Peer Review File

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Reviewer A

I read carefully your manuscript entitled, Characterization of the dynamic changes of comprehensive epigenetic regulation during the development of human primary Sjögren's syndrome'.

The authors designed a study of epigenetic codes in two females suffering from primary Sjögren's syndrome – and later a MALT lymphoma.

I would recommend to the authors to clarify the next points:

- Is there any difference between Ro/La positivity and epigenetic changes? This is usually a relevant issue in pSS and should be further explained. It could be applied to other classical clinical factors to develop lymphoma in pSS, as cryoglobulinemia.

Reply: This is a good and important question. Two patients we focused on in this study were diagnosed as pSS, and mainly presented the oral symptoms without any other organs involved such as cryoglobulinemia, hypergammaglobulinemia or allergic purpura. In this study, Ro/La in serum was positive in both of them and there may be other clinical risk factors for pSS to develop into lymphoma. In this study, the two patients were found to have one of the risk factors was repeated or continuous parotid gland enlargement. It has been reported that 12% of pSS patients with only one risk factor could develop into lymphoma (Medicine (Baltimore). 2009 Sep; 88(5):284-293). Our results so far can provide dynamic epigenetic patterns for the progression and development of the specific type of pSS, and it could be potentially applied to predict the progression of pSS with the changes of differential histone modification on some target genes. Nevertheless, due to the limited sample size and unitary sample type, our data so far cannot do more application to other classical clinical factors. But from the results of H3K4/9/27/36/79me3 on known target genes, we can detect them in more samples and attempt to achieve the potentials in clinical studies.

- The abstract term of serious pSS is confusing. The research is about MALT lymphoma development, one of pSS complications – there are other extraglandular serious clinical entities.

Reply: The comment is right. We remove "serious" in abstract and figure 4, and

correct as “Transcriptome landscapes indicated two destinies of pSS progression with or without MALT lymphoma representing the distinct populations of differential expressed genes and functions.”

- The authors should consider some comments about connections with clinical and laboratory parameters (translational).

Reply: We added the connection between gene expression and clinical phenotypes in Discussion section. “The secretory function of salivary gland being used to reliably evaluate the severity and classification of pSS supports our GO enrichment analysis that salivary glands atrophy and cavitation usually appear in PG_NC compared to early stage of LG_pSS including reduced expressions of Ccnd2, Sfrp1, and IGF-1. Nevertheless, the secretory function may not be appropriate to the glands with MALT lymphoma. In turn, parotid gland swelling accompanied with recurrent infection and persistent pain is tightly associated with MALT, displaying the irregular expressions such as IL-17, and Bcl-2. Consistently, H3K9me3 and H3K27me3 of Bcl-2 are observed significantly decreased in PG_MALT compared to PG_NC and LG_pSS in our data, which indicates that histone alterations indeed affect gene expression in pSS progression.”

- The gene routes described should be explained in more detail. How these genes could be related to MALT lymphoma?

Reply: The basic principle of PCA analysis is to map the expression of global genes to a certain dimension to assess the differential expression profiles among samples. The given Route 1 and 2 similar with an analogous pseudotime analysis is drawn according to the approximation of these gene profiles of LG_NC, LG_pSS, PG_NC, PG_MALT. These genes not only include MALT related pathways, but also contain other functions.

- Citing the figures might not suitable in the discussion section. The results of the research were shown in the corresponding section. I recommend omitting this Figure’s comments in the discussion section

Reply: Figure 4 is not an experimental result, but a graphic conclusion of this manuscript. We believe it is suitable to put in Discussion section.

- It’s rather relevant to report the limitations of the manuscript at the end of the discussion section, and before final conclusions.

Reply: We added a paragraph in it. “Of course, there are still shortcomings in our study, including the limited sample size as well as less abundant types of pSS. Besides

MALT lymphoma, other extraglandular complications of pSS are likely to reflect other routes of pSS development. Additionally, more epigenetic signatures may help advanced understand the molecular mechanism on pSS pathogenesis and development. The target genes and signaling pathways affected by differential epigenetic modifications will be validated in future study to develop the therapeutic strategy of pSS by epigenetic drugs."

- Minor typo mistake in figure 4: More serious pSS.

Reply: We change figure 4 "With or without MALT"

Sincerely,

Reviewer B

The paper "Characterization of the dynamic changes of comprehensive epigenetic regulation during

Development of hum1an primary Sjögren's syndrome" by N Cao et. Al. evaluated gene expression in labial glands with or without Sjogren's syndrome (SS), parotid gland with tumor and paracancerous tissues from two patients with MALT lymphoma using RNA-seq and ChIP-seq of H3K4/9/27/36/79mc3.

Line 46-49: the statement that 30-50% of pSS patients have lymphomas is not true

Reply: In this article, we have no statement that 30-50% of pSS patients have lymphomas, but 30-50% of pSS patients have extra-glandular manifestations including non-Hodgkin lymphoma of the B-cell series, such as mucosa-associated lymphoid tissue (MALT) lymphoma. MALT is only a small part of the extraglandular manifestations.

This study involves 2 patients in which labial glands were evaluated at first diagnosis and then at second evaluation parotid glands were excised (with or without lymphoma) and MALT lymphoma was identified. It is hard to know with the differences in the tissues studied whether there is a direct progression from the genetic changes observed in one tissue to that observed in another. It is unclear whether the changes noted are primary or secondary. The overall concept of the paper is interesting, but the data cannot be used to make any conclusions regarding pathophysiology

Reply: First, the specimens we harvest are from the same patients with different stages, which eliminates individual differences as much as possible, and emphasizes the epigenetic and genetic importance for disease development. Moreover, we majorly focus on the patients who finally develop to MALT but without any other

complications involved in other organs, which also simplifies the experiment and analysis model. The objective of this study is to clarify the role of some important histone hallmarks in regulating gene expression during the long scale of pSS development. With the regards to pathophysiological relativity, we have to admit that it cannot give more information to indicate the diagnosis or prognosis of pSS so far due to the limited sample size. But from the given genes with differential epigenetic modifications, they can be considered as therapeutic targets for reference in future study.

Reviewer C

This is an excellent article that tries to piece the puzzle between autoimmunity & malignancy, but the following points are worth exploring in the discussion.

1. What was the time gap between sample analyses?
2. Did both the patients have autoantibodies to Ro and La at diagnosis?
3. What was the focus score at diagnosis?

   Reply: We add the Table 1 for the clinical information to address Questions 1 to 3.

4. Discuss why whole genome methylation not done and specifically these were chosen?

   Reply: Genome methylation means DNA methylation. Here, we only focused on H3K4/9/27 tri-methylation. Due to short of funding, we cannot conduct all epigenetic modification in this diseases. H3K4/9/27me3 as we know were important for gene transcription, and they are usually catalyzed by the same type of enzymes, which may be benefit to developing clinical treatment. Previous studies have reported the connections between H3K27ac, H3K4me1, H3K36me3 and pSS (Second paragraph of Introduction), but are not systematic and comprehensive enough to figure out the epigenetic role in pSS.

5. Discuss the role of c-myc and Bcl-2 with the trimethylation findings, if any

   Reply: We check the data. Transcription and epigenetic modifications of c-myc have no significant change in our data. While RNA levels of Bcl-2 are slightly increased in MALT compare to other groups (most significant FC=1.42, p=0.053). But H3K9me3 and H3K27me3 of Bcl-2 are observed significantly decreased in PG_MALT compared to PG_NC and LG_pSS., and the changes of H3K4/36/79me3 of Bcl-2 are not obvious. We have pay attention to the common genes on apoptosis and tumorigenesis. Unfortunately, c-myc and Bcl-2 are not the typical genes in our data. In discussion section, we added some contents on Bcl-2 “In turn, parotid gland swelling accompanied with recurrent infection and

persistent pain is tightly associated with MALT, displaying the irregular expressions such as IL-17 and Bcl-2. Consistently, H3K9me3 and H3K27me3 of Bcl-2 are observed significantly decreased in PG_MALT compared to PG_NC and LG_pSS in our data, which indicates that histone alterations indeed affect gene expression in pSS progression."

6. Did any of the patients have EBV infection?

   Reply: EBV infection is negative either in these two patients. We have replenished in Table 1.