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Buenos Aires, February 21st, 2019

ESPS Manuscript No.: 44797
World Journal of Hepatology

Ya-Juan Ma
Director, Editorial Office

Ke-Qin Hu
Editor-in-Chief

Dear Dr. Ma and Dr. Hu,

We are enclosing the full version of our revised manuscript in Word format (file name: WJH-44797-Revised Manuscript.docx), entitled: **"MicroRNAs contribute to ATP-binding cassette transporter- and autophagy-mediated chemoresistance in hepatocellular carcinoma"** authored by María V. Espelt, María L. Bacigalupo, Pablo Carabias and María F. Troncoso (ID 02447122), to be considered for publication as a Minireview in *World Journal of Hepatology*.

We would like to thank the reviewers for the revision of our manuscript, for their advice and for bringing to our attention important points to be considered in our analysis that undoubtedly improved our manuscript. We made our best to appropriately address all the reviewers' concerns and resubmit the modified manuscript. We are sending the revised manuscript with changes underlined.

A point-by-point response to the reviewers' and editor's comments is included in the following pages.

Briefly, our manuscript was carefully revised by a native English speaker. We submitted a copy of the full approved grant application form. We also changed our manuscript title to a more informative and specific one. We also modified the abstract and included two references as requested.

Hoping that you will find our article suitable for publication in *World Journal of Hepatology*, we thank you very much for your consideration.

Kind regards,

María Fernanda Troncoso, Ph.D

Researcher, National Research Council (CONICET)
Assistant Professor, University of Buenos Aires (UBA)

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Comments from Reviewer No.ID 02523682:

Comment: "The authors described that modulation of ABC transporter and/or autophagy-related gene expression or function by miRNAs could be determinant for HCC chemoresistance. Especially, they emphasized that miRNAs may regulate the biological processes, signaling pathways and/or molecular mechanisms in HCC therapy, and thus miRNA-based therapy together with conventional chemotherapeutic drugs has a great future in cancer therapy. However, some suggestions should be considered. The abstract should be modified and more concise according the main content of this manuscript: miRNA functions in HCC chemoresistance. The title "New insights into molecular mechanisms of chemoresistance in hepatocellular carcinomais" seems to be big and not specific, and is not consistence to the main content of this manuscript, and should be revised. Actually, autophagy plays very important role in tumorigenesis, metastasis, targeted therapy and drug resistance of HCC (World J Gastroenterol. 2018, 24(41):4643-4651). In addition, autophagy is also important mediator for oncolytic virotherapy for HCC (Biochem Biophys Res Commun. 2017, 491(2):469-477)."
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision

Reply to reviewer: We thank you for taking the time to review our manuscript.

-In the revised version, the abstract was modified as indicated below. Changes are underlined. Now, the abstract is more concise and related to our analysis focused on the novel evidence that relates miRNAs, ABC transporters, autophagy and chemoresistance in HCC.

Therefore, the **abstract** was modified as follows (changes are underlined): "Hepatocellular carcinoma (HCC) has an elevated mortality rate, largely because of high recurrence and metastasis. Additionally, the main obstacle during treatment of HCC is that patients usually develop resistance to chemotherapy. Cancer drug resistance involves many different mechanisms, including alterations in drug metabolism and processing, impairment of the apoptotic machine, activation of cell survival signaling, ~~and escape of~~ decreased drug sensitivity and autophagy, among others. ~~Drug efflux is mediated by ABC (ATP binding cassette) transporters which limit cell exposure to chemotherapeutics, allowing tumor cell survival and resistance to death. Autophagy occurs mainly as a response to cellular stress, acting as a cytoprotective mechanism. In normal liver, damaged mitochondria and mutated cells are removed through autophagy, and this mechanism suppresses tumor initiation. But once the tumor is established, autophagy promotes tumor growth and confers protection to HCC cells against antineoplastic drugs.~~ Nowadays, miRNAs are emerging as master regulators of normal physiology- and tumor-related gene expression. In HCC, aberrant expression of many miRNAs leads to chemoresistance. Herein, we particularly analyzed miRNA impact on HCC resistance to drug therapychemoresistance~~, by modulating ABC transporter family protein expression or function and autophagy. Emerging findings reveal that certain miRNAs regulate ABC transporter expression by~~

~~specifically targeting, for instance, *ABCB1* and/or *ABCC1* genes. As most of these miRNAs are downregulated in HCC tissues and cells, P-gp and/or MRP1 expression levels increase and intracellular therapeutic drug accumulation decrease, turning HCC cells less sensitive to death. Further, others miRNAs target autophagy-related gene expression, inhibiting autophagy and acting as tumor suppressors. Nevertheless, many of these molecules are downregulated in HCC, or their levels are reduced after therapeutic drug treatment, thus these miRNAs do not inhibit autophagy or tumor growth and, resistance is favored~~. Certain miRNAs target ABC (ATP-binding cassette) transporter genes. As most of these miRNAs are downregulated in HCC, transporter levels increase and intracellular drug accumulation decrease, turning cells less sensitive to death. Others miRNAs target autophagy-related gene expression, inhibiting autophagy and acting as tumor suppressors. Nevertheless, due to its downregulation in HCC, these miRNAs do not inhibit autophagy or tumor growth and, resistance is favored. Concluding, modulation of ABC transporter and/or autophagy-related gene expression or function by miRNAs could be determinant for HCC cell survival under chemotherapeutic drug treatment. Undoubtedly, more insights on the biological processes, signaling pathways and/or molecular mechanisms regulated by miRNAs are needed. Anyway, miRNA-based therapy together with conventional chemotherapeutic drugs has a great future in cancer therapy."

-Besides, the **title** of the previous version of our manuscript: "New insights into molecular mechanisms of chemoresistance in hepatocellular carcinoma" was replaced by the following: "MicroRNAs contribute to ATP-binding cassette transporter- and autophagy-mediated chemoresistance in hepatocellular carcinoma". We consider that the title of the revised manuscript is more specific than the preceding one and includes the main content of the manuscript.

-Moreover, as suggested by the reviewer we incorporated two articles (References No. 48 and 51 of the revised version).

Therefore, in the **"Autophagy in the development of HCC chemoresistance**" section we incorporated this information as follows (changes are underlined): "In normal liver, damaged mitochondria and mutated cells are removed through autophagy, and this mechanism suppresses tumor initiation. But once the tumor is established, autophagy acts as a pro-oncogenic factor, as it promotes tumor growth[~~47~~] ~~and confers protection to HCC cells against antineoplastic drugs,~~ metastasis and resistance to therapeutic drugs (revised in[47, 48]). Actually, inhibition of autophagy in HCC cells incubated with sorafenib and bevacizumab enhanced cell lethality[~~48, 49~~] [49, 50]. Moreover, autophagy modulation participated in oncolytic virotherapy in a liver cancer stem cell model[51]. Thus, appropriate autophagy regulation could effectively suppress HCC growth and metastasis. In this section, we describe the recent findings concerning the effect of miRNAs in autophagy and chemoresistance in HCC." (page 15 of the revised version).

Accordingly, in the corresponding section the following references were incorporated:

48 **Huang F**, Wang BR, Wang YG. Role of autophagy in tumorigenesis, metastasis, targeted therapy and drug resistance of hepatocellular carcinoma. *World J Gastroenterol* 2018; **24**(41): 4643-4651 [PMID: 30416312 PMCID: PMC6224467 DOI: 10.3748/wjg.v24.i41.4643]
51 **Zhang J,** Lai W, Li Q, Yu Y, Jin J, Guo W, Zhou X, Liu X, Wang Y. A novel oncolytic adenovirus targeting Wnt signaling effectively inhibits cancer-stem like cell growth via metastasis, apoptosis and autophagy in HCC models. *Biochem Biophys Res Commun* 2017; **491**(2): 469-477 [PMID: 28698142 DOI: 10.1016/j.bbrc.2017.07.041]

Comments from Reviewer No. ID 03656580:

Comment: "The precise mechanisms regarding miRNA involvement in resistance will lead us to find new ways of making HCC treatment more effective. The development of miRNAs, miRNAs mimics or anti-miRNA with long half lives and their use in combination with chemotherapeutic drugs could be a powerful option. Undoubtedly, more insights on the biological processes, signaling pathways and/or molecular mechanisms regulated by miRNAs are needed. Anyway, miRNA-based therapy together with conventional chemo-therapeutic drugs has a great future in HCC therapy."
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)

Reply to reviewer: We thank you for taking the time to review our manuscript.

Editor's comments:

Comment 1: "Please provide a certificate letter from a professional English language editing company. For manuscripts submitted by non-native speakers of English, please provide a language certificate from one of the professional English language editing companies mentioned in 'The Revision Policies of BPG for Article'."
Reply to comment 1: We have read the section about Manuscripts submitted by non-native speakers of English included in the "BPG's Revision Policies for Article". Nevertheless, as one of the reviewers classified our manuscript as Grade A, and according to what is declared in the "2.1 Language evaluation" paragraph we do not consider it necessary to make use of a professional English language editing company. Instead, the revised manuscript was carefully checked and corrected by a native English speaker. We are confident that our manuscript will reach Grade A in the language evaluation. The corresponding author signs as guarantee.

Comment 2: "A copy of the full approved grant application form(s), consisting of the information section and body section, should be provided to the BPG in PDF format."

Reply to comment 2: The copy of funding agency approval document was provided in PDF format, as requested. Our grants are from the Argentinean governmental Agency of Science and Technology (Agencia Nacional de Promoción Científica y Tecnológica-Fondo para la Investigación Científica y Tecnológica, ANPCyT-FONCYT), the National Research Council (Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET) and from a State University (Universidad de Buenos Aires, UBA).

Comment 3: "Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039, Fax: +86-10-59080039."
Reply to comment 3: Our telephone number is now in the correct format.

María Fernanda Troncoso