Reviewer’s report

Title: Inhibiting Inducible miR-223 Enhances Anti-Proliferative Effects of Celastrol on Human Cancer Cell Lines MCF-7 and PC3

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Reviewer: Marco Folini

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In the present paper, Cao Lu et al have investigated at molecular level the anti-proliferative effects of celastrol in breast and prostate cancer cells. Specifically, they found that exposure of cancer cells to celastrol results in increased miR-223 expression levels, activation of mTOR and induction of HSF-1/Hsp70. On the basis of these observations, the authors conclude that celastrol-mediated modulation of these three factors plays a role in protecting cancer cells to the toxic effects of the anti-cancer substance itself.

Though the question posed by the authors sounds scientifically appropriate, the study results unfocused and characterized by different weaknesses. The manuscript does not add any novel insight into to the field, as the involvement of mTOR/HSF-1/Hsp70, along with NFKB, TGFb and other factors, in mediating the effects of celastrol has been extensively investigated in both breast and prostate cancer cell models. On the contrary, the authors missed the opportunity to add novelty to their study by deeply investigating the molecular/biological nature of the mutual relationship between the three identified factors (especially the functional role of miR-223) involved in the response of cancer cells to celastrol and to provide suggestions for a possible therapeutic exploitation.

Major compulsory revision

1) the rationale for the use of MCF-7 and PC-3 cells for the analysis of cell response to celastrol exposure should be better defined.

2) data reported in Fig 1 do not provide support for the functional involvement of miR-223 (the only novel aspect of the study) in the response of breast and prostate cancer cells to celastrol. For instance, down-regulation of miR223 upon transfection with an antagonir results in a negligible enhancement of celastrol toxic effect. This evidence raises concern on the biological meaning of celastrol-dependent up-modulation of miR-223, which may simply reflect a drug-dependent global perturbation in the endogenous expression levels of miRNAs. For this reason, the levels and functional role of additional miRNAs, relevant for either cancer types or already reported to be associated to the response of cancer cells to celastrol (e.g., miR-21, miR-224, miR-326), should be investigated in the context of the response of breast and prostate cancer cells to celastrol.

3) the authors state several times that cancer cells uses three mutually promoted responses (miR223, mTOR and HSF-1/Hsp70) to survive and proliferate.
However, the increase in cell survival or proliferation has not been properly addressed. To ascertain whether or not the modulation of those factors/pathways is related to a true protective effect upon exposure to celastrol, long-term cytotoxicity assay (i.e., clonogenic assay) should be performed under the different experimental conditions reported in Fig 1, 2 and 4. Moreover, the biological events (e.g., inhibition of apoptosis, autophagy induction, modulation of epithelial-to-mesenchymal transition, endogenous redox levels, MDR phenotype) associated to such a protective effect should be deeply investigated at cellular level.

4) considering that miR-223 has been reported to affect the viability of different cancer cells (see the statement at page 8, lines 205-206) it is no clear at all why down-regulating or over-expressing it showed no obvious effect on MCF-7 and PC-3 cell growth (see statement at page 8, lines 212-213 and Fig. 1B). This point must be addressed.

5) The manuscript requires extensive revision for English.

Minor essential revision
1) data reported in Fig. 1B are in contrast to those reported in Fig. 1A in terms of IC50s for celastrol.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**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