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ROLE OF THE PROGESTERONE RECEPTOR (PR) IN THE REGULATION OF INFLAMMATORY RESPONSE PATHWAYS AND AROMATASE IN THE BREAST

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Abstract

There is convincing evidence to suggest that estrogen and inflammatory mediators play important roles in growth and progression of breast cancer. Moreover, local conversion of androgens to estrogens by aromatase (product of *CYP19* gene) occurs in 70% of all breast cancers. The actions of aromatase in both the breast tumor and in surrounding adipose stromal and endothelial cells can result in high local levels of estrogen production that stimulate tumor growth. The efficacy of current endocrine therapies is predicted only if the tumor contains significant amounts of ER. Presence of PR in the tumor also is an important predictor of tumor aggressiveness and responsiveness to endocrine therapy. Immunoreactivity for aromatase in human breast tumors is highly correlated with that for cyclooxygenase 2 (COX-2), the rate-determining enzyme in prostanoid biosynthesis. COX-2 expression also is correlated with expression of HER-2/neu, an oncogene expressed in >30% of breast tumors. In this manuscript, we will review findings suggest that induction of COX-2 by inflammatory cytokines acting through NF- κ B contributes to the increase in *CYP19* expression and breast cancer progression, and that PR plays a dominant protective role in breast cancer cells by antagonizing NF- κ B activation of COX-2.

1. Introduction

In addition to its vital role in reproduction, estrogen acting through the estrogen receptor (ER) has been implicated in the pathophysiology of a number of diseases, including cancer of the breast. Estrogen synthesis from C₁₉-steroids is catalyzed by aromatase P450 (product of the *aromatase/CYP19* gene). Aromatase is upregulated in 70% of all human breast cancers [1] and is expressed both in tumor and in surrounding adipose stromal and endothelial cells [1]. Thus, induction of aromatase within and surrounding the breast tumor can result in high local levels of estrogen production that stimulate tumor growth. In this regard, third-generation aromatase inhibitors have greater efficacy than tamoxifen in treatment of early and late-stage breast cancer [2]. However, responsiveness to such therapies is only predicted if the breast tumor contains significant amounts of ER [3]. The presence of progesterone receptor (PR) in a breast tumor

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also is an important predictor of responsiveness to therapy [4]. It has long been thought that this is due to the fact that PR is an estrogen-induced target gene that serves as an indicator of ER functional capacity and differentiation status of the tumor [5]. In this article we will provide support for the hypothesis that PR also serves a critical and protective role in the breast by inhibiting NF- κ B activation, thereby blocking inflammatory response pathways, expression of oncogenic growth factor receptors and aromatase induction.

2. The aromatase (CYP19) gene and its tissue-specific expression

Human (h) *CYP19* is a single-copy gene expressed in a number of tissues, including placenta [6], gonads [7-11], discrete nuclei of brain [12], adipose stromal cells [13] and in breast cancer epithelial and stromal cells [1]. Expression of aromatase in each of these tissues is controlled by tissue-specific promoters that lie upstream of tissue-specific first exons encoding the 5'-untranslated regions (UTRs) of the mRNAs. Thus, aromatase mRNA transcripts in gonads, brain, adipose and placenta contain different first exons (*Ila*, *If*, *I.4/I.3* and *I.1*, respectively) that are alternatively spliced onto a common site just upstream of the translation initiation codon in *exon II* [13] (Fig. 1A). These tissue-specific promoters and first exons lie at various distances upstream of *exon II* (*exons II - X* encode the aromatase P450 protein). Sequencing of the human genome has revealed that *hCYP19* encompasses ~130 kb of DNA. The start site of transcription in placenta (*exon I.1*) lies ~100,000 bp upstream of the start site of translation [14,15]. Adipose tissue has two alternative tissue-specific first exons; one is ~70,000 bp upstream (*exon I.4*) [16], and the other is ~250 bp upstream (*exon I.3*) [16,17] of the translation start site. The brain-specific first exon (*If*) [18] lies ~40,000 bp upstream [15], whereas in the gonads, the start site of transcription (in *exon Ila*) lies only 39 bp upstream of the start site of translation [19] (Fig. 1A). In human breast tumors, the start site of transcription corresponds to that used in gonads (*exon Ila*) or just upstream in *exon I.3* [17]. Thus, the aromatase mRNAs in these different tissues have unique 5'-untranslated regions, while the sequences encoding the aromatase protein are identical.

The presence of unique, tissue-specific UTRs in hCYP19 transcripts is exemplified by the study shown in Figure 1B, in which quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR) was carried out using validated primers to specifically amplify the 5'-UTRs of hCYP19 transcripts in ovary (*Exon Ila*), adipose (*Exon I.4*), placenta (*Exon I.1*), and breast cancer (*Exon I.3*). These primers were designed to hybridize with equivalent efficiency; thus, the relative levels of *CYP19* expression in each of these tissues can be compared. As can be seen, *exon Ila*-containing transcripts were predominant in the ovary, *exon I.4*-containing transcripts were predominant in adipose tissue, *I.1*-containing transcripts predominated in placenta and *I.3*-containing transcripts were highest in T47D breast cancer cells. When the relative levels of *exon Ila*- and *I.3*-containing hCYP19 mRNA transcripts were compared in the T47D cells, they were found to be roughly equivalent (data not shown).

3. Regulation of *hCYP19* gene expression in breast cancer

In human breast tumors and surrounding adipose stromal cells, aromatase was found to be upregulated via switching from the weak adipose-specific promoter (*I.4*) to the strong ovarian (*Ila*) [20,21] and so-called 'breast cancer' (*I.3*) [17] promoters. *Promoter I.3* lies just upstream of *promoter Ila*; consequently, both share important response elements, including a CREB-like sequence (CLS) [22-24], two nuclear receptor response elements (NRE-A and NRE-B) [23,25,26], which likely bind the orphan nuclear receptors SF-1 and LRH-1 [23,24,26-28], and two GATA-binding sites [29,30].

Promoter switching is likely mediated by induction of cyclooxygenase 2 (COX-2) in the breast tumor [1,31], resulting in increased prostaglandin E₂ (PGE₂) synthesis by breast tumor

epithelial cells and infiltrating macrophages [20]. In the study shown in Figure 2, forskolin, which stimulates adenylyl cyclase and cAMP formation, and PGE₂ had marked effects to stimulate aromatase activity in cultured human breast adipose stromal cells [20]. PGE₂ acts via receptors that activate protein kinase A (PKA) and Ca²⁺/PKC pathways in the breast tumor and surrounding stromal cells. Previously, we observed that cAMP and phorbol esters acted synergistically to increase aromatase activity and expression in human adipose stromal cells [32]. The finding that PGF₂α (which acts exclusively via receptors that mediate increased cytoplasmic calcium and PKC activation) had little effect on aromatase activity in these cells, supports our findings that upregulation of cAMP mediated pathways are essential for aromatase induction [33]. In this study, it was also observed that PGE₂ stimulation of aromatase was associated with switching from the weak adipose-specific *promoter I.4* to the strong ovarian and breast cancer promoters, *I.1a* and *I.3*, respectively [20]. Similar findings regarding promoter switching were obtained by Harada and colleagues in breast cancer cells [21].

Many of the same transcription factors that mediate cAMP stimulation of *hCYP19* expression in ovarian granulosa cells likely cause its upregulation in breast cancer. Cyclic AMP/PKA induction of *promoter I.1a* activity in ovarian granulosa cells is mediated by CREB transcription factor, which binds to the CREB-like sequence (CLS) [15] and by the orphan nuclear receptors steroidogenic factor-1 (SF-1) and liver receptor homologue-1 (LRH-1), which bind to NRE-A and NRE-B sites [26-28]. The *hCYP19 exon I.1a* 5'-flanking region also contains enhancers that bind the transcription factors GATA3 and 4 [30,34], which are expressed in some breast cancer cell lines and act synergistically with LRH-1 in a PKA-dependent manner to increase *promoter I.1a* activity in transfected breast cancer cells [34]. In a panel of breast tumors, LRH-1 was found to be upregulated 4-5 fold in the tumor and surrounding stromal cells, as compared to normal tissues [35]. Furthermore, in cultured human adipose stromal cells, LRH-1 and aromatase expression were found to be induced by treatment with PGE₂ [35]. Notably, LRH-1 is localized to human chromosome 1q32.1, a region amplified in primary breast tumors and breast cancer cell lines [35]. LRH-1 has been suggested to contribute to intestinal tumorigenesis by serving as a potent coactivator of β-catenin-mediated induction of the *cyclin D1* and *c-Myc* promoters and as a direct transcriptional activator of the *cyclin E1* promoter in intestinal epithelial cells [36]. The orphan nuclear receptor, estrogen-related receptor (ERR)α, which is structurally and functionally similar to the classical ERs but does not bind estrogen, was also found to interact with the NRE-A element and to induce aromatase expression via *promoter I.3* [37]. ERRα expression in human breast tumors is significantly associated with risk of recurrence and adverse clinical outcome [38]. Presence of ERRα also is correlated with markers of tumor aggressiveness, such as absence of ERα and increased HER-2/neu [39]. Thus, a number of the transcription factors that are known to increase *CYP19* gene expression are found to be upregulated in malignant breast tumors and in breast cancer cell lines.

4. COX-2 and breast cancer

There is convincing evidence that COX-2, the rate-determining and highly regulated enzyme in prostanoid biosynthesis, is overexpressed in breast cancer [40]. COX-2 upregulation is associated with increased aromatase [1] and enhanced expression of the tumorigenic marker, HER-2/neu. HER-2/neu and COX-2 appear to exist in a positive feedback loop, whereby HER-2/neu increases COX-2 transcription and elevated COX-2, via PGE₂, induces HER-2/neu expression [41]. In a study of >6,000 women, use of nonsteroidal anti-inflammatory drugs was found to reduce the risk of breast cancer [42]. Furthermore, COX-2 inhibition decreased aromatase expression in human breast cancer cells in culture [43]. Consequently, clinical trials of COX-2 inhibitors alone and in conjunction with aromatase inhibitors are in progress. It is, therefore, evident that activation of inflammatory response pathways in the breast can lead to induction of COX-2 and aromatase, as well as growth factor receptors, such as HER-2/neu, which promote tumorigenesis.

5. NF- κ B and breast cancer

The transcription factor NF- κ B is a critical mediator of the inflammatory response and of cell survival, and plays an important role in cellular transformation and oncogenesis. The NF- κ B family is comprised of the RelA (p65), RelB and c-Rel proteins, which are synthesized in their mature forms [44]. Other family members include NF- κ B1 (p100) and NF- κ B2 (p105), which are synthesized as larger precursors that are proteolytically processed at their C-terminal ends to form p50 and p52, respectively [44]. NF- κ B is typically comprised of a heterodimer of p50 and p65 proteins [44]. In the inactive state, p50/p65 heterodimers are retained in the cytoplasm in a complex with I κ B α or I κ B β . Upon stimulation of the cell by a variety of signals, including proinflammatory cytokines and bacterial LPS, there is activation of a specific I κ B kinase (IKK β), resulting in the phosphorylation, ubiquitination and proteolytic degradation of I κ B [44]. This permits release of NF- κ B heterodimer, its translocation to the nucleus and transcriptional activation of target genes involved in the inflammatory response, immunoregulation, anti-apoptosis and in enhanced cell proliferation [45].

There is increasing evidence to suggest that NF- κ B plays an important role in normal mammary gland development and in breast carcinogenesis. Increased levels of IKK activity, nuclear NF- κ B and NF- κ B DNA-binding activity have been observed in human breast cancer cell lines and in breast cancer specimens (see [45] for review). On the other hand, a dominant-negative mutation of IKK β in mouse mammary tumor cells inhibited NF- κ B activation and decreased tumorigenic potential of the cells [46]. In a recent study of human breast tumors, NF- κ B activation was increased in ER(-) as compared to ER(+) tumors and was predominately upregulated in ER(-)/HER-2/neu(+) tumors [46]. Additionally, increased NF- κ B p50 DNA-binding activity was found to be associated with poor clinical outcome in ER(+) breast tumors [47]. Furthermore, increased levels and DNA-binding activity of NF- κ B proteins RelB and p52 were observed in mouse mammary tumors induced by treatment with 7,12-dimethylbenz (a) anthracene (DMBA) [48].

Evidence also exists for a cooperative interaction between NF- κ B- and ER-mediated inflammatory response pathways in the breast. Estrogen action to stimulate breast cancer growth is mediated, in part, by nongenomic mechanisms involving interaction of ER α with growth factor receptors and signaling cascades that originate at the plasma membrane. This involves activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase pathways, with enhanced estrogen-independent phosphorylation and activation of nuclear and membrane-associated ER α [49]. Interestingly, the coactivator SRC-3 (AIB1), which is upregulated in metastatic human breast cancer, is phosphorylated in response to estradiol-17 β , MAPK [50], and HER-2/neu tyrosine kinase [51]. Phosphorylation of SRC-3 causes its nuclear translocation, increased interaction with ER and the resulting enhancement of ER transcriptional activity [50]. SRC-3 also is phosphorylated by IKK in response to inflammatory cytokines, which causes its nuclear translocation; SRC-3 acts in concert with IKK to enhance NF- κ B-mediated gene expression [52]. The finding that ER α stimulation of target gene transcription in MCF-7 cells is dependent upon SRC-3 phosphorylation by MAPK and IKK signaling pathways [50] suggests that SRC-3 may serve as a potentially harmful integrator of ER- and NF- κ B-mediated inflammatory signaling pathways in the breast.

6. PR and breast cancer

It has long been appreciated that the presence of PR in a breast tumor is an independent predictor for benefit from adjuvant endocrine therapy and of disease-free survival [53-55]. Breast tumors that are PR(-) have a much higher proliferation rate and are more likely to manifest increased expression of the tumorigenic prognostic indicators, HER-2/neu and EGFR, than PR(+) tumors [56-59]. PR exists as three major isoforms, PR-A (94 kDa), PR-B (114 kDa) [60] and PR-C

(60 kDa) [61]. PR-B and PR-A both bind to progesterone response elements (PREs) in DNA; however, PR-A lacks one of three transcriptional activation domains that are present in PR-B [62]. In this regard, PR-A can act as a potent dominant-negative repressor of PR-B in a cell- and promoter-specific context [63]. PR-A also may inhibit PR-B transcriptional activity by competing for limiting amounts of coactivators. PR-A and PR-B are present in roughly equivalent amounts in normal breast tissue and are distributed in luminal cells throughout the intralobular ducts and alveoli [64]. Interestingly, in invasive breast tumors the ratio of PR-A to PR-B has been reported to increase [65]. However, studies of gene targeted mice where PR-A or PR-B were independently ablated suggest that both isoforms function in a tissue-specific manner as distinct transcriptional activators [66-68]. In cell types and gene contexts where PR-A does not antagonize PR-B, coactivators may not be limiting [63].

The third PR isoform, PR-C, is an N-terminally truncated form of PR, which lacks the DNA binding domain and two activation (AF) domains near the aminoterminal, but contains the ligand-binding domain [61]. PR-C inhibits PR-B transcriptional activity in transfected cells [69]. Since PR-C cannot bind DNA but binds progesterone [70], PR-C may inhibit PR function by sequestering progesterone and coactivators away from the PR-B-isoform. PR-C also binds to PR-B, thereby reducing the capacity of PR-B to interact with PREs in progesterone-responsive genes [71]. Interestingly, we recently observed that PR-C expression was increased in T47D breast cancer cells as a result of increased NF- κ B activation and binding to the *PR* gene promoter [69].

7. PR as a potential inhibitor of NF- κ B-mediated breast tumorigenesis

In recent studies, we observed that progesterone caused pronounced inhibition of IL-1 β induction of COX-2 mRNA levels in cultured T47D human breast cancer (Fig. 3) and myometrial cells [72]. An antagonistic effect of progesterone on IL-1 β -induced COX-2 expression also was observed in studies of human amnion epithelial cells, LUS fibroblasts [73], and in human fetal lung type II cells [74]. The finding that the inhibitory effects of progesterone in T47D cells were blocked by the PR antagonist, RU486 (Fig. 3), suggests that this effect is mediated by the PR.

The human *COX-2* gene contains two well-characterized NF- κ B response elements in its promoter that bind NF- κ B *in vitro* and *in vivo* [75-77]. In studies using human myometrial cells in culture, we found using chromatin immunoprecipitation (ChIP) that the effects of IL-1 β to stimulate recruitment of NF- κ B p65 to both proximal and distal NF- κ B elements of the *COX-2* promoter were markedly diminished by co-incubation with progesterone [72]. These findings suggest that PR either directly interacts with NF- κ B to block its binding to DNA, or inhibits NF- κ B activation and translocation to the nucleus. A mutual antagonism between the PR and the p65 subunit of NF- κ B has also been reported in COS-1 and HeLa cells, in which activation of NF- κ B by TNF- α repressed PR transcriptional activity, while PR also repressed p65-mediated transcription [78]. Also, in amnion epithelial cells, PR overexpression repressed NF- κ B transcriptional activity, while IL-1 β activated NF- κ B and inhibited PR transcriptional activity [79]. Based on findings that PR and p65 interact *in vitro* [78], it was suggested that mutual repression resulted from formation of an inactive complex of these proteins on the DNA that was incapable of interacting with essential coregulators.

While the mechanisms for such antagonistic effects of PR and NF- κ B on expression of specific genes are incompletely defined, there is abundant evidence to suggest that inflammatory and immunosuppressive actions of glucocorticoids are mediated by glucocorticoid receptor (GR) antagonism of NF- κ B transactivation via several cell type- and promoter-specific mechanisms [80]. These include: (1) direct [81] or indirect (through CREB-binding protein [CBP]) [82] interaction between the N-terminus of p65 and DNA-binding domain of GR; (2) competition

between GR and NF- κ B for limiting amounts of the coactivators, CBP/p300 and steroid receptor coactivator (SRC)-1 [83] and/or; (3) glucocorticoid/GR induction of *I κ B α* gene expression [84]. An action of progesterone to inhibit NF- κ B activation by induction of *I κ B α* expression was previously observed in human myometrial cells [72], macrophage cell lines [85] and in T47D cells [86]. Since PR is structurally very similar to GR, it is likely that similar mechanisms of NF- κ B crossrepression apply to both nuclear receptors.

8. PR acts in a ligand-independent manner to inhibit NF- κ B activation and COX-2 expression in human breast cancer cells

To further define the mechanisms for PR-mediated inhibition of NF- κ B activation and COX-2 expression, T47D cells, which express PR-A, PR-B and PR-C isoforms, were transfected with a siRNA specific for full-length PR (PR-B), with siRNAs for both PR-B and the truncated PR-A, or with an PR-B siRNA mismatch, as control. Cells incubated with the transfection reagent in the absence of siRNA also were studied, as control (Lipid). After PR knockdown was achieved (Fig. 4A), the cells were incubated for 6 h in the absence or presence of IL-1 β , progesterone or the two agents in combination, and COX-2 mRNA levels were analyzed by Q-RT-PCR (Fig. 4B). As observed in studies shown in Figure 3, in control cells transfected with PR-B siRNA mismatch or with transfection reagent alone, IL-1 β stimulated COX-2 expression, while progesterone diminished this induction (Fig. 4B). Reduction of PR-B protein levels using siRNA specific for PR-B mRNA did not alter basal COX-2 mRNA levels or the ability of progesterone to impair IL-1 β -induced increases in COX-2 expression, as compared to cells incubated with transfection reagent alone or transfected with a siRNA mismatch. By contrast, complete ablation of PR-A plus PR-B using siRNA directed against both PR subtypes greatly enhanced both basal and IL-1 β stimulated COX-2 mRNA (Fig. 4B). This also was associated with a pronounced increase in nuclear NF- κ B p65 protein levels (Fig. 4A). Furthermore, in the T47D cells lacking PR-A and PR-B, progesterone was unable to diminish COX-2 expression (Fig. 4B). Moreover, none of the PR siRNA treatments had an effect on basal expression of *I κ B α* protein (Fig. 4A).

These intriguing findings indicate that PR has a dominant ligand-independent action to inhibit NF- κ B activation and COX-2 expression. Whereas, siRNA directed against PR-B alone had no effect on NF- κ B activation or on COX-2 mRNA levels, complete knockdown of PR-A plus PR-B enhanced NF- κ B p65 nuclear translocation and caused a >30-fold increase in COX-2 mRNA levels. These effects were observed in the absence of exogenous progesterone treatment, and prevented progesterone-mediated inhibition of COX-2 expression. Collectively, these findings suggest that PR plays a dominant protective role in human breast cancer by blocking induction of *COX-2*. The effect of PR to inhibit NF- κ B activation likely occurs by direct interaction with p65 [78]. This ligand-independent mechanism may be of great importance in breast tissues of postmenopausal women, where PR may serve a protective role in the presence of negligible circulating levels of progesterone. On the other hand, progesterone clearly is required for PR induction of *I κ B α* expression. Studies are ongoing to determine whether abrogation of PR expression in breast cancer cells results in a coordinate increase in expression of aromatase, *HER-2/neu* and on cellular proliferation.

9. Conclusions

As discussed above, it is well recognized that human breast cancer is associated with an inflammatory response, involving increased macrophage invasion, with resulting enhanced cytokine production, NF- κ B activation and increased expression of *COX-2*. As outlined in Figure 5, we propose that the increased levels of prostaglandins within the tumor and stroma, in turn, upregulates expression of the genes encoding aromatase and *HER-2/neu*. The elevated levels of estrogen formed within the tumor and surrounding stromal cells, acts together with

the increased prostaglandins and HER-2/neu to further enhance the inflammatory response and tumor cell proliferation, leading to tumor progression. We further propose that the progesterone receptor serves a crucial role in blocking breast tumor formation and progression by its action to inhibit NF- κ B activation via ligand-dependent (*i.e.* induction of I κ B α expression) and independent (*i.e.* direct interaction) mechanisms. In this manner, the presence of increased levels of PR in breast epithelial cells inhibits inflammatory response pathways and tumor formation and progression by preventing induction of aromatase and oncogenic tyrosine kinase-linked growth factor receptors. Conversely, a decline in PR expression and/or an impairment of PR function in the breast may facilitate the initiation of tumor growth and progression.

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Reference List

- [1]. Brodie AM, Lu Q, Long BJ, Fulton A, Chen T, Macpherson N, DeJong PC, Blankenstein MA, Nortier JW, Slee PH, van de Ven J, van Gorp JM, Elbers JR, Schipper ME, Blijham GH, Thijssen JH. Aromatase and COX-2 expression in human breast cancers. *J. Steroid Biochem. Mol. Biol* 2001;79:41–47. [PubMed: 11850206]
- [2]. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. *N. Engl. J. Med* 2003;348:2431–2442. [PubMed: 12802030]
- [3]. Chang J, Powles TJ, Allred DC, Ashley SE, Makris A, Gregory RK, Osborne CK, Dowsett M. Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients. *Clin. Cancer Res* 2000;6:616–621. [PubMed: 10690547]
- [4]. Clark GM, McGuire WL, Hubay CA, Pearson OH, Marshall JS. Progesterone receptors as a prognostic factor in Stage II breast cancer. *N. Engl. J. Med* 1983;309:1343–1347. [PubMed: 6633596]
- [5]. Horwitz KB, Koseki Y, McGuire WL. Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. *Endocrinology* 1978;103:1742–1751. [PubMed: 748014]
- [6]. Fournet-Dulguerov N, MacLusky NJ, Leranth CZ, Todd R, Mendelson CR, Simpson ER, Naftolin F. Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. *J. Clin. Endocrinol. Metab* 1987;65:757–764. [PubMed: 3308940]
- [7]. Steinkampf MP, Mendelson CR, Simpson ER. Regulation by follicle-stimulating hormone of the synthesis of aromatase cytochrome P-450 in human granulosa cells. *Mol. Endocrinol* 1987;1:465–471. [PubMed: 3155262]
- [8]. Krasnow JS, Hickey GJ, Richards JS. Regulation of aromatase mRNA and estradiol biosynthesis in rat ovarian granulosa and luteal cells by prolactin. *Mol. Endocrinol* 1990;4:13–12. [PubMed: 1970119]
- [9]. Levallet J, Bilinska B, Mitre H, Genissel C, Fresnel J, Carreau S. Expression and immunolocalization of functional cytochrome P450 aromatase in mature rat testicular cells. *Biol. Reprod* 1998;58:919–926. [PubMed: 9546721]
- [10]. Carreau S. Germ cells: a new source of estrogens in the male gonad. *Mol. Cell Endocrinol* 2001;178:65–72. [PubMed: 11403896]
- [11]. Lanzino M, Catalano S, Genissel C, Ando S, Carreau S, Hamra K, McPhaul MJ. Aromatase messenger RNA is derived from the proximal promoter of the aromatase gene in Leydig, Sertoli, and germ cells of the rat testis. *Biol. Reprod* 2001;64:1439–1443. [PubMed: 11319149]
- [12]. Roselli CE, Abdelgadir SE, Ronnekleiv OK, Klosterman SA. Anatomic distribution and regulation of aromatase gene expression in the rat brain. *Biol. Reprod* 1998;58:79–87. [PubMed: 9472926]
- [13]. Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, Graham-Lorence S, Sun T, Fisher CR, Qin K, Mendelson CR. Aromatase expression in health and disease. *Recent Prog. Horm. Res* 1997;52:185–213. [PubMed: 9238853]

- [14]. Means GD, Mahendroo MS, Corbin CJ, Mathis JM, Powell FE, Mendelson CR, Simpson ER. Structural analysis of the gene encoding human aromatase cytochrome P-450, the enzyme responsible for estrogen biosynthesis. *J. Biol. Chem* 1989;264:19385–19391. [PubMed: 2808431]
- [15]. Kamat A, Hinshelwood MM, Murry BA, Mendelson CR. Mechanisms in tissue-specific regulation of estrogen biosynthesis in humans. *Trends Endocrinol. Metab* 2002;13:122–128. [PubMed: 11893526]
- [16]. Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. *J Biol. Chem* 1993;268:19463–19470. [PubMed: 7690033]
- [17]. Zhou C, Zhou D, Esteban J, Murai J, Siiteri PK, Wilczynski S, Chen S. Aromatase gene expression and its exon I usage in human breast tumors. Detection of aromatase messenger RNA by reverse transcription-polymerase chain reaction. *J. Steroid Biochem. Mol. Biol* 1996;59:163–171. [PubMed: 9010331]
- [18]. Sasano H, Takashashi K, Satoh F, Nagura H, Harada N. Aromatase in the human central nervous system. *Clin. Endocrinol. (Oxf)* 1998;48:325–329. [PubMed: 9578823]
- [19]. Means GD, Kilgore MW, Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific promoters regulate aromatase cytochrome P450 gene expression in human ovary and fetal tissues. *Mol. Endocrinol* 1991;5:2005–2013. [PubMed: 1724289]
- [20]. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE₂ via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology* 1996;137:5739–5742. [PubMed: 8940410]
- [21]. Harada N, Honda S. Molecular analysis of aberrant expression of aromatase in breast cancer tissues. *Breast Cancer Res. Treat* 1998;49(Suppl 1):S15–S21. [PubMed: 9797013]
- [22]. Michael MD, Michael LF, Simpson ER. A CRE-like sequence that binds CREB and contributes to cAMP-dependent regulation of the proximal promoter of the human aromatase P450 (CYP19) gene. *Mol. Cell. Endocrinol* 1997;134:147–156. [PubMed: 9426158]
- [23]. Fitzpatrick SL, Richards JS. Cis-acting elements of the rat aromatase promoter required for cyclic adenosine 3',5'-monophosphate induction in ovarian granulosa cells and constitutive expression in R2C Leydig cells. *Mol. Endocrinol* 1993;7:341–354. [PubMed: 8387157]
- [24]. Fitzpatrick SL, Richards JS. Identification of a cyclic adenosine 3',5'-monophosphate-response element in the rat aromatase promoter that is required for transcriptional activation in rat granulosa cells and R2C Leydig cells. *Mol. Endocrinol* 1994;8:1309–1319. [PubMed: 7854348]
- [25]. Michael MD, Kilgore MW, Morohashi K, Simpson ER. Ad4BP/SF-1 regulates cyclic AMP-induced transcription from the proximal promoter (PII) of the human aromatase P450 (CYP19) gene in the ovary. *J. Biol. Chem* 1995;270:13561–13566. [PubMed: 7768959]
- [26]. Hinshelwood MM, Repa JJ, Shelton JM, Richardson JA, Mangelsdorf DJ, Mendelson CR. Expression of LRH-1 and SF-1 in the mouse ovary: localization in different cell types correlates with differing function. *Mol. Cell. Endocrinol* 2003;207:39–45. [PubMed: 12972182]
- [27]. Falender AE, Lanz R, Malenfant D, Belanger L, Richards JS. Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary. *Endocrinology* 2003;144:3598–3610. [PubMed: 12865342]
- [28]. Liu DL, Liu WZ, Li QL, Wang HM, Qian D, Treuter E, Zhu C. Expression and functional analysis of liver receptor homologue 1 as a potential steroidogenic factor in rat ovary. *Biol. Reprod* 2003;69:508–517. [PubMed: 12672674]
- [29]. Bouchard MF, Taniguchi H, Viger RS. Protein kinase A-dependent synergism between GATA factors and the nuclear receptor, liver receptor homolog-1, regulates human aromatase (CYP19) PII promoter activity in breast cancer cells. *Endocrinology* 2005;146:4905–4916. [PubMed: 16109788]
- [30]. Tremblay JJ, Viger RS. GATA factors differentially activate multiple gonadal promoters through conserved GATA regulatory elements. *Endocrinology* 2001;142:977–986. [PubMed: 11181509]
- [31]. Brueggemeier RW, Richards JA, Petrel TA. Aromatase and cyclooxygenases: enzymes in breast cancer. *J. Steroid Biochem. Mol. Biol* 2003;86:501–507. [PubMed: 14623550]
- [32]. Evans CT, Corbin CJ, Saunders CT, Merrill JC, Simpson ER, Mendelson CR. Regulation of estrogen biosynthesis in human adipose stromal cells. Effects of dibutyryl cyclic AMP, epidermal growth

factor, and phorbol esters on the synthesis of aromatase cytochrome P-450. *J. Biol. Chem* 1987;262:6914–6920. [PubMed: 3032980]

- [33]. Mendelson CR, Corbin CJ, Smith ME, Smith J, Simpson ER. Growth factors suppress and phorbol esters potentiate the action of dibutyladenosine 3',5'-monophosphate to stimulate aromatase activity of human adipose stromal cells. *Endocrinology* 1986;118:968–973. [PubMed: 3004902]
- [34]. Bouchard MF, Taniguchi H, Viger RS. Protein kinase A-dependent synergism between GATA factors and the nuclear receptor, liver receptor homolog-1, regulates human aromatase (CYP19) PII promoter activity in breast cancer cells. *Endocrinology* 2005;146:4905–4916. [PubMed: 16109788]
- [35]. Zhou J, Suzuki T, Kovacic A, Saito R, Miki Y, Ishida T, Moriya T, Simpson ER, Sasano H, Clyne CD. Interactions between prostaglandin E₂, liver receptor homolog-1, and aromatase in breast cancer. *Cancer Res* 2005;65:657–663. [PubMed: 15695411]
- [36]. Botrugno OA, Fayard E, Annicotte JS, Haby C, Brennan T, Wendling O, Tanaka T, Kodama T, Thomas W, Auwerx J, Schoonjans K. Synergy between LHR-1 and β -catenin induces G1 cyclin-mediated cell proliferation. *Mol. Cell* 2004;15:499–509. [PubMed: 15327767]
- [37]. Yang C, Zhou D, Chen S. Modulation of aromatase expression in the breast tissue by ERR α -1 orphan receptor. *Cancer Res* 1998;58:5695–5700. [PubMed: 9865725]
- [38]. Suzuki T, Miki Y, Moriya T, Shimada N, Ishida T, Hirakawa H, Ohuchi N, Sasano H. Estrogen-related receptor α in human breast carcinoma as a potent prognostic factor. *Cancer Res* 2004;64:4670–4676. [PubMed: 15231680]
- [39]. Ariazi EA, Clark GM, Mertz JE. Estrogen-related receptor α and estrogen-related receptor γ associate with unfavorable and favorable biomarkers, respectively, in human breast cancer. *Cancer Res* 2002;62:6510–6518. [PubMed: 12438245]
- [40]. Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res* 2002;62:632–635. [PubMed: 11830510]
- [41]. Benoit V, Relic B, Leval X, Chariot XA, Merville MP, Bours V. Regulation of HER-2 oncogene expression by cyclooxygenase-2 and prostaglandin E₂. *Oncogene* 2004;23:1631–1635. [PubMed: 14985703]
- [42]. Cotterchio M, Kreiger N, Sloan M, Steingart A. Nonsteroidal anti-inflammatory drug use and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev* 2001;10:1213–1217. [PubMed: 11700271]
- [43]. Diaz-Cruz ES, Shapiro CL, Brueggemeier RW. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *J. Clin. Endocrinol. Metab* 2005;90:2563–2570. [PubMed: 15687328]
- [44]. Baldwin ASJ. The NF- κ B and I κ B proteins: new discoveries and insights. *Annu. Rev. Immunol* 1996;14:649–683. [PubMed: 8717528]
- [45]. Cao Y, Karin M. NF- κ B in mammary gland development and breast cancer. *J. Mammary Gland Biol. Neoplasia* 2003;8:215–223. [PubMed: 14635796]
- [46]. Biswas DK, Dai SC, Cruz A, Weiser B, Graner E, Pardee AB. The nuclear factor κ B (NF- κ B): A potential therapeutic target for estrogen receptor negative breast cancers. *Proc. Natl. Acad. Sci. U S A* 2001;98:10386–10391. [PubMed: 11517301]
- [47]. Zhou Y, Eppenberger-Castori S, Marx C, Yau C, Scott GK, Eppenberger U, Benz CC. Activation of nuclear factor- κ B (NF- κ B) identifies a high-risk subset of hormone-dependent breast cancers. *Int. J. Biochem. Cell Biol* 2005;37:1130–1144. [PubMed: 15743683]
- [48]. Demicco EG, Kavanagh KT, Romieu-Mourez R, Wang X, Shin SR, Landesman-Bollag E, Seldin DC, Sonenshein GE. RelB/p52 NF- κ B complexes rescue an early delay in mammary gland development in transgenic mice with targeted superrepressor I κ B- α expression and promote carcinogenesis of the mammary gland. *Mol. Cell. Biol* 2005;25:10136–10147. [PubMed: 16260626]
- [49]. Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ. The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor α to the plasma membrane. *Proc. Natl. Acad. Sci. U S A* 2004;101:2076–2081. [PubMed: 14764897]
- [50]. Wu RC, Qin J, Yi P, Wong J, Tsai SY, Tsai MJ, O'Malley BW. Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic responses to multiple cellular signaling pathways. *Mol. Cell* 2004;15:937–949. [PubMed: 15383283]

- [51]. Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SA, Wong J, Allred DC, Clark GM, Schiff R. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J. Natl. Cancer Inst* 2003;95:353–361. [PubMed: 12618500]
- [52]. Wu RC, Qin J, Hashimoto Y, Wong J, Xu J, Tsai SY, Tsai MJ, O'Malley BW. Regulation of SRC-3 (pCIP/ACTR/AIB-1/RAC-3/TRAM-1) coactivator activity by I κ B kinase. *Mol. Cell. Biol* 2002;22:3549–3561. [PubMed: 11971985]
- [53]. Osborne CK, Yochmowitz MG, Knight WA III, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer* 1980;46:2884–2888. [PubMed: 7448733]
- [54]. Ravdin PM, Green S, Dorr TM, McGuire WL, Fabian C, Pugh RP, Carter RD, Rivkin SE, Borst JR, Belt RJ. Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study. *J. Clin. Oncol* 1992;10:1284–1291. [PubMed: 1634918]
- [55]. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J. Clin. Oncol* 2003;21:1973–1979. [PubMed: 12743151]
- [56]. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177–182. [PubMed: 3798106]
- [57]. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL. HER-2/neu oncogene protein and prognosis in breast cancer. *J. Clin. Oncol* 1989;7:1120–1128. [PubMed: 2569032]
- [58]. Lebeau A, Unholzer A, Amann G, Kronawitter M, Bauerfeind I, Sendelhofert A, Iff A, Lohrs U. EGFR, HER-2/neu, cyclin D1, p21 and p53 in correlation to cell proliferation and steroid hormone receptor status in ductal carcinoma in situ of the breast. *Breast Cancer Res. Treat* 2003;79:187–198. [PubMed: 12825853]
- [59]. Arpino G, Weiss H, Lee AV, Schiff R, De Placido S, Osborne CK, Elledge RM. Estrogen receptor-positive, progesterone receptor-negative breast cancer: Association with growth factor receptor expression and tamoxifen resistance. *J. Natl. Cancer Inst* 2005;97:1254–1261. [PubMed: 16145046]
- [60]. Horwitz KB, Francis MD, Wei LL. Hormone-dependent covalent modification and processing of human progesterone receptors in the nucleus. *DNA* 1985;4:451–460. [PubMed: 4092571]
- [61]. Wei LL, Gonzalez-Aller C, Wood WM, Miller LA, Horwitz KB. 5'-Heterogeneity in human progesterone receptor transcripts predicts a new amino-terminal truncated "C"-receptor and unique A-receptor messages. *Mol. Endocrinol* 1990;4:1833–1840. [PubMed: 2082185]
- [62]. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol. Endocrinol* 1993;7:1244–1255. [PubMed: 8264658]
- [63]. Chalbos D, Galtier F. Differential effect of forms A and B of human progesterone receptor on estradiol-dependent transcription. *J. Biol. Chem* 1994;269:23007–23012. [PubMed: 8083200]
- [64]. Clarke RB, Anderson E, Howell A. Steroid receptors in human breast cancer. *Trends Endocrinol. Metab* 2004;15:316–323. [PubMed: 15350603]
- [65]. Mote PA, Bartow S, Tran N, Clarke CL. Loss of coordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. *Breast Cancer Res. Treat* 2002;72:163–172. [PubMed: 12038707]
- [66]. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr. Shyamala G, Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 1995;9:2266–2278. [PubMed: 7557380]
- [67]. Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science* 2000;289:1751–1754. [PubMed: 10976068]
- [68]. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc. Natl. Acad. Sci. U S A* 2003;100:9744–9749. [PubMed: 12897242]

- [69]. Condon JC, Hardy DB, Kovacic K, Mendelson CR. Upregulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of NF- κ B may contribute to the onset of labor through inhibition of PR function. *Mol. Endocrinol* 2006;20:764–775. [PubMed: 16339279]
- [70]. Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL, Quinn PG. An amino-terminal truncated progesterone receptor isoform, PRc, enhances progestin-induced transcriptional activity. *Mol. Endocrinol* 1996;10:1379–1387. [PubMed: 8923464]
- [71]. Wei LL, Norris BM, Baker CJ. An N-terminally truncated third progesterone receptor protein, PR (C), forms heterodimers with PR(B) but interferes in PR(B)-DNA binding. *J. Steroid Biochem. Mol. Biol* 1997;62:287–297. [PubMed: 9408082]
- [72]. Hardy DB, Janowski BA, Corey DR, Mendelson CR. Progesterone receptor (PR) plays a major anti-inflammatory role in human myometrial cells by antagonism of NF- κ B activation of cyclooxygenase 2 (COX-2) expression. *Mol. Endocrinol.* 2006in press
- [73]. Loudon JA, Elliott CL, Hills F, Bennett PR. Progesterone represses interleukin-8 and cyclooxygenase-2 in human lower segment fibroblast cells and amnion epithelial cells. *Biol. Reprod* 2003;69:331–337. [PubMed: 12672669]
- [74]. Hardy DB, MacDonald E, Smith M, Mendelson CR. Developmental regulation of the eicosanoid pathway mediates the induction of surfactant protein-A (SP-A) expression in the fetal lung. *J. Soc. Gynecol. Invest* 2006;13(Supplement2):242A.
- [75]. Tazawa R, Xu XM, Wu KK, Wang LH. Characterization of the genomic structure, chromosomal location and promoter of human prostaglandin H synthase-2 gene. *Biochem. Biophys. Res. Commun* 1994;203:190–199. [PubMed: 8074655]
- [76]. Newton R, Kuitert LM, Bergmann M, Adcock IM, Barnes PJ. Evidence for involvement of NF- κ B in the transcriptional control of COX-2 gene expression by IL-1 β . *Biochem. Biophys. Res. Commun* 1997;237:28–32. [PubMed: 9266823]
- [77]. Soloff MS, Cook DL Jr, Jeng YJ, Anderson GD. In situ analysis of interleukin-1-induced transcription of COX-2 and IL-8 in cultured human myometrial cells. *Endocrinology* 2004;145:1248–1254. [PubMed: 14645117]
- [78]. Kalkhoven E, Wissink S, van der Saag PT, van der Burg B. Negative interaction between the RelA (p65) subunit of NF- κ B and the progesterone receptor. *J. Biol. Chem* 1996;271:6217–6224. [PubMed: 8626413]
- [79]. Allport VC, Pieber D, Slater DM, Newton R, White JO, Bennett PR. Human labour is associated with nuclear factor- κ B activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Mol. Hum. Reprod* 2001;7:581–586. [PubMed: 11385114]
- [80]. Dumont A, Hehner SP, Schmitz ML, Gustafsson JA, Liden J, Okret S, van der Saag PT, Wissink S, van der Burg B, Herrlich P, Haegeman G, De Bosscher K, Fiers W. Cross-talk between steroids and NF- κ B: what language? *Trends Biochem. Sci* 1998;23:233–235. [PubMed: 9697408]
- [81]. Wissink S, van Heerde EC, Schmitz ML, Kalkhoven E, van der Burg B, Baeuerle PA, van der Saag PT. Distinct domains of the RelA NF- κ B subunit are required for negative cross-talk and direct interaction with the glucocorticoid receptor. *J. Biol. Chem* 1997;272:22278–22284. [PubMed: 9268377]
- [82]. McKay LI, Cidlowski JA. CBP (CREB binding protein) integrates NF- κ B (nuclear factor- κ B) and glucocorticoid receptor physical interactions and antagonism. *Mol. Endocrinol* 2000;14:1222–1234. [PubMed: 10935546]
- [83]. Sheppard KA, Phelps KM, Williams AJ, Thanos D, Glass CK, Rosenfeld MG, Gerritsen ME, Collins T. Nuclear integration of glucocorticoid receptor and nuclear factor- κ B signaling by CREB-binding protein and steroid receptor coactivator-1. *J. Biol. Chem* 1998;273:29291–29294. [PubMed: 9792627]
- [84]. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS. Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* 1995;270:283–286. [PubMed: 7569975]
- [85]. Miller L, Hunt JS. Regulation of TNF- α production in activated mouse macrophages by progesterone. *J. Immunol* 1998;160:5098–5104. [PubMed: 9590261]

- [86]. Deroo BJ, Archer TK. Differential activation of the $\text{I}\kappa\text{B}\alpha$ and mouse mammary tumor virus promoters by progesterone and glucocorticoid receptors. *J. Steroid Biochem. Mol. Biol* 2002;81:309–317. [PubMed: 12361720]

Figure 1.

Structural organization of the human CYP19 (aromatase) gene and tissue-specific expression of CYP19 mRNA transcripts. **Panel A:** Exons II-X, which encode the aromatase protein, and their introns comprise a region of ~34 kb in size. The heme-binding region (HBR) and two polyadenylation signals in the 3'-untranslated region (UTR) are encoded in exon X. The alternative first exons, I.1, I.3/I.4, I.2 and I.2a, encode the 5'-UTRs of the aromatase P450 mRNAs in the placenta, adipose tissue, brain and gonads, respectively. These tissue-specific first exons are alternatively spliced onto a common site (shown by vertical dotted line) just upstream of the ATG codon in exon II. The region containing these alternative first exons

encompasses ~100 kb. Based on the Celera Human Genome database (hcg 39857CYP19GA_x2HTBL5L7WT), the human *CYP19* gene lies on chromosome 15, spanning q14→q15. [15]. **Panel B:** Q-RT-PCR using primers specific for the 5'-UTRs of *CYP19* transcripts in ovary (*Exon IIa*), adipose (*Exon I.4*), placenta (*Exon I.1*), and breast cancer (*Exon I.3*) were used to amplify *CYP19* mRNA transcripts containing these alternative first exons in human adipose tissue, ovary, placental syncytiotrophoblast and T47D breast cancer cells. These hybridize with equivalent efficiency; thus, the relative levels of *CYP19* expression in each of these tissues can be compared.

Figure 2.

Confluent monolayers of human adipose stromal cells were cultured for 24 h in the absence (control) or presence of forskolin (25 μ M) (cAMP), PGE₂ or PGF₂α (1 μ M each) and aromatase activity was assayed by the incorporation of tritium from [³H]androstenedione into water.

Modified from Y. Zhao, V.R. Agarwal, C.R. Mendelson, E.R. Simpson. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE₂ via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene, *Endocrinology* 137 (1996) 5739-5742 [20], with permission. Copyright 2006, *The Endocrine Society*.

Figure 3.

RU486 Blocks the Progesterone-induced Suppression of COX-2 mRNA in T47D Cells. T47D cells were incubated for 6 h the absence or presence of RU486 (10^{-7} M) with and without IL-1 β (10 ng/ml), progesterone (10^{-7} M), or both. RNA was isolated and the expression of COX-2 was analyzed by Q-RT-PCR. The data are expressed as fold increase over vehicle control cells. The bars represent the mean $\pm$ S.E.M. of values from three independent experiments. From D.B. Hardy, B.A. Janowski, D.R. Corey, C.R. Mendelson. Progesterone receptor (PR) plays a major anti-inflammatory role in human myometrial cells by antagonism of NF- κ B activation of cyclooxygenase 2 (COX-2) expression. Mol. Endocrinol. (2006), in press [72], with permission. Copyright 2006, *The Endocrine Society*.

Figure 4.

Knockdown of PR-A and PR-B markedly enhances expression of COX-2 mRNA in T47D cells. T47D cells were transfected with siRNA targeted against PR-B alone or with siRNAs against both PR-B and PR-A for 5 days. Cells were cultured for 6 h in the absence or presence of IL-1 β (10 ng/ml) with or without progesterone (10⁻⁷ M). Cell lysate proteins were analyzed for levels of PR, NF- κ B p65, I κ B α , and β -actin by immunoblotting (**Panel A**). RNA was isolated and the expression of COX-2 (**Panel B**) mRNA transcripts were analyzed by Q-RT-PCR. Data are the mean $\pm$ SEM of values from four independent experiments and are expressed as arbitrary units. From D.B. Hardy, B.A. Janowski, D.R. Corey, C.R. Mendelson. Progesterone receptor (PR) plays a major anti-inflammatory role in human myometrial cells by antagonism of NF- κ B activation of cyclooxygenase 2 (COX-2) expression. *Mol. Endocrinol.* (2006) in press [72], with permission. Copyright 2006, *The Endocrine Society*.

Figure 5. *Progesterone/PR serves a protective role in the breast by inhibiting NF- κ B activation of inflammatory response pathways.* Human breast cancer is associated with an inflammatory response, with increased macrophage invasion, enhanced cytokine production, NF- κ B activation and increased expression of *COX-2*. The increased production of prostaglandins, in turn, upregulate expression of the genes encoding aromatase and HER-2/neu. The elevated levels of estrogen formed within the tumor and surrounding stromal cells, act together with the increased prostaglandins and HER-2/neu to further enhance the inflammatory response and tumor cell proliferation, leading to tumor progression. On the other hand, we also postulate that the progesterone receptor serves a crucial role in blocking breast tumor formation and progression by its action to inhibit NF- κ B activation.