Reviewer’s report

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

Version: 2 Date: 02 May 2019

Reviewer: Richard E. Davis

Reviewer’s report:

The authors have satisfactorily addressed my previous concerns, with two following exceptions:

1) I previously stated that "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 authors’ response focused on whether Gm40600 was actually produced by the exogenous vector, which was confirmed by qRT-PCR comparing vector-transduced cells with Gm40600 vs. the empty vector. However, that does not answer the underlying question, which can be stated thusly: "Regarding the amount of Gm40600 expressed in SP2/0 cells by exogenous means, was it physiological or supraphysiological? In other words, was it similar to the amount of Gm40600 expressed in LPS-induced PB/PC, or was it much greater? If it was physiological, then I am more inclined to believe that the results support the downregulation of Gm40600 in SP2/0 cells as a necessary part of tumor development. If it was supraphysiological, then the toxic effect of exogenous Gm40600 may not be relevant.

The authors should use qRT-PCR to compare the expression of Gm40600 in LPS-induced PB/PC vs. SP2/0 cells with exogenous Gm40600.

2) I previously stated that "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."

In response, the authors have done these things:
A. They have re-examined their RNA-Seq data and found both spliced and unspliced Xbp-1 mRNA in SP 2/0 cells, with no difference between empty vector vs. Gm40600 overexpression.

B. They have used a reporter assay to show that Bcl2 transcriptional activity is promoted by Blimp1 overexpression.

These responses are not adequate to address concerns about whether protein levels of Blimp1, Xbp-1, and Bcl2 proteins in SP 2/0 cells are truly reduced by Gm40600, and whether the transcriptional effects of Blimp1 and Xbp-1 on Bcl2 are relevant. The RNA-Seq data in Table II show no significant effect of Gm40600 on Blimp1 or Xbp-1. Table II shows fewer transcripts for Bcl2 with Gm40600, but levels are so low for both Vector and Gm40600 that this difference may not be statistically significant.

The authors should do one or more of the following experiments:

-- Use their Bcl2 promoter assay to compare SP 2/0 cells with empty vector vs. Gm40600 overexpression.

-- Use qRT-PCR to quantify Bcl2 expression in SP 2/0 cells with empty vector vs. Gm40600 overexpression.

-- Use a more precise technique than western blotting, such as flow cytometry or electrochemiluminescence, to establish whether Gm40600 reduces protein levels of Blimp1, Xbp-1, or Bcl2 proteins in SP 2/0 cells.

Lacking these experiments or comparable equivalents, or positive results, the authors should reduce or remove their assertion that Gm40600 has a negative effect on Blimp1, Xbp-1, and Bcl2.

I commend the authors for having included results using Stch-/ B cells from mice. However, these results raise the following questions:

1) Loss of Stch causes remarkable 50% reductions in mRNA for Blimp1 and Xbp-1. Even more remarkable is the >95% reduction in mRNA for Gm40600. The authors state that "these data suggest that Gm40600 expression is positively related to Prdm1 and Xbp1 expression." Even though "suggest" is used, that statement implies that Prdm1 and Xbp1 have a positive effect on Gm40600 expression, for which there is no direct proof. The authors should either attempt to provide proof, or qualify their statement to say that there may not be a causal link between Prdm1 or Xbp1 and Gm40600.

2) B cell-specific knock-out of Stch did not affect B-cell mature and activation but reduced PC production (data not shown). From this, the authors again "suggest" that "Gm40600 expression…is involved in nonmalignant PC generation/maintenance." Even though "suggest" is used, this statement raises two issues:
A. There is no direct proof. That could be provided by forced Gm40600 expression in Stch-/- mouse B cells, but such an experiment is beyond the scope of the present report. Instead, their statement should be qualified to say that there may not be a causal link between Gm40600 expression and PC production from Stch-/- mouse B cells.

B. It changes the way in which the function of Gm40600 should be viewed. Previously, it appeared as if Gm40600 is a tumor suppressor, whose loss is necessary for the development and/or proliferation of malignant PCs, as modeled by SP 2/0 cells. This new finding suggests something different: that Gm40600 expression may be necessary for nonmalignant PC generation/maintenance. Both may be true, but the authors should add this to the list of "unsolved questions" at the end of the Discussion.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
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