CJanuary 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8067-review.doc).

Title: Human Liver stem/progenitor cells decrease serum bilirubin in hyperbilirubinemic Gunn rat

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Name of Journal: *World Journal of Gastroenterology*

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

1 Format has been updated: columns category, core tip, comments including background, research frontiers,… and PMID reference number were added. All of the modifications are identified by grey color police, briefly all the abbreviations are detailed, the term "implantation rate" was changed for "repopulation ratio" and figure 4 was changed.

2 Response to reviewer:
"This study shows an interesting result that ADHLSC (adult stem/progenitor cells from adult human liver) engraftment into Gunn rat's liver can significantly reduce the serum bilirubin levels, suggesting that ADHLSC might be a promising candidate for treatment of Crigler-Najjar type I syndrome.

1. I hope the authors may further state that as an adult stem/progenitor cells, whether ADHLSC may show stem cell markers? And why these cells do not express biliary markers and do not differentiate into biliary cell?"

   ADHLSCs used in the current study were extensively characterized for their stemness expression profile as well as their plasticity. These cells as mentioned in the manuscript were produced under GMP setting for human transplantation purposes [1]. ADHLSC were obtained after primary cultures of liver cell suspensions predominantly constituted by hepatocytes (more than 95%). ADHLSC became predominant because of their proliferation potential and their population homogenous. The process may be related to the existence of an original liver resident cell present in the initial cell

suspension and/or to a reprogramming process of isolated hepatocyte that may happen during the in vitro de-differentiation process of the primary culture.
ADHLSC express several markers of adult mesenchymal stem/progenitor cells and of liver origin. Indeed, ADHLSC are immunopositive for membrane markers, like for the well described bone marrow MSC, among which CD 29 (integrin β1), CD 44 (receptor for hyaluronic acid), CD 73 (5'-nucleotidase), and CD 90 (Thy1).
The mesenchymal expression profile was also confirmed by the non-expression of hematopoietic markers like CD133 and CD117.
The hepatic origin of ADHLSC was demonstrated by the immunopositivity for albumin and alpha-antitrypsin, glucose-6-phosphatase (G6P), Gamma-glutamyltransferase (GGT),…[2]. However, ADHLSC did not express any of the hepatic cytokeratins (both hepatocytic and biliary), which is also in accordance with their non-epithelial shape (in contrary to epithelial biliary cells). We have additional evidences of their endodermic origin like for sox17 and GATA4 expression (manuscript in preparation).

Compared to other MSC of extra-hepatic origin, we further demonstrated that ADHLSC displayed an advanced hepatogenic differentiation potential both at the expression and the functional level. In vivo, we did not detect human biliary markers staining in the livers of animal transplanted with ADHLSC

Altogether, this supports the hypothesis that ADHLSC are liver stem/progenitor cells exhibiting an advanced hepatocytic and not a biliary lineage.

2. "What is the outcome of ADHLSC after 60 days post-transplantation into Gunn rats (UGT1A1 deficient animal)?"

   In this paper we conducted a long-term study and showed that the serum unconjugated bilirubin levels were significantly decreased 27 weeks post-transplantation. These effects were already noticed after 8 weeks whereas no effect was seen before, suggesting that transplanted ADHLSC were progressively implanted, differentiated in situ before becoming functional and able to conjugate UCB. These data were in accordance with those of Khuu et *al*.[3] illustrating the engraftment and in situ differentiation potential of ADHLSC in an immunodeficient SCID mouse model 2 months post transplantation.

3. "I hope the authors may further discuss the limitation or potential risk of ADHLSC-based cell therapy, and your strategy."

   The safety issues related to ADHLSC have been extensively addressed at different level:
   - Scheers et *al*.[4] illustrated the presence of appropriate gatekeeper mechanisms against transformation in long term culture. Indeed, 3 of the 12 populations exhibited cytogenetic anomalies, these anomalies were not correlated with tumorigenic potential in vitro or in vivo, and expression of cell cycle-related genes was appropriately upregulated, inducing senescence. In parallel, although chromosomal anomalies may occur in long-term cell cultures, neither transformation nor alteration of their characteristics was noted during in vitro expansion. All ADHLSCs reached senescence and growth arrest.

- We confirmed the genetic and phenotype stability of our cells after large scale production.

- Ten millions ADHLSC were injected in Nude mice and no tumor formation was detected after 24 weeks post-subcutaneous injection

- We confirmed the liver homing of ADHLSC using ^{111}Indium marked cells in a model of catheterized rats after 1h, 24h and 72h; by confirming that less than 5% of the whole body signal was localized outside of the liver [1, 5, 6].

1. Sokal EM., S.X., Ottolenghi C., Jazouli N., Clapuyt P., Lacaille F., Najimi M., de Lonlay P., Smets F., *Liver Engraftment and Repopulation by In Vitro Expanded Adult Derived Human Liver Stem Cells in a Child with Ornithine Carbamoyltransferase Deficiency.* JIMD Reports, 2014. **In press**.
2. Najimi, M., et al., *Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes?* Cell Transplant, 2007. **16**(7): p. 717-28.
3. Khuu, D.N., et al., *Adult human liver mesenchymal stem/progenitor cells participate in mouse liver regeneration after hepatectomy.* Cell Transplant, 2013. **22**(8): p. 1369-80.
4. Scheers, I., et al., *Adult-derived human liver progenitor cells in long-term culture maintain appropriate gatekeeper mechanisms against transformation.* Cell Transplant, 2012. **21**(10): p. 2241-55.
5. Tatiana Tondreau, C.M., Stephan Walrand, Muriel Helbo, Nawal Jazouli, Mustapha Najimi , Vinciane Wouters, Eddy Rommel, Fran çois Jamar, Etienne Sokal. , *In vivo biodistribution of Adult Human Liver Stem/Progenitor Cells following intraportal transplantation in catheterized rats.* Plos One, 2014. **Submitted**.
6. Defresne F., T.T., St éphenne X., Smets F., and N.M. Bourgois A., Jamar F., Sokal EM., *Biodistribution of adult derived human liver stem cells following intraportal infusion in a 17 years-old patient with glycogenosis type 1A.* Nuclear Medicine and Biology, 2014. **In press**.

Revision has been made according to the suggestions of the reviewer

3 References and typesetting were corrected

4 The copyright assignment will be sent this week

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

Sincerely yours,

Cédric Maerckx Etienne Sokal