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The role of NF-kappaB in endometriosis

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1. ABSTRACT

The nuclear factor kappaB (NF-kappaB) is a ubiquitously expressed transcription factor playing vital roles in innate immunity and other processes involving cellular survival, proliferation, and differentiation. This review highlights the importance of NF-kappaB in the pathophysiology of endometriosis. Constitutive activation of NF-kappaB has been shown in endometriotic lesions. Complex interactions of NF-kappaB with steroid receptors and apoptotic molecules in endometriosis resulting in opposing roles of NF-kappaB are discussed. NF-kappaB regulates the expression of cytokines mediating autocrine self-amplifying cycles of cytokine release and NF-kappaB activation, leading to maintenance of inflammatory reactions in endometriosis. NF-kappaB can contribute to the increased ability of endometriotic cells to invade and adhere to the peritoneal surface by regulating the expression of matrix metaloproteinases. We are presenting the role of NF-kappaB to regulate vascularization and oxidative stress in endometriotic cells. Effects of drugs used for the treatment of endometriosis on NF-kappaB pathway are presented and we show how drugs that inhibit the NF-kappaB can mediate the progression of endometriosis. Novel therapeutic strategies involving the NF-kappaB and applied in endometriosis are also discussed.

2. INTRODUCTION

Endometriosis (derived from the Greek words *endo*, "inside", and *metra*, "womb") was first described by Daniel Shroen in 1690 in a book entitled "*Disputatio Inauguralis Medica de Ulceribus Ulceri"* (1). He stated categorically: "This is a female disorder, characteristic of those who are sexually maturing". He also described the lesions as inflammations, that they had a tendency to form adhesions that linked visceral areas together (2). Nowadays, endometriosis is a common, benign gynecological disorder 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 (3). Although the high incidence of endometriosis and the fact that more than eighty years have passed since the theory described by Sampson of menstrual dissemination of endometrial tissue into the peritoneal cavity, our current understanding of the aetiology and pathophysiology of endometriosis remains obscure (4).

A general accepted concept regarding the pathogenesis of endometriosis is that an inflammatory process with altered function of immune-related cells exists in the peritoneal environment in women who developed the

disease. We and others have showed an increased number of macrophages and increased levels of pro-inflammatory cytokines in the peritoneal fluid of patients with endometriosis (5-7). The main source of cytokines is thought to be the macrophages. However, endometriotic cells produce cytokines independent of macrophages contributing to their survival into the peritoneal cavity (8). We found that interleukine-8 (IL-8) significantly enhanced proliferation of stromal cells derived from ovarian endometriomas, suggesting that IL-8 may promote the progression of endometriosis (9). We also showed that TNFa promoted the proliferation of endometriotic stromal cells by inducing IL-8 gene and protein expression (10). It is therefore possible that an altered peritoneal microenvironment with increased expression of pro-inflammatory cytokines and growth factors may enhance the capability of endometrial cells to implant and grow into the peritoneal cavity. Nowadays, endometriosis is considered to be an inflammatory-like phenomenon. Inflammatory responses are now thought to be mediated by the activation of the transcription factor, nuclear factor-kappaB (NF-kappaB).

NF-kappaB is involved in numerous pathways mediating cell proliferation, survival, apoptosis, adhesion, invasion, and neo-vascularization in various cell types (11). These pathways are also altered in endometriosis. Moreover, NF-kappaB is constitutively activated in endometriosis suggesting the importance of this pathway for the development and progression of the disease (12). Interestingly, all existing and nearly all investigational medications for endometriosis appear to act through suppression of NF-kappaB activation (13). In the current review we will describe briefly the signaling pathways activated the NF-kappaB and the following post-transcriptional effects. Evidence for its role in normal endometrium and in ectopic endometriotic tissues will be presented. Finally, therapeutic implications targeting the NF-kappaB will also be discussed.

3. THE NF-KAPPAB SIGNALING PATHWAY

The transcription factor NF-kappaB was first discovered in 1986 as a factor in the nucleus that binds the DNA promoter of the kappa chain of immunoglobulins in B cells (14,15). Activation of NF-kappaB leads to the expression of products that are both pro-inflammatory and anti-inflammatory, as well as pro-apoptotic and anti-apoptotic. NF-kappaB may allow cells to be protected and proliferate, and at the same time can initiate an inflammatory response through the recruitment and activation of effector cells of the immune system (16). Thus, NF-kappaB is a unique transcription factor regulating dual and opposing roles in different cells, tissues, and physiological or pathophysiological conditions.

Five members of the NF-kappaB family, p50, p52, p65 (RelA), c-Rel, and RelB, form various homo- and heterodimers, where the most common active forms of which are the p50/RelA or p52/RelA heterodimer. In most cell types inactive NF-kappaB complexes are sequestered in the cytoplasm, whereas, constitutive activation occurs in B cells, some monocyte cell lines, and malignant cells (17). The NF-kappaB dimers bind to specific inhibitors of NF-kappaB (IkappaB), forming a complex, which is inactive because it is unable to bind to DNA. There are several IkB proteins, which have different affinities for individual Rel/NF-kB complexes, are regulated slightly differently, and are expressed in a tissue-specific manner. The IkB proteins include, at least, p105, p100, IkBa, IkBb, IkBg, IkBe, IkBz, Bcl-3, and the Drosophila Cactus protein (18,19). In response to multiple stimuli, including cytokines, viral and bacterial pathogens, and stress-inducing agents, activation of the cytoplasmic NF-kappaB occurs, mainly through IkappaB kinase (IKK)-dependent phosphorylation and degradation of the IkappaB inhibitory proteins (18,19). The IKK complex consists of two catalytic subunits (IKK-alpha and IKK-B) and the NF-kappaB essential modulator NEMO or IKK-γ (18-20). NEMO serves as the regulatory subunit, being essential for IKKB activation. IKK activation induces IkappaB peptides polyubiquitination and fast proteolysis by the 26S proteasome leading to liberation and activation of NF-kappaB. The active NF-kappaB translocates into the nucleus where it regulates gene expression through binding to the kappaB elements in the enhancer or promoter regions of the genes (14-18,21).

The ability of many different signal transduction pathways originating from a wide variety of inducing mechanisms to converge on a single target (NF-kappaB induction) highlights the importance of this transcription factor to control various cellular functions. Three major pathways of NF-kappaB activation have been identified. The canonical pathway depends on the inducible degradation of inhibitory IkappaB proteins, which retain most NF-kappaB dimers (except those that contain RelB) in the cytoplasm (22). This pathway can be induced by proinflammatory stimuli such as TNF-a, IL-1, and lipopolysaccharide (LPS). The non-canonical pathway is triggered by stimuli activating IKK-alpha and mainly associated with presence of RelB in the cytoplasm (22). Finally, atypical pathways are induced by diverse stimuli and activate different forms of NF-kappaB dimers with distinct functions (23). In the classical pathway, one of the target genes activated by NF-kB is that which encodes IkBa. Newly-synthesized IkBa can enter the nucleus, remove NF-kB from DNA, and export the complex back to the cytoplasm to restore the original latent state. Thus, the activation of the NF-kB pathway is generally a transient process, lasting from 30-60 minutes in most cells (17).

The activation of NF-kappaB is required for the regulation of many crucial inflammatory and immune response genes; genes involving in cellular proliferation, cell adhesion, and genes regulating apoptosis (13). A complete list of genes whose expression is regulated by NF-kappaB is available at http://www.nf-kb.org. The physiologic role of NF-kappaB is to regulate B-cell development, proliferation and effector functions (17). NF-kappaB also controls the expression of many cytokines and various T-cell functions (24-26).

4. INTERACTIONS BETWEEN SEX STEROID HORMONES AND NF-KAPPAB PATHWAY

The observation that pregnancy can lead to remission of rheumatoid arthritis initiated attempts to identify

the "anti-rheumatic substance X", as well as the female sex hormones, which might be responsible for these remissions (27). Nowadays, we know that this phenomenon no doubt exemplify the negative cross-talk of sex steroid hormones with NF-kappaB (27,28). Because of its central role in signal transduction in immunological and inflammatory responses, inhibition of NF-kappaB activity by steroids could provide an explanation for their anti-inflammatory and immunosuppressive activity.

4.1. Progesterone and progesterone receptor

Progesterone has been shown to increase the expression of IkappaBalpha (29). Reciprocal negative cross-talk has been observed between NF-kappaB and the progesterone receptor (PR). Activation of the PR can lead to inhibition of NF-kappaB-driven gene expression and NF-kappaB activation antagonizes also PR-activated target genes (30). Direct interaction between p65 and PR has been described resulting in the formation of inactive complexes that cannot bind to DNA or activate other essential co-factors (30). However, more recent studies have shown that progesterone acts through multiple mechanisms to inhibit NF-kappaB: i) PR directly inhibits NF-kappaB dimmer binding to its consensus DNA response elements; ii) progesterone/PR down-regulate the transcription of TNF-related apoptosis-inducing ligand (TRAIL) and its receptor TRAIL2 which activate NF-kappaB; and iii) progesterone/PR up-regulate the transcription of A20 and A20 binding inhibitor of NF-kappaB 2 (ABIN-2), binding proteins that inactivate NF-kappaB (31) (Figure 1).

4.2. Estrogen receptors

Mutual repression of signalling between NF-kappaB and estrogen receptor (ER) has also been reported in various studies (32-34). Loss of ER function has been associated with constitutive NF-kappaB expression and hyperactive Mitogen Activated Protein Kinase (MAPK) pathway, because of constitutive secretion of cytokines and growth factors (27,35,36). In vitro studies revealed that estrogens can block DNA binding of NF-kappaB to kappaB site promoters (37,38). In addition, estradiol alters NF-kappaB activation through regulation of IKK activation and IkappaB expression (39-40).

However, the negative interaction between steroid receptors and NF-kappaB cannot explain why during pregnancy when both estrogens and progesterone levels are increased, NF-kappaB is also activated. Estrogens can both activate and inhibit the NFkappaB. ERalpha and ERB can associate with NF-kappaB and with steroid hormone co-activators at the promoter region of NF-kappaB-regulated gene. It is possible that co-factors, previously described as co-activators or co-repressors, can switch functionality depending on the promoter context (27,41). It is known, that eukaryotic transcriptional regulation is accomplished by multiprotein complexes that assemble at response elements embedded in DNA sequences close to target promoters (41). The precise arrangements of sequences within response elements and the expression levels and activities of regulatory factors within a cell are key determinants of the composition and function of a given regulatory complex. The effects can be profound: within different regulatory complexes, a given co-factor may activate transcription, repress, or display no regulatory activity (41). For instance, Hirano and co-workers found that nanomolar concentrations of estradiol for 6h enhanced NF-kappaB activation with TNFalpha-stimulation in primary human T cells (42). The enhanced activation was in part due to ERB association with the p65, leading to the recruitment of glucocorticoid receptor interacting protein (GRIP); a co-factor originally identified as a co-activator of the p160 family member that in this case worked as a co-activator. GRIP can also acts as co-repressor as was proven in the case of estrogen-dependent inhibition of NF-kappaB that requires its function (43). Additionally, it is possible that NF-kappaB activation is not exclusively depending on estrogen or progesterone concentration and the expression of steroid hormones. Other molecules, such as pro-inflammatory cytokines (IL-1, TNFalpha) and other nuclear receptors such as, peroxisome proliferator activated receptor (PPAR) may also involve in NF-kappaB activation in human endometrium.

Positive interactions between the ER and NF-kappaB signaling pathways have been described in some cell lines. In the T47D breast cancer cell line, estradiol induces cell proliferation and cyclin D1 expression by a NF-kappaB-dependent mechanism, which is enhanced in the presence of TNFa and involves the formation of a protein complex containing ER, p65, and the co-activator ras-related C_3 botulinum substrate 3 (RAC3) (44). In another experiment, related to gender-specific regulation of longevity genes, an estradiol-mediated increase in MAPK activation was found to activate also the NF-kappaB signaling pathway (45,46). Through a non-genomic ER action, NF-kappaB-dependent gene product cyclooxygenase-2 (COX-2) leads to production of the atheroprotective prostacyclin, PGI_2 (47).

In endometrial stromal cells (ESCs), ERs were shown to inhibit DNA binding of p50 and p65 subunits of NF-kappaB (23). In addition, NF-kappaB activation significantly reduced estrogen responsiveness of ER-alpha-transfected ESCs (48). However, p50 did not impair ER-alpha DNA binding, suggesting possible indirect mechanisms for this type of interaction (48). Recently, King and co-workers described a positive interaction between ERs and NF-kappaB signaling pathway in an endometrial epithelial cell line (49). Estradiol and IL-1B treatment of EECs enhances ERE activity by NF-kappaB and ER-dependent mechanism; this interaction could be mediated by both ERs (49). In endometriotic stromal cells, we have shown that estradiol enhanced IL-8 production induced by TNFalpha, via activation of the NF-kappaB pathway (50).

It seems that interactions of steroid receptors and NF-kappaB pathway are not only cell-type specific but various co-factors may be involved in each cell-type and produce differential results. Steroid receptors can prevent the interaction of NF-kappaB with some required co-activators, but no others, providing the basis for signal- and promoter-specific interactions. It is also possible that different steroid family members recruit different co-factors to cross-talk with NF-kappaB (27).

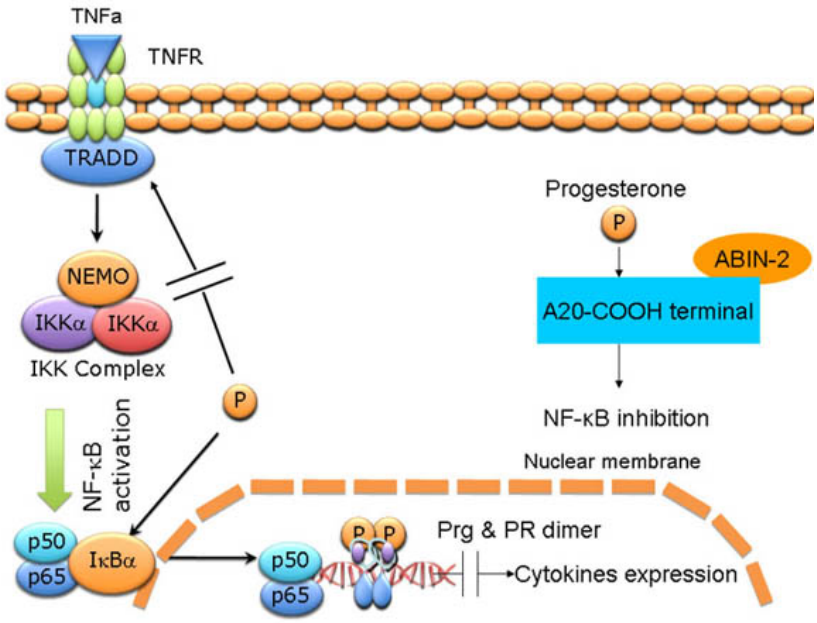

Figure 1. Progesterone acts through multiple mechanisms to inhibit NF-kappaB. Although direct interaction between p65 and PR resulting in the formation of inactive complexes that cannot bind to DNA cannot be excluded, recent studies (Davies et al., 2004) have shown that: i. Progesterone receptor and ligand acts as a competing transcription factor displacing NF-kappaB from the binding site of gene; ii. Progesterone can stimulate the synthesis of the inhibitory binding protein IkappaBalpha; iii. Progesterone/PR down-regulate the transcription of TNF-related apoptosis-inducing ligand (TRAIL) and its receptor which activate NF-kappaB; and iv. Progesterone/PR up-regulate the transcription of A20 and A20 binding inhibitor of NF-kappaB (ABIN2) which inactivate NF-kappaB.

5. NF-KAPPAB EXPRESSION IN NORMAL ENDOMETRIUM

Several functions of the human endometrium are associated with inflammatory-like responses, e.g. implantation and menstruation (51). NF-kappaB may have an important role to regulate these processes. Human endometrial cells can constitutively express NF-kappaB proteins (52,53). The immunocytochemical analysis in cultured epithelial endometrial cells revealed that the staining intensity of p65 was low during the proliferative phase, but increased during the secretory phase and was maximal at the time of implantation (52). During secretory phase and implantation there was also decreased staining of the inhibitory proteins IkappaB (53,54). These differences in the expression of NF-kappaB family members were not so pronounced in human endometrial stromal cells (54). It is of interest to note that the PR-B expression is in the opposite direction as that of NF-kappaB: lower in the secretory phase but higher in the proliferative phase (55).

NF-kappaB is involved in the inflammatory events associated with menstruation (53,56). Progesterone withdrawal induces vasoconstriction with associated hypoxia which will in turn, induce IkappaBalpha phosphorylation and ubiquitination and subsequently NF-kappaB activation (53,57). NF-kappaB activation will further induce the production of pro-inflammatory cytokines, which will affect lytic enzyme activity and production of matrix metalloproteinases (MMPs) which are involved in tissue breakdown (58). In addition, mediators known to activate NF-kappaB are present in endometrium during menstruation (e.g., IL-1, TNFalpha) and these are likely to contribute to the stimulation of NF-kappaB at this time (53).

The IKK complex is also expressed in human endometrium. IKKalpha is expressed in the endometrium throughout the menstrual cycle with increased expression in decidua whereas IKKB was decreased in decidua (53). IKKalpha may be involved via NF-kappaB and COX-2, in the expression of mediators, e.g., prostaglandin E_2 which are vital for implantation and successful pregnancy (53,59). The reduced amount of IKKB in decidua may suggest a mechanism to decrease proinflammatory signaling to NF-kappaB at a time when local immunosuppression is necessary.

6. NF-KAPPAB EXPRESSION IN WOMEN WITH ENDOMETRIOSIS

Although the role of NF-kappaB in endometriosis pathway seems to be inevitable, data in the literature evaluating NF-kappaB activation in human eutopic endometrium from endometriosis patients are scarce. NF-kappaB dysfunction was observed during the late secretory phase in eutopic endometrium of endometriosis patients. IkappaB kinase was strongly reduced coincidently with the

reduction of NF-kappaB DNA binding when compared with the normal endometrium at the same stage of the menstrual cycle (60). In addition, eutopic endometrium from women with adenomyosis the NF-kappaB expression was recently evaluated (61). Higher expression of nuclear p65 and p52 together with lower expression of PR-B and cytoplasmic IkappaBalpha was observed comparing with the expression of these molecules in normal endometrium (61). The latter findings suggest that the eutopic endometrium of women with adenomyosis may already have shown signs of progesterone resistance and constitutive NF-kappaB activation.

NF-kappaB alterations in eutopic endometrium of women with endometriosis may be involved in other processes contributing to the establisment and the development of the disease. Activation of NF-kB has been implicated in the early development of endometriotic lesions in vivo (12,13,23). Nuclear factor-kB (NF-kB) plays a key role in the immune and inflammatory response and modulates cell proliferation, apoptosis, adhesion, invasion, and angiogenesis in many cell types. These cell processes are involved in the development of endometriosis (23). It is possible that perpetuating up-regulation of NF-kappaB expression may synergise with activation of as yet unknown factors (strong candidates are oxidative stress and iron overload) to trigger the inflammatory response, impairing macrophage phagocytic activity and facilitating endometriosis development. Furthermore, local immune mechanisms and also deranged innate immune abnormalities in the peritoneal cavity may support the development of endometriosis.

In vivo, constitutive activation of NF-kappaB has been shown in ectopic endometrial cells and pelvic macrophages in women with peritoneal endometriosis (12,62). In a study conducted by Gonzalez-Ramos and co-workers (2007) red, active endometriotic lesions showed a significantly higher degree of activation of the NF-kappaB pathway than black, inactive lesions (12). According to the authors, the lesion type was the only factor influencing NF-kappaB activation in endometriotic lesions (12). Transcriptional active p50/p65 heterodimer expression were abundant in red endometriotic lesions (12,23). Both the canonical and the atypical NF-kappaB activation pathways can produce p50/p65 dimer; the canonical pathway in response to inflammatory stimuli (TNF-alpha, IL-1B) in endometrial and endometriotic stromal cells and the atypical pathway in response of ROS, hypoxia, and genotoxic stress (63-67). Therefore, it is possible that both pathways are activated in red endometriotic lesions.

6.1. The role of NF-kappaB to promote inflammation in women with endometriosis

6.1.1. Cross-talk between NF-kappaB and cytokines in endometriosis. A positive auto-regulatory loop

Cytokines produced by many cell types in peritoneal fluid, play a diverse role in constructing the peritoneal environment that induces the development and progression of endometriosis. NF-kappaB can be activated by proinflammatory cytokines. We previously showed that IL-6, IL-8, and TNF-alpha are significantly elevated in the peritoneal fluid of women with endometriosis compared with that of women without endometriosis (5,9).

TNF-alpha elicits inflammatory gene expression from epithelial cells (68). The TNF-alpha-induced activation of pro-inflammatory NF-kappaB and other transcription factors can set off a cascade of changes in expression of their target genes, resulting in increased production of pro-inflammatory cytokines and chemokines, and increased anti-apoptotic, angiogenic and invasive capabilities (13). In addition, TNF-alpha enhances mitogenic activity and up-regulates the IL-8 gene and protein expression through NF-kappaB activation in endometriotic stromal cells (50). Specifically, TNF-alpha induced the expression of phosphorylated IkappaB resulting in its degradation; eventually leads in the activation of NF-kappaB (50).

Involvement of NF-kappaB activation in lipopolysaccharides(LPS)-inducible TNF-alpha and IL-8 up-regulation was verified in experiments using an inhibitor for NF-kappaB (N-alpha-tosyl epsilon-phenylalanyl-chloromethyl ketone-TPCK) (69). TPCK reduced LPS-induced IL-8 protein production in endometriotic stromal cells (69). The fact that IL-8 enhanced the proliferation of ectopic endometriotic cells, activates angiogenesis and neutrophil migration and differentiation suggesting that this cytokine may promote the progression of endometriosis (9). Progesterone and progestational compounds (dienogest) were attenuate the expression of IL-8 by reducing TNF-alpha-induced NF-kappaB activation in endometriotic stromal cells (70).

TNF-alpha, by mediating the NF-kappaB pathway, can alter cytokine expression in endometriotic cells contributing to the progression of the disease. NF-kappaB and activator protein (AP-1) activation is critical for TNF-alpha-induced IL-6 gene and protein expression in endometriotic stromal cells (71). Increased serum and follicular fluid levels of IL-6 attenuates aromatase activity and estrogen biosynthesis affecting the fertility of women with endometriosis (72). TNF-alpha gene silencing resulted in the attenuation of expression of *BIRC3* (cIAP-inhibitor of apoptosis protein) and *IL-8* genes, which are major markers genes of the NF-kappaB pathway (73).

Attempting to define the characteristics of the TNFalpha-induced signaling pathway and gene expression in endometriotic stromal cells we observed a novel signaling molecule, transforming growth factor B-activated kinase 1 (TAK1) (Figure 2). TAK1 is a member of the MAPK kinase kinase (MAPKKK) that can be activated by various cytokines, such as TGFB, IL-1B, TNFalpha, and toll-like receptor ligands (74,75). MAPKK kinases are involved in the phosphorylation of the IKK complex. TAK1 activates the NF-kappaB pathway by interacting with the TNFalpha receptor-associated factor (TRAF) 6 and phosphorylating the NF-kappaB inducing kinase (NIK). In addition, TAK1 activates MAPK pathway. TNF-alpha plays a major role in constitutive activation of NF-kappaB via TAK1-IKK pathway and TNF-alpha-activated-TAK1 leads to the activation of downstream kinases, including ERK1/2, p38, and IKK (76,77). We have showed that

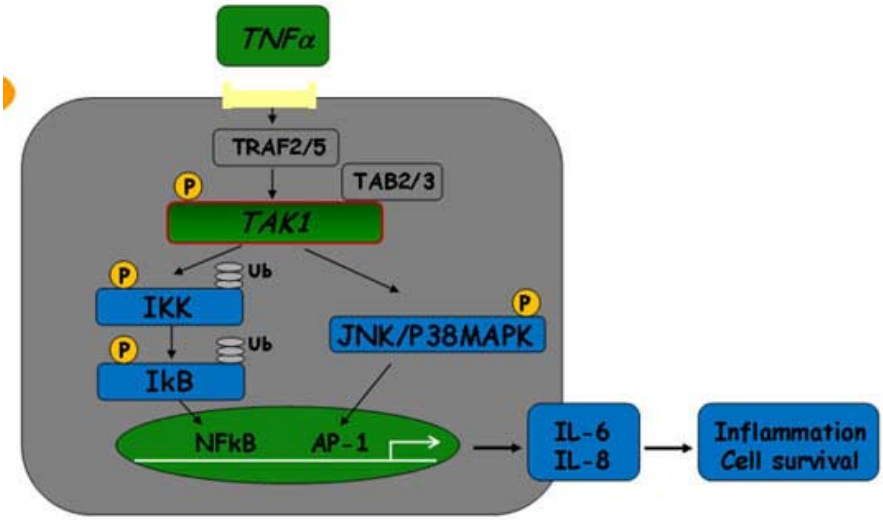

Figure 2. TAK1 is a member of the MAPK kinase kinase (MAPKKK) that can be activated by various cytokines, such as TGFB, IL-1B, TNFalpha, and toll-like receptor ligands. TAK1 activates the NF-kappaB pathway by interacting with the TNFalpha receptor-associated factor (TRAF) 6 and phosphorylating the NF-kappaB inducing kinase (NIK). In addition, TAK1 activates MAPK pathway. TNF-alpha plays a major role in constitutive activation of NF-kappaB via TAK1-IKK pathway and TNF-alpha-activated-TAK1 leads to the activation of downstream kinases, including ERK1/2, p38, and IKK.

endogenous TAK1 silencing repressed TNFalpha-enhanced phosphorylation of IkappaBalpha or MAPKs in ESCs, subsequently, attenuated IL-6 and IL-8 synthesis, as well as the cell proliferation in ESCs (78). It is therefore possible that TAK1 possess the ability to regulate proinflammatory cytokine synthesis and the mitogenic activity of ESCs and this mechanism depends mainly on the activation of the NF-kappaB and MAPK pathways.

Not only TNF-alpha, but also the proinflammatory cytokine IL-1B can induce IL-8 and IL-6 gene and protein expression via the NF-kappaB activation pathway (79,80). RANTES (Regulated upon activation normal T-cell expressed and secreted), a chemokine secreted from a variety of cell types, is also produced and secreted from IL-1B via activation of the proximal NF-kappaB response element in the RANTES gene promoter in endometriotic stromal cells (63). Afterwards, RANTES induce the recruitment of leukocytes, especially macrophages, to sites of ongoing inflammation, e.g., active endometriotic lesions.

Recent studies have shown that NF-kappaB mediates macrophage Migration Inhibitory Factor (MIF) gene activation in response to IL-1B or TNF-alpha in ectopic and eutopic endometrial stromal cells (66,81,82). MIF is a multifunctional proinflammatory cytokine that activates a number of immunocompetent cells, promote endothelial cells proliferation, stimulate angiogenesis in vivo, and induce the synthesis and secretion of matrix metalloproteinases in different cell types (83-86). The NF-kappaB-dependent transcriptional activation of MIF in endometriotic stromal cells may provide a possible mechanism by which ectopic endometrial cells resist apoptosis, proliferate and exacerbate the local peritoneal endometriosis-associated inflammatory reaction (82).

In contrast, an anti-inflammatory cytokine IL-10 can interfere with the NFkappaB-mediated pathway by blocking the nuclear translocation of the transcription factor and the binding to DNA (87,88). We have shown that IL-10 attenuates TNF-alpha-induced IL-6 synthesis in cultured endometriotic cells via a mechanism that is definitively dependent on the activation of NF-kappaB pathway (89). Specifically, administration of exogenous IL-10 reduced the intranuclear concentration of p65 in endometriotic stromal cells suggesting that IL-10 possesses anti-inflammatory effects through the NF-kappaB pathway (89). In addition, other authors have proved that IL-10 inhibits TNF-alpha expression by interfering with NF-kappaB (90,91). Another factor with anti-inflammatory effects caused by antagonizing the activities of NF-kappaB is the PPAR-γ ligand, pioglitazone. We have observed that pioglitazone effectively reduced TNF-alpha-induced IL-8 expression in endometriotic stromal cells, probably through a NF-kappaB-dependent pathway (92). In addition, the intranuclear concentration of p65 protein is reduced after the addition of pioglitazone in these cells (88). Ligands of the PPAR-γ have been shown to inhibit the expression of various cytokines in macrophages and other cell lines, principally by preventing the activation of NF-kappaB (93,94).

The above findings suggest that endometriotic cells might have characteristics that cannot be controlled to maintain the balance between pro- and anti-inflammatory cytokines. Mechanisms that alter the cytokines profiles in these cells are mainly modulated through the NF-kappaB pathway. NF-kappaB regulates the inducible expression of many cytokines, chemokins, adhesion molecules, and acute phase proteins contributing to the progress and evolution of endometriosis (95). The production of these pro-

inflammatory cytokines can further promote NF-kappaB activation leading to an altered gono- and phenotype of endometriotic cells.

6.1.2. NF-kappaB and macrophages

Peritoneal macrophages have been identified as key cells in the regulation and promotion of the pelvic inflammatory disease in women with endometriosis. Once activated, macrophages can release a wide range of factors (cytokines, growth factors, angiogenic factors) and express COX-2 and nitric oxide synthase (iNOS) contributing to the maintenance and progression of endometriosis. A statistically significant higher proportion of NF-kappaB nuclear translocation was found in peritoneal macrophages from women with endometriosis compared with women without the disease (62). According to a research group, iron overload may be one of the factors explaining the increased activation of NF-kappaB in peritoneal macrophages of women with endometriosis (23). Iron is one of the mediators of the endometriosis-associated inflammatory response and oxidative stress and is a known inducer of the NF-kappaB pathway (96-98).

The increased activation of NF-kappaB in peritoneal macrophages may alter their physiological functions. Macrophages from women with endometriosis have increased expression of MIF that probably contributes to the increased macrophages infiltration in PF of these women (80-82). Activated macrophages can induce the production of proinflammatory cytokines that in turn are able to potentiate the activation of NF-kappaB, thereby providing a kind of positive feedback (99).

6.1.3. Interactions between NF-kappaB and PR in endometriosis

A functional PR is important for the physiological regulation of inflammatory processes in the endometrium during the menstrual cycle by mediating the NF-kappaB activity and cytokine expression (53,56). Genetic alterations of the PR result in the increased expression of proinflammatory cytokines in eutopic and ectopic endometrium of women with endometriosis. A preponderance of PR gene polymorphisms (+331G/A and PROGINS) lead to dysfunctional PR proteins (100,101). A distorted PR-A/PR-B ratio has been associated with polymorphisms in the PR gene (102). This could explain the diminished PR-mediated suppression of NF-kappaB-associated genes and supports the theory of progesterone resistance in women with endometriosis (103). Environmental toxicants, such as dioxin, were shown to influence the PR-A/PR-B ratio in co-cultures of endometrial stromal and epithelial cells leading to a functional progesterone withdrawal and increased cytokines (RANTES) expression via the NF-kappaB pathway (104,105).

6.1.4. NF-kappaB can regulate COX-2 and prostaglandins expression

A growing body of evidence indicates that prostaglandins (PGs) contribute to the pathophysiology of endometriosis. The concentration of PGE_2 in PF is higher in women suffering from the disease and COX-2 is more abundantly expressed in ectopic endometriotic tissues compared with eutopic endometrial tissues (106). LPS, an inducer of the NF-kappaB pathway, promoted the proliferation and invasion of endometriotic stromal cells via up-regulation of COX-2 and PGE_2 expression (107). COX-2 is an NF-kappaB-target gene and can be up-regulated via NF-kappaB activation. An inhibitor of NF-kappaB activation was able to decrease COX-2 mRNA and protein expression and PGE_2 levels in endometrial stromal cells (108). A region of the COX-2 promoter gene (-360/-218-bp) contain a variant nuclear factor NF-kappaB site at -222 to -213 that, when mutated, completely abolished COX-2 promoter activation (109). Binding of NF-kappaB p65 to this site is, in part, responsible for the COX-2 promoter activation (109).

However, PGE_2 could integrate multiple cell survival signaling pathways that promote the survival of endometriotic cells via the action of its receptors EP2 and EP4 (110). Banu and co-workers (2009) have proposed a mechanism of action of these receptors: PGE_2→EP2/EP4→Src(a tyrosine kinase)/B-arrestin 1 complex→Epidermal Growth Factor Receptor (EGFR) and/or TNF-alpha/IL-1B→Extracellular signal Regulated Kinases (ERK)1/2 and/or protein kinase AKT and/or NF-kappaB and/or Beta-catenin (Figure 3) (110). It is possible that selective inhibition of these receptors could potentially suppress the adverse effects of most of the pathways contributing to the propagation of endometriosis.

6.2. NF-kappaB and angiogenesis

The expression of several angiogenic factors is regulated by NF-kappaB. Macrophages as well as tumour cells have been reported to produce vascular endothelial growth factor (VEGF) under the control of NF-kappaB activation (111). In women with endometriosis peritoneal macrophages and endometriotic cells showed increased VEGF gene and protein expression (112,113). It is therefore possible that neo-angiogenesis in endometriosis is a NF-kappaB-dependent process. However, deletion of putative NFkappaB-binding sites from the VEGF promoter did not affect LPS-induced VEGF promoter activity, suggesting that NFkappaB is not solely and directly involved in VEGF transcription (114). Other factors can linked the NF-kappaB activation and the increased expression of VEGF. Leptin and stress-activated protein kinases (p38 MAPK and c-jun NH_2-terminal kinase-JNK) can serve this role as was proven in human embryonic kidney and breast cancer cells and additionally in hamster fibroblasts (115,116).

Other angiogenic factors are also NF-kappaB target genes, such as Monocyte Chemoattractant Protein 1 (MCP-1), Intercellular Adhesion Molecule 1 (ICAM-1), IL-8, and MIF (50,69,81,82,117,118). Recently, in a rat endometriosis model, a significant reduction in microvessel density was achieved by inhibiting the NF-kappaB pathway (23,119). These evidences suggest the importance of NF-kappaB in neoangiogenesis in endometriosis. However, additional *in vivo* experiments are needed to unravel the exact mechanism of NF-kappaB-mediated action in these angiogenic factors in endometriosis.

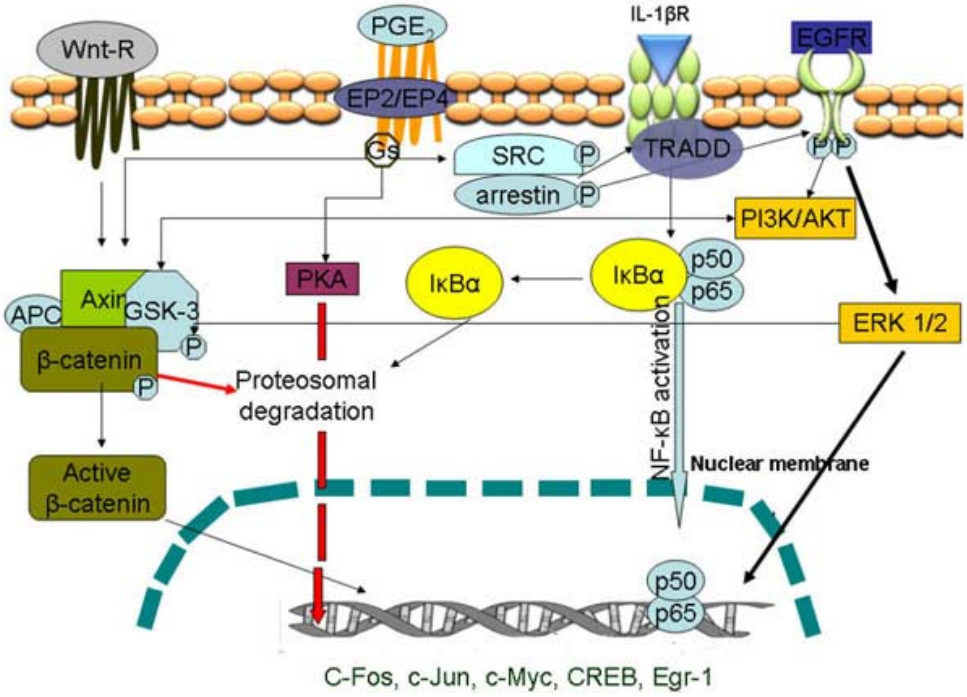

Figure 3. Via the action of the receptors EP2 and EP4, PGE2 could integrate multiple cell survival signaling pathways that promote the survival of endometriotic cells. According to Banu and co-workers (2009) EP2 and EP4 interact with: i. cSRC kinase and B-arrestin 1 complex and trans-activates EGFR, which in turn activates: ERK1/2 and AKT pathways; ii. EP2/EP4 interacts with cSRC kinase and B-arrestin 1 complex and trans-activates TNFR and IL-1BR, which consecutively activates NF-kappaB pathway; and iii. EP2/EP4 activates Beta-catenin pathways by disestabilishing Gs and axin complex and inhibiting GSK3B through AKT and ERK1/2 pathways. Activation of specific transcriptional DNA complexes is the result of interaction between the three different pathways upon activation of EP2/EP4.

6.3. NF-kappaB and matrix metalloproteinases, plasminogen activator system

Proteolysis of the extracellular matrix is a crucial event for endometriotic cells to invade the surrounding tissue. Matrix metalloproteinases (MMPs) are key players in degradation of extracellular matrix and basement membranes, and thus are important for cellular invasiveness of endometriotic cells. It was shown that the pattern of MMPs expression in endometrium and peritoneal fluid of women with endometriosis significantly differ from that in healthy women (120,121). Immunohistochemical expression of MMP-1, -2, -3, and -9, tissue inhibitors (TIMPs) 1 and 2, and cathepsin-D is well correlated with the clinical severity of endometriosis (122). In co-cultures with normal endometrial stromal cells, endometriotic epithelial cells increased MMPs expression and secretion via an NFkappaB-dependent pathway (123). This cross-talk between epithelial cells and stromal cells may facilitate the implantation and extension of the ectopic foci and favour the development of the disease.

NF-kappaB regulates the transcription of many MMPs (MMP-1, MMP-3) in various cells (124,125). However, activation of NF-kappaB is insufficient but absolutely required for upregulation of other MMPs. Complex interactions with additional factors may be needed. AP-1 is an attractive possibility, because a functional NF-kappaB binding element exists in the MMP-9 promoter in a region that cooperates with the proximal AP-1 element (126,127). On the other hand, overexpression of IkappaBalpha completely inhibited NF-kappaB binding and both MMP-9 protein and mRNA expression, demonstrating an absolute requirement for NF-kappaB activity in upregulating MMP-9 (128). Another way of NF-kappaB action on MMPs is by inducing the expression of membrane type metalloprotease (MT-MMP) that produce active MMP-2 from a precursor form (129).

Together with MMPs, other proteolytic factors contributing to the endometrial invasion to the peritoneum is the plasminogen activator system. There is an increase in the expression of urokinase plasminogen activator (uPA) in endometrial tissue and in peritoneal fluid from patients with endometriosis (130,131). This increase might contribute to the invasive potential of endometriotic cells. NF-kappaB–responsive elements are present in uPA, uPA Receptor and phosphorybosilanthranylate isomerase 1 (PAI-1) promoters suggesting that this transcription factor plays an important role to the expression of uPA in endometriotic cells (132). These results suggest that both endometrial and peritoneal factors of women with endometriosis via NF-kappaB activity can enhance the proteolytic capability of ectopic tissue facilitating the adhesion and the invasion process of endometriotic cells into the peritoneal cavity.

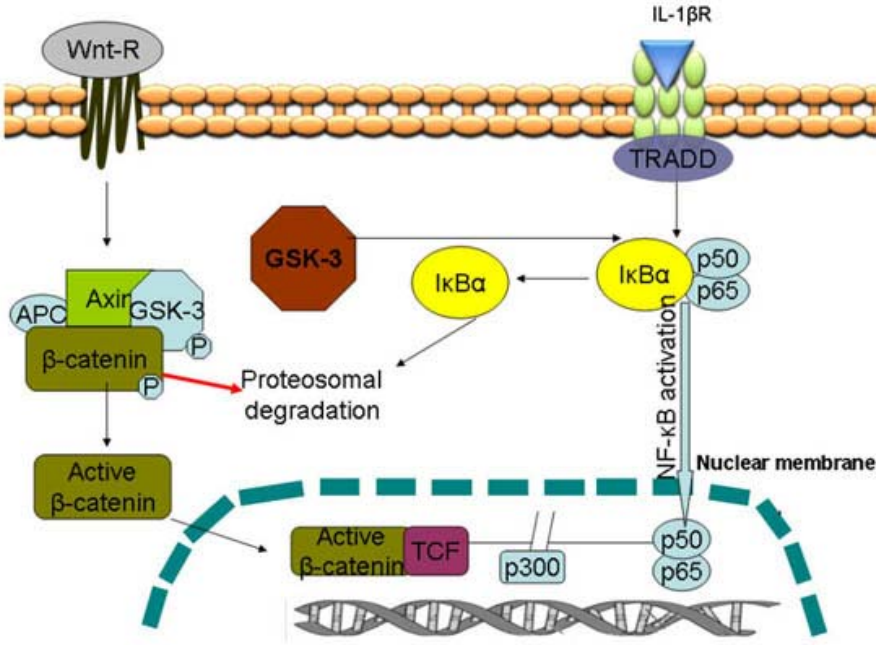

Figure 4. NF-kappaB physically interacts with Beta-catenin, resulting in a reduction of NF-kappaB nuclear translocation, DNA binding, and target gene expression. An inverse correlation exists between the two pathways. The Beta-catenin physically forms a complex with NF-kappaB, resulting in the reduction of NF-kappaB transactivation activity. Nuclear p65 specifically suppress Beta-catenin/TCF-dependent transcription. Transcription is regulated by the competitive binding of specific transcription factors to p300. Endogenous p300 is required for both p65- and Beta-catenin-mediated transcription. In the cytoplasm these pathways probably interact through the GSK3B. GSK3B can inhibit basal and TNF-alpha-induced NF-kappaB activity, probably through stabilization of IkappaBalpha expression.

6.4. NF-kappaB and Beta-catenin

NF-kappaB physically interacts with Beta-catenin, resulting in a reduction in NF-kappaB nuclear translocation, DNA binding, and target gene expression (133). In addition, Beta-catenin-dependent transcriptional activation can be regulated by IKKs (134). A growing body of evidence verifies this relation in normal endometrium and in endometrial carcinoma cells (106,135). An inverse correlation exists between the two pathways in endometrial cancer cells since down-regulation of the active nuclear p65 results in the nuclear accumulation of Beta-catenin (135). Beta-catenin physically form a complex with NF-kappaB, resulting in a reduction of NF-kappaB DNA-binding and transactivation activity (133). On the other hand, p65 was showed to specifically suppress Beta-catenin/T-Cell Factor (TCF)-dependent transcription, suggesting that NF-kappaB modifies signal pathways after the binding of Beta-catenin/TCF4 complexes with target DNA (Figure 4) (135,136).

The functional importance of Beta-catenin signaling during progression of endometriosis could be attributed to the promotion of cellular proliferation by stimulating expression of several target genes for proliferation (c-myc and cyclin D1) invasion (MMP-7, MT1-MMP) and angiogenesis (VEGF) (137-141). Recently, it was found that inhibition of Beta-catenin leads to an up-regulation of the uPA/uPAR system through NF-kappaB cooperation, thus promoting cell invasion (142). Specifically, treatment of Beta-catenin small interfering RNA (siRNA)-transfected cancer cells with a specific inhibitor of NF-kappaB, SN50, significantly reduced enhancement of uPA, uPAR and PAI-1 expression and cancer cell invasion (142). Furthermore, Beta-catenin siRNA-treated cells exhibited NF-kappaB nuclear accumulation (142). The importance of these findings in endometriotic cells remains to be elucidated. Both pathways could be involved in cellular transformation from normal to endometriotic phenotype, cellular proliferation and cell-cell adhesion.

6.5. NF-kappaB and oxidative stress

Oxidative stress is considered to be involved in the establishment and development of endometriosis. Many previous studies have reported that levels of oxidative stress and antioxidant biomarkers found in serum and peritoneal fluid are significantly different between patients with and without endometriosis (143,144). In the presence of oxidative stress, reactive oxygen species (ROS) increase growth and adhesion of endometrial cells in the peritoneal cavity (143). The known correlation between ROS and proliferation of cancer cells, along with the increased production of ROS in response to chronic inflammation in endometriosis suggests a possible role for ROS in the regulation of endometriotic cell proliferation. Endometriotic cells display a high endogenous oxidative stress with a profound alteration of ROS detoxification pathways associated with increased cellular proliferation

and activation of the MAP kinase ERK1/2, a phenomenon very close to what is observed in tumour cells (145).

Oxidative stress is a strong inducer of NF-kappaB activation and NF-kappaB regulates the gene coding for inducible nitric oxide synthase (iNOS) (146,147). NO generated by iNOS is converted to reactive nitrogen species, which can exert an altered cellular phenotype including direct DNA damage, oncogenic mutations, inhibition of apoptosis, and may also promote angiogenesis (148). NF-kappaB activation induced by ROS could stimulate COX-2 and PGs expression in endometrial stromal cells (149). Inflammatory-cytokines cause superoxide radical generation and damage cells, whereas manganese superoxide dismutase (Mn-SOD), located in the mitochondria, protects cells by scavenging superoxide radicals. TNF-alpha induces NF-kappaB-mediated expression of Mn-SOD and attenuates ROS accumulation in human endometrial stromal cells providing a self-defense mechanism of these cells against TNFalpha-mediated oxidative stress (64). It is possible that this mechanism is not active in endometriotic cells; additional studies are needed in order to clarify the role of NF-kappaB-mediated ROS production in endometriosis.

6.6. The two faces of NF-kappaB in apoptosis of endometriotic cells

A common characteristic of endometriotic cells is their ability to evade the apoptotic machinery. Paradoxically, growth factors leading to cell survival and DNA damaging agents leading to apoptosis seem to activate the same NF-kappaB signalling pathway. NF-kappaB activation traditionally considered to confer a resistant to apoptosis phenotype in endometriotic cells. In vivo, NF-kappaB inhibition in early-stage endometriotic lesions induced in nude mice was found to decrease proliferation of endometriotic cells and stimulate their apoptosis (150). This may be resulted by down-regulation of the anti-apoptotic Bcl-2 and Bcl-X_L with simultaneous activation of caspases-3, -8, and -9, as was observed in endometriotic stromal cells after treatment with the NF-kappaB inhibitor, BAY 11-7085 (151). Suppression of NF-kappaB activity by proteasome inhibitors also suppresses proliferation of endometriotic cells in vitro (13). A group of anti-apoptotic genes, including *Bcl-2, Bcl-xL, cIAP1, cIAP2, XIAP, A20, c-FLIP,* and *TRAF-2,* can be up-regulated by NF-kappaB leading to protection of the cells from apoptosis in response to a variety of DNA-damaging signals (21,152).

NF-kappaB can protect endometriotic cells from apoptosis by interaction with other signalling pathways. Akt promotes cell survival through the activation of the NF-kappaB (153). NF-kappaB activates iNOS that provides protection from TNF-alpha- and Fas-mediated apoptosis, as was proven in mouse hepatocytes (154). According to some authors, the ability of endometriotic cells to circumvent apoptotic signals can be the result of the increased PGE_2 signaling which is associated with abundant expression of the anti-apoptotic Bcl-2 and Bcl-$_{XL}$ 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, NF-kappaB, Beta-catenin) (110). NF-kappaB signaling, MAPK/ERK signaling, and PI3K/Akt signaling were all involved in the proliferation of endometriotic cells (155).

NF-kappaB can also alter genes expression that regulates the proliferation of endometriotic cells. Administration of estrogens induced decreased PTEN expression in eutopic and ectopic endometria of women with endometriosis via mediation of the NF-kappaB pathway (154). The PTEN gene is a negative regulator of the PI3K pathway and is consider a tumor suppressor. The loss of PTEN has been reported in endometriosis malignant transformation and as an early event in endometriosis-related ovarian carcinomas (156,157). In endometriosis the presence of a positive feedback loop involving the NF-kappaB has been recently described: a high estrogen environment→ high PI3K/Akt activity→ high NF-kappaB activity and transcription of anti-apoptotic genes→ low PTEN expression→ high PI3K/Akt activity (155). This auto-regulatory positive loop may lead to constant cell proliferation and to the survival of ectopic foci of endometriosis (Figure 5).

NF-kappaB also acts upon the control of the cell cycle, which is critical in determining the degree of cellular apoptosis and proliferation. Inhibition of NF-kappaB activation can reduce cyclin D1 activity, a positive regulator of G1-to-S-phase progression, leading to delayed cell cycle progression (158). NF-kappaB has also implicated in the transcriptional up-regulation of the oncogenic microRNA-155 that stimulates the expression of anti-apoptotic cytokines (159). In addition, the upstream kinases of NF-kappaB, IKKalpha and IKKB are able to modulate cell growth and anti-apoptotic responses in a NF-kappaB independent manner (160,161).

Of potential interest regarding inhibition of apoptosis is the observation that NF-kappaB can antagonize p53 function, possibly through the cross-competition for transcriptional coactivators (p300) (162). NF-kappaB activation antagonizes the function and stability of p53 protein in a variety of cellular models (163).

However, the general view that NF-kappaB activation promotes cell survival has been challenging by recent evidence indicating pro-apoptotic-like effects of NF-kappaB under many circumstances. It was proven that NF-kappaB is an essential component in mediating p53-induced cell death. Inhibition of NF-kappaB abrogates p53-mediated apoptosis (21). In various cell lines, T cells, B cells, fibroblasts, neuronal, and HeLa cells, overexpression of c-Rel and RelA induces apoptosis, whereas inhibition of NF-kappaB protects these cells from apoptosis (21). It was suggested that NF-kappaB can promote the expression of p53, Fas, FasL, Bax, Bcl-xL, and other pro-apoptotic molecules. The pro-apoptotic nature of NF-kappaB activation has not yet been shown in endometriotic cells. These opposite effect may be attributed to various cross-talk of NF-kappaB with other pathways and/or different transcription binding sites that their accessibility from NF-kappaB based on the degree of their histone acetylation and methylation (21).

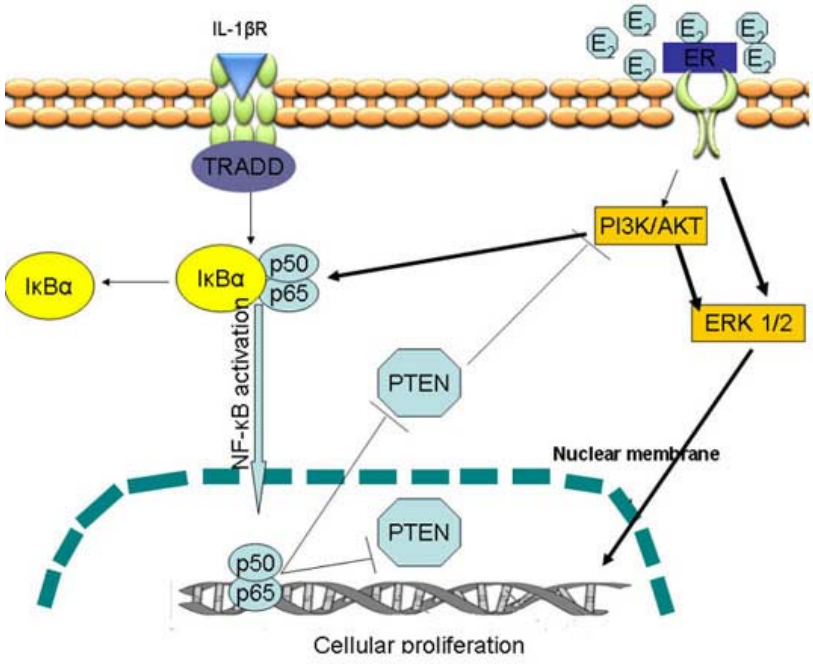

Figure 5. NF-kappaB signaling, MAPK/ERK signaling, and PI3K/Akt signaling were all involved in the proliferation of endometriotic cells. However, only the NF-kappaB pathway was accompanied by estradiol-induced decreased PTEN expression in eutopic and ectopic endometria of women with endometriosis. In endometriosis the presence of a positive feedback loop involving the NF-kappaB has been described: a high estrogen environment→ high PI3K/Akt activity→ high NF-kappaB activity and transcription of anti-apoptotic genes→ low PTEN expression→ high PI3K/Akt activity. The activated PI3K/Akt can further promote the DNA-binding activity of NF-kappaB. GSK3B: glycogen synthase kinase 3B, TCF: T cell factor

Recently, Gangadharam et al. suggested a novel pathway for doxorubicin-mediated cell death through the following signalling 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 (164). 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 (164).

7. THE ROLE OF NF-KAPPAB IN THE TREATMENT OF ENDOMETRIOSIS

7.1. Medical treatment for endometriosis affects the NF-kappaB pathway

Endometriosis is an inflammatory, estrogen-dependent disease. Current therapeutic alternatives consist of various treatments aimed at decreasing circulating estrogen to postmenopausal levels and to decrease the inflammatory process. Whereas all the established drugs for treating endometriosis do not target NF-kappaB specifically, but probably most of them they do affect the NF-kappaB pathway.

Progesterone, a physiological inhibitor of NF-kappaB in the uterus, suppresses NF-kappaB activity indirectly by stimulating the synthesis of IkappaB and/or directly by inducing binding of PR with NF-kappaB subunits (30,53). Prolonged progestin exposure down-regulates endometrial RANTES gene transcription in human endometrial stromal cells, in vitro. The effect is mediated through the NF-kappaB pathway (165). Danazol, progesterone, progestins, and thalidomide have been shown to attenuate TNFalpha-induced IL-8 and IL-6 production via inhibition of NF-kappaB activity in endometriotic stromal cells (70,166). The antiprogestin RU486, increases NF-kappaB activation in human endometrial stromal cells (167). This activation resulted in increased apoptosis of endometrial stromal cells due to up-regulation of *bax* and down-regulation of *Bcl-2* gene. In the same study, inhibition of NF-kappaB with PDTC suppressed the RU486-induced growth inhibition and apoptosis of the endometrial cells (167). The down-regulation of Bcl-2 expression induced by NF-kappaB activation in endometrial cells is inconsistent with the view that binding of NF-kappaB proteins to promoter sites results in activation rather than suppression of gene expression. NF-kappaB has showed a cell type specificity with usually opposing results possibly attributed to the fact that different signaling pathways may be evoked in the different cell system.

GnRHa treatment attenuated TNF-alpha-induced IL-8 expression, inhibited the expression of phosphorylated IkappaB, and consequently suppresses NF-kappaB activation in endometriotic cells (50). GnRH participates in

the macrophage function and the NF-kappaB signalling pathway may be responsible for GnRH-mediated immune system modulation. The activity of NF-kappaB was suppressed by GnRH exposure in freshly isolated peritoneal macrophages (168). On the other hand, NF-kappaB subunit p65 acts as a potent repressor of *hGnRH II* promoter in various cells expressing GnRH receptors showing that NF-kappaB can influence the *GnRH* gene expression (169).

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 (170). Although the exact mechanism of raloxifene action has not been determined, treatment with raloxifene at micromolar concentrations suppressed the production of nitric oxide (NO) by down-regulating expression of the inducible nitric oxide synthase (iNOS) gene in LPS-activated macrophages (171). The decreased expression of iNOS and subsequent reduction of NO were due to inhibition of NF-kappaB. In addition, pretreatment with raloxifene reduced LPS-induced Akt phosphorylation, as well as, NF-kappaB DNA binding activity and NF-kappaB-dependent reporter gene activity. These findings indicate that raloxifene exerts its anti-inflammatory action in LPS-stimulated macrophages by blocking the PI 3-kinase-Akt-NF-kappaB signaling cascade, and eventually reduces expression of pro-inflammatory genes such as iNOS (171).

Experimental drugs for treating endometriosis highlighted also the role of NF-kappaB in the initiation and progression of the disease. A selective ERB agonist has been shown to cause lesion regression in an experimentally induced model of endometriosis in mice (172). The exact mode of action is not known. However, more recently, another study by Xiu-li and co-workers (2009) has shown that this ERB agonist inhibits iNOS production in LPS-activated human peritoneal macrophages of women with endometriosis via suppression of NF-kappaB activation (173). In addition, urinary human chorionic gonadotropin (hCG) attenuated inflammation-dependent NF-kappaB activation and cytokine expression (IL-1B and TNF-alpha) in endometriotic stromal cells alleviating disease-related pain in women with endometriosis (174). Further studies are needed to delineate the exact role of hCG on endometriosis.

7.2. Drugs mediating the NF-kappaB pathway affect also endometriosis progression

NF-kappaB has an important regulatory role in the proinflammatory response of endometrial stromal cells from women with endometriosis. Thus, NF-kappaB is an excellent potential candidate to target the inflammatory response in endometriotic cells (50). Efforts have been made for the development of specific drugs targeting the NF-kappaB pathway. The inhibition of the NF-kappaB could be achieved by: i) direct inhibition of NF-kappaB; ii) prevention of NF-kappaB precursors from processing into mature forms by proteasome inhibitors; iii) prevention of IkappaB degradation; iv) inhibition of IKK; and v) inhibition of NF-kappaB nuclear translocation (13,119).

In a recent review study more than 785 NF-kappaB inhibitors has been described including a variety of synthetic and natural molecules (e.g., NSAIDs, antioxidants, peptides, small RNA/DNA, microbial and viral proteins, phytochemicals, and so on) (175). Some of them have been used in women with endometriosis or in *in vitro* experiments in endometrial cells. An anti-inflammatory (COX-2 inhibitor) drug (sulindac) exerts its effects by suppression of NF-kappaB nuclear translocation and inhibition of NF-kappaB-mediated gene transcription in normal and endometriotic stromal cells (176). Newer NSAIDs and COX-2 inhibitors, were shown to attenuate the implantation of eutopic endometrium and growth of implants in rodent models (177,178).

A soluble inhibitor of NF-kappaB, BAY 11-7085, was used to examine the potential application for the treatment of endometriosis. It was shown that BAY 11-7085 significantly inhibited the endometriotic stromal cells proliferation and induced apoptosis (151). In a different study, two NF-kappaB inhibitors, BAY 11-7085 and SN-50, induced a significant reduction of endometriotic lesions in a mouse experimental model of endometriosis (150). NF-kappaB inhibition reduced intercellular adhesion molecule (ICAM-1) expression and cellular proliferation while increased the apoptosis of endometriotic lesions (150). Another synthetic NF-kappaB inhibitor, TPCK, decreases the IL-8 production and cell proliferation of endometriotic cells supporting the theory that drugs targeting the NF-kappaB pathway may be beneficial in the treatment of endometriosis (69).

Decoy oligonucleotides bear the consensus binding sequence of a specific transcription factor. When introduced into cells, decoys impair the authentic interaction between the target transcription factor and genomic DNA, with subsequent inhibition of gene expression (179,180). Transfection of endometriotic stromal cells with NF-kappaB decoy inhibited the IL-1B-induced NF-kappaB activation, monocyte chemotactive activity, and the RANTES expression of these cells (181).

Proteasomes are responsible for the degradation of many intracellular proteins, thus helping to maintain cellular homeostasis during biological processes. The proteasome is responsible for constitutive activation of NF-kappaB (119,182). Inhibition of the proteasomal function results in stabilization and accumulation of its substrates, which include cyclins, transcriptional factors, tumour suppressor proteins, and IkappaB which inhibits NF-kappaB (183). In vivo, Celik et al., reported that intraperitoneally administered pyrrolidine dithiocarbamate (PDTC) and bortezomib, proteasome inhibitors, inhibit the development of experimental endometriotic implants in rats (119). Recently we had shown that another NF-kappaB inhibitor, apigenin, inhibits TNF-alpha-induced cell proliferation and prostaglandin E_2 synthesis in human endometriotic stromal cells (184). The in vitro application of various other NF-kappaB inhibitors (e.g., curcumin, IKK-2 inhibitor, PPAR-γ ligand, IL-10) on endometrial and endometriotic cells resulted in decreased proliferation and invasion and increased apoptosis of these cells

(23,70,81,89,92,185,186). Introduction of some of these NF-kappaB modulators in clinical practice will hopefully enable more effective treatment in women with endometriosis.

We have to emphasize that new medical treatment inhibiting the NF-kappaB pathway needs to be tested after in vitro and rodent studies firstly in the baboon model for endometriosis to ensure safety and effectiveness before application in human trials. Spontaneous endometriosis occurs only in women and in non-human primates (187). Inbred rhesus monkeys kept in colonies offer an attractive preclinical model to study the inheritance of spontaneous endometriosis and additionally the pathogenesis, the pathophysiology, and new medical treatment options. In baboons, induction of endometriosis after intrapelvic injection of menstrual endometrium leads to biological changes in peritoneal cavity and in endometrium. This induction process may allows the study of cause-effect relationships which may lead to the discovery of new biomarkers for the development of new non-invasive diagnostic tests and drugs that may prevent or treat endometriosis (187).

8. CONCLUSIONS AND FUTURE PERSPECTIVES

The role of NF-kappaB in the pathogenesis of endometriosis seems to be undisputable. In women with the disease, NF-kappaB is highly expressed in endometriotic tissues compared with normal endometrium. Activation of NF-kappaB is responsible for increased production of pro-inflammatory cytokines and chemokines, prostaglandins, ROS, MMPs, and estrogen that alter the phenotype of endometrial cells. These cells with increased activation of NF-kappaB have greater capacity for invasiveness, neovascularization and are presented with resistance to apoptosis.

A lot of efforts have been made to manufacture compounds that decreased the NF-kappaB activation in endometriosis and in patient with tumors. However, NF-kappaB is a major transcription factor involving in many signaling cellular pathways that are essential for the normal cellular function. Its activation results in the physiologic transcription of different genes. Nowadays, compounds that suppress NF-kappaB activation are already available. In clinical practice these compounds may result in severe side effects and cellular toxicity. Strong inhibition of NF-kappaB might not be practical since its absence can result in severe immunodeficiency. Investigators should be cautions considering the dual role of this transcription factor and existing crosstalk between individual signaling pathways. Great challenges should be the manufacture of drugs that i) normalize and not suppress the activity of NF-kappaB or drugs that ii) specifically block NF-kappaB activation in target cells leaving unaffected the normal cells or drugs that iii) targeting the function of specific genes whose expression is regulated by the NF-kappaB transcription factor.

The introduction of cognate double-stranded small interfering RNA (siRNA) for silencing genes at the mRNA level via the RNA interference (RNAi) cellular pathway is very promising. siRNA is a highly specific and efficient tool for diminishing the expression of a target gene and also has the potential to be used as a targeted therapeutic agent. Recently, the intraperitoneal administration of NF-kappaB p65 siRNA and paclitaxel prolonged the survival of mice with peritoneal metastasis of gastric cancer and inhibited the cancer growth (188).

The epigenetic regulation of gene expression has now received great attention. Chromatin structure and epigenetic settings appear to be the ultimate integration sites of both environmental and differentiative inputs, determining proper expression of each NF-kB dependent gene (189). Recently, regulation of the NF-kappaB pathway by CpG methylation and histone modifications at the IkappaBalpha promoter was achieved in intestinal epithelial cells (190). Inhibition of IkappaBalpha methylation resulted in decreased expression of IL-8 due to decreased NF-kappaB activation (189). These data suggest that the inhibition of DNA methylation should be explored as a therapeutic approach to inhibit the chemotaxis of immune cells whose recruitment depends on the secretion of NF-kappaB.

The study of NF-kappaB pathway help us to understand better the pathophysiology of endometriosis and promises to develop new diagnostic tools and therapeutics for the treatment of the disease. However, additional clinical studies are needed to further elucidate the role of these compounds for the treatment of endometriosis.

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Abbreviations: NF-kappaB: nuclear factor-kappa beta, IkappaB: inhibitors of nuclear factor kappa beta, IKK: inhibitor of nuclear factor kappa beta kinase, MMPs: matrix metalloproteinases, COX-2: cyclooxygenase-2, MAPK/ERK: mitogen activated protein kinase/extracellular signal regulated kinase, PI3K/Akt: phosphoinositide 3 kinase/protein kinase B, PTEN: phosphatase and tensin homolog, TAK1: TgfB activated kinase 1, TGFB: tumor growth factor-B, IL-8: interleukine-8, IL-1B: interleukin-1B, IL-6: interleukine-6, IL-10: interleukine-10, LPS: lipopolysaccharide, TNF-alpha:tumor necrosis factor-alpha, ERK1/2: extracellular signal regulated kinase1/2, cSRC kinase: cellular sarc kinase; EGFR: Epidermal growth factor receptor, AKT: Protein kinase B, TNF-alphaR: tumor necrosis factor-alpha receptor, IL-1BR: interleukine-1B receptor, Gs: G protein signaling, GSK3B: Glycogen synthase kinase 3B, TRAIL: TNF-related apoptosis-

inducing ligand, PR: progesterone receptor, ABIN-2: A20 binding inhibitor of NF-kappaB-2, ER: estrogen receptor, GRIP: glucocorticoid receptor interacting protein, PPAR: peroxisome proliferator activated receptor, TPCK: N-alpha-tosyl epsilon-phenylalanyl-chloromethyl ketone, AP-1: activator protein-1, TRAF: TNFalpha receptor-associated factor, RANTES: regulated upon activation normal T-cell expressed and secreted, MIF: migration inhibitory factor, iNOS: nitric oxide synthase, PGE_2: prostaglandin E_2, VEGF: vascular growth endothelial factor, MCP-1: monocyte chemoattractant protein 1, ICAM-1: intracellular adhesion molecule 1, TIMPs: tissue inhibitor matrix metalloproteinase, uPA: urokinase plasminogen activator, PAI-1: phosphorybosilanthranylate isomerase 1, siRNA: small interfering RNA, ROS: reactive oxygen species, Mn-SOD: manganese superoxide dismutase

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