Format for ANSWERING REVIEWERS

October 31, 2019

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 51691-revised variant.doc).

First title: High protein and low circulating levels of arylsulfatases A and B can predict the invasive potential of colorectal cancer

Modified tile (based on suggestion of reviewer 1)**: Interaction of arylsulfatases A and B with maspin: a possible explanation for dysregulation of tumor cell metabolism and invasive potential of colorectal cancer**

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Name of Journal: *World Journal of Clinical Cases*

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Reviewer`s 1 opinion

Overall, the paper is well conceived, but in my opinion requires several revisions, outlined below.

1) Although low expression of the ARSB gene is a risk, there is a contradiction that it becomes a risk when all three of the ARSB/ARSA/Maspin proteins are positive, but how do you think about the mechanism?

2) In this paper the correlation between the immunohistochemical (IHC) and gene expression of ARSB has not yet been examined in CRC (Introduction line 7), but later that ARSA's role in CRC is unclear (Introduction line 29). I feel that the reason for investigating the dynamics of ARSB in this paper (not ARSB) is unclear. You should elaborate on the reasons for this survey. Also, the reason for choosing Maspin for quantification is not clear. You should state that in the introduction. Do you need to change the title accordingly?

3) Is there a possibility for concrete clinical application? For example, this time you used surgical specimens, but can you say the same way with biopsy tissue specimens?

Authors' answers

1. Your valuable question added value to the paper because we have determined us to emit a hypothesis, based on our results and literature data. Thank you, indeed!

   Firstly, the gene expression was detected from blood, whereas IHC positivity was checked in tumor tissues. As no previous papers were previously published, it is difficult to explain the mechanism. We might try detecting an interaction among the three markers but experimental studies are necessary to confirm or infirm this hypothesis. Till now, it is known that loss of ARSA/ARSB induce metabolic disorders and decreases in carcinomas. Based on these facts and our result, we can suppose that, in CRC, the arylsulfatases are firstly lost, as result of

metabolism imbalance. In tumor tissue, in parallel with the gene expression, decreased levels are seen in early stages but, in parallel with lymph vessels invasion (presence of lymph node metastases), when the tumor cells metabolism is augmented (metabolic reprogramming), as it was previously described in literature, arylsulfatases might be regained and induce tumor cells aggressively behavior. As regarding maspin, it was previously shown that its expression is correlated with hypoxic-induced angiogenesis and, subsequently, metabolic adaptation of tumor cells and risk for local recurrence or lymph node metastases. This is the first study that showed that, a Maspin-arylsulfatases interaction might exist, and, based on your valuable comments, an interesting hypothesis was released. We have added these data in red in the main text and prove them with bibliographic references.

2. In the introduction, we have added that both of the arylsulfatases can be involved in CRC and we examined IHC both of these enzymes, with gene expression study of the ARSB gene. On the same page, we have clarified the using of maspin, in blue. The title of the article was also modified, to be more attractive.
3. Yes, further studies needed to be done for gene expression in surgical or biopsy material. It could be performed from both, using paraffin-embedded tissues. The clinical application might consist on identification of a substance which might restore the metabolic-induced disorders.

Reviewer's 2 opinion

The authors investigated the role of Arylsulfatase A and B in the development and progression of colorectal cancer. They found that IHC expression of ARSA and ARSB might have a predictor for the prognosis of colorectal cancer.

Authors' answers

Thank you for your precious time spent on doing this review.

Reviewer's 3 opinion

The present study aimed to evaluate the possible prognostic value of arylsulfatase A and/or arylsulfatase B in colorectal cancer, at circulating and protein levels. This is the first study relating expression of ARSB gene in serum with CRC risk. In addition, it is shown that triple positivity for maspin/ARSA/ARSB and ARSB gene expression seems to be indicators of CRC aggressive behaviour, independent of lymph node status. The work is very interesting, since CRC remains one of the leading causes of cancer mortality. Although the number of patients included in the study was rather small, considering the CRC incidence, the results obtained are highly encouraging for further and deeper examination. The m/s is well written, all methods and results are adequately described and discussed. However, there are some points requiring correction and revision to be the m/s acceptable for publication. Major points Page 6, line 15: Chondroitin sulfate and dermatan sulfate are the targets of ARSB, which are glycosaminoglycans. Therefore, this sentence must be corrected to: ARSB is mostly involved in breaking down glycosaminoglycans (GAG), such as dermatan sulfate and chondroitin sulfate. Minor points (grammar/typo errors) General: it is better to use "behavior" in all cases (page 5, lines 1, 13, 17 and elsewhere). Page 4, line 17: "were" instead of "was". Page 5, line 14: literature. Page 7, line 19: aggressiveness. Page 14, line 20: metastasis.

Authors' answers

All the suggested modifications have been done.

Thank you again for publishing our manuscript in the *World Journal of Clinical Cases*

Sincerely yours,

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