Author's response to reviews

Title: B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome

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Author's response to reviews: see over
Date: May 30th, 2014

Dear Editor,

We highly appreciate the opportunity to resubmit the manuscript “B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome”.

Please find enclosed a new version of the manuscript, and see below for our comments to the reviewers.

Sincerely,

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Our response to the reviewers’ comments is as follows:

**Reviewer 1**
In this article, the authors have just expand the sample size of the patients compared with the article they published last year (B7-H3 expression in colorectal cancer: nuclear localization strongly predicts poor outcome in colon cancer. Int J Cancer. 2012 Dec 1; 131(11):2528-36), and there is no change in the substance. Experiments should be done to prove the function of B7-H3 and its possible reason that effect the clinical outcome.

**Reply:**
We agree that it is of great interest to explore the functional role of B7-H3 in experimental model systems, and we have been and are currently studying functional aspects of B7-H3 in cancer (Nygren et al, Identifying microRNAs regulating B7-H3 in breast cancer: the clinical impact of microRNA-29c; Tekle et al, B7-H3 contributes to the metastatic capacity of melanoma cells by modulation of known metastasis-associated genes; Liu et al, B7-H3 silencing increases paclitaxel sensitivity by abrogating Jak2/Stat3 phosphorylation). However, the aim of the present study was to evaluate whether the prognostic impact of nuclear B7-H3 found in the paper published last year, could be validated in an independent cohort of colorectal cancer patients. A prognostic biomarker must be thoroughly validated before it can be considered utilized in clinical decision making and a prime step towards this is to examine whether its prognostic impact can be reproduced in an independent cohort. We believe that our manuscript adds important information to the B7-H3 puzzle, in which contrasting roles of tumour B7-H3 has been reported. The role of B7-H3 as a prognostic marker in cancer in general is undetermined, and its molecular function is subject to extensive studies.

**Reviewer 2**
**Point 1:**
Figure 1 is very poor! For example, the author said that Panel A shows predominantly cytoplasmic/membrane and stromal staining. However, the reviewer did not agree this conclusion because I did not find positive cells through the whole section. At the same time, I did not agree that nuclear and cytoplasmic/membrane staining in panel B. Moreover, nuclear B7-H3 staining was also very weak in Panel C. I strongly recommend the author replace these pictures. Additionally, please add some pictures demonstrated that B7-H3 staining in cytoplasmic/membrane/nuclear for positive controls.
Reply:
We agree that membrane staining is not clearly present in these panels, and we have now changed the figure text accordingly. However, we do not agree that Figure 1 is very poor. Please see the modified figure 1 below, with arrows indicating positive cancer cells in panel A, B and C.

Point 2:
It seems that most B7-H3 positive cells are not cancer cells that described in Figure 4. We think that cells (like macrophages) infiltrated with these tissues are positive for B7-H3. I recommend the author detect the exact cell types that are positive for B7-H3, like immunofluorescent double staining.

Reply:
As described in the manuscript, Figure 4 shows B7-H3 immunohistochemical staining in two simulated tissue microarray cores from distinct segments of the whole tissue sections from two separate patients in the cohort used in our previous paper (B7-H3 expression in colorectal cancer: nuclear localization strongly predicts poor outcome in colon cancer. Int J Cancer. 2012 Dec 1; 131(11):2528-36). The whole tissue sections from this previously studied cohort were simply utilized in the present manuscript to demonstrate heterogeneity of nuclear B7-H3 expression, which was not revealed in the small TMA cores evaluated in this study. These cores are taken from morphologically representative areas of tumour tissue and
the amount of tumour-infiltrating immune cells in each core is insignificant (Figure 1). Therefore, and although we agree that it would be interesting, we consider it inappropriate to evaluate B7-H3 staining in the tumour microenvironment any further than scoring the tumour-infiltrating stroma as B7-H3 positive or negative as we already did. Also, such analyses are not within the scope of this paper, in which the primary aim was to examine the prognostic role of nuclear B7-H3 in an independent colorectal cancer cohort.