June 3, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10351-Review.doc).

**Title:** Increasing Expression and Possible Role of Chitinase 3-like-1 in Colitis-Associated Carcinoma Model

**Author:** Ma JiaYi, Li RunHua, Huang Kun, Tan Gao, Li Chen, Zhi FaChao

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 10351

The manuscript has been improved according to the suggestions of reviewers:

1 Figure format has been updated and figure legends has been revised according to editor's suggestion(highlighted).

2. We have made additional remarks to statistical method(highlighted in the Statistical analysis section), and this does not bring differences to the existing conclusion. The statistic certificate has been provided.

3. This manuscript has been reviewed and edited by professional English editing experts and the language certificate has been provided.

4. Questions has been answered according to the comments of the reviewer

(1)Answers about the role of oxidative stress in marker expression through the effect of caffeine

We thanked for the instructive comment. However, we are afraid that there may be some misunderstandings. The effect of caffeine is to inhibit CHI3L1 expression and to scavenge reactive oxygen species (ROS). Based on this finding, we further investigated the role of CHI3L1 in ROS production. As we have showed, CHI3L1 indeedly increased oxidative stress in colon cell line, and thus we suggested a pathogenetic role of CHI3L1 in the course of inflammation-based carcinogenesis. The role of ROS in CHI3L1 expression was not our major focus.

(2)Answers about the experiment design

We apologize for the confusion. Our aim was to investigate the possible role of CHI3L1 molecular in the progression of colitis-associated carcinoma. Accordingly, we used chronic colitis model(DSS model) and CAC model(AOM/DSS model) in this experiment and thus 5 groups were included: control, CAC control, CAC+caffeine, colitis control and colitis+caffeine. AOM alone was not able to induce inflammation(Onizawa M et.al, Am J Physiol Gastrointest Liver Physiol 296: G850–G859, 2009.). So, single use of AOM , which can not mimic chronic colitis-based carcinogeneisis, was not included.

(3)Answers about the dose of caffeine

2.5mM caffeine was applied in the experiment as the CHI3L1 inhibitor and a treatment agent. One previous study has made it quite clear that 2.5mM is equivalent to 2-3 cups of coffee(ref 10, Mozoguchi group). We have added such information in the materials and methods section.

(4)Answers about causal relationship between increasing expression of CHI3L1 and the development of CAC

We understand the reviewer's concern. Though CHI3L1 can be up-regulated during inflammation, the increasing expression of CHI3L1 in the progression of carcinogenesis cannot be solely explained by inflammation. The induction regimen constisted of repeated cycles of DDS and water. The inflammation was relieved in the rest period, and could not explain the increasing trend in the whole period. Furthermore, the proximal and distal colon were in the same inflammed environment, which was inconsistent with the higher expression of CHI3L1 in the distal colon. Thus, a strong connection has been built between CHI3L1 expression and carcinogenesis.

(5)Answers about the causal relationship between CHI3L1 expression, oxidative stress change and carcinogenesis

Oxidative DNA damage is an important and irreversible event in the ongenetic transformation of

colon epithelial cells. And that's why we examined the oxidative DNA damage marker --8-OHdG in specimens from colitis control group and colitis+caffeine group. Furthermore, the in vitro experiment result suggested a direct impact of CHI3L1 on the intracellular reactive oxygen species . The combination of in vivo and in vitro expriments led to the conclusion about the possible oncogenic role of CHI3L1.

(6)Answers about the successful rate of colitis and DSS tolorance in mice

We thanked the reviewer's question. DSS administration was a classic and mature method to induce colitis in mice. According to clinical manifestation (blood diarrhea and body weight loss) and pathological examination, all animals in CAC group and colitis group in this experiment suffered from moderate to severe inflammation. Two animals in colitis control group(2/5) died in the experiment period, compared with none in colitis+caffeine group (0/5). However, the exact cause of deathes were unknown, because specimens has been dissolved when we find the bodies. As a result, we did not include this part into the manuscript.

(7)The future study plan about CHI3L1 , its linkage with colorectal cancer, and data from clinical patients

In this report, we described the impact of CHI3L1 on intracellular ROS production. In the future, we will further study the underlying mechanism of the imbalance between generation of ROS and decreased antioxidant defense systems. We are also dedicated to provide more concrete evidence of the CHI3L1 oncogenic role in colitis-associated colorectal cancer, for intance, by using CHI3L1-knockout animals or more specific CHI3L1 inhibitor. We agree with the reviewer that it is very important to investigate whether experimental observations hold true in human studies. Regrettingly, specimens from clinical patients who suffered from CAC were hard to obtain. The data was by far stricted in animal studied and *in vitro* studies.

(8) Answers about the major contribution of HT29 cell line study

Previous CAC studies usually focus on the inflammtory cytokines(e.g.:TNF-α,IL-6,etc.). In this manucript, we focused on the oxidative DNA damage, which provided a new perspective of the pathogenic role of CHI3L1 in CAC.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,
JiaYi Ma, MD
1st Dept. of Medicine
Southern Medical University
Guangzhou, Guangdong
E-mail: ponymjy@gmail.com