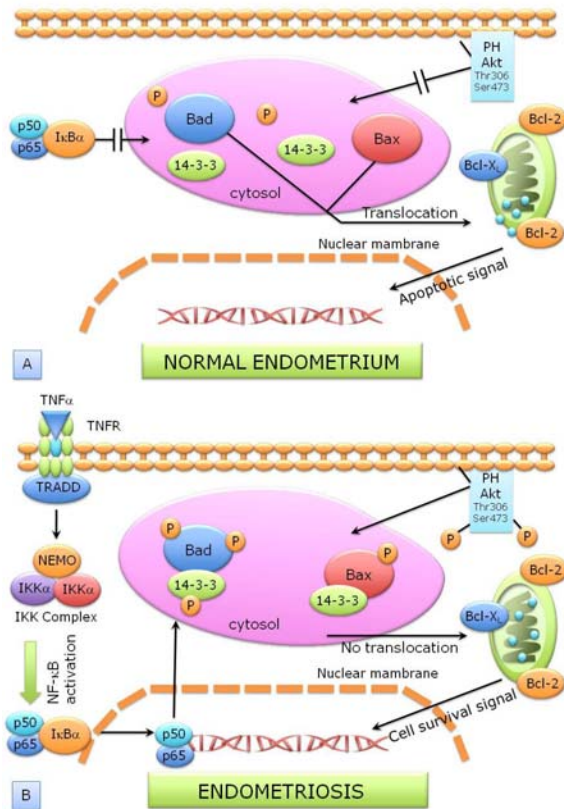
Figure 2. Effects of Akt and NF-kappaB pathways on cellular apoptosis in normal endometrium and endometriosis. A: In normal endometrium, NF-kappaB and Akt pathways inactivation leads to dephosphorylation of Bad and Bax proteins, dissociation of Bad and Bax from 14-3-3 proteins, permits translocation of Bad and Bax proteins from the cytosol to the mitochondria, and thereby augments interaction between Bax/Bad and Bcl-2/Bcl-XL proteins. These sequential interactions result in release of cytochrome c from the mitochondria into the cytosol and activation of caspase-3 and PARP enzymes that eventually provoke apoptosis of human endometrial cells. B: In endometriotic cells, NF-kappaB and Akt activation leads to cell survival. These activated molecules can induce phosphorylation of Bad protein,sequestration of Bad and Bax proteins in the cytosol with 14-3-3 proteins. Eventually, leads to the prevention of Bad and Bax translocation from the cytosol to the mitochondria. This prevents interactions between Bax/Bad and Bcl-2/Bcl-XL and thereby promotes survival of endometriotic cells.

different apoptotic pathways and etiopathologies. In ovarian endometriosis, the weak Bcl-2 expression was shown (77, 81). The Bcl-2 expression was lower in ovarian endometriosis than in peritoneal and colorectal endometriosis (82). Moreover, as observed by Beliard *et al* the peritoneal endometriosis exhibited a higher Bcl-2 expression than those found in eutopic endometrium of women with or without endometriosis (83).

Braun *et al* analyzed the expression of apotosis-

regulating genes in eutopic endometrium from women with or without endometriosis. The $Bcl-X_L/Bcl-X_S$ (anti-apoptotic/pro-apoptotic) ratio was substantially higher in eutopic endometria from women with endometriosis compared to endometria from women without endometriosis (64). This result could be consistent with apoptotic resistance and enhanced survival of endometrial cells in endometriosis.

Increased prostaglandin E_2 (PGE_2) signaling was observed in ectopic endometriotic tissues compared with eutopic endometrial tissues during the menstrual cycle (84). According to the authors, the ability of endometriotic cells to circumvent apoptotic signals can be the result of the increased PGE_2 signalling which is associated with abundant expression of the anti-apoptotic Bcl-2 and $Bcl-X_L$ proteins, low expression of pro-apoptotic Bax protein, phosphorylation/inactivation of pro-apoptotic Bad protein, and activation of multiple cell survival signalling pathways (ERK1/2, Akt, nuclear factor-kappaB, beta-catenin) (Figure 2) (84).

5.3. Fas/FasL system in endometriosis

Fas and its ligand are diminished in eutopic endometrium from endometriosis patients when compared with control women (70). Few studies have been published on the expression of Fas in endometriotic tissues. According to our knowledge, there are no studies showing quantitative comparison of Fas expression between endometriotic tissues and endometrium from disease-free women. Fas is expressed randomly in both eutopic and ectopic endometrial tissues (85). In accordance with this finding, Watanabe *et al* also observed Fas expression in glandular cells of both ectopic and eutopic endometrium (54). These findings suggest that the expression of Fas antigen may be less involved in apoptosis of eutopic and ectopic endometrial tissues as an apoptosis-regulator. In contrast, with the cyclic expression pattern of Bcl-2, Fas expression was constant in both tissues throughout the menstrual cycle. Differences in the expression of Fas were found between ovarian, uterine, cervical, and endometrial carcinoma tissues comparing with normal tissues. Tumor cells had significantly decreased levels of Fas (86). Eidukaite *et al* found abundant expression of Fas antigen in NK-cells of PF of women with early stages of endometriosis (87). The authors suggested that the activated PF NK-cells can be intensively eliminated via the Fas/FasL apoptosis, thus providing conditions for the survival of ectopic endometrial cells and the development of the disease at the initial stages of endometriosis.

In contrast with Fas, many studies indicated that higher expression of FasL by endometriotic tissues contributes to their survival and the development of endometriosis. The levels of soluble/active FasL are higher in serum and PF in women with moderate to severe endometriosis than in women with early-stage disease or in disease-free women (88). Higher levels of soluble FasL in the PF of women with endometriosis may contribute to increased apoptosis of Fas-bearing immune cells in the peritoneal cavity, leading to their decreased scavenger activity (79). This may result in prolonged survival of

endometrial cells into the peritoneal cavity. Several authors have shown that endometrial glandular and stromal cells express FasL. In addition, PF leukocytes are another plausible source for high levels of soluble FasL in women with endometriosis, because human-activated peripheral blood mononuclear cells were shown to express FasL messenger RNA (89).

Macrophage-derived growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF) are increased in the PF of women with endometriosis (90). Garcia-Velasco *et al* showed that macrophage-conditioned media containing PDGF and TGF-alpha induced FasL expression in endometrial stromal cells, suggesting that peritoneal macrophages in endometriosis might stimulate a Fas-mediated apoptosis of immune cells (91). FasL expression in the endometriotic cells may protect them from attack by T lymphocytes. Consequently, ectopic endometrial cells escaping from immune surveillance in the peritoneal cavity of women with endometriosis may contribute to the maintenance of the disease.

It is, therefore, possible that many endometriotic cells not only become resistant to Fas-mediated apoptosis, but additionally they have acquired the ability to utilize this pathway to their advantage by launching a "Fas counterattack" against the host's immune system. The increased expression of FasL by endometriotic cells coincides with their inherent resistance to Fas-mediated apoptosis which protects them from a "suicidal" death.

Up-regulation 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 (92). Metalloproteinases (MMPs) have been implicated in the conversion of FasL to active/soluble forms, suggesting that these molecules can activate or release factors involved in the apoptotic process (93). MMP-2, MMP-9 and Mt1-MMP messenger RNA expression levels are higher in endometriotic lesions compared with normal eutopic endometrium, implying that endometriotic tissue has a greater capacity for invasion. Early lesions of endometriosis reportedly invade the extracellular matrix of the peritoneum (94). FasL expression that occurs when endometrial stromal cells attach to the extracellular matrix may be one of the critical events in the development of endometriosis. Supporting this theory, Wisniewski *et al* found that blocking of Fas signalling with FasL Interfering Protein (FLIP) impaired MMP-2 activity, resulting in reduction of invasiveness and motility in glioma cells (95). Under these observations, we could speculate that the expression levels of soluble/active FasL may be enhanced in shedding endometrial cells presenting in the peritoneal environment and protect endometrial cells from the immune effectors cells of the peritoneal cavity.

Interleukin-8 (IL-8), a chemokine for neutrophils and a potent angiogenic agent, is elevated in the PF of women with endometriosis (96,97). Selam *et al* (98)

examined whether IL-8 upregulate FasL expression in endometrial cells and may be relevant for the development of a local immunotolerance in endometriosis. They demonstrated a concentration-dependent increase in IL-8-induced FasL expression in endometrial stromal cells. The authors speculated that elevated IL-8 levels in PF, via stimulation of FasL-induced apoptosis in activated T lymphocytes, contribute to an immune-privileged environment around the endometriosis implants supporting their survival. IL-8 exerts a chemotactic activity primarily on neutrophils and inhibits their apoptosis even in the presence of Fas engagement (99). Kwak *et al* investigated the effects of plasma and PF from patients with advanced endometriosis on apoptosis of neutrophils (100). Adding plasma and PF in neutrophils culture reduced spontaneous apoptosis. Neutralizing IL-8 antibody abrogated the delay of neutrophil apoptosis induced by PF, suggesting that IL-8 is one of the neutrophil survival factors in the PF of endometriosis patients. The impaired clearance of cells responsible for innate immunity in the PF of patients with endometriosis may be associated with the development of the disease (100).

5.4. Apoptosis in peritoneal macrophages from patients with endometriosis

The peritoneal cavity, the commonest site of endometriosis (101), contains fluid whose major cellular constituents are macrophages (102). In endometriosis, the number and secretory activity of these cells increase (4, 103) and evidence suggests that these cells play an important role in developing and maintaining of endometriosis (20, 104). The function of macrophages is altered in several aspects, as well. The cytotoxic power of peritoneal macrophages in endometriosis patients with respect to the endometrium is reduced (105). The diminution of cytotoxicity of peritoneal macrophages could be more significant than that of the circulating macrophages (106). Recent studies have suggested that the decreased cytotoxicity of peritoneal macrophages could be resulted from iron overload. Iron overload can increase NF-kappaB activity in peritoneal macrophages from women with endometriosis (107). NF-kappaB-activated macrophages release proinflammatory cytokines which may activate NF-kappaB in endometriotic cells, consequently promoting cytokine production from endometriotic cells, enhances the synthesis of antiapoptotic factors and eventually contributes to cell survival in the peritoneal cavity (108). Another potent regulator of phagocyting activity of peritoneal macrophages in women with endometriosis is the increased expression of PGE_2 which inhibits the expression of CD36 scavenger receptor in these cells (109). Reduced expression of this receptor may result in loss of macrophage's phagocytic ability. The reduced capacity of peritoneal macrophages from women with endometriosis to mediate lysis of endometrial cells together with the increased resistance of ectopic endometrial cells to macrophage-mediated cytolysis may promote survival of the endometrial cells in the peritoneal cavity of women with endometriosis (110).

McLaren *et al* reported an increased percentage of Bcl-2-positive macrophages in PF of women with

endometriosis compared with the non-endometriotic group, resulting in an increased number of cells surviving the process of activation and thus delaying apoptosis (81). This may explain the increased numbers of macrophages found in the PF of patients with endometriosis. Immunohistochemical staining revealed a population of Bcl-2-positive and Bax-negative tissue macrophages present only in ectopic tissue during both phases of the menstrual cycle (81). The expression of Bcl-2 and the absence of Bax may confer on these macrophages a decreased susceptibility to apoptosis, given the known properties of Bcl-2 and Bax, and may result in an extended life expectancy of these cells.

5.5. Apoptosis in pathophysiology of endometriosis
Accumulating evidences suggest that the endometrial cells from women with and without endometriosis have fundamental differences. Endometrial cells from women with endometriosis has enhanced proliferation and increased ability to implant and survive in ectopic locations. Impaired sensitivity of endometrial tissue to spontaneous apoptosis contributes to the abnormal implantation and growth of endometrium at ectopic sites. The inability of endometrial cells to transmit a "death" signal or their ability to avoid cell death is associated with increased expression of anti-apoptotic factors (e.g., Bcl-2) and decreased expression of pro-apoptotic factors (e.g., Bax) (80). It remains unclear whether the abnormal apoptosis in the eutopic endometrium from patients with endometriosis is primary in origin or secondary after establishment of pelvic endometriosis process. This could be attributed to the fact that at the time of clinical presentation and diagnosis most women have already established disease and therefore, it is very difficult to investigate the early developmental stages of the endometriosis.

Reflux of endometrial fragments during menstruation into the peritoneal cavity is a common phenomenon. Under normal conditions, cells that do not adhere to their extracellular matrix enter apoptosis as they receive different signals from their adhesion receptors (111). However, in women with endometriosis these cells have the ability to adhere to mesothelial cells of peritoneum, to proliferate, and to produce neoangiogenesis resulting in the development of active endometriosis. The effect of MMPs on apoptotic factors and their regulation by steroid hormones may provide a link between endometrial turnover and the invasive process necessary for the development of endometriosis.

It is possible that intrinsic abnormalities in transplanted eutopic endometrium are contributed to the pathogenesis of pelvic endometriosis. Abnormal signaling pathways in the eutopic endometrium of women with endometriosis have been recently reported. Two different groups demonstrated increased activity of the protein kinase A and B pathways regulating the function of many cellular proteins involved in apoptosis and proliferation (112, 113). It was suggested that increased Akt phosphorylation may be related to the altered apoptosis/proliferation in endometriosis, and therefore Akt

may play a critical role in the pathogenesis of endometriosis. Another pathway whose activation confers a resistant to apoptosis phenotype in endometriotic cell is the NF-kappaB (108) (Figure 2). This pathway is an important regulator of cell proliferation and apoptosis of endometriotic lesions. NF-kappaB activation can promote the proliferation of endometriotic cells. On the other hand, NF-kappaB inhibition was found to decrease proliferation of endometriotic cells and stimulate their apoptosis, in early-stage endometriotic lesions induced in nude mice (114).

cDNA microarray analysis has provided interesting insight for altered gene expression profiles in patients with endometriosis. Using this method, Arimoto *et al* found 97 up-regulated and 337 down-regulated genes in women with endometriosis (115). Genes related to apoptosis (GADD34, GADD45A, GADD45B, PIG11) and the tumor suppressor TP53 gene, were down-regulated in endometriotic tissues. These findings are in consistent with the decreased spontaneous apoptosis observed in eutopic endometrium from women with endometriosis.

Survivin is a member of the inhibitors of apoptosis family. Inhibitors of apoptosis proteins directly inhibit the terminal effector caspase 3 and 7, and thus protect the cells from apoptosis. Ueda *et al* showed that endometriotic cells express more survivin genes than normal endometrial cells without endometriosis (116). Susceptibility to drug-induced apoptosis in endometriotic cells was attenuated compared to eutopic endometrial cells (117, 118), implying that endometriotic cells may have the ability to survive and develop in the ectopic sites. Increased survivin expression was present in eutopic and ectopic epithelial cells but only ectopic epithelial cells lost the cyclic variation of survivin expression throughout menstrual cycles. Ectopic stromal cells lost their regulating functions on survival signaling, PI3K/Akt/Survivin signaling, in epithelial cells and also lost the ability to mediate the inhibitory effect of progesterone on survival signalling in epithelial cells (119). We recently demonstrated that survivin plays a critical role in susceptibility of endometriotic stromal cells to apoptosis and the survivin inhibitor may be effective as a treatment for endometriosis (120). YM155, a novel small-molecule survivin suppressant, induces regression of hormone-refractory prostate cancer (HRPC) (121). Phase II clinical studies of YM155 for HRPC, melanoma, and non-small cell lung cancer are in progress. Moreover, inhibition of survivin by RNA interference reduced cell proliferation and induced apoptosis in endometrial Ishikawa cancer cells by activating caspase-3 and caspase-8 thus suggesting that survivin may be an attractive target for endometriosis and endometrial cancer treatment (122).

5.6. Apoptosis and treatment of endometriosis
5.6.1. Hormonal treatment
Endometriosis is an estrogen-dependent disease. Current therapeutic alternatives consist of various hormone treatments aimed at decreasing circulating estrogen to postmenopausal levels. Incubation with GnRH-agonists (GnRH-a) increased the apoptotic rate in eutopic and

ectopic endometrial cells from women with endometriosis (123-125). Medical strategies to intervene anti-apoptotic or pro-apoptotic pathways may lead to identify effective treatment modalities for the treatment of endometriosis. It has been proposed that the anti-proliferative effect of GnRH-a treatment may be mediated by the activation of Fas/FasL system. The increase in apoptotic rate may be due to alterations in the expression of apoptosis-related genes after GnRH-a administration. Treatment with GnRH-a was found to affect the expression of a diverse range of genes, including those that encode apoptotic factors (126). GnRH-a treatment was able to markedly reduce the inflammatory reaction and angiogenesis and to significantly induce apoptosis, as was shown from the increased expression of activated caspase-3, in eutopic endometrial cells and lesion from women with endometriosis (127). GnRH-a was found to increase the expression of the pro-apoptotic proteins Bax and FasL and to decrease the expression of the anti-apoptotic protein Bcl-2 in eutopic endometrial cells (128).

Aromatase overexpression has recently been observed in endometriotic tissues (129, 130). Aromatase catalyzes the conversion of androgens to estrogens. Local estrogen production by endometriotic implants may contribute to the progression of endometriosis in unfavorable condition. Aromatase inhibitors have been successfully used for the treatment of endometriosis (131, 132). Aromatase inhibitors, letrozole and anastrozole, have increased apoptosis and reduced cell proliferation of epithelial endometrial cells from patients with endometriosis (133). Treatment with either anastrozole or letrozole, in a mouse endometriosis model, did not prevent lesion establishment; however, it significantly decreased the size of the endometriotic lesion (134). Anastrozole was more effective to induce apoptosis of endometriotic cells compared to letrozole (134). These findings support further investigation of aromatase inhibition for the treatment of endometriosis.

Combined oral contraceptives (OC) can administer in women with endometriosis in order to reduce pain symptoms (135) and to maintain the status quo by preventing the progression or recurrence of the disease (136). In histological studies, there was an arrest in endometrial gland proliferation resulted in progressive atrophy of the endometrium after long-term use of OC (137). Meresman *et al* have demonstrated that OC can enhance apoptosis (decreased Bcl-2/Bax expression ratio) in the eutopic endometrium of women with endometriosis (138). Another study has shown the inhibitory effects of progestogens on endometrial proliferation and the authors proposed that these compounds enhance apoptosis in the endometrium (69).

Clinically, the use of progestins or OC was also suggested as efficacious treatment for endometriosis (139,140). Induction of pseudopregnancy using a levonorgestrel-releasing intrauterine device has been proposed for the treatment of endometriosis (141, 142). Levonorgestrel increased Fas expression and enhanced the apoptotic index in eutopic and ectopic endometrium of patients with endometriosis (142). In order to avoid the systematic side effects of progestogens, levonorgestrel-microspheres were directly injected into endometriotic cysts (143). Reduced size of endometriotic lesions and endometrial atrophy was observed six months after the injection.

5.6.2. Other treatment alternatives
Although recent medical management of endometriosis is almost exclusively accomplished through the use of GnRH-a or steroidogenic compounds, these treatments are far away to consider ideal. There is definitely room for improvement of medical treatment of endometriosis with respect to the desire to avoid the adverse side effects associated with the hypoestrogenic environment induced by the current GnRH-a therapies. Modulation of apoptotic factors may result in the effective treatment of endometriosis. However, a detailed understanding of the molecular mechanism by which these drugs and hormones induce cell death should provide a fundamental approach for increasing the sensitivity of endometriotic cells to these drugs.

Several new compounds have been investigated as new treatment modalities for endometriosis. Bufalin is a major digoxin-like immunoreactive component isolated from the skin and parotid venom glands of toad and is known as an apoptosis inducing agent. Adding bufalin in culture of ovarian endometriotic cyst stromal cells (ECSC) significantly inhibited the cell proliferation and DNA synthesis of the cells and induced apoptosis (144). Another apoptosis inducing agent, beta-hydroxyisovalerylshikonin (beta-HIVS), was also examined using ECSCs. Beta-HIVS significantly inhibited the proliferation of the ECSCs and induced apoptosis (145). These agents may be potential therapeutic drugs for the treatment of endometriosis.

Sakamoto *et al* compared IL-8 expression in endometriotic stromal cells of patients treated with GnRH-a and those of patients without treatment before surgery (146). They showed that GnRH-a attenuated the IL-8 expression by reducing TNF-alpha-induced NF-kappaB activation. A soluble inhibitor of NF-kappaB (BAY 11-7085) was used to examine the potential application for the treatment of endometriosis. It has been shown that BAY 11-7085 significantly inhibited the cell proliferation and induced apoptosis (147).

Recently, Gangadharam *et al* suggested a novel pathway for doxorubicin-mediated cell death through the following signaling cascades: early induction of NF-kappaB→ increased IL-8 expression→ increased intracellular Ca^{2+}→ activation of calcineurin→ nuclear translocation of nuclear factor of activated T lymphocytes (NF-AT) → expression of NF-AT-dependent FasL→ FasL-mediated caspases activation→ cell death (148). The fact that proapoptotic Bax expression is also increasing at early time of doxorubicin treatment support its role as an NF-kappaB-dependent gene product which may facilitate FasL-dependent apoptosis through cascade-dependent pathway (148). However, a lot of concerns have to be raised for the administration of a tumor-suppressive agent in order to treat a benign disease.

Othman *et al* showed that mutants of estrogen receptor genes delivered to endometriotic cells *via* an adenovirus vector decreased cell proliferation, induced apoptosis and decreased cytokine production, suggesting that adenovirus–mediated gene therapy may represent a potential therapeutic option for endometriosis in the future (149).

A selective estrogen receptor modulator, raloxifene, has been investigated for the treatment of endometriosis. Altindas *et al* administered raloxifene in a rat endometriosis model and found significantly reduced endometriotic implants after eight weeks of therapy (150). Although the exact mechanism of raloxifene action has not been determined, Morishima *et al* showed that act via induction of apoptosis (151). Specifically, raloxifene administration in human endometrial carcinoma cells (Ishikawa) increased the number of apoptotic cells, the activities of caspases-3, -8, -9, and cytosolic cytochrome c levels were also elevated. In contrast, cleavage of Bid was not observed. Thus, raloxifene induced mitochondrial-mediated apoptosis in endometrial cancer cells but not via the Bid-mitochondrial pathway (151). Clinical studies are needed to evaluate if raloxifene can be used as an alternative drug for the treatment of endometriosis.

Recently, Banu *et al* have shown that selective inhibition of cyclooxygenase-2 prevented survival, migration, and invasion of human endometriotic epithelial and stromal cells, which was due to decreased PGE_2 production (152). PGE_2 signalling components are more abundantly expressed in ectopic endometriosis tissues compared with eutopic endometrial tissues during the menstrual cycle. PGE_2 promoted the survival of human endometriotic cells through EP2 and EP4 receptors by activating ERK1/2, Akt, nuclear factor-κB, and β-catenin signaling pathways. Selective inhibition of EP2 and EP4 suppressed these cell survival pathways and augmented interactions between proapoptotic proteins (Bax and Bad) and antiapoptotic proteins (Bcl-2/Bcl-X_L), facilitated the release of cytochrome c, and activated caspase-3/poly (ADP-ribose) polymerase-mediated intrinsic apoptotic pathways (84).

6.CONCLUSIONS

Apoptosis play an important role in the development and progression of endometriosis. Manipulation of cell death processes could be used to treat endometriosis. However, it is important to remember that no biochemical pathway stands on its own. Apoptosis represents the final execution step that defines the fate of a cell. However, the decision for the survival or the death of a cell has been decided earlier, through various and complicated gene regulations. Advances in molecular biology and genetics will help us to understand these issues and may yield prevention and treatment modalities for the endometriosis in the near future.

7. REFERENCES

1. B. Eskenazi, and M.L. Warner: Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 24, 235-238 (1997)

2. G. Leyendecker, L. Wildt, and G. Mall: The pathophysiology of endometriosis and adenomyosis: tissue injury and repair. *Arch Gynecol Obstet* 280, 529-538 (2009)

3. M. Quinn: Endometriosis: the elusive epiphenomenon. *J Obstet Gynaecol* 29, 590-593 (2009)

4. J. Halme, M.G. Hammond, J.F. Hulka, S. Raj, and L.M. Talbert: Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet. Gynecol* 64, 151-154 (1984a)

5. W.C. Keettel, and R.J. Stein: The viability of the cast-off menstrual endometrium. *Am. J. Obstet. Gynecol* 61, 440 (1951)

6. P. Vercellini, O. De Giorgo, G. Aimi, S. Panazza, A. Uglietti, and P.G. Crosignani: Menstrual characteristics in women with and without endometriosis. *Obstet. Gynecol* 90, 264-268 (1997)

7. M. Nisolle, M. Berliere, B. Paindaveine, F. Casanas-Roux, A. Bourdon, and J. Donnez: Histologic study of peritoneal endometriosis in infertile women. *Fertil.Steril* 53, 984-988 (1990)

8. K. Arumugam, and J.M. Lim: Menstrual characteristics associated with endometriosis. *Br. J. Obstet. Gynecol* 104, 948-950 (1997)

9. R.F.P.M. Kruitwagen, L.G. Poels, W.N.P. Willemsen, I.J. de Ronde, P.H. Jap, and R. Rolland: Endometrial epithelial cells in peritoneal fluid during the early follicular phase. *Fertil. Steril* 55, 297-303 (1991)

10. D. Vinatier, G. Orazi, M. Cosson, and P. Dufour: Theories of endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol* 96, 21-34 (2001)

11. L.C. Kao, A. Germeyer, S. Tulac, S. Lobo, J.P. Yang, R.N. Taylor, K. Osteen, B.A. Lessey, and L.C. Giudice: Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* 144, 2870-2881 (2003)

12. K.M. Eyster, O. Klinkova, V. Kennedy, and K.A. Hansen: Whole genome deoxyribonucleic acid microarray analysis of gene expression in ectopic versus eutopic endometrium. *Fertil Steril* 88, 1505-1533 (2007)

13. K.L. Sharpe-Timms: Endometrial anomalies in women with endometriosis. *Ann. N.Y. Acad. Sci* 943, 131-147 (2001)

14. C. Jolicoeur, M. Boutouil, R. Druin, I. Paradis, A. Lemay, and A. Akoum: Increased expression of monocyte chemotactic protein-1 in the endometrium of women with endometriosis. *Am. J. Pathol* 152, 125-133 (1998)

15. L.S. Noble, K. Takayama, K.M. Zeitun, J.M. Putman, D.A. Johns, M.M. Hinsshelwood, V.R. Agarwal, Y. Zhao, B.R. Carr, and S.E. Bulun: Prostaglandins E2 stimulates aromatase expression in endometriosis-derived stromal cells. *J. Clin. Endocrinol. Metab*82, 600-606 (1997)

16.G. Leyendecker, G. Kunz, M. Noe, M. Herbertz, and G. Mall: Endometriosis: a dysfunction and disease of the archimetra. *Hum. Reprod. Update*4, 752-762 (1998)

17. P.R. Koninckx, S.H. Kennedy, and D.H. Barlow: Endometriotic disease: the role of peritoneal fluid. *Hum. Reprod. Update*4, 741-751 (1998)

18. W. Kupker, A. Schulze-Mosgau, and K. Dietrich: Paracrine changes in the peritoneal environment of women with endometriosis. *Hum. Reprod. Update*4, 719-723 (1998)

19. T. Harada, A. Enatsu, M. Mitsunari, Y. Nagano, M. Ito, T. Tsudo, F. Taniguchi, T. Iwabe, M. Tanikawa, and N. Terakawa: Role of cytokines in progression of endometoriosis. *Obstet. Gynecol. Invest*47(suppl 1), 34-40 (1999)

20. T. Harada, T. Iwabe, and N. Terakawa: Role of cytokines in endometriosis. *Fertil. Steril*76, 1-10 (2001)

21. J.F.R. Kerr, A.H. Wyllie, and A.R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*26, 239-257 (1972)

22. M. Izawa, and J. Yeh: Apoptosis in human reproductive tissues:emerging concepts. *Reprod Med Rev*6, 23-36 (1997)

23. T. Harada, A. Kaponis, T. Iwabe, F. Taniguchi, G. Makrydimas, N. Sofikitis, M. Paschopoulos, E. Paraskevaidis, and N. Terakawa: Apoptosis in endometrial and endometrotic tissues. *Hum Reprod Update*10, 29-38 (2004)

24. J.F.R. Kerr, C.M. Winterford, and B.V. Harmon: Apoptosis: its significance in cancer and cancer therapy. *Cancer*73, 2013-2026 (1994)

25. G.S. Hotamisligil: Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*140, 900-917 (2010)

26. L.A. Tartaglia, T.M. Ayres, G.H.W. Wong, and D.V. Goeddel: A novel domain within the 55 kd TNF receptor signals cell death. *Cell*74, 845-853 (1993)

27. L.A. O'Reilly, and A. Strasser: Apoptosis and autoimmune disease. *Inflamm. Res*48, 5-21 (1999)

28. K. Cain, D.G. Brown, C. Langlais, and G.M. Cohen: Caspase activation involves the formation of the apoptosome, a large caspase-activating complex. *J Biol Chem*274, 22686-22692 (1999)

29. K. Cain, S.B. Bratton, C. Langlais, G. Walker, D.G. Brown, X.M. Sun, and G.M. Cohen: Apaf-1 oligomerizes into biological active approximately 700-Kda and inactive approximately 1.4-Mda apoptospme complex. *J Biol Chem*275, 6067-6070 (2000)

30. C. Angello, L. Caruso, M. Maggioni, M. Donadon, M. Montorsi, R. Santambrogio, G. Torzilli, V. Vaira, C. Pellegrini, M. Roncalli, G. Coggi, and S. Bosani: Inhibitors of apoptosis proteins (IAPs) expression and their prognostic significance in hepatocellular carcinoma. *BMC Cancer*9, 125 (2009)

31. N. Roy, M.S. Mahadevan, M. McLean, G. Shutler, Z. Yaraghi, R. Farahani, S. Baird, A. Besner-Johnston, C. Lefebvre, X. Kang, M. Salih, H. Aubry, K. Tamai, X. Guan, P. Ioannou, T.O. Crawford, P.J. de Jong, L. Surh, J.E. Ikeda, R.G. Korneluk, and A. MacKenzie: The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscularatrophy. *Cell*80, 167-178 (1995)

32. P. Liston, N. Roy, K. Tamai, C. Lefebvre, S. Baird, G. Cherton-Horvat, R. Farahani, M. McLean, J.E. Ikeda, A. MacKenzie, and R.G. Korneluk: Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature*379, 349-353 (1996)

33. N. Roy, O.L. Deveraux, R. Takahashi, G.S. Salvesen, and J.C. Reed: The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J*16, 6914-6925 (1997)

34. O.L. Deveraux, and J.C. Reed: IAP family proteins-suppressor of apoptosis. *Gene Dev*13, 239-252 (1999)

35. J.A. Garcia-Velasco, and A. Arici: Apoptosis and the pathogenesis of endometriosis. *Sem. Reprod. Med*21, 165-172 (2003)

36. I. Budihardjo, H. Oliver, M. Lutter, X. Luo, and X. Wang: Biochemical pathways of caspase activation during apoptosis. *Ann Rev Cell Dev Biol*15, 269-290, (1999)

37. M.O. Hengartner: The biochemistry of apoptosis. *Nature*407, 770-776 (2000)

38. S.G. Rolland, Y. Lu, C.N. David, and B. Conradt: The BCL-2-like protein CED-9 of C. Elegance promotes FZO-1/Mfn1,2- and EAT-3/Opa1-dependent mitochondrial fusion. *J Cell Biol*186, 525-540 (2009)

39. A. Autret, and S.J. Martin: Emerging role for members of the Bcl-2 family in mitochondrial morphogenesis. *Mol Cell*36, 355-363 (2009)

40. M. Karbowski, K. Norris, M.M. Cleland, S.Y. Jeong, and R.J. Youle: Role of Bax and Bak in mitochondrial morphogenesis. *Nature*443, 658-662 (2006)

41. C. Sheridan, P. Delivani, S.P. Cullen, and S.J. Martin: Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome C release. *Mol Cell*31, 570-585 (2008)

42. P. Delivani, C. Adrain, R.C. Taylor, P.J. Duriez, and S.J. Martin: Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol Cell*21, 761–773 (2006)

43. D. Arnoult, A. Grodet, Y.J. Lee, J. Estaquier, and C. Blackstone: Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. *J Biol Chem*280, 35742–35750 (2005)

44. S. Frank, B. Gaume, E.S. Bergmann-Leitner, W.W. Leitner, E.G. Robert, F. Catez, C.L. Smith, and R.J. Youle: The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell*1, 515–525 (2001)

45. R.J. Youle, and M. Karbowski: Mitochondrial fission in apoptosis. *Nat Rev Mol Cell Biol*6, 657–663 (2005)

46. D. Hopwood, and D.A. Levison: Atrophy and apoptosis in the cyclical human endometrium. J. *Pathol*119, 159-166 (1976)

47. K. Kokawa, T. Shikone, and R. Nakano: Apoptosis in the human uterine endometrium during the menstrual cycle. *J. Clin. Endocrinol. Metabol*81, 4144-4147 (1996)

48. T. Shikone, M. Yamoto, K. Kokawa, K. Yamashita, K. Nishimori, and R. Nakano: Apoptosis of human corpora lutea during cyclic luteal regression and early pregnancy. *J. Clin. Endocrinol. Metabol*81, 2376-2380 (1996)

49. X.J. Tao, K.I. Tilly, D.V. Maravei, J.L. Shifren, S. Krajewski, J.C. Reed, J.T. Tilly, and K.B. Isaacson: Differential expression of members of the bcl-2 gene family in proliferative and secretory human endometrium: Glandular epithelial cell apoptosis is associated with increased expression of Bax. *J. Clin. Endocrinol. Metabol*82, 2738-2746 (1997)

50. T.E Vaskivuo, F. Stenback, P. Karhumaa, J. Risteli, L. Dunkel, and J.S. Tapanainen: Apoptosis and apoptosis-related proteins in human endometrium. *Mol. Cell. Endocrinol*165, 75-83 (2000)

51. J. J. Brosens, and B. Gellersen: Death or survival – progesterone-dependent cell fate   decisions in the human endometrial stroma. *J Mol Endocrinol*36, 389-398 (2006)

52. J.C. Reed: Double identity for proteins of the Bcl-2 family. *Nature*382, 773-776 (1997)

53. Y. Otsuki, O. Misaki, O. Sugimoto, Y. Ito, Y. Tsujimoto, and Y. Akao: Cyclic bcl-2 expression in human uterine endometrium during menstrual cycle. *Lancet*344, 28-29 (1994)

54. H. Watanabe, H. Kanzaki, S. Narukawa, T. Inoue, H. Katsuragawa, Y. Kaneko, and T. Mori: Bcl-2 and Fas expression in eutopic and ectopic human endometrium during the menstrual cycle in relation to endometrial cell apoptosis. *Am. J. Obstet. Gynecol*176, 360-368 (1997)

55. R. Konno, H. Yamakawa, H. Utsunomiya, K. Ito, S. Sato, and A. Yajima: Expression of survivin and Bcl-2 in the normal human endometrium. *Mol. Hum. Reprod*6, 529-534 (2000)

56. Y. Otsuki: Apoptosis in human endometrium: apoptotic detection methods and signaling. *Med. Electron. Microsc*34, 166-173 (2001)

57. M. Ulukus, H. Cakmak, and A. Arici: The role of endomtrium in endometriosis. *J Soc Gynecol Investig*13, 467-476 (2006)

58. O. Bozdogan, P. Atasoy, S. Erekul, N. Bozdogan, and M. Bayram: Apoptosis-related proteins and steroid hormone receptors in normal, hyperplastic, and neoplastic endometrium. *Int J Gynecol Pathol*21, 375-382 (2002)

59. E. Daikoku, Y. Ito, and Y. Otsuki: The induction of apoptosis in ovaries and uteri of bcl-2 deficient mice. *Med. Electron. Microsc*31, 68-76 (1998)

60. P.A. Rogers, F. Lederman, D. Plunkett, and B. Affandi: Bcl-2, Fas, and caspase-3 expression in endometrium from levonorgestrel implant users with and without breakthrough bleeding. *Hum. Reprod*15(Suppl. 3), 152-161 (2000)

61. Z.N. Oltvai, C.L. Milliman, and S.J. Korsmeyer: Bcl-2 heterodimerizes *in vitro* with a conserved homolog, Bax, the accerelated programmed cell death. *Cell*74, 609-619 (1993)

62. M. Hanada, C. Aime-Sempe, T. Sato, and J.C. Reed: Structure-function analysis of Bcl-2 protein. Identification of conserved domains important for homodimerization with Bcl-2 and heterodimerization with Bax. *J. Biol. Chem*270, 11962-11969 (1995)

63. T. Chittenden, E.A. Harrington, R. O'Connor, C. Flemington, R.J. Lutz, G.I. Evan, and B.C. Guild: Induction of apoptosis by the Bcl-2 homologue Bak. *Nature*374, 731-733 (1995)

64. D.P. Braun, J. Ding, F. Shaheen, J.C. Willey, N. Rana, and W.P. Dmowski: Quantitative expression of apoptosis-regulating genes in endometrium from women with and without endometriosis. *Fertil. Steril* 87, 263-268 (2007)

65. M. Narkar, S. Kholkute, S. Chitlange, and T. Nandedkar: Expression of steroid hormone receptors, proliferation and apoptotic markers in primate endometrium. *Mol Cell Endocrinol*246, 107-113 (2006)

66. X.J. Tao, R.A. Sayegh, J.T. Tilly, and K.B. Isaacson: Elevated expression of the proapoptotic BCL-2 family member, BAK, in the human endometrium coincident with apoptosis during the secretory phase of the cycle. *Fertil. Steril*70, 338-343 (1998)

67. R. Rotello, R.C. Lieberman, R.B. Lepoff, and L.E.

Gerschenson: Characterization of uterine epithelium apoptotic cell death kinetics and regulation by progesterone and RU 486. *Am. J. Pathol*140, 449-456 (1992)

68. S. Tabibzadeh: Signals and molecular pathways involved in apoptosis with special emphasis on human endometrium. *Hum. Reprod*1, 303-323 (1995)

69. H.O.D. Critchely, S. Tong, S.T. Cameron, T.A. Drudy, R.W. Kelly, and D.T. Baird: Regulation of bcl-2 gene family members in human endometrium by antiprogestin administration *in vivo*. *J. Reprod. Fert*115, 389-395 (1999)

70. M. Bilotas, R.I. Baranao, R. Buquet, C. Sueldo, M. Tesone, and G. Meresman: Effects of GnRH analogues on apoptosis and expression of Bcl-2, Bax, Fas, and FasL proteins in endometrial epithelial cell cultures from patients with endometriosis and controls. *Hum Reprod*22, 644-653 (2007)

71. X. Peng, T. Maruo, H. Matsuo, S. Takekida, and J. Deguchi: Serum deprivation-induced apoptosis in cultures porcine granulosa cells is characterized by increased expression of p53 protein, Fas antigen and Fas ligand and by decreased expression of PCNA. *Endocr. J*45, 247-253 (1998)

72. H. Abe, M.A. Shibata, and Y. Otsuki: Caspase cascade of Fas-mediated apoptosis in human normal endometrium and endometrial carcinoma cells. *Mol Hum Reprod*12, 535–541 (2006)

73. H.M. Gebel, D.P. Braun, A. Tambur, D. Frame, N. Rana, and D.P. Dmowski: Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. *Fertil. Steril* 9, 1042-1047 (1998)

74. K. Szymanowski: Apoptosis pattern in human endometrium in women with pelvic endometriosis. *Eur J Obstet Gynecol Reprod Biol*132, 107-110 (2007)

75. M.C. Johnson, M. Torres, A. Alves, K. Bacallao, A. Fuentes, M. Vega, and M.A. Boric: Augmented cell survival in eutopic endometrium from women with endometriosis: expression of c-myc, TGF-beta1 and bax genes. *Reprod Biol Endocrinol*3, 45 (2005)

76. W.P. Dmowski, J. Ding, J. Shen, N. Rana, B.B. Fernandez, and D.P. Braun: Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis. *Hum. Reprod*16, 1802-1808 (2001)

77. A.G. Goumenou, I.M. Matalliotakis, M. Tzardi, Y.G. Fragouli, N.G. Mahutte, and A. Arici: Apoptosis and differential expression of apoptosis-related proteins in endometriotic glandular and stromal cells. *J Soc Gynecol Investig*11: 318-322 (2004)

78. R.K. Jones, R.F. Searle, and J.N. Bulmer: Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. *Hum. Reprod*13, 3496-3502 (1998)

79. A. Fujishita, R.O. Chavez, P.K. Nakane, T. Yamabe, T. Koji, T. Ishimau, and H. Masuzaki: Expression of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: an immunohistochemical and *in situ* hybridization study. *Fertil. Steril*67, 856-864 (1997)

80. G.F. Meresman, S. Vighi, R.A. Buquet, O. Contreras-Ortiz, M. Tesone, and L.S. Rumi: Apoptosis and expression of Bcl-2, Bax in eutopic endometrium from women with endometriosis. *Ferti. Steril*74, 760-766 (2000)

81. J. McLaren, A. Prentice, D.S. Charnock-Jones, A.M. Sharkey, and S.A. Smith: Immunocolonization of the apoptosis regulating proteins Bcl-2 and Bax in human endometrium and isolated peritoneal fluid macrophages in endometriosis. *Hum. Reprod*12, 146-152 (1997)

82. C. Dufournet, C. Uzan, R. Fauvet, A. Cortez, J.P. Siffroi, and E. Darai: Expression of apoptosis-related proteins in peritoneal, ovarian and colorectal endometriosis. *J Reprod Immunol*70, 151-62, (2006)

83. A. Beliard, A. Noel, and J.M. Foidart: Reduction of apoptosis and proliferation in endometriosis. *Fertil Steril*8, 80-85 (2004)

84. S.K. Banu, J.H. Lee, V.O. Speights, A. Starzinski-Powitz, and J.A. Arosh: Selective Inhibition of Prostaglandin E2 Receptors EP2 and EP4 Induces Apoptosis of Human Endometriotic Cells through suppression of ERK1/2, Akt, NF_B, and β-Catenin Pathways and activation of intrinsic apoptotic mechanisms. *Mol Endocrinol*23, 1291–1305 (2009)

85. M. Harada, N. Suganuma, M. Furuhashi, T. Nagasaka, N. Nakashima, F. Kikkawa, Y. Tomoda, and K. Furui: Detection of apoptosis in human endometriotic tissues. *Mol. Hum. Reprod*., 2, 307-315 (1996)

86. H. Das, T. Koizumi, T. Sugimoto, S. Chakraborty, T. Ichimura, K. Hasegawa, and R. Nishimura: Quantitation of Fas and Fas ligand gene expression in human ovarian, cervical and endometrial carcinomas using real-time quantitative RT-PCR. *Br. J. Cancer*82, 1682-1688 (2000)

87. A. Eidukaite, A. Siaurys, and V. Tamosiunas: Aberrant Expression of CD95 and CD69 Molecules among CD56+ Cells in Women with Endometriosis. *Am J Reprod Immunol*55, 276-281 (2006)

88. J.A. Garcia-Velasco, N. Mulayim, U.A. Kayisli, and A. Arici: Elevated soluble Fas ligand levels may suggest a role for apoptosis in women with endometriosis. *Fertil. Steril*78, 855-859 (2002)

89. T. Suda, T. Okazaki, Y. Naito, T. Yokota, N. Arai, S. Ozaki, K. Nakao, and S. Nagata: Expression of the Fas ligand in T cell lineage. *J. Immunol*154, 3806-3813 (1995)

90. D.J. Oosterlynck, C. Meuleman, M. Waer, and P.R. Koninckx: Transforming growth factor beta activity is increased in peritoneal fluid from women with

endometriosis. *Obstet Gynecol*84, 287-292 (1994)

91. J. Garcia-Velasco, A. Arici, T. Zreick, F. Naftolin, and G. Mor: Macrophage-derived growth factors regulate FasL expression in endometrial stromal cells: a role in endometriosis. *Mol. Hum. Reprod*.5, 642-650 (1999)

92. B. Selam, U.A. Kayisli, J.A. Garcia-Velasco, and A. Arici: Extracellular matrix-dependent regulation of Fas ligand expression in human endometrial stromal cells. *Biol. Reprod*66, 1-5 (2002a)

93. N. Kayagaki, A. Kawasaki, T. Ebata, H. Ohmoto, S. Ikeda, S. Inoue, K. Yoshino, K. Okumura, and H. Yagita: Metalloproteinase-mediated release of human Fas ligand. *J Exp Med*182, 1777-1783 (1995)

94. M.D. Spuijbroek, G.A. Dunselman, P.P. Menheere, and J.L. Evers: Early endometriosis invades the extracellular matrix. *Fertil. Steril*58, 929-933 (1992)

95. P. Wisniewski, A. Ellert-Miklaszewska, A. Kwiatkowska, and B. Kaminska: Non-apoptotic Fas signaling regulates invasiveness of glioma cells and modulates MMP-2 activity via NFκB-TIMP-2 pathway. *Cellular Signalling*22, 212–220 (2010)

96. T. Iwabe, T. Harada, T. Tsudo, M. Tanikawa, Y. Onohara, and N. Terakawa: Pathogenetic significance of increased levels of interleukin-8 in the peritoneal fluid of patients with endometriosis. *Fertil. Steril* 69, 924-930 (1998)

97. T. Iwabe, T. Harada, T. Tsudo, Y. Nagano, S. Yoshida, M. Tanikawa, and N. Terakawa: Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J. Clin. Endocrinol. Metab*85, 824-829 (2000)

98. B. Selam, U.A. Kayisli, J.A. Garcia-Velasco, G.E. Akbas, and A. Arici: Regulation of Fas Ligand expression by IL-8 in human endometrium. *J. Clin. Endocrinol. Metabol*8, 3921-3927 (2002b)

99. S. Leuenroth, C. Lee, P. Grutkoski, H. Keeping, and H.H. Simms: Interleukine-8-induced suppression of polymorphonuclear leukocyte apoptosis is mediated by suppressing CD95 (Fas/Apo-1) Fas-l interactions. *Surgery*124, 409-417 (1998)

100. J.Y. Kwak, S.W. Park, K.H. Kim, Y.J. Na, and K.S. Lee: Modulation of neutrophil apoptosis by plasma and peritoneal fluid from patients with advanced endometriosis. *Hum Reprod*17, 595-600 (2002)

101. S. Jenkins, D.L. Olive, and A.F. Haney: Endometriosis: pathogenetic implications to of the anatomic distribution. *Obstet. Gynecol*67, 335-338 (1986)

102. A. Eischen, B. Duclos, M. Schmit-Goguel, N. Rouyer, J.P. Bergerat, M. Hummel, R. Oskam, and F. Oberling: Human resident peritoneal macrophages: phenotype and

histology. *Br. J. Haematol*88, 712-722 (1994)

103. J. Halme, S. Becker, M.G. Hammond, M.H. Raj, and S. Raj: Increased activation of pelvic macrophages in infertile women with endometriosis. *Am. J. Obstet. Gynecol*145, 333-337 (1983)

104. J.W. Ramsey: Peritoneal fluid: its relevance to the development of endometriosis. *Fertil. Steril*60, 1-14 (1993)

105. J. Halme, S. Becker, and R. Wing: Accentuated cyclic activation of peritoneal macrophages in patients with endometriosis. *Am. J. Obstet. Gynecol*148, 85-90 (1984b)

106. D.P. Braun, H. Gebel, C. Rotman, N. Rana, and W.P. Dmowski: The development of cytotoxicity in peritoneal macrophages from women with endometriosis. *Fertil. Steril*57, 1203-1210 (1992)

107. J.C. Lousse, A. Van Langendonckt, S. Defrere, E. Renkin, and G. Donnez: Increased activation of nuclear factor-kappaB (NF-κB) in isolated peritoneal macrophages of patients with endometriosis. *Fertil Steril*90, 217-220 (2008)

108. R. Gonzales-Ramos, A. Van Langendonckt, S. Defrere, J.C. Lousse, S. Colette, L. Devoto, and J. Donnez: Involvement of the nuclear factor-κB pathway in the pathogenesis of endometriosis. Fertil *Steril* (in press) (2010)

109. P.C. Chuang, Y.J. Lin, M.H. Wu, L.Y.C. Wing, Y. Shoji, and S.J. Tsai: Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. *Am J Pathol*176, 850–860 (2010)

110. D.P. Braun, H. Gebel, N. Rana, and W.P. Dmowski: Cytolysis of eutopic and ectopic endometrial cells by peritoneal blood monocytes and peritoneal macrophages in women with endometriosis. *Fertil. Steril*6, 1103-1108 (1998)

111. A.E. Aplin, A. Howe, S.K. Alahari, and R.L. Juliano: Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobin-cell adhesion molecules, and selectins. *Pharmacol. Rev*50, 197-263 (1998)

112. O. Cinar, Y. Seval, Y.H. Uz, H. Cakmak, M. Ulukus, U.A. Kayisli, and A. Arici: Differential regulation of Akt phosphorylation in endometriosis. *Reprod Biomed Online*19, 864-71 (2009)

113. L. Aghajanova, J.A. Horcajadas, J.L. Weeks, F.J. Esteban, C.N. Nezhat, M. Conti, and L.P. Giudice: The Protein Kinase A Pathway-Regulated Transcriptome of Endometrial Stromal Fibroblasts Reveals Compromised Differentiation and Persistent Proliferative Potential in Endometriosis. *Endocrinology*151, 1341–1355 (2010)

114. R. Gonzalez-Ramos, A. Van Langendonckt, S.

Defrere, J.C. Lousse, M. Mettelen, A. Guillet, and J. Donnez: Agents blocking the nuclear factor-kappaB (NF-κB) pathway are effective inhibitors of endometriosis in an *in vivo* experimental model. *Gynecol Obstet Invest*65, 174-186 (2008)

115. T. Arimoto, T. Katagiri, K. Oda, T. Tsunoda, T. Yasugi, Y. Osuga, H. Yoshikawa, O. Nishii, T. Yano, Y. Taketani, and Y. Nakamura: Genome-wide cDNA microarray analysis of gene-expression profiles involved in ovarian endometriosis. *Int. J Oncol*22, 551-560 (2003)

116. M. Ueda, Y. Yamashita, M. Takehara, Y. Terai, K. Kumagai, K. Ueki, K. Kanda, H. Yamaguchi, D. Akise, Y.C. Hung, and M. Ueki: Survivin gene expression in endometriosis. *J Clin Endocrinol Metab*87, 3452-3459 (2002)

117. M. Nishida, K. Nasu, T. Ueda, J. Fukuda, N. Takai, and I. Miyakawa: Endometriotic cells are resistant to interferon-gamma-induced cell growth inhibition and apoptois: a possible mechanism involved in the pathogenesis of endometriosis. *Mol Hum Reprod* 11, 29-34 (2005)

118. M. Izawa, T. Harada, I. Deura, F. Taniguchi, T. Iwabe, and N. Tearakawa: Drug-induced apoptosis was markedly attenuated in endometriotic stromal cells. *Hum Reprod* 21, 600-604 (2006)

119. H. Zhang, M. Li, X. Zheng, Y. Sun, Z. Wen, and X. Zhao: Endometriotic stromal cells lose the ability to regulate cell-survival signalling in endometrial epithelial cells *in vitro*. *Mol Hum Reprod*15, 653–663 (2009)

120. A. Watanabe, F. Taniguchi, M. Izawa, K. Suou, T. Uegaki, E. Takai, N. Terakawa, and T. Harada: The role of surviving in the resistance of endometriotic stromal cells to drug-induced apoptosis. *Hum Reprod*24, 3172-3179 (2009)

121. T. Nakahara, M. Takeuchi, I. Kinoyama, T. Minematsu, K. Shirasuna, A. Matsuhisa, A. Kita, F. Tominaga, K. Yamanaka, and M. Kudoh: YM155, a novel small-molecule surviving suppressant, induces regression of established human hormone-refractory prostate tumor xenografts. *Cancer Res*67, 8014-8021 (2007)

122. Z. Ai, L. Yin, X. Zhou, Y. Zhu, D. Zhu, Y. Yu, and Y. Feng: Inhibition of survivin reduces cell proliferation and induces apoptosis in human endometrial cancer. *Cancer*107, 746–756 (2006)

123. A. Imai, A. Takagi, and T. Tamaya: Gonadotropin-releasing hormone analog repairs reduced endometrial cell apoptosis in endometriosis *in vitro*. *Am. J. Obstet. Gynecol*182, 1142-1146 (2000)

124. G.F. Meresman, M.A. Bilotas, E. Lombardi, M. Tesone, C. Sueldo, and R. Baranao: Effect of GnRH analogues on apoptosis and release of interleukin-1β and vascular endothelial growth factor in endometrial cell cultures from patients with endometriosis. *Hum Reprod*18,

1767-1771 (2003)

125. G.F. Meresman, M.A. Bilotas, R.A. Buquet, R. Baranao, C. Sueldo, and M. Tesone: Gonadotoropin-releasing hormone agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis. *Fertil Steril*80, 702-707 (2003)

126. S.S. Kakar, S.J. Winters, W. Zacharias, D.M. Miller, and S. Flynn: Identification of distinct gene expression profiles associated with treatment of L T2 cells with gonadotropin-releasing hormone agonist using microarray analysis. *Gene*308, 67-77 (2003)

127. K.N. Khan, M. Kitajima, K. Hiraki, A. Fujishita, I. Sekine, T. Ishimaru, and H. Masuzaki: Changes in tissue inflammation, angiogenesis and apoptosis in endometriosis, adenomyosis and uterine myoma after GnRH-a therapy. *Hum Reprod*25, 642–653 (2010)

128. M. Tesone, M. Bilotas, R.J. Barañao, and G. Meresman: The Role of GnRH Analogues in Endometriosis-Associated Apoptosis and Angiogenesis. *Gynecol Obstet Invest*66(Suppl.1), 10-18 (2008)

129. L.S. Noble, E.R. Simpson, A. Johns, and S.E. Bulun: Aromatase expression in endometriosis. *J Clin Endocrinol Metab*81:174-179 (1996)

130. J. Kitawaki, T. Noguchi, T. Amatsu, K. Maeda, K. Tsukamoto, T. Yamamoto, S. Fushiki, Y. Osawa, and H. Honjo: Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. *Biol Reprod*57:514-519 (1997)

131. K. Takayama, K. Zeitoun, R.T. Gunpy, H. Sasano, B.R. Carr, and S.E. Bulun: Treatment of severe postmenopausal endometriosis with aromatase inhibitor. *Fertil Steril*69, 709-713 (1998)

132. S. Soysal, M.E. Soysal, S. Ozer, N. Gul, and T. Gezgin: The effects of post-surgical administration of goserelin plus anastrozole compared to goserelin alone in patients with severe endometriosis: prospective randomized trial. *Hum Reprod*19, 160-167 (2004)

133. G.F. Meresman, M. Bilotas, V. Abello, R. Buquet, M. Tesone, and C. Sueldo: Effects of aromatase inhibitors on proliferation and apoptosis in eutopic endometrial cell cultures from patients with endometriosis. *Fertil Steril*84, 459-463 (2005)

134. M. Bilotas, G. Meresman, I. Stella, C. Sueldo, and R.I. Barañao: Effect of aromatase inhibitors on ectopic endometrial growth and peritoneal environment in a mouse model of endometriosis. *Fertil Steril* (in press) (2009)

135. T. Harada, M. Momoeda, Y. Taketani, H. Hoshiai, and N. Terakawa: Low-dose oral contraceptive pill for dysmenorrhea associated with endometriosis: a placebo-controlled, double-blind, randomized trial. *Fertil Steril*90,

1583-1588 (2008)

136. B.A. Lessey: Medical management of endometriosis and infertility. *Fertil Steril* 73, 1089-1096, (2000)

137. E.T.A. Koh, P.J. Illingworth, W.C. Duncan, and H.O. Critchley: Immunolocalization of Bcl-2 protein in human endometrium in the menstrual cycle and simulated early pregnancy. *Hum. Reprod* 10, 1557-1562 (1995)

138. G.F. Meresman, L. Auge, R.I. Baranao, E. Lombardi, M. Tesone, and C. Sueldo: Oral contraceptives suppress cell proliferation and enhance apoptosis of eutopic endometrial tissue from patients with endometriosis. *Fertil. Steril* 77, 1141-1147 (2002)

139. K.S. Moghissi: Treatment of endometriosis with estrogen-progestogen combination and progestogen alone. *Clin Obstet Gynecol* 31, 823-828 (1988)

140. L.M. Kettel, A.A. Murphy, A.J. Morales, and S.S.C. Yen: Preliminary report on the treatment of endometriosis with low-dose mifepristone (RU486) *Am J Obstet Gynecol* 178, 1151-1154 (1998)

141. L. Fedele, S. Bianchi, G. Zanconato, A. Portuese, and R. Raffaelli: Use of a levonorgestrel-releasing intrauterine device in the treatment of rectovaginal endometriosis. *Fertil Steril* 75, 485-488 (2001)

142. M.K.O. Gomes, J.C. Rosa-e-Silva, S.B. Garcia, A.C. Japur de Sa´ Rosa-e-Silva, A. Turatti, C.S. Vieira1, and R.A. Ferriani: Effects of the levonorgestrel-releasing intrauterine system on cell proliferation, Fas expression and steroid receptors in endometriosis lesions and normal endometrium. *Hum Reprod* 24, 2736–2745 (2009)

143. P. Yuan, Y. Huang, H. Wu, Z. Teng, J. Zhang, and X. Xin: Induction of a local pseudo-pregnancy via levonorgestrel-loaded microspheres for the treatment of endometriosis in a rabbit model. *Hum Reprod* 25, 462-469 (2010)

144. K. Nasu, M. Nishida, T. Ueda, N. Takai, S. Bing, H. Narahara, and I. Miyakawa: Bufalin induces apoptosis and the G0/G1 cell cycle arrest of endometriotic stromal cells: a promising agent for the treatment of endometriosis. *Mol Hum Reprod* 11, 817-823 (2005)

145. M. Nishida, K. Nasu, T. Ueda, A. Yuge, N. Takai, and H. Narahara: Beta-hydroxyisovalerylshikonin induces apoptosis and the G0/G1 cell cycle arrest of endometriotic stromal cells: a preliminary *in vitro* study. *Hum Reprod* 21, 2850-2856 (2006)

146. Y. Sakamoto, T. Harada, S. Horie, Y. Iba, F. Taniguchi, S. Yoshida, T. Iwabe, and N. Terakawa: Tumor necrosis factor- -induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-κB activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. *J. Clin. Endocrinol. Metabol* 88, 730-735 (2003)

147. K. Nasu, M. Nishida, T. Ueda, A. Yuge, N. Takai, and H. Narahara: Application of the nuclear factor-κB inhibitor, BAY 11-7085, for the treatment of endometriosis: an *in vitro* study. *Am J Physiol Endocrinol Metab* 293, E16-23 (2007)

148. C. Gangadharan, M. Thoh, and S.K. Manna: Late phase activation of nuclear transcription factor kappaB by doxorubicin is mediated by interleukine-8 and induction of apoptosis via FasL. *Breast Cancer Res Treat* 120, 671-683 (2010)

149. E.E. Othman, S. Salama, N. Ismail, and A. Al-Hendy: Toward gene therapy of endometriosis:adenovirus-mediated delivery of dominant negative estrogen receptor genes inhibits cell proliferlation, reduces cytokine production, and induces apoptosis of endometriotic cells. *Fertil Steril* 88, 462-471 (2007)

150. D. Altintas, A. Kokcu, B. Kandemir, M. Tosun, and M.B. Cetinkaya: Comparison of the effects of raloxifene and anastrozole on experimental endometriosis. *Eur J Obstet Gynecol Reprod Biol* (in press) (2010)

151. S. Morishima, M.A. Shibata, M. Ohmichi, and Y. Otsuki: Raloxifene, a selective estrogen receptor modulator, induces mitochondria-mediated apoptosis in human endometrial carcinoma cells. *Med Mol Morphol* 41, 132–138 (2008)

152. S.K. Banu, J. Lee, V.O. Speights Jr, A. Starzinski-Powitz, and J.A. Arosh: Cyclooxygenase-2 regulates survival, migration and invasion of human endometriotic cells through multiple mechanisms. *Endocrinology* 149, 1180–1189 (2008)