Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 93 (2005) 201–208
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

The role of progesterone metabolites in breast cancer: Potential for new diagnostics and therapeutics☆

J.P. Wiebe*, M.J. Lewis, V. Cialacu, K.J. Pawlak, G. Zhang

Department of Biology, Hormonal Regulatory Mechanisms Laboratory, University of Western Ontario, London, Ont., Canada N6A 5B7

Abstract

Proliferative changes in the normal breast are known to be controlled by female sex steroids. However, only a portion of all breast cancer patients respond to current estrogen based endocrine therapy, and with continued treatment nearly all will become unresponsive and experience relapse. Therefore, ultimately for the majority of breast carcinomas, explanations and treatments based on estrogen are inadequate. Recent observations indicate that 5α-pregnane and 4-pregnene progesterone metabolites may serve as regulators of estrogen-responsive as well as unresponsive human breast cancers. The conversion of progesterone to the 5α-pregnanes is increased while conversion to the 4-pregnenes is decreased in breast carcinoma tissue, as a result of changes in progesterone metabolizing 5α-reductase, 3α-hydroxysteroid oxidoreductase (3α-HSO) and 20α-HSO activities and gene expression. The 5α-pregnane, 5α-pregnane-3,20-dione (5αP) stimulates, whereas the 4-pregnene, 3α-hydroxy-4-pregnen-20-one (3αHP), inhibits cell proliferation and detachment, by modulation of cytoskeletal and adhesion plaque molecules via the MAP kinase pathway and involving separate and specific plasma membrane-based receptors. The promotion of breast cancer appears to be related to changes in in situ concentrations of cancer-inhibiting and cancer-promoting progesterone metabolites. New diagnostic and therapeutic possibilities for breast cancer are suggested.

Keywords: Progesterone metabolites; Breast cancer; Steroid receptors; Membrane steroid receptors; Gene expression; Human breast cell lines; MCF-7, MCF-10A, T47D, MDA-MB-231; 5α-Reductase; 3α-Hydroxysteroid oxidoreductase, 20α-hydroxysteroid dehydrogenase; 5α-Dihydroprogesterone; 3α-Dihydryprogesterone; MAP kinase; Actin Vinculin; Proliferation; Adhesion

1. Introduction

Breast cancer is one of the most widespread malignancies of women in Western society. In spite of extensive investigations, there is currently no adequate endocrine explanation for the majority of breast cancer cases. Although both female sex steroids, estradiol-17β (estradiol) and progesterone, are known to be involved in normal breast development as well as in the proliferative changes that occur during the menstrual cycle, pregnancy and lactation [1,2], current endocrine therapy is based almost exclusively on suppression of estradiol action. However, this estrogen-based therapy is effective in only a fraction of all breast cancer patients. Moreover, a large proportion of those patients with advanced neoplasia who respond initially, will eventually experience relapse and fail to respond to additional anti-estrogen therapy [3]. Thus, for the large number of breast cancers that are unresponsive to estrogen-based therapy, as well as for those showing relapse, there is currently no adequate hormonal explanation and hence no hormone-based treatment. The role of progesterone in breast cancer is not understood and although progesterone metabolism is known to occur in breast tissue, the potential role of progesterone metabolites has only recently begun to be explored [4].

The impetus for research on the metabolites of progesterone stemmed from the numerous conflicting reports about the effects of progesterone and other (synthetic) progestins on breast cancer cells. For example, some studies showed tumour regression with some doses of progestin treatment [5], while others reported increased epithelial proliferation [6]. Retrospective studies suggested that surgery performed

☆ Proceedings of the 16th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology, "Recent Advances in Steroid Biochemistry and Molecular Biology" (Seefeld, Tyrol, Austria, 5–8 June 2004).

* Corresponding author. Fax: +1 519 661 3935.
E-mail address: jwiebe@uwo.ca (J.P. Wiebe).

0960-0760/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.12.003

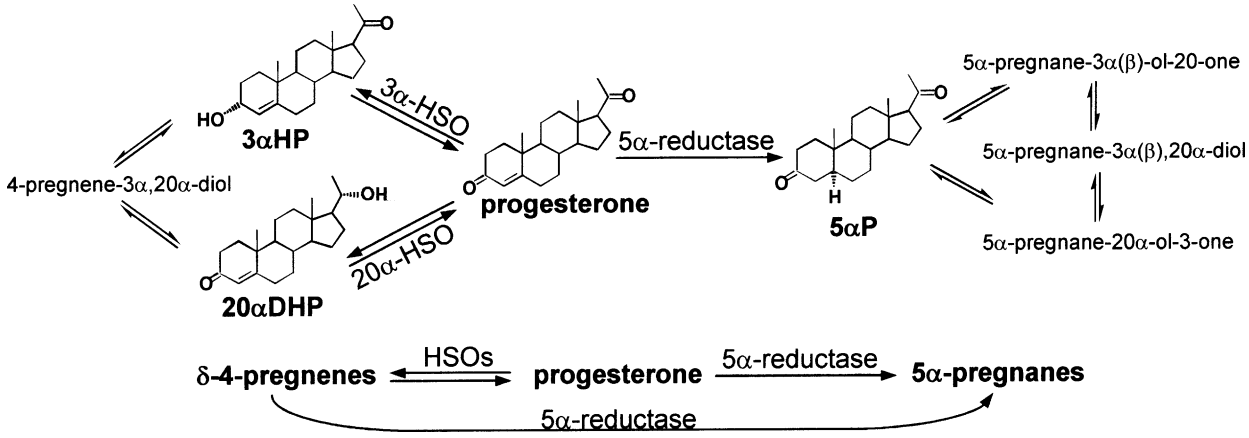

Fig. 1. Progesterone conversion to d-4-pregnenes and 5α-pregnanes by human breast tissue.

during the luteal phase of the menstrual cycle (when progesterone levels are higher) resulted in higher disease-free and overall survival rate than when surgeries were performed during the follicular phase [7]. In vivo, progestins either stimulate or decrease tumour growth [8] and in vitro either stimulate or inhibit cell proliferation [9], and cell cycle progression [10]. These reported contrary, and seemingly paradoxical, actions of progesterone in breast cancer cells, suggested to us the possibility that progesterone may be converted within breast tissue to two types of metabolites, those that stimulate and those that inhibit cell proliferation and tumorigenesis. If these could be shown to exist, they might also provide the basis of an endocrine explanation for all those breast cancer cases which are unresponsive to the anti-estrogen therapy. In our studies we identified the progesterone metabolites produced by breast tissue, the differences in progesterone metabolite production and in metabolizing enzyme activity and mRNA expression in normal and carcinoma tissue, the effects of the metabolites on cell proliferation and adhesion, cellular and molecular mechanisms of action and the unique receptors for the progesterone metabolites. The findings suggest potential roles of the progesterone metabolites in breast cancer.

2. Progesterone metabolism in normal and tumor breast tissue

Tumorous and non-tumorous tissues from the operated breasts of patients were used to determine their relative capacities for converting [^{14}C]progesterone. All the breast tissues in the study converted [^{14}C]progesterone into at least ten different metabolites [4], regardless of estrogen (ER) and progesterone (PR) receptor concentrations or a woman's age and ovarian state. The metabolites were rigorously identified by procedures that included TLC, HPLC, chemical derivatization, gas chromatography and mass spectrometry [4,11]. The results showed that breast tissues convert progesterone into two classes of metabolites (illustrated in Fig. 1): those which retain the double bond of progesterone in the carbon-4 position of ring A (delta-4-pregnenes; 4-pregnenes) and those that are 5α-reduced (5α-pregnanes). In the progesterone conversion pathway, the first 5α-reduced metabolite is 5α-pregnane-3,20-dione (5αP), catalyzed by 5α-reductase activity. The two 4-pregnenes resulting from direct progesterone conversion are 4-pregnen-3α-ol-20-one (3αHP) and 4-pregnen-20α-ol-3-one (20αDHP), catalyzed by the actions of 3α-hydroxysteroid oxidoreductase (3α-HSO) and 20α-HSO, respectively (Fig. 1). The conversion to 5αP is irreversible, whereas the conversions to 3αHP and 20αDHP are reversible reactions. Each of the 4-pregnenes can be further reversibly converted to 4-pregnene-3α,20α-diol. 5αP can be altered to 3- and 20-hydroxy pregnanes by the reversible actions of 3α-HSO, 3β-HSO, and 20α-HSO. Each 4-pregnene can also be irreversibly 5α-reduced to the respective 5α-pregnane by the action of 5α-reductase (Fig. 1). Thus the ratio of 5α-pregnanes:4-pregnenes can be altered by changes in activities of the progesterone metabolizing enzymes (PMEs), 5α-reductase, 3α-HSO and 20α-HSO.

3. Progesterone conversion to 5α-pregnanes is increased and conversion to 4-pregnenes is decreased in tumorous compared to normal (nontumorous) breast tissues

Although both normal and tumorous breast tissues convert progesterone to the two classes of metabolites, there are significant quantitative differences [4]. In normal (nontumorous) breast tissue, conversion of progesterone to 4-pregnenes greatly exceeds the conversion to 5α-pregnanes, whereas in tumorous tissue, production of 5α-pregnanes is higher than that of 4-pregnenes (Fig. 2a). The average ratio of 5α-pregnanes/4-pregnenes increases more than five-fold, from about 0.3 in non-tumorous to about 1.6 in tumorous breast tissue. The differences in amounts of 4-pregnenes and 5α-pregnanes are mainly due to changes in the amounts of the metabolites, 3αHP and 5αP, and the ratio of 5αP:3αHP is nearly 30-fold higher in tumorous than in nontumorous breast tissues (Fig. 2b).

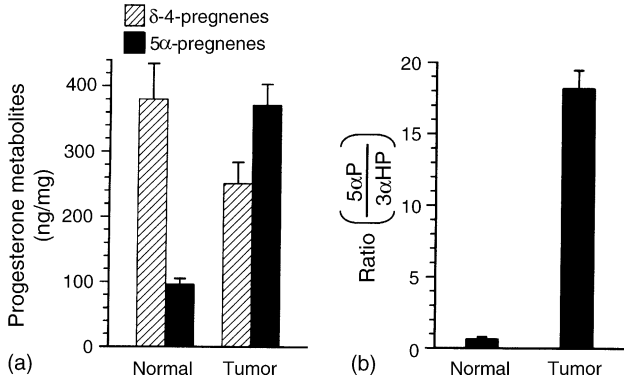

Fig. 2. The difference in progesterone metabolism between normal (non-tumorous) and tumorous breast tissue represented: (a) as total mass (ng) of 4-pregnenes and 5α-pregnanes produced per mg of breast tissue protein; (b) as the ratio of 5αP:3αHP. Tumorous tissue exhibits nearly a four-fold increase in 5α-pregnane amounts (a) and nearly a 30-fold increase in 5αP:3αHP ratio (b). Data from [4].

4. Breast tumor tissues exhibit increases in 5α-reductase and decreases in 3α-HSO and 20α-HSO activities and mRNA expression

In vitro enzyme studies showed that the activity of 5α-reductase is higher, whereas that of 3α-HSO and 20α-HSO is lower in tumorous than in normal breast tissue [4] (Fig. 3a). Metabolism studies show the capacity of tissue to synthesize or interconvert compounds; they do not show what is actually present in tissues. Measurements of 3αHP and 5αP by radioimmunoassays [12] and by selected ion mode (SIM) gas chromatography–mass spectrometry (GC–MS) show that the ratios of 5αP:3αHP are about 0.1 and 1.4 in non-tumorous and tumorous breast tissues, respectively, on a per mg protein basis [4]. These values suggest that the observed in vitro metabolic shift towards increased 5αP and decreased 3αHP in tumorous breast tissue may have in vivo relevance.

Altered levels of expression of the PME genes paralleled the observed changes in levels of enzyme activities (Fig. 3b and c). Studies on 11 paired (normal and tumor) breast biopsies using reverse transcription (RT) polymerase chain reaction (PCR) showed higher levels of expression of 5α-reductase type 1 (SRD5A1) and type 2 (SRD5A2) and lower levels of expression of 3α-HSO type 2 (AKR1C3), 3α-HSO type 3 (AKR1C2) and 20α-HSO (AKR1C1) in the tumor tissues (Fig. 3b; [13,14]). The ratios of tumor/normal expression levels for 5αR1 and 5αR2 were about 35–85-fold higher than the ratios of tumor:normal expression levels for the HSOs (Fig. 3c). The selective expression increases in 5α-reductase mRNA and decreases in 3α- and 20α-HSO mRNAs help to explain the parallel selective changes in PME activities in breast carcinoma. These changes may be responsible for the increases in 5α-pregnane:4-pregnene ratios observed in breast tumor tissue.

5. The activities and mRNA expression of progesterone metabolizing 5α-reductase(s) are higher whereas those of 3α- and 20α-HSOs are lower in tumorigenic than in nontumorigenic breast cell lines

Four breast cell lines were examined: nontumorigenic MCF-10A cells and tumorigenic MDA-MB-231, MCF-7, and T47D cells. The MCF-10A cell line is ER/PR-negative; MCF-7 and T47D cells are ER/PR-positive and estrogen-dependent for tumorigenicity, whereas MDA-MB-231 cells are ER/PR-negative and estrogen-independent for tumorigenicity. The results showed that 5α-pregnane production is significantly higher in each of the three tumorigenic cell lines than in the nontumorigenic cells [15]. The ratios of 5α-pregnanes:4-pregnenes were between 6.5-fold and 9.6-fold higher in the tumorigenic cell lines than in the nontumorigenic cells. The 5α-reductase activity was significantly

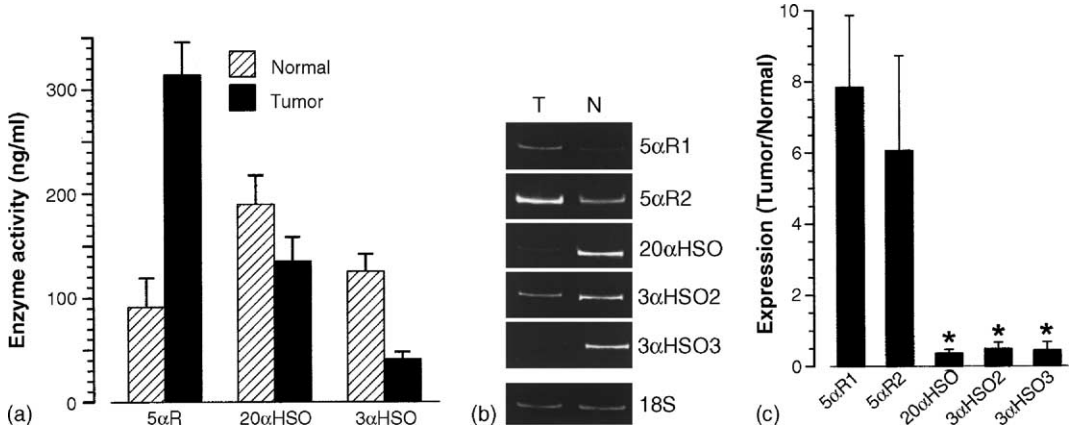

Fig. 3. Activity and expression of progesterone metabolizing enzymes in paired normal and tumor breast tissues. (a) Relative activities of 5α-reductase, 20α-HSO and 3α-HSO. (b) Representative RT-PCR results (gels) for one of 11 tumor and paired normal breast tissue samples. For each tissue, separate cDNA samples were amplified with primers specific to 18S rRNA (internal standard), 5αR1 (27 cycles), 5αR2 (33 cycles), 20α-HSD, 3α-HSD2 and 3α-HSD3 (27 cycles each). Note that intensity and abundance of 5αR1 and 5αR2 bands is greater, whereas that of the HSO bands is less in tumor than paired normal tissue samples. (c) Expression level of each gene (in relation to 18S rRNA) calculated as a tumor/normal ratio for each patient. Each bar and line represents the mean ± S.E.M. of 11 paired tissue samples. (*) indicates significantly different from 5αR1 at $p < 0.01$ and from 5αR2 at $p < 0.05$ (data from [13,14]).

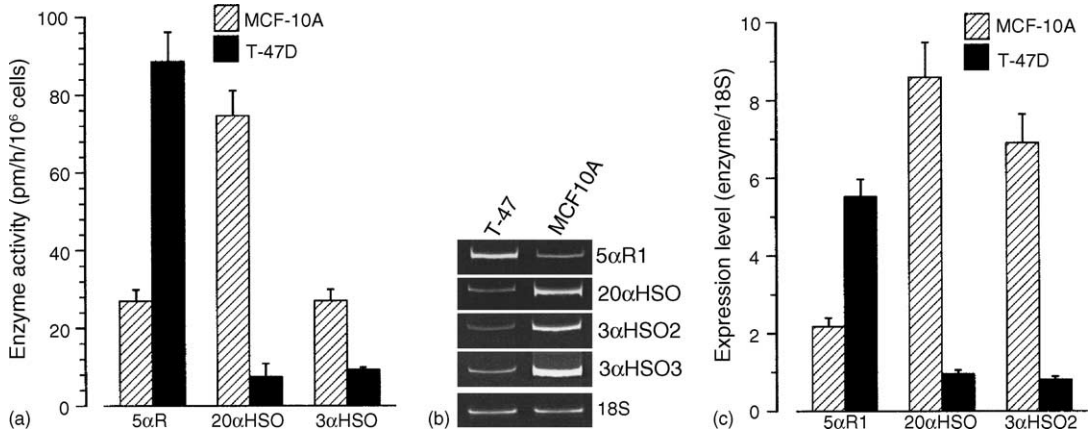

Fig. 4. Activity (a) and expression (b and c) of progesterone metabolizing enzymes in nontumorigenic (MCF-10A) and tumorigenic (T-47D) human breast cell lines. (a) Activities of 5α-reductase (5αR), 20α-HSO and 3α-HSO were calculated (as pmol/h/10^6 cells) from [^{14}C]progesterone metabolism studies. (b) Representative gels from RT-PCR showing the relative expression levels of 5αR type 1, 20α-HSO, 3α-HSO type 2 and 3α-HSO type 3, compared with internal standard, 18S rRNA. In each case aliquots of cDNA were amplified with gene-specific primers and run on 9% polyacrylamide gels. (c) Computer-assisted quantification of expression levels calculated as ratios against 18S rRNA (mean ± S.E.M.) from six separate experiments. Activity and expression levels for T-47D cells are all significantly different from those of MCF-10A cells at $p < 0.001$ (modified from [15]).

higher and the activities of 3α-HSO and 20α-HSO were significantly lower ($p < 0.001$) in the tumorigenic than in nontumorigenic cells [15] (Fig. 4a shows MCF-10A and T-47D cells for comparison). The differences in PME activities correspond to differences in gene expression. RT-PCR showed significantly ($p < 0.001$) higher expression of 5α-reductase type 1 (5αR1), and lower expression of 20α-HSO (AKR1C1), 3α-HSO type 2 (AKR1C3) and type 3 (AKR1C2) in tumorigenic than in nontumorigenic breast cell lines [15] (Fig. 4b and c).

Overall the progesterone metabolizing enzyme activity and expression studies suggest that 5αR1 has been stimulated whereas the HSOs have been suppressed in breast cancer tissue and tumorigenic cells.

6. 5α-Pregnanes act as cancer-promoting hormones; 4-pregnenes act as cancer-inhibiting hormones

Two cardinal aspects of cancer involve uncontrolled cell replication (leading to neoplasia) and altered cell adhesion (leading to metastasis); the progesterone metabolites have been demonstrated to affect both.

6.1. Cell replication

Uncontrolled cell replication (proliferation) is one of the hallmarks of cancer and factors which affect cell proliferation rates are known to affect cancer rates [2,16]. The effects of the progesterone metabolites on proliferation have been examined on several (MCF-7, MCF-10A, ZR-75-1, MDA-MB-231, T-47D) breast cell lines. The results showed that the 4-pregnene, 3αHP, exerts anti-proliferative effects, whereas the 5α-pregnane, 5αP, stimulates breast cell line proliferation (Fig. 5a shows effect on MCF-10A cell proliferation; other details in [4]). The suppression of cell proliferation exerted by any of the 4-pregnenes studied was changed to stimulation when the 4-pregnene was reduced to its respective 5α-pregnane [4]. From these results we concluded that breast cell proliferation can be significantly modulated by the metabolites of progesterone, being inhibited by the 4-pregnenes and stimulated by the 5α-pregnanes, such that the final result may depend on the relative abundance of each of the two classes of compounds. The effect is evident in all cell lines investigated, regardless of absence or presence of ER, PR or tumorigenicity.

6.2. Cell adhesion

In vitro, normal cells of either mesenchymal or epithelial origin usually depend on adhesion (anchoring) to, or spreading on, a solid substratum for cell division. Cell migration and invasion are fundamental components of tumor cell metastasis and require changes in cell adhesion. As cells become neoplastic, they become less dependent on support of solid substrates for cell proliferation [17]. In vivo, these changes in adhesion that enable tumour cells to depart from the primary site of growth constitute the first step toward invasion and cancer metastasis. Therefore, the identification of endogenous factors that contribute to a change in cell adhesion is important for establishing natural causes that prevent or promote the acquisition of metastatic potential. Having shown that the endogenous progesterone metabolites effect changes in cell proliferation, we tested their potential for altering adhesion in breast cell lines [4,18]. The results showed that the metabolite, 5αP, which caused significant stimulation of cell proliferation, also significantly decreased attachment (and increased detachment) of cells from the substratum (detachment effect shown in Fig. 5b). The opposite effect was observed with the proliferation-inhibiting progesterone metabolite, 3αHP, which promoted cell attachment and decreased cell detach-

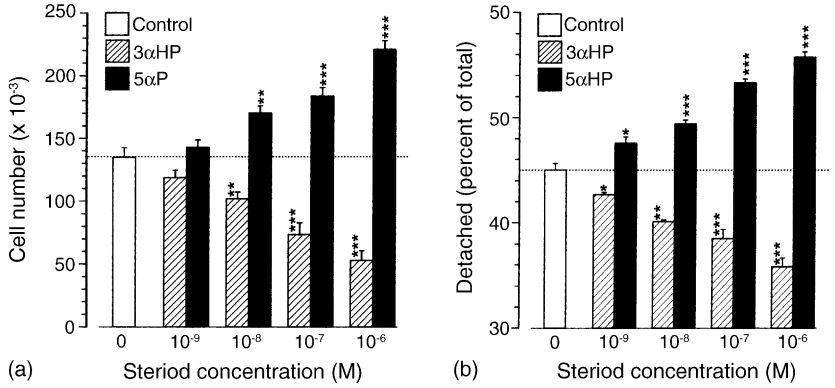

Fig. 5. Dose-dependent stimulatory and inhibitory effects of the progesterone metabolites, 5αP and 3αHP, respectively on proliferation (a) and detachment (b) of MCF-10A breast cells. Data are the means (±S.E.M.) of four separate experiments in which the cells were exposed for 72 h to 0 (control), 10^{-9} to 10^{-6} M 5αP or 3αHP. Significantly different from control at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (modified from [4]).

ment (Fig. 5b). The opposing actions of 5αP and 3αHP on both cell anchorage and proliferation further strengthen the hypothesis that the direction of progesterone metabolism in vivo toward higher 5α-pregnane and lower 4-pregnene concentrations could promote breast neoplasia and lead to malignancy.

6.3. The 5α-pregnane, 5αP, decreases adhesion plaques, vinculin expression and polymerized F-actin in breast cancer cells

To determine the mechanisms of action of 5αP on cell adhesion, MCF-7 cells were grown without or with 5αP (10^{-9} to 10^{-5} M), and the effects on cell and nuclear morphology, adhesion plaques, vinculin and actin expression, actin polymerization, and microfilament distribution were examined by immunohistochemistry, morphometry and Western blotting [18]. Treatment of cells with 5αP resulted in dose-dependent decreases in cell area, cell-to-cell contacts, attachments to the substratum, and increases in variation in nuclear area. These changes in the 5αP-treated cells were accompanied by decreases in vinculin-containing adhesion plaques (Fig. 6A–C), vinculin expression (Fig. 6C, gel), polymerized actin stress fibres (Fig. 6D and E) and decreases in ratio of insoluble/soluble actin (Fig. 6F). The results suggest that 5αP may modulate cytoskeletal and adhesion molecules and that the observed decreases in adhesion and increases in cell proliferation following 5αP treatment may be related

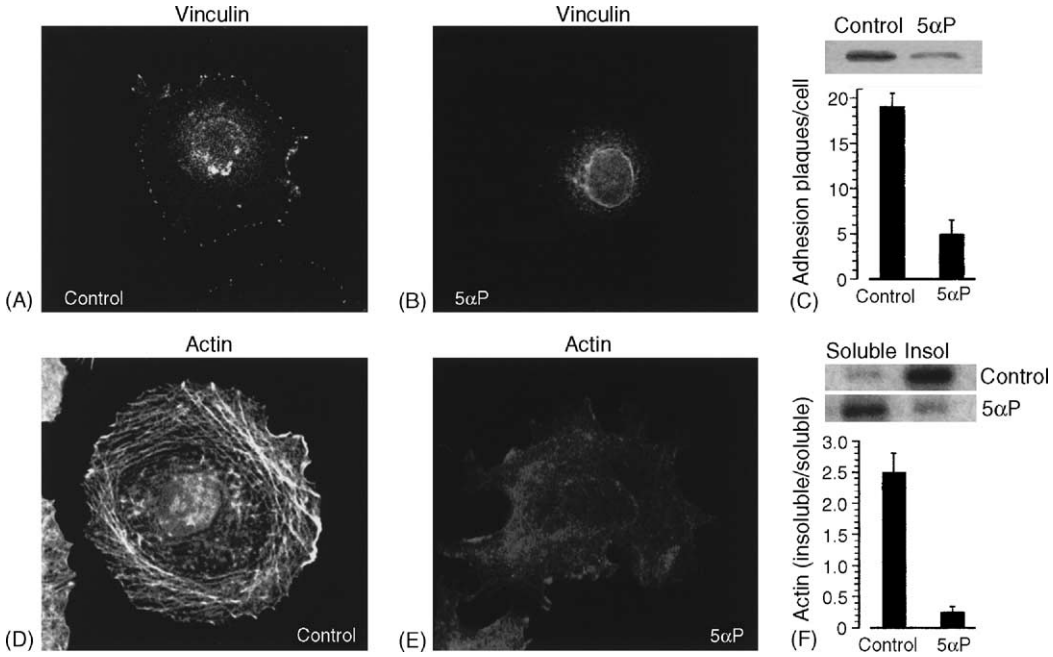

Fig. 6. Effect of 5αP on (A–C) vinculin and (D–F) actin stress fibers in MFC-7 cells. Vinculin-containing adhesion plaques appear conspicuously in the perimeter and basally in untreated (Control) cells (A), whereas 5αP-treated cells (B) show a marked reduction in adhesion plaque number (C, bar graph) and in vinculin expression (C, gel). Actin stress fibers are prominent in the control cells (D), whereas 5αP-treated cells show marked loss of observable actin fibers (E) and great reduction in insoluble (Insol; polymerized) and concomitant increases in soluble (Sol; depolymerised) actin (F) (modified from [18]).

to depolymerization of actin and decreased expression of vinculin.

7. Membrane-based receptors for 5α-pregnane and 4-pregnene progesterone metabolites in human mammary cells

Current dogma is that the obligatory first step in the mechanism of action of a steroid hormone is the binding to a receptor molecule. To identify potential binding sites for the progesterone metabolites, we synthesized [^{3}H]-labeled 5αP and 3αHP and conducted binding studies on the nuclear, cytosolic and plasma membrane fractions of MCF-7 cells using radioreceptor assays [19]. The studies demonstrated that binding of 5αP and 3αHP occurs in the plasma membrane fraction (Fig. 7a). The binding was specific for the respective steroid (i.e. not displaced by other steroids at 200–500-fold concentrations). Saturation and Scatchard analyses indicated separate (single classes) and distinct high-affinity, low capacity receptors for 5αP and 3αHP. These membrane-based binding sites (receptors) are not only distinct from each other but also from the well-studied nuclear/cytosolic PR, ER, androgen and corticosteroid receptors. The studies provide the first demonstration of specific receptors for 4-pregnene and 5α-pregnane progesterone metabolites in human mammary cells.

7.1. Regulation of membrane-based 5αP receptors by estradiol and 3αHP

Since estradiol has well known mitogenic effects on breast cancer cells, experiments were performed to determine if the 5αP membrane binding sites are influenced by exposure to estradiol. Incubations of the MCF-7 cells in charcoal-stripped growth medium with estradiol or 3αHP resulted in significant increases and decreases, respectively, in 5αP binding sites as compared to control cells (Fig. 7b). The effect appears to be time-dependent, with maximum increase in 5αP receptor density after 24 h exposure to estradiol [19]. In contrast to this estradiol-mediated increase in 5αP binding sites, preliminary experiments in which MCF-7 cells were exposed to 1.0 nM estradiol for 24 h showed a 60% decrease in 3αHP binding sites. These results suggest that the putative tumorigenic actions of 5αP may be significantly augmented by the estradiol-induced increases in 5αP binding and decreases in 3αHP binding. In addition, 5αP receptor density also appears to be increased by 5αP itself (a form of auto up-regulation). Of particular interest are observations that the estradiol-mediated increase in 5αP receptor density can be dose-dependently blocked by exposure to 3αHP (Fig. 7b; [20]), suggesting the possibility that 3αHP, if present in sufficient concentrations, may function in abating the stimulatory effects of estradiol and 5αP. The suggested implications for breast cancer from these results are that the stimulatory and inhibitory effects of 5αP and 3αHP, respectively, on cell replication and cell detachment, might be significantly modified by exposure to estradiol, 4-pregnenes, and 5α-pregnanes. The location of the receptors for the progesterone metabolites on the mammary cell membrane suggests involvement of nongenomic mechanisms of action via cell signaling pathways.

8. Action of 5αP via the mitogen-activated protein kinase (Erk/MAPK) pathway

Signaling pathways that control cell proliferation and adhesion are often initiated from surface receptors and may converge on the MAPK cascade, a module consisting of MAP kinase kinase (MEK) and MAPK [21,22]. To determine if the effects of 5αP on cell replication and adhesion involve activation of MAPK, MCF-7 cells were serum-starved for 36 h and then incubated for 4 h with various concentrations of 5αP (10^{-10} – 10^{-5} M); MAPK activation was then deter-

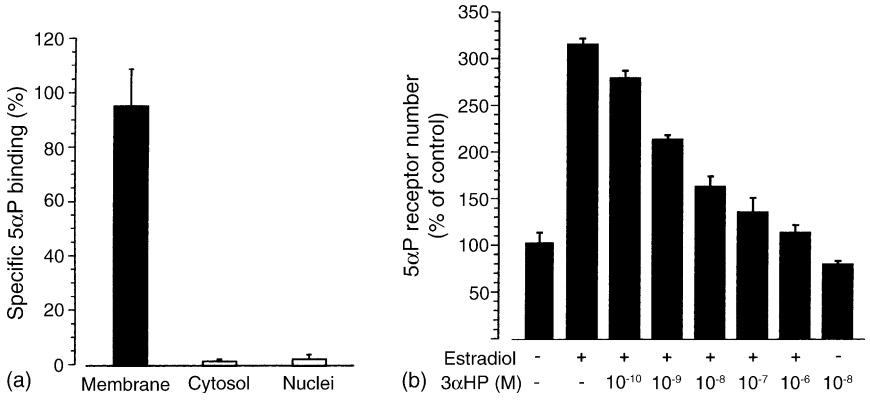

Fig. 7. Specific binding of [^{3}H]5αP to membrane fraction of MCF-7 cells (a) and induction and suppression of 5αP receptor number by estradiol and 3αHP, respectively (b). In (a) each bar (mean ± S.E.M.) represents percentage of total [^{3}H]5αP binding that was displaced by unlabeled 5αP. Displacement in cytosol and nuclei is essentially zero. (b) Exposure to estradiol (10^{-8} M) for 24 h results in marked (>3-fold) increases and exposure to 3αHP results in significant decreases in 5αP receptor numbers. Concomitant exposure to 3αHP causes significant dose-dependent decreases in estradiol-stimulated 5αP receptor numbers [20].

mined in cell lysates after SDS-PAGE protein separation and anti-MAPK (Erk1/2) antibody chemiluminescence probing. The results showed that 5αP treatment resulted in significant, dose-dependent increases in activated Erk1/2 when considered as percentage of total MAPK (Fig. 8; [23]). The maximum activation of Erk1/2 occurring at a 5αP concentration of 10^{-6} M was 2–2.5-fold higher than controls (untreated). Treatment of cells with the MEK inhibitor, PD98059, resulted in significant ($p < 0.001$) suppression of the 5αP-induced MAPK activation (Fig. 8a). Similarly, in cell replication and detachment assays, 5αP (10^{-6} M) resulted in significantly ($p < 0.001$) increased replication and detachment of MCF-7 cells, whereas PD98059 significantly suppressed ($p < 0.01$) the 5αP-induced replication and detachment Fig. 8b and c). The data suggest that the action of 5αP on breast cancer cells may involve modulation of the MAPK pathway.

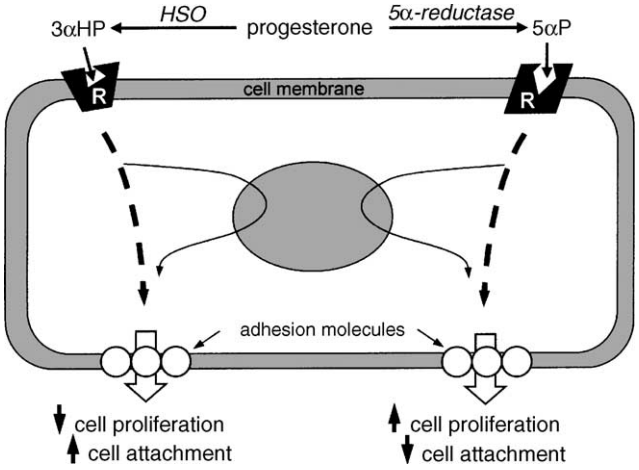

Fig. 9. Summary of the sites of action of the progesterone metabolites 5αP and 3αHP on a stylized breast cell resulting in cancer-promoting or -inhibiting alterations in cell proliferation and adhesion.

9. Potential for new diagnostics and therapeutics

Current evidence suggests that breast carcinoma is accompanied by changes in in situ progesterone metabolism which result in increased concentrations of cancer-promoting 5α-pregnanes and decreased concentrations of cancer-inhibiting 4-pregnenes. Fig. 9 summarizes our current understanding of the possible involvement of progesterone metabolites, 3αHP and 5αP, in a stylized breast cell during carcinoma progression. Local levels of 5αP are increased, whereas those of 3αHP are decreased, as a result of changes in expression and activity of 5α-reductase and 3α-HSO. Separate and specific membrane-based receptors for 5αP and 3αHP exist. The 5αP receptors are up-regulated by 5αP and estradiol and down-regulated by 3αHP. The progesterone metabolites modulate the MAP kinase pathway, the cytoskeleton and adhesion complexes in breast cell lines. The actions of 5αP result in increased cell proliferation and decreased cell attachment, whereas 3αHP actions result in decreased cell proliferation and increased cell attachment. The findings suggest new diagnostic tests based on measurements of 5α-pregnane concentrations, changes in 5α-reductase and HSO activities and gene expression, and 5αP-receptor concentrations. Therapeutic regimens might involve (a) decreasing 5αP and increasing 3αHP by blocking 5α-reductase and stimulating HSO activities and gene expression, (b) blocking the binding of 5αP to its receptor, and (c) down-regulating the 5αP receptor concentration.

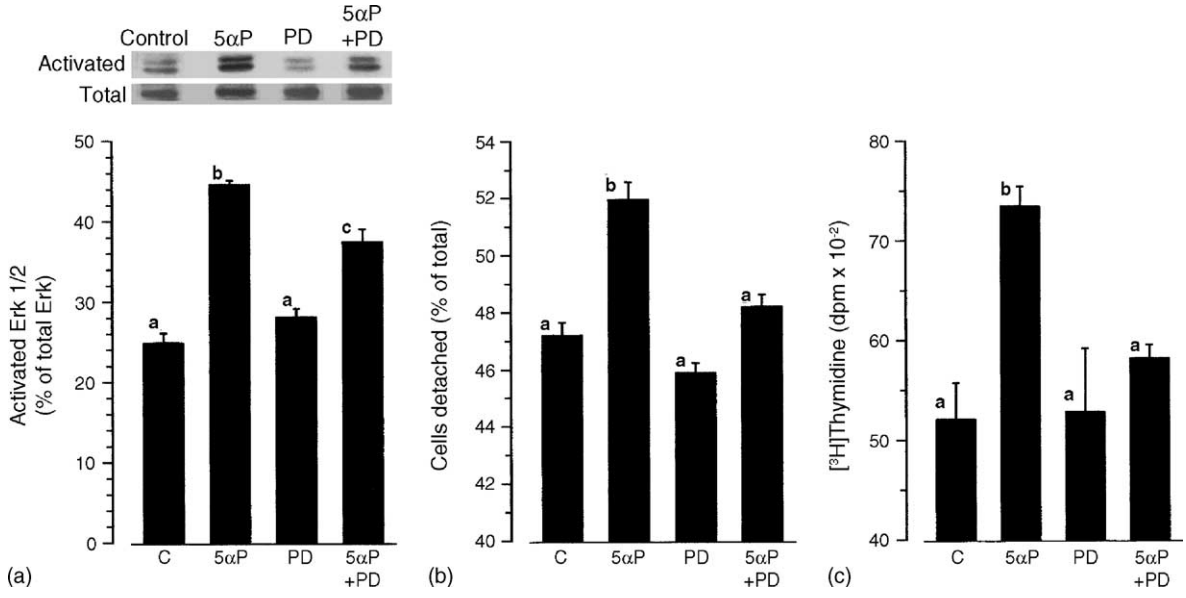

Fig. 8. The effect of 5αP and mitogen-activated protein kinase (MAPK) inhibitor, PD98059 (PD), on (a) activation of MAPK (Erk1/2), (b) detachment and (c) replication of MCF-7 cells. Different letters on bars indicate significant differences at $p < 0.05$ (from [23]).

Acknowledgements

The research was supported by grants from The Susan G. Komen Breast Cancer Foundation, The Natural Sciences and Engineering Research Council of Canada, and the Canadian Institutes of Health Research.

References

[1] J.J. Going, T.J. Anderson, S. Battersby, C.C.A. MacIntyre, Proliferative and secretory activity in human breast during natural and artificial menstrual cycles, Am. J. Pathol. 130 (1988) 193–204.
[2] M.C. Pike, D.V. Spicer, L. Dahmoush, M.F. Press, Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk, Epidem. Rev. 15 (1993) 17–35.
[3] W.L. McGuire, Prognostic factors for recurrence and survival in human breast cancer, Breast Cancer Res. Treat. 10 (1987) 5–9.
[4] J.P. Wiebe, D. Muzia, J. Hu, D. Szwajcer, S.A. Hill, J.L. Seachrist, The 4-pregnene and 5α-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion, Cancer Res. 60 (2000) 936–943.
[5] R.J. Santen, A. Manni, H. Harvey, C. Redmond, Endocrine treatment of breast cancer in women, Endocr. Rev. 11 (1990) 221–265.
[6] T.J. Anderson, S. Battersby, R.J.B. King, K. McPherson, J.J. Going, Oral contraceptive use influences resting breast proliferation, Hum. Pathol. 20 (1989) 1139–1144.
[7] Z. Saad, V. Bramwell, J. Duff, M. Girotti, T. Jory, J.G. Heathcote, I. Turnbull, B. Garcia, L. Stitt, Timing of surgery in relation to the menstrual cycle in premenopausal women with operable breast cancer, Br. J. Surg. 81 (1994) 217–220.
[8] C.L. Clark, R.L. Sutherland, Progestin regulation of cellular proliferation, Endocr. Rev. 11 (1990) 266–302.
[9] R.J.B. King, A discussion of the roles of estrogen and progestin in human mammary carcinogenesis, J. Steroid Biochem. Mol. Biol. 39 (1991) 811–818.
[10] E.A. Musgrove, R.L. Sutherland, Cell cycle control by steroid hormones, Semin. Cancer Biol. 5 (1994) 381–389.
[11] M.A. Glasier, J.P. Wiebe, R. Hobkirk, Progesterone metabolism by guinea pig intrauterine tissues, J. Steroid Biochem. Mol. Biol. 51 (1994) 199–207.
[12] J.P. Wiebe, K.J. Barr, K.D. Buckingham, A radioimmunoassay for the regulatory steroid, 3α-hydroxy-4-pregnen-20-one (3αHP), J. Steroid Biochem. Mol. Biol. 38 (1991) 505–512.
[13] M.J. Lewis, J.P. Wiebe, Expression of 5α-reductase is higher in tumorous whereas expression of 20α- and 3α-hydroxysteroid oxidoreductases is higher in matched nontumorous breast tissue biopsies, Endocr. Soc. Ann. Meet. Abstracts 85 (P1) (2003) 139–167.
[14] M.J. Lewis, J.P. Wiebe, J.G. Heathcote, Expression of progesterone metabolizing enzyme genes (AKR1C1, AKR1C2, AKR1C3, SRD5A1, SRD5A2) is altered in human breast carcinoma, BMC Cancer 4 (27) (2004) 1–12.
[15] J.P. Wiebe, M.J. Lewis, Activity and expression of progesterone metabolizing 5α-reductase, 20α-hydroxysteroid oxidoreductase and 3α(β)-hydroxysteroid oxidoreductases in tumorigenic (MCF-7, MDA-MB-231, T-47D) and nontumorigenic (MCF-10A) human breast cancer cells, BMC Cancer 3 (9) (2003) 1–15, http://www.biomedcentral.com/1471-2407/3/9.
[16] S.M. Cohen, L.B. Ellwein, Cell proliferation in carcinogenesis, Science 249 (1990) 1007–1011.
[17] A. Raz, Adhesive properties of metastasizing tumor cells, Ciba Found. Symp. 141 (1988) 109–122.
[18] J.P. Wiebe, D. Muzia, The endogenous progesterone metabolite, 5α-pregnane-3,20-dione, decreases cell-substrate attachment, adhesion plaques, vinculin expression, and polymerized F-actin in MCF-7 breast cancer cells, Endocrine 16 (2001) 7–14.
[19] P.J. Weiler, J.P. Wiebe, Plasma membrane receptors for the cancer-regulating progesterone metabolites, 5α-pregnane-3,20-dione and 3α-hydroxy-4-pregnen-20-one in MCF-7 breast cancer cells, Biochem. Biophys. Res. Commun. 272 (2000) 731–737.
[20] K.J. Pawlak, J.P. Wiebe, Plasma membrane receptors for the cancer-promoting progesterone metabolite, 5α-pregnane-3,20-dione, are modulated by other endogenous steroid hormones, Endocr. Soc. Ann. Meet. Abstracts 85 (P1) (2003) 136–166.
[21] G. Pearson, F. Robinson, G.T. Beers, B. Xu, M. Karandikar, K. Beerman, M.H. Cobb, Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions, Endocr. Rev. 22 (2001) 153–183.
[22] R.J. Santen, R.X. Sing, R. McPherson, R. Kumar, L. Adam, M.-H. Jeng, W. Yue, The role of mitogen-activated protein (MAP) kinase in breast cancer, J. Steroid Biochem. Mol. Biol. 80 (2002) 239–256.
[23] V. Cialacu, G. Zhang, J.P. Wiebe, Induced detachment of MCF-7 breast cancer cells by the progesterone metabolite, 5α-pregnane-3,20-dione, is linked to activation of the mitogen-activated protein kinase (Erk/MAPK) pathway, Endocr. Soc. Ann. Meet. Abstracts 85 (P1) (2003) 138–166.