**Reviewer's report**

**Title:** Atypical antipsychotics induce human osteoblasts apoptosis via Wnt/β-catenin signaling

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**Reviewer:** Eric Hay

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In this paper call "Atypical antipsychotics induce human osteoblasts apoptosis via Wnt/β-catenin signaling" written by Peifan Li, interesting results about the effect of atypical antipsychotics on osteoblastic survival. Because is known that atypical antipsychotics treatment is associated with a low BMD. In the aim to have a better comprehension of this observation authors use an in vitro model: they used one human osteoblastic cells line (hFob1. 19) in which they measure the apoptotic effect of 4 different AP drugs. Then they show an unbalance between pro apoptotic and anti-apoptotic protein associated to a decrease on b-catenin protein expression at both cytoplasmic and nuclear compartment. This effect is reduced when cells are co treated with Ap's and Resveratrol. This co treatment enhances the b-catenin protein and reduces the pro apoptotic effect of Ap. Even the experiment are well designed, since some details still remain unclear.

Major comment

Page 11 line 56 authors must explain how apoptosis rate was calculated; the valor on bar graph seems to be different than the value that appears on cell sorting graph? I got the same question for the drug tested.

Page 15 line 9 "However, the current study suggest that Aps may induce low BMD by sustaining elevate the secretion of prolactin and subsequently inhibit estrogen secretion as their dopamine D2 receptor-blocking effect" I don't see how the data presented in this article can demonstrate this effect?

Page 18 concerning the conclusion authors don't really demonstrated that b-catenin variation observed with Aps treatment is responsive for the increases of apoptosis. The only things that authors can conclude with the presented data is that there is a correlation between the decrease of b-catenin in the nucleus and the increases of apoptosis but if authors want to demonstrated the hypothesis suggested in the cartoon more assays must be done to bring more evidence to confirm this hypothesis. For example authors claim that apoptosis is induced by the unbalance between bax/bcl2-MCl-1 that drive to the activation of caspase 3 but caspase 3 could be also activated by the extrinsic pathway via caspase 8, and it seems to me that this pathway must checked to improve the evidence of the involvement of bax/bcl-2 on the apoptosis activation. Analysis of caspase 9 (associated with the release of cytochrome C ) will be an another interesting evidence. For the more the use of resveratrol, a polyphenol, as wnt canonical pathway activator is not really relevant. Because Y. Zou in 2015 have published that resveratrol decreases the WNT
canonical path way. More interestingly Chen HJ, in 2012 have demonstrated that resveratrol: "did not affect the accumulation and nuclear targeting of β-catenin. In contrast, co-immunoprecipitation and in vitro binding analyses substantiated that resveratrol was capable of disrupting the binding between β-catenin and TCF4, contributing to the decreased Wnt signaling." This important data tell us that nuclear β-catenin measure is not sufficient to conclude about the transcriptional activity of the Wnt canonical pathway. So to be sure that the anti-apoptotic effect of the resveratrol is due to the Wnt canonical pathway I suggest to measure apoptosis during the Aps/ resveratrol treatment in presence of wnt inhibitor, or test the transcriptional activity of β-catenin/TCF complex with transactivation assay (top flah). Without this data authors must modify their conclusions.

Minor comment

Method for nuclear separation must be describe in materials an methods

Page 8 line9 "were without treated….." this sentence is not clear.

Page 8 line 12 'Risperidone (control) and treated with Risperidone at different concentrations…” authors must explicit exactly what they use as control in this form is nor really clear.

Page8 line 59 why author speak about "ribo nuclease RNAse"?

Page 10 line 28 " for Ku 70" what authors want to say?

page 13 line 45 "similar result that " this sentence is not clear

Figure 2 B in the graph representation of cell sorting with respiridone the dose 5µM appear but in the bar graph is this value did not exist authors must correct the mistake.

Figure 3 C magnifications must be specified. In material and method the type (optical, apotome, confocal) and the reference of the microscope must be specified because some device don't allow to measure a real colocalisation.

What is the Aps concentration used in human therapy (or serum concentration detected) and what's the concentration used in the culture cell during this assay? is this value is in the same range?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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