Response to reviewers' comments

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled **"NCAPD2/3 induces inflammation via the IKK/NF-κB pathway in ulcerative colitis" (Manuscript NO: 51579)**. We have carefully revised the manuscript and made correction which hoping to get approval. All modifications have been highlighted in yellow. The main corrections in the paper and the responses to the reviewer's comments are as follows:

Reviewer 1

Dear Associate Editor, Thank you for sending me the article entitled "NCAPD2/3 induces inflammation via the IKK/NF-κB pathway in ulcerative colitis" for review. This cross sectional study compared the Levels of NCAPD2/3 in intestinal tissue between UC patients and control group. The result showed that NCAPD2 and NCAPD3 expression was significantly upregulated in UC patients. Moreover, IKK and NF-κB protein expression in the si-NCAPD2, si-NCAPD3 and si-NCAPD2+si-NCAPD3 groups was significantly decreased. The research concluded that NCAPD2/3 plays an important role in improving ulcerative colitis-induced inflammation induced via the IKK/NF-κB pathway. There are some comments as the followings:

1-The aim of study is expressed as: "To evaluate the role and underlying mechanisms of NCAPD2/3 in the development and progression of ulcerative colitis (UC)." This cross sectional design would only show the NCAPD2/3 correlation between UC intestinal tissue and healthy controls. It could not evaluate the development or progression of UC.

Reply: Thanks for your comments. Based on your opinion, we have carefully read the manuscript and modify the **Objective** as below: To determine the level of NCAPD2/3 in intestinal mucosa and explore the mechanisms of NCAPD2/3 in the ulcerative colitis (UC). We have modified in manuscript and highlighted in yellow.

2- In the conclusion section is said: "NCAPD2/3 plays an important role in improving ulcerative colitis-induced inflammation induced via the IKK/NF-κB pathway.". Again the design of study could not prove this fact.

Reply: Thanks for your suggestion. In order to objectively express the results of the study, we have modified the **Conclusion** as below: NCAPD2/3 is highly expressed in the intestinal mucosa of patients with ulcerative colitis. Overexpression of NCAPD2/3 promotes the release of pro-inflammatory cytokines by modulating the signaling pathway of the IKK/NF-κB. We have modified in manuscript and highlighted in yellow.

3- There are some points regarding the UC selection criteria. Some of the UC patients' exacerbations could be caused by CMV or C.difficile. There is no data regarding the charachteristics of included UC patients. Were they treated with 5-ASA or biologics? Were they diagnosed for the first time? The treatment affects the inflammation in intestine.

Reply: Thanks for your comments. In this study, we recruited 30 patients with active UC confirmed by colonoscopy (Mayo endoscopic score > 2), and characteristics of patients were listed in the table 1, including ongoing treatment. Although some of the UC patients may be exacerbated by CMV or C.difficile, CMV and C.difficile have typical features in colonoscopy, and patients who had typical characteristics of CMV or C.difficile were excluded in this study. We have added the information in manuscript and highlighted in yellow.

Reviewer 2

Dear Authors, I read your manuscript titled "NCAPD2/3 induces inflammation via the IKK/NF-κB pathway in ulcerative colitis". In this study, the expression level of NCAPD2/3 was evaluated in the intestinal tissue of the patients with ulcerative colitis. It was found that the expression of NCAPD2/3 increased in the mucosa of the ulcerative colitis compared with that of healthy control. after the knock-out of the NCAPD2/3, it was shown that the level of inflammatory cytokines decreased. At the same time, NFkB pathway that is the central point of inflammatory reactions, was supressed after the NCAPD2/3 knock-out. Overall it is a globally good presented study with an interesting and novel topic. Abstract was well described and clear. Introduction was sufficient but, there were some deficiencies. The study is well designed and conducted. Methods are well described and clear. Conclusions are justifiable and prudent enough with some deficiencies. It can be acceptable for publication but still I have suggested some issues for revision;

1. In the introduction section, reasons for the study of NCAPD2/3 in the ulcerative colitis were not

expressed clearly. need for the study was not clarified. It must be explained.

Reply: Thanks for your comments. At present, there are few studies on NCAPD2/3 in intestinal diseases. Yin et al. found that NCAPH, a NCAPD3 homologous complex, high expression promotes colonic cancerous cell proliferation and Schuster et al. reported that NCAPD3 plays an important role in microbial immunity and in the process of human intestinal epithelial cells to clear bacteria. We have added these in the manuscript and highlighted in yellow.

2. In the results section, (3.1 Clinical and Analysis), the sentence of "Based on these results, we inferred that NCAPD2/3 might play an important role in inducing UC development" must be removed, because this sentence contains interpretation. In the results, obtained results were given without interpretation.

Reply: Thanks for your suggestion. We have deleted it in the manuscript.

3. In the discussion section, limitations and future directions were not mentioned. They must be briefly considered.

Reply: Thanks for your comments. Given the exploratory and retrospective design of our study, an the clinical sample was too small, the result of these findings will need to be further validated. Future work will be aimed to deeply investigate the role of NCAPD2/3 in the onset and progression in inflammatory bowel disease, including ulcerative colitis and Crohn’s disease. We have added these in the manuscript and highlighted in yellow.

4. In the discussion section, the statements that were mentioned the association between NCAPD2/3, NfkB pathway and ulcerative colitis include exact descisions, they may be softened.

Reply: Thanks for your suggestion. We have revised this, such as “ In summary, we found for the first time that NCAPD2/3 upregulation in the intestinal mucosa of patients with active ulcerative colitis and the mechanism may involve stimulating the secretion of inflammatory factors IL-1β, IL-6 and TNF-α by activating IKK/NF-κB pathway. We have revised it in manuscript and highlighted in yellow.

Your sincerely, Bolin Yang M.D.

Nov 10, 2019