Reviewer's report

Title: Comparative actions of progesterone, medroxyprogesterone acetate, drospirenone and nestorone on breast cancer cell migration and invasion

Version: 5 Date: 2 March 2008

Reviewer: Mariana Salatino

Reviewer's report:

- Major Compulsory Revisions:

1) The authors indicate in Figure 1 that the addition of any of the four progestin induced formation of cell membrane structures involved in cell adhesion and movement, including ruffles, focal adhesion complexes and pseudopodia. This result would be greatly reinforce with the addition of the progestin inhibitor ORG 31710 in combination with the four compounds to evaluate whether the formation of this cell membrane structures is now impaired.

2) The authors indicate in Fig 2 that E2 induced actin rearrangement in T47-D breast cancer cells. However they claimed that each progestin, when added to E2, did not significantly change the effect of E2 itself, although the cells often displayed a somewhat more evident rearrangement of actin fibers and cell membrane structures formation as compared to treatment with the progestin alone. This affirmation is too vague and the effect of the two hormones together should be addressed more precisely using each progestin in combination with an ER inhibitor such as ICI 182,780.

3) Page 8 paragraph 4: the authors proposed that the PR antagonist ORG31710 significantly reduced cell migration associated with the four tested progestins (Fig. 6A-E), but also significantly decreased the effect of the combination of E2 with any of the progestins (Fig. 6A-E). However if they were not able to find any significant additive effects of E2 on cell migration during co-treatment with any of the progestins the correct form of this affirmation should as follows.

Interestingly, the PR antagonist ORG31710 (1⁄4M) significantly reduced cell migration associated with the four tested progestins both in the absence and in the presence of E2 (Fig. 6A-E).

4) The authors state that the induction of breast cancer cell horizontal migration and invasion by progestins was related to the differential ability of these steroids to activate the actin-binding protein moesin, leading to effects on actin cytoskeleton remodeling and on the formation of cell membrane structures that mediate cell movement. However, this affirmation is not based on these group pf results. A sounder demonstration should be given. Authors should evaluate the effect of blocking moesin expression using moesin specific asense-oligodeoxinucleotides or iRNA on cell fate or the migration and invasion capacity induced by progestins.
5) The discussion is particularly confusing, with many ideas that are thorny to follow, presenting some difficulties in reaching a conclusion based on the results. Specifically paragraph 5 and 6 should be rewritten paying particular attention in the concentration discussion which is the most unclear issue.

In paragraph 8 the idea of the ORG mediated- inhibition of the additive effect of E2 on progestin regarding the migration and invasive capacities of T47D cells in not clearly explained.

The grammar should be check in all the discussion.

- Minor Essential Revisions

Abstract:
1) The word “Little” should be replaced for “limited or lacking”.
2) In the results paragraph the affirmation “differences were found in terms of potency, with MPA being the most active and DRSP being the least” the word “active” should be replace for “effective” or something similar.
3) In the conclusion paragraph the word “tendency” should be replace for “ability” or something similar.
4) The sentence “we characterized the signaling steps recruited by these progestins” lacks of experimental bases and should be removed. There is no characterization of any signaling steps in the results, only some assumptions based on inhibitor assays.

Introduction
5) Page 3 Line 2 delete the phrase “in women with an uterus” and explain more ie. an inappropriate endometrial proliferation caused by estradiol administration.
6) Page 3 Line delete “On the other hand, progestins are not equal”.
7) Revise the sentence “As for the risk of breast cancer, the French cohort study as well as the E3N-EPIC cohort study show that synthetic progestins, but not natural progesterone, increase the relative risk of breast cancer in postmenopausal women receiving continuous-combined HRT. It is confused.”
8) Link the paragraph 4 with 5 using a period.
9) Paragraph 6 line 3 the word “little” should be replaced for “limited or lacking”.
10) Paragraph 6 line 4 the word “Plus” should be replaced for “moreover”.
11) Last paragraph line 2 add the phrase “in combination with E2.”

Material and methods
12) Page 5 paragraph 2 line 4 delete the word “or”.
13) In the immunobloting protocol a detailed composition of the lysis buffer or an indicative reference is missed.
Results

14) The sentence should be revised for an English expert. Underline parts are wrong. As P, MPA and DRSP have binding affinities for progesterone receptor (PR) in the same range, while NES is about a 100-fold more effective compared to P in inducing endometrial transformation or binding to PR [11], we used a 100-fold lower concentration of NES.

Figure Legends

15) Figure 3:
First line moesin should not appear between parentheses.
The indication of the use of the inhibitor Y-27632 and the concentration used is missing in the legend.

Discussion

16) Paragraph 1; the word Little should be replaced for limited or lacking.
17) Paragraph 5 line 3 induce instead of inducing.
18) Last paragraph, rewrite the sentence These differences in biological efficacy are to some extent linked to partially discrepant recruitment of extra-nuclear signaling pathways by PR in the presence of each progestin using potential.
19) Last paragraph; delete the last part of the sentence from the coma.

- Discretionary Revisions

20) What happened with moesin expression or activation at 24 or 48 hs? I would be interesting to evaluate the effect of progestin addition at long times to differentially characterized short and long terms effects.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'