

Dear Editor and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled " High expression of autophagy-related genes EIF4EBP1 could promote tamoxifen resistance and predict poor prognosis in breast cancer" (Manuscript ID: 84499). 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. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors:

1. Are there controversies in this field? What are the most recent and important achievements in the field? In my opinion, answers to these questions should be emphasized. Perhaps, in some cases, novelty of the recent achievements should be highlighted by indicating the year of publication in the text of the manuscript.

**Reply 1:** Thanks for your suggestion. Some scholars have conducted some studies on the role of EIF4EBP1 in tamoxifen resistance, and the conclusions of these studies are similar, and we have supplemented this section.

**Change in the text:** In this study, we investigated the role of EIF4EBP1 in tamoxifen resistance. For the role of EIF4EBP1 in tamoxifen resistance, some scholars have done some studies, and the conclusions of these studies are similar and there is no controversy. Jun-Xian Du et al pointed out in a 2020 study that EIF4EBP1 has significant prognostic value in breast cancer, indicating poor prognosis<sup>[1]</sup>. The 2019 study noted that EIF4EBP1 is located within the 8p11-p12 genomic locus, which is frequently amplified in breast cancer and is known to predict poor prognosis and resistance to endocrine therapy<sup>[2]</sup>. Another study from

2022 indicated that consumption of EIF4EBP1 significantly reduced the proliferation and metastasis of TNBC cells.<sup>[3]</sup> These studies demonstrate that EIF4EBP1 plays an oncogene role in breast cancer, and are the latest and most important achievement in the field. However, most of these conclusions are based on bioinformatics analysis. The role of EIF4EBP1 in tamoxifen resistance has not been experimentally demonstrated.

2. The results and discussion section is very weak and no emphasis is given on the discussion of the results like why certain effects are coming in to existence and what could be the possible reason behind them?

**Reply 2:** Thanks for your suggestion. We have made modifications to the results and discussion section, emphasizing that EIF4EBP1 may increase the resistance of T47D-R cells to tamoxifen by regulating autophagy.

**Change in the text:** This study indicate that EIF4EBP1 could enhance increase the resistance of T47D-R cells to tamoxifen. EIF4EBP1 was autophagy-related genes (ARGs), Some studies have demonstrated the role of EIF4EBP1 in autophagy. It has been reported that in CACO-2 cells exposed to cetuximab, EIF4EBP1 expression and autophagosome formation increased, and autophagy increased the efficacy of cetuximab in colorectal cancer[4]. Moreover, Chin-Yu Lai et al indicates that Lamictal ketoneses YXM110 is a kind of new synthetic drugs, exhibit excellent antitumor activity in many cancer cells, the effect with EIF4EBP1 depletion and the regulation of autophagy[5]. In this study, we suggest that EIF4EBP1 may increase the resistance of T47D-R cells to tamoxifen by regulating autophagy.

This study shown that EIF4EBP1 was overexpressed in the breast cancer, which increased cell proliferation, invasion and migration in BC cells. And we hypothesized that EIF4EBP1 may have a cancer-promoting effect in breast cancer by regulating the above pathways.

3. Conclusion: not properly written.

**Reply 3:** Thanks for your suggestion. We have made modifications to the conclusion section.

**Change in the text:** This study indicated that EIF4EBP1 was overexpressed in the breast cancer and tamoxifen-resistant cell line, which increased cell proliferation, invasion, migration and tamoxifen resistance in BC cells.

4. Results and conclusion: The section devoted to the explanation of the results suffers from the same problems revealed so far. Your storyline in the results section (and conclusion) is hard to follow. Moreover, the conclusions reached are really far from what one can infer from the empirical results.

**Reply 4:** Thanks for your suggestion. We have made modifications to the discussion section.

**Change in the text:** This study indicated that EIF4EBP1 was overexpressed in the breast cancer and tamoxifen-resistant cell line, which increased cell proliferation, invasion, migration and tamoxifen resistance in BC cells.

5. The discussion should be rather organized around arguments avoiding simply describing details without providing much meaning. A real discussion should also link the findings of the study to theory and/or literature.

**Reply 5:** Thanks for your suggestion. We have made revisions to the discussion section by linking the research results with theory and literature around the argument.

**Change in the text:** This study indicate that EIF4EBP1 could enhance increase the resistance of T47D-R cells to tamoxifen. EIF4EBP1 was autophagy-related genes (ARGs), Some studies have demonstrated the role of EIF4EBP1 in autophagy. It has been reported that in CACO-2 cells exposed to cetuximab, EIF4EBP1 expression and autophagosome formation increased, and autophagy increased the efficacy of cetuximab in colorectal cancer[4]. Moreover, Chin-Yu Lai et al indicates that Lamictal ketoneses YXM110 is a kind of new synthetic drugs, exhibit excellent antitumor activity in many cancer cells, the effect with EIF4EBP1 depletion and the regulation of autophagy[5]. In this study, we suggest that EIF4EBP1 may increase the resistance of T47D-R cells to tamoxifen by regulating autophagy.

This study shown that EIF4EBP1 was overexpressed in the breast cancer, which increased cell proliferation, invasion and migration in BC cells. And we

hypothesized that EIF4EBP1 may have a cancer-promoting effect in breast cancer by regulating the above pathways.

6. Spacing, punctuation marks, grammar, and spelling errors should be reviewed thoroughly. I found so many typos throughout the manuscript.

**Reply 6:** Thanks for your suggestion. We thoroughly checked for spacing, punctuation mark, grammar and spelling errors.

7. English is modest. Therefore, the authors need to improve their writing style. In addition, the whole manuscript needs to be checked by native English speakers.

**Reply 7:** Thanks for your suggestion. We searched for native English speakers for polishing.

**The proof of polishing is as follows:**

Reviewer #2:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade D (Rejection)

Conclusion: Rejection

Specific Comments to Authors: I think this paper results do not show any link to autophagy research, specifically the studied protein EIF4EBP1. The figures are poor specifically figure one How do you consider this protein as autophagy-related?

**Reply 1:** Human Autophagy Database (HADb, <http://www.autophagy.lu/>) was a web-based database, which provides a complete list of human genes and proteins that are directly or indirectly involved in autophagy, and we found EIF4EBP1 from this database. Some studies have demonstrated the role of EIF4EBP1 in autophagy. It has been reported that in CACO-2 cells exposed to cetuximab, EIF4EBP1 expression and autophagosome formation increased, and autophagy increased the efficacy of cetuximab in colorectal cancer[4]. Moreover, Chin-Yu Lai et al indicates that Lamictal ketoneses YXM110 is a kind of new synthetic drugs, exhibit excellent antitumor activity in many cancer cells, the effect with EIF4EBP1 depletion and the regulation of autophagy[5]. In this study, we suggest that EIF4EBP1 may increase the resistance of T47D-R cells to tamoxifen by regulating autophagy.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: In the manuscript, the authors reported a study that showed that the overexpression expression of EIF4EBP1 was associated with lymph node metastasis, endocrine therapy status and metastasis stage in breast cancer patients. In addition, the authors found that EIF4EBP1 knockdown could reverse tamoxifen resistance, whereas overexpression of EIF4EBP1 increased tamoxifen resistance in breast cancer cells. Despite the present study is preliminary, it may give some important information about the relationship between the overexpression of EIF4EBP1 and poor prognosis and metastasis in breast cancer patients. The new information may support that EIF4EBP1 could be a diagnosis and therapeutic target. The manuscript could be further improved before it could be recommended for publication. Some points are listed as follows:

1. Please add line numbers and page numbers in the manuscript. It helps reading and comment.

**Reply 1:** Thanks for your suggestion. We have added line numbers and page numbers to the manuscript.

2. Many typos or grammatical errors found, such as Page 1: “cells. Which”, “Key Words”, Page 2: CO2, Page 5: “samples was”, Page 11: “were redcued”, Page 12: “the expression of EIF4EBP1 were upregulated” ... etc.

**Reply 2:** Thanks for your suggestion. We thoroughly checked for spacing, punctuation mark, grammar and spelling errors and we searched for native English speakers for polishing.

**The proof of polishing is as follows:**

3. Fig. 1: The content in the picture is too small read. The resolution is too low.

**Reply 3:** Thanks for your suggestion. We have improved the resolution of Figure 1 and uploaded a PDF version of Figure 1.

4. Fig. 2: It is unclear breast cancer tissues coming from which group of patients or what sort of patients.

**Reply 4:** Thanks for your suggestion. In Figure 2, we selected the T47D cell line and the T47Dtamoxifen resistant cell line, and we have annotated the content of Figure 2 again.

5. Fig. 3: (B), What is the title of x-axis? Concentration? (C), what is/are the treat conditions for the cells?

**Reply 5:** Thanks for your suggestion. The title of x-axis in Fig.2B is concentration of tamoxifen. In Fig.2C, The NC group was transfected with no substance into T47D-R cells, while the si-NC group was transfected with a negative control siRNA into T47D-R cells. The si-EIF4EBP1 group was transfected with a short interfering RNA of EIF4EBP1 into T47D-R cells. Subsequently, logarithmically growing cells were inoculated into a 6-well plate at a density of 400 cells per well. T47D-R cells are replenished with 5  $\mu$  Cultivate in tamoxifen medium. After 2 weeks, the cell colonies were fixed with 4% paraformaldehyde and observed with 0.1% crystal violet staining. Then count the cell colonies and take photos. The experiment was conducted in triplicate.

6. Fig. 4: (C), what is/are the treat conditions for the cells?

**Reply 6:** Thanks for your suggestion. The NC group does not transfect any substance into T47D-R cells, the ov-NC group is the negative control vector that we transfect into T47D-R cells, and the ov-EIF4EBP1 group is the plasmid that transfects EIF4EBP1 into T47D-R cells. Subsequently, logarithmically growing cells were inoculated into a 6-well plate at a density of 400 cells per well. T47D-R cells are replenished with 5  $\mu$  Cultivate in tamoxifen medium. After 2 weeks, the cell colonies were fixed with 4% paraformaldehyde and observed with 0.1% crystal violet staining. Then count the cell colonies and take photos. The experiment was conducted in triplicate.

7. In section 3.4, the authors studied the effect of overexpression of EIF4EBP1 in tamoxifen-resistant T47D-R cells. It is unclear why the authors did not examine the patient breast cancers that overexpressed EIF4EBP1.

**Reply7:** The experiment of supplementing the overexpression of EIF4EBP1 in breast cancer patients is indeed helpful to explain the role of EIF4EBP1, but because of the problem of the experiment cycle, we will verify it in subsequent projects.

## References

- [1] Du JX, Chen C, Luo YH, et al. Establishment and validation of a novel autophagy-related gene signature for patients with breast cancer. *Gene*. 2020. 762: 144974.
- [2] Rutkovsky AC, Yeh ES, Guest ST, et al. Eukaryotic initiation factor 4E-binding protein as an oncogene in breast cancer. *BMC Cancer*. 2019. 19(1): 491.
- [3] Yan C, Liu Q, Jia R. Construction and Validation of a Prognostic Risk Model for Triple-Negative Breast Cancer Based on Autophagy-Related Genes. *Front Oncol*. 2022. 12: 829045.
- [4] Guo GF, Wang YX, Zhang YJ, et al. Predictive and prognostic implications of 4E-BP1, Beclin-1, and LC3 for cetuximab treatment combined with chemotherapy in advanced colorectal cancer with wild-type KRAS: Analysis from real-world data. *World J Gastroenterol*. 2019. 25(15): 1840-1853.
- [5] Lai CY, Pan SL, Yang XM, et al. Depletion of 4E-BP1 and regulation of autophagy lead to YXM110-induced anticancer effects. *Carcinogenesis*. 2013. 34(9): 2050-60.