ANSWERING REVIEWERS

March 05, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (ESPS Manuscript NO: 8938).

Title: Characterization of Monocarboxylate Transporters activity in acidic metabolism of primary and metastatic hepatocellular carcinoma microenvironment

Author: Peter Laszlo Lakatos, Gabriella Gyori, Judit Halasz, Peter Fuszek, Janos Papp, Balazs Jaray, Peter Lukovich, Laszlo Lakatos

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8938

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
(1)

1. Venancio et al., demonstrated the expression levels/activities of MCTs, CD147, and GLUT1 in HCC. By IHC analysis, they showed overexpression of MCT4 and GLUT1 is increased in HCC progression, whereas MCT1, MCT2 and CD147, the chaperone of MTCs, decreased during hepatocarcinogenesis. The authors suggest that it may exist another chaperone for MCT4, and link the hypoxia pathway, metabolic pathway, and MCTs expression pattern in HCC development. These results are important and valuable. There are some technical and conceptual points that the authors should be addressed before acceptance of this manuscript.

1. Figure 1 presents the immunostaining of MTCs, CD147 and GLUT1. However, these images are collected from different specimens. It will be nice to show these results by serial sections. Especially the expressions of MCT4 and GLUT1; the expressions of MCT1, MCT2, and CD147.

Authors: This is very nice idea, but unfortunately, for technical reasons this option is not available for the current work.

2. The authors claim that expression levels of MCTs have a prognostic value for HCC. However, there were no any figures/tables to correlate the relationship between MCTs and survival rate.

Authors: The referee is right. The phrase was modified. The casuistic was studied in patients of necropsy routine. Data about survival rates were not available.

Other points

1. First section of Results, lines 2 and 10, “cervical” lesions should be “hepatic” lesion.

Authors: The correction was made.

(2)

General comment: The manuscript entitled “Characterization of monocarboxylate transporters activity in acidic metabolism of primary and metastatic hepatocellular carcinoma microenvironment” documented the expression of monocarboxylate transporters (MCT) in non-neoplastic tissue, primary HCC and metastatic HCC. They found that plasma membrane expression of MCT4 and overall expression of GLUT1 showed progressively increased expression from non-neoplastic to primary HCC to metastatic HCC. Moreover, MCT2 expression negatively correlated to the progression from non-neoplastic, primary to metastasis. Therefore, they concluded that MCT4 and GLUT1 appear to play a role in HCC progression, while MCT2 is lost during progression and associated with better prognosis Specific comments:

1. The title “acidic metabolism” seems not involve in research findings.

Authors: The term acidic is currently implicit in cancer cells expression of MCTs family and other markers as GLUT1, because the direct involvement of these molecules with ionic transport that results in “acidic medium.

2.Figure 1 did not show the position of MCTs and other markers. It could be useful for readers to know where is plasma membrane and where is total expression by labeling with arrow.

Authors: The Figure C is an exception among the other stains because represents a faintly positive reaction of MCT2 that is generally weak in carcinomas; the others reactions were mostly positive in membrane, which is demonstrated. Arrows were included in Figure 1.C.

3. More discussion between plasma membrane and cytoplasm might be useful to understand their findings in the study.

Authors: We added an additional phrase to explain this point at Discussion section. *It is important to emphasize that MCTs and their chaperones are predominantly expressed in plasma membrane, their natural location, in both normal and neoplastic cells. Not surprisingly that the hyper-expression of MCTs and CD147 corresponded more consistently with clinical data in different solid tumours* [3]

(3)

In this manuscript, Alves et. al. examined immunohistochemistry profiles of several monocarboxylate transporters in hepatocellular carcinomas and their metastatic lesions collected from an autopsy series. This is an extension of the group’s previous works on a different tumor type. While the work is somewhat of interest, the following major issues prevent the manuscript from being published. Major issues: 1. The authors examined the IHC profiles using specimens from autopsy cases. However, as the post mortem interval may vary a lot and can be long, its impact on hypoxia metabolites is unknown. In addition, large proportions of patients might have suffered from ischemic events prior to death. It is well documented in the literature that increase expression of stress proteins and up-/down-regulation of downstream targets happen within a short period of time after the onset of hypoxia. Results from autopsy cases thus can be subject to artifacts of global alterations of gene expression in hypoxia related genes. Therefore, the study needs to be validated in resection and transplant specimens.

Authors: This is an interesting point, but despite of these comments, autopsy material is an important tool to evaluate the impact of the tumours in terms of invasion and spread along the body. The major concern regarding the post-mortem alterations mentioned is not a bias since the markers were similarly demonstrate in all cases. The autopsy is performed after 6 hours after the time of death. We did not observe morphological alterations compatible with cellular degeneration. We are consolidating the clinical data of surgical and transplant specimens for validate the results we have obtained in this study.

The treatment modalities of HCC include locoregional therapies (e.g. transarterial chemoembolization, ethanol injection, etc.), and one of the therapeutic mechanisms is to induce tumor hypoxia. It is therefore critical to document whether the patients have had therapies prior to death, the type of therapy, and how were the sections selected (e.g. whether close to tumor necrosis).

Authors: These informations will be now subject of relevant study with surgical and transplant specimens. Many cases in autopsy have no precise information regarding treatment.

Additional minor issues: 1. The authors used tissue microarray as the only tissue source for immunostains and marker expression analysis. Tissue microarray data should be validated by immunostains on regular tissue sections in at least a subset of the cases (especially the negative cases).

Authors: The reactions were previously (data not shown) validate with regular paraffin sections.

2. The authors state that the expression of certain marker correlates with prognosis. This data is not shown. 3. The manuscript contains numerous typo and grammar errors, and needs to be edit by an English-speaking author.

Authors: This information was modified along the text.

3 References and typesetting were corrected

Authors: The references were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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