Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “MiR-145-5p inhibits gastric cancer progression via the serpin family E member 1- extracellular signal-regulated kinase-1/2 axis” (Manuscript NO: 91958, Basic study). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Furthermore, we would like to show the details as follow:

Reviewer 1#

MicroRNAs that regulate gene expression are important in cancer physiology. However, their involvement in gastric cancer (GC) is not yet well known. The aim of the study presented here was to understand the molecular mechanism of miR145-5p on proliferation, apoptosis, invasion, epithelial-mesenchymal transition and metastasis formation in gastric cancer. To do this, the authors demonstrated the expression of miR-145-5p and serpin family E member 1 (SERPINE1) in gastric cancer tissues. The work concluded that miR-145-5p could affect proliferation, migration and invasion by negatively regulating SERPINE1 levels and controlling the extracellular protein-regulated kinase-1/2 (ERK1/2) pathway. This important work, which seems to me to be well done, provides useful elements for understanding gastric cancers. However, the manuscript requires some improvements before considering its publication.

Q1: Page 5, line 85. “Following the provided guidelines” seems to me to be not sufficient for a scientific article: briefly describe the method used.

R: Thank you very much for this useful suggestion. We have described more details as follow: Transfections were performed using Lipofectamine2000 (Invitrogen, USA) following the provided guidelines: When the cells reached the logarithmic growth phase, trypLE was used to digest the cells. The cells were then centrifuged, resuspended, and counted on a 6-well plate (with a fusion degree of about 80% during cell transfection. The mixed siRNA (100 nM) and
an appropriate amount of Lipofectamine2000 dissolved in serum free Opti MEM I was added to the plate. After transfection, the culture medium was replaced after culturing the cells in a CO₂ incubator at 37°C for 4–6 hours. The cells were incubated for 48 hours before proceeding with additional experiments.

**Q2. Page 7, line 32.** “0.1% crystal violet”: what was the diluent? Methanol? Ethanol? Buffer? Water? Please specify.

**R:** In this experiment, 0.1% crystal purple from Seville Biology in China was used, and the product number is G1014-50ML. 0.1% crystal purple was dissolved in 20% alcohol.

**Q3. Page 7, line 35.** “According to the instructions”: briefly describe the method used (see above).

**R:** Thank you very much for this professional advice. We have described the method used as follow: Flow cytometry was performed according to the Annexin V-FITC/PI apoptosis kit (Multi Sciences, China) instructions. To test the samples, $5 \times 10^5$ cells, including those in the culture supernatant, were collected by centrifuging with pre-cooled PBS at 4°C and 1500 rpm for 5 minutes. Annexin V-FITC (5 μL) and 10 μL PI were added to each tube, mixed gently, and incubated in the dark at room temperature for 5 minutes. A CytoFLEX (BECKMAN, USA) was used to analyze the proportion of apoptotic cells in each sample. Annexin V-FITC was detected using the FITC detection channel (Ex = 488 nm; Em = 530 nm), and PI was detected using the PI detection channel (Ex = 535 nm; Em = 615 nm).

**Q4. Page 7, line 37.** “were collected by centrifugation”: what were the characteristics of centrifugation? Speed (in g number), duration?

**R.** Thank you very much for this comment. The cells were collected by centrifugation at 4°C, 1500rpm for 5 minutes with pre-cooled PBS.

**Q5. Page 12, line 51.** “and EMT”: also write EMT in full if it is not written above. Generally speaking, given the number of abbreviations used, it would be useful to have a table of abbreviations at the beginning or end of the text.

**R.** Thank you for this comment. We have used the full name of EMT.
(epithelial-mesenchymal transition) on p. 3 line 45, so we used EMT in this place.


R. Thank you very much for this professional comment. We have changed it in the revision.

Q7. “D. Differentially expressed of SERPINE1 mRNA”: explain the micrographs: is it an immunohistochemical reaction? Was DAB used as a chromogen? Is the technique described in the materials and methods? Page 19, line 98. “Expression of Ki67”: is this technique described in the material and methods? In general, carefully check whether all the techniques (visualization and others) have been described in the materials and methods;

R. Thank you very much for this comment. We have added the following paragraph in our article:

**Immunohistochemistry staining assay**

Tumor tissue from human or nude mice was fixed with 4% formaldehyde, dehydrated, embedded in paraffin, and cut into 4 μm thick tissue sections. After heating at 60°C for 1 hour, the slices were dewaxed and rehydrated with a concentration gradient of alcohol (100, 95, 90, 80, and 70%). The tissue sections were boiled in 10 mmol/L sodium citrate buffer (pH 6.0) at a high temperature to recover the antigens, and incubated for 20 min with 3% hydrogen peroxide to block endogenous peroxidase activity. BSA (5%) was added to the sections and sealed for 30 min. The slices were incubated overnight with primary anti-SERPINE1 (Proteintech, USA,1:500) and anti-Ki67 (Proteintech, USA,1:10000) antibodies at 4°C. The tissue was then incubated with a secondary antibody and observed under a microscope. After the slices were slightly dried, the freshly prepared DAB chromogenic solution (TIANGEN, PA140212) was dripped onto the circle. The coloration time was controlled under the microscope, with brown color representing positive staining. The slices were then washed with tap water to stop the coloration. Harris hematoxylin (BOSTER, AR1108) was used to restain the samples for 5 min (the time was controlled by the degree of staining). The samples were then washed in tap water, differentiated with 1% hydrochloric acid alcohol for a few seconds, and rinsed in tap
water. When the ammonia water was blue, the samples were rinsed in running water, dehydrated, and sealed. The images were observed and captured using a fluorescence microscope.

**Q8.** Page 18, line 85 “D. Transwell invasion “: specify the staining used to detect the cells. Page 19, line 95: in figure 8, add the staining used.

**R.** Thank you very much for this comment. 0.1% crystal violet was used to staining the cells and we have described it in the “Invasion assay” in the revised Materials and methods.

Thank you very much for your attention and time. Look forward to hearing from you.

Yours sincerely,

Jianqiang Guo

15 Feb., 2024