

Peer Review File

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Comments from Reviewer A:

Question 1: Please clarify the purpose and hypothesis of the research.

Reply 1: There were many studies focusing on the role of FOXP3 in breast cancer, but the significance of FOXP3 in this disease is still insufficient, for there were some Inconsistent findings. Moreover, the the exact disease process from normal to primary and metastasis which it involved in hasn't been revealed until now. Therefore, The aim of this study was to evaluate the expression level of FOXP3 in interstitial tissues in matched normal breast tissues, primary breast cancer tissues and axillary lymph node metastatic cancer tissues of 44 breast cancer patients, so as to better clarify FOXP3's role in the process of breast cancer tumorigenesis and progression. In addition, we also investigated the relationship between stromal FOXP3 expression and axillary lymph node status to illustrate the clinical significance of FOXP3 expression in the disease. We have modified the describe of the the purpose and hypothesis of the research in the revised manuscript, and have highlighted the changes in yellow in the introduction section.

Changes in the text: We have modified our text as advised (see Page 3, line 55-57,65-68; Page 4, line 78-90; Page 5, line91-95 and Page16, line353,354,368-371)

Question 2: Please describe the analysis method of this study in detail in material and methods section.

Reply 2: In the revised manuscript, we have enriched the content of the statistical analysis part in material and methods section, and described all methods we had used in this paper, which has been highlighted in yellow.

Changes in the text: We have modified our text as advised (see Page 8, line 161 and 166-168)

Question 3: The figure of immunohistochemistry is ambiguous. Please replace it.

Reply 3: There are too many similar images presented in Figure1 leading to the figure of immunohistochemistry is ambiguous, for the images are very poor quality and too small. All those images in Figure1 were presented to show the characteristics of immunohistochemical staining of intratumoral FOXP3 in breast tissues. Therefore, we selected three representative images as Figure 1, and modified the Legends for figures accordingly, which has been highlighted in yellow.

Changes in the text: We modified our text as advised (see Figure 1, and Page 17, line 403-408)

Question 4: Does this FOXP3 evaluate the Treg function? This paper has not been analyzed and discussion of the problem. And, please describe the discussion in more detail.

Reply 4: Although many studies focused on discovering the expression of FOXP3 in non-T-regulatory cells, such as cancer cells and macrophages, FOXP3 was still the most specific marker of regulatory T cells (Tregs), which was critical for their development and function. Loss of FOXP3 function lead to Tregs deficiency. Thus the foxp3 detected in our study may evaluate the Treg function to some degree. However, some studies recently reported that there were multiple isoforms of FOXP3 and different isoforms located in different subcellular sites might display variable influence on the development and function of Tregs. Further studies on the isoforms and subcellular locations may provide more in understanding the true function of FOXP3 in cancers. In this study, we examined the whole nuclear expression level of FOXP3 Without distinguishing its specific isoforms or subcellular locations. Despite the above limitations, the study is still meaningful for it has revealed the exact development

process of breast cancer in which FOXP3 plays roles. We have added this discussion in the revised version highlighted in yellow.

Changes in the text: We modified our text as advised (see Page 10, line 220,221; Page11, line 222-224;and Page13, line 275-284)

Question 5: The sentence of this paper has many careful mention errors. Please review it.

Reply 5: We have reviewed the manuscript very carefully and found some errors which have been corrected in the revised manuscript. These changes have been marked in yellow.

Changes in the text: We modified our text as advised (see Page 1, line 22,23; Page2, line 25,32,39,40,41; Page3, line 52,53; Page 5, line 104,110; Page6, line 115,116,118,119; Page 8, line 173; Page 9, line 182,184,185,190,194; Page 10, line 206,210,213,214,216; Page 11, line 228,237; Page 12, line 246,252,254,265 and Page13, line 266,269,272,286)

Comments from Reviewer B:

Question 1: The histo pathology reporting forming the basis for all data presented needs revision. The scoring system has been badly devised it is non-reproducible and is certainly not standardised scoring (page 7). One pathologist scoring five fields or view is questionable. Maybe have dual scoring and think about using image analysis to get a value. Hence all data statistically presented here not valid

Reply 1: It's our fault for forgetting cite the origin of the scoring system. As the editor said the scoring system may not be a standardised one, but the scoring system we used in this study was suggested by pathologists according to previous studies which had been used in many cancers and also in our other indicators' researches. The objectivity as well as the value of this scoring system had been recognized widely. Therefore, we selected this scoring system to report our IHC results and we believed that the data obtained from the scoring system were credible. However, the description of scoring

process was confused as suggested by the reviewer, thus we modified this part in the revised version. The modified contents and the references referred to the provenance of the scoring system and our previous study were added in the revised manuscript, which had been marked in yellow in the references section.

Changes in the text: We modified the text (see Page 7, line 149-150) and added two references (see Page 8, line 157, and Page 16, line 374-377)

Question 2: The images submitted are of very poor quality and too small. No staining can be differentiated for region or specificity !! The legends are not providing the information required, language like “obviously” is of poor choice and magnification is just x400 for instance.

Reply 2: There are too many similar images presented in Figure 1 leading to the figure of immunohistochemistry is ambiguous, for the images are very poor quality and too small. All those images in Figure 1 were presented to show the characteristics of immunohistochemical staining of intratumoral FOXP3 in breast tissues. Therefore, we selected three representative images as Figure 1, and modified the Legends for figures accordingly, which has been highlighted in yellow.

Changes in the text: We modified our text as advised (see Figure 1, and Page 17, line 403-408)

Question 3: The methods need to provide more detail regarding IHC. There is no mention of retrieval & not enough about the detection kit which is only available in China.

Reply 3: We have enriched the contents of the IHC method as suggested by the reviewer, which had been marked in yellow in the Immunohistochemical analyses part of the materials and methods section.

Changes in the text: We modified our text as advised (see Page 6, line 130-134 and Page 7, line 135-140)

Question 4: The choice of statistical presentation is not clear Figure 3 nothing is readable.

Reply 4: In the revised manuscript, we have enriched the content of the statistical analysis part in material and methods section, and described all statistical methods we had used in this paper, which has been highlighted in yellow.

In addition, there might be some mistakes in last manuscript for there were only two figures, Figure 3 was not included in this paper.

Changes in the text: We have modified our text as advised (see Page 8, line 161 and 166-168)

Question 5: Reference #5 too old more recent literature in this area is available

Reply 5: We have replaced the reference #5 with more recent literature in this area as suggested by the reviewer, which marked in yellow in the Reference section. And because we have added some references in this version, the reference #5 in previous manuscript refers to reference #10 now.

Changes in the text: We have modified our text as advised (see Page 16, line 343-344)