Research Article

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Transplantation of a human iPSC-derived hepatocyte sheet increases survival in mice with acute liver failure

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Background & Aims: Hepatocyte transplantation is one of the most attractive approaches for the treatment of patients with liver failure. Because human induced pluripotent stem cell-derived hepatocyte-like cells (iPS-HLCs) can be produced on a large scale and generated from a patient with liver failure, they are expected to be used for hepatocyte transplantation. However, when using conventional transplantation methods, i.e., intrasplenic or portal venous infusion, it is difficult to control the engraftment efficiency and avoid unexpected engraftment in other organs because the transplanted cells are delivered into blood circulation before their liver engraftment.

Methods: In this study, to resolve these issues, we attempted to employ a cell sheet engineering technology for experimental hepatocyte transplantation. The human iPS-HLC sheets were attached onto the liver surfaces of mice with liver injury.

Results: This method reduced unexpected engraftment in organs other than the liver compared to that by intrasplenic transplantation. Human albumin levels in the mice with human iPS-HLC sheets were significantly higher than those in the intrasplenically-transplanted mice, suggesting the high potential for cell engraftment of the sheet transplantation procedure. In addition, human iPS-HLC sheet transplantation successfully ameliorated lethal acute liver injury induced by the infusion of carbon tetrachloride (CCl_4). Moreover, we found that the hepatocyte growth factor secreted from the human iPS-HLC sheet played an important role in rescuing of mice from acute hepatic failure.

Conclusions: Human iPS-HLC sheet transplantation would be a useful and reliable therapeutic approach for a patient with severe liver diseases.

Keywords: iPS cells; Hepatocyte; Cell sheet; Transplantation.
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Introduction

Hepatocyte transplantation is one of the most attractive approaches for the treatment of patients with liver failure, including patients with hepatocellular carcinoma, and drug-induced liver injury. However, the use of human hepatocytes presents difficulties due to the limited variety of donor sources, limited cell proliferation potential, and allogenic immune rejection in the transplantation setting. Because human induced pluripotent stem cells (iPSCs) [1] can proliferate infinitely and can be generated from a patient with liver failure, human iPSC-derived hepatocyte-like cells (iPS-HLCs) have the potential to resolve these problems, and are expected to be used in hepatocyte transplantation. We have recently succeeded in generating highly functional human iPS-HLCs by a combination of overexpression of hepatic transcription factors and three-dimensional (3D) culture [2–5]. The hepatic gene expression levels of the human iPS-HLCs were comparable to those of primary human hepatocytes [4]. Therefore, we anticipate that our human iPS-HLCs may be a potential cell source for hepatocyte transplantation.

Experimental investigations using rodent models are expected to contribute technical improvements to the clinical methods of hepatocyte transplantation. In previous reports, human hepatocytes and human iPS-HLCs were transplanted into the rodents with liver injury by either intrasplenic or portal venous infusion [6–8]. These hepatocyte transplantation methods have some disadvantages, including difficulties with the regulation of

engraftment efficiency and the potential for engraftment in other organs, since the transplanted hepatocytes are distributed into other organs as well as the liver following delivery through blood circulation [9,10]. Although several groups have transplanted human iPS-HLCs into mice [8,11,12], the engraftment efficiencies in the transplanted mice were limited. Moreover, researchers recently reported that the transplanted cells were engrafted at an unexpected site; the peritoneum [8]. Therefore, the methods of iPS-HLC transplantation must be improved before their clinical use in human transplantation.

Recently, a cell sheet-based tissue engineering approach has attracted much attention in cell transplantation therapy [13–17] because of its potential for delivering a large number of cells to the desired organ without loss of transplanted cells. A previous study has shown that the cell viability after islet cell sheet transplantation was higher than that after direct cell infusion [18]. Moreover, cell sheet transplantation of myoblasts and cardiac cells provided higher therapeutic values and achieved greater amelioration of heart failure in mice than a direct cell infusion approach [19,20]. Therefore, it might be reasonable to expect that a cell sheet engineering approach would realize improvements in the control of engraftment efficiency and the avoidance of unexpected site engraftment of human iPS-HLCs. To date, several researchers have conducted hepatocyte sheet transplantation into ectopic sites (e.g., the subcutaneous space) and succeeded in achieving therapeutic values in experimental animal models [14–16]. However, the feasibility and therapeutic values of human iPS-HLC sheet transplantation on the liver surface (orthotropic transplantation) have yet to be clarified.

In the present study, we first created the human iPS-HLC sheets by culturing the human iPS-HLCs in Temperature-Responsive Culture Dishes (TRCDs) [21] and then harvesting them in a sheet format using temperature change-dependent cell harvesting. We then transplanted the human iPS-HLCs to two-thirds partially hepatectomized mice either by a cell sheet transplantation or direct cell infusion method (infused intrasplenically). To compare the safety of these two transplantation methods, the distribution of the transplanted cells in these recipient mice was assessed by semi-quantitative PCR. In addition, the human albumin (ALB) levels in these recipient mice serum were measured to assess the engraftment efficiency of the human iPS-HLCs in these two transplantation methods. Moreover, we conducted an additional experiment to determine the therapeutic value of human iPS-HLC-based cell transplantation. To induce lethal acute liver failure, we administered carbon tetrachloride (CCl_4) to the mice before transplantation of the human iPS-HLC sheet or the human iPS-HLC suspension, and then we assessed the survival rates in each groups.

Materials and methods

Cell sheet-harvesting procedure

On day 35, human iPS-HLCs were seeded onto a 24-well TRCD (CellSeed). Two days after the seeding, the human iPS-HLC sheet was harvested as a contiguous cell sheet by using a CellShifter (CellSeed) support membrane according to the manufacturer's instructions with some modifications. CellShifter was developed exclusively for harvesting cells as a cell sheet. Briefly, on day 37, a CellShifter support membrane was cut in half (approximately 0.95 cm^2/piece), and one of the half sheets was placed on top of the human iPS-HLCs. The culture temperature was then decreased to 20 °C for 30 min, and the CellShifter support was removed. The human iPS-HLCs were thus harvested as a monolayer cell sheet attached to the CellShifter support.

Transplantation of the hepatocyte-like cells

Before transplantation, the liver capsules of intended sites were removed by rubbing the liver surface using a swab. To transplant the harvested human iPS-HLC sheet, the sheet was placed onto the liver surface of recipient mice with the CellShifter. Approximately 5 min later, the CellShifter was removed while leaving the human iPS-HLC sheet on the liver surface of the recipient mice. These operations were repeated. The number of human iPS-HLCs transplanted in this manner was approximately 8×10^5. In sham-operated mice, an unused CellShifter support (i.e., without attached iPS-HLCs) was placed onto the liver surface and removed approximately 5 min later, and this procedure was also repeated in the same way as in the experimental group. To prepare the human iPS-HLC suspension, the harvested human iPS-HLC sheet was suspended into the hepatocyte culture medium (Lonza) using 0.05% trypsin (Invitrogen). A suspension of 8×10^5 viable human iPS-HLCs was then infused into the inferior splenic pole of recipient mice. Note that the total cell number transplanted per mouse was equal between the human iPS-HLC sheet and human iPS-HLC suspension group. These procedures were finalized by closing the skin wound.

Results

To generate the human iPS-HLCs, hepatic differentiation was performed using a combination of stage-specific transient transduction of FOXA2/HNF1α and monolayer/3D culturing as shown in Fig. 1A [4]. To evaluate the hepatic characteristics of the human iPS-HLCs, the gene expression levels of hepatocyte-related markers were examined (Fig. 1B–E). The results showed that the gene expression levels of alpha-1-antitrypsin (*αAT*), cytochrome P450 3A4 (*CYP3A4*), and *CYP1A2* in the human iPS-HLCs were comparable to those in primary human hepatocytes that were cultured 48 h after plating the cells (PHH48hr) (Fig. 1B, D, and E, respectively). The gene expression level of *ALB* in the human iPS-HLCs was significantly higher than that of PHH48hr (Fig. 1C). Moreover, the percentages of both ALB- and asialoglycoprotein receptor 1 (ASGR1)-positive cells were approximately 80% (Fig. 1F). In contrast, the percentage of keratin 7 (CK7; a cholangiocyte marker)-positive cells was approximately 20% (Supplementary Fig. 1) Thus, these results suggested that the human iPS-HLCs were efficiently and almost homogeneously generated from human iPSCs in this study, just as in our previous study [4,22]. On day 35, the human iPS-HLCs were passaged and cultured on a TRCD for 2 days, and then the human iPS-HLC sheets were harvested using a CellShifter by reducing the culture temperature to 20 °C for 30 min. At that time, the vertical sections of the harvested human iPS-HLC sheet were prepared (Fig. 1G–I). We found that the human iPS-HLCs were successfully harvested in a monolayer cell sheet format (Fig. 1G). Immunofluorescence staining revealed that the human iPS-HLCs in a sheet format were positive for hepatic markers (cytokeratin (CK) 18 and alpha-1-antitrypsin (αAT)) (Fig. 1H and I, respectively). Therefore, we confirmed that the human iPS-HLC sheets were successfully fabricated using a TRCD and a CellShifter. Based on this information, we further conducted transplantation experiments to determine the feasibility and the therapeutic values of cell transplantation using the human iPS-HLC sheets. The transplantation procedure is schematically shown in Fig. 1J. An image of the human iPS-HLC sheets attached onto the mouse liver surface is shown in Fig. 1K.

In order to determine the usefulness of the human iPS-HLC sheets as a novel form of hepatocyte transplantation, the human iPS-HLC sheets or human iPS-HLC suspension were transplanted into the two-thirds partially hepatectomized mice (PHx mice). First, the distribution of the transplanted cells was examined by