Dear Editor:

We highly appreciate all of the insightful comments from the reviewers and editor of our work: Manuscript NO.: 73148, Review entitled " KAI1/CD82 Gene and ATX-LPA Axis in Gastrointestinal Cancers". We believe that the revised version of our paper addresses all concers by the referees in detail. In what follows the referees'comments are in black and the authors'responses are in blue. Those changes are highlighted within the manuscript.

Responses to the comments of Reviewer #1

1. The typesetting of the description of the abbreviated paragraph is not clear.

Thank you for your valuable suggestions which are very important to our research. We followed the relevant requirements of Author Guidelines and reviewed the reviews published and found that the abstract of the review did not have a clear fixed segmented format. KAI1 and ATX-LPA signaling pathways are currently the research hotspots of various cancers. However, KAI1 has not been reported to inhibit cancer metastasis by inhibiting ATX-LPA axis. Our group's previous preliminary results showed that KAI1 inhibited cancer metastasis by inhibiting the ATX-LPA axis. This is a new idea, and we have funding from the National Nature Science Foudation of China. Therefore, we reviewed the research progress of KAI1, ATX-LPA axis and Gastrointestinal cancers respectively.

2. The article describes the four gastrointestinal diseases separately in the KAI1 CD82 and ATX-LPA axis, but it briefly summarizes the targeted therapy. To make the article top-heavy is the lack of a paragraph in the main content of the article.

Thank you for your valuable suggestions which are very important to our research. I am ashamed and sorry for our previous incomplete literature review of this department. After reviewing the literature on ATX and LPA targeted therapy, we added some of the content to enrich it. The added content is in the highlighted section of the revised article.

However, as a tumor suppressor gene, KAI1 was discovered in 1995, most of the current studies are still focused on its cancer inhibition mechanism and retrospective cohort studies. We searched PUBMED and Web of Science again with the keywords KAI1 and (antibody or antigen or targeted therapy or antagonists), trying to find new relevant literature. But unfortunately, there is little literature on relevant reports.

3. In the "Comparative analysis of LPAR-mediated signals in tumour", The description of the receptors is also too general. Although the general attributes may be the same, there are subtle differences between them in different diseases. For example, in hepatocellular carcinoma, the location and role of LPAR2, LPAR6 and other 1, 3, and 5 receptors are slightly different, and there are also differences between the 2, 6 receptors, which are all negative effects. If the authors want to narrate, they should also check the literature in detail and describe clearly.

Thank you for your valuable suggestions which are very important to our research. I am ashamed and sorry for our previous incomplete literature review of this department. We reviewed relevant literature and added content, which is in the highlighted part of the article. If the added part is still not appropriate, we will revise it immediately.

4. Specific details evaluation: 1、In the "Abbreviations", "Vascular Endothelial Growth Factor C, VAGFC"is wrong.

Thank you for your valuable suggestions which are very important to our research and we have made corrections.

Responses to the comments of Reviewer #2

1. However, despite the details provided, there is a lack of integration, the reader access to an unconnected list of data and it is really challenging to extract a clear physiological/pathological picture.

Thank you for your valuable suggestions which are very important to our

research. I am ashamed and sorry for that. No studies have been reported on KAI1-ATX-LPA signaling pathway at present. I suppose that the most valuable physiological/pathological picture is probably the mechanism by which KAI1 inhibits ATX-LPA in cancer. Our research team has been committed to studying the mechanism of KAI1 inhibiting ATX-LPA signaling pathway to inhibite cancer metastasis, which has been funded by the National Natural Science Foundation of China. The purpose of this review is to provide a new research target for KAI1-ATX-LPA to inhibit cancer metastasis. We drew the Figure 1 to show that ATX-LPA axis plays a key role in the pathophysiology of tumor cells. Figure A shows the anabolism and catabolism of tumor extracellular LPA. ATX/lysoPLD catalyzes the generation of LPA from LPC, and LPPs promotes the hydrolysis of LPC. Figure B shows that LPA activates multiple pathological processes in tumor cells by binding GPRs (LPARs) to promote the occurrence and development of tumors. The mechanism of KAI1-ATX-LPA tumor inhibition is still under study, and we expect to provide new targets for cancer diagnosis and treatment.

2. Critical vision and discussion of the presented evidences is needed to provide the reader with a critical perspective

Thank you for your valuable suggestions which are very important to our research. In this paper, the molecular composition of KAI1/CD82 gene and ATX-LPA axis, their physiological functions in tumours, and their roles in gastrointestinal cancers and target therapy are reviewed. KAI1 inhibits metastasis of cancer, and its correlation with prognosis has been confirmed by most articles. ATX-LPA axis expression promotes cancer proliferation and metastasis, which has been confirmed by most articles. However, the related mechanisms inhibit/promote cancers still need to be further explored. This study attempts to review the relevant mechanisms. The controversial part is how much LPAR1-6 is expressed in different cancers and its mechanism. I am ashamed and sorry for not being able to elaborate on the controversial part. We also searched the relevant literature on Pubmed and Web of Science and added some discussion contents. The additions are highlighted in the text. If the added part is still

not appropriate, we will revise it immediately.

2．KAI1/CD82 is presented as a factor that can down regulate HGF (page 12) while it induces up-regulation of HGF-induced Sprouty2 (page 13). This apparent contradiction deserves some discussion.

Thank you for your valuable suggestions which are very important to our research. The title of Page 12 is *KAI1 inhibits HGF-induced invasion of pancreatic cancer by sphingosine kinase activity*, and the title of Page 13 is *KAI1/CD82 suppresses hepatocyte growth factor-induced migration of hepatoma cells via upregulation of Sprouty2.* In fact, both of the two studies are the results of our research group. Although one is for pancreatic cancer, the other is for liver cancer, both studies concluded that KAI1 inhibits HGF to inhibits cancer metastasis. However, the article in Page13 confirmed that KAI1 inhibited hepatocyte growth factor-induced migration of hepatoma cells by up-regulating Sprouty2. We reviewed the literatures again and found no new literature about KAI1 and HGF. So we didn't add new discussions.

4. The sentence on page 14 "the decreased expression of KAI1 in CRC might be a therapeutic target for CRC" is confuse. The gene/protein might be a target but not the decrease.

Thank you for your valuable suggestions which are very important to our research. We have changed the original sentence to “KAI1 may be a new therapeutic target for CRC.” The change sentence is highlighted in the revised manuscript.

5. According to Human Protein Atlas (www.proteinatlas.org), CD82 has no prognostic value in cancer. It might be worth to discuss these evidences as well.

Thank you for your valuable suggestions which are very important to our research. I am very sorry that we cannot find this sentence in the original article. In fact, Zhu et al.

completed a meta-analysis included 31 studies that analyzed the prognosis of KAI1 and multiple human malignancies in 2017[1]. High CD82 expression was significantly associated with overall survival (HR =0.56, 95%CI: 0.47-0.67) and disease/recurrence/progression-free survival (HR =0.42, 95%CI: 0.30-0.59). In addition, they performed a subgroup analysis showing that CD82 is associated with a good prognosis in cancer patients. In conclusion, CD82 may be a promising biomarker for predicting the prognosis of patients with malignant tumors, and its biological function has important research value for this topic.

Responses to the comments of Re-reviewer

1. Regarding the issue: "KAI1/CD82 is presented as a factor that can down regulate HGF (page 12) while it induces up-regulation of HGF-induced Sprouty2 (page 13). This apparent contradiction deserves some discussion", the two sentences in the text are: "KAI1 may inhibit the metastasis of PANC1 and MiapACA-2 PC cells by downregulating Hepatocyte growth factor (HGF)." "Mu et al found that KAI1/CD82inhibits the migration of HCC cells by upregulating HGF-induced Sprouty2[125]." If from the second sentence the reader should understand that Sprouty up-regulation occurs through a mechanisms involving the increase of HGF then, there is an apparent inconsistency, please revise.

Thank you for your valuable suggestions which are very important to our research. This is indeed a mistake caused by our inadequate understanding of the original text. We fully read the original text and made corrections. The point of the paper on page 12 (Page 14 of the revised manuscript) is that KAI1 may inhibit the metastasis of pancreatic cancer cells PANC-1 and Miapaca-2 caused by hepatocyte growth factor by down-regulating SPK expression. After infection with the KAI1 gene, decreased invasive ability in the Boyden Chamber assay was observed in PANC-1 and Miapaca-2 cells that were induced by hepatocyte growth factor. Over-expression of KAI1 in the cells led to the deactivation of SPK and a decreased level of intracellular sphingosine-1-phosphate. The point of the paper on page 15 (Page 15 of

the revised manuscript) is that KAI1/CD82 suppresses hepatocyte growth factor-induced migration of hepatoma cells via down regulation of SphK1. Hepatocyte growth factor (HGF) induces the migration of hepatoma cells through activation of cellular sphingosine kinase 1 (SphK1). Adenovirus-mediated gene transfer of KAI1 (Ad-KAI1) downregulates the SphK1 expression and suppresses the HGF-induced migration of SMMC-7721 human hepatocellcular carcinoma cells. In fact, the opinion of the two articles is that KAI1 inhibits HGF induced metastasis of cancer cells. We have corrected and highlighted the revised manuscript.

2. The Human Protein Atlas is an outstanding initiative associated to the Human Proteome Project that has made available valuable information about functional and pathological aspects of about 17,000 proteins. In particular, based on the expression levels of these proteins in healthy and diseased tissues, they are able to propose scores that suggest the prognostic value of proteins in diseases. As I pointed out in my previous comment, CD82 has not prognostic value in cancer, according to their analysis. My suggestion is to mention this data and to discuss accordingly the data from the author's studies.

Thank you for your valuable suggestions which are very important to our research. I am very sorry that I misunderstood your meaning before. The Human Protein Atlas found that CD82 has not prognostic value in cancer, which I have added to the revised manuscript. Page 8 is changed below and highlighted in the revised manuscript: In 2017, a meta-analysis involving 31 studies showed that high KAI1 expression is significantly associated with overall survival (OS; HR = 0.56, 95% CI: 0.47–0.67) and disease-free/relapse-free/progression-free survival (PFS; HR = 0.42, 95% CI: 0.30–0.59) in cancer patients. In addition, they performed a subgroup analysis showing that KAI1/CD82 is associated with a good prognosis in cancer patients. KAI1/CD82 may be a promising biomarker for predicting the prognosis of patients with malignant tumors, and its biological function has important research value for this topic[27]. However, the Human Protein Atlas is an outstanding initiative associated to the Human

Proteome Project that has made available valuable information about functional and pathological aspects of about 17,000 proteins. In particular, based on the expression levels of these proteins in healthy and diseased tissues, they are able to propose scores that suggest the prognostic value of proteins in diseases. Considering that only 31 studies were included in the meta-analysis, more studies may be needed in the future to verify whether KAI1 can be used as a prognostic factor.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

With best regards!

Yours sincerely

Professor. Xiaozhong Guo

Department of Gastroenterology,

General Hospital of Northern Theater Command of China Medical University

No. 83 Wenhua Road, Shenyang, China.

Tel: 86-24-28897603;

Fax: 86-24-28851113;

Email: guoxiaozhong1962@126.com

[1] Zhu J, Miao C, Liu S, Tian Y, Zhang C, Liang C, *et al*. Prognostic role of CD82/KAI1 in multiple human malignant neoplasms: a meta-analysis of 31 studies. Onco Targets Ther, 2017, 10(5805-5816. https://doi.org/10.2147/ott.S150349.