Author's response to reviews

Title: Prognostic significance of fascin expression in advanced colorectal cancer: an immunohistochemical study of colorectal adenomas and adenocarcinomas

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Author's response to reviews: see over
Re: BMC-Cancer

Prognostic significance of fascin expression in advanced colorectal cancer: an immunohistochemical study of colorectal adenomas and adenocarcinomas

Yosuke Hashimoto, Marek Skacel, Ian C. Lavery, Abir L. Mukherjee, Graham Casey and Josephine C. Adams

Dear Ms. Puebla,

Thank you for your letter of 3rd Oct, regarding the provisional acceptance of our manuscript.

I have amended the Abstract and methods to indicate that the CCF IRB is referred to, and to give the CCF IRB number in the methods. These changes are incorporated in the final submission that accompanies this letter.

We have gone again through the BMC checklist and believe that all formatting is appropriate.

We hope this paper is now finally acceptable for publication in BMC-Cancer and look forward to seeing it on the Web soon. We are pleased to publish this study in BMC-Cancer.

Yours sincerely,

Josephine Adams.

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Responses to Referees comments.

Reviewer 1.
While we agree with the reviewer that moving on to a detailed analysis of the relationship between fascin expression and MSI status is an important next step, this goal goes beyond the scope of the present study. To draw statistically meaningful conclusions we will need to again analyse a large set of tumors and this represents a major effort and commitment of resources in terms of scoring and data analysis. The research presented here establishes for the first time the status of fascin expression in colorectal tumors and its potential clinical significance.
We have specified in the Discussion that hMLH1 would be an appropriate marker for future studies of the relationship between fascin expression and MSI status (p16).

Reviewer 2.
Major.

1. We have retained the wording of “colorectal tumors” and “carcinomas” in the Background section, where colorectal cancer and carcinomas from different tissues are discussed in general. Our study was of colorectal adenomas and adenocarcinomas and this wording has now been standardised throughout the text. “Polyp” has been replaced by “adenoma” throughout.

2a. We used two sets of specimens in order to incorporate both clinical annotation (with regard to the TMA set) and a large number of adenomas in the study. Only 15 adenomas were included on the TMAs (stated on p7). In addition, the collection of additional adenocarcinomas allowed us to compare results from the TMA sections with conventional sections. No mesenchymal tumors were included. With regard to point 1 above, this is now clarified in the text.

2b. Adenomas or adenocarcinomas with high grade dysplasia were not included in the study group. This is now stated in the Methods (p6). The size of the adenomas used in our study is now stated on p6. Most were less than 2cm in diameter.

2c. Of the stage I and II patients, only 5 received any adjuvant therapies and of these five, only one was a fascin-high patient. Inclusion of this information in the statistical analyses does not change our original conclusions. Of the 62 stage III and IV patients, 19 received no adjuvant therapy, 27 received chemotherapy and 16 received combined adjuvant chemotherapy and radiotherapy. This is now stated in the Methods (p7). We conducted additional Kaplan-Meier analyses to compare the survival of each of these subsets of patients with other stage III/IV patients and found no statistically significant differences. The fascin-high patients include patients with and without adjuvant treatments. These points are now specified in the results (p12/13). Overall, considering adjuvant therapy in relation to the survival of patients with high-fascin tumors does not alter our original conclusions and has not led to any changes in the figures.

2d. We agree that the description of the clinical followup was confusing in the original version. The mean clinical followup period for surviving patients was 5 years, but the mean clinical followup period for all patients was 38 months. This figure is now stated in the Abstract (p2) and Methods (p7).

3a, 3b. It has indeed been a concern to us in conducting the study whether the TMA specimens accurately report fascin staining, given its patchy distribution in the conventional sections. We analysed this point extensively prior to the original submission. The proportion of fascin-positive tumors scored from the TMA is similar to that from the analysis of conventional sections (17% vs 26%, respectively). The TMAs are prepared from cores that cover, for each specimen, 3.5mm² of the tumor area. Within
the conventional sections, we have measured tumors to cover areas from 30 mm$^2$ to 100 mm$^2$. Thus, the TMA cores sample from 3.5% to 10% of tumor areas. This is sufficient to identify all tumors with >10% of fascin-positive cells. Tumors with less than 10% of fascin-positive cells can be missed and scored as fascin-negative, however, it is only the tumors with >10% of fascin-positive cells (2+, 3+ in the scorings) that show the significant clinical correlation with patient survival.

3c. We cannot make statements about the expression of fascin and Ki67 in relation to the histological differentiation of tumors because the majority of adenocarcinomas in our study were moderately-differentiated. Six tumors in the TMA set were poorly-differentiated. This is now explained in the methods (p6 and p7).

3d. “Data not shown” is now indicated on p11.

Minor.
1. References have been added into the indicated sentence (on p4, refs. 13 and 14).
2. Mention of the colon has been removed from this sentence.
3. The typo has been removed.
4. Our standard scoring is explained in the Methods with regard to the conventional sections. We have emphasised that it is this scoring method that was modified for the TMA (p8/9).

Discretionary.
We agree that a larger scale analysis of fascin expression in relation to tumors in the proximal colon is now an important next step, however this goes beyond the focus of the present study. The need to pursue this question in depth is indicated in the Discussion (p16).