Author's response to reviews

Title: Infrequent cyclooxygenase-2 overexpression in serrated colorectal polyps

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Author's response to reviews: see over
14 November 2007

Melissa Norton, MD
Editor-in-Chief, BMC Cancer

Deborah Saltman, M.D., Ph.D.
Editorial Director, BMC Cancer

RE: Manuscript (ID 6460975891506196. Version 2) entitled “Cyclooxygenase-2 overexpression is common in serrated and non-serrated colorectal adenoma, but uncommon in hyperplastic polyp and sessile serrated polyp/adenoma”

Dear Professor Norton and Professor Saltman,

We are submitting a revised version of the original contribution entitled as above to BMC Cancer. All of the authors contributed to the manuscript preparation and approved its contents. This manuscript acknowledges all sources of support for the work. No conflicts of interest are present. The manuscript has not been published previously and is not being considered concurrently by any other publication.

Reply to reviewers is described below.

I look forward to hearing from you.

Sincerely,

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Reply to reviewers (Manuscript ID 6460975891506196. Version 2)

The reviewer’s comments are in square brackets [ ] and italicized. Changes in response to the reviewers’ comments are underlined in the revised manuscript. Thanks to reviewers’ valuable comments, our manuscript has been much improved.

Reviewer #1

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

[1. The frequency of COX-2 overexpression of SA is similar to that of non-serrated adenoma, but that of HP and SSA were significantly less than that of non-serrated adenoma. From these observations, authors concluded that COX-2 may play a less significant role in serrated pathway in colorectal carcinogenesis. However, in considering that HP and SSA are considered to be precursors of SA and show lower grade of dysplasia, SA showed similar COX-2 overexpression to ordinary adenoma, in ordinary adenoma frequency of COX-2 overexpression depended on grade of dyaplasia (Dis Colon Rectum 2003;46:786-792), COX-2 may play a some role of carcinogenesis even in serrated pathway. The authors should discuss about it.]

We have added discussion and cited the Dis Colon Rectum article (page 11, paragraph 1). We have also modified in Abstract (page 3) and Discussion (page 12 paragraph 3, page 13 top), emphasizing that COX-2 may still play a role in later stage of the serrated pathway.

[2. The positive and negative controls for COX-2 immunohistochemistry should be described.]

We have put descriptions of positive and negative controls (pages 7-8 top).

[3. Fig. 1 includes only highly magnified photos, and it is difficult to identify which is HP, SSA and SA.]

We have replaced the old photo with a 100x photo of SSA (panel A), so that microscopic features (abnormal architecture of glands) of SSA are clearly seen. We did not provide a photo for HP. The photo of SA (panel C) shows classic features of abundant eosinophilic cytoplasm and serrated appearance, which has been demonstrated well with the original panel C.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)


[1. "proximal tumor" and "distal tumor" should be "tumor of the proximal colon" and "tumor of the distal colon", respectively.]

We used the term “tumor of the proximal colon” and “tumor of the distal colorectum” (page 10, paragraph 2).

[2. Page 4: "COX-2 specific inhibitor" should be "COX-2 selective inhibitor"]

We have changed to “COX-2 selective inhibitor” (page 4, paragraph 1).

[3. Legend of Fig. 1B: "Mixed adenoma" should be "Mixed polyp"]

We have changed to “Mixed polyp” in Fig. 1B legend.

Reviewer #2

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

[1. The scoring criteria for the immunohistochemical stain should be described more specifically. How were the thresholds set for weak vs. strong overexpression? The controls should be described in more detail. Does a "negative" score (i.e. "no overexpression") imply that there is no staining at all, or could there be weak staining (a baseline level of "expression" as opposed to "overexpression")? If yes, how is it distinguished from “weak overexpression”. Was there a minimal percentage of cells that had to be positive? If the distinction between COX-2 negative and weak overexpression is not explained more clearly, one could ask whether the weak overexpression of COX-2 in traditional serrated adenomas could also be interpreted as negative in the sense of low baseline expression, which would change the overall findings of the study.]

Usually, there is very little, if any, “baseline COX-2 staining” in adenomas. Thus, focal staining in adenoma/polyp cells can be regarded as overexpression – there is no minimal percentage. We admit that a distinction between weak and strong staining is arbitrary and subjective. However, we tried our best to categorize weak vs. strong staining in a consistent manner. We have put descriptions of positive and negative controls. Accordingly, the Method section has been revised, incorporating these changes (pages 7-8 top).
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

[1. In this manuscript the term “serrated colorectal polyp” is restricted to SSA and mixed polyps but does not include traditional serrated adenomas. This might be confusing to the reader, as traditional serrated adenomas have elsewhere also been called “serrated polyps”. Therefore, the term “serrated colorectal polyp” should be avoided in order to prevent misunderstandings. The distinction is essential, because according to this manuscript and other publications, traditional serrated adenomas behave differently when COX-2 expression is compared to SSA and mixed polyps.]

We agree that the term “serrated polyp” is indeed confusing and has no clear definition. Accordingly, we have changed the title and replace “serrated polyp” with more specific terms in the text (page 9, paragraph 2) and deleted “serrated polyps” from Table 1 and Table 2.

[2. The fact that traditional serrated adenomas as well as non-serrated adenomas show COX-2 overexpression with about the same frequency is not sufficiently emphasized in the manuscript, where the low levels of COX-2 expression in HP, SSA and mixed polyps are repeatedly compared to non-serrated adenomas only even though COX-2 expression in serrated adenomas is very much like the one in non-serrated adenomas.]

We emphasized this fact in Results section as “Despite the presence of serrated appearance, traditional serrated adenomas showed the frequency of COX-2 overexpression similar to non-serrated adenomas” (page 9, paragraph 2).

[3. It is not quite clear whether the mixed polyps are mixed SSA and tubular/tubulo-villous adenoma or mixed SSA and traditional serrated adenoma or possibly either.]

In the original version, we described in Results as “5 mixed polyps (MPs) with SSA and non-serrated adenoma” in the first paragraph of Results section (now page 8 bottom). We did not have MPs with SSA and serrated adenoma.

Discretionary Revisions (which the author can choose to ignore)

[1. In this study as in other publications, COX-2 overexpression seems to be as common in adenocarcinoma with serration as in adenocarcinoma without serration. Also, COX-2 expression in traditional serrated adenomas seems to be similar to non-serrated adenomas. As the authors point out, these findings suggest that COX-2 becomes more frequent in higher-grade neoplasias. Looking at COX-2 expression in adenomatous polyps]
depending on the degree of dysplasia could help support these conclusions.}

We separated adenoma with intramucosal carcinoma from ordinary adenomas. However, we did not grade the degree of dysplasia in adenomas/polyps without intramucosal carcinoma.

[The low frequency of COX-2 expression seem to be restricted to the more uncommon SSA and mixed polyps as well as hyperplastic polyps (in this study only lesions with a size of >1cm are included). In this context it would be of interest to also specifically discuss COX-2 expression in the serrated vs. the adenomatous component of the mixed polyps.]

Among the 5 mixed polyps examined, only 1 showed weak COX-2 expression in adenomatous portion as well as SSA portion. Because this was only one case, we did not describe it.

[The overall question is, whether the low frequency of COX-2 expression in hyperplastic polyps, SSAs and mixed polyps is a function of the serrated pathway or due to COX-2 overexpression being a "later" event in carcinogenesis.]

This is still an unresolved question. Further studies are necessary to solve this problem. Thus, we have modified Abstract (page 3) and Discussion (page 12 paragraph 3, page 13 top), emphasizing that COX-2 may still play a role in later stage of the serrated pathway.

[2. It is suggested that a second pathologist review the slides to determine the histologic type of the serrated lesions to minimize subjectivity in the classification of these lesions.]

A second pathologist and coauthor M.M. examined all serrated lesions and discrepant diagnoses were resolved by discussion and consensus. We have added description in Methods (page 6, paragraph 2).

[3. Figure 2 might benefit from higher resolution]
It seems difficult to see the photos because these are not stained with hematoxylin, but we believe that COX-2 staining is well visible.

Reviewer #3

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

[This reviewer has only one issue with the paper, which is that the title is misleading. As the authors’ introduction states, serrated polyps usually include the class of serrated adenomas. In serrarted adenomas the authors found the same level of COX-2 over-expression as they did in tubular adenomas. To many readers, who may only skim the title of the study, the exact opposite conclusion will be communicated. The authors should consider changing the title to something akin to:  
“COX-2 overexpression characterizes late but not early serrated colorectal neoplasias.”  
Or some other title that more accurately represents the findings of the study.]

It is indeed confusing and misleading to use the poorly defined term “serrated colorectal polyps”. We have changed to a more explicit title, “Cyclooxygenase-2 overexpression is common in serrated and non-serrated colorectal adenoma, but uncommon in hyperplastic polyp and sessile serrated polyp/adenoma”