December 12, 2014

Dear Editor,

Title: SGK1 inhibits cellular apoptosis and promotes proliferation via the MEK/ERK/p53 pathway in colitis

Author: Jian'an Bai, Guifang Xu, Lijun Yan, Weiwen Zeng, Qianqian Ji, Jindao Wu, Qiyun Tang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14561

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

1. Format has been updated.
2. Revision has been made according to the suggestions of the reviewer

First, please accept my sincerely gratitude for your kindly warm heart to review our paper, it is a tremendous encouragement and chance for us. Based on these comments, we have made careful modifications on the manuscript. In addition, the two authors listed in the previous manuscripts are the main contributory authors, other contributory authors have been added and wish for your permission. Most changes made to the text are in red color. In addition, we have consulted AJE for paper revision and gained a certificate before the submission this time. We hope the new manuscript will meet your magazine's standard. Below you will find our point-by-point responses to the reviewers' comments:

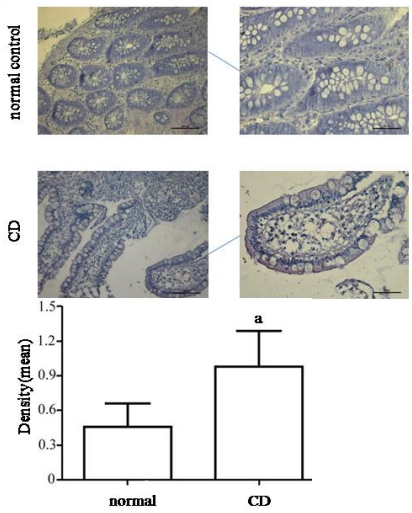
2.1 The first reviewer (Professor)

First, please accept my sincerely gratitude for your kindly warm heart to review our paper, it is a tremendous encouragement and chance for us. Based on your valuable comments, we have made careful modifications on the manuscript. We hope that these revisions are satisfactory.

(1) The title needs to be rewritten since the authors are not studying Crohn's disease. In fact, the authors have used the model BALB/c mice for their experimentation, with TNBS as the chemical to induce colitis.

We are sorry about the presentation of our content about the theme of the experiment. We have designed the experiment with the purpose to uncover the mechanism of SGK1 in the protection of apoptosis on intestinal epithelial cells (IECs). And TNBS was used to induce acute colitis in BALB/c mice. TNF-α was used to induce Th1-immunoreaction on IECs. Crohn's disease (CD) is a chronic, relapsing and debilitating colitis. The pathology involves the Th1-immunoreaction and apoptosis of IECs which is a key component of intestinal mucosal barrier. Therefore, we revised the directly mention of CD to colitis marked in red color, and added an experiment of SGK1 expression in patients with active CD or not which was served in Figure 1. We hope that the revision is satisfactory.

Figure 1



(2) The manuscript does have many grammatical mistakes, and many sentences need reframing.

We are sorry about the problems in the expression of English. Thus, we have consulted AJE for paper revision and gained a certificate submitted following this letter. We hope that the revision is satisfactory.

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Manuscript title:
SGK1 inhibits cellular apoptosis and promotes proliferation via MEK/ERK/p53 pathway in Crohn's disease

Authors:
Jian'an Bai, Guifang Xu, Lijun Yan, Weiwen Zeng, Qianqian Ji, Jindao Wu, Qiyun Tang

Date Issued:
December 9, 2014

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(3) A drawback of the study is that the authors have failed to discuss the possible confounding factors and limitations of the study in their discussion.

Thanks for your careful observation and comment. We have a little problem here about the mean of confounding factors. In our opinion, the confounding factors you mentioned here indicate the solvent of the pathway inhibitor (U0126)? We have designed the solvent as DMSO and carried out the experiment successfully. The results indicate a similar variation with negative control siRNA and share the similar statistical significance. When we put the images in the paper, we found the inaesthetic vision as a whole. At last, we decided to exclude these images. Of course, we can provide them in supplement if necessary.

There are some limitations of our study, such as the specific interaction mechanism of SGK1 and MEK1, the further variation of ERK1/2 pathway with SGK1 inhibition. The most important limitation may be the indirect relationship between SGK1 and apoptosis of IECs. We consider the role of SGK1 overexpression in the regulation of IECs apoptosis may be more directly explanation of the relationship. These designs inclding overexpression assay were not carried out in the current paper and would be listed in our further study. We are really sorry and expect about that. We hope that the revision is satisfactory.

(4) Should not the legend for Figure 3 C, D be that SGK1 expressions at different times and doses on TNF treated IEC-6 cells (rather than HCT-116 cells)?

We must apologize for the careless about the detail. Now it has been revised to IEC-6 cells in red color in Figure 4 C, D (That is a figure was added for Figure 1). Thanks for your circumspection sincerely.

We hope that these revisions are satisfactory. Thank you for your valuable comments sincerely.

2.2 The second reviewer (Professor)

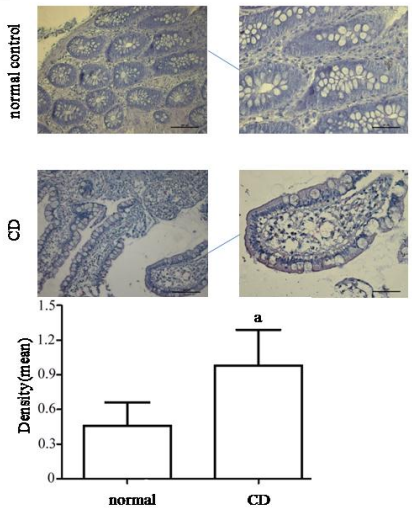
Please accept my sincerely gratitude for your kindly warm heart to review our paper, it is a tremendous encouragement and chance for us. Based on your valuable comments, we have made careful modifications on the manuscript. We hope that these revisions are satisfactory.

(1) SGK1 is ubiquitously expressed in almost all tissues of digestive tract, such as esophagus, stomach, liver, intestine, and pancreas. This paper did not offer evidence of abnormal

expression of SGK1 in Crohn's disease (CD), so its hypothesis that SGK1 inhibitors may be potentially therapeutics in the treatment of CD is not reasonable. Because this a model study, so it can not reflect the real CD. Thus, it is better to offer this evidence literally in the introduction or experimentally in CD samples. Otherwise, the results in this paper can not conclude that GK1 inhibits cellular apoptosis and promotes proliferation via MEK/ERK/p53 pathway in Crohn's disease.

We are sorry about the presentation of our content about the theme of the experiment. We have designed the experiment with the purpose to uncover the mechanism of SGK1 in the protection of apoptosis on intestinal epithelial cells (IECs). And TNBS was used to induce acute colitis in BALB/c mice. TNF-α was used to induce Th1-immunoreaction on IECs. Crohn's disease (CD) is a chronic, relapsing and debilitating colitis. The pathology involves the Th1-immunoreaction and apoptosis of IECs which is a key component of intestinal mucosal barrier. Therefore, we revised the directly mention of CD to colitis marked in red color, and added an experiment of SGK1 expression in patients with active CD or not which was served in Figure 1. We hope that the revision is satisfactory.

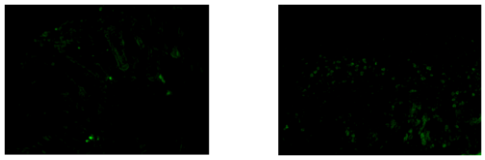
Figure 1

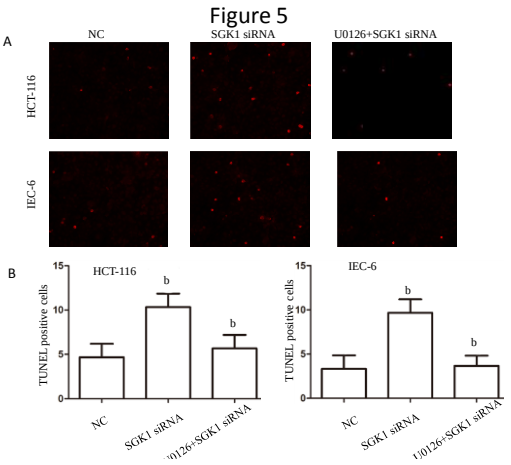


(2) The figure2 and figure 4 showing apoptosis is not specific and the apoptosis cells can not be seem, it is better give another images.

We are sorry to show the vague images here from the microscope which is unsatisfactory on green fluorescent. Thus, we carried out the TUNEL assay of IECs again with red fluorescence. However, the samples of paraffin section limited the repeated experiment, and we provided some better images here. The images were listed below. We hope that the revision is satisfactory.

Figure 5





(3) This paper concluded that SGK1 inhibits cellular apoptosis and promotes proliferation via MEK/ERK/p53 pathway by interfering of SGK1, this is not a direct result. Thus it is better to give the direct results (for example, overexpression cause apoptosis and inhibition of proliferation) if consider acceptation.

Thanks for your careful observation and comment. Following your opinion, we consider the role of SGK1 overexpression in the regulation of IECs apoptosis as more directly explanation of the relationship. There are some other limitations of our study, such as the specific interaction mechanism of SGK1 and MEK1, the further variation of ERK1/2 pathway with SGK1 inhibition. These designs inclding overexpression assay were not carried out in the current paper and would be listed in our further study. We are really sorry and expect about that. We hope that the revision is satisfactory.

(4) This sentence "For further investigation, MEK1 inhibitor (U0126) was used to prove the MEK/ERK-dependent reaction" is not a result. Many English errors are found in the paper.

Follow your comment, we consider this sentence was inappropriate here. Thus, we revised it to "Cells treated with MEK1 inhibitor (i.e., U0126) before siSGK1 transfection showed a reversal of the siSGK1-induced cellular apoptosis" and consulted AJE company for grammar revision. We hope that the revision is satisfactory.

Many English errors are found in the paper.

At last, we are sorry about the problems in the expression of English. Thus, we have consulted AJE for paper revision and gained a certificate submitted following this letter. We hope that the revision is satisfactory.

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3 References and typesetting were corrected.

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

Sincerely yours,
Jian'an Bai