Reviewer’s report

Title: Gm40600 suppressed SP 2/0 isograft tumor by reducing Blimp1 and Xbp1 proteins

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Reviewer: Richard E. Davis

Reviewer’s report:

The authors have done extensive work and made interesting observations suggesting that restoration of the predicted gene Gm40600, if it can be achieved therapeutically, could be effective for the treatment of multiple myeloma (MM). However, there are many shortcomings in the manuscript and study.

Major criticisms

1) A significant area of uncertainty concerns why SP 2/0 cells were selected as the model of MM in this study. The ATCC website does not provide information, since the ATCC has stopped offering it. Other sources of information state that mouse myeloma SP2/0-AG14 is a hybrid between BALB/c spleen cells and the myeloma cell line P3X63AG8, and is used as a fusion partner for the production of hybridomas secreting specific monoclonal antibodies. Therefore, SP2/0 cells have 2 features that may make them less than ideal for the way in which they were investigated in this study:

--They lack immunoglobulin production, which has great relevance to the unfolded protein response (UPR) and MM biology. This is particularly important regarding the potential role of Xbp-1. Overexpression of Gm40600 in an immunoglobulin-secreting mouse MM cell line, or in SP 2/0 cells after conversion to a state of immunoglobulin production (e.g., PMID: 17308121), could be informative.

--They likely have features of the BALB/c. strain. This calls into question why C57BL/6 mice were used as the source for LPS-stimulated B cells; elsewhere in the study, the authors use Balb/c mice, which could have provided B cells.

2) There are some faults in methods or analysis:

--Unless it has been shown that fusion to GFP does not affect Gm40600 function, it is not justified to use GFP fusion to select cells for studies of the effect of Gm40600 on other proteins (Bcl6, Irf4, etc.). Cells with the LV201 vector and Puro selection should be used for this purpose, unless studies are done to show that the effects of Gm40600-GFP overexpression are the same as
for Gm40600 overexpression. The only justification for using Gm40600-GFP is to determine subcellular localization by imaging.

--Table 2 should show the expression level of Gm40600, so that it can be determined whether the exogenous vector is producing physiological or supraphysiological levels.

--The analysis of RNA-sequencing data is very limited. Gm40600 has simply been "cherry-picked" out of the results. Although the data have been deposited with ArrayExpress, they are not yet publicly available. At a minimum, the authors should place Gm40600 in some context, and explain why it was chosen for further investigation. E.g., was it the most highly downregulated gene, or just one among others? Was any pathway analysis (such as GSEA) done in the comparisons, either between LPS cells vs. SP 2/0 cells (Table I), or between SP 2/0 cells with vector vs. Gm40600 (Table II)?

3) Figure 3B is said to show that Gm40600 reduced protein levels of Blimp1, Xbp-1, and Bcl2 proteins in SP 2/0 cells, but the differences are not convincing. The authors claim, or propose, that

A. The reduced Bcl2 mRNA levels with Gm40600 are due to the following mechanism: Reduced protein levels of Xbp-1, possibly the result of lower protein levels of Blimp1, lead to reduced Bcl2 transcription, since Xbp-1 normally promotes Bcl2 transcription.

B. Reduced Bcl2 protein leads to the increased spontaneous apoptosis observed after Gm40600 overexpression.

The authors should do experiments to validate these findings and interpretations. This is especially the case because Xbp-1 has a distinctive biology associated with the UPR, which is highly relevant to MM biology. Some examples of possible experiments:

--Show that Xbp-1 mRNA is spliced in SP 2/0 cells, and therefore likely to be active. A simple RT-PCR assay is well-established for this purpose, as in PMID: 29062910.

--Clarify whether the Xbp-1 shown in Figure 3B is the unspliced form (~33 kDa) or the spliced form (~ 54 kDa).

--Use a reporter assay, as in PMID: 20408817, to show that Xbp-1 transcriptional activity is reduced by Gm40600 overexpression.

--Show that exogenous expression of Bcl2 reduces apoptosis caused by Gm40600 overexpression.

4) The manuscript would benefit from some further experimental work, because there is virtually nothing known about Gm40600. In particular, the question arises as to what is the normal expression of Gm40600 at different stages of B-cell development. Is Gm40600 expression
increased by LPS stimulation in normal B cells? Is it expressed in normal mouse PCs? This would lend some insights into the significance of the lack of Gm40600 expression in SP 2/0 cells, and whether they have downregulated it as part of normal PC differentiation, vs. as part or consequence of their derivation as an immortalized PC line. These data could be generated by qRT-PCR of sorted B cell populations, and would be a valuable resource.

Minor criticisms:

1) The English writing needs to be substantially improved. BMC Cancer may offer a service for that.

2) The Abstract is complicated by BMC Cancer’s requirement to conform to the Background/Methods/Results/Conclusions format, in that the Methods needs to state that Gm40600 was investigated, before the Results explains why it was investigated (i.e., because it is expressed less in SP 2/0 cells). Nonetheless, the Abstract needs to state that Gm40600 is a predicted gene, when Gm40600 is first mentioned in the Methods; otherwise, it could easily be assumed to be some sort of reagent. The Abstract also needs to state the method by which Gm40600 is overexpressed.

3) The last paragraph of the Introduction needs to make clear that at this point, the Introduction has changed from a presentation of background information to a brief description of the present study and its findings.

4) In the Methods section, the "RNA-sequencing" subsection needs to be divided into 2 parts, or 2 subsections, that present:

   --How the cellular samples were prepared. The description for LPS-stimulated B cells is sufficient, since it cites reference 16. However, the description for SP 2/0 cells is inadequate. That information is provided in the legend to Table 1, but should be in this section instead.

   --Some minimum detail about the RNA sequencing: RNA preparation, kits, etc.

5) Other flaws of writing in the Methods section. It is necessary to:

   --State that a cDNA for Gm40600 was used

   --Describe what is LV201 (a lentiviral vector with Puromycin selection)

   --State that a control form of LV201 was used, and specify what it was (presumably an empty vector)

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
No

**Does the work include the necessary controls?**
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No

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