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: Benjamin Verillaud

Reviewer's report:

In this manuscript, Cao et al describe the effects of celastrol on the level of miR-223 in 2 cancer cell lines, and try to enhance the anti-proliferative potential of celastrol by regulating the level of miR-223, mTOR and HSP70. The question posed by the authors is well defined (is it possible to increase the cytotoxic effect of celastrol?), the methods and results are well described. However, several issues need to be addressed:

• Major Compulsory Revisions

General comments:

- 1. The authors conclude that inhibiting miR-223, mTOR or down-regulating HSP70 could enhance the anti-proliferative effects of celastrol. However, the results of figure 2B and 5 only show a very little (sometimes less than additive) effect of these combinations on cell growth. To support their conclusion, the authors should use at least one other cell growth assay (clonogenic assay…) and quantify the synergistic effect of 2 cytotoxic compounds, for example with the Bliss additivism index.

- 2. The rationale for targeting mTOR and HSP70 should be explained more clearly, as well as the interest of studying NFkB. The objective of the study is to explore new ways to increase the effects of celastrol: the interactions between miR-223, NFkB, mTOR, HSF-1 and HSP70 (in figure 4 and in the discussion section) are therefore probably off the topic, and would probably deserve to be explored into more details in another study.

Specific issues:

- 3. Abstract and title: the title should be modified, as it only reflects a small part of the results. The conclusions of the abstract need to be either supported by more data or brought down as the “synergistic” effects of celastrol and miR-223/mTOR inhibition/HSP70 appear to be very limited in the cell viability assays.

- 4. Introduction: the biological effects of celastrol, as well as the rationale for further investigation on mTOR, NFkB and HSP 70 throughout the study should be explained in the introduction section.

- 5. Materials and methods: for most of the reagents, it would be easier to describe the origin of the product in the appropriate paragraph of the M and M
section (for example, in “cell culture”: cells were grown in RPMI (PAA laboratories), etc…).

- 6. Results:
  o first section (line 188): the title should be modified to include the fact that celastrol displays cytotoxic activity on both cell lines AND induce miR-223 expression. The term “IC50” should be used rather than “cells were reduced to half of the control”
  o second section (line 203): once again, the results are statistically significant but too modest to draw a general conclusion, and should be confirmed with another cell viability assay
  o third section (line 220): the results displayed in figure 3 are interesting, though they remain difficult to interpretate, as the mechanistic aspects are not explored in this study. These results are maybe a little off the topic, but they could probably be integrated in figure 5. A densitometric quantification of the dots would be useful.
  o fourth section (line 243): this section as well as figure 4 could be skipped and further explored in another study.
  o fifth section (line 270): the results also seem to show a modest effect of the combination celastrol/other treatment. A quantification of the synergistic effect (like the Bliss index) should be provided to the reader. Ideally, another cell viability assay should be performed.

- 7. Discussion section: the discussion about interactions between NFkB/mTOR/HSF1/HSP70 should be shortened.

• 8. Minor Essential Revisions: the manuscript should be edited for English grammar and syntax, to correct a few remaining mistakes.

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