Reviewer's report

Title: Gonadotropin-Releasing Hormone Receptor Activates GTPase RhoA and Inhibit Cell Invasion in the Breast Cancer Cell Line MDA-MB-231

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Reviewer: Patrizia Limonta

Reviewer's report:

In tumor cells in which it is expressed (such as breast cancer cells), the GnRH receptor (GnRHR) is endowed with a strong antiproliferative and antimetastatic activity. Aim of this work was to underline some of the molecular mechanisms whereby GnRH agonists (Buserelin) inhibit breast cancer cell invasion. It is reported that, in breast cancer MDA-MB-231 cells transiently transfected with either the wild-type GnRHR or the mutant GnRHR-DesK191, the GnRH agonist triggers decreased invasiveness, together with actin cytoskeleton remodeling, increase in stress fibers, and increase in cell adhesion to substrate. These effects are mediated by activation of RhoA GTPase. The question posed by the authors is well defined and the methods are appropriate and well described.

Major Compulsory Revisions:

1) As underlined by the authors, different breast cancer-derived cell lines express specific binding sites for GnRH and respond to GnRH agonists with reduced proliferation and metastatic behavior (references n. 20, 21). An antimetastatic effect of GnRH agonists has been shown to reduce metastasis formation by MDA-MB-231 breast cancer cells in vivo (reference n. 21). The results reported in this paper demonstrate that the GnRH agonist Buserelin inhibits the invasive potential of MDA-MB-231 breast cancer cells only when they are artificially forced to overexpress either the wild-type or the mutant GnRHR. In MDA-MB-231 cancer cells transfected with the empty vector, the GnRH agonist does not apparently affect the invasive behavior of the cells. These results should be confirmed by experiments performed in breast cancer cells expressing high levels of GnRHR in basal conditions (without the need to artificially overexpress these receptors). These data would improve the relevance of the paper.

2) Abstract, Conclusions, third sentence: 'These observations offer new insights into the molecular mechanisms whereby this malignant cell line expresses its invasion potential'. According to the data reported in the paper, the GnRH agonist does not efficiently affect the invasive behavior of MDA-MB-231 cells in basal conditions (i.e., when transfected with an empty vector). These malignant cells need to be transfected with the GnRHR in order to respond to the GnRH agonist with a decreased invasive behavior. Thus, in line with comment 1), this sentence does not seem to be supported by the results here described.

3) Which is the effect of the GnRH agonist on the proliferation of MDA-MB-231 breast cancer cells, transfected with either the empty vector or with the wild-type/mutant GnRH receptors?
3) Radioligand binding assays: which is the value of the binding affinity constant for the overexpressed GnRH receptors?

4) In MDA-MB-231 breast cancer cells overexpressing either the wild-type or the mutant GnRHR, Buserelin stimulates intracellular production of IP3. According to the literature, a Galphai protein also seems to be deeply involved in the direct antitumor activity of GnRH analogs (Limonta et al., Endocrinology 1999, 140:5250-5256; Imai and Tamaya, Vitam Horm 2000, 59:1-33; Grundker et al., Endocrinology 2001, 142:2369-2380). This point should be addressed in the 'Discussion'.

Minor Essential Revisions:
1) Methods, Constructions, second sentence: 'As previously shown [60]'. Reference n. 60 does not exist in the 'References' section.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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