Author's response to reviews

Title: Involvement of hyaluronidases in colorectal cancer

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Author's response to reviews: see over
Dear Sir,

Please find attached the thirdly revised form of our manuscript, written by H. Bouga et al., which was corrected according to the reviewers’ comments. In the next page you will find the corrections/answers to the comments, which are also highlighted in the text. We believe in this form the paper is suitable for publication in “BMC Cancer”, as a regular research paper.

Looking forward to hearing from you soon,

Sincerely Yours

Demitrios H. Vynios
Professor of Biochemistry
Reviewer comments:

Although the authors have tried to answer the points raised, still they should clarify the following points before the final version could be accepted for publication. As such the role of hyaluronidases is difficult to interpret.

1. The explanation offered by the authors regarding the progression of cancer by hyaluronidases (for example, create small fragments of hyaluronan initially and removal of ECM finally is hypothetical in nature) need confirmation by experiments. Instead, to avoid complication the authors simply state the role of hyaluronidases colon cancer progression is confirmed here on the basis of the results.

The explanation of our results (end of page 11 and first para of page 12) is based in our previous observations in various cancers and observations by other investigators. The sentence “It would be interesting to identify the size distribution of hyaluronan at early stages of cancer…” indicates one series of experiments that can be applied to verify our hypothesis. Additional experiments that may be applied include the expression of hyaluronan synthases and CD44 and are refered at the end of the same para.

2. The authors stressed on sequential extraction of hyals. Moreover, Hyal-3 was extracted under highly denaturing condition with 4M Guanidium HCl-1% Triton X-100. There are references in the literature that Hyal-3 is separated by Percoll gradient from Sucrose-HEPES-EDTA homogenate. The authors should explain why they have chosen denaturing condition.

When the purification of a membrane-bound or intracellular enzyme is required, a homogenization step is first applied to disrupt the cellular membrane. This is a mechanical denaturation. Thereafter, a variety of protocols can be applied for its characterization. In our work, to understand the role of hyaluronidases in colon cancer progression, the localization of the various isoforms of hyaluronidases was studied using the three-step extraction procedure. This is clearly described in page 12, second line. In addition, as it is also clearly indicated in the results section (page 10, second line), Hyal-3 was solubilized only in detergent-containing denaturing buffer, as they also mentioned, indicating its different localization in the tissue than the other isoforms found, and, of course, the advantage of the applied procedure compared to others involving direct extraction of all enzymes present in a tissue sample. The sentence “This three-step extraction procedure was applied to permit the differential extraction of Hyals isoforms, since accumulated evidence suggests that they are present in either soluble or membrane-bound form [11, 12].” was included in the methods section to describe more clearly the methodology choosen.