Reviewer's report

Title: Integrative analysis of next generation sequencing for small non-coding RNAs and transcriptional regulation in Myelodysplastic Syndromes

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Reviewer: Rui Chen

Reviewer's report:

The manuscript titled “Integrative analysis of next generation sequencing for small non-coding RNAs and transcriptional regulation in Myelodysplastic Syndromes” by Beck et al. had described results of ncRNA profiling of MDS low and high risk patient primary cells by deep sequencing. By comparing MDS patient samples to the control, the author had identify a set of miRNA and other ncRNA that are differentiated expressed among these samples. In addition, extensive integrated analysis of ncRNA profiling, mRNA expression, miRAN predicted targets, and TF downstream targets had been performed to identify potential key regulators that are specific to different stage of MDS. As the first ncRNA profiling for MDS samples, coupled with extensive and detailed analysis of the data, I believe this work has generated many hypothesis and will be generally interested to this research field, therefore suitable for publication after addressing the following concerns.

Major Compulsory Revisions

First, MDS, like other type of cancers, is a heterogeneous disease. Therefore, it seems that the author only profiled one sample from each group (control, low, and high grade MDS sample). It would be very difficult for me to assess significance of the differential expressed ncRNAs. Either additional samples need to be profiled or the expression level of selected set of ncRNA that are differentiated expressed need to be examined in additional samples to confirm the findings.

Second, the author found 39 novel miRNA. In addition, they found 24 miRNA* were only expressed in RA and hypothesize that they can be potentially used as biomarkers. However, the author did not show validation of any of these findings with different technique, such as Taqman assay, and in additional samples. I feel experimental validation of a subset of these findings is essential to give confidence of the results.

Discretionary Revisions

Third, the bone marrow contains several cell types and cell type composition of bone marrow is different between MDS patient and control and also among MDS patient at different stages. It seems to me that profiling of sorted cell types will be much more informative and the data will be easier to interpretate.
Fourth, the author performed extensive data integrative analysis and, very interestingly, end up a small number specific findings. For example, "In RA, two subtype-specific expressed miRNAs were selected as most dominant regulators. Whereas the differentially expressed target genes of hsa-mir-1977** regulate hematopoiesis and apoptosis, hsa-miR-130a has previously been associated with the regulation of angiogenesis and platelet physiology". To assess the accuracy of the finding, I feel some quick follow up studies of these two genes in additional MDS patients at different stages should be examined to see if these are key regulators and/or biomarkers. In addition, what is the effect of change of expression of these two genes in primary cells?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests