

Format for ANSWERING REVIEWERS



November 7, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5096-Scaggiante et al.doc).

Title: Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials

Author: Scaggiante Bruna, Maryam Kazemi, Gabriele Pozzato, Barbara Dapas, Rosella Farra, Mario Grassi, Fabrizio Zanconati and Gabriele Grassi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5096

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

Please note that all modifications are reported in green in the revised manuscript.

REFEREE 1

1-The clinical and experimental data is mixed. The authors should report in a tabular form the different prognostic markers that have proven to distinguish between high risk and low risk HCC and data regarding correlation with survival for specific markers.

In the revised manuscript three novel tables addressing the referee request have been added.

2.It has been mentioned at multiple places in the manuscript about potential therapeutic use of some of the markers. Again, a table including status of different therapeutic manipulations and any reported result including result in cell culture / animal models, etc. is desirable.

See answer to referee point 1.

3. A number of different mirna's that have been reported to have association with HCC by other groups are missing. These should be included.

In the revised manuscript we have expanded the miRNA section 3 (page 11 line 1 from bottom, page 12 lines 1-2 from top, page 12 lines 5-11 from bottom, page 13 lines 7-16 from top). Additionally, on page 12 line 1 from top we have also introduced a novel and recent reference (no. 68) which describes the state of the art of miRNA and HCC. We did not expand further the miRNA section 3 as our manuscript is mainly focused on the description of the most novel HCC markers (specified in the revised title, on page 2 line 14 from top, page 3 line 3 from bottom) and it is not intended to cover all the known possible HCC markers.

4.Language and grammatical errors require correction

In the revised manuscript we have corrected the grammatical and language errors.

REFEREE 2

1. Grassi et al., presented a nice overview of the current knowledge on prognostic and therapeutic molecules

involved in HCC. Authors focused on 5 topics due to the broad nature of the subject. However, the topics covered are still very broad. For instance, it is now possible to write a comprehensive review on just one of these topics, such as the microRNAs.

See the response to point 3 of referee 1.

2a. Because this review is broad, it only gives a brief overview on each of 5 topics, rather than a deep critical analysis on every one of them. It would be useful to the readers if the authors can expand it further to include deep discussion on all topics covered

According to the referee request, the revised manuscript has been significantly expanded. In particular:

- In section 2 the following novel lines have been introduced: page 4 lines 6-11 from bottom, page 5 lines 1-5 from top, page 5 lines 11-18 from bottom, page 9 lines 1-10 from bottom, the entire page 10, page 11 lines 1-8 from top;
- In section 3 the following novel lines have been introduced: page 11 line 1 from bottom, page 12 lines 1-2 from top, page 12 lines 5-11 from bottom, page 13 lines 7-16 from top.
- In section 5 the following novel lines have been introduced: page 17 lines 16-18 from top, page 17 lines 27-28 from top, page 17 lines 3-5 from bottom, page 18 lines 6-13 from top, page 18 lines 1-5 from bottom, page 19 line 1-2 from top, page 19 lines 7-8 from top, page 19 lines 10-19 from top, page 19 lines 1-10 from bottom, page 21 lines 7-10 from top, page 22 lines 8-10 from top, page 22 lines 11-20 from bottom.

All together in the revised manuscript four additional pages have been added compared to the first submitted manuscript, thus expanding the text of about 25%. Despite this increment, we again would like to point out that our manuscript is mainly focused on the description of the most novel HCC markers (specified in the revised title, on page 2 line 14 from top, page 3 line 3 from bottom) and it is not intended to cover all the known possible HCC markers.

2b...or reduce it to 2 or 3 most exciting topics and expand them further.

We decided to expand the manuscript.

3. In addition, it would be useful if the authors could include a table for each topic. While it may not be possible to discuss on each marker that was published, a comprehensive listing of markers in tables would certainly benefit the reader.

In the revised manuscript we have introduced three novel tables addressing this request; obviously we have not included all the known HCC markers, just those mentioned in the text of the review.

REFeree 3

1) The work is a list of virtually every factor potentially involved in hepatocellular carcinoma with minimal insight into the studies that have been performed on each.

Our intent was not to list all described HCC markers/target molecules; we are sorry the referee did not appreciate our effort to prepare an update of recent HCC markers/target molecules.

2) Factors are included that cannot possibly serve as biomarkers for this disease (e.g., TGF-beta)

Whereas it is possible that in the next future TGF- β will turn out not to be a relevant marker/target in HCC, several works are in favor of its direct/indirect implication in HCC as indicated in our review (Ref 70 Wang B, Hsu SH, Majumder S, Kutay H, Huang W, Jacob ST, Ghoshal K. TGF β -mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3; Ref 123 Lee D, Chung YH, Kim JA, Lee YS, Lee D, Jang MK, Kim KM, Lim YS, Lee HC, Lee YS. Transforming growth

factor beta 1 overexpression is closely related to invasiveness of hepatocellular carcinoma. Oncology 2012;82 :11-8; Ref 124 Okumoto K, Hattori E, Tamura K, Kiso S, Watanabe H, Saito K, Saito T, Togashi H, Kawata S. Possible contribution of circulating transforming growth factor-beta1 to immunity and prognosis in unresectable hepatocellular carcinoma Liver Int 2004;24 :21-8; Ref 126: Giannelli G, Bergamini C, Fransvea E, Sgarra C, Antonaci S. Laminin-5 with transforming growth factor-beta1 induces epithelial to mesenchymal transition in hepatocellular carcinoma. Gastroenterology 2005;129 :1375-83; Ref 134 Wang YP, Yu GR, Lee MJ, Lee SY, Chu IS, Leem SH, Kim DG. Lipocalin-2 negatively modulates the EMT in hepatocellular carcinoma through the EGF (TGF-beta1)/Lcn2/Twist1 pathway 1. Hepatology 2013 20).

3) *There are no unifying or summary figures. Thus, the work is merely a summary of the literature that sheds little light on the area*

Please see response to referee 2 point number 3.

5) *Finally, the manuscript suffers from major language problems.*

Please see the response to referee 1 point number 4.

We trust that our revised manuscript will meet your and the reviewer's approval.
Looking forward to hearing from you soon, please receive my best regards

Sincerely yours,



Gabriele Grassi, M.D., Ph.D.

Department of Life Sciences
University Hospital of Cattinara,
Strada di Fiume 447,
34100 Trieste, Italy;
Tel: +39-040-3996227;
Fax: +39-040-3994593
e-mail: ggrassi@units.it