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August 27, 2017

Barbara Rattner
Executive Editor

Manel Esteller
Editor-in-Chief
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Dr. Rattner,

Enclosed, please find our revised manuscript entitled “UBE3A-mediated regulation of imprinted genes and epigenome-wide marks in human neurons” for consideration as a Research Paper for publication in *Epigenetics*. I am the corresponding author for this manuscript and my contact information is listed below.

We thank the editor and reviewers for taking the time to review our submission. We were overall quite pleased by the favorable comments by the reviewers. All comments and criticisms were taken into consideration and the manuscript has been amended and improved in response. Please find the list of comments and changes below:

Comments by reviewer #1
1. The wording in the results section of p. 5 is a bit confusing. For example, the sentence "Differentially expressed gene lists showed no significant overlaps except a modest significance with differential H2A.Z genes in the KDSH comparison," is difficult to understand. It seems like the KDSH comparison shows overlap between RNA, DMR, K4me3 and H2A.Z.

Reworded: “Differentially expressed genes did not significantly overlap with other datasets except in the KDSH comparison group, where it overlapped with differential H2A.Z genes.”

2. In addition, the data presented in Fig. 5 are only moderately informative. While this is a time saving way to show genome-wide data, it is relatively non-interesting in terms of informational content. Is there an alternative way to display these data? Or are there other data that the readers would want to include (perhaps Table 10 or the imprinted gene network described in the discussion?). If the data in Fig. 5 are critical to interpreting the findings presented, the Fig should be enlarged to be clearly visible without the aid of magnification.

Figure 5 has been removed from the main text and included as a Supplemental figure (Figure S2) with accompanying legend.

3. Does the conclusion that monoubiquitinated H2A.Z is representative of a poised sate comport with previous findings that show that H2A.Z and DNA methylation are mutually antagonistic? This does not need to be addressed explicitly, but it is useful to keep in mind.

The link between H2A.Z poising, DNA methylation, and epigenetic silencing is explored in paragraph 3 of the discussion. But to emphasize this point, further text regarding monubiquitinated H2A.Z has been added to the Introduction and the new 4th paragraph of the Discussion.

Minor concerns:
p. 5, second line- 'contract' should be 'contrast.'
Corrected
p. 7. SAGA- should be spelled out = Spt-Ada-Gcn5-acetyltransferase complex.
Corrected
p. 11. 2nd paragraph - the authors state that UBE3A is paternally imprinted in post-natal neurons. This seems to run counter to what is stated in the introduction.
We have changed the wording in the Introduction to read “Neuronal maturation” rather than “development”.

Comments by reviewer #2
1. In this manuscript, the authors did not describe how they define the differentially methylated regions (DMR), differential expression, and differential histone peak levels for H3K4me3 or H2A.Z. Without these information, it is difficult to judge the data quality and make any conclusions. Please add these information to the result section for Figure 1C.
Definition cutoffs have been added to the Results section on p. 5.

2. Although the authors observed differences in DNA methylation, histone H3K4me3 or H2A.Z modification and genes expression in the four comparison groups, these differences could be due to technical issues or culture effects. How many replicates have the authors performed?
Knockdowns were performed in triplicate cultures. This has been mentioned in the Methods and Results sections, as well as the figure legend for Figure 1.

3. Please add some descriptions of the quality of all the sequencing data in the method section or as a supplementary table.
Sequencing data summaries are presented in Supplemental Data S.Table 1.

4. The authors concluded that UBE3A regulate imprinted genes, however, the statistics of the overlap between the sequencing datasets and the imprinted gene list were not impressive (Table 2), especially for RNA-seq and H2A.Z chip-seq. Only a few imprinted genes have been altered (about 1% of the total altered genes). This data does not support the authors' conclusion.
The DNA methylation changes were highly significantly enriched for imprinted genes in all comparison groups, supporting our conclusion that UBE3A regulates methylation of imprinted genes. The wording of the abstract and first paragraph of the Discussion makes it clear that this conclusion was based on DNA methylation changes. While imprinted genes are a small proportion of genes affected in our datasets, we observed methylation changes in the majority of known imprinted genes and some of these also overlapped with additional transcriptional and/or epigenetic changes (Figure 4B).

5. The authors observed that across all comparisons, genes with DMRs were the most numerous, while genes showing differential H2A.Z peaks were least prevalent (Figure 2). Could the authors provide some explanations? Since UEB3A does not directly regulate DNA methylation, what is the mechanism that altering UEB3A levels result in the most changes in DNA methylation?
The proposed *UBE3A* mechanism of alterations to DNA methylation is regulation of H2A.Z deposition and monoubiquitination. This alters the chromatin state leading to changes in DNA methylation. This hypothesis is explained in the discussion (p.10) and has also been included in the Introduction. Also, since quantitating the number of epigenetic differences between conditions is inherently related to the width (genomic size) of these features, abundance of the changes is not necessarily an indication of significance, which is why statistical methods were employed in Tables 1 and 2 to assess the significance.

6. The authors observed that the KDSH comparison showed the most unique pattern of overlapping epigenetic marks with the most H3K4me3 changes (Figure 2). Could the authors provide some explanations?
We have added two sentences to the Discussion on p. 11 discussing these results in the context of UBE3A’s ascribed function in degrading estrogen receptors transiently bound to promoters.

7. For the DNA methylation data, are the DMRs near the imprinted genes from four comparison groups overlapped with known imprinting control regions (ICRs)?
The observed DMRs are primarily in promoters and within the gene body. However, a quick intersect of DMR data with known, ubiquitous ICRs has shown a number of ICRs that contain DMRs in each comparison group. This has been included in the manuscript and a list of affected ICRs has been included in the Supplemental Data as S.Table 12.

8. The authors observed changes in H2A.Z marks both at promoter and at gene bodies (Figure 4). However, the function of H2A.Z at promoters was previously reported to be different from those at gene bodies. Could the authors provide more discussions?
We have added four additional sentences to the Discussion on p. 11 that speculate on the different functions of promoter vs gene body H2A.Z and how our data show regulation by UBE3A at both positions.

9. There is no description for Figure 5 in the result section. What is the message that the authors would like to deliver through Figure 5?
Figure 5 has been removed from the main text and included as a Supplemental figure (Figure S2) with accompanying legend.

All of the authors have agreed to the submission and have no conflicts to declare. This manuscript has not been submitted for publication elsewhere. If accepted for publication, we agree to pay the page and color figure charges. There are no restrictions to depositing all sequencing data in full upon publication. Because of the large quantity of genomic data contained in 13 Supplementary tables, these have been uploaded as a single file with separate tabs in an xls file.

We have also uploaded a possible cover image that we hope you will consider.

Sincerely,

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