Author's response to reviews

Title: Co-culture of primary human tumor hepatocytes from patients with hepatocellular carcinoma with autologous peripheral blood mononuclear cells: Study of their in vitro immunological interactions.

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Author's response to reviews: see over
Athens 29.11.12

To the editor,

We submit the revised manuscript entitled “Co-culture of primary human tumor hepatocytes from patients with hepatocellular carcinoma with autologous peripheral blood mononuclear cells: Study of their in vitro immunological interactions.”

We include the answers of the comments raised by the reviewers.

The content of the manuscript has not been published or submitted elsewhere.

This work has been accepted as a poster in the International Liver Congress™ 2009 - 44th annual meeting of the European Association for the Study of the Liver April 22 - 26, 2009 - Copenhagen, Denmark.

All authors have contributed significantly and agree with the content of the manuscript.

Furthermore no conflict of interest applies to all authors.

We enclose a full point by point description of the changes made and our answers to reviewers’ comments. We believe now that all the comments have been clarified.

We also want to thank once again the reviewers for their valuable comments.

Thank you for considering the revised manuscript for publication in your journal.

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Point-by-point description

Answers to comments

Reviewer 1

Comment 1: The new figure 3 is not convincing, because the two control conditions are lacking: isotype control and positive control (T-cells/lymphocytes and macrophages, respectively). Without these data, the “negative” staining is not interpretable.

Answer to comment 1: In addition, to the new Figure 3 we send you the positive controls of CD3 and CD14 flow cytometric staining, respectively. In order to determine the positive as well as the negative flow cytometric staining of CD3 and CD14 surface markers in the hepatocyte population, we used as positive control the respective staining in the PBMCs population. We send you the positive staining of CD3-FITC and CD14-PE antibodies in the PBMCs population by flow cytometry. The positive staining of both CD3 and CD14 in the PBMCs population is clearly shown in the Figure below. We had performed many experiments in order to define the negative staining of those two antibodies in the hepatocytic population by flow cytometry.
Comment 2: I am furthermore not fully convinced by the MHC-II staining. However, I think it should be easy to confirm this point by real-time PCR.

Answer to comment 2: We have performed the MHC-II staining by flow cytometry in many experiments for both hepatocytes and PBMCs. We have carefully set up the flow cytometric parameters in order to estimate the MHC-II staining. We believe that we have managed to answer (address) our questions concerning the MHC-II expression on hepatocytes and that real-time PCR is not necessary to confirm that at this point.

Answers to comments

Reviewer 2

Minor comment: As a minor comment I recommended to depict standard deviation in their graphs. Although the authors responded to my comment by providing standard deviations the revised changes are not acceptable yet. In figures 4c and 5c graph scales are not correct thus standard deviations are not illustrated completely or seem to be truncated. I recommend changing the graphs’ scales to show standard deviations as a whole. Furthermore SD levels are very high so significant changes between some groups appear remarkable but due to a collective of 11 patients/cell preparations it may be possible.

Answer to comment: The graph’s scales have been changed in Figures 4c and 5c. The SD levels are clearly now illustrated.