Reviewer 00503587

This work focused on two mRNA molecules and their potential relevance to IBD Specific

Comments

1. The title requires revision. It states a decreased risk of active CD. This could mean a decreased risk of a flare of disease, or a change in this marker during active disease or a decreased risk with the development of CD. It should be revised to be more clear and accurate.

Answer: Thank you for your comments. Indeed, we meant "a change in this marker during active disease" but not "a flare of disease" (we didn't assess the risk of flare, active disease CD patients might not be only due to disease flare but also on account of recent disease development). And as your suggestion, we have amended the title to "Circulating miR-125a but not miR-125b is decreased in active disease status and negatively correlates with disease severity as well as inflammatory cytokines in patients with Crohn's Disease" highlighted in the manuscript with red color.

2. The Methods section of the ABSTRACT contains numbers that should be in the Results section

Answer: Thank you for your comments. We have put the number contained in the "Methods Section" into the "Results Section" in the ABSTRACT according to your review and highlighted in red color.

3. There are numerous errors of English language (grammar and word usage) in every section of the work that all require correction

Answer: Thank you for your comments. We have thoroughly reviewed the manuscript and corrected the grammar and word usage errors, in the meanwhile, we have invited a language editing company (American Journal Experts, www.aje.com) as the journal suggested to polish the language and correct grammars to make the article more readable. The corrections were highlighted in red color in the manuscript.

4. What is meant by a converse association (ABSTRACT)?

Answer: Thank you for your comments. "Converse association" means a negative association, in order to avoid ambiguity, we have amended the "converse association" to "negative association" and highlighted in red color in the manuscript.

5. The word "dramatically" would be better replaced with a word more suitable to a scientific publication

Answer: Thank you for your comments. We replaced the "dramatically" with the word "gradually" and highlighted in red color, which more suitable for a scientific publication. In the manuscript, it becomes "MiR-125a level was gradually increased in A-CD patients who achieved clinical remission".

6. The ABSTRACT comments on 3 months of treatment, but does not specify the nature of this. Later in the RESULTS section, this is also not well described

Answer: Thank you for your comments. The description of treatment duration in A-CD patients was described in the RESLUT - "MiR-125a/b expression after treatment in A-CD patients"-"First

paragraph"- "After 3 months' treatment in A-CD patients", and we have highlighted it in red color.

7. The INTRODUCTION suggests a direct relationship between GDP and development of IBD. Is this well-documented? If so, an appropriate reference should indicate this

Answer: Thank you for your comments. The direct relationship between GDP and development of IBD is well –documented. We added two appropriate references indicating that IBD is associated with the improvement of GDP and the economies [1. Gastroenterology. 2013 Jul;145(1):158-165.e2; 2. J Dig Dis 2010; 11(2): 76-82] in the manuscript.

8. The INTRODUCTION includes the abbreviations of terms (e.g. NF-kB) without explanation of the full term

Answer: Thank you for your comments. We have added the full term of abbreviations in INTRODUCTION and highlighted in red color including transforming: growth factor-β (TGF-β), nuclear factor-kB (NF-kB), systemic lupus erythematosus (SLE) and rheumatic arthritis (RA) in the revised manuscript.

9. Any company that was the source of reagents should be provided with full details (as per standard protocol)

Answer: Thank you for your comments. We have added the detailed description of the source of the reagents used in this study in the MATERIALS AND METHODS as follows: TRIzol reagent (Invitrogen, CA, USA); PrimerScript Real-time reagent kit (TAKARA BIO Inc. Shiga, Japan), SYBR Premix Ex TaqTM II (TAKARA BIO Inc. Shiga, Japan), IL-17, TNF-α and IFN-γ ELISA kits (eBioscience, CA, USA). They were highlighted in red color in the manuscript.

10. Were the cytokines also measured in the control patients as well? This is not indicated

Answer: Thank you for your comments. We did not measure these cytokines in the control patients (HCs), we have added this description in MATERIALS AND METHODS and highlighted in red color: "The measurement of interleukin 17 (IL-17), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) expressions in plasma samples from A-CD patients and R-CD patients **(but no HCs)** were carried out by commercial ELISA kit according to the manufacturer instructions (all from eBioscience, CA, USA)."

11. One outcome was repeat assessment of CDAI at 3 months. Were bloods (as more objective assessments) not measured at this time?

Answer: Thank you for your comments. miR-125a/b in plasma were detected at 3 months in only active CD patients (n=29), since it was a point that treatment response assessment for the active patients. But in R-CD patients (remission patients), the miR-125a/b expressions were not assessed. Cytokines were not assessed at 3 months in all patients. And we have added the description in MATERIALS AND METHODS as follows: "After 3-month treatment, CDAI and blood of patients were assessed again, and change of miR-125a/b expression was analyzed based on clinical remission achievement or not."

12. It is unclear what a "great diagnostic value" is (Results). This should be revised.

Answer: Thank you for your comments. We meant to state that a good diagnostic value, but indeed

there was not standard for “great”, thus we deleted the word “great” in the manuscript.

13. The DISCUSSION suggests that this is a retrospective study, yet the methods appears to indicate prospective recruitment, and subsequent assessment of key outcomes. This should be corrected/consistent

Answer: Thank you for your comments. It was our written mistake, this study was a retrospective study, we have corrected the “retrospective” to “prospective”.

14. The titles/legends for Table 1 and Figure 1 should be revised to include more specific details (so that they are more independent of the text of the MS)

Answer: Thank you for your comments. We revised the titles/legends for Table 1 and Figure 1 to include more specific details, as well as the other Figures to make sure they are more independent of the text.

15. Some of the Figures (e.g. on Fig 4) contain unhelpful negative data - is this necessary? Inclusion as negative results in the text of the RESULTS section may be adequate (with a supplementary online table if required)

Answer: Thank you for your comments. We have emphasized the positive results in the RESULTS section to make the article more readable, but all negative data to be included in Sup. file might not be suitable, since negative data was also very important for us to make a conclusion, and there would be 7 other Sup figures existed if we put all the negative results in Sup files. Thanks again for your suggestion.

Reviewer 02618027

Crohn’s Disease (CD) is an important clinical problem, and microRNAs have been shown to be implicated in the inflammatory bowel seen in CD. Decreased levels of miR-125a were found in the plasma of patients with active CD (A-CD), compared to patients with remission CD (R-CD) or healthy controls (HC). However, circulating miR-125b levels remained unchanged between patient groups. While the results are interesting, several concerns are noted:

Major comments:

1. What tissue or cell type might be the largest contributor of miR-125a in the circulation of HCs?

Answer: Thank you for your comments. The largest contributor of miR-125a in the circulation of HCs may be germinal center (GC) and hematopoietic stem cells (HSC). Shaham et al. suggestes that miR-125a is enriched in HSCs (up to 23-fold over total bone marrow), particularly in long-term HSCs (up to 6-fold). Besides, miR-125a is not restricted to the stem cell population, and its cluster members, miR-99b and let-7e were found to be preferentially expressed by the centroblasts in the GC [Leukemia. 2012 Sep;26(9):2011-8]. We have added this in the DISSCUSSION. Thanks again for your suggestion.

2. What are the differences between miR-125a and miR-125b that may be responsible for the dichotomy of the observations seen in A-CD and R-CD?

Answer: Thank you for your comments. We think the differences may be on account of that (1) the powerful anti-inflammation role of miR-125a in CD resulting in high correlation of miR-125a

with disease inflammation, thus miR-125a was decrease greatly in A-CD patients compared with R-CD patients. (2) miR-125b had dual effects of both anti-inflammation and pro-inflammation which was reported by analysis of previous studies [1.BMB Rep. 2016 Jun;49(6):311-8; 2. J Hematol Oncol. 2013 Jan 15;6:6; 3. Leukemia. 2012 Sep;26(9):2011-8], and its role in regulating inflammation in CD was not obvious. Based on this frame, we illuminated that MiR-125a but not miR-125b was decreased the risk of active disease status and negatively correlated with disease severity as well as inflammatory cytokines in CD patients. We have added this in the DISCUSSION. Thanks again for your suggestion.

3. What were the circulating levels of miR-29b, let-7, miR-192, miR-142-3p, miR-21, miR-495-5p, and miR-19b in A-CD, compared to R-CD and HC patients in this study? Do any of those miRs also correlate with severity of CD and inflammatory cytokine expression?
Answer: Thank you for your comments. We did not detect the plasma levels of miR-29b, let-7, miR-192, miR-142-3p, miR-21, miR-495-5p, and miR-19b in this study, and due to the long duration from the sample obtaining to the present date (The enrollment was from May 2014 to Jun 2016), the samples were not available to be used for further miRNAs detection. Besides, we meant to understand the role of plasma miR-125a/b in CD patients instead of these reported miRNAs that we referred in the INTRODUCTION since there are a great amount of miRNAs dysregulation reported previously and we only presented a few examples. Thanks again for your suggestion.

4. How was plasma extracted from blood? The centrifugation details are important to ensure that red blood cell lysis has not occurred to contaminate the plasma samples used for analysis.
Answer: Thank you for your comments. We have added the detailed procedure of plasma extracted in the MATERIALS AND METHODS as follows: Blood samples were collected from all participants in EDTA tubes at the time enrolled in this study and after 3-month treatment in A-CD patients. After collection, blood samples were centrifuged at 1000 g for 15 mins at 4℃ temperature, then the superstratum were obtained as plasma and stored in at -80℃ for further detection. If the red blood cell lysis was discovered, a repeat blood sample was obtained from the patients or HCs. Thanks again for your suggestion.

5. How did the cytokine levels of IL-17, TNFa, and IFNg in R-CD patients compare to HC?
Answer: Thank you for your comments. We feel sorry that we did not detect the IL-17, TNF-α, and IFN-γ expressions in HCs, and the description has been put in MATERIALS AND METHODS in the original manuscript. As to further detection, the plasma samples were all expired for the detection, thus we added this in the limitation section. Thanks again for your suggestion.

6. Do miR-125a and miR-125b have different targets?
Answer: Thank you for your comments. They have different targets, we have analyzed the target genes of miR-125a and miR-125b by Validated Target Module-MicroRNA-gene targets on miRWalk 2.0 [Journal of Biomedical Informatics, 44: 839-7, 2011.], which revealed that miR-125a had 234 reported target genes while miR-125b had 391 reported target genes, and they shared 110 similar target genes, while the majority of the target genes were different between miR-125a and miR-125b, this might be a possible explanation of the discrepancy between

miR-125a and miR-125b in CD. We have added this in the DISCUSSION.

7. Were levels of Tregs and TH17 measured in the plasma collected? Since miR-125a correlated with severity of CD, did Tregs and Th17 (known targets of miR-125a) also correlate with severity of CD?

Answer: Thank you for your comments. Levels of Tregs and Th17 were not measured in this study, and the plasma samples were all expired for the detection. However, many previous studies have been reported that Tregs and Th17 levels were correlated with the disease severity of CD [reviews: 1. Discov Med. 2012 Oct;14(77):253-62. 2. Gut. 2009 Aug;58(8):1152-67. 3. Lancet. 2012 Nov 3;380(9853):1590-605.]. We have added these in the main part and limitations in DISCUSSION. Thanks again for your suggestion.

Minor comments:

1.The manuscript text should be reviewed for proper use of grammar and syntax.

Answer: Thank you for your comments. We have thoroughly reviewed the manuscript and corrected the grammar and word usage errors, in the meanwhile, we have invited a language editing company (American Journal Experts, www.aje.com) as the journal suggested to polish the language and correct grammars to make the article more readable. The corrections were highlighted in red color in the manuscript.

2. Contractions should not be used in formal scientific publications, e.g. “didn’t” (Abstract Results) and “couldn’t” (Results).

Answer: Thank you for your comments. We have revised the manuscript carefully and ensured that no such contractions existed in the revised manuscript. Thanks again for your suggestion.

3. CRP, CDAI, IL-17, TNF-a, IFN-g, etc. should be defined in the Abstract.

Answer: Thank you for your comments. We have added the full name of C-reaction protein (CRP), Crohn’s disease activity index (CDAI), interleukin 17 (IL-17) and interferon-γ (IFN-γ), etc in the ABSTRACT. Thanks again for your suggestion.

4. If used only once in the text, an abbreviation is unnecessary, e.g. “(GDP)” is not needed after “gross domestic product”.

Answer: Thank you for your comments. We realized it and deleted the abbreviation “(GDP)” according to your comments. Thanks again for your suggestion.

5. CRP, ESR, and CDAI should be defined in the Materials and Methods.

Answer: Thank you for your comments. We have added the full name of C-reaction protein (CRP), (erythrocyte sedimentation rate) ESR, and Crohn’s disease activity index (CDAI) in the Materials and Methods, as well as interleukin 17 (IL-17), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in the MATERIALS AND METHODS.