Author’s response to reviews

Title: Therapeutic potential of placental mesenchymal stem cells after transplantation through portal vein into Chinese miniature pigs with acute liver failure

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Author’s response to reviews: see over
Dear Dr. Lee,

In response to your email communication on February 21, 2012 regarding our manuscript (MS: 1537165508601985), we have completed the experiments suggested by the reviewer and would like to address her comments point-by-point as follows:

**Reviewer: Dr. Tamara Vanhaecke**

**Major revisions:**

- in order to further characterize the hPMSC-obtained hepatocyte-like cells, the authors have, as requested by both reviewers, additionally investigated LDL uptake, glycogen storage, urea synthesis and CYP3A4 activity. However, the measured values are compared versus HepG2 cells, which are hepatoma cells and thus not normal primary human hepatocytes at all. The levels for e.g. CYP3A4 activity they find for the hPMSC-obtained hepatocyte-like cells are even significantly lower than the HepG2 values, which exhibit several magnitudes lower activity compared to normal primary human hepatocytes. Therefore, the only good reference that should be used here are primary human hepatocytes and thus values of the latter for all extra activity measurements performed should be included so that the reader does not get the false impression that 'fully functional' hepatocytes were obtained out of the hPMSC! Also in the discussion section this huge difference in activity level should be discussed.
Response: We concur with the points and suggestions made by the reviewer, and conducted additional experiments examining LDL uptake, glycogen storage, urea synthesis and CYP3A4 activity of the normal primary human hepatocytes and compared the hMSCs-derived hepatocytes-like functions with them. As the reviewer rightly predicted, our results do show that hepatocyte-like cells derived from hMSCs had much lower levels of LDL uptake and CYP-450 enzymatic activities compared with primary human hepatocytes. It is of note that in order to keep the total cell number equivalent and comparable, we plated $5 \times 10^5$ hepatocytes (per well) which were cultured for only one day versus previously $6 \times 10^3$ C3A cells (per well) were plated but cultured for longer time (six days).

We think there are at least three explanations for the differences. Firstly, the functionality of hepatocytes in vivo relies on a 3-dimensional (3D) environment which differs significantly with the 2-dimensional (2D) culture conditions in vitro. Primary hepatocytes will quickly lose activities during in vitro culture. For example, our previous data showed that at the sixth day of in vitro culture, the hepatic CPY3A4 activity dropped down to one-fifth of that at the second day. 3D culture environment promotes cell-cell interactions that may further enhance maturation and function. Secondly, although the majority of the cells in the liver are hepatocytes, the liver also contains other cell
types, such as Kupffer cells, liver endothelial cells, and so forth. These cells may have an effect on hepatocyte maturation and liver function. The isolated primary human hepatocytes under \textit{in vitro} culture conditions lack such supporting system which may partially account for their lower enzymatic activities and functionalities as compared to hepatocytes \textit{in vivo}. Thirdly, it also remains a possibility that our current culture conditions may not be optimal for further hepatocyte maturation, and future work on maturation will need to take these limiting factors into account.

In response to the reviewer’s suggestions, we made appropriate changes to the ‘Methods’ (p. 13–15), ‘Results’ (p. 23) and ‘Discussion’ (p. 29-30) sections, and Fig. 6 in the revised manuscript.

We thank the reviewer for her helpful comments and suggestions, and thank you very much for offering us with the opportunities to improve our work. We look forward to hearing from you further.

With best regards,

Lanjuan Li

February 24, 2012