

# Triple-Negative Breast Cancer (TNBC) Cell Metastasis is Controlled by the Opposing Actions of 5alpha-dihydroprogesterone (5alphap) and 3alpha-dihydroprogesterone (3alphahp) on Expression of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases

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## Research article

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# Abstract

## Background

Triple receptor-negative breast cancers (TNBC) comprise up to 20% of all breast cancers and are the most aggressive mammary cancer subtype, marked by early-onset metastasis, high tumor recurrence rate, and low overall survival. Because TNBC cells lack expression of the estrogen receptor (ER), progesterone receptor (PR) and excess human epidermal growth factor receptor 2 (HER2), there have been no hormone-based explanations and therapeutics for TNBC. Our previous studies had shown that the endogenously produced hormones, 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ P) and 3 $\alpha$ -dihydroprogesterone (3 $\alpha$ HP), respectively, stimulate and inhibit TNBC cell proliferation and primary tumor development. Here we examined the role of 5 $\alpha$ P and 3 $\alpha$ HP in controlling metastasis of TNBC (MDA-MB-231) human breast cells. The objectives were to determine the effects of 5 $\alpha$ P and 3 $\alpha$ HP on TNBC cell (a) adhesion, migration and invasion and (b) expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).

## Methods

MDA-MB-231 human breast cells were incubated in medium without (control) or with 5 $\alpha$ P or 3 $\alpha$ HP. Effects on adhesion were examined by previously validated detachment assays. Effects on migration and invasion were measured by transwell assays employing modified Boyden chambers. Effects on expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 were determined by reverse transcription polymerase chain reaction (RT-PCR).

## Results

5 $\alpha$ P stimulated, whereas 3 $\alpha$ HP suppressed, TNBC cell detachment, migration and invasion. 5 $\alpha$ P stimulated expression of MMP-2 and MMP-9, and inhibited expression of TIMP-1 and TIMP-2, whereas 3 $\alpha$ HP inhibited expression of MMPs and stimulated expression of TIMPs.

## Conclusions

Here we show for the first time that 5 $\alpha$ P stimulates and 3 $\alpha$ HP suppresses the key elements of metastasis (adhesion, migration, invasion) in TNBC cells, and that these opposing actions correlate with the opposing effects of these two hormones on MMP and TIMP expression. The results indicate that the relative concentrations of the cancer-promoter hormone (5 $\alpha$ P) and the cancer-suppressor hormone (3 $\alpha$ HP) in the breast microenvironment control TNBC cell metastasis, and suggest that blocking 5 $\alpha$ P synthesis and increasing 3 $\alpha$ HP concentrations in the breast microenvironment could provide the first hormone-based therapeutic regimen to arrest TNBC progression and metastasis.

## Background

Breast cancer is the most frequently diagnosed cancer in women [1] and triple-negative breast cancer (TNBC), the subtype which tests negative for expression of the estrogen receptor (ER), progesterone

receptor (PR) and excess human epidermal growth factor receptor 2 (HER2) [2] comprises up to 20% of all breast cancers [2,3]. TNBC is the most aggressive mammary cancer subtype, marked by early-onset metastasis, high tumor recurrence rate, and low overall survival [4,5,6]. The etiology of TNBC is not understood and the lack of receptor expression means that the current hormone-based targeted therapies which are applicable for receptor-positive breast cancers are ineffective, and the only proven method for systemic management of TNBC is chemotherapy [7,8], which provides only limited survival for the patients, along with considerable untoward side-effects. There is, therefore, an urgent need to understand the cause(s) and regulation of TNBC, in particular the progression to metastasis, which is the leading cause of death from all breast cancers.

Previous studies demonstrated that breast tissues [9] and human breast cell lines [10] readily convert progesterone to 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ P) and 3 $\alpha$ -dihydroprogesterone (3 $\alpha$ HP) by the actions, respectively, of 5 $\alpha$ -reductase and 3 $\alpha$ -HSO (Figure 1) and that tumors and tumorigenic breast cell lines produce more 5 $\alpha$ P and less 3 $\alpha$ HP than normal (non-tumorous) breast tissue and non-tumorigenic breast cell lines, due to up-regulation of 5 $\alpha$ -reductase expression and down-regulation of 3 $\alpha$ -HSO expression [9-12]. Particularly noteworthy with respect to the present studies are the prominent differences in expression between adjacent triple receptor-negative normal and tumor breast tissues [11].

*In vitro* studies with various breast cell lines, including MDA-MB-231 cells (which have been characterized as human TNBC cells [13]), showed that 5 $\alpha$ P acts as a breast cancer-promoter hormone by stimulating cell proliferation and inhibiting apoptosis and adhesion, whereas 3 $\alpha$ HP acts as a cancer-suppressor hormone by having the opposite effects [9,14]. The pro-cancer and anti-cancer actions, respectively, of 5 $\alpha$ P and 3 $\alpha$ HP were confirmed with *in vivo* xenograft studies [15] in which MDA-MB-231 cells were implanted in mammary fat pads of immunosuppressed mice treated with 5 $\alpha$ P and/or 3 $\alpha$ HP. The results showed that 5 $\alpha$ P stimulated, whereas 3 $\alpha$ HP inhibited, initiation and subsequent growth of primary human TNBC cell tumors; in addition, 3 $\alpha$ HP resulted in regression of tumors that were already established.

Having demonstrated that TNBC cell proliferation and the onset and growth of primary TNBC tumors are controlled by the relative concentrations of 5 $\alpha$ P and 3 $\alpha$ HP in the microenvironment, it was of interest to determine the effects of 5 $\alpha$ P and 3 $\alpha$ HP on metastasis, the process that involves the escape of activated tumor cells from the neoplasm followed by migration to, and invasion of, distant locations where they form the malignant growths which are the primary cause of cancer deaths [16]. The metastatic cascade depends on changes in adhesion properties between cells and between cells and the extracellular matrix (ECM) [17], and the changes in adhesion involve recruitment of proteases to degrade the ECM, basement membrane and cell junctions. The matrix metalloproteinases (MMPs) are the main proteases involved in remodelling the ECM [18,19] and thus have a direct role in potentiating migration and invasion of metastatic cells [19,20]. The MMPs are secreted in a latent form, bound to specific inhibitors called the tissue inhibitors of metalloproteinases (TIMPs) [21]. Activation of MMPs results when the TIMPs are proteolytically cleaved from them. Since the TIMPs inhibit the enzymatic activity of the MMPs, the balance between the level of active MMPs and available TIMPs is a pivotal determinant of ECM turnover

[22]. Consequently, factors that result in changes in expression levels of MMPs and/or TIMPs can affect adhesive, migratory and invasive properties [ 23,24] and correlate with breast cancer prognostic factors [25].

The objectives of the current studies were to determine the effects of 5 $\alpha$ P and 3 $\alpha$ HP on triple-negative (MDA-MB-231) breast cells in terms of (a) adhesion, migration and invasion and (b) expression of MMP-2, MMP-9, TIMP-1 and TIMP-2. Here we show for the first time that 5 $\alpha$ P stimulates, whereas 3 $\alpha$ HP inhibits, receptor-negative breast cell detachment, migration and invasion, and that these opposing actions are via opposing effects of these two hormones on MMP and TIMP expression. The results provide further evidence that the hormones, 5 $\alpha$ P and 3 $\alpha$ HP, play important roles in stimulating or suppressing various stages leading to deregulation from the normal state to onset and growth of primary tumors and to development of metastatic lesions of TNBC. The results also indicate that therapeutics based on raising the 3 $\alpha$ HP:5 $\alpha$ P concentration ratio in the microenvironment have the potential to suppress TNBC progression and metastasis.

## Methods And Materials

### Chemicals

The steroid hormones, 3 $\alpha$ HP and 5 $\alpha$ P, were obtained from Steraloids Inc (Wilton, N.H., USA). Cell culture media, insulin, penicillin and streptomycin were obtained from Sigma Chemical Co. (Oakville, ON). Calf serum was purchased from Invitrogen (Burlington, ON). Other chemicals were of analytical grade and purchased from Sigma Chemical Co., BDH Inc., (Toronto, ON) VWR (Mississauga, ON) or Fisher Scientific Ltd., (Toronto, ON). Ethanol was double glass distilled.

### Cell Culture

The human breast cell line, MDA-MB-231, was obtained from the American Type Culture Collection (Rockville, MD). The cells were grown in a 1:1 Ham's F12 Medium and Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM glutamine, 100 units/ml penicillin, 78 units/ml streptomycin, 1.2 mg/ml sodium bicarbonate and 10% full calf serum as previously described [14]. Cells were grown in T-75 flasks (Sarstedt) in a humidified incubator at 37°C and a 5% CO<sub>2</sub> atmosphere, and were harvested at approximately 80% confluence using 0.025% trypsin and EDTA (0.2 M) in phosphate-buffered saline (PBS) solution (pH 7.3).

**Cell adhesion.** Cell adhesion was determined by a detachment assay described previously [9]

### Cell Migration and Invasion Assays

Cell migration and invasion transwell assays were carried out in modified Boyden chambers [26,27] using 24-well cell culture plates with inserts containing 8  $\mu$ m pore polycarbonate membranes (BD Biosciences, Canaan, CT). The treatment media contained dextran-coated charcoal-treated (DCC-treated)

calf serum at 2% for 5αP studies, and 5% for 3αHP studies along with double distilled ethanol at a final concentration of 0.1% without (control) or with either 5αP ( $10^{-6}$ M) or 3αHP ( $10^{-6}$ M), respectively. The reason for using higher (5%) serum concentrations for the 3αHP studies, was to have higher numbers of migrating/invading cells in the controls, thereby facilitating better detection of the suppressive effects of 3αHP.

**(a) Migration.** Migration was defined as the ability of cells to move through the 8 μm pores. Cells were seeded at  $1 \times 10^5$ /200 μl of treatment medium in each of the upper chambers and 800 μl of the respective treatment medium was added to the bottom wells. After incubation of the plates for 6 hours (37°C; 5% CO<sub>2</sub>), non-migrated cells were gently removed from the top of the filter using cotton swabs. The bottom of each filter was gently rinsed with Earl's Balanced Salt Solution (BSS) and the cells that had migrated to the lower surface of the polycarbonate membrane were fixed in 2% formaldehyde and stained with Giemsa stain. The filters were detached from the bottom of the inserts, mounted on glass slides, and viewed microscopically. The number of migrated cells per well was determined by counting the cells within a standardized grid of 16 equal-sized fields, which together covered about 90% of the membrane surface. Each treatment consisted of 4 or 5 replicate wells and experiments were repeated at least three times.

**(b) Invasion.** Invasion was defined as the ability of cells to penetrate the 8 μm pores of polycarbonate membranes coated with 2.5 mg/mL of matrigel (BD Biosciences, Canaan, CT), a reconstituted basement membrane extract of the Englebreth-Holm-Swarm mouse sarcoma [28]. The gel was allowed to polymerize for 1h at room temperature. Then  $1 \times 10^5$  cells in 200 μl of respective treatment medium were seeded in the upper chambers and 800 μl of the same treatment medium was added to the bottom wells. Incubation was for 24 hours at 37°C, and cells having invaded the bottom of the polycarbonate membrane were scored as described above for migration. Each treatment consisted of 4 or 5 replicate wells and experiments were repeated at least three times.

### **MMP and TIMP expression studies**

Cells were seeded in 35 mm culture dishes (Falcon) at  $5 \times 10^4$  cells per dish in the respective control/treatment media and allowed to attach for 24 hours. They were then incubated for 48 hours in medium containing serum at 5% for the 3αHP studies and 2% for the 5αP studies. After 48 hours cells were harvested and RNA was isolated.

**RNA Isolation & Reverse Transcription.** RNA was extracted from the cells using RNeasy Mini kit (Qiagen) following the manufacturer's protocol. Complimentary DNA (cDNA) was obtained from 10 μl of RNA using Oligo(dT) (Invitrogen), SuperScript II Reverse Transcriptase (Invitrogen) in a total volume of 20 μl following the manufacturers protocol.

**RT-PCR and Quantification.** RT-PCR was performed on a GeneAmp PCR System 9600 (Perkin Elmer) in triplicate. PCR primers were purchased from Invitrogen with the sequences given in Table 1. PCR

conditions were as follows: 95°C denaturing for 2 minutes followed by 30 seconds of annealing at 42°C followed by extension for 1 minute and 30 seconds at 72°C. For each reaction, a 1 µl aliquot of cDNA product was amplified in a 50 µl total volume using 1 U of Platinum Taq DNA Polymerase (Invitrogen), 50 mM MgCl<sub>2</sub>, 10 mM dNTP's, and 4 µl of each primer. The same cycling conditions and PCR reagent concentrations were used for each set of primers. PCR products were confirmed by separation on a 1.5% agarose gel and quantification using the Quantity One (BioRad) program.

### ***Statistics***

Results are given as mean ± SEM. Data were analyzed by unpaired Student's *t*-test with InStat software (GraphPad, San Diego, CA). Differences were considered significant if *p* < 0.05.

**Table 1. PCR primers (upstream; downstream) used in the MMP and TIMP expression studies.**

Name	Primer sequence
MMP-2	5'-GCT GGC TGC CTT AGA ACC TTT C-3' 5'-GAA CCA TCA CTA TGT GGG CTG AGA-3'
MMP-9	5'-GCA CGA CGT CTT CCA GTA CC-3' 5'-GCA CTG CAG GAT GTC ATA GGT-3'
TIMP-1	5'-CTG CGG ATA CTT CCA CAG GT-3' 5'-GTT TGC AGG GGA TGG ATA AA-3'
TIMP-2	5'-GAT GCA CAT CAC CCT CTG TG-3' 5'-GTG CCC GTT GAT GTT CTT CT-3'

## **Results**

### **Effects of 5aP and 3aHP on proliferation and cell adhesion.**

Proliferation studies (Figure 2A) showed that 5aP dose-dependently stimulated, and 3aHP inhibited, [<sup>3</sup>H]thymidine incorporation, confirming that the MDA-MB-231 cells used for this study were responsive to 5aP and 3aHP as previously reported [14]. The effects of 5aP and 3aHP on adhesion were determined by detachment assays (Figure 2B) which showed that detachment is dose-dependently increased by 5aP, and decreased by 3aHP.

## Effects of 5αP and 3αHP on cell migration and invasion

Figure 3 shows representative microscopic fields of undersides of Boyden chamber membranes from control (Figure 3Ai) and 5αP-treated (Figure 3Aii) MDA-MB-231 cells, indicating the 8μm membrane pores and cells that have migrated through the pores and attached to, and spread on, the underside, which were scored as 'migrated'. 5αP resulted in significant increases (Figure 3B), whereas 3αHP resulted in significant decreases (Figure 3C), in number of migrated cells. Similarly, 5αP increased, whereas 3αHP decreased, the invasive activities of the cells (Figures 3D & 3E).

## Effects of 5αP and 3αHP on expression of MMPs and gelatinase activity.

Since MMPs are the main proteases involved in remodelling the ECM and thus have a direct role in migration and invasion, we examined the effects of 5αP and 3αHP on expression levels of MMP-2 and MMP-9.

The results showed that 5αP increased expression of MMP-2 and MMP-9 (Figures 4A & 4C), whereas 3αHP decreased expression of MMP-2 and MMP-9 (Figures 4B & 4D). As a measure of the active proteolytic MMP levels, gelatinase expression was determined; 5αP resulted in stimulation (Figure 4E), while 3αHP resulted in suppression (Figure 4F) of gelatinase expression.

## Effects of 5αP and 3αHP on expression of TIMPs

Since TIMPs inhibit the enzymatic activity of MMPs, a decrease in TIMP expression can lead to increased MMP activity and *vice versa*. Results from experiments to determine the effects of 5αP and 3αHP on MDA-MB-231 cell expression of TIMP-1 and TIMP-2 are shown in Figure 5. 5αP suppressed (Figures 5A & 5C), whereas 3αHP stimulated (Figures 5B & 5D) expression of TIMP-1 and TIMP-2.

## Discussion

Previous studies showed that proliferation of TNBC cells *in vitro* [14], and initiation and growth of primary TNBC tumors *in vivo* [15] are stimulated by 5αP and inhibited by 3αHP. The opposing effects on TNBC cell proliferation result from opposing actions of 5αP and 3αHP on breast cell mitosis and apoptosis [14]. The findings described here show for the first time that 5αP stimulates, whereas 3αHP suppresses, TNBC cell detachment, migration and invasion, the cardinal processes involved in cancer cell metastasis. In addition, the studies show that these opposing effects are directly correlated with the opposing effects of 5αP and 3αHP on expression and activation of MMPs, which regulate adhesion properties between cells and between cells and ECM, and thereby control the changes in migration and invasion. Corroborating our findings are results from other studies which have shown that up-regulation of MMPs facilitates increased migration and invasion [23, 29- 31], whereas down-regulation of MMPs by anti-cancer and/or anti-metastatic agents inhibits cell migration and invasion [24,32,33].

The relevance of the findings that 5αP stimulates and 3αHP inhibits the deregulation of TNBC cells from a state of normalcy to one of tumorigenesis and metastasis, is enhanced by observations that TNBC

tissues [9] and tumorigenic cells [14] convert more progesterone to 5 $\alpha$ P, and less to 3 $\alpha$ HP, than normal tissue and nontumorigenic breast cells, as a result of higher expression of 5 $\alpha$ -reductase (SRD5A1; SRD5A2) and lower expression of 3 $\alpha$ -HSD (AKR1C2; AKR1C3) genes [10; 11]. The resulting increases in 5 $\alpha$ P:3 $\alpha$ HP concentration ratios in the breast microenvironment correlate with the observed increases in TNBC cell mitogenic and metastatic activities [9, 15].

### ***Molecular mechanisms of action of 5 $\alpha$ P and 3 $\alpha$ HP***

The opposing actions of 5 $\alpha$ P and 3 $\alpha$ HP can be explained, in part, by studies which have identified separate receptors for 5 $\alpha$ P (5 $\alpha$ PR) and 3 $\alpha$ HP (3 $\alpha$ HPR) and their interactions with cell signalling cascades. The 5 $\alpha$ PR and 3 $\alpha$ HPR are located on plasma membranes of breast cells [34]. They exhibit high specificity and are distinct from each other and from known receptors of other steroid hormones [34,35]. Binding of 5 $\alpha$ P to its receptor results in activation of MAPK/ERK [35] which is known to upregulate MMP expression/activity [36,37] leading to decreases in adhesion plaques, vinculin expression and polymerized actin [38] and promoting increased detachment, migration and invasion [39-40]. On the other hand, 3 $\alpha$ HP can inhibit protein kinase C (PKC) activity ([41], and unpublished results) which, in turn, can lead to suppression of MMP activation, and the resulting inhibition of cell invasion and migration [42, 43].

## **Conclusions**

Based on previous and current findings we propose the following model (Fig. 6) to explain how 3 $\alpha$ HP and 5 $\alpha$ P, can regulate the adhesive, migratory, and invasive processes integral to metastasis of triple- negative breast cancers. 3 $\alpha$ HP and 5 $\alpha$ P, derived from autocrine and/or paracrine sources, couple with their respective plasma membrane-based receptors, triggering suppression or activation, respectively of PKC or MAPK signaling pathways. Binding of 5 $\alpha$ P to its receptor activates the MAPK/ERK1/2 signaling cascade resulting in increased expression of MMPs and/or decreased expression of TIMPs, leading to stimulation of proteolytic degradation of the ECM and providing enhanced ability of cells to detach, migrate and invade. Binding of 3 $\alpha$ HP to its receptor results in down regulation of PKC activity which leads to decreased expression of MMPs-2/9 and/or increased expression of TIMPs-1/2, resulting in decreased proteolytic degradation of the surrounding ECM, thereby suppressing cell detachment, migration, and invasion. The relative concentrations of 3 $\alpha$ HP and 5 $\alpha$ P in the microenvironment determine whether local clusters of breast cells maintain a state of "normalcy" or proceed toward metastasis. A higher 3 $\alpha$ HP-to-5 $\alpha$ P concentration ratio promotes normalcy, whereas a higher 5 $\alpha$ P-to-3 $\alpha$ HP concentration ratio promotes mitogenesis and metastasis of TNBC. The findings suggest (a) that tests which measure the relative concentrations of 5 $\alpha$ P:3 $\alpha$ HP in a patient's blood, saliva or tissue samples could serve as prognostics/diagnostics for the status of a TNBC patient, and (b) that blocking synthesis of 5 $\alpha$ P and increasing 3 $\alpha$ HP concentrations in the breast microenvironment can provide a hormone-based therapeutic measure to block TNBC onset, progression and metastasis.

## **Abbreviations**

**TNBC:** triple-negative breast cancer; **ER:** estrogen receptor; **PR:** progesterone receptor; **5alphaP:** 5alpha-pregnane-3,20-dione (5alpha-dihydroprogesterone); **3alphaHP:** 4-pregnen-3alpha-ol-20-one (3alpha-dihydroprogesterone); **MAPK:** mitogen-activated protein kinase; **PKC:** protein kinase C; **MMP:** matrix metalloproteinase; **TIMP:** tissue inhibitor of metalloproteinase

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

### Competing interests

The authors declare that they have no competing interests.

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### Author's contributions

JPW conceived the study, created the study design, performed data analyses, assisted in preparation of the figures and drafted the manuscript. JJ assisted in the study design, performed the migration and invasion studies, data analyses and assisted in manuscript editing. AK performed the MMP and TIMP expression studies and assisted in data analyses and manuscript preparation.

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## Figures

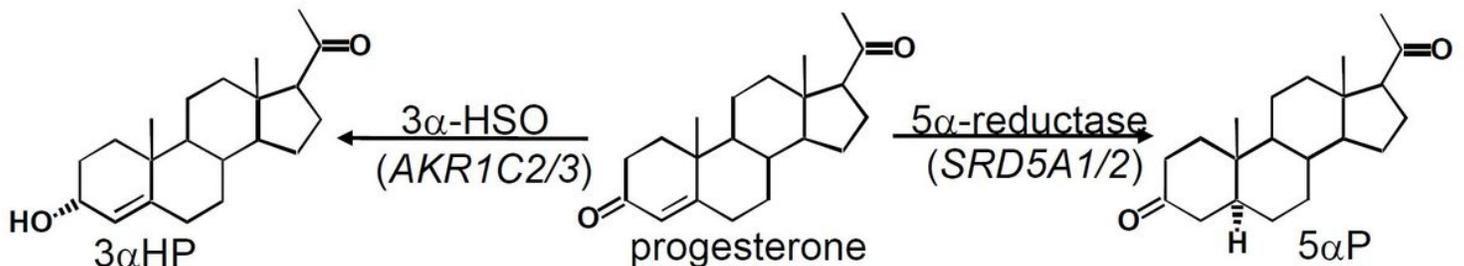
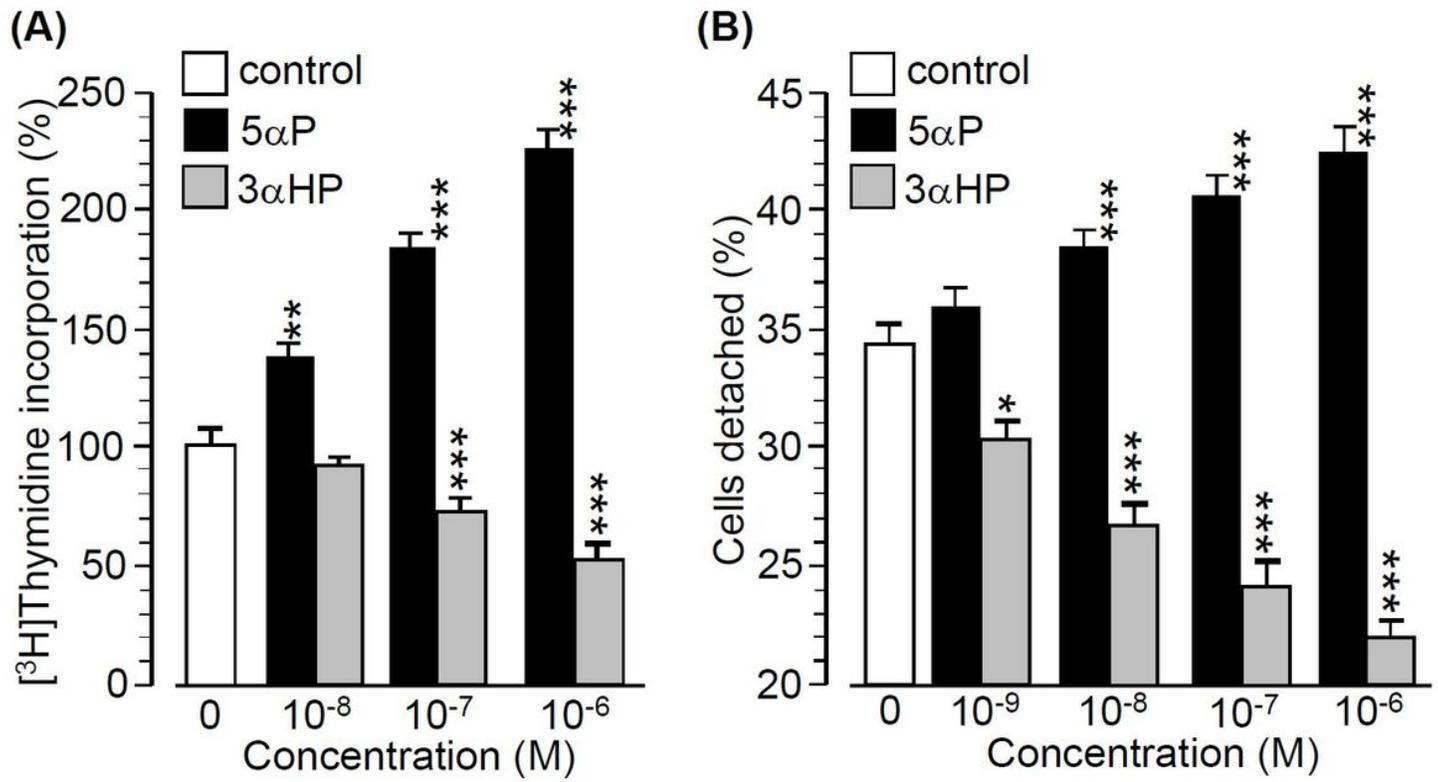


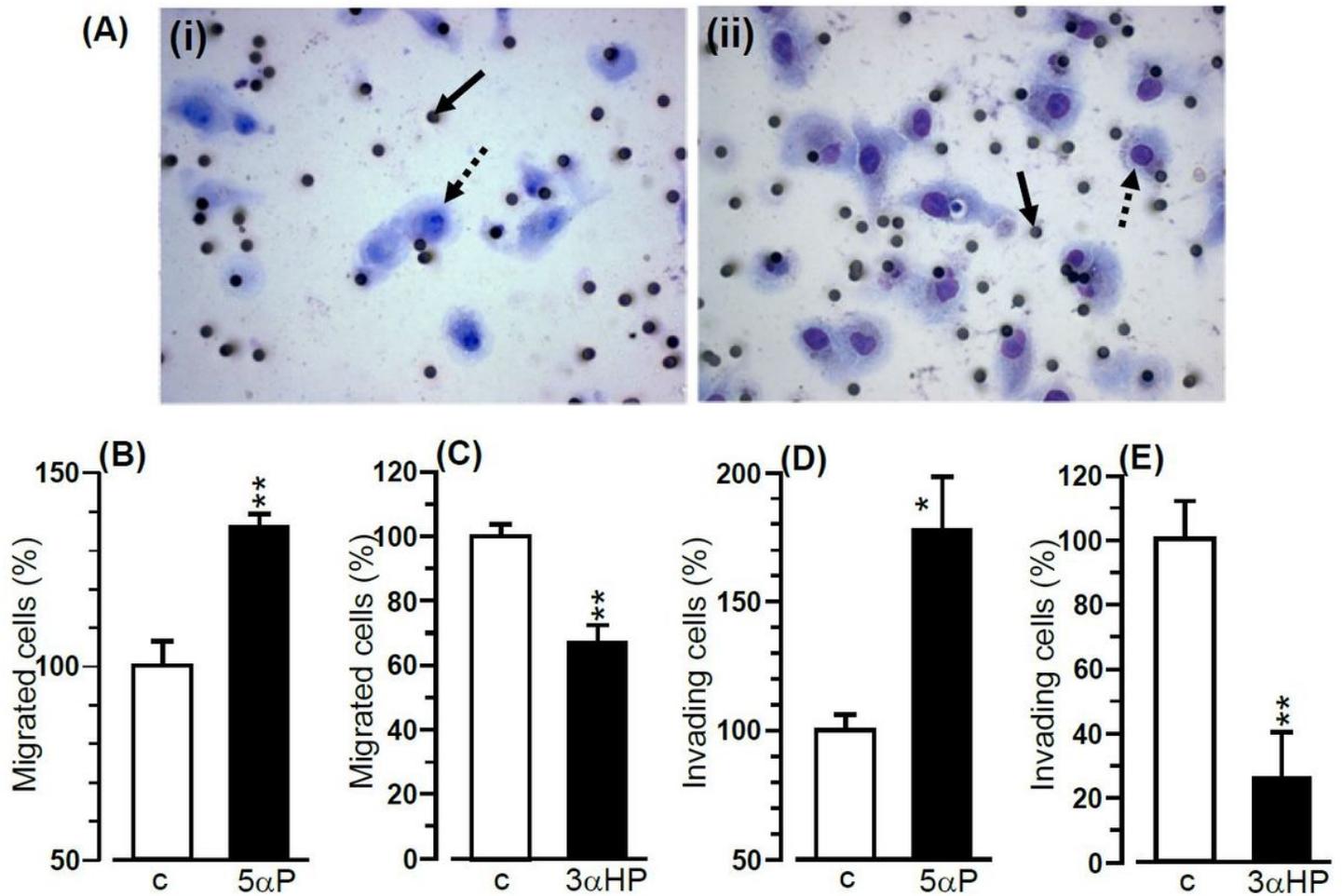
Figure 1

TNBC (MDA-MB-231) cells convert progesterone to 3 $\alpha$ HP and 5 $\alpha$ P by the actions of 3 $\alpha$ -hydroxysteroid oxidoreductase (3 $\alpha$ -HSD) and 5 $\alpha$ -reductase (5 $\alpha$ -R) expressed by the genes AKR1C2/3 and SDR5A1/2, respectively



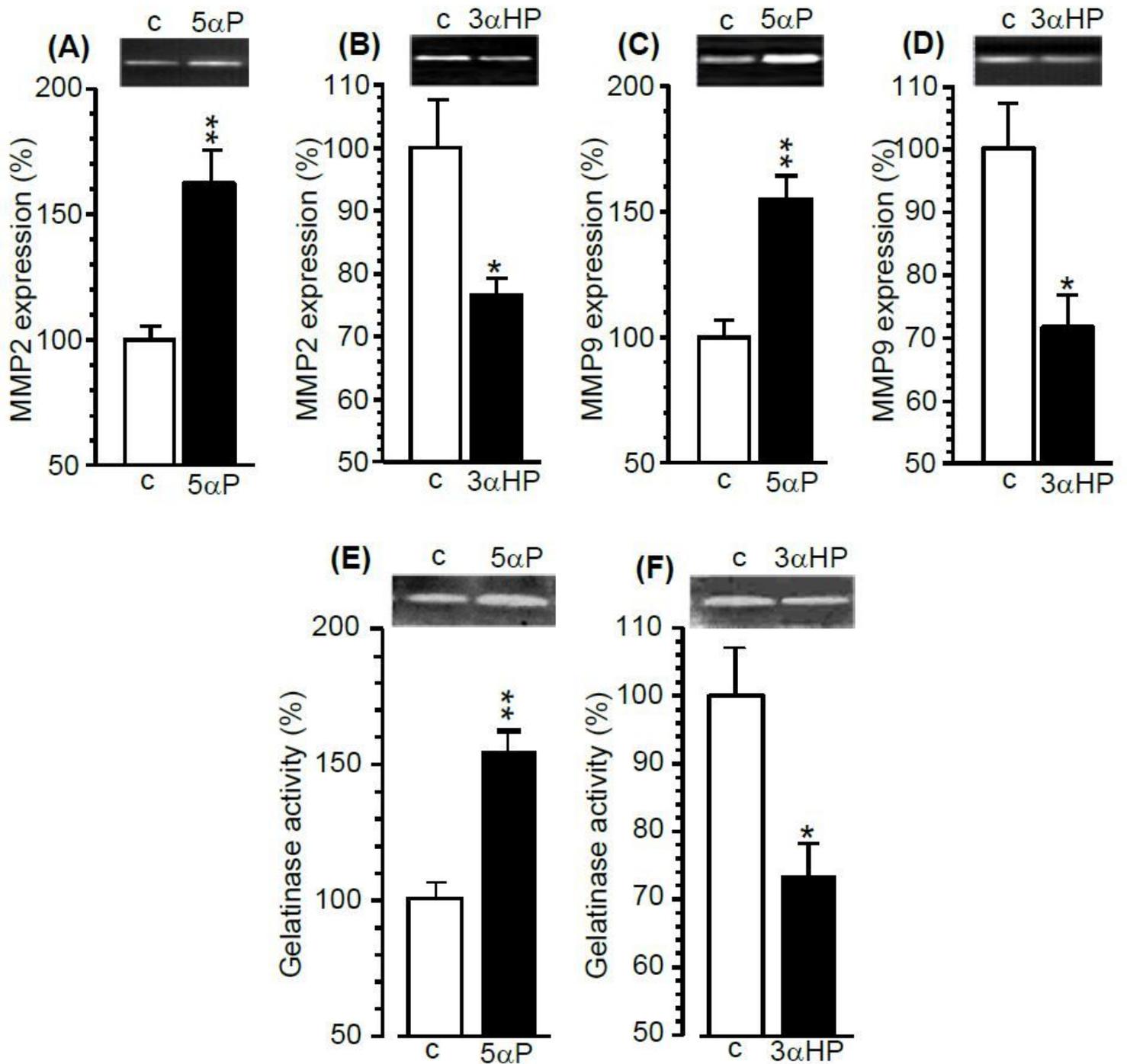
**Figure 2**

Dose-dependent effect of 5 $\alpha$ P and 3 $\alpha$ HP on proliferation (A) and adhesion (B) of MDA-MB-231 cells. Cells were exposed to 0 (control), 10<sup>-8</sup>, 10<sup>-7</sup>, or 10<sup>-6</sup> M of either 3 $\alpha$ HP or 5 $\alpha$ P. Proliferation was determined by [3H]thymidine incorporation, and adhesion was determined by a cell detachment assay as described in [9]. Results are presented as mean  $\pm$  SEM, calculated as percent in relation to control from 4 separate experiments, each treatment having 4-6 replicates. \*, \*\*, \*\*\*, significantly different from controls at p < 0.05, p < 0.01, p < 0.001, respectively.



**Figure 3**

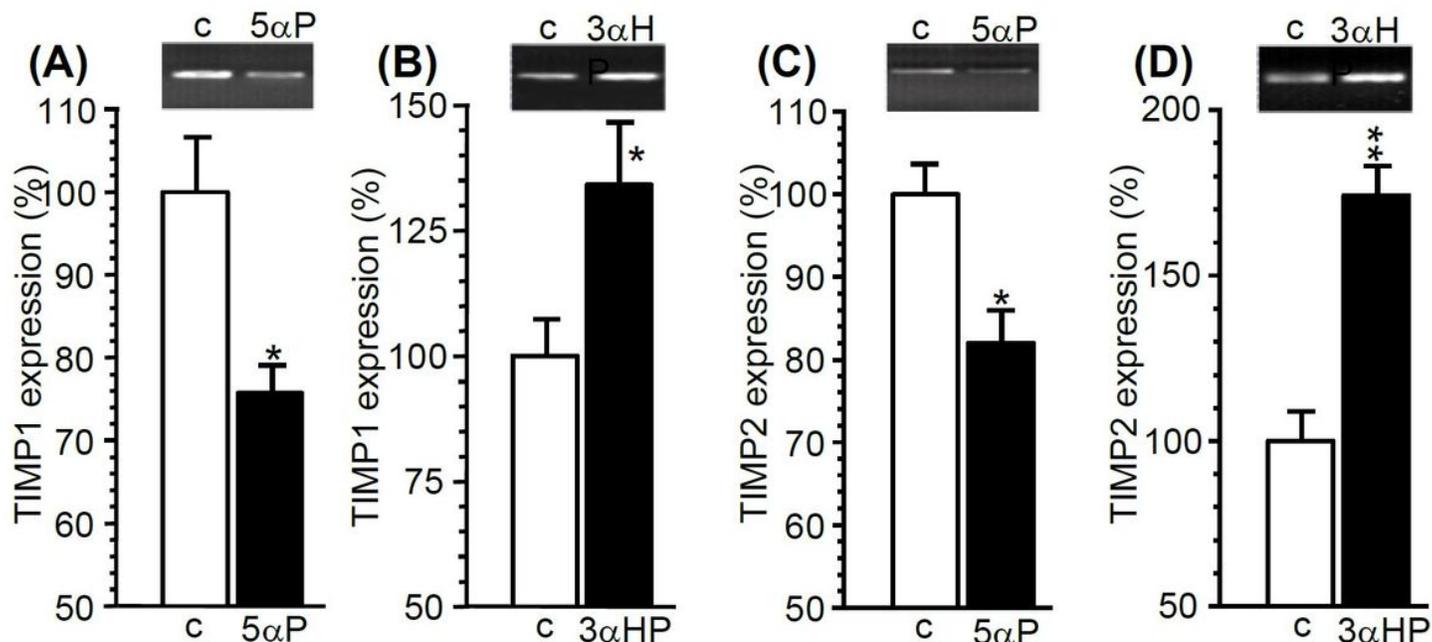
Effect of 5αP and 3αHP on MDA-MB-231 cell migration and invasion. (A) Photomicrographs of sample areas of porous polycarbonate membranes showing migrated control (Ai) and 5αP-treated (Aii) cells. Solid arrows point to the 8 μm pores in the membrane, and dashed arrows point to cells that have migrated through pores. Cells were incubated for 72h without (control, c) or with either 10-6M 5αP or 3αHP, and then transferred to the modified Boyden transwell chambers and effects on migration (B & C) and invasion (D & E) were quantified as described in Methods. Each bar and line represents the number of cells (mean ± SEM) having migrated or invaded, standardized to 100% for controls, from 3 independent experiments with 3-5 replicates per treatment. \*, \*\*, significantly different from controls at  $p < 0.05$ , and  $p < 0.01$ , respectively.



**Figure 4**

Effect of 5αP and 3αHP on expression of MMP-2 (A & B) and MMP-9( (C & D) and on gelatinase activity (E & F). For the MMP-2 and MMP-9 expression studies, cells were incubated for 48 h in medium without (control, c) or with either 5αP or 3αHP (at 10<sup>-6</sup> M), RNA was isolated, and RT-PCR quantification was as described in Methods. For the gelatinase activity studies (E & F), cells were cultured for 24 hours in serum-free medium with/without 5αP or 3αHP, medium was collected, concentrated and aliquots subjected to gelatin zymography and bands quantified as described in Methods. Data for the MMP-2 and MMP-9 expression studies are from 4 independent experiments, with 4 replicates in each. Data for the gelatinase

activity studies are from 3 replicate incubations. Each bar and line represent the mean  $\pm$  SEM, standardized to 100% for controls. Representative gels are shown above each graph. \*, \*\*, significantly different from controls at  $p < 0.05$ , and  $p < 0.01$ , respectively.



**Figure 5**

Effect of 5 $\alpha$ P and 3 $\alpha$ HP on expression of TIMP-1 (A & B) and TIMP-2 (C & D). Cells were incubated in medium without (control, c) or with either 5 $\alpha$ P or 3 $\alpha$ HP (at  $10^{-6}$  M) for 48 h, RNA was isolated, and RT-PCR quantification was as described in Methods. Each bar and line represents the mean  $\pm$  SEM of expressed TIMP-1 or TIMP-2 standardized to 100% for controls. Data are from 4 independent experiments, with 4 replicates in each. Representative RT-PCR gels are shown above each figure. \*, \*\*, significantly different from controls at  $p < 0.05$  and  $p < 0.01$ , respectively.

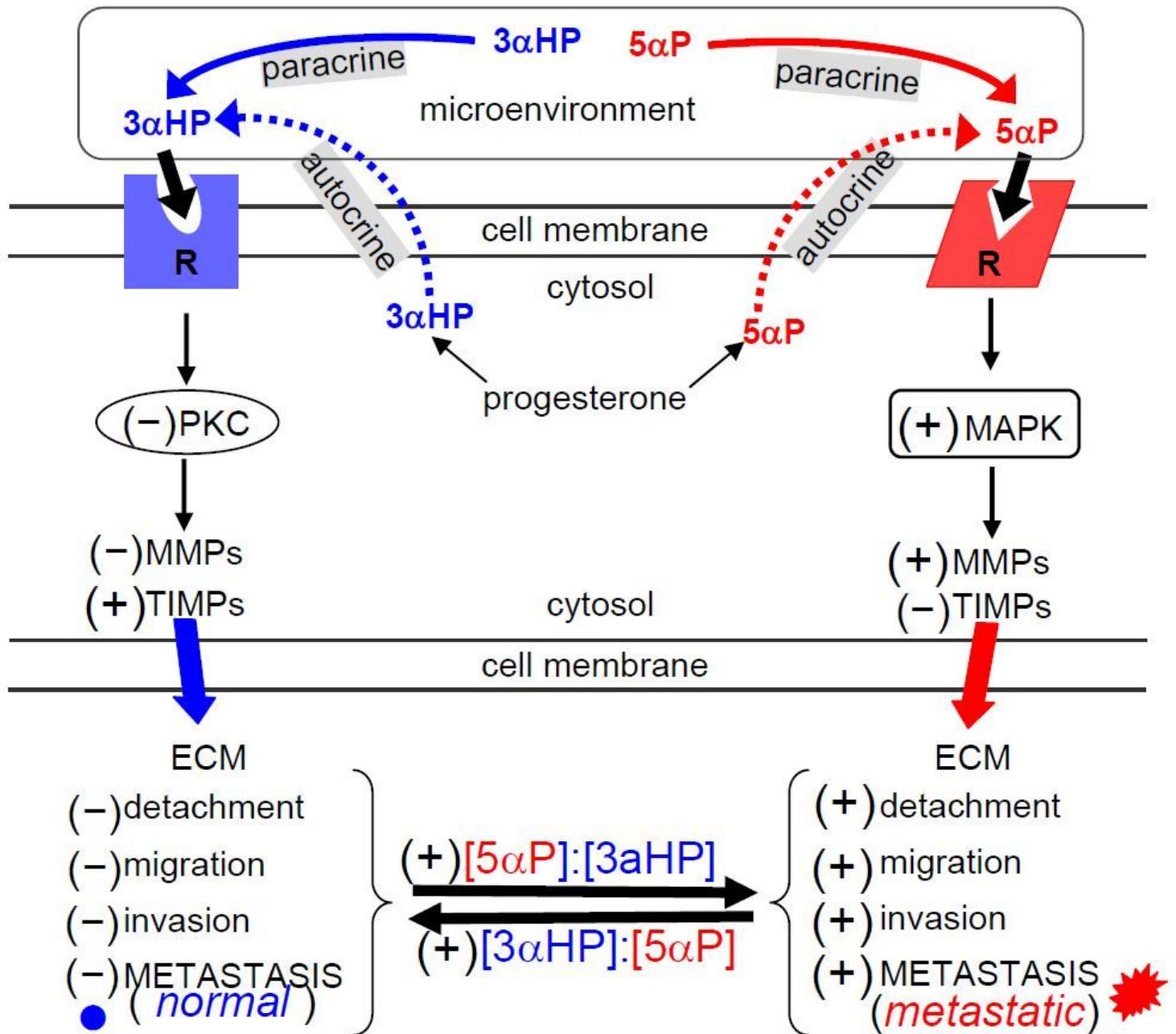


Figure 6

Summary of opposing effects of 3αHP and 5αP on expression of MMP-2/9 and TIMP-1/2 leading to opposing effects on TNBC cell metastatic actions (detachment, migration and invasion). 3αHP suppresses (-), whereas 5αP promotes (+), metastasis. A higher 5αP-to 3αHP concentration ratio in the microenvironment promotes metastasis, whereas a higher 3αHP-to 5αP concentration ratio suppresses metastasis and promotes normalcy. (See text for details)