Reviewer's report

Title: A Her2-let-7-beta2-AR circuit affects prognosis in patients with Her2-positive breast cancer

Version: 2  
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Reviewer: Marco Fiocchetti

Reviewer's report:

In this manuscript, authors are aimed to understand the molecular mechanism underlying the up-regulation of #2-AR in Her2 overexpressing breast cancer cells and its clinical significance. Reported results and discussion are sustained by data affirming the pivotal role of let-7f in the up-regulation of #2-AR level in Her2 overexpressing breast cancer cells and the association between high level of #2-AR with lymph node metastasis and poor prognosis in Her2-positive breast cancer patients. However the manuscript presents diverse criticisms.

Major Compulsory Revisions:

1) The aims are not clear, authors introduce Her2 and in particular let-7f without any further explanation at the end of introduction; whereas the definition of Let-7f as miRNA is assessed for the first time only at line 167 page 8. For reader’s help, Her2 receptor and its association with the more aggressive phenotype and poor prognosis of breast cancer should be better described to strongly sustained the experimental work. Furthermore, the description of let-7f should be also reported in the background section and not only in the results. In the background authors should answer to several question about let-7f, like as: which is known about the function of let-7f in particular in breast cancer and in the regulation of #2-AR? Why do authors choose to study let-7f in the Her dependent up-regulation of #2-AR?

2) Authors identify mechanism underlying the up-regulation of #2-AR in Her2 overexpressing breast cancer cell by using only Her2 transfected or not transfected MCF-7. The breast cancer cell line SK-BR-3 endogenously expresses higher levels of Her2 than MCF7. SK-BR-3 cell model represents a better model to study the mutual and reciprocal interaction between Her2, #2-AR, and let-7f. Some experiments should be performed in this cell line.

3) In the figure 1A and B the relative mRNA expression of Her2 (A) and ADRB2 (B) in normal and cancer breast tissue samples are shown, however, in the figure legend it is not reported which samples, normal or cancer, are identified by 1 and 2 panels.

4) In the results section that refers to the let-7f dependent regulation of #2-AR expression in breast cancer cells the authors reports the cell treatment with let-7 inhibitors and transfection with let-7f mimics. Authors should clarify the compounds which are used as inhibitors or mimetics.
5) Moreover, in the same results section at line 175-179, authors indicates that “Fig. 2A and 2B show that the treatment with the let-7 inhibitors caused a concentration-dependent increase of the #2-AR expression in both MCF-7 and MCF-7/Her2 cells (upper panel). In contrast, the transfection with the let-7f mimics exhibited a marked inhibitory effect on the #2-AR expression in a concentration-dependent manner (lower panel)”. First of all authors should indicate the concentrations which have been used in these experiments. Furthermore, in the western blot image what is indicated as + or ++ should represent the different concentrations of inhibitor or mimics which should be explained in the figure legend. Also, in the Figure 2A western blot related to MCF7 only the lanes indicated as “-“ or “+” are reported suggesting that in these case no concentration dependent effect has been analyzed, authors should clarify this mistake. In the same figure 2 in both A and B panel the #2-AR protein level at basal condition in the experiment with let-7f inhibitor appears to be lower than that reported in the same condition in the experiment with let-7f mimics. These differences should be discussed. Furthermore, authors in the material and methods section which refers to western blot and in the figure 2 legend stated that immunoblotting experiments were repeated twice. The authors should repeat experiment at least one time more in order to clarify the different level of #2-AR at basal condition in MCF-7 and in Her2 overexpressing MCF-7 and they should report also densitometry analysis to determine the fold differences in protein expression. At the same time in the Fig. 2A, the quality of GAPDH western is very low, thus it is difficult to discriminate the GAPDH level of a single sample. A better quality western should be performed.

6) In the figure 3 let-7f mRNA relative level in MCF-7/Her2 is around a value of 0.3-0.4 as reported in the figure A and B, whereas in the figure C the let-7f mRNA level at time 0, which should be comparable with the above reported values, is around 1.0. Authors should explain this discrepancy and which method they used to calculate the relative levels of each mRNA.

Discretionary Revisions

1) In material and methods authors do not report how MCF-7 breast cancer cells have been cultured (medium, antibiotics, other). A more detailed description of culture method is needed.

2) In the section Western Blot authors report only the antibodies used for immunoblotting. A more detailed description of Western Blot protocol is required.

3) In lines from 287 to 289 authors report “A recent study showed that single nucleotide polymorphisms of the #2-AR gene were associated with LNM, poor prognosis, and high expression levels of #2-AR, EGFR, VEGF, and MMP-2”. The reported study is about pancreatic carcinoma, but it could be understood only by reading the related reference. For reader’s help authors should modify the sentence.
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