Reviewer's report

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

Version: 3 Date: 14 July 2012

Reviewer: valter D longo

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Shi et al BMC Cancer

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

Summary:

In the presented manuscript, Shi et al. demonstrate that serum starvation sensitizes malignant cells to the chemotherapeutic drug cisplatin (CDDP) while protecting normal cells. In BrdU labeled cancer cells, serum starvation decreased the fraction of S-phase cells by 40% compared to untreated control. To explore the underlying effects on cell cycle arrest, the authors focused their interest on the serine/threonine protein kinase Ataxia telangiectasia mutated (ATM). Serum starvation in ZL55 cancer cells caused a 5-fold increase in ATM phosphorylation and increased activation/ phosphorylation of Chk2 and elevation of p53 protein levels, both downstream targets of ATM. The ATM-mediated p53 activation was shown to be dependent on AMPK activation. Combining CDDP with serum starvation in vitro further increased the activation of ATM/Chk2/p53 signaling when compared to either single treatment alone, which was proposed by the authors to be the underlying mechanism for the enhanced sensitization of cancer cells to CDDP. In vivo, the authors showed a growth delay and also complete remission in 60% of the animals with mesothelioma xenografts, and in 40% of the animals with lung carcinoma xenografts, caused by the combined treatment of CDDP and short-term food starvation (STS). When investigating the effects of starvation in normal cells, the authors found that serum starvation resulted in a complete arrest of cellular proliferation and that the proliferation arrest in normal cells is due to p53/p21 activation, which is AMPK-dependent but ATM-independent.

This is an important study that: 1) extends previous results by other to the combination of cisplatin and different cancer types, 2) provides potential mechanisms for the effects starvation in the protection of normal and killing of cancer cells.

Major:

1) In addition to serum starvation the authors should consider modulating glucose levels to mimick the glucose changes caused by starvation in vivo.
2) The background of the manuscript should focus at least a little bit on the
connection of cell cycle progression and ATM/Chk2/p53 to allow the reader to establish why the authors focused on this key component of cellular stress. The authors should consider moving the sentence: “ATM is the key component in cellular stress responses to DNA damage and oxidative stress.” (page 5) to before “In ZL55 cells serum starvation induced a five-fold increase of phosphorylation of ATM…” (page 4) as this establishes a link that allows the reader to understand why the authors analyze cell cycle arrest and move on to investigate ATM.

3) In figure 2B, the error bars for the serum starved and CDDP/serum starved HCT116 cells are very close and seem to almost overlap. The authors have indicated in the figure legend that the data is significantly different (*P<0.02, **P<0.01) but give no information how the significance has been calculated. This should be included.

4) The authors describe that “the combined treatment of CDDP and serum starvation synergistically reduced the clonogenicity of human mesothelioma ZL55 cells in comparison to either treatment alone (Figure 1D)”. However, figure 1D does not indicate synergistic effects because CDDP treatment caused a ~60% reduction in colonies and serum starvation resulted in a ~35% reduction. The combination of treatments results in a 9% reduction and thus demonstrates additive (and not synergistic) effects on ZL55 cancer cells.

Further the legend for figure 1 states that “colony formation assay was performed with untreated control, or treated with CDDP alone, serum starvation alone, or both together”. The graph in figure 1D does not show the untreated control. In my opinion, the control should be included into the graph (with error-bars) or the legend should explain that the data is shown in percent relative to untreated control. The same is true for figure 5D.

5) The authors evaluated histone H2AX phosphorylation for A549 cells but did not present similar data for ZL55 cells? It would have been interesting to establish changes in histone modification for both malignant cell types.

6) For all in vivo experiments, with either the ZL55 human mesothelioma xenografts or A549 human lung adenocarcinoma cells, the authors describe a complete remission (60% and 40% respectively). In the material and method section but also in the results section, the authors should describe how this is established. I am wondering (based on the error-bars for the tumor-progression in vivo), if all mice actually grew a tumor or if the remission criteria is based on animals that were included into the study but had a very small, non progressing tumor from the beginning of the experiment? It is important to be clear on whether 1) all mice had established tumors at the beginning of the treatments, 2) all mice were tested for evidence of tumors at the end of the experiment. Also it should be clear when the last measurement was made.

7) The MTT assay is a colorimetric assay that extrapolates cell number according to the reduction of MTT via mitochondrial dehydrogenases and is known to be affected by the metabolic activity of the cells. STS likely affects mitochondrial activity and thus could the measurement of cell survival using MTT assay lead to misinterpretation. Therefore, the authors should use additional techniques to
corroborate the in vitro data for normal SDM104 cells; ex. colony formation as used for the ZL55 and HCT116 cells.

Minor:
The authors should write “short-term” consistently throughout the manuscript. I found different versions ranging from “short-term”, “short term” and “shortterm”.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.