Reviewer's report

Title: Tumor Necrosis Factor-alpha Attenuates Starvation-Induced Apoptosis through Upregulation of Ferritin Heavy Chain in Hepatocellular Carcinoma Cells

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Reviewer: Joanne Hildebrand

Reviewer's report:

This manuscript reports that the TNF mediated rescue of Hepatocellular Carcinoma cell (HCC) lines from serum starvation induced apoptosis is dependent on the induction of Ferritin Heavy Chain (FHC). While the TNF induced upregulation of FHC has been shown to be protective in other cellular contexts, characterizing this phenomenon in the context of HCC and nutrient deprivation is important and interesting work.

Figures 1, 4, 5 and 6 directly address this phenomenon but on balance I feel there are too many holes to fill for me to recommend publication at this time. I have tried to list these in detail below and hope they are useful.

The prospect of autophagy somehow modulating the early stages of the NF-kB signaling cascade is intriguing. While this manuscript describes some interesting observations I suggest the studies described in figures 2 and 3 should be pursued more rigorously and in a separate publication. The role of autophagy is only supported by studies with the PI3 kinase inhibitor 3-MA and is purely corollary. It is possible that 3-MA interferes with the TNF-a induced signaling cascade via a mechanism that is completely independent of autophagy. This idea should also be pursued using alternative means of inhibiting autophagy eg. Beclin-1 knockdown.

Major Compulsory Revisions

General points:

There many occasions in the introduction and discussion sections where published studies are discussed but not referenced (eg. asbestos exposure in mesothelial cells).

There are several typographical errors and incomplete or grammatically incorrect sentences that require correction, i have pointed out some but not all.

Specific Points:

Figure 1.

• Starvation induces a 40% drop in viability at 24hrs as measured by MMT but apoptosis is only 13.2% at this point. Please address this in your discussion.
• The 24hr time post 6 hour ‘pre-starvation’ was chosen throughout the paper and as a result, differences in apoptosis being measured are very small (ie 17% vs 5% of a total cell population). Is it possible to sample a wider time course of serum pre-starvation that shows a greater proportion of cells undergoing apoptosis 24 hours post TNF treatment? This would form a better alternative to the experiment shown in Fig. 1c and d. If there is some precedent for the use of these time points please discuss this in the results or discussion section.

* Figure 1c and 1d – in text authors say that ‘TNF-a could protect cells in a time dependent manner’ – this conclusion cannot be made from this experiment. There is not a significant amount of death at 12 hours to measure rescue, and the proportion of rescue (calculated as white bar divided by (black bar minus white bar) does not change significantly between 24 and 48 hours.

Figure 2.
• Figs d-g. Does this show that TNF induced rescue is dependent on autophagy, or that the inhibition of autophagy just adds to the serum starvation induced stress and prevents cells from being rescued by TNF via a different mechanism? Correlation not proof of causation.

In the main text there is reference to ‘FITC-PI’ staining, replace with Annexin V-PI staining.

Figure 3.
• main text – replace ‘signaproteins’ with signaling proteins
• provide details of experiment performed in Fig 3 in figure legend or main text. How long was TNF stimulation? Were cells pre-treated with 3-MA and for how long?
• Perturbation of the TNF induced signaling pathway in the absence of autophagy as measured by IKK phosphorylation and IkBa degradation must be examined over a variety of time points (only one time point was examined in this study and it was not clear what point this was).
• Fig 3.e. Show quantitation of nuclear p65 over independent experiments or several cells per field.

Figure 4.
• An alternative to the chemical inhibitor BAY11-7082 should also be included to demonstrate the importance of NFKB transactivation in the rescue from apoptosis and to exclude other pro-survival pathways induced by TNF. Eg; Transient transfection with some form of NF-kB super-repressor
• For all western blots showing Caspases 8 and 3, please do not exclude portions of the membrane. Pro and cleaved forms should be shown, and please indicate position of molecular weight markers.
Figure 5.

• There appears to be a decrease in serum starvation induced apoptosis in Fig 5g and h compared to the same conditions in Fig 1e. For example - 15% to 10% apoptosis - it may seem minor, but this equates to a 50% difference in results from experiment to experiment and a major change in the % of these cells that are rescued by TNF. This example highlights the difficulty on testing rescue of such a small proportion of apoptotic cells.

Figure 6.

• Test that ROS are actually the effectors of cell death by testing if serum starved cells are rescued from apoptosis by NAC.

Minor Essential Revisions

• Fig 1e - Please specify in the legend that the X axis represents Annexin V-FITC.

• Fig 2a - There is no mention of p62 blots in the main text or legend.

• Part of the Y axis in Fig 4a is obscured

• The word ‘starvation’ is often used alone, please specify that this is ‘serum starvation’.

• Several times the word ‘activity’ is used alone – what kind of activity? Say ‘cell viability’ or ‘metabolic activity as measured by MTT assay’. On the same note, I would recommend against the use of statements like ‘x resulted in an increase of activity’, as it implies a ‘gain’ in viability over untreated cells, instead use ‘X prevented the loss of cell viability as measured by the MTT assay’.

Level of interest: An article of importance in its field

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

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