November 5, 2018

Dear Editor,

We appreciate the opportunity to revise our article „**HuR mediated post-transcriptional regulation of inhibitors of apoptosis proteins in pancreatic cancer**" (no. 42941). All comments by reviewers have been addressed, with corresponding changes made directly to the manuscript where appropriate. All corrections are highlighted in the revised manuscript. Detailed point-by-point responses to the reviewers:

Reviewer 1 (code 03408355):

Reviewer: In the methods, 61 PDAC tissues were obtained, but 20 tissues were analyzed by IHC.

Answer: As pancreatic carcinoma tissues were obtained from patients undergoing a partial pancreatodeduodenectomy, only the best tumor's section and slides were used for further immunohistochemistry analysis. However, due to lack of specificity of IAP1 and IAP2 antibodies to non-specific binding, there were inconclusive expression of IAP1 and IAP2 on immunohistological examination so further analysis of more samples was discontinued. Similar results are frequently reported in the protein analysis data bases and/or research papers.

Reviewer: Only one PDAC cell line was used, which was not quite sufficient for providing more reliable conclusions.

Answer: We accept this issue as a limitation of our study due to financial abilities to use more PDAC cell lines. However, we acknowledge this weakness in the "limitation of the study" section in our article and suggest that further investigation must be carried out to use more than one PDAC cell line. In our experiments we used PANC1 cell line that is known to typically reflect the cellular phenotype and genotype of PDAC.

Reviewer: In the statistical analysis, one tailed or two-tailed p value was not mentioned.

Answer: We used two-tailed p value and now according to reviewers' comment, we made correction and mention this in materials and methods' "statistical analysis" section.

Reviewer: The location of PDAC were not described in the results.

Answer: All samples of pancreatic carcinoma were located in the head of the pancreas. Now according to reviewers' comment, we made correction and mention this in material and methods' "Human pancreatic cancer tissues and data collection" section.

Reviewer: In figure 1, two donors had relatively high IAP2 expression. Thus, normal tissue and matched PDAC tissue from the same patient should be collected and more appropriate for the analysis of IAP2 expression.

Answer: This very good remark, that we were considering before, however due to our previous work and some other authors' observations[1], the control group "normal tissue" used from the same cancer patients more accurately known as "tumor adjacent non-tumor tissues" is debatable and raises many questions. Adjacent "normal" tissue could be already influenced by cancer cells that interact extensively with the surrounding micro-enviroment of the tumor and altered pathways could misrepresent some genes and proteins expression. Another limitation would be the lack of detailed information about distances between tumor-adjacent tissues and tumors. Without this, the researchers could not determine whether the characteristics they observed in tumor-adjacent tissues were unique to these cells or part of the disease process of entire organs affected by the development of cancer[1]. Additionally, National Cancer Institute in its article "Study Uses Open Data to Analyze "Normal" Tissue Near Tumors" raises question if normal tissue from another donor that someone collected during surgery could be more accurate control then adjacent normal tissue from the same cancer patient[2]. Therefore, for this reason, in our study we chose normal pancreatic tissue samples obtained through an organ donor as more accurate control group.

Reviewer: A diagram illustrating the role of HuR in pancreatic cancer could be drawn, which may help the readers understand better.

Answer: We included the diagram (Figure 6) illustrating the role of HuR and IAP's in pancreatic cancer.

Reviewer 2 (code 03104467):

As the reviewer had not provided any specified questions, we revised some results and made some corrections according to other's reviewers comments.

Reviewer 3 (code 00698109):

Reviewer: In the text (Fig. 3, 4) described that IAP2 is related with HuR as well as IAP1, but the IAP2 results are not shown. IAP2 results are required in Fig 3, and 4.

Answer: As the correlation of HuR and IAP2 was not very strong compared with IAP1, we didn't include it in the figure. However, now according to reviewer's remarks, the results of HuR correlation with IAP2 are added to the article in figure 3 D. Additionally, the correlation of IAP2 and HuR is mentioned in the results "HuR expression and correlation with IAP1 and IAP2" section.

Figure 4 demonstrates immunoprecipitation experiment that magnetic beads with anti-HuR antibody and protein precipitates showed clear HuR signals, while GAPDH was undetectable. And only then total RNA bound to the precipitated HuR proteins obtained from the PANC-1 cells was isolated and analyzed by qRT-PCR using IAP1 and IAP2 primers that revealed strong only qualitative not quantitative expression that meant the fact that HuR binds to IAP1 and IAP2. This is explained in details in results "HuR protein binds to IAP1 and IAP2 mRNA in pancreatic cancer cells"section.

Reviewer: In Figure 5, IAP2 increased significantly with HuR inhibition, additional explanation and mechanisms should be identified.

Answer: However, we don't know further underlying explanation or mechanisms. According to other authors, HuR has diverse functions and could act by functioning as either an oncogene or a tumor suppressor that regulates the expression of various target genes[3], that might have happened with IAP2 and HuR post-transcriptional regulation. However, as the mechanism underlying HuR and IAP2 mediated carcinogenesis is still unclear, more studies should be done in the future.

We have already notated these contradicted findings in discussion's 3th paragraph: "These finding contradict in part with Jeong-Dan Cha study, where oral cancer cells were transfected with HuR siRNA, HuR and cIAP2 expression were reduced. However, it might be due to different tumor's features. On other hand, it is well established that mRNA stabilizing proteins could exert opposite effects for different

target molecules". Due to reviewer's comment, further supplemented sentences were added in discussion's 3th paragraph: "HuR could act by functioning as either an oncogene or a tumor suppressor, that might have happened with HuR and IAP2 regulation. However, as the mechanism underlying HuR and IAP2 mediated carcinogenesis is still unclear, more studies should be done in the future.

References:

1. Aran D, Camarda R, Odegaard J, Paik H, Oskotsky B, Krings G, Goga A, Sirota M, Butte AJ. Comprehensive analysis of normal adjacent to tumor transcriptomes. *Nat Commun* 2017;[PMID: 29057876 DOI: 10.1038/s41467-017-01027-z]
2. https://www.cancer.gov/news-events/cancer-currents-blog/2017/tumor-adjacent-tissue
3. Wang J, Guo Y, Chu H, Guan Y, Bi J, Wang B. Multiple functions of the RNA-binding protein HuR in cancer progression, treatment responses and prognosis. Int. J. Mol. Sci.2013;**14**:10015–41 [PMID: 23665903 DOI: 10.3390/ijms140510015]

The content of the revised manuscript is original and it has not been published or accepted for publication, either in whole or in part, in any form. No part of the manuscript is currently under consideration for publication elsewhere.

Thank you again for considering to publish our manuscript in the World Journal of Gastroenterology.

Sincerely,

Ausra Lukosiute-Urboniene, MD

Institute for Digestive System Research
Lithuanian University of Health Sciences
Eiveniu str. 4, 50161 Kaunas

Lithuania

E-mail: ausra.urboniene2@lsmuni.lt

Telephone: +370-674-29833

Fax: +370-37-326375