

## ***Response to the comments of Reviewer #1 (06269450)***

**Comment:** Title: The title reflects the main subject of the manuscript.

**Response:** Thanks for your suggestion.

**Comment:** Abstract: The abstract basically summarizes and reflects the work described in the manuscript.

**Response:** Thanks for your suggestion.

**Comment:** Key words: The key words reflects the focus of the manuscript.

**Response:** Thanks for your suggestion.

**Comment:** Background: The epidemiology of gastric cancer in China or in the world and the current 5-year survival rate should be described in more detail. The mechanism of EMT in GC and how IL-34 affects tumor proliferation through EMT should be reviewed in detail.

**Response:** Thank you for your suggestion, we have revised the epidemiology and the current 5-year survival rate of GC to make it more detailed. In addition, we sought to describe in more detail the mechanism of EMT in GC and how IL-34 affects tumor proliferation through EMT.

**Comment:** Methods: The manuscript describes materials and methods in adequate detail. The research objectives adequately achieved by the experiments used in this study.

**Response:** Thanks for your suggestion.

**Comment:** Results: The results showed that the expression of IL-34 is increased in GC tissues and cell lines, IL-34 is positively correlated with the proliferation and metastasis of gastric cancer cells. The mechanism may be related to the regulation of EMT related protein expression.

**Response:** Thanks for your suggestion.

**Comment:** Discussion: There was little analysis of the results in the discussion. The relationship between EMT and the proliferation and metastasis of gastric cancer and the role of IL-34 in it can be discussed.

**Response:** Thank you for your valuable suggestions, and we have revised the discussion section based on your suggestions.

**Comment:** Illustrations and tables: The figures, diagrams and tables sufficient are good quality and appropriately illustrative of the paper contents.

**Response:** Thanks for your suggestion.

**Comment:** Biostatistics: The manuscript meets the requirements of biostatistics.

**Response:** Thanks for your suggestion.

**Comment:**Units:The manuscript meets the requirements of use of SI units.

**Response:**Thanks for your suggestion.

**Comment:**References:References meet requirements.

**Response:**Thanks for your suggestion.

### ***Response to the comments of Reviewer #2 (05429043)***

**Comment:**1."Trastuzumab is the main drug for targeted therapy of gastric cancer, but the percentage of HER2-positive patients(1) " the sentence seems to be vivid.

**Response:**Thank you for the correction, we have corrected the logic error.

**Comment:**2.The methodology should be systematically arranged ...Ethical clearance number for clinical sample and animal study are importance.

**Response:**We have re-optimized the method section. In addition, we have also provided Ethical clearance number for clinical sample(Quick-PJ2019-09-11) and Ethical clearance number for animal study(SC20200513) based on your suggestions.

**Comment:**3.All figures need 300DPI resolution and a visible size..now it looks no clarity....

**Response:**Thank you for your suggestions. All figures are at a resolution of 300 DPI.

**Comment:**4.Figure legends need to be re-written with more clarity.

**Response:**Thank you for your suggestion, the legend has been rewritten.

**Comment:**5.Flow is lacking in the manuscript.Please correct it.

**Response:**Thanks to your suggestions, we have polished the language of the manuscript to make it flow better.

**Comment:**6.Result part need to be modified with more recent reference.

**Response:**We have updated the references in the results section.

**Comment:**7.Quantification of blots is needed.

**Response:**All WB results were quantified using ImageJ software.

**Comment:**8.No mention about statistical analysis in detail.

**Response:**We provide further explanation of the statistical methods in the Statistical analyses section.

### ***Response to the comments of Reviewer #3 (03252941)***

**Comment:**Li et al investigated the effect of IL-34 on the proliferation, migration, and epithelial-mesenchymal transition (EMT) of gastric cancer cells by using cell lines

and animal models. This is a well-designed and well-performed study and the presented data presented are persuasive and convincing. I have some comments.

**Comment:**1.(p.6, 1.7) Dilution rate of anti-IL-34 antibody should be described.

**Response:**page6, line7: Thank you for your valuable suggestions, we have added dilution rate of anti-IL-34 antibody.

**Comment:**2.(p.8, 1.9) cds should be spelled out.

**Response:**page8, line9: Thank you for your suggestions, we have added the full name of cds(coding sequence).

**Comment:**3.(Figure 1A) In Tumor, background stromal cells are also stained with anti-IL-34 antibody. To fairly compare staining intensity between Normal and Tumor, section containing transition between Tumor and Normal should be presented.

**Response:**Figure 1A: We have reselected a set of sections containing both tumor tissue and normal paracancerous tissue.

**Comment:**4.(Figure 3A) It would be helpful to present pathology of tumors transfected with IL-34 cds and mock so as to demonstrate that IL34 overexpression results in EMT.

**Response:**Figure 3A:Thank you for your suggestion. At the time of animal experiments, we did not perform further pathological testing on the tumors in nude mice. Unfortunately, the laboratory where we are currently located is off limits due to the epidemic, so we wre unable to perform subsequent pathological examinations.

#### ***Response to the comments of Reviewer #4 (06286468)***

**Comment:** In this work, the role of IL-34 on the proliferation and epithelial-mesenchymal transition of gastric cancer cells was studied. The results showed that the expression of IL-34 is elevated in GC tissues and cell lines compared to normal gastric tissues or cell lines and IL-34 promotes the proliferation, clone formation, migration and invasion by regulating EMT-related protein expression in GC cells. The study is logically designed, the idea is new and very interesting. Although, there are several concerns that need to be addressed.

**Comment:**1.It is suggested to added plotting scale in the results of clone formation, migration and invasion assays.

**Response:**Thank you for your suggestions, we have added the plotting scale as requested.

**Comment:**2.IL-34 is a cytokine which mainly secreted out of the cell, why the authors only studied the role of IL-34 in the cell?

**Response:**At the time of designing the experiment, we planned to collect serum from tumor patients to test the expression level of IL-34. Because venous blood collection is an invasive procedure, which led some patients to refuse the procedure. Therefore,

we terminated the project prematurely.

**Comment:**3. More work should be added in the discussion section. An in-depth mechanism study is lacking in this work. The authors should add more mechanism study in the manuscript, or add your research plan at least.

**Response:** Thank you for your valuable suggestions. Due to financial constraints and insufficient time for experiments caused by the epidemic lockdown, some experiments, especially those involving mechanistic aspects, could not be carried out. However, we have added subsequent experimental plans to the discussion section.

**Comment:**4. The concept of EMT was first reported in the embryology area. EMT is a biological process which is of great importance in embryogenesis and organ development. I suggest that the research process and some discoveries of EMT could be added in the introduction section. Some references could be cited, “Exosomes Regulate the Epithelial-Mesenchymal Transition in Cancer”, “EMT Transition States during Tumor Progression and Metastasis” and “Pinin Induces Epithelial-to-Mesenchymal Transition in Hepatocellular Carcinoma by Regulating m6A Modification”, for example, or any other similar references.

**Response:** We have added relevant literature as you suggested.

**Comment:**5. I think language polishing from a native speaker of English is necessary.

**Response:** We have followed your suggestions for further language polishing.

### ***Response to the comments of Reviewer #5 (00505755)***

**Comment:** This study demonstrates that IL-34 promotes the proliferation and EMT in gastric cancer. Section 2.1 Patients and samples may be revised to describe the detailed information on grade in line 6 of the section.

**Response:** Page 9, Section 2.1, Line 6: Thank you for your suggestion, we have revised the grade of tumors (well, moderate, and poor) to make it more detailed.