Reviewer's report

Title: Nuclear-encoded mitochondrial MTO1 and MRPL41 are regulated in an opposite epigenetic mode based on estrogen receptor status in breast cancer

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Reviewer: Yoshitomo Chihara

Reviewer's report:

The authors presented that MTO1 and MRPL41 were epigenetically regulated, and showed an opposite expression pattern according to ER status. They formulated consecutive experiments and performed them carefully. These results are potentially interesting

Major criticisms

1. How to select the target genes for this study (MTO1 and MRPL41) were unclear and confused. Page 6, Line 1 to 4, they mentioned that they selected the genes which were overrepresented in breast tissue. Whereas, in Page 10, they mentioned that they identified highly upregulated genes in cancer tissue. Furthermore, in Table S2, 7 genes did not show the enrichments in cancer tissue. Which is true?

Another thing, why they did not compare breast cancer and mammary gland profile to compare genetic and epigenetic alterations occurring in cancer?

Why they did not address to down regulated genes to refer to epigenetic regulation in cancer tissues?

2. In Discussion, they mentioned that TSA treatment increased MTO1 and MRPL41 expression because TSA induced demethylation. This hypothesis was based on ref.29. Ref.29 showed that hypermethylation of 5-cytosine was induced by l-methionine treatment in mice’s frontal cortex, and HDAC inhibitor promoted demethylation during l-methionine withdrawal in vivo. In cancer cells, DNA methylation status and expression change in many genes endogenously. In cancer cells, the hypothesis that acetylation regulated DNA methylation is uncommon. For this reason, the changes of DNA methylation status before and after TSA treatment should be shown.

3. In Introduction, they should not summarize the results of this study in the end. Please write down “what is being reported in the article”.

4. Figure 4 and 5, please indicate CpG island, and CpG sites that they assayed for readers to follow easily.

Minor criticisms

1. Several gene names including MTO1 and MPRL41 are written in italic type. Consolidate them.
2. Figure 1, 2 and 3. DNA methylation status was assayed by semi-quantitative methods. Thereby the Y axis should be DNA methylation levels or status.

3. Figure 1B and Figure S1C, a total number of patients exceed 45.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.