Author's response to reviews

Title: The association between RCAS1 expression in laryngeal and pharyngeal cancer and its healthy stroma with cancer relapse

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Author's response to reviews: see over
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BMC Cancer

Editor-in-Chief

Dear Editor-in-Chief

I would like to resubmit a revised version of the manuscript entitled: “The association between RCAS1 expression in laryngeal and pharyngeal cancer and its healthy stroma with cancer relapse” to the BMC Cancer, which we have revised in accordance with the Reviewers helpful suggestions. We are pleased, that all suggestions and requests for clarification could be incorporated into a final manuscript. The corrections in the text were marked with red color. I would like to thank the editors and reviewers for analyzing and understanding the paper’s message in depth and for the constructive suggestions.

With best regards,

Magdalena Dutsch-Wicherek, M.D., Ph.D

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Below, please find attached the response to reviewers’ comments:

Reviewer 1

**Reviewer’s report**

**Title**: The association between RCAS1 expression in laryngeal and pharyngeal cancer and its healthy stroma with cancer relapse

**Version**: 1  **Date**: 21 November 2008

**Reviewer**: Kenzo Sonoda

**Reviewer’s report**:

Comments:

The authors immunohistochemically investigated RCAS1 expression not only in pharyngeal and laryngeal cancer but also in its clear surgical margin in order to evaluate an involvement of RCAS1 in local cancer recurrence. RCAS1 was identified in all cancer tissues. Moreover, RCAS1 was detected even in clear surgical margin samples at lower level than cancer. Intriguingly, RCAS1 expression was significantly strong in clear surgical margins in case of cancer relapse. The authors concluded that immune cell suppression connecting with tumor RCAS1 expression might be important for cancer recurrence. So far, there was no report regarding RCAS1 expression in surgical margins, making the results of this work valuable. However some suggestions are made below to be criticized:

(Major Compulsory Revisions)

1. In “Results” section, the authors described that “RCAS1 presence was also revealed in immunohistochemistry in all (96.5%) samples derived from
histopathologically negative surgical margins” (page 9, line 15). However, RCAS1 is positive in 96.5% (50 positive cases in 51 total cases) in Table 2. Additionally, Fig. 3 shows RCAS1 immunoreactivity in cancer but not in clear surgical margins. The authors should revise the text in order to make readers understand clearly. 

**Reply:** In accordance with the Reviewer’s suggestion, we have changed the text in order to make it clearer: “The presence of RCAS1 was also revealed in immunohistochemistry in almost all (96.5%) the samples derived from histopathologically negative surgical margins” (page 9, line 15).

2. In “Results” section, the authors did not mention Fig. 4 and 5. The authors should describe the significance shown by these figures. 

**Reply:** We have added the following commentary concerning Figures 4 and 5: RCAS1 immunoreactivity in the stratified squamous epithelium from healthy surgical margin was present mostly in the superficial layers of the epithelium, while RCAS1 immunoreactivity identified in the stratified columnar epithelium was present only in the margins of epithelium as a very intense brown reaction. The immunoreactivity of RCAS1 remained at the highest level in the close vicinity of the cancer and was observed to decrease as the distance from the tumor increased (Fig.4). The type of staining pattern in the healthy epithelium was granular and membranous (Figure 5).

3. In “Methods” section, the authors described that 20 tissue samples ware also obtained from the healthy mucous membranes of patients without cancerous lesions. However, there was no result concerning these healthy tissues. The authors should add data by using healthy tissues. 

**Reply:** We have also evaluated 20 tissue samples derived from the healthy mucous membranes of the upper respiratory tract of patients without cancerous lesions. The samples were assessed using the Western blot method. We did not detect the presence of RCAS1 in these samples. In accordance with the Reviewer’s suggestion, we have added these results to the Results Section.

4. The authors discussed that RCAS1 expression in clear margins may contribute for cancer relapse, therefore I would suggest a consideration to characterize RCAS1 positive cells in order to understand biological significance of RCAS1. 

**Reply:** In accordance with the Reviewer’s suggestion, these parts of the Results and Discussion Sections were revised and rewritten. The information concerning the biological role of RCAS1 in the clear surgical margin was also added.

5. The authors described that RCAS1 stromal expression might be responsible for tumor invasion (page 12, line 8-11). However, RCAS1 expression was
evaluated in cancer cells but not in stroma of uterine cervix (Sonoda K et al. Gynecol Oncol 2005; 99: 189-198). The authors should revise the text correctly. **Reply:** We have revised the text according to the Reviewer’s suggestion and rewritten the Discussion section.

(Minor Essential Revisions)

1. In “Methods” section, several typographical errors (for examples, 50l1/4g; I1/4m, etc.) should be corrected (page 6-7). **Reply:** The errors in the Methods Section were corrected according to the Reviewer’s suggestion.

2. In Table 1, the numbers were indicated for positive lymph node metastasis with or without extracapsular spread. However, these numbers do not sum to 27. The author should correct numbers. **Reply:** We have corrected the numbers corresponding to the positive lymph nodes with extra-capsular spread according to the Reviewer’s suggestion.

3. In Table 1, the numbers were indicated for tumor size, however, these numbers do not sum to 51. The author should correct numbers. **Reply:** We have corrected these numbers according the Reviewer’s suggestion.

4. In Table 2, the authors should indicate an asterisk which was described in a footnote. **Reply:** We have indicated the footnote in Table 2 with an asterisk, according the Reviewer’s suggestion.

5. In Table 2, “in six specimens (20%)” should be corrected to “in six specimens (12%)” (line 1 in a footnote). **Reply:** In accordance with the Reviewer’s suggestion, we have corrected the percentage of patients in Table 2.

6. In Tables, the word indicating statistical difference “p” should be written as “P” (italic capital). **Reply:** In accordance with the Reviewer’s suggestion, we have changed the “p” describing statistical difference to “P.”

(Discretionary Revisions)

No suggestions.

That is all.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.

Reviewer 2

Reviewer’s report

Title: The association between RCAS1 expression in laryngeal and pharyngeal cancer and its healthy stroma with cancer relapse

Version: 1 Date: 21 November 2008

Reviewer: Munechika Enjoji

Reviewer’s report:

Authors examined the RCAS1 expression in laryngeal and pharyngeal cancers and investigated its clinical availability for predicting recurrence, prognosis, and so on. The study is really significant. If their presentation and explanation are improved in some points, this article becomes more persuasive.

Comments

1. Fig. 1 (Western blot): There was no heading for each column. Therefore, we cannot evaluate the result. Please supply. Reply: The figure was corrected according to the Reviewer’s suggestion.

2. Results (page 9, line 15): in all (96,5%) samples # in almost all (96.5%) samples. Reply: The Results Section was corrected according to the Reviewer’s suggestion:

The presence of RCAS1 was also revealed in immunohistochemistry in almost all (96.5%) samples derived from histopathologically negative surgical margins.

3. Table 3-7: In this style, expression levels seem not to be quantitative. The expression levels of RCAS1 (Western blot) should be shown as the
RCAS1/#-actin ratio. **Reply:** The RCAS1/beta-Actin ratios were added to the tables according to the Reviewer’s suggestion.

4. Table 5, 6: Show the data in clear surgical margin. The results are more important, I think. **Reply:** The tables were revised according to the Reviewer’s suggestion.

5. Results (page 11, line 9): Authors said “RCAS1 immunoreactivity was observed to decrease in accordance with the distance from the tumor”. However, no data were presented at least in the RESULTS. Fig. 4 may represent the sentence, but it may be only one specific case. **Reply:** In accordance with the Reviewer’s suggestion, the following sentences were added to the Results Section:

> RCAS1 immunoreactivity in the stratified squamous epithelium from healthy surgical margin was present mostly in the superficial layers of the epithelium, while RCAS1 immunoreactivity identified in the stratified columnar epithelium was present only in the margins of epithelium as a very intense brown reaction. The immunoreactivity of RCAS1 was at its highest level in the close vicinity of the cancer and was observed to decrease as the distance from the tumor increased (Fig.4). The type of staining pattern in the healthy epithelium was granular and membranous (Figure 5).

6. Fig. 4 (immunostaining): Which side is cancer-side? Please write down. **Reply:** According to the Reviewer’s suggestion, an arrow was added to the figure to indicate the cancerous side.

7. Fig. 5 is not mentioned in the text. **Reply:** According to the Reviewer’s suggestion, the description of Figure 5 along with a short commentary was added to the Results Section.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published **Reply:** The language correction of the manuscript was performed by a native speaker.

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'