Human hepatobiliary progenitor cells as new candidates for liver cell therapy
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Introduction
Orthotopic liver transplantation is the only treatment for patients with end-stage liver diseases; however, this methodology is limited due to a shortage of organ donors and liver cell therapy has been suggested as an alternative treatment. Injection of hepatocytes has been tested but this method suffers from practical limitations (difficulty in expansion and cryopreservation). The present work describes the isolation and culture of human hepatobiliary and mesenchymal progenitor cells called H2Stem cells starting from unsuitable organ for transplantation. These cells can differentiate in vitro into hepatocytes in both 2D and 3D culture system. Furthermore, liver derived H2Stem stem cells showed in vivo engraftment and in situ differentiation in pre-clinical models of liver regeneration (Figure 3).

Results

H2Stem cells isolation and use

Material and Methods

H2Stem isolation
For H2Stem cells isolation, human liver parenchymal cell suspensions are seeded on type I rat collagen coated dishes, in William’s E Medium supplemented with FBS, Dexamethasone, Insulin and Splethall Growth Factor. After 7-12 days of culture, clusters formed by H2Stem cells appear and are trypsinized within the next 2-3 days. H2Stem cells are then cultivated at 1,000 cells/cm² in the same medium supplemented with Hepatocytes Growth Factor.

Cells identity and characterization
Identity of H2Stem cells has been verified for different liver sources. Expression of positive (CD90/CD73/CD166/CD146/Akines) and negative markers (CD34/CD3/CD133) defining the cell population is assessed after 2 passages in culture, using flow cytometry and immunofluorescence stainings.

2D Differentiation
H2Stem cells were cultivated on Collagen Type I coated plates at a density of 10,000 cells/cm² in the proliferation medium. Hepatic differentiation is started upon 95-100% confluence by change of media into the differentiation medium composed of IMDM containing 20ng/ml HGF, 20ng/ml EGF, 1µM Dexamethasone and 1% Insulin:Transferrin:Cholecalciferol. The cell culture medium for hepatic differentiation is changed twice a week until the latest 2 weeks. CYP3A4, Albumin, Alpha-fetoprotein and albumin-secretion were analyzed using commercially available kits (Promega and Abcam).

3D Cultures
H2Stem cells Spheroids were produced by spontaneous aggregation of cells in low attachment 96 well plates. After 10-20 days incubation in the proliferation (Undifferentiated spheres) or in the differentiation medium (Differentiated spheres) spheres were harvested for analysis. Expression of Vimentin, CD34, CD3, CD133 and HNF4A was assessed by immunostaining in the differentiated spheres. CYP3A4 activity was evaluated on both undifferentiated and differentiated 3D cultures, using a commercially available kit (Promega).

Injection in FRG mice model
To evaluate H2Stem engraftment and functionality in vivo, the FRG mouse model was used. The FAD/- phenotype decreases hepatic function and to an almost complete if hepatic function is not restored through reopulation with healthy cells. This model has been largely applied for evaluating reopulation with human hepatocytes as well as with hepatocytes derived from iPSC. In this study, FRG mice were injected with 500,000 cells/animal by the intra-portal route. Human albumin in blood was measured every 2 weeks and immunohistochemistry performed on mice livers to show H2Stem cells engraftment and functionality.

Discussion
In this study we describe the isolation of hepatobiliary and mesenchymal progenitor cells called H2Stem from human livers unsuitable for transplantation. These cells can present the ability to grow in culture and can be differentiated in vitro into hepatocyte like cells in both 2D and 3D. In addition, H2Stem cells can engraft in livers of FRG mice showing a mature hepatic phenotype in vivo. Based on this set of data we postulate that H2Stem cells could be the perfect cell candidate for liver cell therapy, proposed as an alternative to orthotopic liver transplantation. Developments are currently ongoing to further expand H2Stem stem cells in vitro under GMP-like conditions before evaluating safety and efficacy in patients affected by liver diseases.