Takayuki Sumida Tsukuba, MD, PhD

Editor-in-Chief

Modern Rheumatology

July 4, 2016

Dear Dr. Tsukuba:

Thank you for your letter of May 10th, 2016 regarding our manuscript entitled “Genome-wide DNA methylation patterns in CD4+ T cells from Chinese Han patients with rheumatoid arthritis”. Our appreciation also goes to the reviewers for their helpful comments. We have revised the manuscript following the reviewer’s comments and your instructions. All the update and change were red color bolded so that you and reviewers could recognize them quickly.

Enclosed please find the revised version of the manuscript along with a point by point description of our responses to the reviewer’s comments. We hope that the manuscript is now acceptable for publication in Modern Rheumatology. Thank you again for your letter and for your editorial assistance.

Sincerely yours,

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**Responses to Reviewer 2’s comments**

We first thank the reviewer for the helpful comments. In the revised manuscript, we have incorporated the reviewer’s comments. In the following, a detailed reply to the general and specific comments by the reviewers are provided in which the reviewer’s comments are reported in normal style while the corresponding authors’ reply is reported in red color.

here are some points that I suggest authors should discuss.  
  
1. Authors discussed the roles of differentially methylated genes, such as HDAC4 and ITIH3, in the pathogenesis of RA. The discussion still seems insufficient. They identified these aberrant genes in RA CD4 T cells. It would be critical in this report whether these genes play important roles in RA CD4 T cells. I recommend they review the previous reports about dysfunctions of RA CD4 T cells and discuss the roles of the differentially methylated genes in RA CD4 T cells. If there is not any evidence, I recommend they describe it.

Response:

Thank you for the suggestion. We introduce more discussion on the probable functions for the DMR genes as follows.

Histone deacetylases (HDACs) have pleiotropic effects within the immune system. At least 11 histone deacetylase (HDAC) exist in human genome and could be divided into 4 sub-families including class I (HDAC1,2,3,8), II (HDAC4,5,6,7,9,10), III ([Sirtuin 1](https://en.wikipedia.org/wiki/Sirtuin_1)-7) and IV (HDAC11) on the basis of size, homology and assembly. HDAC4 belongs to the class IIa HDACs and which are predominantly playing the role on regulating adaptive immunity. Previous evidence shown nuclear export of HDAC4 is quite important for induced expression of IL­5 in activated T cells indicating the methylation status aberrant would cause compressive influence to immune-response. Meanwhile, methylation change of HDACs might also cause isoform-selective difference in immune cells and therefore caused different response to the corresponding drugs for different RA patients.

The above contents have been added into the manuscript with red colors. In terms of other DMRs as we mention in the manuscript, they are almost novelty identified DMRs in RA and don’t have enough previous research. We have the evidence will come out in the future to support our result and we will also conduct further study to validate these biomarkers with some specific strategies.   
  
2. Generally, epigenetic mechanisms are functional through the change in gene expression. Analyzing not only epigenetic changes but also gene expression is required in the epigenetic studies. If epigenetic changes affect gene expression, they are functional. In addition, a part of differentially methylated genes might be false positive as written in discussion site. To solve these problems, the analysis of gene expression is needed. I understand why authors do not analyze gene expression. Their data about differentially methylated genes in RA CD4 T cells is still worth publishing. If they plan another study of DNA methylation and gene expression, I recommend they describe it.

Response:

Yes. We appreciated your understanding. This is our first stage for the epigenetics research on RA. We are planning to use WGBS, RRBS and MBD-seq technique to construct more accurate and creditable methylome for RA. And the RNA-seq would be conducted simultaneously with methylation detection. We are planning to identify the epigenetic regulated RA related etiological genes and factors so that these biomarkers can be used in the coming personalized medicine and precision medicine.