Reviewer's report

Title: Stat1 activation attenuates IL-6 induced Stat3 activity but does not alter apoptosis sensitivity in multiple myeloma

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Reviewer: Ulrich Nachbur

Reviewer's report:

A) General Comments:
1) This article aims at exploring the role of Stat1 in IFN mediated apoptosis in the multiple myeloma cell line U-266-1979. The question is well defined and stated clearly in the third paragraph of the introduction and in the abstract.

2) The methods used in this article are well chosen and controlled and adequately described.

3) The data is mostly sound and well presented. However, Dimberg et al tend to over interpret their data. Changes in band intensities on Western blots seem hardly relevant and yet the authors claim differences. For instance in figure 2A the authors claim that phosphorylated Stat1 is ‘more abundant in the U-266-Stat1C cells’ compared to the U-266-pCIneo control cells. This is based on quantification of this single Western blot and on the basis on Actin quantification between samples for the supernatant and the cytosol (supplemental Figure S1).

Whereas the statement that pStat1 is present in both, cytoplasmic and nuclear fraction is correct, it is not adequate to quantify these small differences based on one Western Blot.

4) The figures are well laid out and easy to follow. Molecular weights on Western blots have to be included. Also it would be helpful to see more of the blots, even if there are unspecific bands appearing (which, in most cases, will further confirm equal loading between lanes). It would be helpful to be able to access array data presented in figure 3. Also, the HTSC revealed 2 drugs whose sensitivity is changed in cells overexpressing Stat1C. It would be helpful and interesting to see this data set, in an additional supplemental figure or incorporated in figure 7.

5) As mentioned above, Dimberg et al have a tendency to over interpret their data. The changes observed in this study are mostly small and changes in mRNA levels of Mcl-1 and other Bcl-2 family members are not reflected in protein levels. Also, overexpression of Stat1C did not alter drug sensitivity to most tested drugs in an unbiased high throughput screen.

In the conclusion section of the abstract, the authors state ‘Stat1 alters … the expression of pro-apoptotic genes’. Looking at figure 4 this statement is unjustified as protein levels of the short form of Mcl-1 are not altered between cell lines (and Harakiri/Noxa are not shown).
Albeit negative results, this is important information for the scientific community which should be published.

6) The entire study is based on one cell line and over expression of Stat1C. This should be addressed or stated clearly in the discussion.

7) Work which form the base of this study are well cited and described. The referencing in the Introduction section can be improved.

8) The title should include that the study is based on the use of the cell line U-266-1970 cells (except figure 1A where U3A cells were used, but this is the only piece of data not from U-266-1970 cells).

9) The writing is acceptable, but can be improved by careful proofreading. A few typos have to be corrected.

B) Major Compulsory Revisions

1) In Figure 2A, levels of total Stat1 should be included. Figure 2A and S1 are based on one Western Blot. For quantification in figure S1, two additional biological repeats are required to show quantification. As mentioned above, it is not particularly necessary to quantify this Western Blot if the conclusion is changed to ‘pStat1 is present in both, the cytoplasm and the nucleus’ without quantification.

C) Minor Essential Revisions

1) Molecular Weights on Western blots have to be added. If possible, more of the gels should be shown.

2) For consistency, in figure 2B, the control cells should be shown on the left and the Stat1C cells on the right, as otherwise throughout the manuscript.

3) A color-coded legend for figure 3 should be provided, describing what the colors represent (delta ct values?).

4) Figure 2C has been repeated only twice and one of these data sets is shown. What do the error bars represent? This experiment should be repeated at least once more and the error bars have to be described.

5) Correct a few typos throughout the manuscript.

D) Discretionary Revisions

1) Figure 1A shows the cell line U3A, transiently transfected with Stat1 and Stat1C. Given that the authors have experience with this cell line, some crucial experiments could be repeated using U3A cells, strengthening the manuscript by using an second cell line. Particularly, Figure 2A, 2C and 5 could be backed up with additional data using U3A cells transiently over expressing Stat1 and Stat1C.

2) Western Blots for protein levels of Noxa and Harakiri should be presented as part of figure 4.

3) Given that IFN regulates cell surface expression of Fas and this upregulation is paralleled by Stat3 deactivation, Stat1 induced downregulation of Stat3 activity
might change cell surface levels of Fas. Even though Figure 6 suggests that this is not the case (only a slight increase in cell death in Stat1C cells compared to pClNeo cells), it might be interesting to show cell surface levels of Fas in these cells.

4) The results of the high throughput screen, especially for drugs that showed resistance in Stat1C expressing cells (gitoxin and gitoxigenin) would be interesting to see and be discussed. Also, resistance to Doxorubicin has been reported to be associated with increased Stat1 activation (reference 32, Fryknas et al), so the resistance to Doxorubicin could be particularly shown and discussed in more detail.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.