Reviewer's report

Title: Celastrol targets mitochondrial respiratory chain complex I to induce reactive oxygen species-dependent cytotoxicity in tumor cells

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Reviewer: Marijeta Kralj

Reviewer's report:

Although numerous studies have reported the potential of Celastrol for the treatment of autoimmune, neurodegenerative and malignant diseases, the exact target has yet to be elucidated, but many cellular effects have been documented. The present study links these documented cellular effects and further proposes a new target and mechanism of action for its anti-cancer activity. Namely, the study demonstrates that celastrol targets MRC complex I and thus induces ROS accumulation, which acts as the key intermediate to induce cell cycle arrest, inhibits Hsp90 chaperone function, promotes the degradation of Hsp90 client proteins, activates JNK and finally initiates apoptotic and necrotic cell death.

This is a in general well designed study investigating the anti-tumor effects of a potential chemotherapeutic agent derived from Chinese traditional medicine. The authors proposed a well-defined hypothesis and performed relevant experiments to support it. The abstract, background, methods, results and discussion sections are adequate and concise. The paper is supported by sufficient amount of published and unpublished work. Also, all potential conflicting evidence (previous studies) is mentioned and discussed.

Major Compulsory Revisions

1. Fig. 1. Figure 1A appears to conflict with the conclusions derived from 1B: at 6 uM Celastrol there is increased ROS production (Fig1A), yet at this concentration, no viable cells are present (Fig 1B). Still, the authors present another experiment (cells were stained with DCFH-DA and analyzed by flow-cytometry ; Fig3, panel A) where there are at least 20 000 viable cells present at 6 uM Celastrol (analyzed at various time points) and the relative ROS-derived fluorescence is showed. Actually, why did the authors used 6 uM of Celastrol for all (most important) experiments, if this concentration completely inhibited the tumor cell growth - this can be concluded from the Fig 1, panel B?!

2. I would highly appreciate if the authors would describe the Mitochondrial Respiratory Chain Complexes Activity Assay Kit principle in more detail. I could not find the protocol or description of this kit (GenMed Scientifics, Shanghai, China) and it is actually the most important experimental evidence for targeting the complex I.

3. I suggest that the authors present a diagram showing the progression of ROS signaling events starting from ROS activation to downstream effects such as apoptosis activation. This is important because the authors present some novel
Findings that should be schematically presented (e.g. the importance of ROS for the degradation of Hsp90 client proteins induced by Celastrol, and the disruption of Hsp90 and Cdc37 interaction).

2. Minor Essential Revisions. I found only minor typo errors: e. g. Abstract/Results “did not disrupting”, should be changed to “did not disrupt”; Background: “a quinine methide”, should be changed to “a quinone methide”; Results/Celastrol induces ROS… “at different time point” to “different time points”; Results/Celastrol-induced activation… since Celastrol does-dependently “ to dose-dependently”; Discussion: “in consistent with this” to “inconsistent with this”

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

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.