Author's response to reviews

Title: Coexpression of VEGF-C and COX-2 and its Association With Lymphangiogenesis in Human Breast Cancer

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Author's response to reviews: see over
Answer to referred comment:

1. The Abstract is not fully comprehensive. The results should be more informative. Lymphatic density is not adequately reported and it is part of the study. It is important to inform the use of double staining.
   Answer1: The Abstract has been revised as the reviewer suggested.

2. Double staining was performed to highlight the lymphatic neovascularization. But in Materials and Methods and Results sections there is no mention how Ki-67 was evaluated. This is important to enhance the comprehension about the lymphangiogenesis activity.
   Answer2: In the present study, we carried out double immunostaining with antibodies to D2-40 and Ki-67 to observe the occurrence of dividing nuclei among lymphatic endothelial cells. The evaluation of Ki-67 was performed according to Beasley et al (Beasley et al. 2002) Intratumoral lymphangiogenesis and lymph node metastasis in head and neck cancer. Cancer Res 62: 1315–1320. Positive staining of Ki-67 was seen in nuclei of lymphatic endothelial cells (Fig 1e-f, black arrows), and proliferating lymphatic endothelial cells were observed in 8 of the 10 cases analyzed. And, as expected, Ki-67-positive nuclei were observed in the tumor cells themselves. The results confirmed Ki-67-positive nuclei in a proportion of lymph vessel endothelial cells, suggesting that there is indeed lymphangiogenesis in breast cancer, the most compelling evidence being the presence of proliferating lymphatic endothelial cells. (Please see Results section.)

3. The authors did not mention if LVD was evaluated in intratumoral and/or peritumoral area. This is important to discriminate lymphangiogenesis. Intratumoral LVD is a concept currently disputable. The Ki-67 figure seems to be taken in peritumoral. Is this correct? This is important to be discussed in Discussion section.
   Answer3: In the present study, LVD was evaluated in peritumoral area and was discussed in Discussion section.


4. Needs some language corrections before being published.
Answer4: We have tried our best to revised our article as the reviewer suggested. And the spelling and typing errors have been checked and corrected.
Answer to referre2 comment:

Major Revision:
The authors well commented about the differences of the antibodies between the present and former study. However, the comments is still thought to be insufficient. If the authors want to comment that another reason of the difference is due to the positive correlation between VEGF-C expression level and histological grade, the authors should show the differences of the distribution (the number of the patients) of each histological grade between two studies.

Answer to the major revision: There is a misunderstanding, and the differences between the present and former study is not due to the positive correlation between VEGF-C expression level and histological grade in our article. Furthermore, the distribution (the number of the patients) of histological grade was not show in the former study (Kinoshita et al. Clinical significance of vascular endothelial growth factor-C (VEGF-C) in breast cancer). In order to avoid such a misunderstanding, we have adjusted the location of the sentence (Furthermore, a significant association between increased VEGF-C expression and advanced histological grading was found, suggesting that poorly differentiated tumor cells may be more capable to secrete VEGF-C, which induced lymphangiogenesis in breast cancer.) by moving it to the next paragraph. Please see the sentence (In the present study, a significant association between increased VEGF-C expression and advanced histological grading was found, suggesting that poorly differentiated tumor cells may be more capable to secrete VEGF-C, which induced lymphangiogenesis in breast cancer.) in Discussion section.

Minor Essential Revisions: (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
We have tried our best to revised our article as the reviewer suggested. And we are sorry that we have not found any missing label on figures after checking the figures carefully.