Author’s response to reviews

Title: EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis

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Version: 3 Date: 5 April 2010

Author’s response to reviews: see over
April 5, 2010

Re: Submission of revised manuscript to BMC Cancer
Title: EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis

Authors: Ranju Ralhan\textsuperscript{1,2*}, Jun Cao\textsuperscript{3*}, Terence Lim\textsuperscript{3}, Christina MacMillan\textsuperscript{3}, Jeremy Freeman\textsuperscript{3}, and Paul G. Walfish\textsuperscript{1,2,3}

Dear Sir,

We are grateful and thank the Editorial Board and Reviewers for supporting our work and a favourable review of our abovementioned manuscript, giving us the opportunity to revise the paper. The manuscript has been revised taking into consideration all the comments of both the Reviewers. The pointwise response to Reviewers comments is also submitted.

We are submitting the revised manuscript for re-consideration for publication in BMC Cancer.
We look forward to a favorable response.

Thanking you.

Yours sincerely,

Paul G. Walfish

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Author’s response to reviews

Title: EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis

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Version 2: April 4, 2010

Author’s response to reviews: see over
Reviewer's report

Title: EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis

Version: 1 Date: 21 January 2010

Reviewer: Francois Le Naour
Following are my comments on the manuscript entitled “EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis” by Ralhan et al.

The manuscript is focused on the distribution of cleavage product of EpCAM especially the nuclear localization of Ep-ICD which is supposed correlated with aggressive thyroid cancers. Although EpCAM is an exciting cancer marker, the manuscript exhibits insufficiencies.

- “The pictures shown are two small for being really demonstrative of the membrane, cytoplasmic or nuclear localization. A higher magnification will greatly help the reader and therefore is needed”.

The photomicrographs in Figure 1 are at 40x but since there were several photomicrographs in one panel these appeared smaller. Figure 1 has been split into two parts for clarity. Figure 2 has been replaced with photomicrographs at 40x magnification as the reviewer indicates.

- “The correlation with beta-catenin is not convincible since there is a poor nuclear labelling. Most nuclei seem not stained”.

As the reviewer indicates all nuclei do not show nuclear beta-catenin; the manuscript has been revised accordingly. However, we did observe a statistical correlation between the nuclear localization of Ep-ICD and beta-catenin proteins in the aggressive thyroid cancers only. We have provided confocal photomicrographs of an ATC patient sample that clearly demonstrates nuclear co-localization of Ep-ICD and beta-catenin at the end of this response letter (Please note that this panel of photomicrographs is for the reviewers only).

- “In the result section, text jumps from Fig1 to Fig3D; the number of figure is disorganized”.

The text has been corrected as indicated by the reviewer.

- “In Fig1: the symbols M, C, N are not explicated in the legend to the figure; I
guess it means membrane, cytoplasm and nucleus”.

Yes, the symbols M, C, N refer to membrane, cytoplasm and nucleus and have been explained in the legends as indicated by the reviewer.

- “In the abstract: “Conclusion In conclusion…” , not really a good writing”.

The conclusions have been revised as the indicated by the reviewer.
Reviewer's report

Title: EpCAM Nuclear Localization Identifies Aggressive Thyroid Cancer and is a Marker for Poor Prognosis

Version: 1 Date: 21 January 2010

Reviewer: Wendy Van Veelen

Reviewer’s report:

Major Compulsory Revisions:

1. “In the more aggressive types of TC, nuclear Ep-ICD expression correlates with nuclear beta-catenin. The authors should mention the relation of nuclear beta-catenin with patient prognosis in the Introduction.

The correlation of nuclear Ep-ICD and beta-catenin with patient prognosis has been mentioned in the Introduction as indicated by the reviewer.

In the Discussion the authors implicate a causal relationship between nuclear Ep-ICD and nuclear beta-catenin expression. This is states too strong as the authors have only observed concomitant nuclear expression of these proteins. Could both proteins serve (equally well) as biomarkers for diagnosis of aggressive TC? Could nuclear Ep-ICD expression (and/or nuclear beta-catenin expression) be used in addition or instead of standard pathological characteristics to improve discrimination between well- and poorly differentiated TC”?

We agree. The statement has been revised as indicated by the reviewer. Our paper focuses on Ep-ICD. As evident from our data, strong nuclear Ep-ICD is observed in most tumor cells in the aggressive thyroid tumors, while nuclear beta-catenin is observed in some nuclei only, so both proteins do not serve as biomarkers of aggressiveness equally well. We are not emphasizing nuclear beta-catenin as a biomarker for thyroid cancer aggressiveness. We used beta-catenin to show its mechanistic relationship to Ep-ICD only. Nuclear Ep-ICD expression can be used in addition to the standard pathological characteristics to improve discrimination between well- and poorly-differentiated TC.

2. “At page. 15 nuclear EpEx is mentioned as an ideal candidate diagnostic marker and therapeutic target for most well- and poorly differentiated TCs, however, EpEx is also expressed in normal thyroid tissue. Therefore, membranous EpEx would not serve as an ideal marker. Instead, nuclear Ep-ICD is specifically expressed in poorly differentiated TCs, like ATC. Therefore, I would say that nuclear Ep-ICD could serve as an ideal target for diagnostic and therapeutic strategies for aggressive TC”.

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Yes, we agree. This change has been made in the revised manuscript as indicated by the reviewer.

3. “The scoring system used for IHC by the authors and shown in fig 3A,B,C is not conventional. Staining intensities on archived paraffin material are not reliable. The data should be presented in a table or diagram (e.g. +/-, +, ++ or percentages), like Fig 3D. There, the percentages of nuclear beta-catenin could be included”.

The detailed scoring data for all the three proteins is given in Table1. This scoring system has been used in all our publications in several cancers. Some of these references are given below:


Minor Essential Revisions:

1. “The IHC pictures in Fig. 1 and 2 should be of a higher magnification. Preferably all at 40x”.

5
The photomicrographs in Figure 1 are at 40x but since there were several photomicrographs in one panel these appeared smaller. Figure 1 has been split into two parts for clarity. Figure 2 has been replaced with photomicrographs at 40x magnification as indicated by the reviewer.

2. “The n, m, and c are not explained in the figure legends of fig 1 and 2 and are not visible in the pictures”.

The symbols in the legend in figure 1 mean M- membrane, C- cytoplasm and N- nucleus as indicated by the reviewer.

3. fig.3. Asterixes with numbers are shown. What do they indicate? Typo in title fig. 3D.

The asterixes with numbers show the outliers and the case number as indicated by the reviewer.

4. fig.4. Lines are not visible.

The figure has been improved as indicated by the reviewer.

5. pag.9. A correlation between beta-catenin and overall survival is not shown in figure 4.

This paper focuses on the prognostic significance of Ep-ICD, therefore, a correlation between beta-catenin and overall survival is not shown in figure 4 as indicated by the reviewer.

6. pag.9/10. The results mentioned in the text would be clearer in a table.

The results have been given in Table 1 as indicated by the reviewer.

7. pag.14. beta-catenin is not a marker for proliferation.

We agree. This has been corrected as indicated by the reviewer.

8. pag.19. Panel CI depicts membranous staining and CIII depicts nuclear staining.

This has been corrected as indicated by the reviewer.

Discretionary Revisions:
1. “It would be interesting to show whether the activity of the proteases TACE or Presenilin-2 is increased in aggressive TC”.

Yes, we agree with the reviewer. However, these studies will be the subject of our future work.
ATC: A, β-catenin; B, Ep-ICD; C, A and B merged; D, DAPI; E, A and D merged; F, B and D merged; G, A,B and D merged.