Reviewer 2

Overall I found this paper interesting and clinicaly valuable because of potential applicability of the results. I would like to address some comments to authors:

Comment 1:

1. Authors enrolled only patients in stage III and IV therefore the title is a bit confusing, authors should address title to advanced CRC.

Author's Reply:

Thank you for your recommendation. It was considered and changes were made in the title of the manuscript, accordingly:

"New Circulating Circular RNAs in Colorectal Cancer Patients with Diagnostic and Prognostic Potential"

changed to

"New Circulating Circular RNAs with Diagnostic and Prognostic Potential in Advanced Colorectal Cancer".

Comment 2:

2. I am interested in that are there any differences in the tests accuracy between stage III and IV, separately for all studied molecules. Moreover it will be also interesting to combine circ and circ+miRNA into one diagnostic panel.

Author's Reply:

Probably, because of the problem with the visualization of Figure 2 it was not clear that we found differences in AUCs between stage III and stage IV but only for hsa_circ_001445 and hsa_circ_0007915.

We compared the accuracy of the couple hsa_circ_001445 and miR-181b, and of couple hsa_circ_0007915 and miR-106a as diagnostic tests. We found differences between AUCs of ROC curves only for first couple – hsa_circ_001445 and miR-181b (Difference=0.112, St. Error=0.044, Z-statistic=2,579, p=0.010). When we combined hsa_circ_001445 with miRNA-181b and performed ROC analysis we found that this panel could have better accuracy (AUC=0.929 95% CI: 0.884-0.974, p<0.001, Fig. 1, here). Unfortunately, the limited available number of patients (61) with data for miRNAs expression makes the results for such a panel unconvincing. This is a post-hoc analysis and it was not planned in our study it is only hypothesis-generating therefore let us not include it in the revisited manuscript. Also, it includes information only for metastatic patients. We could try to evaluate the circ+miRNA diagnostic panel/s in a larger cohort involving also patients at earlier stages of the disease in our next study.

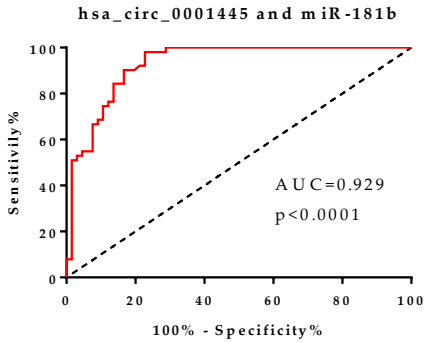


Figure 1. Product of values for hsa_circ_001445 and miRNA-181b for the discrimination between healthy controls and metastatic CRC patients

Comment 3:

3. Authors found one circRNA involved in patients´ survival, please more carefully discuss why exactyl this molecule could be ralated to this event as well as the simple bioninformatics such as KEGG or GO are welcomed

Author's Reply:

Thank you for your comment. Unfortunately, at this stage of our study, we could only speculate why high expression of hsa_circ_0001445 was related to higher survival. We could comment on different other studies that performed functional analyses and investigated different target molecules. For example, Yu et al. (2018) have found that in hepatocellular carcinoma hsa_circ_0001445 sponges miR-17-3p and miR-181b-5p, thus promoting the expression of a well-known tumor suppressor of TIMP3 and a study by Tian & Liang (2018) have reported that hsa_circ_0001445 downregulates the cancerogenic miR-620 in cervical cancer. There are no functional analyzes for hsa_circ_0001445 in CRC, which is a good opportunity to include such analyzes in our future research.

As to GO and KEGG analyses, we thank you for that proposal. Indeed, we are planning to use them to complement functional analyses in our next study. We did not present them in the current manuscript, because it was focused on the levels of investigated circRNAs and whether they have any significance for the clinics, do they have diagnostic and/or prognostic potential.

References

1. Yu, J., Xu, Q. G., Wang, Z. G., Yang, Y., Zhang, L., Ma, J. Z., Sun, S. H., Yang, F., & Zhou, W. P. (2018). Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma. Journal of hepatology, 68(6), 1214–1227. https://doi.org/10.1016/j.jhep.2018.01.012
2. Tian, J., & Liang, L. (2018). Involvement of circular RNA SMARCA5/microRNA-620 axis in the regulation of cervical cancer cell proliferation, invasion and migration. European review for medical and pharmacological sciences, 22(24), 8589–8598. https://doi.org/10.26355/eurrev_201812_16622

Comment 4:

4. Authors should more carefully explain why exactly these circRNAs were selected to the study protocol.

Author's Reply:

Thank you for your comment. Our work in the field of ncRNAs began with a study of the expression of circulating miRNAs in CRC patients. As a result, we detected several miRNAs with abnormal expression in patients' plasma samples. In an attempt to find out the reason for this, we examined our CRC patients for SNP in miRNA genes and whether there are any circRNAs, which act as sponges for miRNAs, involved in tumorigenesis. Therefore, we focus on the selected four circRNAs – three of them have never been studied in CRC before, and only one has been investigated in other studies with CRC patients. The results obtained for these circRNAs encouraged us to summarize our findings in the current manuscript. Our aim was to present their clinical significance.

We are planning to deepen the research on these and other circRNAs, in terms of their functionality, by including new analyzes. We believe that the analysis of circRNAs in the circulation has a future since there is a small number of studies with liquid biopsy samples and even smaller studies with Caucasian cohorts.

Comment 5:

5. Figure no 2 is invisible, perhaps it is a fault of the file conversion, but should be checked before revision

Author's Reply:

We apologize for this inconvenience. We did not notice that Figure 2 was not displayed because we submitted the manuscript as a word file template. Probably the reason Figure 2 is not visible is that we did not paste it correctly in the template. We hope that this technical problem was avoided in the revised manuscript.

We are very thankful for your observations and comments. We are hoping that we have understood your comments and our answers and the corrections in the manuscript are acceptable.