Author's response to reviews

Title: Napsin A as a marker of clear cell ovarian carcinoma

Authors:

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Author's response to reviews: see over
Dear Dr. Stephan Polterauer
Editor, BMC Cancer

Manuscript reference number MS: 1064094041023330
Research article entitled “Napsin A as a marker of clear cell ovarian carcinoma” by Skirnisdottir et al.

Thank you for the valuable comments made by the reviewers on our manuscript. We have, as far as possible, addressed the concerns of the reviewers and made appropriate changes to the manuscript and added figures. Changes are indicated in yellow and our responses to reviewers are given below in italic.

Reviewer #1:
**Reviewer's report**
**Title:** Napsin A as a marker of clear cell ovarian carcinoma  
**Version:** 1  
**Date:** 24 August 2013  
**Reviewer:** Zannoni Gian Franco

**Reviewer's report:**
The authors evaluate the role of Napsin-A as marker of ovarian clear cell carcinoma. As correctly pointed out in the manuscript, the differential diagnosis between high grade serous and clear cell ovarian cancer can be very difficult. Therefore, it is certainly useful to identify novel biomarkers able to help pathologists in achieving a final correct diagnosis. For these reasons, the novelty and scientific relevance of the manuscript seem to be acceptable.

On the other hand, some major criticisms have to be raised, in particular:

- Although the authors investigated a quite large series of advanced ovarian cancer, only 16 patients were affected by clear cell carcinoma, thus potentially biasing the reliability of provided results.

*Out of the 131 patients with FIGO-stage I-II epithelial ovarian cancer (EOC) included in this study 16 patients (12.2 %) had clear cell carcinoma. This can be compared with an earlier study from Sweden (reference 28) including 28 (12.4 %) patients with clear cell carcinoma out of 226 EOC patients also in FIGO-stages I-II. There are significant geographic differences in the prevalence of clear cell carcinoma. Studies in North America and Europe quote prevalence of 1–12% whereas the prevalence in Japan is as high as 15–25% according Anglesio MS et al (reference 7).*
However, a higher number than 16 patients in our study could potentially prevent the biasing effect in a study including a higher number of patients.

-Please, consider extension of study population; otherwise, emphasize this study limitation in the manuscript.

We have added a section to the Discussion (manuscript page 19 the, last paragraph), where we emphasize this study limitation.

-The authors provide a multivariate analysis using Napsin-A as end-point (Table 4 A-B). However, it is more appropriate to evaluate the role of Napsin A, and other clinico-pathological factors as predictor of clear cell histology. For these reasons, I recommend to reformulate data analysis choosing clear cell histology as primary end-point.

In the logistic regression analysis the model was not significant with clear cell histology as endpoint. Therefore we preferred Napsin A as primary endpoint.

-It seems that the "p21+p53- phenotype" could be associated with Napsin A expression, and clear cell histology. Therefore, it remains unclear what Napsin A really add to the "p21+p53- phenotype" along the process of differential diagnosis. Please consider this specific point in the discussion section.

-Since the main goal of the manuscript is to evaluate the predictive role of a novel biomarker, it could be useful to provide a more advanced statistical analysis, including ROC curves, focused on evaluating the predictive role of Napsin A for clear cell histology.

ROC curves have been constructed for the “Napsin A phenotype”, "p21+p53- phenotype" and the "p21+p53-Napsin A+ phenotype". The findings are demonstrated in three new figures which (mentioned in “Results” and “Discussion”) show that the "p21+p53- phenotype" did not add to the “Napsin A” phenotype along the process of differential diagnosis between clear cell ovarian tumors and histological subtypes. It is shown in Figure 2 (A-C) that AUC for Napsin A alone is 0.882, and for p21+p53- it is 0.720. However, for concomitant p21+p53- and Napsin A+, AUC decreases to 0.795.

In conclusion: Napsin A alone might better help along the process of differential diagnosis between clear cell ovarian tumors and histological subtypes than p21+p53- status or concomitant Napsin A and p21+p53- status.
Reviewer #2:

**Reviewer's report**

**Title:** Napsin A as a marker of clear cell ovarian carcinoma  
**Version:** 1  
**Date:** 2 September 2013  
**Reviewer:** Toshihiko Torigoe

**Reviewer's report:**  
The authors examined the positivity of Napsin A, p21, p27 and p53 in ovarian cancer tissues by immuno-histochemistry. The results showed that positivity of Napsin A was significantly associated with clear cell carcinoma. It was also associated with concomitant positivity for p21 and negativity of p53. They concluded that Napsin A should become a marker of clear cell ovarian carcinoma. The rationale of the study is clear and the experiments were conducted well. The study should contribute to the pathological diagnosis of ovarian cancer.

Minor essential comment:  
1. English grammar and spellings should be checked precisely.

*We have checked English grammar and spellings throughout the manuscript.*

Discretionary comments:  
1. The authors proposed that Napsin-A positivity of clear cell carcinoma might predict about platinum sensitivity in the discussion (page 16). Is there any evidence supporting the proposal?

*Recently, it was shown in genome-wide screening for genes involved in chemo-resistance, that NAPA consistently was over-expressed in cisplatin-resistant cells. The corresponding protein Napsin A is an aspartic peptidase and was found to represent an anti-apoptotic protein that promotes resistance to cisplatin (reference 12, 14).*

For example, is there any report showing that Napsin A-positive lung cancers or renal cell cancers are resistant to platinum chemotherapy?

*We are sorry, but we have not been able to find any reports about this issue.*

2. It is informative if the authors discuss the positivity of Napsin A and p21/p53 status of other cancers such as renal cell cancers and lung cancers.

*Both p21 and p53 have previously been reported to show a variation in expression in different kinds of adenocarcinoma with clear cell features, such as renal cell carcinoma and lung carcinoma. Also Napsin-A has been shown to be expressed in a large proportion of clear cell adenocarcinomas of other organs.*
Thus, none of these markers can be described as specific for any special type of carcinoma. The immunoprofile, Napsin A-positivity, p21-positivity and no positivity for p53, of the majority of the clear cell ovarian carcinomas in this study is not unique. However, it differs considerably from the findings in the other ovarian carcinomas and provides valuable information for the diagnosis of these tumors.

References which support the answer:

3. Representative IHC pictures of Napsin A +, ++, +++; and negative cases should be presented in Figure 1.

If the reviewer would like IHC pictures of Napsin A +, ++, +++; and negative cases is important for publication of the manuscript, of course all these IHC pictures could be uploaded as an additional file.

Sincerely,

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