Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-826

Reviewer A

This is an interesting study investigating PTEN/AKT expression and breast cancer cell proliferation/apoptosis mechanisms. Results are interesting and showed that PTEN expression is inversely correlated with proliferation and AKT activation. However, there are some points that need to revise, especially methodology.
Dear editor: We thanks for your positive feedback. We have revised the manuscript accordingly.

1. The last paragraph of the introduction says that they are exploring mechanisms of breast cancer occurrence. However, the study is looking at proliferation and expression of PTEN/AKT but it doesn´t have the methodology for looking at breast cancer occurrence.
Reply 1: We have revised the introduction. See page 5, line 123-124.

2. It also states that it provides a foundation for the early diagnosis and treatment of breast cancer, but this is not included in objectives, results or discussion.
Reply 2: We have deleted it. See page 5, line 123-124.

3. Methodology is not complete described. Authors are presenting results on breast cancer tissue and normal tissue with different breast cancer subtypes and stages. This has not included in the methodology where it is only described cell experiments.
Reply 3: We have added it in the methodology. We thank for your help in improving the quality of the present study. See page 5, line 131-133.

4. In the methods it is not described how many repetitions were done per each experiment (in tables it says n=3).
Reply 4: We have added in the methods. See page 5, line 152.

5. ER and HER2 expression of cell cultures was not included. This is important since the mechanisms of proliferation might be different depending on breast cancer subtypes. It was not discussed as well.
Reply 5: ER、PR、HER2 and Ki-67 expression of cell cultures are proved to be influencing factors of the prognosis of patients with breast cancer. We found relationship between the luminal type (Determined by ER、PR、HER2 and Ki-67 expression) and gene PTEN. See page 8, line 250-252.

6. It is not clear why the expression of PTEN decreased with tumor stage and histological grade but not with lymph node metastasis. Please clarify
Reply 6: In the results, paragraph 3 and 4, we found significant association between PTEN gene and lymph node metastasis. Page 8, line 230-252.

7. Please clarify what is WHO grade.

Reply 7: We have added. See page 8, line 248.

8. In the last part of the results authors are presenting results with AKT inhibitors but this was not included in the methodology and not in the discussion
Reply 8: We have added it in the methodology and discussion. See page 7, line 201-206.

9. Line 331 is incomplete.
Reply 9: We have revised it. See page 12, line 362.

10. Figure 2: Please clarify why authors decided to have a separate experiment for patients with lymph node metastasis) stage II, III and IV might have lymph node metastasis as well.
Reply 10: The TNM stage and lymph node metastasis are important factors influencing the prognosis. We have added in the discussion. See page 11, line 336-337.

11. Limitations are missing.
Reply 11: We have added. See page 11, line 351-356.

Reviewer B

The manuscript "The effects of the tumor suppressor gene PTEN on the proliferation and apoptosis of breast cancer cells via AKT phosphorylation" is dedicated to the AKT-mTOR signaling pathway in breast cancer. The manuscript written in good English.
I would like to make recommendations that rather need to be edited. At the beginning of the article, the authors pose 2 questions (What is known and what is new? What is the implication, and what should change now?). However, in conclusion, the answers to these questions are not clear. This signaling pathway has been previously studied and the conclusion is written as a statement of known facts. Please highlight what's new and what needs to change according to your research.
Reply: We thanks for your positive feedback. We have revised the introduction accordingly. See page 4, line 118-121.

Reviewer C

1) First, the abstract needs some revisions. In the background, the authors did not describe the knowledge gaps on the role of PTEN and what the potential clinical significance of this research focus is. In the methods, the authors need to briefly describe the questions to be answered by these experimental procedures. The results need to quantify the findings by reporting statistics and accurate P values, such as survival rates. In the conclusion, please have a more detailed comment for the clinical implications of the findings.

Reply 1: We have revised the abstract accordingly. Page 2-3, line 33-60.

2) Second, in the introduction of the main text, please have a brief review on what has been known on the biomarkers associated with the pathological-physiological mechanisms of BC

and have comments on their limitations and knowledge gaps. Please further explain why PTEN-AKT mechanism deserved to be studied. Please tone down the sentence "This information provides a foundation for the early diagnosis and treatment of breast cancer" otherwise please further explain and clarify this.

Reply 2: We have revised the introduction accordingly. See page 4-5, line 118-125.

3) Third, in the methodology of the main text, the authors need to describe the research design and have an overview of the experimental procedures and the research questions to be addressed by them. In statistics, please ensure $P<0.05$ is two-sided.

Reply 3: We have revised it accordingly. See page 5-7, line 130-214.

Reviewer D

The proliferation and apoptosis of cancer cells play important roles in breast carcinomas. In the manuscript "The effects of the tumor suppressor gene PTEN on the proliferation and apoptosis of breast cancer cells via AKT phosphorylation", authors investigated the effects of the PTEN gene on the proliferation and apoptosis of breast cancer cells through AKT phosphorylation. Couple questions are required to be answered before it will be accepted.

(1) What were the roles of AKT phosphorylation in breast cancer? Please state in the introduction.

Reply 1: We have added in the introduction. See page 4, line 118-121.

(2) In the introduction, it was advised to add related reference (DOI: 10.21037/jgo-21-235) about the PTEN/AKT/mTOR signal pathway.

Reply 2: We have added. See reference 7.

(3) In the methods, it was showed that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reference antibodies were used. But, in the figure 2, -actin was showed. Why? And the mTOR antibodies were used. But no results about mTOR.

Reply 3: We have corrected the mistake. Thanks for your help. See page 5, line 134.

(4) How to construct the pcDNA3.0-PTEN plasmid? Please state clearly. And detection of PTEN and AKT mRNA expression by polymerase chain reaction (PCR) was showed. But there were no results.

Reply 4: We have revised the mistake and deleted it. Thanks.

(5) Please state clearly the breast cancer tissues were obtained from where? And provide the ethical statement.

Reply 5: We have added it. See page 5, line 137-141.

(6) In the figure 1 and 2 legend, please supplement the descriptions of A and B.

Reply 6: We have added figure 1 and figure 2 legend. See page 16 and 17.

(7) It was more convincing to identify the pathway by AKT/mTOR signal pathway inhibitor.

Reply 7: We have added it as a limitation in the discussion. See page 11, line 352-356.

(8) How about the effects of PTEN on the migration or invasion of breast cancer cells? It was better to test these effects by experiments.
Reply 8: We have discussed it as a limitations in the discussion. See page 11, line 352-356.

(9) It was the conclusion that overexpression of the PTEN gene can promote AKT phosphorylation by inhibiting the AKT-mTOR signaling pathway. Please provide the results about P-AKT and mTOR.
Reply 9: We have revised the conclusion. See page 3, line 57-60.

(10) The minor question was that what was the meaning of "preambulation" and "A549 amino acids" in the introduction? Please state clearly.
Reply 10: These are meaningless, we have deleted these.

Reviewer E

1. Figure 2
Please explain PTEN and AKT in the legend.
Reply: We have added. See page 18, line 519-520.

2. Figure 3
Please explain PTEN and HE in the legend.
Reply: We have added. See page 18, line 525-526.

3. Figure 4
Please explain PTEN and AKT in the legend.
Reply: We have added. See page 19, line 531-532.

4. References/Citations
Please double-check if more studies should be cited as you mentioned "studies".

93 functions. Studies have found that most proteins involved in signal transduction are
94 products of oncogenes or tumor suppressor genes, and their abnormal expression is
102 closely related to the occurrence and development of tumors (10). The AKT pathway

351 Previous studies have also revealed that low expression of PTEN can enhance the
352 activity of PI3K kinase, activate the PI3K/AKT/mTOR signaling pathway, and
353 facilitate the proliferation of tumor cells (30).

Reply: We have revised them. See page 4, line 90 and page 10, line 316-317.

5. Tables

Please add the description to the table footnote that how the data are presented in table.

Reply: We have added. See page 17, line 500-505.