Reviewer’s report

Title: Caffeine citrate enhanced cisplatin antitumor effects in osteosarcoma and fibrosarcoma in vitro and in vivo

Version: 0 Date: 08 May 2019

Reviewer: Avudaiappan Maran

Reviewer's report:

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In this manuscript, Abe et al have investigated the effects of caffeine, citrate or caffeine citrate along with the chemotherapeutic agent cisplatin on osteosarcoma and fibrosarcoma cells. The authors have studied the in vitro actions using HOS, human osteosarcoma, LM8, mouse osteosarcoma and HT1080, human fibrosarcoma cell lines. The study design includes measurement of cell survival, proliferation and apoptosis. In addition, the authors have validated their findings using in vivo models of osteosarcoma and fibrosarcoma. Based on their evaluations, the authors show that a combination of cisplatin and caffeine citrate has the strongest anti-tumor effect in blocking osteosarcoma and fibrosarcoma growth both in vitro and in vivo. The study also demonstrates the effect is synergistic in nature. It has been shown that this combination is more effective compared to cisplatin in with caffeine or citrate. The authors have evaluated a novel treatment method to enhance the chemotherapeutic effects of cisplatin for sarcomas. It is a well-thought out study. Quantitative information will be essential to evaluate the various treatment effects. Also, there are additional concerns that need to be addressed before this manuscript is further considered.

Specific Comments:
1. The manuscript is not focused as it tries to investigate both osteosarcoma and fibrosarcoma in the same study.
2. If the authors want to use both the cancer models, it would have been beneficial to study the differential effects of combination therapy on fibrosarcoma and osteosarcoma. This could be addressed by looking at molecular mechanisms and some protein markers.
3. The conclusion section in the "Abstract" needs to be rephrased and rewritten as it reads like a statement on observed results.
4. The manuscript has included statistical analysis. However, quantitative information (fold change or percent change) will be required for all the figures.
5. The "Results" should have titles and presentations explaining the observed effects. It has been titled like "Methods" section. For example, instead of presenting as "cell proliferation assay" it should be presented as" Treatment effects on cell proliferation"
6. There are many grammatical and typographical errors throughout the manuscript (some examples are given below) which need to be visited and edited by professional proofreading. Some examples from the manuscript text:
   a. Methods section: Page 13, line 19: change "activatity" to "activity"
   b. Page 10, line 5: "Inhivited" to "inhibited"
   c. Page 14, line 15: "upregurated" to "upregulated"

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**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.

Unable to assess

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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