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Progestins induce catalase activities in breast cancer cells through PRB isoform: Correlation with cell growth inhibition

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ABSTRACT

Reactive oxygen species (ROS) have been suggested to participate in tumor emergence due to their mitogenic and apoptotic signaling, and as contributors to DNA structural damage. Here we report that progesterone and various synthetic steroids with progestin potencies (norethisterone acetate, MPA, and Tibolone) counteract cell growth induced by hydrogen peroxide (H_2O_2), through a potent induction of catalase activities, in breast cancer cells and normal human epithelial breast cells. At physiological concentrations, progesterone and the pure progestin, Org2058, displayed the most potent H_2O_2 detoxification ability suggesting its effect was characteristic of its progestin potency. We also report on the enhancement of catalase activities by progesterone receptor isoform B (PRB), as determined from experiments using antiprogestins and MDA-MB-231, cells engineered for the selective expression of progesterone receptor isoform A or B. The potent action of progesterone on catalase activities indicates its contribution to a beneficial role in breast cell homeostasis.

1. Introduction

ROS are known to severely affect cellular proliferation and apoptosis regulation [1,2]. ROS include multiple molecular species (H_2O_2, peroxides, hydroxyl radicals, nitric oxide) and are continuously generated in mammalian cells as a consequence of aerobic respiration. The initiation of ROS generation occurs through the reduction of oxygen to produce superoxides (O_2^-). Superoxides can spontaneously dismutate into H_2O_2 or under the control of superoxide dismutase (SOD). The main step in H_2O_2 cellular detoxification relies upon catalase activities, where these enzymes transform H_2O_2 into H_2O and O_2. ROS are also involved in growth factor transduction pathways through the alteration of protein-tyrosine phosphorylation, and modulate the function of specific proteins involved in apoptosis, such as p53, and caspases [3,4]. The external addition of low concentrations of H_2O_2 and/or O_2^- were shown to promote cell growth in various cell types (fibroblasts, smooth muscle cells, aortic endothelial cells) [5,6] whereas over expression of catalase or SOD inhibits cell proliferation and cell death [6,7]. ROS are involved in tumorigenesis initiation and progression caused by the disruption of these pathways and its direct DNA toxicity [8,9].

A number of studies concerning ROS concentration and their catabolism indicate a direct role of ROS in breast cancer progression. Large quantities of hydrogen peroxide (H_2O_2) are released, most likely derived from superoxide, in breast cancer cells [10]. Serum markers for oxidative DNA damage were shown to increase in women diagnosed with breast cancer [9]. More recently, the detoxification pathway, particularly catalase expression and activation, was associated with the control of breast cancer progression. For example, acatalasemic and hypocatalasemic mice, which have drastically decreased catalase levels in the blood and tissues, are more susceptible to mammary carcinoma than their wild type counterparts [11]. In humans, a C/T polymorphism at base pair 262 was identified in the promoter region of the catalase gene (CAT) with higher catalase activities with the CC genotype. The authors observed that the CC allele was linked with an overall 17% reduction in risk of breast cancer as compared to its T variant. This effect was even more pronounced in women with high consumption of fruits and vegetables [12]. Another recent publication, showed an increased risk related to HRT use in women with the CT or TT CAT genotypes [13]. In addition, estradiol decreases catalase activities involved in the antioxidant effects in breast cancer cells. This effect was mediated by the estradiol receptor alpha, suggesting that

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steroid hormones may play a role in ROS metabolism and breast cancer occurrence [14,15]. Progesterone is the natural ligand for PR and progestins are non-selective synthetic steroids which display mixed effects due to their various hormonal activities. The effect of progestins on these pathways has never been investigated. We thus investigated the actions of progesterone and synthetic progestins on the effects of hydrogen peroxide on growth. Catalases play an important role in hydrogen peroxide metabolism. We thus studied the catalase activities and its regulation in breast cells, as caused by the type of progesterone receptor (PR). The effects of progesterone and a pure pregnane progestin, Org2058, were compared to norethisterone acetate (NETA) and medroxyprogesterone acetate (MPA) on catalase activities and its regulation. Finally, we also studied the effect of Tibolone and its metabolites. Our results reveal that progestins inhibit growth through a potent induction of catalase activities in normal and breast cancer cells, and this regulation is mediated via the progesterone receptor B isoform.

2. Materials and methods

2.1. Chemicals, reagents and steroids

Roswell Park Memorial Institute 1640 (RPMI), SVF, Hams' F10 medium (Ham F10), 5.6% $NaHCO_3$ solution, glutamine, Hanks' balanced salt solution (HBSS), 0.25% sterile trypsin solution, Lipofectamine Plus Reagent, and geneticin (G418) were provided by Invitrogen Life-Technologies (Cergy-Pontoise, France). Penicillin (10,000 U), streptomycin (10 mg), choleratoxin, transferrin, insulin, triodothyronine (T3), cortisol (F), epidermal growth factor (EGF), 17β-estradiol (E2), progesterone (P4), medroxyprogesterone acetate (MPA), norethisterone acetate (NETA), hyaluronidase, bisBenzimide (Hoechst 33258), aminotriazole (AT), isopropyl-β-D-thiogalactopyranoside (IPTG), Igepal, monoclonal anticatalase (CAT 505), and the goat antimouse horseradish peroxydase-conjugated secondary antibodies were obtained from Sigma (St. Quentin-Fallavier, France). The anti-PR mouse monoclonal antibody was obtained from Novocastra Laboratories (Newcastle, UK). The Kit HSII, ATP bioluminescence assay, the protease inhibitor complex MiniComplete, and collagenase were obtained from Roche (Meylan, France). The ECL Plus Western blotting detection system was obtained from GE Healthcare (Orsay, France). Org2058, Tibolone (OrgOD14), its 3α-reduced metabolite (3α-OH Tibolone), its 3β-reduced metabolite (3β-OH Tibolone) and its Δ^4-isomer (Δ^4-Tibolone, OrgOM38), and the antiprogestin Org31710 (AP) were kindly provided by Organon (Oss, The Netherlands).

2.2. Culture procedures

T47-D cell lines were a gift from Dr. C. Mercier-Bodard (Kremlin-Bicêtre, France). T47-D cells were cultured in RPMI 10% fetal calf serum (FCS), containing 2 mM glutamine and the PR and ERα content was regularly checked. T47-DN5 cells were kindly provided by C. Clarke (Sydney, Australia). This cell line is stably transfected with an inducible expression plasmid for the progesterone receptor isoform A (PRA). To induce PRA, cells were treated 48 h with IPTG as previously reported [16]. MDA-MB-231 cells were cultivated in RMPI 10% FCS.

Specimens of normal breast were obtained from 5 women (aged 15–25 years) undergoing for reduction mammoplasty and processed according to the French law on clinical experimentation. The patients had no history of breast disease and pathological study showed only normal breast tissue. The procedure used for the culture human breast epithelial (HBE) cells has been described elsewhere [17].

2.3. Construction of MDA-MB-231 cell lines stably expressing hPRA or hPRB

MDA-MB-231 cells were transfected with 4 μg of progesterone receptor expression vector hPRA or hPRB using Lipofectamine Plus Reagent in serum free medium according the manufacturer procedure. These plasmids contain human PRA or PRB isoform coding sequence cloned in pOP13 vector (kindly provided by C. Clarke). Twenty-four hours after transfection, cells were plated in 100 mm culture dishes in complete medium. Clones were selected by 1 mg/ml G418. Individual colonies were transferred to 48-well plates and expended in the presence of G418. Clones were analyzed for PRA and PRB expression by immunoblotting. Two clones of each were chosen, with a high PR expression (A7, B18) and with a low PR expression (A2, B11). One clone selected by G418 but which do not expressed either PRA or PRB was used as control (C6).

2.4. Steroid treatments

HBE cells (5×10^5), T47-D or T47-DN5 cells, and (6×10^5) MDA-MB-231 cells were plated. 24 h following the seeding, cells were treated with progestins in phenol red free medium containing 5% of FCS charcoal treated. P, Org2058, NETA, MPA and Tibolone and its metabolites were used at 100 nM, 10 nM and 1 μM concentration. P was added twice a day and the synthetic progestin every 48 h. Org2058 was used alone or in combination with the antiprogestin Org31710 (100 nM). Control cells were treated with medium containing 1:10,000 ethanol, corresponding to the steroid dilution buffer. Aminotriazole (AT) was tested at the concentration range from 0.1 to 10 mM alone or with Org2058.

2.5. Growth under progestins

5×10^5 T47-D cells were plated and treated by the various progestins. The number of healthy cells was measured by trypan blue exclusion using a Malassez chamber. Measurements were performed in duplicate, with a minimum of three independent experiments. Results were presented at 48 h and expressed as means ± SEM.

2.6. H_2O_2 and growth index

H_2O_2 may affect both proliferation and apoptosis of breast cells and therefore we have determined the growth index (ratio of mitotic cells over cells with apoptotic features). Experiments were performed using Hoechst 33258 dye (bisBenzimide) as previously described and modified as follows [18]. T47-D and MDA-MB-231 cells were plated, respectively, at the density of 5×10^4 and 2×10^4 per well, in a 12-well culture plate. Cells were treated with H_2O_2 at concentrations range from 0.1 μM to 1 mM with or without Org2058 (100 nM). The numbers of cells displaying mitotic nuclei and apoptotic features were counted by scanning ten different random fields magnification (200×). Measurements were performed in duplicate, with a minimum of three independent experiments. Results were expressed as means ± SEM.

2.7. Catalase activities

Catalase activities were measured each day for 4 days following progestin treatment, as previously described [19]. Briefly, the cell suspension was pelleted and washed several times with PBS buffer. The pellet was resuspended in 50 mM Tris buffer pH 7.8, containing 2 mM $MgCl_2$, 2 mM $CaCl_2$, and sonicated for 20 s at 4 °C × 3 times. The maximum catalase activities were determined with a sensitive Clark electrode measuring O_2 produced after addition of H_2O_2 [20,21]. Since catalase induction was observed with a maximal

Table 1
Effects of progestins and Tibolone on T47-D cell growth.

	Control	Org2058		NETA		MPA		P4		Tibolone	
Concentration (μM)		1	0.1	1	0.1	1	0.1	1	0.1	1	0.1
T47-D cell number ($\times 10^4$)	87 ± 2	38 ± 0.4**	45 ± 0.5**	60 ± 0.4*	70 ± 3*	51 ± 0.5**	63 ± 1*	48 ± 0.7*	50 ± 0.5*	51 ± 0.5**	70 ± 0.6*

The number of cells was measured after a 48-h hormone treatment. Measurements were performed in duplicate with a minimum of three independent experiments. Results were expressed as means ± SEM.

* $p < 0.05$.

** $p < 0.01$.

effect after 48 h of treatment in preliminary experiments, this time of treatment was subsequently used. Catalase activities were measured in duplicate in five independent experiments and expressed as O_2 μmoles produced by hour for 10^6 cells and as means ± SEM.

2.8. ATP production

ATP was measured using the kit ATP. 5×10^5 cells were lysed for 5 min at room temperature. The bioluminescence was quantified on 100 μl of each sample after a 1 s delay and the signal was integrated for 10 s. The maximum value was used.

2.9. Western blots

The Western blots were performed as previously described [22], and modified as follows. Forty μg of T47-DN5 proteins or 50 μg of MDA-MB-231 proteins were loaded and separated using a 10% SDS-PAGE and electro-transferred to PVDF membrane. Membranes were incubated with monoclonal antibody against catalase (1:10,000) or human PR antibody (1:2000 for T47-DN5 cells and 1:500 for MDA-MB-231 cells) and with a horseradish peroxydase-conjugated secondary antibody (1:2000). Catalase and PR immunoreactivities were detected with an enhanced chemiluminescence kit. PRA and PRB expression protein levels were quantified from immunoblots using the quantification software ImageQuant 5.0 (Molecular Dynamics).

2.10. Statistical analysis

Unpaired Student's *t*-tests were performed on the results of proliferation, catalase activities measurements, and ATP production. One-way ANOVA test and multiple range Student–Newman–Keuls tests were performed to compare the relative efficiency of each treatment.

$p < 0.05$ was considered as significant.

3. Results

3.1. Effects of steroids on T47-D and normal breast cell growth

We first evaluated the effect of the steroids on breast cancer cell growth. As shown in Table 1, addition of steroids decreased cell growth. Org2058 and progesterone were the most efficient steroids because the inhibition of growth was already more significant at 100 nM as compared to NETA, MPA and Tibolone. At higher concentration (1 μM) all steroids caused a potent decrease on cellular growth as compared to control cells. Similar decreases (30%) on cell growth were seen in HBE cells treated with 100 nM Org2058 ($77 \pm 10 \times 10^4$ for control cells and $54 \pm 5 \times 10^4$ for treated cells ($p < 0.05$, $n = 5$)).

3.2. Effect of H_2O_2 on growth index with and without Org2058

H_2O_2 has been reported to promote proliferation and apoptosis in various cell types. We firstly performed experiments to determine the endogenous concentration of H_2O_2 in T47-D cells. H_2O_2 level was too low for being measured with the Amplex Red Hydrogen Peroxide kit and remained undetectable in this cell line whereas we found it in a positive control. Thus, we studied responses of T47-D cells to increasing concentrations of H_2O_2 and the link between the progestin antiproliferative effect and the oxido-reductive status of the cells. Increasing concentrations of H_2O_2 induced a biphasic effect on the T47-D cell growth index as quantified by comparing the ratio between mitosis and apoptosis. H_2O_2 stimulated growth with a maximal effect at 1 μM in the control cells (Fig. 1). At 100 μM H_2O_2, the rate of apoptosis increased (2-fold) and mitosis decreased (1.5-fold). At 1 mM H_2O_2, most of the cells died (data not shown). Interestingly, addition of 100 nM Org2058 blunted the effect of H_2O_2 on cell growth index (Fig. 1). Confirming this inhibitory effect, the progestin produced a shift of the biphasic effect of H_2O_2, since a modest but significant ($p < 0.05$) increase in growth index was observed at 10 μM H_2O_2, immediately followed by its decrease (Fig. 1). As catalases are the major effectors in the dismutation of H_2O_2, we concentrated our studies on the catalase activities regulated by progestins.

3.3. Effects of steroids on catalase activities and correlation with growth

Effects of progestins, Tibolone, and its metabolites on catalase activities in T47-D and normal breast cells were quantified by measuring in the cellular extract, the quantity of O_2 produced after addition of H_2O_2. Progestins induced catalase activities in T47-D cells (Fig. 2) with progesterone and Org2058 being the most efficient, as the effect was detected at 100 nM concentrations. MPA, NETA and Tibolone also induced catalase activity but only at 1 μM. The Tibolone metabolites, Δ^4-isomer and 3β-OH Tibolone also

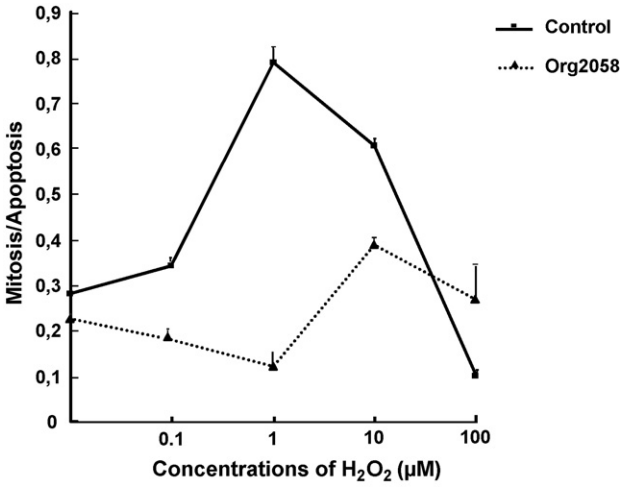

Fig. 1. Effect of H_2O_2 treatment on T47-D cells growth. T47-D cells were treated with a range of H_2O_2 concentrations for 48 h with or without 100 nM Org2058. Mitosis and apoptosis were quantified using Hoechst 33258 as described in Section 2. Results were expressed as means of the ratio ± SD percent of mitotic/apoptotic cells ($n = 3$).

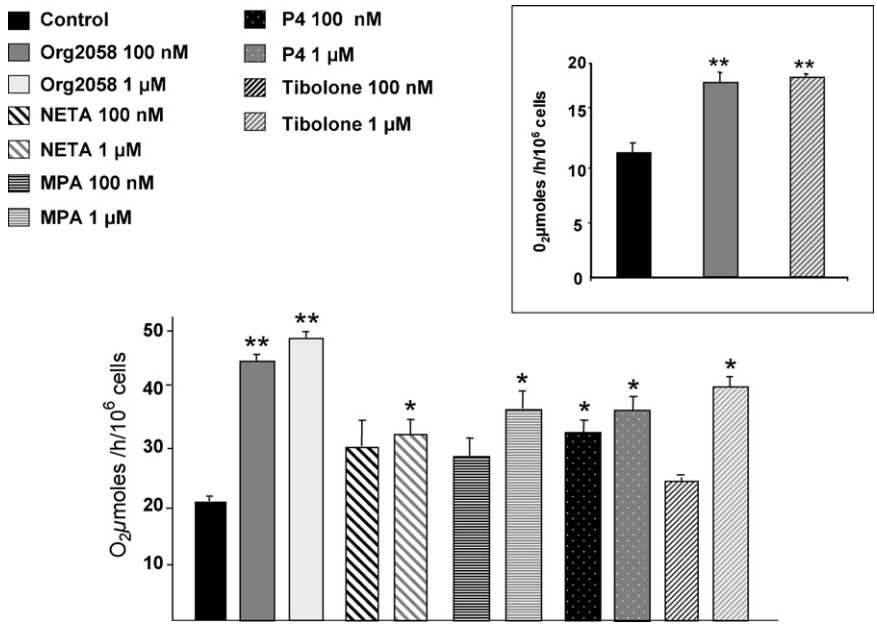

Fig. 2. Catalase activities induced by progestins in human normal and breast cancer cells. T47-D cells were treated with Org2058, NETA, MPA, progesterone and Tibolone at 100 nM and 1 μM for 48 h. Catalase activities were measured following progestin treatments as μmoles of O_2 released by the transformation of H_2O_2. Results are shown as means ± SEM ($n = 5$). Insert: catalase activities were measured in human normal epithelial breast cells after treatment with 100 nM Org2058 or 1 μM Tibolone for 48 h. Results were expressed as means ± SEM ($n = 4$). $^*p < 0.05$; $^{**}p < 0.01$.

stimulated O_2 production. In contrast, the 3α-OH Tibolone had no effect (Fig. 3). Furthermore, Org2058 and Tibolone also induced a significant increase of catalase activities in HBE cells after 48 h of treatment (Fig. 2, insert).

When we correlated the effects of progestins on growth and on catalase activities, the correlation coefficient was $r = -0.95$, suggesting that the range of effects of progestins on catalase activities and inhibition of growth were similar.

3.4. Confirmation of catalase involvement in the effect of Org2058 on cell growth

In addition to O_2 production, ATP is produced by catalase during H_2O_2 transformation [20,23]. Therefore we measured ATP and O_2 formation after stimulation with Org2058 in the presence and absence of a catalase inhibitor. In T47-D cells, ATP production was significantly increased within 24 h of Org2058 treatment compared to control cells as the result of catalase stimulation. Aminotriazol (AT) is a known potent catalase inhibitor [24], and supporting the hypothesis of a progestin effect on catalase activities, we observed that AT inhibited ATP and O_2 production in T47-D cells in the presence or absence of Org2058 (Fig. 4A and B). The increase in catalase activities and the effects of Org2058 on T47-D cells growth was corroborated, as a significant effect of AT on proliferation was observed at 0.1 and 1 mM (data not shown). The maximal effect of AT was observed at 10 mM. As shown in Fig. 4C, a blockade of catalase activities by AT totally reversed the Org2058 effects on cell growth, with a 180% increase in Org2058 + AT treated cells condition compared to Org2058 treated cells ($p = 0.02$).

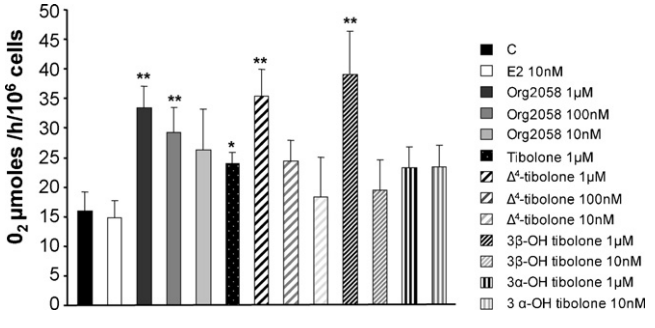

Fig. 3. Catalase activities induced by Tibolone and its metabolites. T47-D cells were treated with Org2058, Tibolone, 3α-OH Tibolone, 3β-OH Tibolone and Δ^4-Tibolone at 100 nM, 10 nM and 1 μM and estradiol 10 nM for 48 h. Catalase activities were measured following progestin treatments as μmoles of O_2 released by the transformation of H_2O_2. Results are shown as means ± SEM ($n = 4$). $^*p < 0.05$; $^{**}p < 0.01$.

3.5. Role of PR in the catalase induction by Org2058

In order to test whether catalase activities were controlled directly via PR, we used the antiprogestin Org31710 in combination with Org2058. Org2058 induced catalase activities after 24 h of treatment of wild type T47-D cells. The effect of Org2058 was fully inhibited by the addition of the antiprogestin Org31710 (AP) confirming a progesterone receptor mediated mechanism (Fig. 5A).

The next step was to look at the involvement of PR isoforms in catalase activities regulation. PRA and PRB isoforms have similar and different target genes and their ratio can be disrupted in 50% of women with breast cancers. In addition, a ratio of PRA over PRB could be a factor of poor prognosis. In order to know if the catalase activities were preferentially dependent upon one or the other isoform or altered by increasing PRA over PRB, we used different engineered breast cell lines which have a different ratio of PRA/PRB isoforms or contain exclusively one of the isoforms.

Firstly we measured catalase activities in T45-DN5 cells expressing an inducible PRA. In T47-DN5 cells, induction of PRA transgene by IPTG revealed a 2-fold increase of PRA over PRB as compared to untreated cells [16] (Fig. 5B). Under these conditions, catalase activities levels did not change in cells in which PRA is over-expressed and when treated with Org2058 or progesterone (Fig. 5B and C). In addition, the experiments revealed that catalase protein levels were not altered by any conditions.

The role of PR isoforms was further explored in MDA-MB-231 cells stably transfected with PRA or PRB. We tested clones with different levels of PRA or PRB expression (Fig. 6A). Basal catalase activities were similar in MDA-MB-231 wild type cells (not shown)

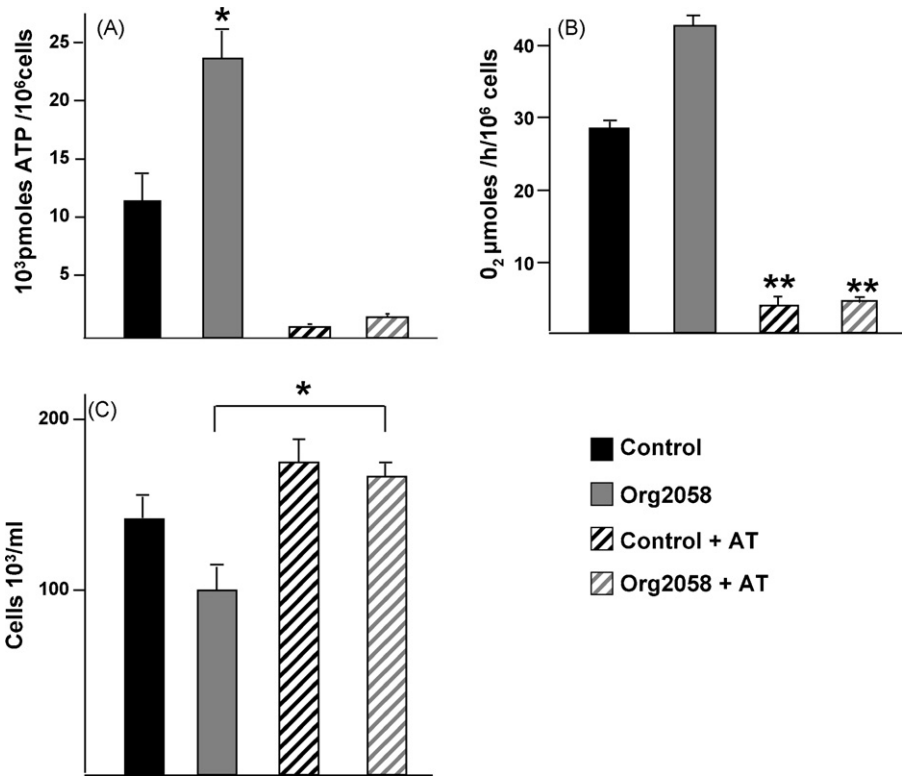

Fig. 4. Correlation between catalase activities, ATP production and cell growth. T47-D cells were treated with 100 nM Org2058 or vehicle (control) with or without 10 nM AT as catalase activity inhibitor for 48 h. (A) ATP production was quantified in duplicate by bioluminescence ($n = 3$). (B) Catalase activities were determined by measuring O_2 production (means ± SEM, $n = 3$). (C) Cell numbers were counted in a Malassez's cell (means ± SEM, $n = 3$). $^*p < 0.05$, $^{**}p < 0.01$.

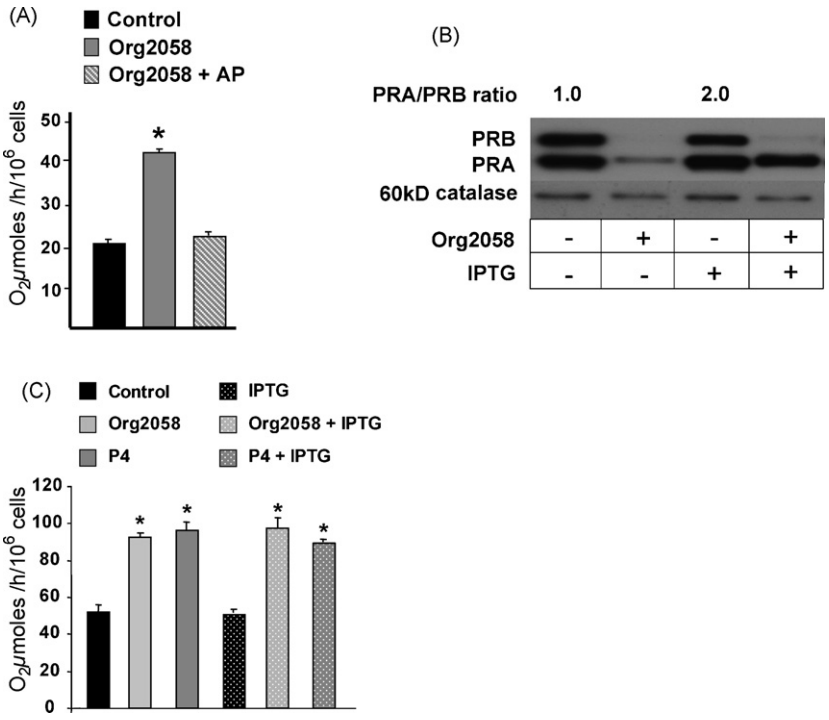

Fig. 5. Catalase activities induction by progestins is not altered by PRA isoform over-expression. (A) Catalase activities were measured in T47-D cells treated with 100 nM Org2058 or vehicle with or without 100 nM AP for 48 h (means ± SEM, $n = 3$; $^*p < 0.05$). (B) T47-DN5 cells were treated for 24 h with 10 nM IPTG or vehicle to induce PRA over expression, then with 100 nM Org2058 or vehicle for 48 h. PRA, PRB and catalase proteins were detected by immunoblotting. Basal and IPTG induced expression of PRA and PRB in progestin treated and non-treated cells were estimated by quantification of signal with ImageQuant 5.0 software and the PRA to PRB ratio was calculated before and after treatment with IPTG. (C) Measurement of catalase activities in T47-DN5 treated for 24 h with 10 nM IPTG or vehicle and then with 100 nM Org2058, 100 nM progesterone (P4) or vehicle for 48 h (means ± SEM, $n = 4$). $^*p < 0.01$.

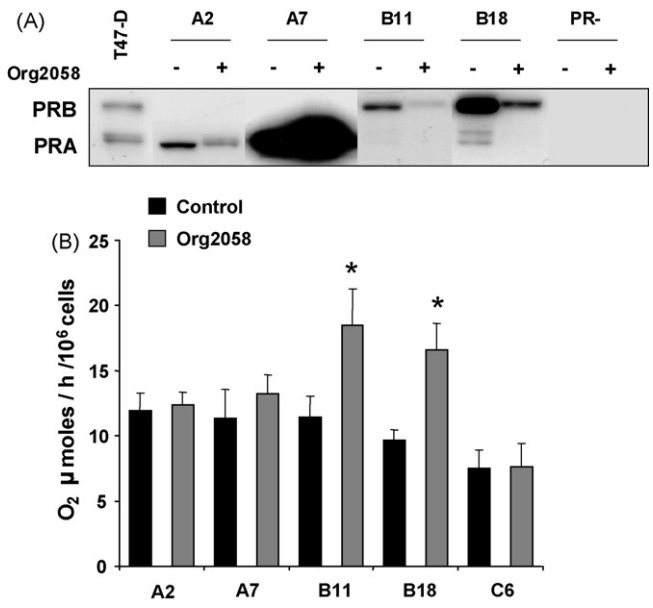

Fig. 6. Catalase activities induction by progestins is mediated through PRB isoform. (A) Stable MDA-MB-231 cell line expressing human PRA (A2 and A7) or PRB (B11 and B18) isoforms were treated with 100 nM Org2058 or vehicle for 48 h. PRA and PRB proteins were detected by immunoblotting. (B) Catalase activities were measured in MDA-MB-231 PRA and PRB clones treated with 100 nM Org2058 or vehicle for 48 h. Results were shown as means ± SEM ($n = 4$), $^{*}p < 0.05$.

and within the control and the transfected clones (Fig. 6B). However under Org2058 exposure, PRA does not mediate an increase in catalase activities, whereas catalase activities were clearly induced in clones both massively and moderately expressing PRB (B11, B18) (Fig. 6B). In these cells, as well as in T47-DN5, expression of catalase protein did not vary under treatment (data not shown). The results on catalase activities were correlated with cell growth. As shown in Fig. 7, Org2058 drastically decreased the cell growth in 1 and 10 µM concentrations of H_2O_2, but only in PRB expressing cells, confirming the modulation of catalase activities through PRB. In PRA over expressing cells (Fig. 7) and control cells (data not shown), Org2058 did not affect cells growth. These data and the result observed with PRA inducible T47-DN5 cells, strongly suggest that PR induced catalase activation is mediated by PRB.

4. Discussion

The antiproliferative and proliferative effects of progestins on breast cells have been reported in various cell culture conditions and according to different metabolites [25]. The effects of progestins in the breast are complex involving cell cycle regulators and various cytoplasmic pathways such as MAP kinase and PI3 kinase activations [26,27]. In this report, we revealed for the first time, a potent antioxidant effect of progestins in breast cells. This effect, the result of an increase of catalase activities, is induced by progestins and mediated through the PRB. This effect was strongly correlated with a negative effect on growth of breast cancer cells.

The different progestins studied displayed different responses on the increase of catalase activities. For example, progesterone and a pure PR ligand (Org2058) produced the most pronounced effects. The correlation of this specific effect on catalase, with an antigrowth effect, may help to explain the differential clinical impact of progesterone and synthetic progestins. In this light, it was reported that progesterone or synthetic progestin may carry different relative risk of breast cancer in postmenopausal women [28,29].

Oxygen species are toxic catabolic products created from respiration. Low concentrations of superoxide and hydrogen peroxide affect the regulation of transcription of factors involved in proliferation of various cell types [1,5,6]. In contrast, high H_2O_2 concentrations induce cell apoptosis and necrosis [3–5,7]. We demonstrated the mitogenic effect of H_2O_2 at low concentrations in breast cells. Catalase controls the H_2O_2 catabolism which limits the action of peroxide. It was subsequently shown that the reduction of catalase activities enhanced the malignant progression [20,30]. Thus, the negative control of estradiol on catalase activities

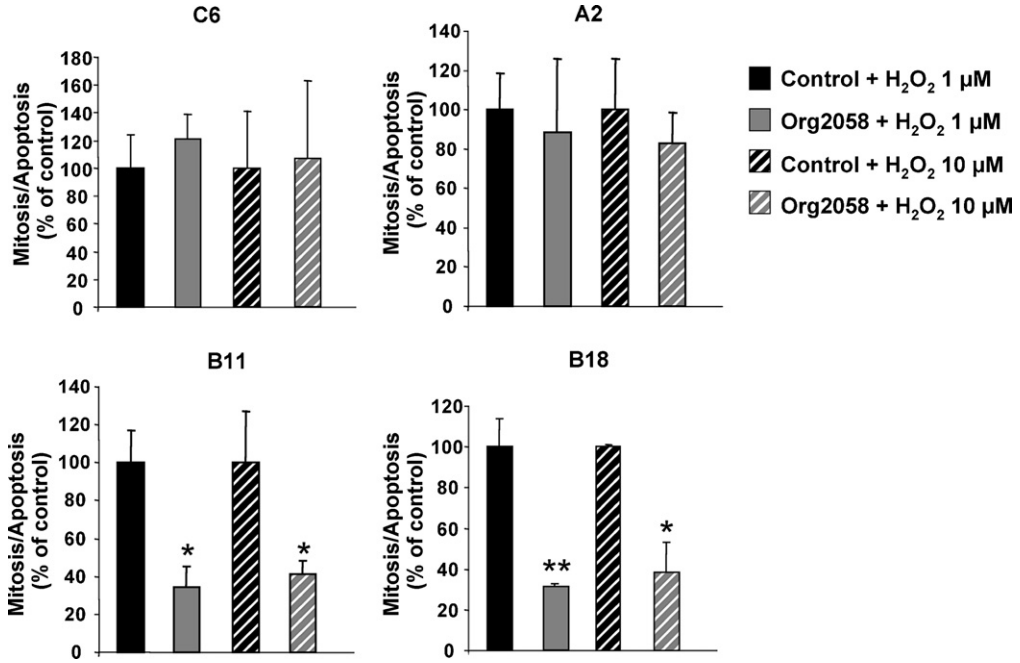

Fig. 7. Effect of H_2O_2 treatment on MDA-MB-231 PRB and PRA clones growth. MDA-MB-231 clones were treated with 1 and 10 µM H_2O_2 concentrations for 48 h with or without 100 nM Org2058. Mitosis and apoptosis were quantified using Hoechst 33258 as described in Section 2. Results were expressed as percentage of control and were shown as means of mitosis/apoptosis ratio ± SEM (C6, B11, B18 ($n = 3$); A2 ($n = 6$)), $^{*}p < 0.05$; $^{**}p < 0.01$.

[14] could contribute to breast cancer progression. However, the inhibitory effect of estrogen on catalase activities previously shown in MCF-7 cells [14] was not observed in T47-D cells (Fig. 3). This discrepancy can be explained by the different levels of ER within both cell lines. Conversely, we revealed progestins to be potent inducers of catalase activities in normal and breast cancer cells associated with a decrease in cell growth. Furthermore, our results suggest that progestin potency is important in the effects observed on catalase activities. Indeed, the basic progestins, Org2058 and progesterone, exhibiting no androgenic or estrogenic activities, were the most potent compounds. Steroids having mixed properties, such as Tibolone and its metabolites, NETA and MPA, were only effective inducers at higher concentrations. The profile of the Tibolone metabolites on catalase activities is in accordance with their previously described specificities: the Δ^4-isomer is a more potent progestin and androgen than Tibolone itself and the 3β-OH Tibolone can be converted to the Δ^4-isomer [31]. The progestagenic effect of the 3β-OH Tibolone is most likely due to metabolism. Steckelbroeck et al. have shown that 3β-OH Tibolone can be back converted back to Tibolone, which may explain its progestagenic activity [32]. Similarly Schatz et al. have shown that the 3β-OH Tibolone stimulates progesterone sensitive parameters in stromal cells [33].

This differential efficacy may also be due to differences in the relative affinity (RBA) for PR between the various progestins. Indeed, Tibolone as compared to Org2058 (100%) has a 1.1% RBA for PR [31], and may be metabolized into its Δ^4-isomer, Org OM38, which has a 10.4% RBA for PR [34]. However, despite a strongest RBA for PR (21.3% and 58.9% compared to Org2058, respectively [35,36]), NETA and MPA were not better inducers of catalase activities than Tibolone and its metabolites. This suggests that the progestin specificity for PR could be more important than the receptor affinity.

Interestingly, the progestin-mediated increase in catalase activities was observed in primary cultures, from reduction mammoplasties for which we previously reported the functional expression of ER and PR [17], and in breast cancer cells. This effect was mediate by progesterone receptor activation because the catalase activities induction by progestins was blocked by an antiprogestin. This result may explain the opposite results of Dabrosin et al. reporting a decrease of catalase activities by progesterone in normal breast cell cultures lacking ER and PR [37].

The effects of the progestins observed herein appeared to be limited to the activity of the enzyme since no variation in the protein level was detected following steroid treatment under all tested conditions (Fig. 5B). Similarly it was recently reported that fatty acids decrease catalase activities without altering protein or RNA levels [38]. The catalase increased activities are mediated selectively through the B isoform of progesterone receptor and the progestin effect on hydrogen peroxide was restricted to the clones expressing PRB. This suggests that the responses of breast cancer cells to progestin may vary according to the relative ratio of PRB and PRA isoforms in tumor cells. The PR isoforms are equally distributed in normal breast tissue and in about half of breast cancers. However, the ratio is disrupted in about half of primary breast cancers and a preferential expression of PRA is associated to a recurrence or tamoxifen resistance [39] (P.A. Mote, A. Gompel, et al., Progesterone receptor A and B expression in primary breast cancer: association with endocrine exposure and disease recurrence, submitted manuscript). This disruption may cause a modification on the cell phenotype, and enhance their oncogenic potential. The consequences of the relative over-expressions of PRA to PRB are still under investigation, and remain controversial (for review [40]).

In conclusion, we showed that hydrogen peroxide increases the growth of breast cancer cells at low concentrations. Progestins significantly increased the catalase activities. This action leads to a decrease in intracellular concentrations of H_2O_2 in breast cancer cells, which by auto-stimulation may enhance proliferation in breast cancer cells. This progestin effects on catalase constitutes a novel mechanism of action. Progesterone was the only clinically used progestin to be significantly efficient at physiological concentrations and its use in clinical practice. Progesterone, acting in a highly potent manner to increase catalase enzyme activities, could contribute in protecting breast cancer cells from the progression to a more malignant phenotype.

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