Author's response to reviews

Title: Clinical Significance of Survivin Expression in Ductal Carcinoma in situ with Microinvasion of the Breast

Authors:

Yasuhiro Okumura (sigeoku@yahoo.co.jp)
Yutaka Yamamoto (ys-yama@triton.ocn.ne.jp)
Zhenhuan Zhang (zhangz@fc.kuh.kumamoto-u.ac.jp)
Tatsuya Toyama (t.toyama@med.nagoya-cu.ac.jp)
Teru Kawasoe (tkawasoe@kumamoto-u.ac.jp)
Mutsuko Ibusuki (pellegrino2002jp@yahoo.co.jp)
Yumi Honda (yumih@kumamoto-u.ac.jp)
Ken-ichi Iyama (iyama@kumamoto-u.ac.jp)
Hiroko Yamashita (hirokoy@med.nagoya-cu.ac.jp)
Hirotaka Iwase (hiwase@kumamoto-u.ac.jp)

Version: 2 Date: 20 March 2008

Author's response to reviews: see over
Manuscript number: 4344176651789266

Answers to the reviewer 1

Thank you for your comments and we are pleased that you are interested in our paper. We hope that we can answer your questions adequately.

1. On the title of our paper, we think our present title is concise and clear and it emphasized on our main result and its clinical importance.

2. Our objective is to study the biological differences between DCIS and DCIS-Mi and try to find some parameters which can be useful in adjuvant systemic therapy indication. We modified our Background part following your suggestions.

3. We deleted the use of abbreviation on first mention and also changed capital writing of survivin throughout the text.

4. We changed the word affecting invasion to associated with invasion.

5. On page 5 from line 3 to line 11, we think it is necessary to make the notion of DCIS-Mi clear and it can not be removed.

6. Previous studies on survivin and prognosis were well established so we need not to display them out. The mechanism on the association between survivin and invasiveness is not our aim in this study and we just found that DCIS-Mi showed higher expression of survivin which suggested that for DCIS-Mi patients need more aggressive treatments.

7. After line 18-20, we added the following the make a proper flow of information: Further biological parameters analyses will make the prediction of local recurrence in DCIS more accurate.

8. On page 5 we deleted line 21 and 22.

9. On endometase/matriplysin-2, we think it is better to have these sentences because it
has also been reported that they are invasive factors.

10. On page 6, we transferred line 11-14 to the upper paragraph.

11. On page 7 in line 4, we added: The ethics committee of Kumamoto University and Nagoya City University approved the study protocol and confirmed with the guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all patients before or after operation.

12. Yes, collagen should be collagenase. We are sorry for misspelling.

13. We confirmed buffer pH 9 for survivin as described in your publication and we will cite your paper (Singapore Med J, 2007;48(7):607-614).

14. skimmed milk is right and the concentration is 5%.

15. We added the references for the work described in Methods.

16. We are sorry for our misspelling of Nederlands and changed it to Netherlands.

17. On page 8 last paragraph we deleted line 23 on antibody recognizing parts.

18. Reference for TUNEL method was added.

19. In the present study we used over 10% as positive cut-off was based on our previous publication (Yamamoto Y. et al. Breast Cancer Res Treat. 2007 Sep 6;).

20. Yes, the immunohistochemistry was read microscopically by 2 independent persons.

21. On page 9 on the last two lines we deleted :Cytoplasmic

22. We re-wrote Statistical Method.

23. All the patients’ information had been obtained from their records.

24. on page 12 we changed more frequently to more frequent, analysis to analyses
invasive focus to invasive tissue and impossible to difficult.

25. On page 13 line 7-23 were deleted.


27. For the lack of nuclear expression in this study remains unclear and must be sought in further study on survivin splice variants expression.

28. On page 15 line 3-4, we used the word *may suggest* to soften this suggestion.


30. We re-wrote in conclusions: survivin may prove to be a useful maker to indicate a difference in biological features between DCIS and DCIS-Mi, and it could become one of the parameters used to determine whether adjuvant systemic or surviving inhibiting therapy should be given to patients with DCIS.

We hope we have addressed these issues satisfactorily. Thank you for your consideration.

Sincerely,

Hirotaka Iwase
Department of Breast and Endocrine Surgery,
Faculty of Medical and Pharmaceutical Sciences,
Kumamoto University, Honjo 1-1-1, Kumamoto 860-8556, Japan.
Phone: +81-96-373-5521; Fax: +81-96-373-5525;
E-mail: hiwase@kumamoto-u.ac.jp.
Manuscript number: 4344176651789266

Answers to the reviewer 2

Thank you for your comments and we are pleased that you are interested in our paper. We hope that we can answer your questions adequately.

1. In this study from 1990 to 2005, 52 cases of DCIS and 28 cases of DCIS-Mi were all the consecutive cases that we could collected and the same fixation/processing protocol was used throughout that time period.

2. When doing multivariate analysis we analyzed all the cases together totally 80 cases.

3. We think it is good to view HER2 positive membrane staining in Figure 1.

4. In situation there are cases less than 5 we used Fisher’s exact test which could show the statistical significance.

5. We will delete reference 13.

6. Yes, nuclear grade refers to nuclear size and variation and we re-write it following your idea.

7. Why should apoptosis and proliferation be associated with microinvasion?
   We hypothesized that the tumor with microinvasion had apoptotic and proliferative abilities.

8. Assessment of HER2 was based on our previous publication in reference (Iwase H. et al. Breast Cancer 2001, 8(2):98-104) and we think it is right.

9. We re-write Discussion part to make it more concise and clear. In addition, because survivin is expressed in both DCIS and DCIS-Mi and the expression level of survivin in DCIS-Mi is significantly higher than that in DCIS so it could be used
clinically as an adjuvant therapy indicator.

We hope we have addressed these issues satisfactorily. Thank you for your consideration.

Sincerely,

Hirotaka Iwase
Department of Breast and Endocrine Surgery,
Faculty of Medical and Pharmaceutical Sciences,
Kumamoto University,
Honjo 1-1-1,
Kumamoto 860-8556, Japan.
Phone: +81-96-373-5521; Fax: +81-96-373-5525;
E-mail: hiwase@kumamoto-u.ac.jp.