

Respected Reviewer (Number ID: 00607640):

Thank you for your valuable comments, and I have revised them in the revised manuscript. Now I will make point-by-point responses to the issues.

1. Abstract: Abbreviations (such as “GC” ..., etc) should be with its full name when it firstly appears and keyword “iTRAQ” is absent in the Abstract.

I have clarified what GC means by showing the full name of gastric cancer when it firstly appears. “iTRAQ” has been added to the third paragraph of the abstract on page 3.

2. “n value” is suggested to be added in all figures’ legend.

“N value” has been added in figure3, 4, 5, and 7.

Respected Reviewer (Number ID: 03976790):

Thank you for your valuable comments, and I have revised them in the revised manuscript. I have made point-by-point responses to the issues.

1. Page 2, line 3: Abstract: clarify what GC means (gastric carcinoma).

I have clarified what GC means by showing the full name of gastric cancer when it firstly appears on page 3.

2. Page 3, immunohistochemical staining. Some details on the IHC technique are missing. Was the staining done on living or fixed cells? In the latter case, what was the fixative? How many sections have been stained, $2 \times 75 = 150$? Have controls been carried out, for example by omission of primary or secondary antibodies? What was the method of staining and counterstaining? What was the method of control used? Peroxidases or others? Was there a method of amplifying the signal? What was the chromogen used? Please complete this part.

I have completed the details of IHC technique on page 7. Staining was done on the section containing 64 pairs of GC tissues and matched adjacent tissues. One section has been stained, $64 \times 2 = 128$. The matched adjacent tissues were used as a control group. The section was incubated with primary antibody and secondary antibody

successively, developed with diaminobenzidine (DAB) reagent, and counterstained with hematoxylin. There was not a method of amplifying the signal. Chromogen is the antigen possessed by the tissue itself.

3. Page 3: What is the sign between “the area of” and “positively”?

The brown-yellow particles in the tissues represent the MRPL35 protein. The degree of staining was scored by the area of positively stained tumor cells relative to the entire tumor area: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The formula used is: the area of stained tumor cells/ the area of the entire tumor tissues (%).

4. Page 4: “coomassie brilliant blue staining”: write “Coomassie” instead “coomassie” (with a capital letter).

I have corrected “Coomassie” instead “coomassie” on page10.

5. Page 7, figure 2A: I counted $16 \times 8 = 128$ stained sections. In the legend and the text, twice 75 sections were stained. Where do the sections in Figure 1A come from? In the same figure, how many tissues come from carcinomas? How many are from adjacent tissues? How many are controls? An insert in figure 2B or 2C showing controls would be useful.

Originally there were 75 pairs of GC tissues and matched adjacent tissues on the section, but some of them were worn out during the IHC process. We performed pathological analysis on the remaining 68 GC tissues, and obtained Table 1. We performed IHC on the remaining 64 pairs of GC tissues and matched adjacent tissues. We read some articles ^[1, 2] and found that the adjacent tissues could be used as a control. So, we have 64 tissues coming from gastric carcinomas, and 64 tissues coming from matched adjacent tissues as controls. I have marked the GC tissues with the red five-pointed star in the figure 2B.

6. Page 8, “The expression of MRPL35 in GC cells”: the number and the legend of the table are missing. In the table: correct “male” instead “meale”

The table was re-attached to the end of the manuscript. I have corrected “male” instead “meale” on page32.

7. Page 13, discussion: no bibliographic reference is given in the discussion?

References have been given in the discussion.

References:

- 1 Ong JR, Bamodu OA, Khang NV, Lin YK, Yeh CT, Lee WH and Cherng YG: SUMO-Activating enzyme subunit 1 (SAE1) is a promising diagnostic cancer metabolism biomarker of hepatocellular carcinoma. *Cells-Basel* 10(1), 2021. PMID: 33477333 DOI: 10.3390/cells10010178
- 2 Sun Z, Zhu Y, Aminbuhe, Fan Q, Peng J and Zhang N: Differential expression of APE1 in hepatocellular carcinoma and the effects on proliferation and apoptosis of cancer cells. *Biosci Trends* 12(5): 456-462, 2018. PMID: 30473552 DOI: 10.5582/bst.2018.01239

The details of IHC technique have already filled in page X. The staining was done on the 68 pairs of GC and matched adjacent tissues which are fixated in 4% paraformaldehyde. Thanks to the tissue microarray (TMA), an effective high-throughput technique for the study of tumor molecular pathology, only one section was stained, but there were 64 pairs ($16 \times 8 = 128$ pieces) of tissues left on this section. It is difficult to carry out a negative control on the same slide because the samples on the tissue microarray are very valuable and very tightly packed. However, we did a control by omission of primary antibody (PBS instead of primary antibody) on another slide that was for testing the appropriate antibody concentration. We did so according to some references, and we are still trying to improve this experimental method.

The method of staining is immunohistochemical streptavidin-peroxidase (SP) method, in short, that is primary antibody + biotinylated secondary antibody + HRP-labeled avidin. The section was incubated with primary antibody and secondary antibody successively, developed with a chromogenic reagent. It was counterstained with hematoxylin. The method of control used was peroxidases. There was not a method of amplifying the signal. Horseradish peroxidase-labeled streptomycin was used after antigen repair.