Author's response to reviews

Title: Ep-ICD subcellular localization index (ESLI) is a novel marker for metastatic papillary thyroid microcarcinoma

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Author's response to reviews: see over
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To
The Editor
BMC Cancer

Sub: Submission of revised manuscript

Ref: 1013651983755189

Title: Immunohistochemical analysis based Ep-ICD subcellular localization index (ESLI) is a novel marker for metastatic papillary thyroid microcarcinoma

Sir,

We are submitting the revised version of the above stated manuscript. We thank the reviewers for favourable review and supporting our work as well as for the constructive suggestions. The manuscript has been revised taking into consideration the comments of all the three reviewers. The pointwise response to reviewers’ comments is also appended.

We hope the revised manuscript will be acceptable for publication in BMC Cancer.

Best regards.

Yours sincerely,

(RANJU RALHAN)
Ref: 1013651983755189

Title: Ep-ICD subcellular localization index (ESLI) is a novel marker for metastatic papillary thyroid microcarcinoma

Reviewer 1:
Reviewer: Sara Simonetti

This is a very interesting article because at the moment knowledge on PTMC behaviour is still scanty. Therefore the present paper gives some new data interesting to better understand this type of tumour. Nevertheless several points should be clarified before publication.

Major Compulsory Revision:
1. The authors study 34 patients and they report the results of IHC expression of EpEx, Ep-ICD and ESLI in 2 separated group of tumors, metastatic and non-metastatic. Moreover they say, in the abstract and in the methods, that this separation of patients was based on the development of metastasis at diagnosis or during follow-up. In the results the authors report the differences of IHC expression of EpEx, Ep-ICD and ESLI in the 2 groups of patients, but they do not correlate this expression with the outcome of the patients. It could be very interesting to explain which is the outcome of the patients (recurrence or not) and which is the immunohistochemical behaviour of EpEx, Ep-ICD and ESLI correlating to the follow-up of the patients.

RESPONSE. We thank the reviewer for the favourable assessment of our work and the suggestions. The small number of cases with recurrence/persistent disease does not permit us to execute the suggested analysis. However, we agree this is an excellent suggestion and will be addressed in future studies.

The authors should introduce the follow-up data in the paragraph "Patient and Material" of the Methods; should add in the Results which is the correlation of IHC expression of EpEx, Ep-ICD and ESLI and the follow-up of the patients, also in a table, and should report in the Discussion their data.

We agree. We have added the patient follow-up details in Page 8, lines 12-14 under "Patients and Materials" in the Methods section as suggested. The follow up data cannot be presented in a table due to the small sample size of events in the follow up period.

Minor Essential Revisions:
1. Title: The title should contain a reference to the used technique in the article. For example "Immunohistochemical expression of Ep-ICD subcellular localization index (ESLI) as a novel marker for metastatic papillary thyroid microcarcinoma".
The title has been changed to reflect the method used as suggested. The revised title is “Immunohistochemical analysis based Ep-ICD subcellular localization index (ESLI) is a novel marker for metastatic papillary thyroid microcarcinoma”.

2. Methods:
- In the paragraph "Immunohistochemistry for EpEx and Ep-ICD expression in PTMCs" it is not reported which kind of counterstaining was used and which tissues have been used a negative and positive control of IHC technique.

The type of controls and counterstaining is added on Page 9, lines 7-10. It has been changed to “Hematoxylin was used as the counterstain for nuclei. The primary antibody was replaced with isotype specific IgG in PTMC used as the negative control. Colon cancer tissue sections known to express Ep-ICD/EpEx were used as a positive control in each batch of IHC analysis.”

- In the paragraph "Evaluation of immunohistochemical staining" a reference of the validation of the applied score is not reported.

The references for the scoring have been added on Page 9, line 19

References

- The title of the paragraph "Ep-ICD Subcellular Localization Index (ESLI)" is in bold and not subtitled as the others.

ESLI subheading has been changed to match those above it on Page 9, line 21.

- In the paragraph "Ep-ICD Subcellular Localization Index (ESLI)" a reference of the validation of the applied score should be reported.

The requested reference has been added on Page 9, line 24.

3. Results:
- The authors should be adding a title to the first paragraph, for example "Patients".

The title has been added to the first paragraph under Results named "Patient follow-up" on Page 10, line 8.

4. Discussion: In the second paragraph "Current AJCC TNM staging recommends using a select few clinicopathological......" the authors should introduce a reference for this data and explain the significance of acronymous AJCC.

The suggested changes have been made to the Discussion on Page 12, lines 11-13

Current American Joint Committee on Cancer (AJCC) TNM staging recommends using a select few clinicopathological variables to determine prognosis in thyroid cancer patients [30]. The reference has been added.


5. Figure 1:- D) The authors report the image as a metastatic PTMC showing strong nuclear and cytoplasmic Ep-ICD accumulation, but this image shows just a cytoplasmic distribution of the protein and not a nuclear one. The authors should modify the figure with a nucleo-cytoplasmic positivity of the protein.

Figure 1D has been replaced to reflect the nuclear and strong cytoplasmic staining in the aggressive PTMC group.

6. Table 1:- In the first line "Gender" how many male patients are included in the study is not reported. In the first column "Metastatic" the percentage in parenthesis is missed.

Table 1a has been modified to reflect the suggestions with the number of male patients reported and percentages provided under the metastatic column.

Reviewer 2:
Reviewer: Antonio Feliciello

The manuscript by Kunavisarut et al. describes the identification of Ep-ICD subcellular localization as a novel marker of metastatic papillary thyroid microcarcinoma (PTMC). The authors found a positive correlation between nucleo-cytoplasmic Ep-ICD localization with loss of membrane staining and the metastatic potential of PTMC lesions. The manuscript is well written and the experiments are appropriately designed and carried out. However, the major problem with this manuscript is the number of patients analyzed and the differences in Ep-ICD intracellular localization between both groups, although statistically significant, are only minimal. Increasing the number of patients analyzed would clearly strengthen the authors’ conclusions.

RESPONSE. We thank the reviewer for the positive comments about our manuscript. Regarding its major limitation in terms of sample size, we appreciate
the difference a large sample would make to this paper. Unfortunately, based on our inclusion and exclusion criteria, we are restricted currently to this small cohort in our hospital. Despite this limitation, our work is important because of the dearth of studies in the field of aggressive papillary thyroid microcarcinoma (PTMC). We recognize that a small study can go a long way in shedding light on the molecular processes that aid distinction of the aggressive counterpart of an otherwise benign disease. Our current PTMC data has set the stage for a larger multicenter study to validate ESLI as a prognostic marker in PTMC.

Reviewer 3:
Reviewer: Maria Quarto
The authors present a study on correlation between nuclear and cytoplasmic Ep-ICD expression and loss of membranous EpEx with metastasis presence in PTMC patients. In general the work is interesting although the sample size is low. My opinion is favorable to the publication with minor corrections. In particular, some point that I would like to get your attention are:
1. The International System of Unit (SI) recommends to use a space for separating the group of three digits and both the comma and period should be used only to denote decimals. So, in page 5, line 4 instead of saying: “...approximately 37,200 new cases....”, it is better to say that “...approximately 37 200 new cases....”. In page 5 line 6 instead of saying: “...for the year 2012 is 56,460...”, it is better to say that “...for the year 2012 is 56 460...”.

RESPONSE. We would like to thank the reviewer for expressing interest in our work and appreciating the same. We agree with all the comments and have made changes to reflect the additions. The suggested SI unit for reporting numerals has been incorporated on Page 5, lines 5-6.

2. I think you don't need to repeat the results of table 2 in the text. (paragraph “Immunohistochemical Ep-ICD and EpEx expression in metastatic PTMC and non-metastatic PTMC”)

For paragraph 1 on Page 11 (Immunohistochemical Ep-ICD and EpEx expression in metastatic PTMC and non-metastatic PTMC), we intended to describe the immunohistochemical trend along with the relevant figures.

3. Figures 2 and 3 are not box plot, but graph bar. The box plot is a graphical representation of the distribution of a sample by means of its central value and variability. This information are not present in your charts. I suggest you to use box plot for this data.

Figure 2 has been changed to a box plot diagram as suggested.

4. Are fig 3a and b a box plot? Authors should explain better because fig 3 is not a scatter plot.
Figure 3 has been correctly renamed as "Dot plot" instead of "Scatter plot", the figure legend has been updated on Page 18, line 17-18 and in text on Page 11, line 14.

5. Data and error must be reported with same significant digits. In table 2 “3 ± 1.67” must be “3.00 ± 1.67”; “1.3 ± 1.07” must be “1.30 ± 1.07”.

Table II has been modified to report data and error in same significant digits on Page 22.