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Dear Editor,

We are hereby resubmitting our revised version of the article: **B2A receptors and morphological changes of the enteric nervous system in colorectal adenocarcinoma**, by Ciurea RN et al., for publication in World Journal of Gastroenterology as an original research article.

First of all, we would like to thank the editors and reviewers for their advices and the opportunity to increase the quality of the current presentation.

Please find attached point-by-point responses to each question and issue raised by the editors and referees.

For increased clarity of the changes that have been performed since the original version of the manuscript, we have also included the manuscript with track changes, as a supplementary material.

As the Editors requested, we have now also included as supplementary data the original images without any text or symbols; funding sourcing has been acknowledged; and the Comments section has been completed at the end of figure legends.

I hereby confirm that all authors have seen and agreed with the content of the manuscript. It has not and will not be published elsewhere. We also declare no conflicts of interest.

Thank you.

Sincerely,

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In the current submission Ciurea RN et al investigated the neuro-neoplastic interrelationship in colon cancer. Authors assessed the expression pattern of beta-2 adrenergic (B2A) receptor and morphological changes of the enteric nervous system in 48 primary colorectal cancer and 9 control non-colon cancer specimens. Using multispectral unmixing microscopy they observed differential expression and localization pattern of B2A receptor in different cell types in normal colon mucosae and colon adenocarcinoma specimen. In particular, they have reported the nuclear membrane staining of B2A receptor in colon cancer cells. Authors found that increased B2A receptor expression directly correlated with increased tumor grading, while relative area of Auerbach and Meissner plexuses negatively correlated with tumor grading. Furthermore, authors observed that B2A receptor area and IOD were significantly associated with clinicopathological features in colorectal cancer including tumor size, tumor invasion and lymph node metastasis. Based on these findings authors concluded that B2A receptors could serve as a prognostic factor in colorectal cancer.

Comments:

1. Authors have not cited their previously published work on B2A receptors “Neuro-neoplastic interrelationships in colorectal level – immunohistochemical aspect in three cases and review of the literature”. Did they include the same 3 patients into this larger cohort? Discuss how are the two studies similar/dissimilar?

Answer: Thank you for this observation. We have now cited the previously published work on B2A receptors “Neuro-neoplastic interrelationships in colorectal level – immunohistochemical aspect in three cases and review of the literature”. We have also discussed this study in the Discussion section. The three patients included in the previous study were not included in the cohort within this study.

2. In the group of 16 patients with undifferentiated colon cancer was the perinuclear expression of B2A receptor uniformly distributed in all cells? What percentage of cells were positive for peri-nuclear localization?

Answer: As indicated by the Referee, we have now calculated the percentage of tumor cells that have intra /perinuclear expression for B2A, and this is now clearly detailed in the Results section.

3. In figure 4, D/E/F axis labeling of IOD is missing.

Answer: We thank the Referee for the observation. Integrated optical density (IOD) reports the average intensity/density of each object (basically area  $\times$  average density). Because optical density is a dimensionless quantity, IOD is also reported as a dimensionless parameter.

4. Although B2A receptors have been mentioned in detail in the discussion section, the introduction section should have a brief description of B2A receptors- why is this the focus of the research and what is its relevance?

Answer: Thank you for the notice. We have made a brief description of B2A receptors in the Introduction section and we have explained why we have focused on them in our research.

5. It would be interesting to perform similar clinical outcome studies as a follow up in this patient cohort to assess the association between tumor recurrence and B2A receptor levels.

Answer: We completely agree with the reviewer's observation, and we are indeed planning to collect any further observational data on these patients. This has been now also presented in the Discussion section.

6. Some minor grammatical errors

Answer: thank you, the manuscript has been proof checked again.