Reviewer’s report

Title: Starvation-induced activation of ATM/Chk2/p53 signaling sensitizes cancer cells to cisplatin

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Reviewer: Jiri Bartek

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The manuscript by Shi et al. presents data on the AMPK and ATM-Chk2-p53 pathway involvement in response of starved and platinum-treated cultured cells, both cancerous and normal, in vitro and in xenograft models. Inhibitors of AMPK and ATM are used to interrogate the respective kinase activities and their contribution to observed responses. It is concluded that normal cells are protected against platinum toxicity due to cell cycle arrest after starvation, while cancer cell lines do not arrest and hence are more sensitive. Furthermore, it is proposed that the critical factor for this differential sensitivity is ATM activation in cancer, but not in normal cells. While the manuscript is potentially interesting despite limited novelty of the concept as such, there are some serious issues with the data and interpretation, in terms of missing controls, and discrepancies in terms of the mechanistic insight, making the present manuscript inconclusive in the parts that are not confirmatory and hence should be strongly supported.

Specific points.

1. Novelty is limited, as also acknowledged by the authors themselves in the Discussion, and the proposed synergism between starvation and chemotherapy is largely confirmatory.

2. To justify publication, all experiments need to be well controlled and the novel aspect of AMPK involvement should be better distinguished from the previous reports that emphasized a role for IGF1 signaling in the synergism of starvation and chemotherapy. Unfortunately, Shi et al. make no attempt to test and exclude that the pathway of IGF1 reported before is distinct from the action of AMPK reported here. Is there additive or epistatic effect of IGF1 vs. AMPK modulation in the present models? Is the AMPK pathway really distinct from the IGF1 pathway, as claimed but not investigated? This needs to be documented.

3. To manipulate AMPK and ATM, only chemical inhibitors are used, which is particularly questionable here as mTOR and ATM are members of the same kinase family, and for the well-known promiscuity of many chemical inhibitors. The controls that are essential, and which are missing, is to test potential impact of the ATM and AMPK inhibitors on the other pathway as well, to exclude cross-inhibition. In addition, at least the critical experiments need to be reproduced with knock-down of ATM and AMPK, respectively, to complement the chemical inhibition by genetic evidence. Otherwise the data remains difficult to
interpret.

4. There is a discrepancy in that p53 is stabilized and activated after cancer cell starvation, however there is apparently no increase of Ser15 phosphorylation of p53, despite ATM is activated. The point that needs to be reconciled is why the activated ATM (a well known kinase targeting ser15 of p53) does not phosphorylate p53 in these cancer cells and how then does p53 get activated and stabilized, without ser15 phosphorylation. At the very minimum, the authors should check acetylation status of p53, which they argue is behind p53 activation, but without any experimental evidence provided.

5. Another discrepancy is using the HCT116 cells, and not testing the ATM involvement to examine whether it is similar to the ZL55 cells. The problem is that the HCT116 cells lack functional MRN complex that is required for ATM activation, and therefore the two cancer cell lines may each rely on a different mechanism to activate p53, in contrast to the unified conclusion of the manuscript. At least the extent of dependency on ATM in clonogenic effects must be compared between HCT116 and ZL55.

6. The third model used in A549 cells, but it is unclear what is the status of AMPK and ATM in these cells.

7. As the main novel aspect of this study is the suggested involvement of AMPK in p53 activation, it is important to at least conclude whether the AMPK-targeted residue(s) of p53 is (are) indeed modulated consistently. This has not been examined.

8. In Figure 1F, examination of total levels of Chk2 is missing, and this control is essential to judge the significance of the phosphorylated form of Chk2.

9. It is also unclear whether the ration of total ATM to phosphorylated ATM is altered, and therefore the blots must be quantified and the ratios established and statistically evaluated. Otherwise the data on ATM phosphorylation is inconclusive.

10. It is unclear whether p53 is indeed elevated in Fig. 1F – the quantification and ratio evaluation under the various conditions is also important for p53.

11. Fig. 2A lacks negative control of untreated cells.

12. Fig. 4A lacks information about several of the proteins involved – including ATM and ATM-P and the ratios, as well as AMPK and p53 ser15-P

**Level of interest:** An article of importance in its field

**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