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The activities of progesterone receptor isoform A and B are differentially modulated by their ligands in a gene-selective manner

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It is known that progesterone receptor (PR) isoform A (PR-A) and isoform B (PR-B) may mediate different effects of progesterone. The objective of this study was to determine if the functions of PR isoforms also vary in response to different PR modulators (PRM). The effects of 7 synthetic PRM were tested in MDA-MB-231 cells engineered to express PR-A, PR-B, or both PR isoforms. The effects of progesterone were similar in cells expressing PR-A or PR-B in which it inhibited growth and induced focal adhesion. On the other hand, synthetic PRM modulated the activity of the PR isoforms differently. RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914 exerted agonist activities on cell growth and adhesion *via* PR-B. *Via* PR-A, however, these compounds displayed agonist effect on cell growth but induced stellate morphology which was distinct from the agonist's effect. Their dual properties *via* PR-A were also displayed at the gene expression level: the compounds acted as agonists on cell cycle genes but exhibited antagonistic effect on cell adhesion genes. Introduction of ERα by adenoviral vector to these cells did not change PR-A or PR-B mediated effect of PRM radically, but it causes significant cell rounding and modified the magnitudes of the responses to PRM. The findings suggest that the activities of PR isoforms may be modulated by different PRM through gene-specific regulatory mechanisms. This raises an interesting possibility that PRM may be designed to be PR isoform and cellular pathway selective to achieve targeted therapy in breast cancer.

Key words: selective progesterone receptor isoforms modulator; progesterone receptor isoform-A and -B

Progesterone is essential for reproductive functions including the development of mammary gland, and it is also implicated in the development of breast cancer.[1,2] The exact function of progesterone on the growth of breast cancer cells; however, has been found to vary depending on experimental models and the test conditions. For example, clinical treatment of patients with high dose of progesterone before breast surgery showed fewer mitotic figures compared with treatment with estrogen alone or estrogen plus progesterone.[3] On the other hand, increase in DNA synthesis is seen in the late luteal phase of the menstrual cycle when both estrogen and progesterone levels are high.[4,5] Similarly, *in vitro* studies have produced conflicting results. Progesterone and progestins have been shown to promote or inhibit cell proliferation.[6–12] These paradoxical findings affect clinical decisions as to whether progestins or antiprogestins would be more appropriate as endocrine therapies for PR-positive breast cancer. While progestin megestrol acetate has been used as second-line endocrine therapy for advanced breast cancer,[13] many studies have been conducted to test the suitability of antiprogestins for breast cancer treatment and some generated positive results.[14–16]

The conflicting effects of progesterone on breast cancer cells in various studies reflect the complexities in the mechanism of the action of progesterone. The effects of progesterone are mediated by progesterone receptors (PR) which exert their effects mainly by regulating the expression of specific target genes. The expression of PR is estrogen-dependent in its target tissues.[17,18] Therefore, the action of progesterone requires the priming treatment of estrogen to induce PR. It is conceivable that the prior treatment of estrogen may overshadow the detection of progesterone's effects. Studies have also indicated that the cross-talk between ER and PR could alter the cellular response to progesterone. For example, transfected PR-B can physically interact with ERα, which then transmit signals received from the agonist-activated PR to the Src/p21(ras)/Erk pathway.[19] Thus, estrogen, ER and its associated signaling pathways may modify PR-mediated effect of progesterone.

Another factor that may contribute to variations in PR-mediated response of progesterone is the presence of two PR isoforms, progesterone receptor-A (PR-A) and -B (PR-B). PR-A lacks the first 164 amino acids at the N-terminus of PR-B.[20] PR-A and PR-B play different roles in modulating cellular response to progesterone treatment by regulating the gene expression differentially.[21,22] It was found in breast cancer cells T47D with PR-A predominance resulted in the acquisition of progestin responsiveness of a subgroup of specific gene targets in signaling pathways that influence cell shape.[23] Variations in PR-A:PR-B ratio in breast tumor also appear to affect clinical outcome. Breast tumor with high PR-A to PR-B ratio is linked to a more aggressive disease and poorer disease-free survival rates.[24,25] Since PR isoforms can modulate gene expression and cell activity differentially in response to PR ligands, they are potential targets for drug intervention.

Previous studies of the differential effect of PR isoforms were mainly conducted using progesterone or R5020 which are pure agonists. PR modulators (PRM) are PR ligands that exhibit agonist, antagonist, or mixed agonist/antagonist effects in progesterone target tissues in cell- and tissues-specific dependent manner.[26] There is evidence that they may regulate gene activity in a promoter-selective manner.[27] The objective of this study was to determine if PR isoforms exhibit different activities in response to different PRM so that we may explore the therapeutic potentials of PRM. This issue was addressed using ER- and PR-negative MDA-MB-231 breast cancer cells that were engineered to express PR-A, PR-B, or both PR isoforms. Seven PRM (CP8668, AED, Dex-Mes, RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914) were tested for their specific cellular effects on cell growth, cell morphology and cell adhesion and on gene expression. Interestingly, the effects of these PRM are cellular activity-selective. In mediating cell cycle progression, all PRM demonstrated agonist activity by inhibiting cell growth through PR-A, PR-B, or both PR isoforms. On the other hand, these compounds induced morphological change, cell adhesion characteristics and cell migration potential that are distinct between cells expressing PR-A and cells expressing PR-B. The findings suggest that the activities of PR isoforms may also be modulated differentially by PR ligands that act on specific aspects of transcriptional activities.

Material and methods

Chemicals and reagents

Progesterone was obtained from Sigma Chemical Co (St. Louse, MO). CP8668 was from Meiji Seika Kaisha (Japan) and

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Dex-Mes and AED were from Steraloids (Newport, RI). RU486 (Mifepristone) was from Siniwest Holdings (San Diego, CA). CDB4124 and 17α-hydroxy CDB-4124 were from Zonegen (Woodlands, TX) and VA2914 was from HRA Pharma (Paris, France). PR antibody was from Neomarkers (Fermont, CA). p21 and Cyclin B antibody were from Transduction Laboratories (San Jose, CA), Cyclin A was from BD Pharmigen (San Jose, CA) and GAPDH was from Ambion (Austin, TX). Real-time PCR reagents and consumables were obtained from Applied Biosystems (Foster City, CA). Tissue culture plastics and reagents were obtained from either Corning (Corning, NY) or Invitrogen (Carlsbad, CA), respectively.

Cell culture

ERα and PR-negative breast cancer cell line MDA-MB-231 were obtained from American Tissue Culture Collection (ATCC) in 1995 at passages 28. MDA-MB-231 cells were cloned using a 96-well plate by single-cell dilution, and clone 2 (MDA-MB-231-CL2) was selected for the transfection studies. All cells were routinely maintained in phenol-red containing Dulbecco's Modified Eagle Medium (DMEM), supplemented with 7.5% fetal calf serum (FCS), 2 mM glutamine and 40 mg/l gentamycin. For all experiments, cells were grown in phenol-red free DMEM supplemented with 5% dextran charcoal-treated fetal calf serum (DCC-FCS) to remove the endogenous steroid hormones that might interfere and complicate the effects of progesterone and progesterone receptor modulators. Cells were treated with 0.1 μM (unless otherwise indicated) test compounds in ethanol. Controls received 0.1% ethanol only.

Transfection of PR into MDA-MB-231 cells

PR expression vectors hPR1 and hPR2 were generous gifts from Professor P. Chambon of IGBMC, France. Vectors hPR1 and hPR2 contain human cDNA coding for isoform B (PR-B) and isoform A (PR-A), respectively, in pSG5 plasmid.[20] hPR1 and/or hPR2 was cotransfected with vector pBK-CMV (Stratagene, La Jolla, CA) containing the neomycin-resistant gene into MDA-MB-231-CL2 cells using Lipofectin reagent (Invitrogen, Carlsbad, CA). Clones were selected in medium containing G418 (500 μg/ml) and these neomycin-resistant clones were further screened for vector pSG5 sequence by polymerase chain reaction using primers flanking the regions of nucleotide 182–405 bp which have little sequence homology to the vector pBK-CMV. The PCR product of expected size was further confirmed by digestion with restriction enzyme NcoI. Three clones of PR-A-(AC2, AC10 and AC35) and 3 clones of PR-B-(BC20, BC38 and BC54) transfected cells were selected for the study of PRM in this report and the PR expression of these clones are shown in Figure 1. ABC28 cells were transfected with both PR-A and PR-B as reported previously.[28] Cells stably transfected with both pBK-CMV and pSG5 plasmid are referred to as CTC15 which was used as transfection control in this study.

Preparation of adenoviruses

The adenovirus carrying ERα was constructed by Transpose-Ad™ system (Qbiogen, Carlsbad, CA). The ERα gene coding sequence from vector HEGO[29] was cloned to shuttle vector pCR259. The recombinant adenoviral plasmids were generated by Tn7 mediated homologous recombination between the Ad5 backbone plasmid and pCR259/ERα. The Pac I linearized recombinant adenoviral plasmids were then transfected into HEK-293A cells by Lipofectamine (Invitrogen) according to manufacturer's instructions. Cells were overlaid with 1.25% agarose and the plaques were harvested 2–3 weeks after the transfection. The control virus (Ad/Φ) was constructed similarly by homologous recombination using empty pCR259 shuttle vector and the Ad5 backbone plasmid. The viruses were propagated in HEK-293A cells and purified with CsCl gradient ultracentrifuge. Titrations of the viruses were determined by $TCID_{50}$ (tissue culture infectious doses) method.

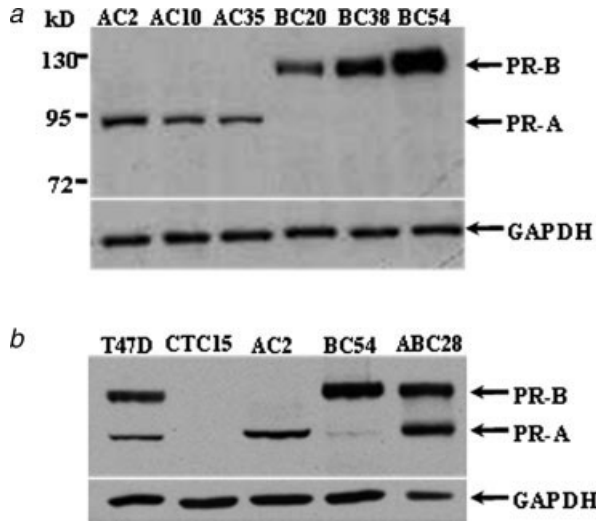

FIGURE 1 – Western blotting analysis of total PR protein. (*a*) Expression of PR isoforms in either PR-A- or PR-B-transfected clones. Lanes AC2, AC10 and AC35 are PR-A-transfected clones while lanes BC20, BC38 and BC54 are PR-B-transfected clones. Molecular weight markers are indicated on the left. The specific bands corresponding to A isoform (M_r 81,000) and B isoform (M_r 116,000) of PR are indicated by arrows. (*b*) Expression of PR isoforms in cells expressing either PR-A (AC2) or PR-B (BC54) or both PR isoforms (ABC28). Vector transfected control CTC15 cells showed no PR expression. For comparison of PR levels, lysate from T47D cells containing the same amount of protein is analyzed. GAPDH was used as a loading control.

Infection of adenoviruses

Cells were infected with desired MOI (multiplicity of infection) of adenoviruses in phenol-red free DMEM supplemented with 5% DCC-FCS. The infection volume for 60 mm Petri dish was 1 ml; the volume for 6-well plate was 0.5 ml/well and for 12-well plate was 0.25 ml/well. Cells with infection media were incubated in a sealed humid box filled with ~5% CO_2 on a rocker shaker at 37°C for 16 hr[30] before the infection media were replaced with fresh media.

Western blotting analysis

Cells (5×10^5) were grown on 60 mm petri dishes in phenol red-free DMEM for 48 hr before being treated (where indicated) with 0.1 μM test compounds in 0.1% ethanol or with 0.1% ethanol only. At designated time point, the cells were lysed with cold lysis buffer (50 mM Hepes, 150 mM NaCl, 1% Triton X-100, 5 μg/ml pepstatin A, 5 μg/ml leupeptin, 2 μg/ml aprotinin, 1mM PMSF, 100 mM sodium fluoride and 1 mM sodium vanadate, pH 7.5). Protein supernatants were collected by centrifugation at 13,000 rpm for 20 min. Quantitation of total protein in cell lysate was determined using BCA Protein Assay Kit from Pierce (Rockford, IL) according to the manufacturer's protocol. Cytosols containing 20 μg of protein were separated in 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membrane (Amersham, Buckinghamshire, UK). The membrane was probed with antibodies against each of the proteins of interest and detected using ECL or ECL Plus™ (Amersham) detection reagents.

Light microscopy

Cells were grown in 6-well plates in phenol red-free DMEM for 48 hr before being treated with 0.1 μM (unless otherwise indicated) test compounds in 0.1% ethanol or with 0.1% ethanol only. After 48 hr, the cells were viewed and photographed either under a Nikon Eclipse TE2000-U phase or Olympus IX71 phase contrast microscope using a 20× objective.

TABLE I – GENE-SPECIFIC PRIMER SEQUENCES THAT WERE USED IN THIS STUDY FOR QUANTITATIVE REAL-TIME PCR

Gene	Forward primer	Reversed primer
36B4	5′-gattggctacccaactgttgca	5′-caggggcagcagccacaaaggc
MMP1	5′-caggttatcccaaaatgatagc	5′-ctgcagttgaaccagctattag
MMP13	5′-cctggagcactcatgtttcctatc	5′-gcaggcgccagaagaatctgtc
UPA	5′-tagccaatgtgggagcagcggttgg	5′-ataagtacattcccaggcactgtcacgt
ADAM8	5′-cggaaccgctgctgcaactctacc	5′-aggcgtcttccgggcactcagg
PPL	5′-actgaaggacaagccaaccaca	5′-acattctctgtaggtgccggga
PKP2	5′-agctgcttccgtccttctgtattc	5′-tgaggtttcttgggctgggtagta
p21	5′-gacaccactggagggtgact	5′-caggtccacatggtcttcct
CCNA2	5′-ttattgctggagctgccttt	5′-ctctggtgggttgaggagag
CCNB1	5′-cacttccttcggagagcatc	5′-caggtgctgcataactggaa
CCNE2	5′-tgttggccacctgtattatctgg	5′-gtcatgaacatatctgctctcc

Immunofluorescence microscopy

Cells were grown on glass coverslips in 6-well plates with phenol red-free DMEM for 48 hr before being treated with 0.1 μM test compounds for 48 hr. Cells were rinsed with phosphate buffered saline (PBS), fixed in 4% formaldehyde for 10 min and permeabilized with 0.2% Triton X-100 for 10 min at room temperature. This was followed by incubation with 2% fetal calf serum in PBS for 1 hr to block nonspecific binding. Subsequent incubations with antibody were carried out in PBS containing 2% fetal calf serum. For costaining of F-Actin and paxillin, antibody to paxillin (BD Biosciences, Franklin Lakes, NJ) was incubated with the cells for 1 hr at 37°C, followed by incubation with Cy5-conjugated sheep anti-mouse antibody (Amersham Pharmacia Biotech, Little Chalfont Bucks, England) and 10 μg/ml FITC-phalloidin in PBS for 1 hr at 37°C. After washing in PBS, the coverslips were mounted on slides with fluorescence mounting media from DAKO (Carpinteria, CA). Stained cells were viewed and photographed using the Zeiss confocal laser scanning microscope model LSM 510.

Wound healing assay

Cells (5×10^5) were plated in 60-mm dishes in phenol red-free DMEM for 48 hr before being treated with 0.1 μM test compounds or control vehicle. After 20 hr of treatment, the monolayer cultures were scratched by scraping with the back of a 200 μl tip across the plate in 4 parallel lines. Cells were then rinsed twice with DPBS and cultured in fresh medium containing test compounds. After 20 hr, each plate was photographed under an Olympus TX71 phase contrast microscope. The cell migration was evaluated for the amount of wound closure by measuring the width of the remaining wound. For each wound, 10 fields were photographed and measured.

Matrigel invasion assay

Cells were grown in 60-mm dishes in phenol red-free DMEM for 48 hr. The cells were then washed and trypsinized. Cells were plated at 3.5×10^4 cells per well on a transwell insert (Corning) with 8 μm pore polycarbonate membrane in media with or without test compound. The membrane in the insert had been precoated with 35 μl Matrigel (BD Biosciences) at dilution of 1:6 (1.5 mg/ml). After incubation for 48 hr in a CO_2 incubator at 37°C, the cells that had migrated through the membrane were trypsinized and counted. Each treatment was performed in triplicates and the experiment was repeated twice.

Statistical analysis

Statistical difference among treatments in cell cycle analysis, wound healing assay and Matrigel invasion assay were analyzed by ANOVA with the Least Significance Test (LSD) as post-hoc test using SPSS (Version 13.0). Statistical difference among treatments between ER-adenovirus- and control virus-infected cells in cell cycle analysis was analyzed by paired *T* test.

Analysis of cell cycle distribution

Cells (1×10^5) were grown in 12-well plates in phenol red-free DMEM. Two days later, the medium was replaced with fresh medium containing 0.1 μM (unless otherwise indicated) test compounds. After 24 hr, the cells were then harvested and stained with propidium iodide (PI) in Vindelov's cocktail (10 mM Tris HCl, pH 8, 10 mM NaCl, 50 mg/l PI and 10 mg/l Ribonuclease A and 0.1% Nonidet P-40)[31] for 1 hr at 4°C in the dark. The stained cells were analyzed in FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) with excitation wavelength of 488 nm. The resulting histograms were analyzed by program MODFIT V3.01 (Becton Dickinson) for cell cycle distribution. The average coefficient of variation (CV) of the analysis is within 7%.

Gene expression by quantitative real-time PCR

Total RNA was extracted after 24 hr of treatment with control vehicle or test compounds using TRIzol reagent according to the manufacturer's protocol (Invitrogen Life Technologies, Carlsbad, CA). cDNA were synthesized from 5 μg total RNA using random primer and SuperScript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA). Gene specific primers were designed using the Primer3 software from Whitehead Institute for Biomedical Research[32] (Table I). Real-time PCR was performed using SYBR Green PCR reagents on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The lengths of PCR products range from 100 to 350 bp. PCR for each gene fragment was performed in triplicates and each primer set was repeated 2–3 times. To ensure PCR product specificity, melting curves were generated after amplification followed by agrose gel electrophoresis. The change in fluorescence of the SYBR Green dye in each cycle were monitored by ABI 7000 system and the threshold cycle (Ct), which is defined as the cycle number at which the amount of amplified target reaches a fixed threshold, was obtained for each gene. The relative amount of PCR products generated from each primer set was determined on the basis of the Ct value. Primer sets for the 36B4 gene, which codes for human acidic ribosomal phosphoprotein PO, were included in each experiment as controls for normalizing the quantity of the cDNA used. The expression difference for each gene between control and PRM-treated samples was calculated by normalizing them with 36B4 gene expression according to the formula:

$$\text{Fold change} = 2^{[[\text{Ct (control) Gene X}-\text{Ct (control) 36B4}]-[\text{Ct (PRM) Gene X}-\text{Ct (PRM) 36B4}]]}$$

RESULTS

Characterization of MDA-MB-231 cells transfected with either PR-A or PR-B

ERα- and PR-negative breast cancer cells MDA-MB-231-CL2 were transfected with PR expression vectors hPR1 or hPR2 coding for human PR-B or PR-A, respectively. Three clones were isolated,

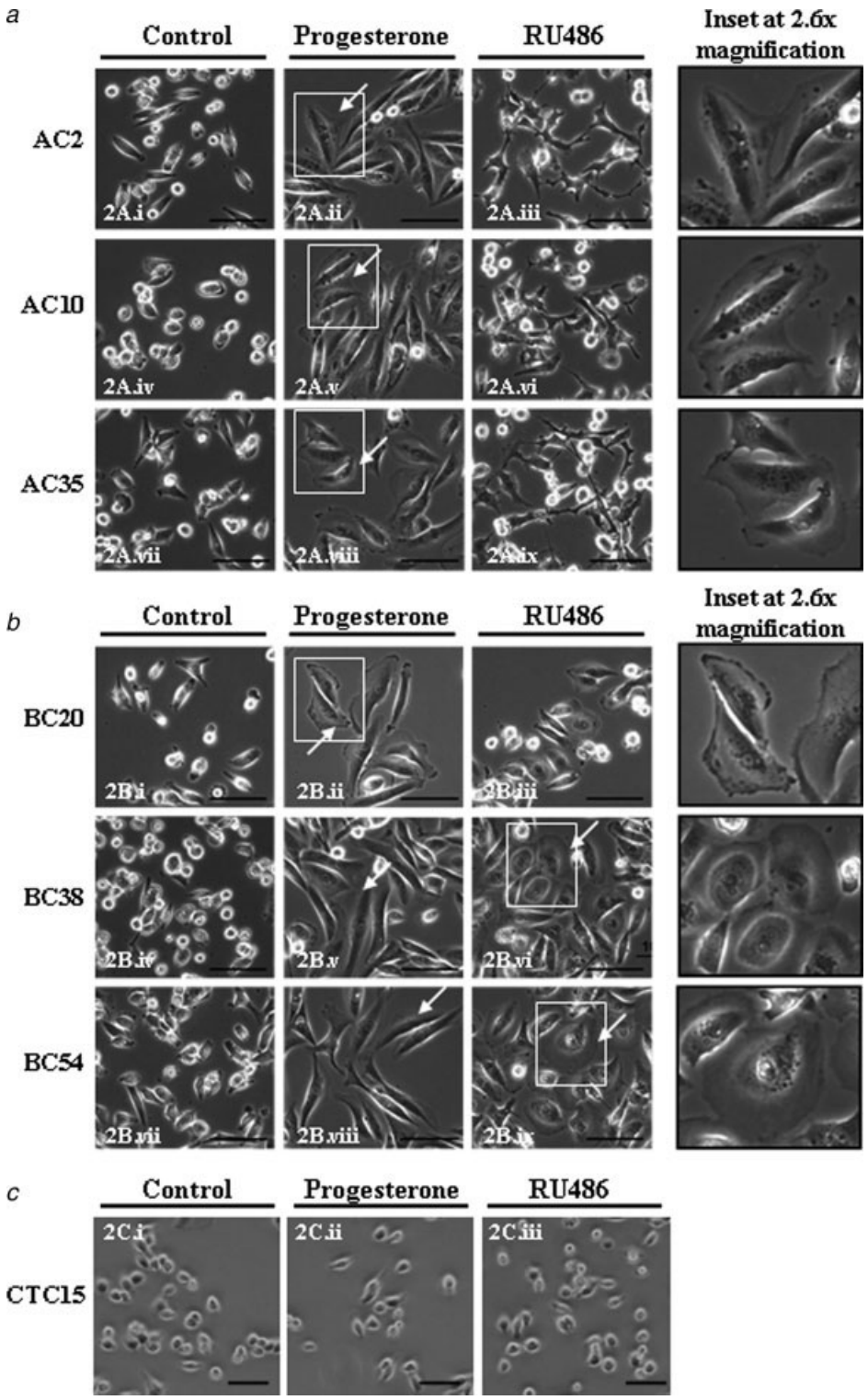

FIGURE 2 – Morphological changes induced by progesterone and RU486 in cell transfected with either PR-A (*a*) or PR-B (*b*) after 48 hr treatment. AC2, AC10 and AC35 were PR-A-transfected cells and BC20, BC38 and BC54 are PR-B-transfected cells. Arrows indicates membrane ruffling and lamellipodia. (*c*) Vector transfected control CTC15 cells did not display notable morphological change after 48 hr treatment with progesterone and RU486. The images were taken under phase contrast microscope using a ×20 objective. Scale bar = 100 μm.

from each PR isoform. The expression levels of PR in cells expressing either PR-A (AC2, AC10 and AC35) or PR-B (BC20, BC38 and BC54) were determined by western blotting analysis (Fig. 1*a*). Characterization of cells expressing both PR isoforms (ABC28) was described previously.[28] The expression level of PR in ABC28 cells, AC2 cells and BC54 cells was about twice as much as that in breast cancer cells T47D which express PR independent of estrogen. The expression of PR was not detected in vector transfected control, CTC15 cells (Fig. 1*b*).

In the absence of treatment, PR-A- or PR-B-transfected cells had similar morphology to vector-transfected control (Fig. 2*ci*) and to the parental cells. Moreover, in the absent of treatment, we did not observe any significant ($p > 0.05$) difference in the growth characteristics between PR-transfected

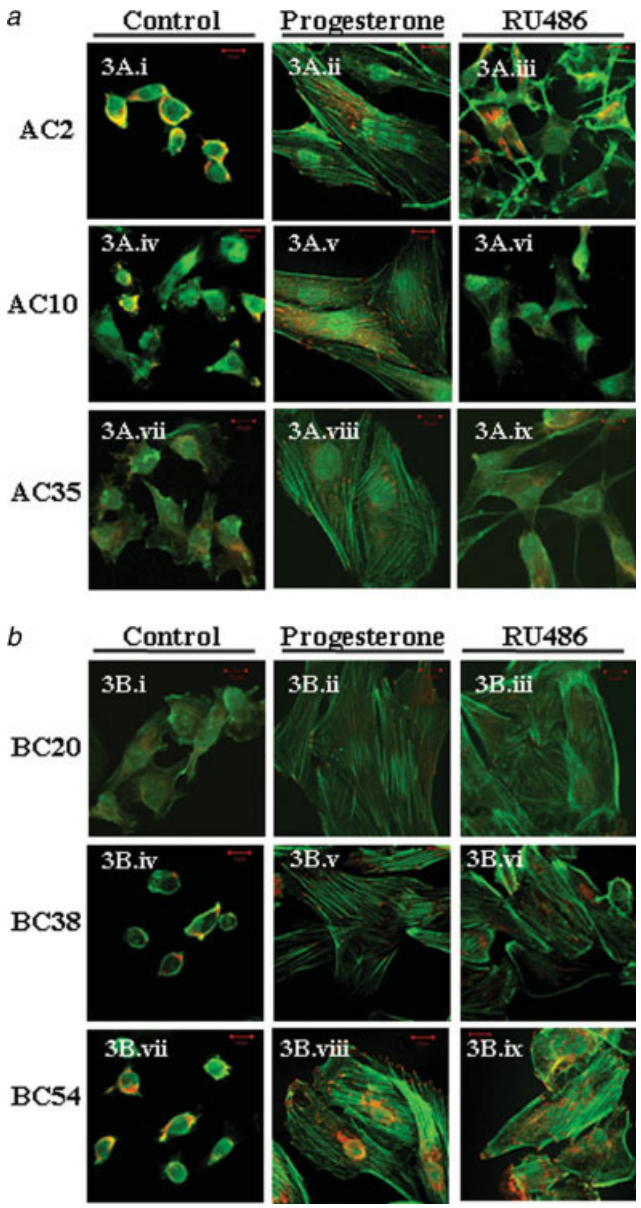

Figure 3 – Effects of progesterone and RU486 on the development of stress fibers and focal adhesion in cells expressing either PR-A (*a*) or PR-B (*b*) represented by cell clone AC2, AC10, AC35 or BC20, BC38, BC54, respectively. Cells were costained with fluorescently labeled phalloidin (green) and antibody against paxillin (red). Scale bar = 10 μm.

cells, vector-transfected cells CTC15 and parental cells MDA-MB-231.

Effect of progesterone and RU486 on cell morphology and the formation of focal adhesions in cells transfected with either PR-A or PR-B

To identify the effects of PRM in either PR-A- or PR-B-transfected MDA-MB-231 breast cancer cells, we first studied the effects of progesterone and putative antiprogestin RU486 in these cells. In all 3 clones expressing either PR-A or PR-B, treatment with progesterone caused changes in cell morphology which was characterized by notable cell spreading and larger cell surface area than vehicle-treated control (Figs. 2*a* and 2*b*, ii, v and viii). Progesterone-induced cell spreading was accompanied by the formation of membrane ruffles and lamellipodia as indicated by further magnified insets. This change in cell morphology was associated with the formation of well-developed stress fibers system with focal adhesion protein paxillin colocalized at the ends of the stress fibers (Figs. 3*a* and 3*b*, ii, v and viii). Although there seems to be some quantitative differences in the formation of the stress fibers and focal adhesions within the respective clones from each PR isoform, the difference is not obvious in the general cell population.

On the other hand, the effect of RU486 on the morphology of cells expressing PR-A is different from that in cells expressing PR-B. In all 3 clones expressing PR-A, RU486-treated cells appeared stellate, with multiprotrusions and long cytoplasmic extensions (Fig. 2*a*iii, vi and ix). These cells had little stress fibers and focal adhesions (Fig. 3*a*iii, vi and ix). In contrast, RU486-treated PR-B expressing cells showed spreading similar to that progesterone-treated cells except that RU486-treated cells were generally rounder (Fig. 2*b*iii, vi and ix). Both progesterone and RU486 induced an extensive formation of stress fibers and focal adhesions in cells expressing PR-B (Fig. 3*b*iii, vi and ix).

Although the images were taken after 48 hr treatment, the morphological changes were noticeable after 8 hr treatment (data not shown). Since the effect of progesterone and RU486 is mediated *via* PR which modulates cell function through regulating gene expression, it is not surprising that the morphological changes were visible only after 8 hr of treatment. This is in contrast to the much more rapid formation of focal adhesions that occurs within an hour due to the activation of integrin or by the addition of ECM proteins which does not involve the mechanism of gene regulation.

Progesterone and RU486 inhibits cell cycle progression in cells transfected with either PR-A or PR-B

Progesterone and RU486 had no significant growth effect on cells transfected with control empty vector, CTC15 cells and the parental cell line MDA-MB-231-CL2 as was reported previously.[28,33] In this study, DNA flow cytometry was performed to determine the effects of progesterone and RU486 on cell cycle progression in cells transfected with either PR-A or PR-B.

Progesterone exhibited significant inhibitory effect on DNA synthesis in all clones transfected with either PR-A or PR-B ($p < 0.05$) (Fig. 4). Similarly, RU486 also demonstrated agonist activity by inhibiting cell growth in cells transfected with either PR isoform (Fig. 4). The growth-inhibitory effect of both progesterone and RU486 on cells transfected with either PR isoform was associated with dose-dependent reduction of percentage of cells in S-phase and was accompanied by accumulation of cells in G_0–G_1 phase (data not shown). Once again, the inhibitory effect of progesterone and RU486 on cell growth was observed in all 3 clones expressing PR-A (AC2, AC10 and AC35) and in all 3 clones expressing PR-B (BC20, BC38 and BC54). The magnitudes of the growth inhibition by progesterone and RU486 were in general positively related to the receptor level. In addition, the growth inhibitory effect of RU486 on clones expressing PR-A was weaker than its effect on clones expressing PR-B, and than the effect of progesterone. Lower levels of PR-A in these clones, particularly in AC10 and AC35 may be 1 of the reasons for the weaker effect of RU486.

Effect of progesterone receptor modulators on cell morphology in cells transfected with PR-A and/or PR-B

In addition to RU486, 6 other PRM were studied for their effects on cells transfected with PR-A and/or PR-B. The reported activities of each PRM are summarized in Table II. The evaluation of the activities of these PR ligands was determined both through *in vivo* and *in vitro* assays.[34–39]

So far, we demonstrated that the response to progesterone and RU486 were similar within the same PR isoform. Therefore, to study the effect of PRM on PR isoform, we selected cell clone AC2 and BC54 to represent the -A or -B isoform, respectively.

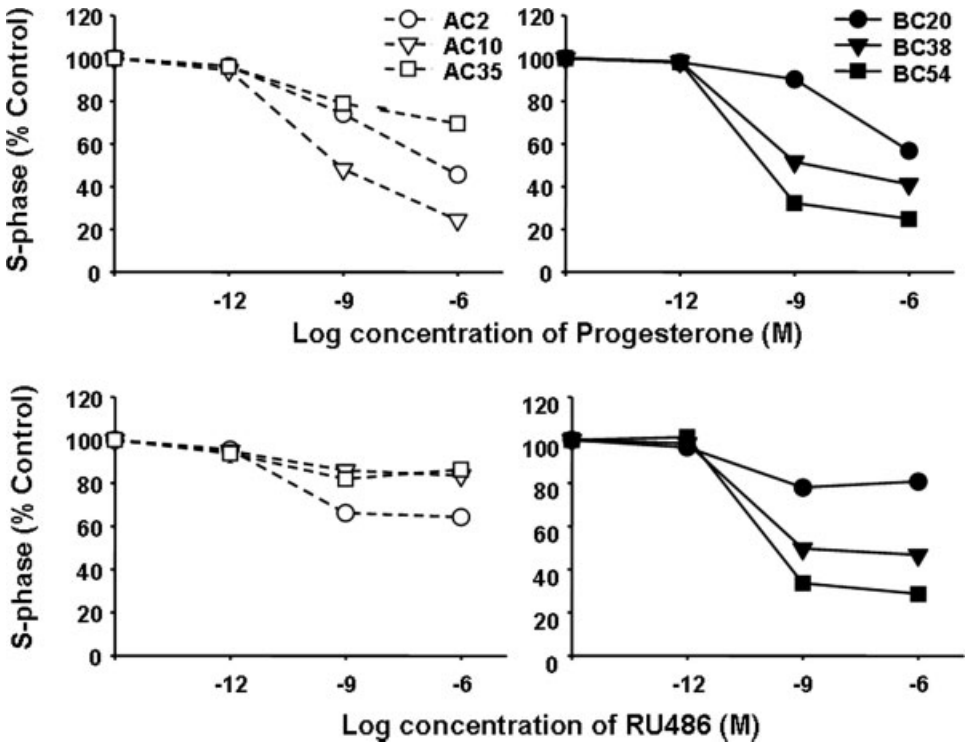

FIGURE 4 – Effects of various concentrations of progesterone and RU486 on the percentage of S-phase fraction in cells transfected with either PR-A (AC2, AC10 and AC35) or PR-B (BC20, BC38 and BC54). Cells were treated for 24 hr and subsequently stained with PI in Vindelov's cocktail. Results are expressed as mean ± SE, $n = 3$. Standard error that is not visible is within the symbol area.

TABLE II – SUMMARY OF PRM THAT WERE USED IN THIS STUDY AND THEIR REPORTED ACTIVITIES

Product name	Chemical name	Reported PR activity
CP8668	(4αR,5R,6R,7R)-7-methoxy-6-(N-propylaminocarbonyl) oxy-4α,5,6,7-tetrahydro-1,3,4a,5-tetramethylbenz[f] indol-2(4H)-one	Progestins, Antiprogestins (Ref. 34)
Dex-Mes	1,4-pregnadien-9α-fluoro-16α-methyl-11β,17,21-triol-3,20-dione21-methanesulphonate (Dexamethasone 21-mesylate)	Antiprogestins, Progestins (Ref. 35)
AED	5-androsten-17α-ethynyl-3β,17β-diol (Ethynlandrostendiol pregnenindiol)	No report
CDB4124	17α-acetoxy-21-methoxy-11β-[4-*N*,*N*-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione	Antiprogestins (Refs. 37, 38)
17α-OH CDB-4124 /CDB-4644	17α-hydroxy CDB-4124	Antiprogestins (Ref. 38)
VA2914/CDB2914	17α-acetoxy-11β-[4-*N*,*N*-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione	Antiprogestins (Refs. 36, 37, 39)

We observed no morphological change in vector transfected control cells CTC15 after 48 hr of treatment with all PRM (Image not shown).

In AC2 cells, CP8668, AED and Dex-Mes treatment led to cell spreading, but to a lesser extent than with progesterone (Fig. 5*a*iii–v). In contrast, CDB4124, 17α-hydroxy CDB4124 and VA2914 induced stellate morphology which is similar to that of RU486-treated cells (Fig. 5*a*vii–ix).

Earlier, we showed that in cells expressing PR-B, treatment with progesterone and RU486 led to cell spreading. In BC54 cells, treatment with all PRM caused cell spreading (Fig. 5*b*), but we noticed some difference in the shape of the cells. BC54 cells treated with CP8668, AED and Dex-Mes were elongated in shape, comparable with that induced by progesterone (Fig. 5*b*iii–v), whereas cells treated with CDB4124, 17α-hydroxy CDB4124 and VA2914 were generally rounder, similar to that induced by RU486 (Fig. 5*b*vii–ix).

Following treatment with CP8668, AED and Dex-Mes, ABC28 cells expressing both PR isoforms exhibited similar morphological change as AC2 cells and BC54 cells, appearing spread and is elongated in shape (Fig. 5*c*iii–v). ABC28 cells treated with CDB4124, 17α-hydroxy CDB4124 and VA2914 adopted stellate cell morphology similar to that observed in AC2 cells (Fig. 5*c*vii–ix). The results suggest that in the presence of putative antagonists RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914, the activity of PR-A was dominant over that of PR-B.

Effect of progesterone receptor modulators on cell motility in cells transfected with either PR-A or PR-B

To delineate whether the changes in cell morphology and in focal adhesion observed with progesterone, RU486 and PRM on the cell cytoskeleton correlated with the cell motility potential, we assessed the motility of cells transfected with either PR-A or PR-B under stimulation with the above test compounds. The cell motility potential was evaluated by wound healing assay and matrigel invasion assay.

Figure 6*a* shows the phase-contrast images of the wound healing after treatment with PRM for 48 hr. The data in Figure 6*b* represent the percentage of wound closure after various treatments compared with that of a freshly scraped wound (0 hr). In AC2 cells, treatment with progesterone and CP8668 resulted in greater

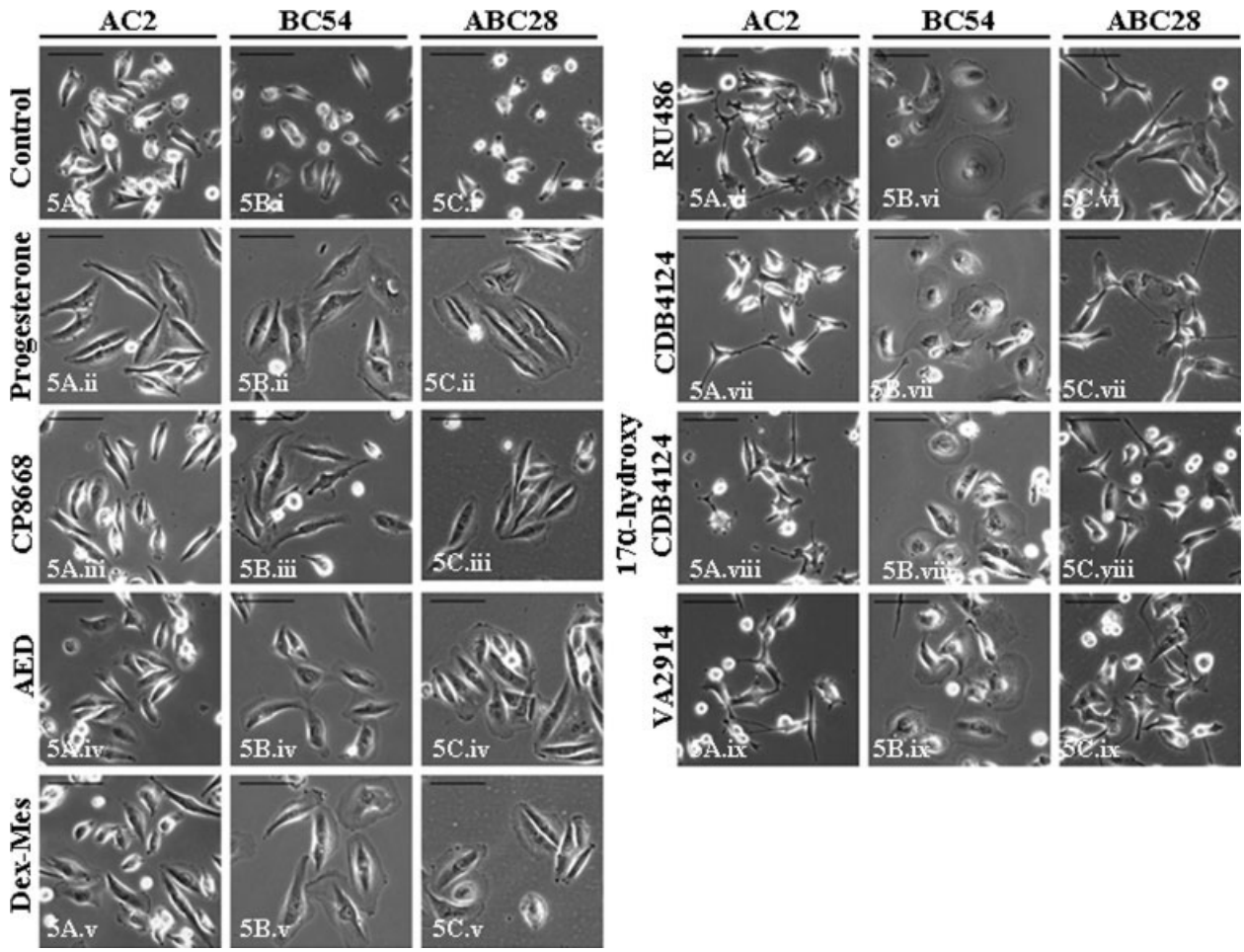

FIGURE 5 – Effects of PRM on morphological changes of cells expressing PR-A (AC2), PR-B (BC54) or both PR isoforms (ABC28). The images were taken after treatment for 48 h with PRM. The concentration of PRM tested was 0.1 μM except that AED and Dex-Mes were tested at 1 μM. Scale bar = 100 μm.

wound closure ($p < 0.05$) than in control cells. In contrast, the migration of AC2 cells treated with RU486, CBD4124, 17α-hydroxy CDB4124 and VA2914 were not significantly different from that of control cells ($p > 0.05$). On the other hand, progesterone, RU486 and all PRM tested induced significant cell migration in BC54 cells ($p < 0.05$).

The results from the matrigel assay echoed those from the wound healing assay (Fig. 7). The migration of AC2 cells treated with progesterone and CP8668 was significantly higher ($p < 0.05$) than vehicle-treated cells. On the other hand, all PRM tested enhanced the migration of BC54 cells.

PRM inhibited cell cycle progression of cells transfected with PR-A and/or PR-B

Flow cytometry analysis was performed to determine the effect of PRM on the distribution of cells in different phase of cell cycle in cell transfected with PR-A, PR-B or both PR isoforms. Before that, we assessed the effect of PRM on vector transfected control CTC15 cells and found that none of the compounds affected the progression of cell cycle at the dose of 10^{-6} M ($p > 0.05$) (data not shown).

All PRM tested induced inhibitory effect on DNA synthesis in cells expressing either PR isoform or both PR isoforms. The growth-inhibitory effect was associated with dose-dependent reduction of percentage of S-phase cells (Fig. 8) and accumulation of cells in G_0–G_1 phase (data not shown).

Generally, progesterone, RU486, CDB4124 and VA2914 were more potent in reducing S-phase fraction than other compounds. They are also generally more potent in BC54 cells and ABC28 cells as compared to AC2 cells. The IC50 values of progesterone, RU486, CDB4124 and VA2914 were all less than 10^{-10} M in BC54 and less than 10^{-9} M in ABC28 cells. In contrast, the IC50 of all compounds were greater than 10^{-6} M in AC2 cells. The weaker response from AC2 cells may be due to the lower receptor level in AC2 cells than in BC54 cells. It is also possible that PR-A is a weaker transactivator than PR-B.[40] 17α-hydroxy CDB4124, a metabolite of CDB4124, is less potent in inhibiting cell growth than the parent compound in all cell lines tested.

Differential regulation of gene expression by PRM in cells transfected with PR-A and/or PR-B

So far, we have demonstrated that the activities of PR-A and PR-B were differentially regulated by PRM. In particular, RU486, CBD4124, 17α-hydroxy CDB4124 and VA2914 induced stellate morphology *via* PR-A, but triggered cell spreading *via* PR-B. These modulators increased cell motility through PR-B, but not PR-A. All PRM tested induced inhibitory effect on DNA synthesis through PR-A and PR-B. We then sought to understand if these differential effects *via* PR-A and PR-B were also reflected at the gene level. It is to be noted that PRM did not affect the expression of the genes tested in vector transfected control, CTC15 cells (data not shown).

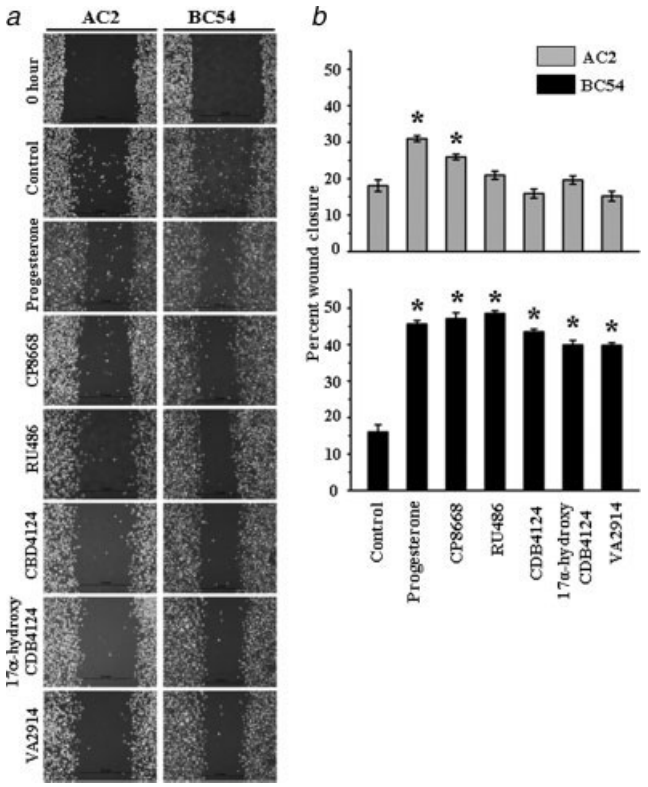

FIGURE 6 – The migration of cells expressing either PR-A (AC2) or PR-B (BC54) was assessed by *in vitro* scratch assay. Cells were treated for 48 hr with 0.1 μM PRM. (*a*) The phase-contrast images of the scratch wound before and after treatment. (*b*) The percentage of wound closure normalized to at 0 hr. Error bars represent the mean ± SE of 10 separate measurements. Asterisks denote significant difference from the control ($p < 0.05$).

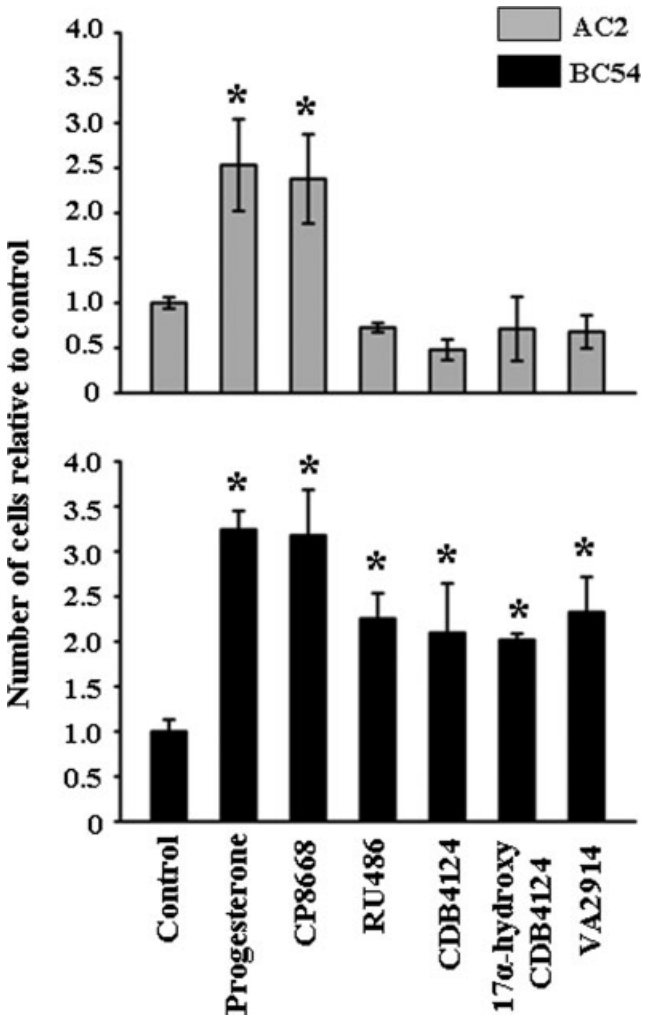

FIGURE 7 – The migration of cells expressing either PR-A (AC2) or PR-B (BC54) was assessed by Matrigel invasion assay. Cells were treated for 48 hr with 0.1 μM PRM. The result is presented as the relative number of cells that have migrated through the transwell inserts when the migration of control-treated cells is given the value of 1. Error bars represent the mean ± SE of 3 replicates. Asterisks denote significant difference from the control ($p < 0.05$).

Expression profiles of genes regulating cell shape or cell adhesion. A total of 6 genes were selected for this study. These genes are known be regulated by progesterone in ABC28 cells[41] and they are matrix metalloproteinase 1 (*MMP1*), metalloproteinase 13 (*MMP13*), plasminogen activator of urokinase (*UPA*), a disintegrin and metalloproteinase domain 8 (*ADAM8*), periplakin (*PPL*) and plakophilin 2 (*PKP2*). The former 4 genes code for enzymes that are involved in degrading the extracellular matrix (ECM), while *PPL* and *PKP2* code for cell–cell adhesion molecules. The regulation of these genes by PRM in cells expressing PR-A (AC2), PR-B (BC54) or both PR isoforms (ABC28) is shown in Figure 9*a*.

In AC2 cells, progesterone down-regulated the expression of *MMP1*, *MMP13*, *UPA* and *ADAM8* but up-regulated the expression of *PKP2* and *PPL*. In general, similar gene expression patterns were observed following treatment with CP8668 and AED while treatment with Dex-Mes did not seem to cause detectable alteration in the expression of all these genes. While RU486 had the opposite effect of progesterone in regulating the gene expression of *MMP1* and *MMP13*, treatment with RU486 caused no significant changes in the gene expression of *UPA*, *ADAM8*, *PPL* and *PKP2*. Similar gene regulation pattern to that of RU486 were observed following treatment with CDB4124, 17α-hydroxy CDB4124 and VA2914.

BC54 cells treated with progesterone, CP8668 and AED exhibited a gene expression pattern similar to that of AC2 cells. Treatment with Dex-Mes effected only the expression of *MMP1*, *ADAM8* and *PKP2*. Generally, treatment with RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914 showed gene expression pattern similar to that of progesterone, although the fold changes were smaller than that after progesterone treatment.

It is also interesting to note that the effects of RU486, CDB4124 and VA2914 on gene regulation in ABC28 cells were generally similar to that in AC2 cells, except that the gene expression of *MMP1* was up-regulated in AC2 cells but down-regulated in ABC28 cells.

Expression profiles of genes involved in cell cycle regulation. We showed earlier that all PRM tested were able to inhibit cell cycle progression, albeit by different magnitudes, in PR-transfected MDA-MB-231 cells. To study if the PRM-mediated growth inhibition induced similar gene expression as progesterone, we examined the expression of cyclin-dependent kinase inhibitor 1A (*p21*), and 3 cyclins [cyclin A2 (*CCNA2*), cyclin B1 (*CCNB1*) and cyclin E2 (*CCNE2*)] in PR expressing cells. The results are shown in Figure 9*b*. Progesterone, RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914 up-regulated the expression of *p21* and down-regulated the gene expression of *CCNA2*, *CCNB1* and *CCNE2* in all three cell lines (AC2, BC54 and ABC28). Of further note is that CP8668 and AED showed little effect on the expression of all 4 genes in AC2 cells, but up-regulated the gene expres-

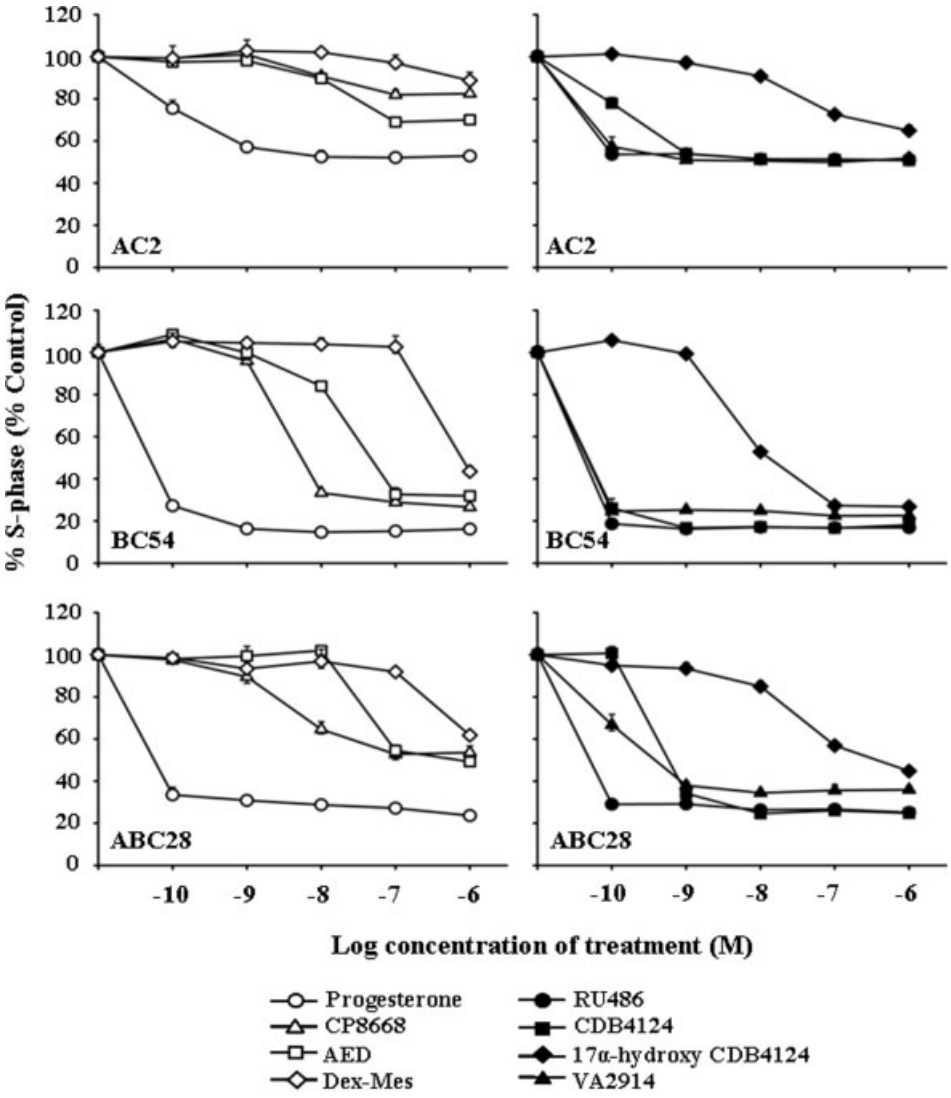

FIGURE 8 – Effects of various concentrations of PRM on the percentage of S-phase in cells expressing PR-A (AC2), PR-B (BC54) or both PR-A and PR-B (ABC28). The percentage of cells was measured by flow cytometry after 24 hr treatment. Cells were stained with PI in Vindelov's cocktail. Results are expressed as percentage of vehicle-treated controls. Error bars that are not visible are within the symbol area. Results are expressed as mean ± SE, $n = 3$.

sion of *p21* and down-regulated the expression of *CCNA2*, *CCNB1* and *CCNE2* in BC54 and ABC28 cells. Dex-Mes did not alter the expression of any of these cell cycle regulating genes in cells expressing either PR isoform except for *p21* which was induced by Dex-Mes in cells expressing PR-B. The PRM-regulated expression of the genes involved in cell cycle regulation was also reflected at the protein level. When proteins from whole cell lysates were analyzed, cyclin A and cyclin B in the cell expressing either PR-A or PR-B were down-regulated by most PRM, whereas p21 was up-regulated (Fig. 9*c*). CP8668 exhibited little effect on the protein levels of cyclin A and B although it increased the level of p21 slightly.

ERα modified the effects of PRM differentially between PR-A and PR-B expressing cells

Both AC2 and BC54 are ERα-negative cells but under most physiological conditions, PR target tissues are also ERα-positive. To test if the presence of ERα may alter PR-A- or PR-B-mediated effects of PRM, we employed adenoviral vector-mediated delivery of ERα into AC2 and BC54 cells. Figure 10*a* shows that the expression of ERα following infection of adenoviral vector-ERα (Ad/ERα) using MOI of 1, 2 and 5 reached peak level after 72 hr infection. After 96 hr, ERα level began to decline. It also seemed that the expression level of ERα is higher in BC54 cells than that of AC2. In consideration of all the time points, Ad/ ERα at MOI of 2 gave similar ERα levels to that of MCF-7 cells and it was therefore chosen for the subsequent study.

Figure 10*b* shows that the adenoviral vector delivered ERα is functionally active as estradiol-17β(L) E2 induced up-regulation of ER target genes *GREB1* and *pS2* in AC2 and BC54 cells. The magnitude of up-regulation is greater in BC54 than in AC2. For example, E2 treatment induced *pS2* gene by 59-fold but it only increased 14 fold in AC2 cells. This may be due to a higher expression level of ERα in BC54 than in AC2 cells.

We then studied the effect of ERα expression on cell cycle progression and on morphological changes in response to PRM. In AC2 cells (Fig. 11), Ad/ERα infection did not affect the growth-inhibitory effect of progesterone and CP8668. In contrast, it strengthened the growth-inhibitory effect of putative antiprogestins (RU486, VA2914 and CBD4124). On the other hand, in PR-B expressing cells, Ad/ERα infection weakened the growth-inhibitory effect of all PRM except VA2914. Although the differences are in the order of 5–10% compared with vector-transfected controls, this is statistically significant in most cases.

Morphologically, Ad/ERα infection caused both AC2 and BC54 cells to become rounder compared with the more elongated morphology in control adenovirus-infected cells (Fig. 12) and in uninfected controls. The magnitude of cell spreading induced by

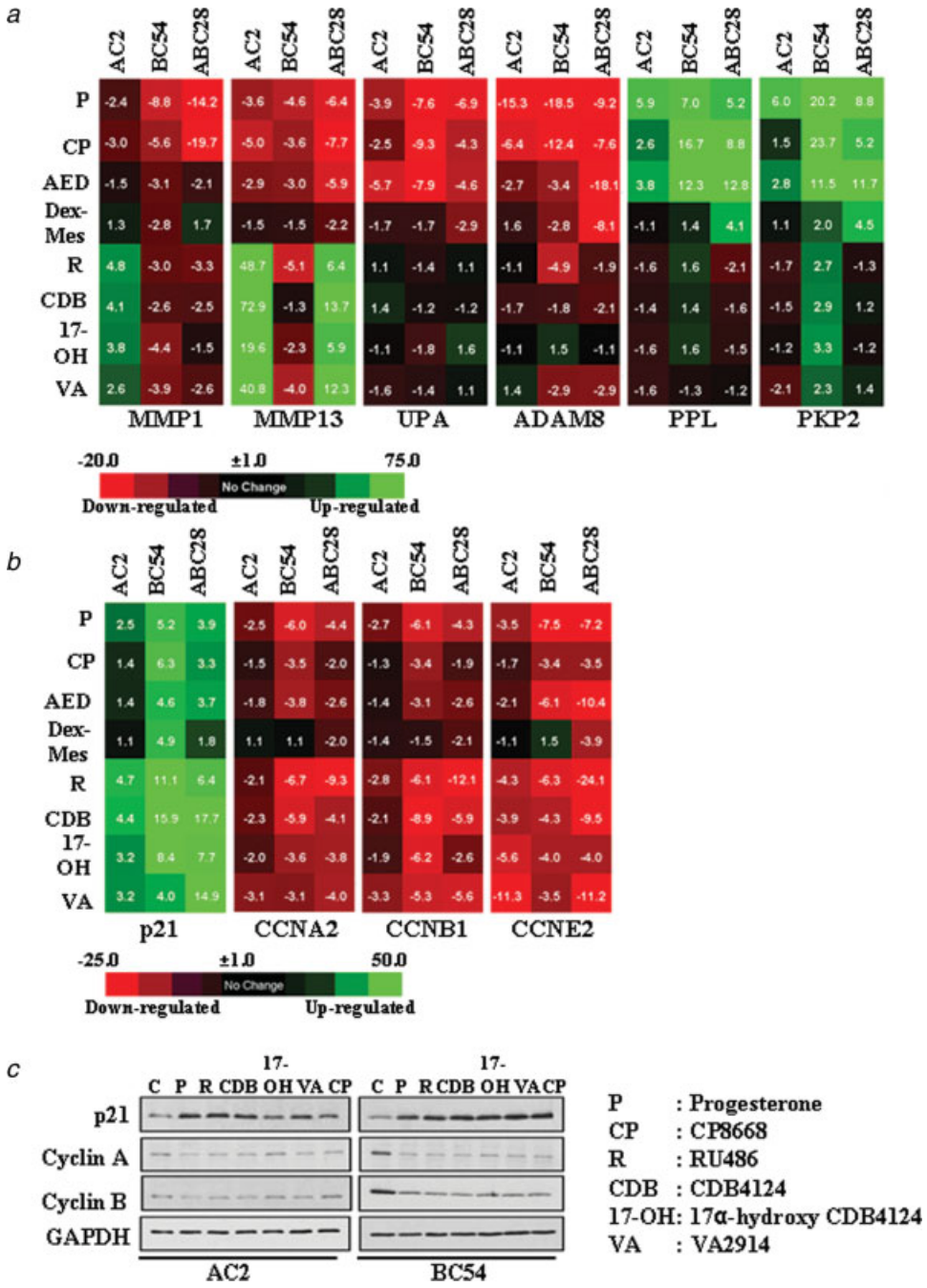

FIGURE 9 – Effects of PRM on the gene expression and protein level in cells expressing PR-A (AC2 cells), PR-B (C54 cells) or both PR isoforms (ABC28 cells). For gene expression data, the results are the average of 2 independent experiments after 24 hr treatment. Numerical numbers denotes the fold change. Negative value represents down-regulation while positive value represents up-regulation. Dendograms depict the expression fold change of *MMP1*, *MMP13*, *UPA*, *ADAM8*, *PPL* and *PKP2* (*a*) or cell cycle regulating genes *p21*, *CCNA2*, *CCNB1* and *CCNE2* (*b*) as detected by quantitative real-time PCR. (*c*) Protein expression of p21, Cyclin A and Cyclin B in cells expressing PR-A or PR-B as detected by Western blot after 48 hr treatment with PRM. The concentration of PRM tested was 0.1 μM except that AED and Dex-Mes were tested at 1 μM.

PRM in BC54 cells were also much less in Ad/ERα infected cells compared with the controls. In AC2 cells, there seems to be a noticeable decrease in cell spreading induced by progesterone and CP8668, yet cells still appeared stellate when treated with RU486, CDC4124 and VA2914. Since the introduction of ERα expression itself caused cell rounding, the reduced spreading in PRM-treated cells is likely due to the effect of ERα.

DISCUSSIONS

Numerous studies have indicated that progesterone receptor isoforms PR-A and PR-B may exert differential biological effects in their target cells. Findings in this study revealed that PR-A and PR-B can also function differently depending on PR ligands. In general, the cellular effects of progestins (CP8668, AED and Dex-Mes) were similar to that of progesterone in cells expressing PR-A and/or PR-B. On the other hand, putative antiprogestins (RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914) modulated the cellular effect of PR-A differently from that of PR-B in an activity-dependent manner. On cell growth, these compounds demonstrated agonist effect by inhibiting cell growth through PR-A and PR-B. In contrast, the effect of these antiprogestins on cell morphology, focal adhesion and cell migration were distinct between the 2 isoforms. Through PR-A, they displayed an antagonist activity; cells appeared stellate with multiple protrusions, less stress fibers and less motile. Through PR-B, these compounds demonstrated agonist-like effects such as the induction of cell spreading, the formation of stress fibers and focal adhesion, and increase in cell motility. In the present study, we found that the changes in the cellular activities mediated by PR following treatment with PRM were consistent with the gene expression study.

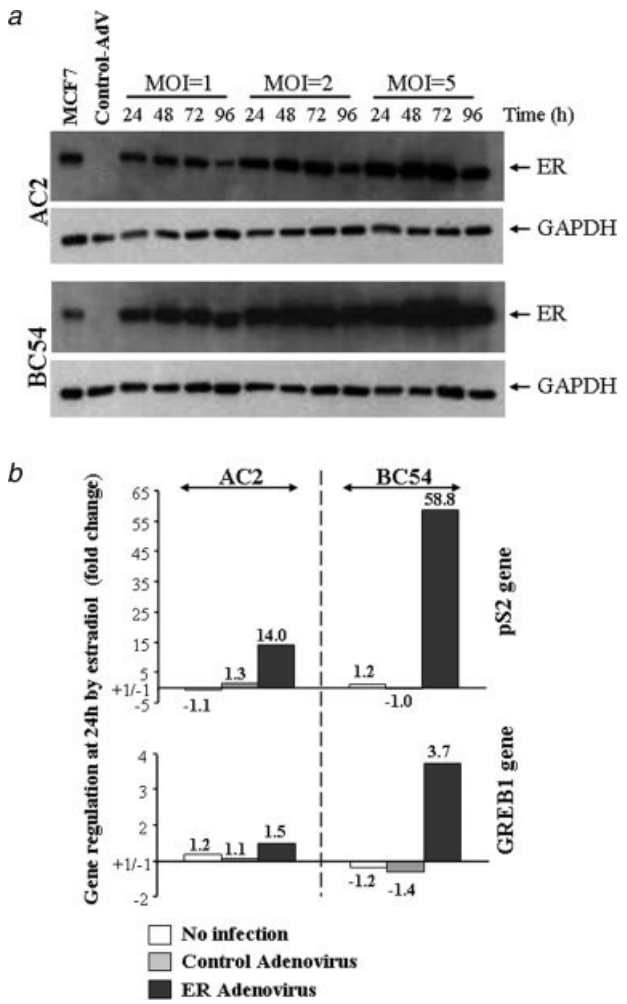

FIGURE 10 – Adenoviral vector-mediated expression of ERα and the functional activity of the ERα in AC2 and BC54 cells. A: The expression of ERα in AC2 and BC54 cells as detected by Western blot after 16 hr of infection with either Ad/ERα or control virus. Cells were infected at the indicated MOI and whole cell lysate was collected after 24, 48, 72 and 96 hr following infection. GAPDH was used as loading control. (*b*) ERα delivered by adenovirus is transcriptional active, as shown by the gene expression level of *pS2* and *GREB1* in AC2 and BC54 cells using quantitative real-time PCR. Cells were infected with either Ad/ERα or control virus at MOI = 2 for 16 hr and treated with 1 nM estradiol-17ß or control vehicle for 24 hr. The parental cells and the control virus-infected cells were used as negative controls.

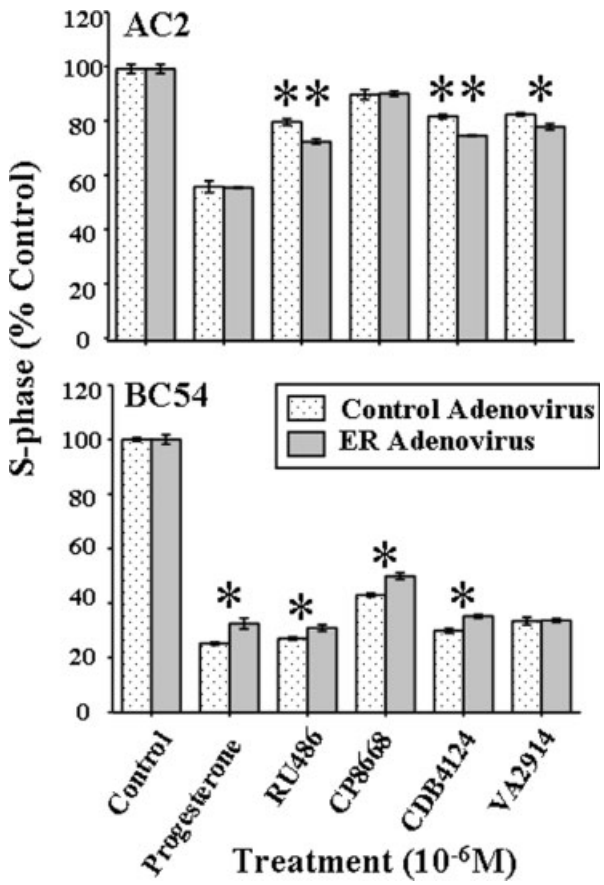

FIGURE 11 – Influence of ERα expression on the percentage of S-phase fraction in PRM-treated AC2 and BC54 cells. Cells were infected with Ad/ ERα at MOI = 2 for 16 h and treated with PRM or control vehicle. The percentage of cells was measured by flow cytometry after 24 hr treatment. Cells were stained with PI in Vindelov's cocktail. Results are expressed as percentage of vehicle-treated controls and as mean ± SE, $n = 3$. Asterisks denote significant difference between control adenovirus- and Ad/ERα-infected cells following PRM treatment. *($p < 0.05$) and **($p < 0.01$).

Several lines of evidence suggest that PRM modulated the activity of PR-A in a gene selective-manner. The absence of an effect on cell cycle regulating genes by CP8668 in AC2 cells is in sharp contrast to its substantial effect on ECM proteases and *PPL*, suggesting that CP8668-bound PR-A may have preferential activity for genes regulating cell shape and cell adhesion. Furthermore, antiprogestins exhibited agonist effect in AC2 cells on the expression of genes regulating cell cycle but induced gene expression changes that was either opposite to that of progesterone or had no effect on the expression of cell shape/cell adhesion regulating genes. Therefore, it seems that PRM regulated the activity of PR-A on cell growth and adhesion characteristics differentially and this gene-selective agonist/antagonist activity of PRM may be promoter-dependent. The promoter-selective effects of antagonist-occupied PR-A have been previously reported in reporter gene assays in which PR-A was transcriptionally active on MMTV promoter but not active on PRE2 thymidine kinase promoter.[40] It is tempting to speculate that antiprogestin-occupied PR-A may adopt different conformations to recruit either corepressors or coactivators depending on the promoter context of specific target genes.

It appears that the effects of agonist-occupied activities of PR-A or PR-B on cell morphology and adhesion characteristics in breast cancer cells are also cell line- and ER status-dependent. Treatment with progestin (ORG2058) led to cell spreading in T47D cells expressing PR-A and PR-B at the ratio of 1:3 but caused cell rounding in cells over-expressing PR-A, suggesting that progestin mediates its effect through PR-A differently from that through PR-B.[42] In our current study using MDA-MB-231 PR-transfected cells, we observed that progesterone treatment induced cell spreading through PR-A and/or PR-B. One difference between the 2 cell lines is that T47D cells are ER-positive whereas MDA-MB-231-derived cells are ER-negative. It is possible that the presence of ER alters the distribution and/or availability of transcription cofactors, which may alter the endpoint effect of PRM.

To determine if the presence of ERα can blunt the cells' response to PRM, we introduced ERα to these cells using adenoviral vector. The introduction of ERα expression in AC2 and BC54 cells resulted in cell rounding in contrast to the more elongated morphology in control cells. This effect of ERα on cell rounding

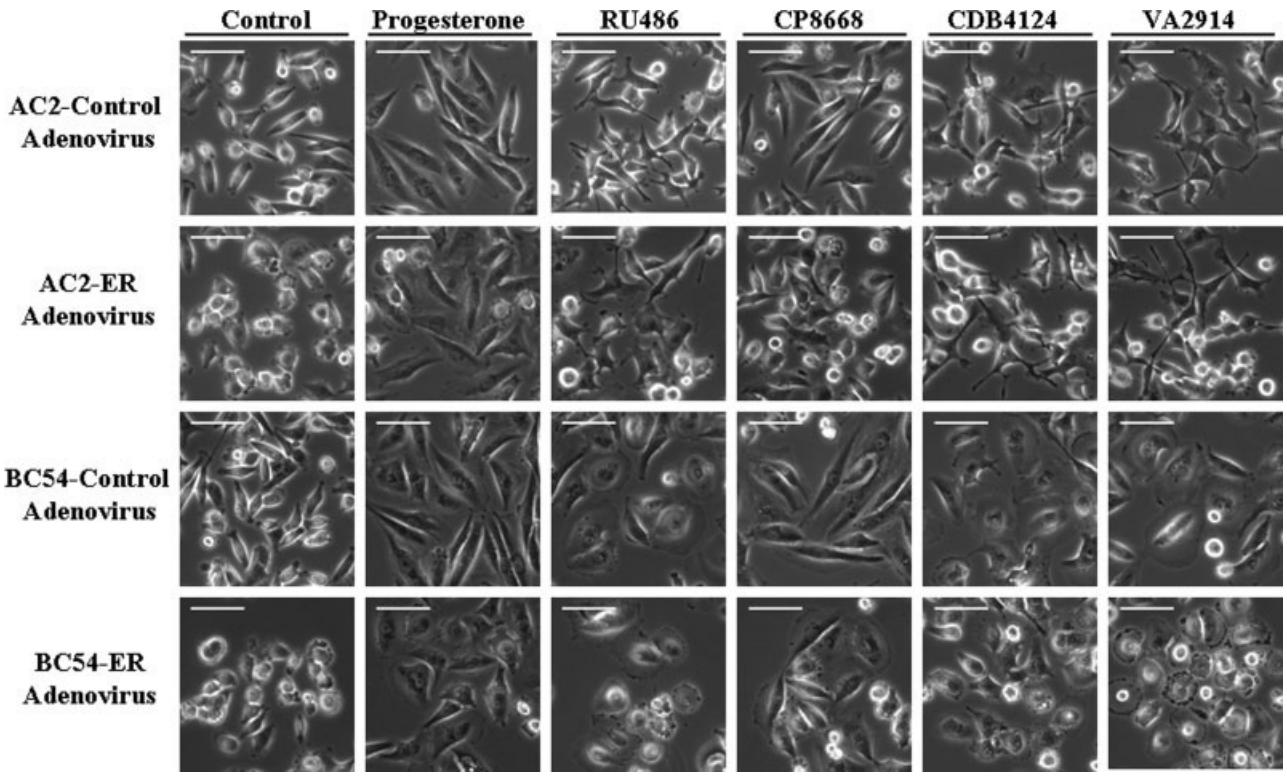

FIGURE 12 – Influence of ERα expression on the morphology of AC2 and BC54 cells. Cells were infected with either Ad/ ERα or control virus at MOI = 2 for 16 hr and treated with PRM for 48 hr. The images were taken under phase contrast microscope using a ×20 objective. Scale bar = 50 μm.

was likely responsible for the reduction in PRM-induced cell spreading in (Fig. 12). In addition, ERα seemed to further enhance the growth-inhibitory effect of antiprogestins in PR-A expressing cells but it clearly did not affect the effect of progesterone and CP8668. One possible mechanism for this observation is that the antiprogestins may also interact with ERα to exert growth-inhibitory effect. Coupled with the growth-inhibitory effect mediated by PR-A alone, the growth-inhibitory effect was further enhanced. This was speculated in view of the findings that RU486 could exert estrogenic effect in MCF-7 cells[43] and in rat uterus.[44] Since estrogen was growth inhibitory in ERα-transfected MDA-MB-231 cells,[45] the ERα-mediated estrogenic effect of antiprogestin in these PR-transfected MDA-MB-231 cells should also be growth-inhibitory. On the other hand, the weakening of PRMs effect by ERα in PR-B expressing cells may be caused by squelching of transcription cofactors. It has long been demonstrated that cotransfection of PR-B with ERα inhibited ERα-mediated reporter gene activity and increase in ERα expression overcame this inhibition.[46] It is conceivable that ERα would also compete with PR-B for transcription cofactors and this competition would result in the attenuation of the effect mediated by PR-B. In sum, although the presence of ERα did not change PR-A or PR-B mediated effect of PRM radically, there are differential functional interactions between ERα and the PR isoforms and these interactions can modify the growth-inhibitory effect of PRM in the opposite directions in PR-A and PR-B expressing cells. It is also to be noted that introduction of exogenous PR and ERα does not necessarily mimic the expression of these 2 receptors under physiological condition. Nonetheless, the models described allowed us to identify the potential interactions between PR and ERα, which are otherwise not possible to identify under physiological conditions.

Focal adhesion and lamellipodia are structures that facilitate the anchoring of the cytoskeleton to the extracellular matrix (ECM) and enable the cells to crawl and adhere.[47,48] It is expected, therefore, that PRM that induced cell spreading and lamellipodia also increased cell motility and migration through Matrigel-coated transwell. This notion applies to progesterone- and CP8668-treated PR-A or PR-B expressing cells, and to PR-B cells treated with RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914. In contrast, compounds (RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914) that induced stellate morphology in PR-A expressing cells decreased cells' motility in both wound healing and matrigel invasion assay. These data suggest that cells with multiple protrusions but without lamellipodia are less motile than flattened cells with lamellipodia. According to this observation, cell morphology may be used to predict the motility and *in vitro* invasiveness of breast cancer cells. It is to be cautioned, however, that increased cell motility does not necessarily indicate increased invasiveness. A number of studies have reported that the Matrigel invasion assays did not correlate with the stages of malignancies or *in vivo* tumorigenicity in breast cancer cells. Using 6 cell lines from human breast cancer, Le Marer and Bruyneel[49] revealed that benign (242A) and primary carcinoma cell lines (341 and 531E) were more motile than cells from late stages of malignancies (ZR75-1 and T47D) in Matrigel barrier. Other studies also showed that nonmalignant human breast epithelial cell lines were more motile than the apparent malignant and invasive breast cancer cells.[50,51] Furthermore, in the present study, progesterone decreased the expression of invasion genes such as *MMP1*, *MMP13* and *UPA* which indicates a less invasive genotype because over-expression these genes are known to be associated with poor prognosis and invasive disease in breast cancer.[52–57] We believe that *in vivo* experimental model would provide a better assessment on the invasiveness of cancer cells.

Progesterone-induced morphological changes and cell motility in PR-A- and PR-B-transfected MDA-MB-231 cells have been reported by Sumida *et al*.[58] However, the study noted stellate morphology in PR-A-transfected cells after progesterone treatment.

This is in contrast to our findings that progesterone induced significant cell spreading and focal adhesion in PR-A-transfected cells. It is to be noted that the observation by Sumida *et al.* was based on 1 cell clone, whereas our observation was based on 3 clones of cells transfected with PR-A, thus eliminating the possibility of clone variation. Furthermore, the effect of progesterone on cell motility in PR-A- and PR-B-transfected cells in our study is the opposite to that reported by Sumida *et al.* who reported that progesterone reduced the migration of PR-transfected MDA-MB-231 cells.[58] We noted a number of differences in the design of experiments between our study and their study. For example, in our experiment the cells were treated with progesterone at the time of plating onto Matrigel whereas in their study, the cells were pretreated with progesterone for 48, 72 and 96 hr before plating onto the matrigel for 16 hr. Furthermore, we coated the membrane with 35 μl of matrigel at ~1.5 mg/ml whereas they used 12 μl mtrigel at 7.5 mg/ml to coat the membrane. It is not possible to conclude if the conflicting results were resulted from these differences. Moreover, since we tested a few PRM, we consistently found that cells with lamellipodia are more motile than those without, confirming that the cells' morphological features affect the cell motility.

In summary, this study revealed that PRM can modulate the activity of PR-A and PR-B differently in cellular activity- and gene-selective manner. In particular, PRM such as RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914 mimicked the agonist effect in modulating the activity of PR-A on cell cycle regulating genes but induced changes in expression that is distinct from that induced by progesterone on genes regulating cell shape/cell adhesion. This raises an interesting possibility that PRM can be designed to be PR isoform- and cellular pathway-selective to achieve targeted therapy with decreased side effect. It is to be noted that RU486, CDB4124, 17α-hydroxy CDB4124 and VA2914 are a group of PRM with similar properties. Other putative antiprogestins may display different properties in modulating the activity of PR-A and/or PR-B. These MDA-MB-231 cells engineered to express either PR-A, PR-B or both PR isoforms are excellent models for evaluating the nature of activity of PRM and to study the PR isoform- and pathway-selective characteristics of various PR modulators.

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