Reviewer's report

Title: MicroRNA regulation and its effects on cellular transcriptome in Human Immunodeficiency Virus-1 (type-1) infected individuals with distinct viral load and CD4 cells

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Reviewer: Nitin Saksena

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I have read with great interest the manuscript by Duskova et al., entitled "MicroRNA regulation and its effects on cellular transcriptome in HIV infected individuals with diverse CD4 counts and viral load.

The question posed by authors is not novel, but the answer to the question posed has been dissected using a better and a more comprehensive approach than other studies published on the same issue to date. It is an elegant dissection and analysis of both mRNA and miRNA from the same group of patients. This truly is the first study on PBMC to do this, apart from a study on HIV infected brain by Zhou L et al., (BMC Genomics, 2012, 13, 677), which authors should cite.

What demarcates this study from others is a highly appropriate patient selection, controlled viral loads and CD4+T cell counts, which other studies have not done. With the robust patient numbers used in this study, it gives a better feel of statistical concordance authors have shown in each case in support of miRNA, mRNA, miRNA-mRNA interactions and pathway analysis. Therefore, it is a very complete study in terms of data, which is sound and methods highly appropriate and innovative.

Duskova et al., show clearly the differential expression between low and high viral loads, which has not been shown before. The findings are more credible because they use PBMC with well controlled viral loads and CD4+T cell counts for all patients, in addition to analyzing in parallel the mRNA and miRNA.

Major compulsory revisions:

1. Although the microRNAs miR-19b, 146a, 615-3p, 382, 34a, 144 and 155, that are known to target innate and inflammatory factors, were significantly upregulated in PBMCs with high viral load, the authors have shown only a very one-sided picture of the targets of these miRNAs in high viral patients. These miRNAs have many more targets and these targets should follow diverse pathways, including cancer which usually predominate. Although it is correct in part, it is essential to educate readers by providing an unbiased data.

2. Similar concern I have about the network analysis and statistical support for
mRNA targets of miRNAs, which are predominantly focused on all pathways relevant to HIV, ignoring the rest. Authors should provide a pathway list of miRNA targets in a more balanced way by categorizing them as top 10 pathways. I am stressing this because a lot of studies are missing out this point. mRNA-miRNA interactions and to interpret them functionally is still at its infancy and these interactions should be viewed with caution as these are complex to interpret.

3. Further, since PBMCs have been analyzed in this study and authors have only briefly mentioned the point about the underlying reasons for differences with other studies, I firmly believe that the authors should bring about all the studies done, to date, on miRNA in relation to HIV infection and summarize in context of their own studies in order to give readers a better feel of differences between PBMCs and other cell types, such as CD4+ and CD8+ T cells types and others during HIV infection.

One of the serious concerns with all miRNA studies in relation to HIV (an for that reason on cancer, neurodegenerative diseases, etc) is that they all show critical differences in DE miRNAs. Why such differences? Authors should bring about a more philosophical discussion on possible reasons for such differences. This will clarify the air regarding possible differences in DE miRNA between different studies. These should include reasons attributed to diverse platforms, parameters, patient selection, cell type selection, individual patient differences, preliminary statistical examination rather than rigor, normalization procedures for patients, adjusted p value vs the observed p values, analytical algorithms, etc.

The discussion and results are overall balanced, except the limitation points highlighted above, and the data are supported by good statistical support. It is a well written paper and very exhaustive!

**Level of interest:** An article of outstanding merit and interest 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