Reviewer’s report

Title: IL-10Ra expression is post-transcriptionally regulated by miR-15a, miR-185, and miR-211 in melanoma

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Reviewer: Yun Ji

Reviewer’s report:

The manuscript "miRNA dysregulation in melanoma cell responsiveness to IL-10" by Venza et al. investigates the expression of IL-10 and its receptors regulated by miRNAs in melanoma cells and the effects of IL-10 on melanoma cell growth. The authors proposed that the growth of melanoma cells was influenced by IL-10 levels in the tumor microenvironment. The different responsiveness of various melanoma cell lines to IL-10 is affected by IL-10a receptor expression, which is post-transcriptionally inhibited by several microRNAs including miR-15a, miR-185, and miR-211. Overall, the data is novel and provides an interesting model of how specific microRNAs regulate cell growth by modulating immunoregulatory cytokine receptor levels in the tumor microenvironment.

There are a number of points the authors need to address before this article can be considered for publication.

Major points:

1. There is a conceptual mistake in this article: microRNAs are considered to regulate gene expression post-transcriptionally. They may confer epigenetic modulations by regulating genes involved in histone and DNA methylation, which is considered as "epigenetic" effects. In this article, it mentioned several times in the text that genes are regulated epigenetically by miRNAs but all the data shown is just posttranscriptional regulation. Please edit the text accordingly.

2. Both G361 and GR-M are cutaneous melanoma lines, but they are very different considering the expression of IL-10Ra and IL-10Rb based on the data presented in this manuscript. Why is G361 more similar to the uveal melanoma cell line OCM-1 with respect to IL-10Ra expression, miRNAs expression and responses to IL-10? Is there any physiological relevance of this difference? It is necessary to include data from primary melanoma tumors (cutaneous and uveal) to evaluate the expression of IL-10, IL-10 receptors and miR-15a, miR-185, and miR-211.

3. miR-15a, miR-185, and miR-211 are all upregulated in G361 and OCM-1, however, the effect of these miRNAs was evaluated individually by luciferase, WB, and growth assays. It would be interesting to know whether there are synergistic or additive effects from all three miRNAs.
4. In Fig. 4A, how is the concentration of IL-10 (50, 100, or 500 U/ml) determined? Is it based on the IL-10 concentration measured in the tumor microenvironment? Please provide reference or justification. Why does 100 U/ml have the best effects on promoting cell growth?

5. Transfecting miR-15a, miR-185, and miR-211 mimics into GR-M line inhibits cell growth; it must be shown that these miRNA mimics reduce IL-10Ra levels. It would be optimal to test the effects of IL-10 on cell growth treated with miRNA inhibitors or mimics to formally test the model that miRNAs regulate IL-10Ra levels to confer response to IL-10 in melanoma cells.

Minor points:

1. There is some mislabeling. For example, the Fig. 2B referred in the text does not match the Figure 2B, which is miRNA array data instead of qPCR data. In Fig. 4A, the labeling should be IL-10 instead of IL-10Ra.

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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