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Identification of a novel mechanism of NF-κB inactivation by progesterone through progesterone receptors in Hec50co poorly differentiated endometrial cancer cells: induction of A20 and ABIN-2

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Abstract

Objective. Nuclear factor kappa B (NFκB) is a strong anti-apoptotic factor, which is constitutively active in human endometrial cancer cells. Progesterone is the principal growth inhibitory hormone in the endometrial epithelium and promotes apoptosis. To identify the pathways through which progesterone controls NFκB function, we explored its genomic and non-genomic effects in endometrial cancer cells.

Methods. PR-negative Hec50co endometrial cancer cells were engineered to express high levels of the A or B isoform of PR (PRA or PRB) by adenoviral infection. Cells were treated with progesterone or vehicle alone, and RNA was isolated. Affymetrix microarrays were performed and transcriptional control of the genes of highest interest was confirmed by semi-quantitative RT-PCR. To assess the non-genomic effects of PR on inflammation associated with NF-κB, electromobility shift assays (EMSAs) were performed.

Results. Expression analysis demonstrated a significant effect of progesterone after 12- and 24-h treatment on several genes; in particular, A20 and ABIN-2 were induced through PRB. These factors bind in a complex and inhibit NFκB transcriptional activity. In addition, EMSAs revealed the complete inhibition of NFκB dimer binding to DNA by both PRA and PRB.

Conclusions. Progesterone is the principal differentiating hormone in the endometrium. We have now identified several down-stream pathways of action, one of which is the control of genes involved in NFκB activity. The tumorigenic inflammatory and anti-apoptotic effects of NFκB are inhibited by progesterone/PRB through the transcriptional control of binding proteins A20 and ABIN-2. This pathway offers interesting targets for future therapeutic development.

Keywords: Progesterone receptors A and B; Endometrial cancer; ABIN-2; A20; NFκB

Introduction

Endometrial cancer is the fourth most common malignancy in women. It is related to over-exposure to estrogen that is not modulated by the differentiating effects of progesterone [1]. Unopposed estrogen stimulation can lead to endometrial hyperplasia, cellular atypia, and endometrial cancer [2]. Endometrial cancer growth is inhibited by progesterone, but when the disease has progressed, the cancer cells lose sensitivity to the hormone, possibly due to the down-regulation of progesterone receptors (PRs) [3]. As PRs are normally up-regulated by estrogens via estrogen receptors (ERs) [4], this implies that failure to induce PRs may be a factor in the genesis and/or progression of endometrial cancer. There are two isoforms of the progesterone receptor, PRA and PRB, which are expressed in the normal endometrium, and both are likely to be required for endometrial differentiation [5]. Mote et al. [6] have demon-

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strated that endometrial carcinogenesis is associated with an imbalance in the expression of one or both PR isoforms. It is also hypothesized that the sensitivity of endometrial cancer to progestin therapy is limited by the loss of PR expression, either due to its natural down-regulation in response to the progestin itself [7], or to some other characteristic of carcinogenesis whereby PR expression is actively inhibited [8].

Loss of sensitivity to the normal signals inducing apoptosis is a characteristic of the transformed cellular phenotype and has been shown to play a role in the onset and/or development of cancer. Tumor necrosis factor (TNF)-α is the principal cytokine that induces apoptosis. TNF-α can also activate, and is in turn induced by NFκB, the central transcription factor that regulates immune and inflammatory responses. In most untransformed cells, NFκB complexes are largely cytoplasmic and remain transcriptionally inactive until the cell is stimulated [9]. Once this occurs, the inhibitory protein bound to NFκB, IκB, becomes phosphorylated on two serine residues and is subsequently proteolyzed by the 26S proteosome. NFκB is then liberated and accumulates in the nucleus where it activates the expression of specific genes involved in immunity, inflammation, and proliferation [10–13]. Activated NFκB is inactivated by binding to accessory proteins, including the newly described A20 and ABIN-2 complex [14,15]. The most common active form of NFκB (p65/p50 dimer) has been found to be present in proliferating endometrium and in endometrial hyperplasia, and its expression has been found to be abnormal in carcinoma [16]. Apart from the expression of the protein subunits, NFκB activity has also been reported to be modulated in endometrial cancer cells, where it is constitutively activated during the process of carcinogenesis [17].

Progesterone is a powerful anti-inflammatory and antiproliferative hormone in the female reproductive tract [18,19]. Dai et al. [20,21] developed an in vitro model for studying the role of the PR in endometrial cancer where Hec50co cells were transfected with PRA and PRB genes to create cell lines expressing PRA or PRB. These studies identified many differentiating pathways through which progesterone inhibits endometrial cancer growth and invasiveness; in particular, progesterone sensitizes cells to apoptosis through an unknown mechanism. We hypothesized that this is due to progesterone's inhibition of NFκB, a powerful inhibitor of Akt phosphorylation that results in cellular insensitivity to apoptotic signals. We propose that progesterone, like glucocorticoids, inhibits NFκB activity by many mechanisms [22]. In this study, we performed a global analysis of gene regulation by progesterone in Hec50co cells transfected with PRA and/or PRB using Affymetrix oligonucleotide microarrays at 12 and 24 h after progesterone treatment initiation. Interestingly, progesterone regulates the expression of several genes including cytokines and binding proteins that are involved in NFκB activation/inhibition. Non-genomic inhibition of NFκB binding to its DNA response element by PR was also demonstrated.

Materials and methods

Cells and reagents

Hec50 cells were provided by Dr. Erlio Gurpide (New York University). Cells were cultured in DMEM (Sigma, St. Louis, MO) supplemented with 10% FBS (Gemini Bio Products, Inc., Calabasas, CA) and antibiotic/antimycotic solution containing 100 units/ml penicillin-G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Gibco Life Technologies, Grand Island, NY). Adenoviral vectors were constructed as described previously [23].

RNA extraction

To assess the genomic effects of progesterone through PRA and PRB, Hec50co endometrial cancer cells were infected with AdCon, AdPRA, or AdPRB (adenovirus carrying no PR, PRA, or PRB genes, respectively) or both PRA and PRB for 15 h and treated with 100 nM progesterone or vehicle (100% ethanol) alone for an additional 12 or 24 h. The multiplicity of infection of viral particles/cell was approximately 10; and conditions were as previously reported [23]. Cells were harvested after treatment by scraping and total RNA was prepared using RNeasy spin columns (Qiagen Corp., Valencia, CA). The procedure was carried out according to the manufacturer's instruction. The RNA quality was checked using the Agilent 2100 Bioanalyzer (Agilent Technologies, Foster City, CA).

Affymetrix microarrays

Independent replicate experiments were performed in duplicate. All procedures were performed according to the instructions from Affymetrix (Santa Clara, CA) using the Affymetrix manual (version 701022 rev 1). Briefly, total RNA (10 μg) was converted into double-stranded cDNA by using the T7-(dT) 24 oligomer and the SuperScript Choice system for cDNA synthesis (Invitrogen Life Technologies). The double-stranded cDNA was cleaned and extracted with phenol/chloroform followed by ethanol precipitation and resuspended in 12 μl RNase-free water. From the cDNA, biotin-labeled cRNA was made and then purified with RNeasy spin columns (Qiagen Corp.) and fragmented. The biotinylated cRNA is then hybridized to an Affymetrix HG-U133A microarray, which carries probes for approximately 22,000 well-substantiated human genes. During the washing step, phycoerythrin conjugated to streptavidin was added and allowed to bind. Excess phycoerythrin is washed from the microarray and then the microarrays are analyzed using a fluorescent scanner. Initial data analysis was performed using Affymetrix Microarray Suite v5.0 software,

setting the scaling of all probe sets to a constant value of 500 for each GeneChip. Additional data analysis was performed using GeneSpring v5.1 (Silicon Genetics Inc., Redwood City, CA). All 12 of the GeneChips that were hybridized with the 24-h samples were normalized against the median of the two control samples (Control vehicle 1 or VEH-1 and Control vehicle-2 or VEH-2). Each measurement for each gene in those specific samples was divided by the median of that gene's measurements in the corresponding control samples. The six 12-h samples were normalized to the single control sample (CV12) and the measurements for each gene in these six samples were divided by the corresponding gene expression value from the control sample. Next, filtering was used to identify the genes that were consistently up- or down-regulated in replicate samples. The gene expression diagrams were generated in GeneSpring, using the smooth correlation function. Name, accession number, and description details were obtained from Affymetrix.

RT-PCR semi-quantitation

Primers for A20 and ABIN-2 were designed by Biosynthesis Inc. (Lewisville, TX) from the paper by Van Huffel et al. [14] and the paper from Dixit et al. [24], respectively. Primers for TRAIL and TRAILR2 were obtained from Clontech (Palo Alto, CA), as was the reverse transcriptase-polymerase chain reaction (RT-PCR) kit used for confirmation. Total RNA was extracted, and cDNA was synthesized from 1 μg of total RNA by random priming. Ten microliters of this cDNA preparation was subjected to 35 cycles of amplification using a Techne thermal cycler. PCR reactions were carried out in 50 μl reaction mixtures, each in the presence of a PCR bead (Amersham Biosciences, Piscataway, NJ) containing 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1.5 U of Taq DNA polymerase and 400 mM of each primer. Reactions were run and 5-μl samples were collected at two-cycle intervals over a 10-cycle span determined to be the optimum for quantitation. All PCR products were run on a 1.5% agarose gel and stained with ethidium bromide. Each reaction included the L13A control amplification gene, which is not regulated by progesterone, to check for constancy in the amount of starting material.

All PCR products from a single experiment were run on an 80-well 1.5% agarose gel and stained with ethidium bromide. Each experiment included amplification of the L13A message as a control. Fluorescence intensity photographs were captured using a BioRad Fluor-S system, and the resulting bands were quantitated using volume integration with the histogram peak background correction method. Data were then plotted to ensure that L13A levels were consistent between the control and P4-treated samples; in no case was there more than a cycle difference between the lines for the control and P4-treated samples (see Fig. 1). P4-induced fold changes in gene expression were estimated by calculating the base 2 antilog of the horizontal distance between the control and P4-treated lines measured in PCR cycles, corrected for any change in L13A level. For example, if the L13A level was one half cycle lower in the progesterone sample than the control and the gene of interest was three cycles less in the progesterone sample than in the control, then the estimated fold-change induced by progesterone would be 2^(−3 − (−1/2))= 2^ −2.5 = 0.177, or almost 6-fold downregulation by progesterone [19].

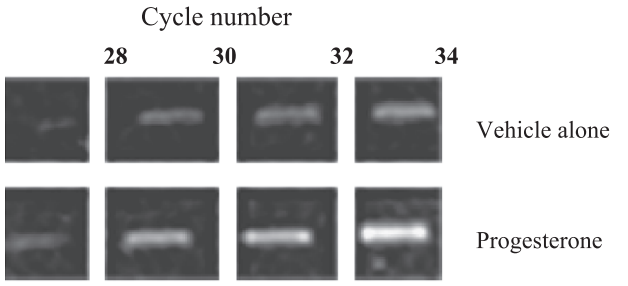

Fig. 1. RT-PCR semi-quantitation of housekeeping gene L13A treated with vehicle (top) and progesterone (bottom) at varying cycle numbers. The band is seen at the same cycle of replication whether or not progesterone is present, indicating no significant change in gene expression in response to progesterone.

Electromobility shift assay (EMSA)

EMSAs were performed to assess the effect of PR expression on the binding of NF-κB to its DNA response element as follows. Hec50co cells were grown to near confluence in dishes or flasks as described, and treated with 4 ng IL-1α to induce cytoplasmic to nuclear shuttling of active NF-κB. After 15 min incubation, cells were washed with PBS, spun at low speed for 5 min, and the supernatant was aspirated to leave the cell pellet. Nuclear extract was then prepared as previously described [25]. All reactions were carried out at 4°C. Briefly, the cell pellet was incubated in hypotonic buffer containing 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM each of EDTA and EGTA to which a protease inhibitor cocktail (Boehringer Mannheim, Indianapolis, IN) was added, followed by lysis with 0.625% Triton X-100. After centrifugation, the cytoplasmic components were removed. The pellet containing the nuclear extract was then suspended in a high-salt buffer containing 20 mM HEPES and 400 mM NaCl plus EDTA, EGTA, and protease inhibitors. This suspension was rocked for 15 min and spun at maximum speed for 5 min. The supernatant containing the nuclear extract was collected and stored at −80°C. Protein concentrations for each extract were determined and equal amounts assayed for DNA binding proteins on a 5% acrylamide gel. The probe was prepared by reacting NFκB consensus oligo with T4 kinase and ^{32}P-γ-ATP. One μl of approximately 200,000 cpm of probe was incubated with 5 μg of each Hec50co cell extract in binding buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5 mM DTT, 1 mM $MgCl_2$

Table 1
Genes upregulated or downregulated by progesterone in PRB or PRA + PRB transfected cells

Gene	Fold Change	PR isoform
A20	2.621	B
ABIN-2	2.486	B
TRAIL	0.340	B
TRAILR2	0.426	A + B

These genes are related to the inhibitory effects of progesterone on NFκB.

0.5 mM EDTA, 0.05 mg/ml poly (di-dC) 4% (v/v) glycerol). The positive control cell line, RAW 264.7, was derived from activated macrophages and was a kind gift from Dr. Gerald Feldman (Food and Drug Administration, Bethesda, MD). All assays were performed in duplicate.

Results

To understand the basis of the distinct effects of progesterone on endometrial cancer cells and to determine which genes are regulated by this hormone, we performed microarray analysis of gene expression in the human endometrial cancer cell line Hec50co transfected with PRA, PRB, or both. Cells were treated with vehicle or progesterone for 12 or 24 h, and total RNA was isolated. Probes generated from this RNA were hybridized to Affymetrix human HG-U133A arrays to analyze expression of approximately 22,000 genes. Two independent replicate experiments were performed in duplicate. Only transcripts found to be consistently regulated on both independent experiments were considered for further study. Of these, several genes were identified that related to the inhibitory effects of progesterone on NFκB (Table 1). These were A20 and ABIN-2, induced by PRB, tumor necrosis factor-α related apoptosis inducing ligand (TRAIL), down-regulated by PRB, and a receptor for TRAIL, TRAILR2, down-regulated by PRA + PRB.

Confirmation of regulated genes with RT-PCR

Several regulated genes identified by the array analysis were selected for further analysis. The genes of interest were A20, ABIN-2, TRAIL, and TRAILR2. To compare our data across experiments, a control housekeeping gene, L13A, was identified, quantitated by RT-PCR, and found not to be significantly regulated by progesterone (Fig. 1). In contrast, the A20 and the ABIN-2 gene products were stimulated by progesterone in cells expressing PRB. These data are shown as an example of the RT-PCR confirmation experiments (Fig. 2), where A20 and ABIN-2 expression is enhanced by at least two cycles (4-fold) by progesterone. In contrast, TRAIL and TRAILR2 were inhibited by progesterone by at least 3-fold in cells expressing PRB and PRA + B, respectively (data not shown). These data are consistent with the approximate changes in gene regulation determined on duplicate expression array experiments using the Affymetrix system.

These experiments confirm that the NFκB inhibitory binding protein A20 and its partner ABIN-2 are under the transcriptional control of progesterone through PRB and are induced with hormonal treatment. On the other hand, TRAIL and its receptor, TRAILR2, which activate NFκB, are down-regulated by progesterone through PRB and PRA + PRB, respectively. These elements constitute two inter-linked downstream genomic pathways through which progesterone inhibits the activation of cytoplasmic NFκB.

Electromobility shift assays (EMSAs)

To assess the ability of progesterone and its receptors to modulate the nuclear functions of NFκB, namely, its ability to bind to the consensus NFκB response element, EMSAs were performed. These revealed the complete inhibition of p65/p50 and p50/p50 NFκB dimer binding to DNA by both PRA and PRB, thereby abrogating the transcriptional activity of this factor (Fig. 3). The effect was independent of the presence of progesterone; hence, hormone is not required to inhibit the binding of NFκB to its DNA response element (compare lanes 5 and 7, Fig. 3, with lanes 6 and 8). In addition, only the NFκB dimers p65/p50 and p50/p50 were affected by PR, not the prominent "nonspecific" protein complex that is not composed of NFκB peptides (Fig. 3). Therefore, the ability of PR to abrogate protein binding to the

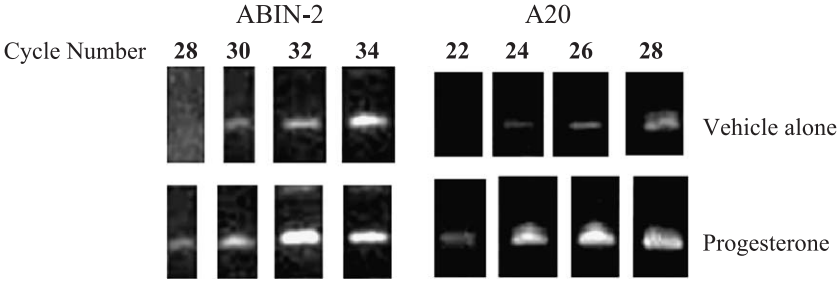

Fig. 2. RT-PCR semi-quantitation of genes ABIN-2 (left) and A20 (right) at varying cycle numbers after treatment with vehicle (top) and progesterone (bottom). Progesterone treatment induces expression at an earlier cycle compared to vehicle, indicating an increase in expression of ABIN-2 and A20 in response to progesterone.

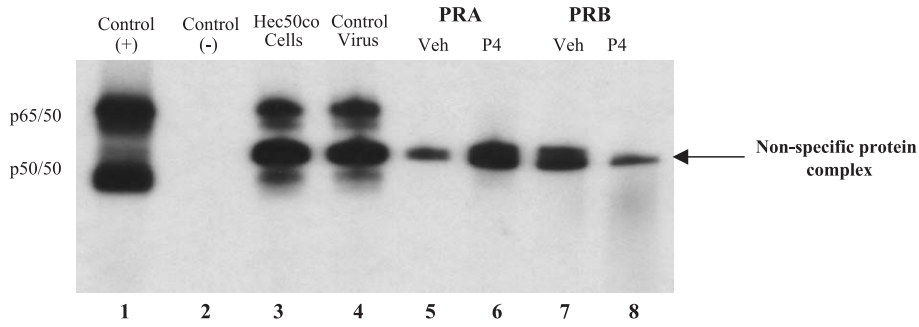

Fig. 3. Negative regulation of NFκB DNA binding by progesterone receptor. Lanes represent the positive control cell line RAW 264.7 (1), negative control with no cell lysate (2), PR-negative Hec50co cells (3), Hec50co cells infected with control virus (no PR) (4), Hec50co cells infected with PRA without and with progesterone treatment (5 and 6, respectively), and Hec50co cells infected with PRB without and with progesterone treatment (7 and 8, respectively).

NFκB DNA response element is somewhat selective to the components of NFκB itself and does not inhibit other proteins from binding to the DNA. These data indicate that PR directly inhibits the binding of NFκB to DNA, likely resulting in a decrease in the transcriptional activity of this factor.

In summary, our findings demonstrate the ability of progesterone and/or PR to inhibit NFκB activity in both the cytoplasm (induction of A20 and ABIN-2, down-regulation of TRAIL and TRAILR2) and the nucleus (direct inhibition of the binding of NFκB to DNA) via genomic and non-genomic mechanisms.

Discussion

Progesterone is known to act by genomic and non-genomic pathways to control cell function and proliferation. The genomic mechanism leads to the transcription of hormone-dependent genes, while the non-genomic effects relate to direct effects of PR on other proteins or transcription factors. Gene expression profiling in response to steroid hormones through their cognate receptors has provided information on many pathways controlled by these factors [26,27] and our data are consistent with these findings. Dai et al. found that progesterone inhibited endometrial cancer cell growth and invasiveness by down-regulation of cellular adhesion molecules through PRB [21]. In preliminary studies, we have also determined that progesterone has a role in inflammation where it induces anti-inflammatory cytokines and inhibits pro-inflammatory cytokines and their receptors.

The functional relationship between inflammation and cancer is not new. Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich with inflammatory cells enhances and/or promotes neoplastic risk [28]. Normal inflammation is self-limiting because the production of anti-inflammatory cytokines closely follows the expression of pro-inflammatory cytokines. Chronic inflammation, which is linked to carcinogenesis, occurs when anti-inflammatory pathways are interrupted. NFκB is a powerful pro-inflammatory modulator that can be constitutively activated in cancer; once activated, it is responsible for the production of an onslaught of pro-inflammatory cytokines. Inappropriate activation of NFκB has been implicated in several types of malignancies where it disrupts the normal cell cycle regulatory pathways, inhibits cellular differentiation, and prevents normal apoptosis [29]. Generally, suppression of NFκB correlates well with inhibition of various damaging cytokines. NFκB is also activated in cancer cells by chemotherapy and radiation, and this response inhibits the ability of these treatments to induce cell death [12]. We propose that one of the protective effects of progesterone as a therapeutic agent in endometrial cancer is its ability to inhibit NFκB. The purpose of these studies was to identify potential mechanisms through which this occurs. We have identified novel genomic anti-inflammatory pathways leading to the expression of cytoplasmic protein inhibitors of NFκB and the down-regulation of inducers of NFκB. We also identified direct, non-genomic effects of PR that abrogate NFκB binding to DNA in the nucleus. These actions predict for a strong inhibition of inflammation by progesterone/PR in endometrial cancer that suppresses the process of carcinogenesis.

To determine the effects of progesterone acting through the PR isoforms A and B on gene transcription, we created poorly differentiated endometrial cancer cell models expressing PRA, PRB, or both as a consequence of gene transfection. Expression array analyses were performed at 12 and 24 h to determine the early and late effects of progesterone through PR on gene expression. For the 24-h time point, two independent experiments were carried out to check data reliability. The correlation coefficients calculated using two different programs were performed and the data were found to be more than 90% concordant (Table 2).

Interesting findings relating to NFκB inhibition were forthcoming from cells expressing PRB alone or PRA + PRB. Through PRB, progesterone induced A20 and ABIN-2 at 12 and 24 h and down-regulated TRAIL at 12 h. In the presence of PRA + PRB, progesterone treatment resulted in the down-regulation of the receptor for TRAIL, TRAILR2, at 24 h. Using semi-quantitative RT-

Table 2
For the 24-h time point, two independent experiments were carried out to check data reliability. The correlation coefficients calculated are shown, and the data between experiments are more than 90% concordant

Samples	R^2 duplicate analysis[a]	Correlation coefficient
Control virus treated with vehicle	0.96	0.98
Control virus treated with progesterone	0.95	0.97
PRA treated with vehicle	0.93	0.97
PRA treated with progesterone	0.91	0.96
PRB treated with vehicle	0.89	0.94
PRB treated with progesterone	0.92	0.96

[a] Pearson product moment correlation coefficient.

PCR, we went on to confirm these initial expression array findings.

The zinc finger protein A20 is a cellular inhibitor of nuclear factor kappa B (NFκB) activation and plays a critical role in terminating NFκB responses; it is involved in the feedback suppression of NFκB activation induced by TNF-α [30]. The underlying mechanism for NFκB inhibition was unknown until Van Huffel et al. [14] characterized another protein, termed the A20 binding inhibitor of NFκB activation-2 (ABIN-2), which binds to the COOH-terminal of A20. Yeast two hybrid studies by Heyninck and Beyaert [31] revealed that the zinc finger domain of A20 also binds to another protein ABIN-1. Like ABIN-2, ABIN-1 interacts with the C-terminal region of A20 and colocalizes with A20 in the cytoplasm. Expression of each ABIN protein inhibits NFκB in a similar manner to A20 itself, suggesting that this function of A20 may be mediated by ABINs. Specifically, ABIN expression induces the binding of NFκB to A20 and inactivates it as a transcription factor. We have now identified A20 and ABIN-2 as genes induced by progesterone. These data are the first to link progesterone/PR with the A20/ABIN-2 pathway and provide a novel explanation for the anti-inflammatory effects of this hormone.

TRAIL was first identified as a pro-apoptosis factor, but it has dual actions and is also known to activate NFκB [32]. We found that TRAIL was down-regulated by progesterone through PRB. Its receptor, TRAILR2, was found to be down-regulated by progesterone through PRA + PRB. As a consequence, NFκB activation is predicted to be inhibited, and this provides another genomic mechanism through which progesterone inhibits inflammation in endometrial cancer cells.

We also showed that PR has non-genomic inhibitory effects on the binding of NFκB to DNA in vitro using EMSAs, an effect that is predicted to suppress the expression of pro-inflammatory cytokines. In these experiments, the ability of PR to inhibit NFκB binding is specific to the p65/p50 and the p50/p50 units, and does not occur for complexes unrelated to NFκB. Therefore, this is not a

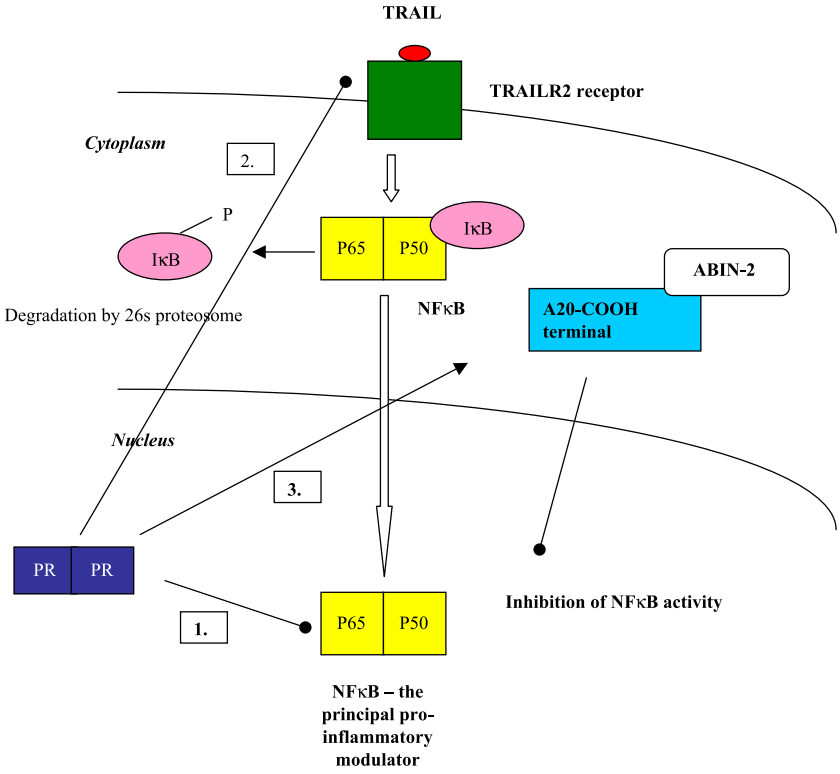

Fig. 4. The NFκB activation pathway and its inhibition by progesterone through its receptor in endometrial cancer. We identified three principal pathways: (1) PR directly inhibits NKκB dimer binding to its consensus DNA response element, (2) progesterone/PR down-regulate the transcription of TRAIL and its receptor TRAILR2 which activate NFκB, and (3) progesterone/PR up-regulate the transcription of A20 and ABIN-2, binding proteins that inactivate NFκB.

generalized squelching mechanism, but has some specificity and selectivity. In addition, the effect is independent of the presence of progesterone and requires only PR. Either isoform of PR (A or B) is equally effective in inhibiting the NFκB/DNA interaction. Also, the effect did not depend upon progesterone-mediated gene transcription. This provides additional evidence for protein/protein interactions between liganded or unliganded PR and NFκB as the mechanism while it has been previously speculated that PR does not exist in the nucleus in the absence of progesterone, in actuality, PR shuttles in and out of the nucleus. This is dependent upon two nuclear localization sequences within the protein, one that is hormone-dependent, and a second that is constitutive [33]. Therefore, PR does exist in the nucleus in the absence of progesterone, as documented by others [34,35], and ligand and unliganded PR both inhibit NFκB.

In conclusion, we have shown that progesterone acts through multiple mechanisms to inhibit NFκB, as shown in Fig. 4. First, there is direct inhibition of NFκB binding to DNA by PR which does not depend upon the presence of ligand and, presumably, is not related to the transcriptional activity of PR. The precise mechanism by which PR prevents NFκB binding to DNA is unknown; however, we propose that this may be due to protein–protein interactions between PR and the p65 subunit of NFκB. Such interactions are reported to be inhibitory to the transcriptional activity of NFκB [22]. Second, through PRB, progesterone inhibits the transcription of TRAIL (an activator of NFκB), and through PRA + PRB, progesterone inhibits the transcription of its receptor, TRAILR2. Finally, progesterone induces the transcription of A20 and ABIN-2 through PRB, resulting in the inactivation of free NFκB in the cytoplasm. These data are of particular importance in light of the availability of new drugs that target the NFκB pathway [9,36]. We propose that, in addition to progesterone, small molecules aimed at interrupting NFκB activation may be useful in the treatment of endometrial cancer.

Acknowledgments

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