Selective Down-Regulation of Progesterone Receptor Isoform B in Poorly Differentiated Human Endometrial Cancer Cells: Implications for Unopposed Estrogen Action

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ABSTRACT

The uterine endometrium responds to unopposed estrogen stimulation with rapid cell proliferation. Progesterone protects the endometrium against the hyperplastic effects of estradiol (E2) through progesterone receptors (PRs), of which two isoforms are expressed: human (h) PRA and PRB. hPRB has a longer NH2-terminus and may function differently from hPRA. Thus, the relative expression of hPRA:hPRB is likely to be important for the action of progesterone. We hypothesized that the hPRA:hPRB ratios may be abnormal in endometrial cancer, leading to a lack of normal progesterone protection against the growth-promoting effects of E2. To test this hypothesis, well-differentiated Ishikawa endometrial cancer cells were compared to poorly differentiated HeC50 and KLE cells. Reverse transcription-PCR was chosen as a sensitive method to detect transcripts for the two forms of PR. The relative expression of PR isoforms under hormonal stimulation was determined by Western blotting. Transient transfections of hPRA and hPRB into endometrial cells allowed the evaluation of the transcriptional activity of each isoform independently on reporter gene transcription under the control of a simple progesterone response element-containing promoter. The effect of coexpressing the estrogen receptor on PR expression was also studied. Ishikawa cells (well-differentiated) express both hPRA and hPRB. Both isoforms, but predominantly hPRB, are up-regulated by E2, and not by tamoxifen or the pure antiestrogen ICI 182,780. HeC50 and KLE cells (poorly differentiated) express only hPRA. No hPRB is present in the poorly differentiated cells, and it is not induced by estrogen receptor expression and/or estrogen treatment. In all cells, hPRB expression, whether endogenous or produced as a result of transfection, acts as a stronger transcription factor than hPRA on a simple progesterone-dependent promoter. We speculate that down-regulation of hPRB may predict for poorly differentiated endometrial cancers that do not respond to progestin therapy.

INTRODUCTION

Cancer of the uterine endometrium is the most common malignancy of the female genital tract, is the third most common cancer in women, and is responsible for 13% of gynecological cancer deaths (1). The induction of endometrial cancer is related to hyperestrogenism unopposed by progesterone. This conclusion is based on studies linking high rates of endometrial cancer to polycystic ovary disease and other hyperestrogenic states in young women (2–4) and to the use of unopposed estrogen replacement therapy in postmenopausal women (5–8), the tumorigenic effects of which can be completely reversed by the addition of a progestational agent (9).

Progesterone counteracts the growth-stimulatory effects of estrogen by inducing glandular and stromal differentiation (10, 11). Endometrial hyperplasia can be reversed by progestin therapy, and progestin treatment is effective in decreasing the growth of endometrial tumors that express PRs (12). The PR content of endometrial cancers is strongly correlated with successful endocrine treatment and survival (13). Although nearly all endometrial tumors express ER at the high levels observed in the proliferative phase of the menstrual cycle, expression of PR is variable; even in the presence of ER, some tumors do not contain PR (14, 15). Because PRs are normally up-regulated by estrogens via ERs (16–19), this implies that failure to induce PR may be a factor in the genesis and/or progression of endometrial cancer.

hPRs are ligand-activated transcription factors that have a hormone-binding domain at the COOH terminus, a DNA-binding domain through which the receptors contact DNA, and two variable-length NH2 termini that produce the two major isoforms (truncated A receptors [20] and B receptors) that contain an additional 164 amino acids at the NH2 terminus (21, 22). Both isoforms have an activation function, AF1, just upstream of the DNA-binding domain and another, AF2, in the hormone-binding domain. Additionally, the unique NH2-terminal sequence of B receptors contains a strong third transcriptional activating function, AF3 (23).

Based on work in T47D breast cancer cells (21) and in some endometrial tumors (24), it was believed that hPRA and hPRB were expressed in approximately equimolar ratios, were dimerized as homo- and heterodimers with equal frequency, and had similar functions. However, recent studies have uncovered provocative differences between hPRA and hPRB expression and function in normal endometrium (25) and breast cancers (22, 26). When hPRA:hPRB ratios were examined in the normal cycling endometrium by immunoblotting (27, 28), hPRB was found to be expressed more highly than hPRB throughout the cycle. However, hPRB expression increased more sharply than hPRA in response to increasing E2 levels at midcycle and disappeared completely in the late secretory phase, whereas hPRA expression continued to be present throughout the cycle. In a recently published analysis of PRs in primary breast tumors, a range of isoform ratios was found, with hPRA exceeding hPRB in 76% of the cases (29).

It has also become increasingly apparent that hPRA and hPRB have different functional characteristics. hPRB is a stronger transcriptional activator of many PRE-containing promoters than is hPRA (23, 16, 30), although the differences are cell specific, and at least two examples exist in which the transcriptional activity of hPRA exceeds that of hPRB (31–33). The magnitude of transcriptional activation by hPRB can be significantly greater than that by hPRA. On the mouse mammary tumor virus promoter, for example, transcriptional activation by hPRB exceeds that by hPRA by 10-fold (20).

Whereas hPRB and hPRA are primarily transcriptional activators of progesterone-responsive genes, hPRA also functions as a transcriptional inhibitor of other steroid hormone receptors, including ER (30) and hPRB (32, 34). The term dominant-negative has been applied to hPRB in response to increasing E2 levels at midcycle and disappeared completely in the late secretory phase, whereas hPRA expression continued to be present throughout the cycle. In a recently published analysis of PRs in primary breast tumors, a range of isoform ratios was found, with hPRA exceeding hPRB in 76% of the cases (29).

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Received 10/7/97; accepted 3/4/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 These studies were supported by NIH Grants R29CA67313 (to K. K. L.), DK48238 (to K. B. H.), and CA26669 (to K. B. H.), the Cancer Research Foundation of America, and the Cancer League of Colorado.

2 To whom requests for reprints should be addressed, at Box B-198, the University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262.
levels are tightly regulated and comprise a dual system to control the actions of progesterone (16).

The studies reported herein were undertaken to determine the relative abundance of the PR isoforms, their expression in response to estrogens and antiestrogens, and their effects on progesterone-dependent reporter gene transcription in cultured endometrial cancer cells. We hypothesized that functional differences between hPRA and hPRB have significance in the endometrium, especially with respect to the ability of PR to counter estrogen-mediated endometrial glandular proliferation. Low expression of either hPRA or hPRB could lead to the potential consequences for tumor development and progression.

**MATERIALS AND METHODS**

**Cell Lines.** Ishikawa and HeC50 cells were a generous gift from Dr. E. Gurpide (Mt. Sinai City University of New York, New York, NY). KLE cells were obtained from the American Type Culture Collection (Bethesda, MD). The cell lines were maintained in Eagle’s MEM (anhydrous; 200 mg/liter), Fe(NO3)3·9H2O (0.10 mg/liter), KCI (400 mg/liter), MgSO4 (anhydrous; 97.67 mg/liter), NaCl (6400 mg/liter), NaHCO3 (3700 mg/liter), NaH2PO4·H2O (125 mg/liter), C2H5OH (1 g/liter), and C3H8O3Na (110 mg/liter); Life Technologies, Inc., Gaithersburg, MD) in the presence of 7.5% fetal bovine serum (Gemini Bio Products, Inc., Calabasas, CA), 2 mm l-glutamine (Life Technologies, Inc.), and an antibiotic/antimycotic solution containing penicillin-G (50 units/ml), streptomycin (50 units/µg), and fungizone (0.125 µg/ml; all from Gemini Bio Products, Inc.).

**RNA Isolation.** Cells were washed and lysed using guanidinium isothiocyanate solution. Total RNA was isolated by phenol-chloroform extraction according to Chomczynski and Sacchi (35).

**RT-PCR.** RT-PCR kits were purchased from Perkin-Elmer Corp. (Branchburg, NJ). cDNAs were synthesized from 1.5 µg of total RNA using random hexamer primers. cDNA synthesis was carried out as suggested by the kit protocol, except that the RNA was incubated at 65°C for 5 min to denature the RNA before the addition of murine leukemia virus reverse transcriptase. The reverse transcription was carried out for 60 min at 42°C. After an incubation at 99°C for 5 min to inactivate the reverse transcriptase, the entire 20-µl cDNA synthesis reaction was used in subsequent PCR reactions with specific primers to amplify regions of PR common to both isoforms and regions unique to hPRB. β-actin cDNA fragments were also amplified as positive controls (Table 1).

**Table 1 Location and sequence of primers**

<table>
<thead>
<tr>
<th>Position</th>
<th>Size (bp)</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>B-unique</td>
<td>429</td>
<td>ACAGAATTTCATTGACTAGCTAAGCTGAAGCCAAAGGGTT</td>
</tr>
<tr>
<td>Reverse-1173</td>
<td>429</td>
<td>ACAGAATTTCATTGACTAGCTAAGCTGAAGCCAAAGGGTT</td>
</tr>
<tr>
<td>Common to A and B</td>
<td>243</td>
<td>ACAGAATTTCATTGACTAGCTAAGCTGAAGCCAAAGGGTT TTTT</td>
</tr>
<tr>
<td>Forward-1239</td>
<td>229</td>
<td>AGGCGGAAACCCGAGAAGGAGAT</td>
</tr>
<tr>
<td>Reverse-1455</td>
<td>229</td>
<td>TGGAGGCTTTGTTTTCAGAGAG</td>
</tr>
<tr>
<td>β-Actin*</td>
<td>771</td>
<td>TGGAGAATGGGAGGCCCCGACTC</td>
</tr>
<tr>
<td>Forward-1386</td>
<td>771</td>
<td>TGGAGAATGGGAGGCCCCGACTC</td>
</tr>
<tr>
<td>Reverse-2811</td>
<td>771</td>
<td>TGGAGAATGGGAGGCCCCGACTC</td>
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* Spans one intron.

**Whole Cell Protein Extraction.** Cells were plated on 15-cm plates. One day after plating, the cells were treated with either vehicle (ethanol) alone; 0.01 µM E2, and trans-tamofoxifen (all from Sigma, St. Louis, MO); or IC 182,870 (Imperial Chemical Industries; a gift of Zeneca Pharmaceuticals, Macklesfield, United Kingdom) dissolved in ethanol. Plates were washed with cold buffer (0.2 g/liter KCl, 0.2 g/liter KH2PO4, 8 g/liter NaCl, and 2.16 g/liter Na2HPO4·7H2O) and scraped with a rubber policeman. The cells were spun down, and the pellets were snap-frozen on dry ice. Cell pellets were then thawed in high-salt buffer (0.4 M KCl, 29 mm HEPES (pH 7.4), 1 mm DTT, and 20% glycerol) plus protease inhibitors for 5-10 min. The extracts were passed through a 28-gauge needle and centrifuged at 50,000 × g for 20 min. Bradford assays were performed in duplicate on the supernatant to quantitate the amount of protein (36).

**Western Blotting.** Enhanced chemiluminescence Western blotting kits were purchased from Amersham (Arlington Heights, IL), and the kit instructions were followed. Briefly, protein extract (800 µg/lane) was loaded on a 7.5% SDS-polyacrylamide gel. This was run at 8 mV overnight in tank buffer (25 mm Tris, 192 mm glycine, and 0.1% SDS (pH 8.3)). The proteins were transferred to nitrocellulose membranes (Amersham) that were then incubated with a mouse monoclonal antibody whose epitope lies in the NH2-terminal region common to both hPRA and hPRB (AB-52; Ref. 33). The membranes were subsequently incubated with a goat antiamouse secondary antibody (Cappel: Organon Teknika Corp., West Chester, PA) followed by luminol reagent (Amersham), and chemiluminescence was detected by autoradiography.

**Transient Transfections and Reporter Assays.** Ishikawa, HeC50, and KLE cells were plated in 10-cm dishes in triplicate and grown to 75–80% confluence. For each plate, 1 µg of the progesterin-dependent reporter plasmid PRE2-TATA-g-CAT (23), containing two consensus PREs cloned upstream of the TATA box from the thymidine kinase gene and the CAT reporter gene was transfected. Additionally, 3 µg of the expression vector for β-galactosidase, pCH110, ± 0.1 µg of the expression vector for ER, pSG5-HEGO (a gift from P. Chambon, CRNS/INSERM/ULP, Cedex France), were introduced into cells by lipofection using 20 µl of LipofectAMINE (Life Technologies, Inc.). In experiments designed to test the expression and transcriptional efficiency of ER introduced into KLE and HeC50 cells, the reporter plasmid vit-tk-CAT was used (a gift from P. Chambon). This vector encodes the consensus estrogen response element from the vitellogenin A2 gene upstream from the thymidine kinase promoter (37). Twenty-four h posttransfection, the cells were treated with vehicle alone or with hormone. At 48–72 h posttransfection, CAT activity was measured in cellular extracts by TLC according to previously published methods (38). Each reaction was normalized to β-galactosidase activity to correct for the efficiency of transfection between plates. Experiments were performed in triplicate and repeated at least three times. The results are shown as representative experiments analyzed by the Student’s t test, and presented as the mean ± SE.

**RESULTS**

**PR Isoform Expression and Regulation by E2**

RT-PCR and Western blotting were used to determine the differential expression of PR isoform transcripts and proteins and whether estrogens induce PR in Ishikawa, KLE, and HeC50 endometrial cancer cells.
mRNAs for hPRA Are Present in Ishikawa, Hec50, and KLE Cells; However, Only Ishikawa Cells Express hPRB mRNAs. Fig. 1a shows data from well-differentiated Ishikawa cells. Fig. 1b shows data from poorly differentiated KLE cells. Results identical to those for KLE were obtained for the Hec50 cells (data not shown). In Fig. 1, a and b, Lane 1 represents amplification of PR mRNAs encoding sequences common to both A and B receptors. A band is seen in both cell types. However, Lane 2 represents B receptors only. Clearly, whereas B-receptors are present in Ishikawa cells, they are missing in KLE cells. When both sets of primers are combined in the same reaction (primers common to both A and B receptors plus primers specific to B receptors) as shown in Lane 3, amplification of transcripts unique to B receptors occurs only in Ishikawa cells. β-Actin products of two different sizes (771 and 229 bp) were used as positive controls. Negative controls, one without RNA (Lane 5) and the other without reverse transcriptase (Lane 6), were included.

hPRA Proteins Are Detectable in Ishikawa, Hec50, and KLE Cells, While hPRB Proteins Are Only Expressed in Ishikawa Cells. Fig. 2 is a representative Western blot showing hPRA and hPRB expression in Ishikawa, KLE, and Hec50 cells in the presence or absence of E₂. The 116–120-kDa triplet band characteristic of hPRB is only present in well-differentiated Ishikawa cells (Lane 1) and is significantly up-regulated after E₂ treatment (Lane 2), whereas the 96-kDa hPRA band is present in all cell lines. A receptors are up-regulated by E₂ in Ishikawa cells (Lane 2) but seem to be constitutively expressed in KLE and Hec50 cells (Lanes 3–6). Whereas E₂ further enhances hPRA expression in Ishikawa cells, it does not induce hPRA expression in KLE or Hec50 cells. These data confirm at the protein level those obtained for mRNA by RT-PCR and indicate that only well-differentiated Ishikawa cells express B receptors, whereas A receptors are present in all three cell lines.

The Up-Regulation of hPRA and hPRB Proteins Requires an Estrogen and Is Not Induced in the Presence of the Antiestrogens Tamoxifen or ICI 182,780. Fig. 3 is a representative Western blot showing the presence of hPR proteins in Ishikawa cells after E₂, E₁, tamoxifen (T), or ICI 182,780 treatment. Note the up-regulation primarily of hPRB and, to a lesser extent, hPRA in response to E₂, E₁ is a weak estrogen in Ishikawa cells (39).

Reporter Gene Transcription by hPRA and hPRB

Transfection experiments were used to answer three questions: (a) What is the transcriptional activity of endogenous hPR on a simple PRE-containing promoter in Ishikawa, KLE, and Hec50 cells? (b) What is the effect of enhancing hPRA versus hPRB expression by transfection on reporter gene transcription under the control of a PRE-containing promoter? (c) In KLE and Hec50 cells that do not express ER, is it possible to reinstate PR-mediated gene transcription by introducing ER and thereby up-regulating endogenous PR expression? The data addressing each question are presented below.

Endogenous Expression of hPRB in Ishikawa Cells Allows Progestin-mediated Transcription of a Reporter Plasmid Controlled by a Simple PRE-containing Promoter; No Transcription Occurs in hPRB-negative Hec50 and KLE Cells. Fig. 4 is a representative experiment showing CAT activity in Ishikawa and KLE cells transfected with PRE-TATA₂₆-CAT in response to E₂ and/or the progesterone agonist R5020. Ishikawa cells demonstrate low baseline CAT activity in the absence of hormone. R5020 alone doubles the transcriptional activity, suggesting that the low constitutive levels of PR are functional. However, the effect of R5020 is significantly enhanced by pretreatment with E₂ for 24 h to further induce PR. When this is followed by R5020 treatment, an additional 2–3-fold increase in CAT activity occurs. No significant induction of CAT activity occurs when the cells are pretreated with ligands that act as antiestrogens, such as tamoxifen or ICI 182,780. Interestingly, the weak estrogen E₁ can mimic the effects of the potent estrogen E₂, with respect to PR induction in Ishikawa cells. We have shown that E₁ is converted to E₂ in these cells; therefore, we speculate that the response to E₁ is actually due to its conversion to E₂.

Based on Western blotting data (Figs. 2 and 3), we hypothesize that...
Fig. 4. E₂ enhances the transcription resulting from endogenous PR in Ishikawa cells. Cells were transfected with a reporter vector, PRE₂-TATA₉-CAT, and then treated with ethanol alone (Lanes 1 and 2), estrogens (E₂ and E₃, Lanes 3 and 4, respectively), or antiestrogens (tamoxifen and ICI 182.780, Lanes 5 and 6, respectively) 24 h posttransfection. Forty-eight h posttransfection, the cells were treated with ethanol alone (Lanes 1 and 2) or R5020, a synthetic progestin (Lanes 3–6). Lysates were normalized to β-galactosidase activity; CAT activity was measured by TLC, and the average of triplicate determinations is shown. 0, ethanol alone; T, tamoxifen; ICI, ICI 182.780.

Fig. 5. Exogenously expressed B receptors activate transcription in KLE cells, whereas A receptors inhibit basal transcription. KLE cells were transfected with PRE₂-TATA₉-CAT and expression vectors for hPRB (Lane 3) or hPRA (Lane 4). Transfections resulted in an increase in PR expression of approximately 40 fm/mg protein. Twenty-four h posttransfection, the cells were treated with ethanol alone (Lane 1) or R5020, a synthetic progestin (Lanes 2–4). Lysates were normalized to β-galactosidase activity; CAT activity was measured by TLC, and the average of triplicate determinations is shown.

The increased CAT activity in Ishikawa cells in response to E₂ is associated with the specific up-regulation of endogenous hPRB, which, in contradistinction to hPRA, is known to strongly activate many PRE-containing promoters (23, 16, 30). Fig. 4 shows that KLE cells exhibit very little baseline CAT activity, and that this is not increased with E₂ treatment, consistent with the Western blot data showing that E₂ cannot up-regulate hPRB expression in these cells. However, KLE cells do express hPRA (Fig. 2). Therefore, the data in Fig. 4 also indicate that hPRA is not a strong activator of PRE₂-TATA₉-CAT, as has been demonstrated previously for other PRE-containing promoters (32). The response of Hec50 cells, which also express only hPRA, is similar to that of KLE cells (data not shown).

Introduction of hPRB, but not hPRA, Restores Progestin-mediated Induction of PRE₂-TATA₉-CAT in KLE Cells. As has been demonstrated, KLE cells express only endogenous hPRA, not hPRB. The experiment in Fig. 5 was designed to test the effect of introducing hPRB into KLE cells. CAT activity controlled by the PRE₂-TATA₉ promoter was used as an indicator of PR action. Fig. 5 is a representative experiment demonstrating CAT activity in KLE cells transfected with expression vectors for hPRB and hPRA. Transfection of the hPRB expression vector led to a 2-fold induction of CAT activity above control levels and a 5-fold induction of CAT over cells transfected with the hPRA expression vector. Therefore, these data confirm the increased trans-activating capability of hPRB compared to hPRA on the PRE₂-TATA₉-CAT vector.

Another finding of interest relates to the magnitude of hPRB induction of CAT in these experiments. KLE cells express relatively high endogenous levels of hPRA. When hPRB expression is introduced, CAT induction is increased, but only modestly, resulting in a 2-fold induction. This is low compared to the 3–4-fold levels observed in Ishikawa cells (Fig. 4), which express relatively higher levels of endogenous hPRB and lower levels of hPRA. We hypothesize that the induction of CAT in KLE cells by hPRB is dampened by the endogenous hPRA. In addition, comparing Lane 1 with Lane 4 in Fig. 5, CAT expression is consistently below baseline when hPRA is overexpressed in KLE cells. These data may be examples of the dominant-negative effects (32) of hPRA on the basal transcription apparatus of the PRE₂-TATA₉ promoter.

Estradiol Responsiveness Can Be Induced in Hec50 and KLE Cells by Transfection of an Expression Vector for ER. Before evaluating whether ER could induce hPRB expression in ER-negative KLE and Hec50 cells, we first determined whether the cells could be successfully transfected with the ER expression vector, pSG5-HEGO, and whether estrogen responsiveness could be restored. The representative experiment shown in Fig. 6 demonstrates that E₂ treatment results in CAT induction when KLE and Hec50 cells are transfected with an estrogen-responsive reporter gene, vit-tk-CAT, and pSG5-HEGO. Estradiol-mediated gene transcription is reinstated by this manipulation in both cell lines. We next asked whether endogenous genes normally regulated by E₂, including PR, could be induced in KLE and Hec50 if ER expression was restored.

ER Expression in KLE Cells Fails to Reinstall PR Expression. We showed in Fig. 2 that no induction of either PR isoform occurs in wild-type KLE and Hec50 cells in response to E₂. This is an expected result because, unlike Ishikawa cells, KLE and Hec50 cells do not express endogenous ER. In the experiment shown in Fig. 7, we wished to test the premise that reintroduction of ER would result in hPRB up-regulation with subsequent induction of the PRE₂-TATA₉ promoter in response to E₂ plus R5020. A representative experiment is shown in which KLE cells were transfected with the ER expression vector and with the promoter/reporter PRE₂-TATA₉-CAT to measure the transcriptional activity of any induced endogenous PR. The cells were treated with E₂ for 24 h to induce PR, followed by R5020 to
activate PR. Interestingly, little significant induction of R5020-driven CAT activity occurs in the E2-treated cells (Lane 3) compared to the untreated cells (Lane 2), suggesting that hPRB expression is not reinstated simply by reintroducing ER into the KLE cells. Similar results were obtained for HecSO cells (data not shown). In contrast, Ishikawa cells show a strong R5020 response after E2 priming. These data suggest that compared to estrogen-responsive Ishikawa cells, KLE and HecSO cells have at least two alterations: (a) the ER is not synthesized; and (b) at least some ER-responsive pathways including PR expression have become desensitized.

**DISCUSSION**

We report an analysis of PR expression and function in well-differentiated endometrial cancer cells, Ishikawa cells, and poorly differentiated HecSO and KLE cells that represents one of the first attempts to determine the consequences of differential PR isoform expression in endometrial cancer. Ishikawa cells are estrogen responsive and express endogenous ER (30–50 fm/mg protein; data not shown). Like most endometrial cells grown in vitro, neither HecSO nor KLE cells express endogenous ER; however, they can be rendered responsive to estrogen (as measured by activation of an estrogen response element-containing promoter) by cotransfecting an expression vector encoding ER and estrogen treatment do not reinstate expression vector for ER, pSG5-HEGO (Lanes 1–6). Cells were treated with ethanol alone (Lanes 1 and 2), E2 or E3 (Lanes 3 and 4, respectively), or antiestrogens (tamoxifen or ICI 182,780, Lanes 5 and 6, respectively). CAT activity driven by PR was measured. 0, ethanol alone; T, trans-tamoxifen; ICI, ICI 182,780.

well-differentiated Ishikawa cells express hPRB; however, poorly differentiated HecSO and KLE cells do not (Figs. 1 and 2). hPRB is up-regulated by estrogen in Ishikawa cells (Figs. 2 and 3) but is not induced by estrogen in HecSO and KLE cells (Fig. 2). This seems to be true even in the presence of ER, because transfection of an expression vector encoding ER and estrogen treatment do not reinstate hPRB-mediated gene expression in KLE cells (Fig. 7).

In experiments designed to test the strength of transcriptional activation of hPRA versus hPRB on the simple PRE2-TATAak promoter, we find that hPRB is a more powerful activator of reporter gene transcription than is hPRA. This is true for cells that are selectively transfected with equal amounts of expression vectors for hPRA and hPRB (Fig. 5). As has been reported in other systems (32), we note that hPRA may be an inhibitor of basal transcription in KLE cells (Fig. 5). It should be noted that unlike PRE2-TATAak, other promoters are more strongly induced by hPRB than hPRA (31, 32, 33), so we anticipate that there are subsets of genes activated primarily by hPRA as well. The study of which progesterone-responsive promoters are induced by each PR isoform is currently underway in our laboratory.

Unopposed estrogen stimulation is the common thread that underlies the development of many endometrial cancers. How does unopposed estrogen stimulation occur? We hypothesize that down-regulation of hPRA or hPRB may contribute to unchecked cell proliferation. It is likely that both isoforms are important to protect the endometrium (hPRA for its dominant-negative effect on ER, and hPRB for its ability to strongly up-regulate the transcription of genes required for differentiation). Poorly differentiated KLE and HecSO cells demonstrate abnormal expression of PR isoforms compared to Ishikawa cells, which are well-differentiated and responsive to progestins (42). KLE and HecSO cells no longer express hPRB but retain expression of hPRA. If the dominant-negative effects of hPRA are indeed important in countering ER-induced transcription (30), this function is likely to be preserved in cells such as KLE and HecSO. However, it is predicted that such cells do not express the hPRB-dependent genes required for progesterone-mediated cell differentiation. These studies indicate that low expression of hPRB portends a poorly differentiated endometrial cancer phenotype that may not respond to progestin therapy.

**REFERENCES**

13. Vecev, N., Nola, M., Marusic, M., Ilic, J., Babic, D., Petrovecki, M., Nikolic, S.,
Novicic, T., Jokic, D., and Vecev, N. Jr. Prognostic value of steroid hormone

14. Polczaski, E. S., Satyaswaroop, P. G., and Mortel, R. Hormonal interactions in
gynecologic malignancies. In: W. J. Hoskins, C. A. Perez, and R. C. Young (eds.),

Cytoplasmic receptors for 17 β-estradiol, 5 α-dihydrotestosterone and progesterone in

16. Horwitz, K. B., and Alexander, P. S. In situ photolinked nuclear progesterone

Ligand-modulated regulation of progesterone receptor, messenger ribonucleic acid and

B. W., and Horwitz, K. B. Multiple human progesterone receptor messenger ribonu-
cleic acids and their autoregulation by progestin agonists and antagonists in breast

19. Savouret, J. F., Rauch, M., Redeuilh, G., Sar, S., Chaucreau, A., Woodruff, K.,
Parker, M. G., and Milgrom, E. Interplay between estrogens, progestins, retinoic acid
and AP-1 on a single regulatory site in the progesterone receptor gene. J. Biol. Chem.,

Human progesterone receptor A and B isoforms across the human menstrual cycle. J. Soc.

21. Graham, J. D., Yeates, C., Balleine, R. L., Harvey, S. S., Milliken, J. S., Bilous,
A. M., and Clarke, C. L. Characterization of progesterone receptor A and B proteins

and B isoforms of the human progesterone receptor operate through distinct signaling

23. Gronemeyer, H., Turcotte, B., Quirin-Stricker, C., Bocquel, M. T., Meyer, M. E.,
Kroowski, Z., Jeltsch, J. M., Lerouge, T., Garnier, J. M., and Chambon, P. The
chenic progesterone receptor: sequence, expression and functional analysis. EMBO J.,

24. Feil, P. D., Clarke, C. L., and Satyaswaroop, P. G. Progesterone receptor structure and
functionally different human progesterone receptor forms A and B. Endocrinology,

Chambon, P. Two distinct estrogen-regulated promoters generate transcripts encoding
receptors concentration in patients with endometrial carcinoma. Acta Obstet. Gy-

26. Polczaski, E. S., Satyaswaroop, P. G., and Mörtel, R. Hormonal interactions in
gynecologic malignancies. In: W. J. Hoskins, C. A. Perez, and R. C. Young (eds.),

27. Wiehle, R. D., Mangal, R., Poinexter, A. H., III, and Weigel, N. L. Human
progesterone receptor A and B expression during the menstrual cycle. Endocr. Soc.

Characterization of functional progesterone receptor A and B forms. Mol. Endocrinol.,

29. Graham, J. D., Yeates, C., Balleine, R. L., Harvey, S. S., Milliken, J. S., Bilous,
A. M., and Clarke, C. L. Characterization of progesterone receptor A and B proteins

and B isoforms of the human progesterone receptor operate through distinct signaling

31. Gronemeyer, H., Turcotte, B., Quirin-Stricker, C., Bocquel, M. T., Meyer, M. E.,
Kroowski, Z., Jeltsch, J. M., Lerouge, T., Garnier, J. M., and Chambon, P. The
chenic progesterone receptor: sequence, expression and functional analysis. EMBO J.,

32. Vegeto, E., Shahbaz, M. M., Wen, D. X., Goldman, M. E., O’Malley, B. W., and
McDonnell, D. P. Human progesterone receptor A form is a cell- and promoter-
specific repressor of human progesterone receptor B function. Mol. Endocrinol.,

33. Tung, L., Mohamed, K., Hoeflref, J. P., Takimoto, G. S., and Horwitz, K. B.
Antagonist-occupied human progesterone B-receptors activate transcription without
binding to progesterone response elements and are dominantly inhibited by A-recep-

34. Kraus, W. L., Weis, K. E., and Katzenellenbogen, B. S. Inhibitory cross-talk between
steroid hormone receptors: differential targeting of estrogen receptor in the repression
of its transcriptional activity by agonist- and antagonist-occupied progesterin receptors.


36. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram

sive element derived from the 5’ flanking region of the Xenopus vitellogenin A2 gene

38. Leslie, K. K., Tasset, D. M., and Horwitz, K. B. Functional analysis of a mutant

39. Leslie, K. K., and Litman, E. The effects of estrogens and antiestrogens on reporter
gene transcription in endometrial and breast cancer cells in vitro: models to study the
consequences of tamoxifen treatment. In: H. Kuramoto and E. Gurpide (eds.). In
Livingstone, 1996.

contraceptive use influences resting breast proliferation. Hum. Pathol., 20: 1139–
1147, 1989.
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